Safety Assessment of Ethyl Tafluprostamide and Isopropyl Cloprostenate as Used in Cosmetics

Status:	Draft Tentative Report for Panel Review
Release Date:	September 11, 2026
Panel Meeting Date:	October 5 – 6, 2026

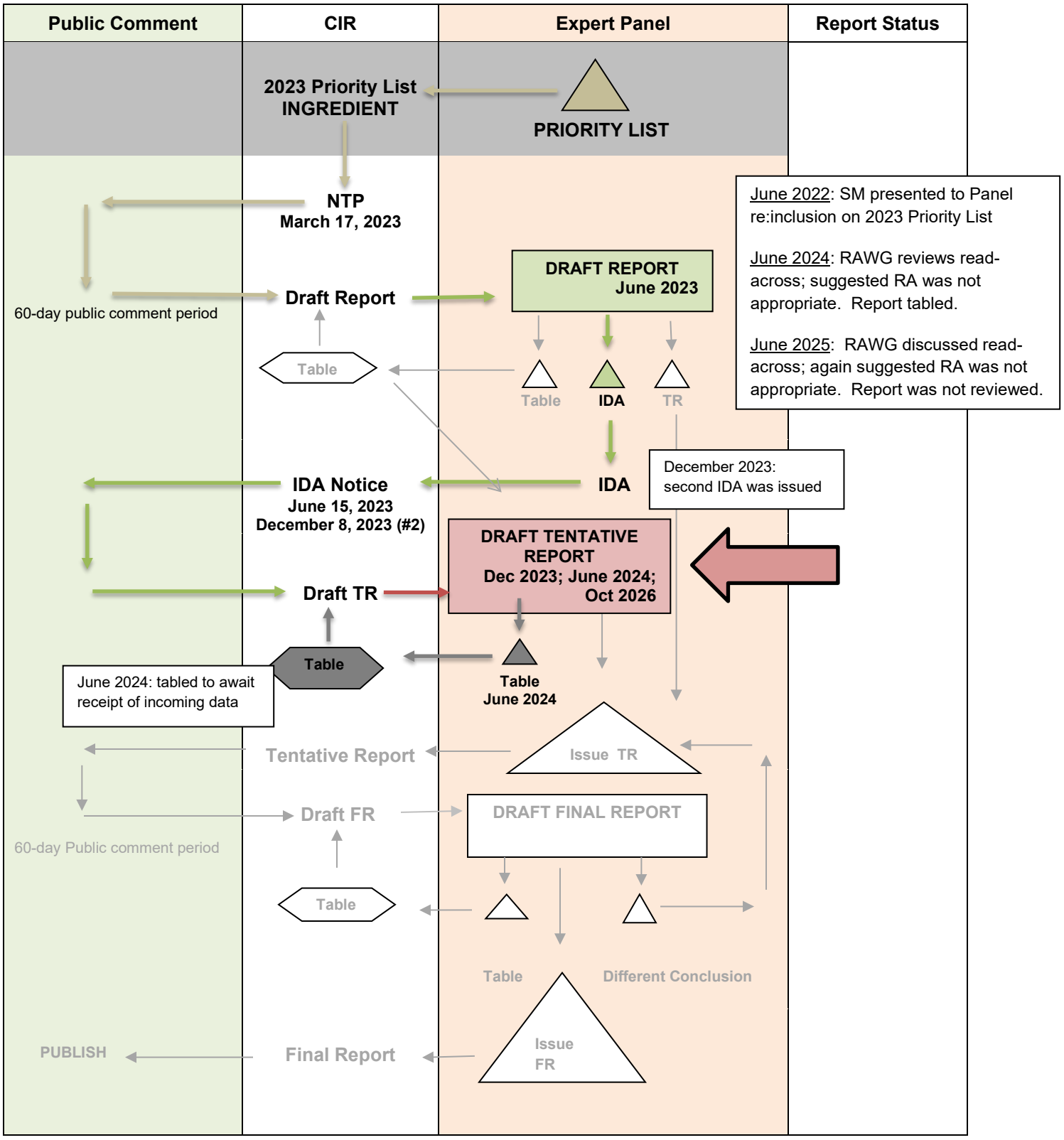
The Expert Panel for Cosmetic Ingredient Safety members are: Chair, Wilma F. Bergfeld, M.D., F.A.C.P.; Donald V. Belsito, M.D.; Bruce A. Brod, M.D., M.H.C.I., F.A.A.D.; Samuel M. Cohen, M.D., Ph.D.; Curtis D. Klaassen, Ph.D.; Allan E. Rettie, Ph.D.; David Ross, Ph.D.; Paul W. Snyder, D.V.M., Ph.D.; and Susan C. Tilton, Ph.D. Previous Panel member involved in this assessment: David E. Cohen, M.D. The Cosmetic Ingredient Review (CIR) Executive Director is Bart Heldreth, Ph.D., and the Senior Director is Monice Fiume, M.B.A. This safety assessment was prepared by Priya Ferguson, M.S., Associate Toxicologist & Senior Scientific Analyst/Writer, CIR, and Jinqiu Zhu, Ph.D., DABT, ERT, DCST, CIR Toxicologist.

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SAFETY ASSESSMENT FLOW CHART

INGREDIENT/FAMILY Ethyl Tafluprostamide and Isopropyl Cloprostenate
MEETING October 2026



COSMETIC
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Memorandum

To: Expert Panel for Cosmetic Ingredient Safety Members and Liaisons
From: Priya Ferguson, M.S., Associate Toxicologist and Senior Scientific Analyst/Writer, CIR
Date: September 11, 2026
Subject: Revised Draft Tentative Report on Ethyl Tafluprostamide and Isopropyl Cloprostenate

Enclosed is the Revised Draft Tentative Report on the Safety Assessment of Ethyl Tafluprostamide and Isopropyl Cloprostenate as Used in Cosmetics. (It is identified as *report_ProstaglandinAnalogues_102026* in the pdf document). Please recall that at the December 2023 meeting, the Panel issued a second Insufficient Data Announcement (IDA) for Ethyl Tafluprostamide and Isopropyl Cloprostenate, and requested the following data:

- for Ethyl Tafluprostamide:
  - acute toxicity data
  - repeated-dose toxicity data
  - developmental and reproductive toxicity (DART) data
  - in vivo genotoxicity data
  - targets and mechanisms 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
  - targets and mechanisms data

Furthermore, at the June 2024 meeting, the Panel decided to table the report for the receipt of incoming data, based on a memo from industry describing on-going studies that would be submitted to the Panel. The data types that were outstanding at the time of that meeting were:

- Data on Ethyl Tafluprostamide:
  - receptor binding potency studies
  - in vitro neutral red uptake assays
  - ReproTracker assay
  - ToxProfiler assay
  - in silico endocrine receptor/activation predictions
  - literature research on endocrine receptor activation by analogues
  - analysis of differences in metabolism due to germinal fluorines in the suitability of analogues in read-across analyses
  - report on read-across analyses
- Data on Isopropyl Cloprostenate:
  - QSAR assessment
  - dermal metabolism and penetration assay

Also at the June 2024 meeting, the Panel requested an ophthalmological analysis (by an independent ophthalmologist) on ocular parameters (e.g., colorimetry) examined in the 8-wk clinical assay previously submitted by industry on Isopropyl Cloprostenate. In addition, the Panel requested information regarding the potential receptors associated with the prostaglandin analogues reviewed in this report, along with binding/potency/K_i values.

The Panel previously received an earlier iteration of this Draft Tentative Report in preparation for the December 2025 meeting; however, review of the report was subsequently postponed. The report is now being presented for review at the October 2026 meeting, and additional updates have been incorporated since the version distributed for December 2025. All changes made since the last iteration formally reviewed by the Panel (i.e., the June 2024 meeting version) are highlighted the report in yellow. Panel members may have notes from the December 2025 version; however, please be aware that the current report contains additional information and revisions not included in that version. New data added to the report since the June 2024 meeting include the following (** indicates data that were not included in the report version originally distributed for the December meeting):

- Data submission regarding Ethyl Tafluprostamide (*data1_ProstaglandinAnalogues_102026*); submission contains the following:
    - An overview on what was previously submitted to Panel
    - Comparative data on tafluprost and bimatoprost with Ethyl Tafluprostamide
    - In vitro tests (ToxProfiler, ReproTracker, and 3T3 NRU assay) on systemic mechanisms of toxicity on Ethyl Tafluprostamide, tafluprost, and bimatoprost
    - PGF2α receptor binding assay for Ethyl Tafluprostamide, tafluprost, and bimatoprost
    - Literature search identifying receptor binding/activation data on Ethyl Tafluprostamide, tafluprost, and bimatoprost
    - In silico analysis using QSAR models to assess similarity of Ethyl Tafluprostamide, tafluprost, and bimatoprost, with respect to receptor binding and activation potential
    - Literature search on in vivo studies for tafluprost and bimatoprost to assess their suitability for assessment of Ethyl Tafluprostamide systemic toxicity
    - Analysis of the validity of using the presented read-across analysis to assess the safety of Ethyl Tafluprostamide as a cosmetic ingredient
    - Analysis of differences in metabolism due to the presence of the 'germinal flourines' functional group in the frame of the read-across approach
    - In-house testing evaluating the amount of cosmetic eyelash product per brush stroke in mink hair
    - Read-across justification report in accordance with the ECHA Read Across Assessment Framework
    - Updated MOS calculation informed by the comparative analysis of new data collected and analyzed on Ethyl Tafluprostamide, tafluprost, and bimatoprost
    - Calculation of toxicological screening value
    - Summarized data on the previously submitted toxicity assays on tafluprost and bimatoprost
- Supplemental toxicological safety assessment of Isopropyl Cloprostenate (at up to 0.005%) in eyelash serums (*data2_ProstaglandinAnalogues_102026);* submission contains the following:
    - Summary of previously provided data for Isopropyl Cloprostenate
    - Summary of the following new data/submissions:
        - Dermal metabolism and penetration assay
        - Independent ophthalmologist analysis of ocular parameters observed in an 8-mo clinical study
    - Updated MOS calculation
    - Enhanced analysis supporting the use of read-across methodologies, including QSAR assessment
    - QSAR toolbox metabolism profiling
- Independent ophthalmologist review of clinical photographs from 8-mo clinical study (*data_3_ProstaglandinAnalogues_102026*) this study has been previously submitted to the Panel, and incorporated into the report, but has also been included in this submission as *data4_ProstaglandinAnalogues_102026* (part 1) and *data5_ProstaglandinAnalogues_102026* (part 2)
- Average sample weight usage testing on lash serums containing Isopropyl Cloprostenate (*data6_ProstaglandinAnalogues102026*)
- Dermal metabolism and penetration assay on a lash serum containing 0.005% Isopropyl Cloprostenate (*data7_ProstaglandinAnalogues_102026*)
- **A letter regarding a "Written Submission for Discussion in December 2025 CIR Meeting – RAWG Position on Animal Testing Related to Prostaglandin Analogs (PGAs)" (*data8_ProstaglandinAnalogues_102026*)
- **A submission from EAS Consulting Group on the Revised Draft Tentative Report (*data9_ProstaglandinAnalogues_102026*); this submission includes suggested edits to the summary of the 8-mo clinical assay performed on 114 subjects using an eyelash serum containing 0.004% Isopropyl Cloprostenate provided in the report. **The Panel should review these edits and determine if these edits should be incorporated into the report.**
- **Updated 2025 RLD (these data are similar to 2024 RLD regarding use categories and frequency of use numbers)

- **Comments on the Revised Draft Tentative Report received from Council (*PCPCcomments_ProstaglandinAnalogues_102026*; responses to these comments included herein as *response-PCPCcomments_ProstaglandinAnalogues_102026)*
- **A study found in published literature on the effect on an eyelash serum containing Ethyl Tafluprostamide on tear film stability, ocular surface inflammation/damage, and intraocular pressure

A search of the newly published literature also identified a recent reference concerning prostaglandin analogues in cosmetic eyelash products.[1] This reference is provided for the Panel's consideration. (This information is not currently included in the report.)

Tables are provided below cross-referencing the data needs identified in the December 2023 IDA for Ethyl Tafluprostamide and Isopropyl Cloprostenate, and whether the requested data were received. While CIR staff have marked below wherein data were received for a particular insufficiency, it is yet up to the Panel to determine if those data are sufficient.

Ethyl Tafluprostamide insufficiencies

Insufficiency	Data received/available?
acute toxicity data	✓*
repeated dose toxicity data	
DART data	
in vivo genotoxicity data	
targets and mechanisms data	✓
information regarding potential receptors/binding/potency/K_i values	✓

*in vitro assay (3T3 NRU)

Isopropyl Tafluprostamide insufficiencies

Insufficiency	Data received/available?
acute toxicity data	✓**
repeated dose toxicity data	✓**
DART data	✓**
in vitro genotoxicity data	✓
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 (0.0075%)	
independent ophthalmologist to assess colorimetric data regarding iris color change for 8-wk use assay	✓
targets and mechanisms data	
information regarding potential receptors/binding/potency/K_i values	

**parenteral study

Also included is a table identifying the data types promised to the Panel at the June 2024 meeting, and whether those data were submitted.

Ethyl Tafluprostamide data promised

Insufficiency	Data received/available?
receptor binding potency studies	✓***
in vitro neutral red uptake assays	✓
ReproTracker assay	✓
ToxProfiler assay	✓
in silico endocrine receptor/activator predictions	✓
literature research on endocrine receptor activation by analogues	✓
analysis of differences in metabolism due to germinal fluorines in the suitability of analogues in read-across analyses	✓
report on read-across analyses	✓

***study initially planned for both prostanoid and prostamide receptors; instead, $PGF_{2\alpha}$ receptors selected as representative target

Isopropyl Tafluprostamide data promised

Insufficiency	Data received/available?
QSAR assessment	✓
dermal metabolism and penetration assay	✓

Furthermore, at the June 2025 meeting, 2 presentations were made regarding these ingredients: 1) Application of Current Read-Across Methodologies to the Safety Assessment of a Cosmetic Ingredient, presented by Dr. Jennifer Ator, ToxServices, and 2) New Data Supporting the Use of Read Across in the Safety Assessment of DDDE as a Cosmetic Ingredient, presented by Ms. Sanghamitra Mishra, ToxMinds. The slides for these 2 presentations are available on the 173rd meeting page (www.cir-safety.org/meeting/173rd-expert-panel-meeting). The Read-Across Working Group (RAWG) reviewed these presentations and the new and previously-submitted data regarding the use of proposed read-across strategies for the review of Ethyl Tafluprostamide and Isopropyl Cloprostenate. In accordance with Section 43 of the *Cosmetic Ingredient Review Procedures and Support to the Expert Panel for Cosmetic Ingredient Safety*, the RAWG has submitted herein a proposal (*RAWG_ProstaglandinAnalogues_102026*) for the Panel's consideration to approve with or without revisions, return for additional work, or disapprove.

In addition, please recall the initial communications regarding potential FDA jurisdiction of eyelash products containing prostaglandin analogues. For clarification, personal communications between CIR and FDA regarding this matter (*communication_ProstaglandinAnalogues_102026*) have been provided herein.

Additional supporting documents for this report package include a flow chart (*flow_ProstaglandinAnalogues_102026*), report history (*history_ProstaglandinAnalogues_102026*), a search strategy (*search_ProstaglandinAnalogues_102026*), a data profile (*dataprofile_ProstaglandinAnalogues_102026*), and transcripts from previous meetings (including the June 2025 RAWG meeting) discussing these ingredients (*transcripts_ProstaglandinAnalogues_102026).*

Previously, it was noted that the SCCS had concluded in a preliminary opinion that the use of Ethyl Tafluprostamide (at a maximum concentration of 0.018%) and Isopropyl Cloprostenate (at a maximum concentration of 0.005%) in eyelash and eyebrow cosmetic products cannot be considered safe for the consumer. This preliminary assessment was based on the potent pharmacological activity of these prostaglandin analogues, even at low concentrations, and the lack of sufficient data to rule out potential reproductive or developmental toxicity, further noting that the proposed read-across strategies therein could not be relied upon for these ingredients. The final opinion, published on February 10, 2026, upheld the same conclusion and further emphasized that this concern is particularly important given that such cosmetic products are likely to be used by women of childbearing age. (See: https://health.ec.europa.eu/publications/sccs-prostaglandin-analogues-methylamido-dihydro-noralfaprostal-mdn-isopropyl-cloprostenate-ipcp_en)

In addition, it should be noted that the SCCS considered that the proposed read-across between Isopropyl Cloprostenate and travoprost, as well as between Ethyl Tafluprostamide and tafluprost, does not satisfy the requirements for a scientifically sound and transparent read-across approach. The SCCS further noted that, in the absence of a suitable point of departure (POD) for Isopropyl Cloprostenate or Ethyl Tafluprostamide, a margin of safety (MOS) cannot be established.

In the RAWG submitted proposal (*RAWG_ProstaglandinAnalogues_102026*), members of the RAWG maintained their previous recommendation - that these same proposed read-across molecules considered by the SCCS (tafluprost and travoprost) are not appropriate sources for the two target ingredients in this report. The risk calculations herein rely on PODs from these read-across sources. Accordingly, if the Panel approves of the RAWG report, then the risk calculations herein are irrelevant to the risk of the cosmetic uses of Ethyl Tafluprostamide and Isopropyl Cloprostenate.

A draft Abstract and Discussion have been included in this report version. The Panel should carefully consider and discuss the data (or lack thereof), and issue a Tentative Report with a safe, safe with qualifications, insufficient data, unsafe, or split conclusion, and identify any additional items for inclusion in the Discussion.

References

1. Couteau C, Coiffard L. Prostaglandin analogues in cosmetic products formulated for eyelash lengthening. *Ann Pharm Fr.* 2026;84(5):889–898.

Prostaglandin Analogues – History

June 2022

Panel reviews Strategy Memo

March 2023

NTP issued March 17

April 2023

Concentration of use survey received – no reported uses for Ethyl Tafluprostamide or Isopropyl Cloprostenate

May 2023

Data received on Isopropyl Cloprostenate – concentration, ocular irritation, dermal sensitization data, and sue studies

Data received on Ethyl Tafluprostamide (several endpoints)

June 2023

Panel issues IDA – needs include: concentration of use, application and packaging, instructions to consumers to prevent skin exposures, 28-d dermal toxicity (other endpoints, if absorbed), sensitization and irritation data, potency data (Ki values of Ethyl Tafluprostamide and Isopropyl Cloprostenate in comparison to bimatoprost)

October 2023

Data received on Ethyl Tafluprostamide (all systemic data in this packet is on tafluprost; Panel will review at December meeting if this data is appropriate for addition)

Data received on Isopropyl Cloprostenate (data in this packet also includes cloprostenol and travoprost data)

November 2023

Instructions for use of a product containing Isopropyl Cloprostenate received

Concentration of use received from Isopropyl Cloprostenate

Comments on draft Tentative Report received from Council

Full study received on 8 mo use study – 0.0044% Isopropyl Cloprostenate lash serum

December 2023

Panel reviews Draft Report and issues an IDA with the following needs:

- 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

The Panel determined that in order for read-across ingredients (tafluprost and travoprost) to be considered, confirmatory data (e.g., receptor interaction studies and downstream profiles of adverse events) is needed.

April 2024

data submitted to CIR indicating information on IPC that will be submitted in the future (QSAR assessment, HET-CAM, EpiOcular assay, HRIPT, 8-wk clinical assay, dermal metabolism and penetration assay)

new data received: Ames assay and in vitro micronucleus assay on IPC

May 2024

W2 data received:

- HET-CAM assay on mascara and eyeliner containing 0.0075% Isopropyl Cloprostenate
- Epi-Ocular eye irritation test on test material containing 0.0075%
- 3 HRIPTs on test material containing 0.0075% Isopropyl Cloprostenate
- Single-center clinical study evaluating the safety and efficacy of a product containing 0.0075% Isopropyl Cloprostenate
- 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

Comments received (W2):

- comments from “Company 1”
- comments from the Council

a read-across memo was sent to the RAWG (Wave 2)

SCCS issued a new a new mandate 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%)

June 2024

Panel reviewed Revised Draft Tentative Report

RAWG reviews potential read-across ingredients; determined read-across was not appropriate

Panel determines to table report for the receipt of new data indicated by a memo submitted by industry

These data include (excluding data that were received as wave 2 at the June 2024 meeting):

- Data on Ethyl Tafluprostamide:
  - receptor binding potency studies
  - in vitro neutral red uptake assays
  - ReproTracker assay
  - ToxProfiler assay
  - in silico endocrine receptor/activation predictions
  - literature research on endocrine receptor activation by analogues

  - analysis of differences in metabolism due to germinal fluorines in the suitability of analogues in read-across analyses
  - report on read-across analyses

- Data on Isopropyl Cloprostenate:
  - QSAR assessment
  - dermal metabolism and penetration assay

March – June 2025

The following data were received:

Submission 1

- An overview on what was previously submitted to Panel
- Comparative data on tafluprost and bimatoprost with Ethyl Tafluprostamide
- In vitro tests (ToxProfiler, ReproTracker, and 3T3 NRU assay) on systemic mechanisms of toxicity on Ethyl Tafluprostamide, tafluprost, and bimatoprost
- PGF2α receptor binding assay for Ethyl Tafluprostamide, tafluprost, and bimatoprost
- Literature search identifying receptor binding/activation data on Ethyl Tafluprostamide, tafluprost, and bimatoprost
- In silico analysis using QSAR models to assess similarity of Ethyl Tafluprostamide, tafluprost, and bimatoprost, with respect to receptor binding and activation potential
- Literature search on in vivo studies for tafluprost and bimatoprost to assess their suitability for assessment of Ethyl Tafluprostamide systemic toxicity
- Analysis of the validity of using the presented read-across analysis to assess the safety of Ethyl Tafluprostamide as a cosmetic ingredient
- Analysis of differences in metabolism due to the presence of the 'germinal flourines' functional group in the frame of the read-across approach
- In-house testing evaluating the amount of cosmetic eyelash product per brush stroke in mink hair
- Reach across justification report in accordance with the ECHA Read Across Assessment Framework
- Updated MOS calculation informed by the comparative analysis of new data collected and analyzed on Ethyl Tafluprostamide, tafluprost, and bimatoprost
- Calculation of toxicological screening value
- Summarized data on the previously submitted toxicity assays on tafluprost and bimatoprost

Submission 2

Supplemental toxicological safety assessment of Isopropyl Cloprostenate (at up to 0.005%) in eyelash serums; submission contains the following:

- Summary of previously provided data for Isopropyl Cloprostenate
- Summary of the following new data/submissions:
  - Dermal metabolism and penetration assay
  - Independent ophthalmologist analysis of ocular parameters observed in an 8-mo clinical study
- Updated MOS calculation
- Enhanced analysis supporting the use of read-across methodologies, including QSAR assessment
- QSAR toolbox metabolism profiling

Submission 3

Independent ophthalmologist review of clinical photographs from 8-mo clinical studiy

Submission 4

Average sample weight usage testing on lash serums containing Isopropyl Cloprostenate

Submission 5

Dermal metabolism and penetration assay on a lash serum containing 0.005% Isopropyl Cloprostenate

June 2025

RAWG reviews potential read-across ingredients and Rev dTR and determines that read-across is not appropriate for this group. Report was not reviewed.

November 2025

Minutes on SCCS meeting received – postponement of Opinion on Prostaglandin Analogues

Comments on report received from Industry

December 2025

Comments on Rev dTR report received from PCPC

October 2026

Panel reviews Rev dTR

Prostaglandin Analogues Data Profile – October 2026 – Writer, Priya Ferguson																																		
				Toxicokinetics			Acute Tox				Repeated Dose Tox				DART				Geno-tox		Carci			Dermal Irritation			Dermal Sensitization				Ocular Irritation		Clinical Studies	
	Reported Use	Method of Mfg	Impurities	log P/log K_{ow}	Dermal Absorption	ADME	Dermal	Oral	Inhalation	Parenteral	Dermal	Oral	Inhalation	Parenteral	Dermal	Oral	Parenteral	In Silico	In Vitro	In Vivo	Dermal	Oral	In Silico	In Vitro	Animal	Human	In Vitro/In silico	Animal	Human	Phototoxicity	In Vitro	Animal	Retrospective/ Multicenter	Case Reports
Ethyl Tafluprostamide	X	X	X	X	X	X		X										X	X				X	X			X		X		X			
Isopropyl Cloprostenate	X		X	X	X					X				X			X	X	X				X	X		X			X		X		X	X

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

Prostaglandin analogues

Ingredient	CAS #	PubMed	FDA	HPVIS	NIOSH	NTIS	NTP	FEMA	EU	ECHA	ECETOC	SIDS	SCCS	AICIS	FAO	WHO	Web
Isopropyl Cloprostenate	157283-66-4	x											x				x
Ethyl Tafluprostamide	1185851-52-8	x											x				

Search Strategy

Search terms below searched in all listed links

Search Terms

- Isopropyl Cloprostenate (2/3 useful hits on PubMed)
- Ethyl Tafluprostamide (1/ hits on PubMed)
- Dechloro Dihydroxy Difluoro Ethylcloprostenolamide (2/2 useful hits on PubMed)
- Isopropyl Cloprostenol (4/42 useful hits on PubMed)
- 157283-66-4 (0 hits on PubMed)
- 1185851-52-8 (0 hits on PubMed)

LINKS

Search Engines

- Pubmed - http://www.ncbi.nlm.nih.gov/pubmed
  - appropriate qualifiers are used as necessary
  - search results are reviewed to identify relevant documents
- Connected Papers - https://www.connectedpapers.com/

Pertinent Websites

- wINCI - https://incipedia.personalcarecouncil.org/winci/ingredient-custom-search/
- FDA databases http://www.ecfr.gov/cgi-bin/ECFR?page=browse
- FDA search databases: http://www.fda.gov/ForIndustry/FDABasicsforIndustry/ucm234631.htm;,
- Substances Added to Food (formerly, EAFUS): https://www.fda.gov/food/food-additives-petitions/substances-added-food-formerly-eafus
- GRAS listing: http://www.fda.gov/food/ingredientspackaginglabeling/gras/default.htm
- SCOGS database: http://www.fda.gov/food/ingredientspackaginglabeling/gras/scogs/ucm2006852.htm
- Indirect Food Additives: http://www.accessdata.fda.gov/scripts/fdcc/?set=IndirectAdditives
- Drug Approvals and Database: http://www.fda.gov/Drugs/InformationOnDrugs/default.htm
- FDA Orange Book: https://www.fda.gov/Drugs/InformationOnDrugs/ucm129662.htm
- (inactive ingredients approved for drugs: http://www.accessdata.fda.gov/scripts/cder/iig/
- HPVIS (EPA High-Production Volume Info Systems) - https://iaspub.epa.gov/oppthpv/public_search.html_page
- NIOSH (National Institute for Occupational Safety and Health) - http://www.cdc.gov/niosh/
- NTIS (National Technical Information Service) - http://www.ntis.gov/
  - technical reports search page: https://ntrl.ntis.gov/NTRL/
- NTP (National Toxicology Program) - http://ntp.niehs.nih.gov/

- Office of Dietary Supplements https://ods.od.nih.gov/
- FEMA (Flavor & Extract Manufacturers Association) GRAS: https://www.femaflavor.org/fema-gras
- EU CosIng database: http://ec.europa.eu/growth/tools-databases/cosing/
- ECHA (European Chemicals Agency – REACH dossiers) – http://echa.europa.eu/information-on-chemicals;jsessionid=A978100B4E4CC39C78C93A851EB3E3C7.live1
- ECETOC (European Centre for Ecotoxicology and Toxicology of Chemicals) - http://www.ecetoc.org
- European Medicines Agency (EMA) - http://www.ema.europa.eu/ema/
- OECD SIDS (Organisation for Economic Co-operation and Development Screening Info Data Sets)- http://webnet.oecd.org/hpv/ui/Search.aspx
- SCCS (Scientific Committee for Consumer Safety) opinions: http://ec.europa.eu/health/scientific_committees/consumer_safety/opinions/index_en.htm
- AICIS (Australian Industrial Chemicals Introduction Scheme)- https://www.industrialchemicals.gov.au/
- International Programme on Chemical Safety http://www.inchem.org/
- FAO (Food and Agriculture Organization of the United Nations) - http://www.fao.org/food/food-safety-quality/scientific-advice/jecfa/jecfa-additives/en/
- WHO (World Health Organization) technical reports - http://www.who.int/biologicals/technical_report_series/en/
- www.google.com - a general Google search should be performed for additional background information, to identify references that are available, and for other general information

Botanical Websites, if applicable

- Dr. Duke's - https://phytochem.nal.usda.gov/phytochem/search
- Taxonomy database - http://www.ncbi.nlm.nih.gov/taxonomy
- GRIN (U.S. National Plant Germplasm System) - https://npgsweb.ars-grin.gov/gringlobal/taxon/taxonomysimple.aspx
- Sigma Aldrich plant profiler- http://www.sigmaaldrich.com/life-science/nutrition-research/learning-center/plant-profiler.html
- American Herbal Products Association Botanical Safety Handbook (database) - http://www.ahpa.org/Resources/BotanicalSafetyHandbook.aspx
- National Agricultural Library NAL Catalog (AGRICOLA) https://agricola.nal.usda.gov/
- The Seasoning and Spice Association List of Culinary Herbs and Spices
- http://www.seasoningandspice.org.uk/ssa/background_culinary-herbs-spices.aspx

Fragrance Websites, if applicable

- IFRA (International Fragrance Association) – https://ifrafragrance.org/
- Research Institute for Fragrance Materials (RIFM) - https://www.rifm.org/#gsc.tab=0 http://fragrancematerialsafetyresource.elsevier.com/

Personal Care Products Council
Committed to Safety, Quality & Innovation

Memorandum

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

FROM: Jaap Venema, Ph.D.
Industry Liaison to the CIR Expert Panel

DATE: December 1, 2025

SUBJECT: Revised Draft Tentative Amended Report: Safety Assessment of Ethyl Tafluprostamide and Isopropyl Cloprostenate as Used in Cosmetics (December 2025 meeting draft)

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

Abbreviations – It is not clear why prostaglandin F is being represented by FP, while the more typical abbreviation, e.g., $PGF_{2\alpha}$, is being used for the other prostaglandins. Please be consistent throughout the document and abbreviate prostaglandin as PG.

Dermal Absorption – For all the in vitro studies, please identify what was used as the receptor fluid as it influences absorption. What was the vehicle for the study on 0.005% Isopropyl Cloprostenate, a formulation?

Developmental and Reproductive, In Vitro, Ethyl Tafluprostamide – With the exception of the first sentence which has units of mg/ml, the rest of the paragraph uses units of µg/ml. Perhaps, mg/ml should be µg/ml.

Endocrine Effects – Please state which pathways were affected.

Ocular Irritation – Please indicate if Isopropyl Cloprostenate (0.0075 and 0.1%) was tested in formulations.

Prostaglandin Analogues – March 2026 – Priya Ferguson	
Comment Submitter: Personal Care Products Council **Date of Submission: February 17, 2026**	
Comment	**Response/Action**
Abbreviations – It is not clear why prostaglandin F is being represented by FP, while the more typical abbreviation, e.g., PGF2α, is being used for the other prostaglandins. Please be consistent throughout the document and abbreviate prostaglandin as PG	Addressed
Dermal Absorption – For all the in vitro studies, please identify what was used as the receptor fluid as it influences absorption. What was the vehicle for the study on 0.005% Isopropyl Cloprostenate, a formulation?	Addressed
Developmental and Reproductive, In Vitro, Ethyl Tafluprostamide – With the exception of the first sentence which has units of mg/ml, the rest of the paragraph uses units of µg/ml. Perhaps, mg/ml should be µg/ml	Addressed
Endocrine Effects – Please state which pathways were affected	Addressed
Ocular Irritation – Please indicate if Isopropyl Cloprostenate (0.0075 and 0.1%) was tested in formulation	it is unknown if Isopropyl Cloprostenate was tested in formulation in these studies

JUNE 2022 PANEL MEETING – PRIORITY LIST DISCUSSION

Belsito Team – June 16, 2022

Dr. Belsito - So I had brought this up because of a colleague of mine who sits on the SCCS as chair, had asked me whether we were looking at this. And then at the last meeting.

We decided that this would probably be more in the purview of the FDA, but then we got, a report back from the FDA indicating that they've looked at the marketing claims and there was nothing that made it look like an OTC drug. So it was back in our court. I do feel like, I guess they're what, three products that have been voluntarily reported? I recall this correctly and yeah, frequency of use in the VCRP. And but, I still think we should reopen this for cause, even if there are three products out there. I mean, there's a prostaglandins and potential side effects or are considerable, depending upon how they're being used, which you haven't looked at, you know. One issue that I have is Bart added a long list of other potential prostaglandin analogues and I'm not sure how to handle that if we do reopen it. It's not what the SCCS looked at. So with that as background, I'll just open it up for everyone's comments.

Dr. Snyder - Well, my comment is I agree that we I can't agree with reopening, but I think we would have to consider all of them, wouldn't we?

Dr. Liebler - So can we only review things that are in the dictionary? Isn't that right?

Dr. Belsito - Yeah, yeah.

Dr. Liebler - We only can review things that are in the dictionaries as I understand it.

Dr. Eisenmann (PCPC) - You can easily review things that are in the VCRP but not the dictionary, but you don't review things that they're that are in neither.

Dr. Rettie - So am I hearing if it's in the dictionary or in the VCRP, then we review it, OK.

Ms. Kowcz (PCPC) - Correct.

Ms. Fiume (CIR) - Yes.

Dr. Liebler - Really. OK. I have no objection. I mean, this is obviously very downstream of my tenure, but I don't object to having any review of any of these that are in the dictionary.

If they're in the VCRP fine, there are a couple of structures that are not in the dictionary, but they on PDF page 4. If that's correct. Those are in the are those VCRP reported?

Dr. Belsito - Page what Dan?

Dr. Liebler - Page 4 of the PDF. But cloprostenate and travopost not in the dictionary, but they're in the VCRP?

Dr. Belsito - Yeah, I think those are actually the ones that are being used.

Dr. Liebler - Yeah. As I last conditioning agents.

Dr. Belsito - That's how they're marketed. But the prescription product, the drug product, but bimatoprost is called Latisse and is marketed as a prescription drug to actually grow the length of the eyelash.

Dr. Liebler - Uh-huh.

Dr. Belsito - A side effect is that if it gets in the eye, it can actually change the color of the eye from blue to brown.

Dr. Liebler - Oh.

Dr. Belsito - Is probably the most disturbing side effect that people do experience. But I don't do any cosmetics or it's not a product that I use at all.

Dr. Liebler - You know. So the question before us. This will work for to add these to the priority list or to proceed to a review of these?

Dr. Belsito - Well, to add them to the 2023 priority list for review, at some point, yes.

Dr. Liebler - Yeah. I mean, I think that's appropriate.

Dr. Belsito - Well, I do too. And then the question is do we add in all of these, I mean that that I couldn't answer.

Dr. Liebler - It seems to me any that are either in the dictionary or in the VCRP.

Dr. Belsito - OK, Carol.

Dr. Eisenmann (PCPC) - But there's a few eyelash conditioning agents that are not prostaglandins that I don't think should be belonged that belong in the report.

Dr. Liebler - Agreed.

Dr. Belsito - Which ones are those, Carol?

Dr. Eisenmann (PCPC) - Towards the end, black Widow Spider Peptide One SP Sr polypeptide. Well, all the ones at the end that that are proteins are mixtures.

Dr. Belsito - Like *(inaudible) adipose stromal cell conditioning media.

Dr. Eisenmann (PCPC) - Correct.

Dr. Belsito - So I guess it would start with glycerin Eitherconic acid peg, four Pinter erythritol crosspolymer, it starts there?

Dr. Eisenmann (PCPC) - I think so.

Dr. Liebler - Yeah. And then the one above it, the isopropyl dimethyl norocarp carbon phosphate. That would seem to potentially belong in the review, and then the one above it at the top of Table 3, the furanyl methylthio methyl sulfinyl triazole.

Dr. Belsito - No, OK. And the one any before that, Dan, that we should delete?

Dr. Liebler - I don't think so.

Dr. Rettie - But. What about the two unavailable on the first page, which certainly one of them sounds like prostaglandin for sure without the structures. Nor Alfa, Prosto and travoprost. They're both prostaglandins, OK.

Dr. Liebler - You know. Yeah, the structures unavailable, I guess, but they're.

Dr. Rettie - Yeah.

Dr. Liebler – Appropriate to include, so all these prostanoid structures, yeah.

Dr. Rettie - That would be 7. Of these.

Dr. Belsito - Yeah, I mean we I think we can use the data for the meta process to read across because that's been extensively studied for drug use. But it wouldn't be something that we would include in the report because it is a drug, not our cosmetic. But I think that data from that can be very helpful. So we would start with Cyclops purple, the bimatoprost. Processed in all travoprost, Roxy *(inaudible) Tanner Prostanoids or and. Nor be. Nor be not appraised, nor to floor Prost. Trifluoromethyl dehydro latanoprost. Method Burnett apros. Neural for procedural travoprostamide. And then we're deleting the fiorinal. We're including the isopropyl dimethyl neuroprosthetic and then from glycerin, itaconic acid peg, four entaerythritol crosspolymer down were eliminating. There was 1,2,3,4,5, 6-7 at the end of the list, so we're limiting those seven plus the. Be there and also that's eight and we're not going to include the metapress because that's a drug, but we'll use data on that to read across.

Dr. Liebler -Yeah. And I would just add that with prostanoids of relatively subtle appearing differences in structures can have dramatic difference in pharmacologic activity. So read across here is going to be a yeah, it's going to be a challenge.

Dr. Belsito - Carol, you still have your hand up. You're muted.

Dr. Eisenmann (PCPC) - No, I don't have any additional comments right now.

Dr. Belsito - OK.

Dr. Rettie - Yeah. So that's something more like 15 structures. I was missing a page when I was counting 7. So yeah, yeah, bigger load.

Dr. Belsito - So. Yes. So Monice were clear on this? We're adding it to the 2023 priority list and the ingredients that we're adding

Ms. Fiume (CIR) - Yes, and I'm assuming it'll be gone over again tomorrow so that Bart can definitely hear all of the names and the rationale behind it.

Dr. Belsito - OK

Dr. Snyder - You're presenting this one, Don.

Dr. Belsito - Okie doke.

Dr. Klaassen - It's going to be a huge task.

Dr. Belsito - Yeah.

Dr. Klaassen - I mean. I think we almost have to start off with the concept that you can't read across. Maybe you can for a few, but I think in general we need to be super, super confident about read across with these chemicals.

Dr. Liebler - Right. I think it'll depend on the endpoint of as usual, but it's going to be a delicate a delicate task.

Dr. Klaassen - Yeah. The good word a delicate task.

Dr. Belsito - OK. I mean, we're not going to know until we dive into it right Dan?

Dr. Liebler - Right.

Ms. Fiume (CIR) - And I do want to point out I'm just seeing it now. For the two that are not in the dictionary, it does say frequency of use not reported. I'm guessing there's suspected use, but I will let Bart speak to that because he is the one that prepared the submission.

Dr. Liebler - OK.

Dr. Belsito - Which two of those Monice that you're talking about are the?

Ms. Fiume (CIR) - Close prostanozol in the travoprost

Dr. Belsito -Yeah.

Ms. Fiume (CIR) - Yeah. So in that first column, he does indicate whether or not this frequency of use or not,

Dr. Belsito - Yeah. Were those in the EU document?

Ms. Fiume (CIR) - I do not know.

Dr. Belsito - Let me just scan that.

Ms. Fiume (CIR) - Yeah, there, there are a couple that are not in the dictionary that says frequency of use not reported.

Dr. Belsito - Yeah. So they actually were looking at there they looked at them. I don't know. They just looked at the whole class. They're not reporting that I can see their use and then they're just. I mean, it's a very helpful report and then and I think it's sort of shows that areas where you may be able to do some read across on PDF page 22 of the SCCS report.

Dr. Liebler - Got it. Thank you.

Dr. Belsito - And they also didn't have the formulas for those two. Yeah, they looked at them. OK. And then use. I like the idea of changing our use table? I don't know what other people thought?

Dr. Snyder - I think it's. I think it's improvement also.

Dr. Liebler - I like. Me too.

Dr. Belsito – Curt?

Dr. Klaassen - Sure.

Dr. Belsito - OK. Okie doke.

Cohen Team– June 16, 2022

Prostaglandins not discussed in this team.

Full Panel – June 17, 2022

Dr. Belsito - Uh. OK. Prostaglandins. Yeah, I still feel we need to open it. You know, we decided previously not to reopen it because we thought it was going to be in FDA issue. Then FDA got back to us and said, well, now that we looked at the marketing, they seemed to be marketing as a cosmetic, not as a drug. I think we need to look at it, you

know, again, VCRP is telling us there are only three products out there. I suspect they're much more. If we do reopen.

Dr. Shank - It's not a reopen, is it?

Dr. Bergfeld - It's a move it up on the priority list.

Dr. Shank - It's to add to the priority list.

Dr. Belsito - Put it on the property list. I'm sorry to put it back on.

Dr. Belsito - May 23 if we do that, there was a whole list of other products planned analog, some of which we did not feel should be in included. We could go through those if we decide to put it on the priority.

Dr. Bergfeld - Bart. Can you make comment and then tell you if the opinion just general open opinion is to put it back on the priority list or reinforce it on the priority list if we need?

Dr. Heldreth (CIR) - Yeah, as Doctor Belsito mentioned, we had brought the first four ingredients listed in this document as a draft priority back in March. And at the time it was unclear if this was within the purview of the panel or under the regulatory authority of FDA drugs. FDA cosmetics got back to us via email. Mentioning that at least some of these prostaglandin derivatives are being used in products that do not appear to make drug claims and therefore could be considered cosmetics. Specifically, they looked at one particular product that contained a ethyl tafluprostamide and the literature that surrounded it did not make any drug claims in particular and therefore it would not be under FDA drugs purview to regulate that product and then falls to this panel to evaluate the safety. So I also included table two as other structurally related prostaglandin derivatives and then only for the sake of being completely inclusive I included table three of other ingredients that are eyelash conditioning agents but are structurally diverse. Was not proposing that we add those. I just wanted to paint the entire picture for the panel.

Dr. Bergfeld - Ok. *(inaudiable)

Dr. Cohen - Yeah, I lost you. Well, I couldn't hear you. It might have been on my side. I.

Dr. Belsito - Yeah, I couldn't hear either.

Dr. Bergfeld - I said, let me see. I'm on. Can you hear me now?

Dr. Belsito - Yeah.

Dr. Heldreth (CIR) - Yes.

Dr. Cohen - Yes, yes.

Dr. Bergfeld - I got my microphone in my hand. My assumption is this was on the priority list. It was questioned. It's now been confirmed that is at cosmetic ingredient at this point in time we do not have to vote it. It's on the priority list. Is that correct?

Dr. Belsito - Are we voted it off for priority list now we have to determine whether it goes back on.

Dr. Bergfeld - Well, I think the clarification that it is a cosmetic ingredient, I guess we can call for emotion. So Don, you want to do that motion?

Dr. Belsito - Yes, put it back on the priority list.

Dr. Bergfeld - Is there a second?

Dr. Cohen - Yeah, a second and Don, do you also as part of your motion wish to include table 2 in in that when we when we review it?

Dr. Belsito - Table three you mean with the list of all the other analogues or potential additions?

Dr. Cohen - No, no. I thought it was.

Dr. Belsito - OK too, yeah.

Dr. Bergfeld - 2.

Dr. Belsito - OK. Yes. I would like to include those. We also did include some others from Table 3 but.

Dr. Liebler - Yeah, all the prosteenoid structures.

Dr. Bergfeld - Yeah.

Dr. Belsito - Yeah. So of table two and Table 3, the only ones we knocked out were purano methylethyl, methylphenol triazole, which was the at the top of PDF page 6. And then we knocked out everything beginning with again PDF Page 6, glycerin it aconitic acid peg, four pentaerythritol, crosslink or crosspolymer, and the remaining 1,2,3,4 products 5,6 below that at the end of the table. But included all the process steps.

Dr. Cohen - So David, you're OK with that grouping as we second the motion for Belsito team? You know that they're appropriately grouped, that we should review those together.

Dr. Ross - So we're looking at tables 1,2?

Dr. Belsito - Table 2 and 3.

Dr. Ross - Structurally the no. Structurally looking similar. Yeah. I mean, I think you could bring those in?

Dr. Bergfeld - OK, Bart, the usual process is that you put it together, look at the chemistry and check with our chemists on the panel to make sure that the chemistry and appropriate ingredients are in it.

Dr. Heldreth (CIR) - Yeah. I mean I think that's whats been confirmed here just now. And so we will include in the draft final priorities list that comes back to the panel in September, we will include all of the ingredients in table one, table 2 and then the one ingredient from table 3 that isopropyl dimethyl norcargo prostate

Dr. Bergfeld - OK.

Dr. Ross - The *(inaudiable) was removed.

Dr. Heldreth (CIR) - Correct everything in Table 3 except for the isopropyl, dimethyl, nor carboprost state was removed.

Dr. Belsito - Yes.

Dr. Ross - Correct.

Dr. Bergfeld - OK, since we've had.

Dr. Heldreth (CIR) - The only reason that the only reason that I put that one in Table 3 instead of Table 2 is that it did not contain a phenylring like all of these structures and stable too.

Dr. Cohen - Yes.

Dr. Liebler - I think the relevant driver structure is at site that dihydroxypropyl entame that prostate piece so the others can be variable. I would expect at the at this point.

Dr. Rettie - Yeah.

Dr. Heldreth (CIR) - Works for me.

Dr. Bergfeld - Uh, is that OK? Alright, then I'm going to call the question. Then the question will go backwards that we're going to put back onto the priority list the prostaglandins with those that were noted earlier to be included and you want to oppose this? Abstaining? Approved. Alright, we're moving forward then. Now we come to the last administrative item and that is the use tables and there have been two proposed the old one and a new one, and Doctor Cohen's going to presently.

SEPTEMBER 2022 PANEL MEETING – PRIORITY LIST DISCUSSION

Cohen Team – September 26, 2022

Dr. Bergfeld - I think that I really want to look at the prostaglandin. So I'm glad everyone's agreeable to keeping them there. They're very much in the world of dermatology, in the topical agents that we use both in cosmetics as well as in prescription drugs. The other thing is that you might want to just briefly discuss that I think the lowest use in this is the hair dye which is 22 and the prostaglandin it is 3 and then 182 the ones following. So we're going to have to decide if there is a line that we can draw. I mean, 3 uses perhaps wouldn't make it if we decide to have a concentration of use minimum.

Dr. Cohen - Yeah, there's a number of things to unpack there, Wilma, to ponder.

Dr. Bergfeld - Yeah.

Dr. Cohen - Susan, any thoughts about the prostaglandin grouping from your from your end? We wanted to get it as comprehensive as possible, but are there any outliers?

Dr. Tilton - No, I don't see anything that I would consider an outlier.

Dr. Cohen - Good to, Wilma

Dr. Tilton - So I'm assuming that some, many of them don't have uses.

Dr. Ross - We don't have.

Dr. Cohen - Many of them don't have what?

Dr. Tilton - Three of them. Three of them have uses. Is that right? Yeah.

Dr. Cohen - Yeah.

Belsito Team – September 26, 2022

Minutes not available.

Full Panel – September 27, 2022

Dr. Belsito - And then just there's a another point that we did discuss was with the Nanumm,Sephora group. There's a flower oil that has a VCRP name but not an INCI name with 9 uses. Which we will include, we just brought that out as how do you deal with an ingredient that is not listed in the cosmetic dictionary? But we'll look at it just as a point of reference. The last and probably the most important was that it was. Recommended in terms of the prostaglandin analogues, of which there are many in the dictionary that we look at, only isopropyl cloprostenate because that's the one that VCRP had data on. However, I sort of felt strongly that we should look at tafluprostamide as well, since the Europeans looked at it specifically at a concentration of .018%, suggesting that it is on the EU, so market and more than likely on our market just not reported to be VCRP during that discussion John Bailey popped up and said there may be some other prostaglandins that industry wanted to add. So if he is online a John, do you want to say something about that?

Dr. Cohen - Someone just raised their hand.

Dr. Bailey (ECG) - OK.

Dr. Belsito - Yeah, it's John.

Dr. Bergfeld - John Bailey.

Dr. Bailey (ECG) - Yeah. No, I think that that's very accurately stated. I think that there is interest in supporting the safety review and that the number of prostaglandins that are established to be used in cosmetics is likely to expand by one or two and those should certainly be added and we will provide try to provide that information. As you know folks, I'm working with develop it and then and then provide that to you for your review. So I think I think it's good to be on there. I think your logic is very sound and we look forward to moving forward on this.

Dr. Belsito - Thanks John.

Dr. Bailey (ECG) - Yeah.

Dr. Belsito - That's all I our group had on the priorities.

Dr. Bergfeld - So it seems to me that we're endorsing the priority list with some addition and expansion of some of the different ingredients?

Dr. Belsito - Yes.

Dr. Bergfeld -Bart, we need to do anything else?

Dr. Heldreth (CIR) - No, I just also just making it quite clear that we're also decreasing the size of the grouping from prostaglandins down to the two.

Dr. Bergfeld - OK.

Dr. Belsito - Or possibly more depending upon industry Bart.

Dr. Heldreth (CIR) - Correct.

Dr. Cohen - Yeah, that's what you meant, right, Don?

Dr. Belsito - Yes.

Dr. Bergfeld - Yeah.

Dr. Cohen - Yeah.

Dr. Bergfeld - So it could be up to five or six maybe. OK. Well, thank you very much. We're going on to our last item of discussion, which is yeast Doctor Belsito and to remind everyone we did have a presentation by the French Group who outlined the class of Yeast that are in cosmetics primarily so Don do you want to carry on?

JUNE 2023 PANEL MEETING – DRAFT REPORT

Cohen Team – June 12, 2023

DR. COHEN: Okay. We can keep muscling through. Prostaglandins. I'm going to need your help here.

DR. ROSS: I would just note here that someone, you know, put prostaglandins, Amphocarboxylates and yeast together on this schedule.

DR. BERGFELD: This is before lunch though.

DR. COHEN: We don't have to -- well, no, we do need to follow that based on the writers. Yes, I know it's a tough run.

DR. ROSS: Let's do it.

DR. COHEN: All right. Ethyl Tafluprostamide and Isopropyl Cloprostenate. So, Priya, this is yours. And these are used as hair conditioning agents and also reported function in cosmetics as nail conditioning agents.

The Isopropyl Cloprostenate in two eyelash serums at 0.0044 and 0.0048 percent. We have a question from the staff. Does the Panel agree that the data on Cloprostenol are not appropriate for inclusion in this report because the data cannot be read across to Isopropyl Cloprostenate? And I'd have to throw that to you guys.

DR. ROSS: I think that concludes -- well, my opinion was that conclusion is valid.

DR. COHEN: That we cannot read across?

DR. ROSS: You cannot read across, yes.

DR. TILTON: Yes. For systemic uptake that would -- and absorption.

DR. COHEN: So we have some impurities, no method of manufacturing, some DART, no genotox. Why don't I open it up for you guys to comment and then we can put our group together.

Actually, before that, I just had a question. On PDF 19, when we have a discussion about the two eyelash serums, it says unknown if these are marked serums. I was determined that 0.0044 and 0.0048 percent, respectively, corresponding to a weight of 8.4 and 13 milligrams of Isopropyl Cloprostenate per usage of each serum, respectively, does that --

DR. ANSELL: No, it's an error.

DR. BAILEY: That's a mistake.

DR. ANSELL: It should be micrograms, not milligrams.

DR. COHEN: Yeah.

DR. TILTON: Okay.

DR. ANSELL: Or nanograms, not milligrams.

DR. COHEN: Okay.

DR. ROSS: Yeah, there was a sentence in there, one or two sentences that didn't quite come together based on the previous one.

DR. COHEN: Yeah, there's one other point where it's four milligrams per brush stroke. And I'm, like, well, that's a lot of -- yeah.

DR. ROSS: Yeah. It didn't come together.

DR. BAILEY: Hi. Can I come up and speak on the mic?

DR. COHEN: Sure.

DR. BERGFELD: John Bailey. I don't know if you all have met John.

DR. BAILEY: Hi. John Bailey.

DR. HELDRETH: Can you announced your affiliation on the microphone please.

DR. BAILEY: My name is John Bailey. I'm currently a consultant with EAS Consulting group. Prior to that I sat in the PCPC chair. Prior to that I sat in the FDA chair. So, I'm very familiar with the activities of this committee.

Regarding the prostaglandins, I think, you've got a report that was mostly extracted from the SCCS. We're fortunate to have some sponsors for this ingredient for use in cosmetics. And some of the data has been submitted in May, so it was late.

But keep in mind that the SCCS is currently reviewing the prostaglandins as well, and some of the data is being generated in conjunction with that, and some of it is not. But it's a work in progress. More data will be coming in that was captured in the reports. And so, it's something that I think will give you guys a much better basis for considering and making a decision regarding the safety.

This is a cosmetic use. It's not a drug use. The products are formulated with primarily two prostaglandins, the Isopropyl Cloprostenate and the DDDE. And the rest of them, as far as I know, are not used in cosmetics. But they may be. But we really have two sponsors.

The use in cosmetics is one where the products are specially formulated so that they're thicker, they're applied in a controlled way. They're applied according to very concise directions, and they're applied with applicators that control -- adding the product in a very controlled way. So, it really becomes an exposure issue mostly. And some of the data will show that the exposure does not cause pharmacological effects, like reducing IOP measurements in eyes.

So, I think that we have some good data coming in. We're going to have more data coming in. And that will be probably be in July and August. So, that can be incorporated in for discussion in a future meeting.

Also, sponsors are going to be bringing in experts, because I'm not an expert on all these different endpoints. And the experts can present data and then be available to answer questions when you get to your reviews.

DR. COHEN: So, should we table this report?

DR. ROSS: There was a list of data coming in on PDF Page 180, according to my notes. It was quite an extensive list.

DR. BAILEY: Yeah. And, of course, we're interested in the Panel's take on what data needs exist. But there is an extensive list of data coming in and that will include the normal toxicological assessment.

DR. COHEN: It sounds like -- because if we start just doing an IDA with needs, knowing that there's other material coming in, maybe we should just table it until that data dump comes and then start the adjudication process.

DR. ROSS: I think we need some direction.

DR. HELDRETH: I mean, you could certainly do that. It seems like we have a timeline for data coming in. That's one option. Another option is to put out your data needs and then John's company would know exactly what you're looking for. And then not bring this report back until, say, December, which would give our staff plenty of time to incorporate what's coming out in July, and any responses to your IDA. So, either way it can work.

DR. COHEN: My only concern is we don't know what's in that data load. And so our IDA will seem ignorant to all the data that we're going to be reviewing the next time. So, that means we'd go out with another IDA. It wouldn't be an IDA, it would be insufficient conclusion. Right?

DR. HELDRETH: Right. Well, I mean, if the data needs change, it's a second IDA.

DR. ROSS: Yeah. And I think there is a list, a fairly comprehensive list on that page. John, has that changed? Do you know?

DR. BAILEY: Pardon?

DR. ROSS: Has that changed? It's a list in your report of the data coming in and when it's about to come in. Has that changed? Is there more data coming?

DR. BAILEY: That list is accurate and there may be some more data coming in, in addition to that. Or additional assessments that -- reports that we added to the body of data that you have available.

DR. COHEN: What PDF is that again, David?

DR. ROSS: 180.

DR. COHEN: And that's specific to DDDE?

DR. ROSS: Yeah. I mean, I went through the report and I've got a list of things that I would consider insufficient, but you know, I don't know if you want to go down that road or not. Why do we have to make that decision first?

DR. COHEN: I think it would be cleaner to have -- I think we'd be better to have the data in the report and then figure out what our data needs are. As opposed to putting data needs on, getting this and then doing another data need. I don't -- that's my gut, but I'm open to suggestions.

DR. BERGFELD: John, do we have any of the medical data on the prostaglandins? How they're used in dermatology for hair growth and eyelash growth. Is that going to be coming in as well?

DR. BAILEY: It may be included in some of the --

DR. BERGFELD: I think that would be helpful, to have it at probably higher concentrations for glaucoma (inaudible).

DR. BAILEY: Yeah. Those analyses will be part of the report.

DR. COHEN: It sounds like these cosmetics are applied the same way a drug is applied,

DR. BERGFELD: Right. Of the new -- the current one, Latisse, in particular.

DR. COHEN: Yeah. It sounds like it's very specifically controlled.

DR. BAILEY: Not quite.

DR. ROSS: I don't think so. I think Latisse is applied to the eyelids. I think these are applied to the eyelashes. Isn't that correct?

DR. BAILEY: Right. That's the way they're applied. It's not to the base.

DR. COHEN: It's not to the base. Right.

DR. ROSS: Yeah.

DR. BAILEY: It's actually applied to the lashes themselves. So, that helps to control the exposure.

DR. COHEN: Yeah.

DR. BERGFELD: But the other thing, it's only applied to one lid. And then the people go like this and it gets on two lids. Both lids are growing hair.

DR. COHEN: Are you talking about Latisse?

DR. BERGFELD: Um-hmm.

DR. COHEN: Oh, people just apply it to both?

DR. BERGFELD: No.

DR. BAILEY: Well, there were some studies to address that exposure, so.

DR. BERGFELD: The original study was to the upper lid first.

DR. ROSS: I see.

DR. BERGFELD: And so, when they blotted, it got on the lower lid.

DR. ROSS: Yeah, I'm not apprised, this is very different, but it's interesting. I did notice -- I think your comment was, it's going to be all about the exposure and I think that's correct.

DR. BAILEY: Yes.

DR. ROSS: Because, you know, the DART studies in here, there were flags, obviously. And there was nothing on the Ethyl Tafluprostamide. You know, there's no repro, no developmental. And the Isopropyl Cloprostenate had some male reproductive effects but, again, no developmental tox. But again, that revolves around the actual exposure.

DR. COHEN: Would there be any objection to us tabling this until these reports come in?

DR. HELDRETH: No objection here. You could also do kind of a combined approach and say it's tabled, but also when we publish our post meeting announcement, put in there, here's things that the Panel is hoping to see based on their review of the documents that they have in front of them, you know, the list that they just went through of everything.

It's a possibility. I'm not saying it's what you have to do. I'm just saying. That may help steer the right data that you want to come in before you see this again.

DR. COHEN: Tom?

DR. SLAGA: Tabling it would be fine.

DR. BERGFELD: Well, I think tabling with the comment that Bart made, is if we already know there's some data that's a little bit weak, the data to point out for the tox, we should include that in our request.

DR. COHEN: Okay. So why don't we enumerate those things?

DR. ROSS: Okay.

DR. COHEN: So, Belsito is going to be presenting this tomorrow and we should just be ready to go. So, we don't have method of manufacturing.

DR. ROSS: That should be an easy one. That's on my list. Yeah.

DR. COHEN: We have impurities.

DR. TILTON: I noted we have predicted absorption rates for Isopropyl Cloprostenate.

DR. BERGFELD: Can't hear you.

DR. SLAGA: We need 28 dermal on both genotoxs.

DR. TILTON: Yeah, we need 28 day, but also experimental data on dermal absorption.

DR. COHEN: On both?

DR. TILTON: We have experimental data for DBTE (phonetic).

DR. BERGFELD: Isopropyl (inaudible).

DR. TILTON: And it looks like there's going to be some additional skin penetration data coming forward.

DR. COHEN: Well, let's see. We can -- it was -- I'm sorry, David, PDF what?

DR. ROSS: 180. You've got some --

DR. TILTON: It's just the prostaglandin. It's just Page 180.

DR. COHEN: It's page 180?

DR. TILTON: Yeah.

DR. COHEN: The IDA is page 180. Plus method of manufacturing and --

DR. ROSS: Yeah, but with respect to potential things that are missing, I think we have to have a discussion whether DART is needed given the exposure. And if DART is needed, then you would need those studies on repro and developmental for Ethyl Tafluprostamide. And you would need developmental on Isopropyl Cloprostenate.

DR. COHEN: What did you need on that, reproductive?

DR. ROSS: Yeah, reproductive toxicology. I think the discussion has to be whether it's needed depending on exposure.

DR. ANSELL: Right.

DR. ROSS: And then, if the answer to that is yes, then you need both repro and developmental of both compounds. Right now there is repro in the document from the Isopropyl Cloprostenate and that's it.

My other question I had on this was, you know, there's a lot of discussion on intraocular pressure. And there's some nice data in there with the Ethyl Tafluprostamide that looked okay, used in a specific product, which was an eyelash product. So, I don't think you need anything more there.

The Isopropyl Cloprostenate was done with a microgram applied directly into the eye. And that saw about a 39 percent decrease in the intraocular pressure.

I think that should be repeated with the eyelash prep to make sure that intraocular pressure is not actually adversely affected when you are using that concentration with the eyelash composition. My guess is, it probably isn't, but I don't know the answer to that.

DR. COHEN: So you want data on intraocular pressure for the eyelash preps?

DR. ROSS: For the Isopropyl Cloprostenate, not for the Ethyl Tafluprostamide. You have that already.

DR. COHEN: We have that. Okay. I think I have those all down now.

DR. BAILEY: Okay. Can I make one additional comment? And that would be, as far as read across goes, is to, you know, not close that door. I think there's going to be an opportunity to make some presentations to support read across later when we bring in the experts to talk about this. Just to keep that on our radar, and I think it'll be worthwhile.

DR. ROSS: Well, people do seem to get very nervous when we talk about read across for prostaglandins.

DR. BAILEY: They do. Yeah.

DR. ROSS: Because You know, the interaction as you know, with the receptors is so specific and stereospecific and so it's tricky to do.

DR. BAILEY: Right.

DR. COHEN: The steroids.

DR. ROSS: Yeah.

DR. COHEN: All right. So we can comment that we can't read across for now pending any new data.

DR. ROSS: And that may change, but right now that's how we see it.

DR. COHEN: Yeah.

DR. HELDRETH: Do you have an idea of what the read across source would be that the experts would talk about?

DR. BAILEY: Pardon? Can't --

DR. HELDRETH: Do you have an idea of which read across source? Like would it be medipros (phonetic) or --

DR. BAILEY: Not specifically at this point.

DR. HELDRETH: Okay. I was just curious. Thanks.

DR. BAILEY: I mean, Cloprostenate is maybe an outlier to some degree. So, I'm not sure how that one might work. But some of the others where we have data might be useful is what I'm hearing.

DR. COHEN: Okay. We'll see how it's presented tomorrow, but it looks like we're going to go for a table with commentary. There's just so much coming in it sounds like.

DR. BAILEY: Thank you very much.

DR. COHEN: Thank you.

DR. ROSS: Thank you.

DR. HELDRETH: Thanks.

DR. BERGFELD: Nice to see you again. You going to be here tomorrow?

DR. BAILEY: Yes.

DR. BERGFELD: Okay, John, thank you.

DR. COHEN: What do you want to do?

DR. ANSELL: It's 11:52.

DR. SLAGA: Break for lunch and come back.

DR. COHEN: Yeah. So what time should we come back? You want to do --

DR. SLAGA: Quarter to.

DR. COHEN: Yeah, I was going to say 12:45. Is that good, or you want 1:00?

DR. ROSS: No, I'm fine.

DR. COHEN: 12:45? 12:45 and we reopened with the Amphocarboxylates.

DR. ROSS: Let's see how many people come back.

DR. COHEN: But we got very far through the list.

DR. BERGFELD: That's nice especially.

DR. COHEN: The backend is not going to be terribly challenging, except for the first two.

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DR. BELSITO: Okay, Prostaglandins. Boy, Priya. Yeast and Prostaglandin, did someone not like you?

MS. CHERIAN: Monice.

DR. BELSITO: Okay. So, again, we got a Wave 2 on the prostaglandins. So, there were a few questions that were asked that I guess we should answer. Do we agree that the data on cloprostenol are not appropriate for inclusion in the report because the data cannot be read across to isopropyl cloprostenate and this is -- I have a question for the team, particularly Allan.

DR. RETTIE: Yeah, I would agree with that. The esters are quite a bit more lipophilic so date of distribution is going to be different. I mean, they're all ultimately going to have to observe the biological effect by being converted to acid to act as a receptor, but the distribution of the more lipophilic ester prodrug that I guess is the question here. So I would agree that the read across is not there for our purposes.

DR. BELSITO: Curt, Paul?

DR. KLAASSEN: Fine.

DR. BELSITO: I had a question on the developmental and reproductive tox study.

DR. SNYDER: What page?

DR. BELSITO: This is page PDF 20. So, it's just they -- you know SCCS is using in silico tools. And in this case, they used an in silico tool to predict that they could be a reasonable certainty of developmental or reproductive toxicity. And of course, we've got the notes of guidance. I don't know if you all read through that as to how the SCCS will be operating in this 2023 and going forward.

And we're not really using any of those tools and should we be using those tools? I mean, they're in silico tools. Can we not buy that software that would allow us to put in structure and look at structural activity relationship and do things like blue screen to give us, you know, genotox alerts and alerts for DART in the absence of data?

DR. RETTIE: I'm only concerned with having some evidence of robustness for the evolving in silico tools. I'm familiar with a few of them, not every one. I've heard that they are performing pretty well, and if we have that kind of information then I'd definitely agree with us using new technology in that regard.

DR. BELSITO: I mean, I think this raises the point and, I mean, we need to enter what will soon be the second quarter of the 21st century. And maybe we need someone to come in on a specific tox endpoint and talk to us. I

mean, we're not going to be doing DPRA's or KeratinoSens we need to understand them. But talk to us about what types of in silico tools can be used to help predict where data is not present or at least give us alerts.

I mean, we can decide what to do with it but, you know, update us on where we are. Because, I mean, I'm on other tox committees and these are being used and they're being accepted. I mean, the SCCS does not use tools that aren't being accepted within the tox world.

DR. KLAASSEN: I think it's a good idea that we look into these. I think I have a similar question, is how we end up using them. You know, if there is no data and we get an alert, and it's for carcinogenicity or something bad, you know really bad, do we then ask for more data or?

DR. SNYDER: Well, I think we want all data available. And then we make a weight of evidence approach to these reports based upon the use, concentration of use -- so it's like we're not just going to take anything standalone -- I mean, I would've bet that they would've flagged prostaglandins. I mean, I would just guess that they would flag it just because the nature of that group.

So, I'm not surprised they flagged it. We do have repo data. So, we look at the repo data and if we have good solid data then we're comfortable with based upon the --

DR. BELSITO: Absorption, use --

DR. SNYDER: -- absorption and use, concentration of use and things like that. So, I agree with both of you. I think we want to see the data because sometimes we don't have that data. And if there's no alerts then we have more confidence to support that we're not concerned for cosmetic of use, so.

DR. RETTIE: So, to some extent it's getting to be a brave new world when we confront new technologies, and I just wondered if it's worth having an expert --

DR. SNYDER: I think it's a great idea.

DR. RETTIE: -- or a group presentation at the beginning of one of our meetings down the line.

DR. SNYDER: Yeah. And have them give us specific examples , like, of how it's utilized. It's utilized in other arenas.

DR. RETTIE: And how it's validated, I'd be interested in that.

DR. BELSITO: I mean, because even if we don't have the software to do this ourselves, we're going to be seeing data that has used this software and we need to know how to interpret that.

I think there are lots of insufficiencies. So, we don't have any use concentrations. We have imputed use concentrations from the products that we were reported on use. The absorption from one of the eye products seems low but we don't have any good systemic toxicity data. We have no DART, no genotox. Sensitization and irritation, to me, seemed okay if those are the concentrations that are actually used.

But, you know, I think in the end my question is are these OTC drugs because I know what they're marketed for, but they do -- they're prostaglandins. We know that there's PGF2 alpha receptors on the hair bulbs. We know that there is some penetration. We know that penetration through hair follicles will be better. If these are getting to the bulb and are causing eyelash or eyebrow growth, then that's not a cosmetic, right? So, we don't have a dose response curve on growth of hair. And an analogue, bimatoprost at 0.03 percent is marketed as a drug.

The trade name is Latisse, it's by prescription. It does cause skin darkening and there's one report of periocular darkening in this paper and it also, in some individuals, has changed the color of the eye from blue to brown. So, I mean, I think we need a lot more data on this. We need dose response in terms of hair growth because we know some of these products can do that. We need DART, we need genotoxicity. Concentration of use. Did you have any other data needs? Allan you're chuckling?

DR. RETTIE: No. I mean, that's just like the whole list here. Yeah, I have the same thing.

DR. SNYDER: Yeah, and we have very little tox data and it's all IP. So, it's not -- there's a lot of data needs.

DR. BELSITO: In Wave 2, I -- that's the problem with Wave 2, I need to keep popping back and forth.

DR. SNYDER: PC comments on the report.

DR. BELSITO: Just -- that was it in Wave 2?

DR. BAILEY: Dr. Belsito?

DR. BELSITO: Yes.

DR. BAILEY: If I could just interject here for a couple minutes.

DR. BELSITO: Sure.

DR. BAILEY: Yeah, there are basically two analogues that are currently being used that we're aware of.

MS. FIUME: Dr. Bailey, would you mind coming to a microphone? Thank you.

DR. BAILEY: Thanks. There are two prostaglandins that we have in the market now that we're aware of. Certainly, that are a step forward to address the CIR review and these are isopropyl cloprostenol and DDDE. And the two companies that are marketing these have submitted data in May, so it was submitted close to the deadline. But more data's being developed, and more reports are going to be coming in over the next two or three months. So, I think that these questions will be addressed and filled out as that data becomes available.

The question of drug versus cosmetic is one that legally these are marketed as cosmetics because they don't make any drug claims and they're used in a very different way than the drugs are used. So, the way I view it is the task here is to look at those cosmetic uses and then determine whether or not they're safe within that context. And if you look at the submissions, the manner of application, the concentrations and things like that are different than the drug uses. And the exposures are going to be very different as well.

So, I think there'll be data forthcoming to answer your questions. I think what we really need is to just have an itemization of the questions that you have. And then, of course, you know this is being reviewed by the SCCS concurrently and some of that information's being developed, or maybe most of it's being developed, for the SCCS as well.

Thinking about the next meeting in September, there is an offer to bring in experts to present and talk about the data that's being developed. So that will give you an opportunity to ask questions and have the experts answer them and enter into a dialog on this. I think that this is kind of a work in progress at this point.

DR. RETTIE: As you're here, can I ask you a question about the cosmetic use or practice amongst cosmetic users across -- because I believe that Latisse when it's being given under the guidance of a practitioner and indeed the dosing recommendation is it's only applied once a day, is that the common practice amongst cosmetic users?

DR. BAILEY: Those are the directions of -- and these products are characterized by directions, clear directions for how to use them and how to apply them and the applicators are designed so that it's applied to the hair and not the skin. So, there are a number of factors that address the exposure. And those calculations, some of them were in the submissions that we made.

DR. RETTIE: I was just curious about it because I read this in a submission that the application, the way that you apply it would localize it to the upper eyelid.

DR. BAILEY: Yeah. On the hair above the base of the --

DR. RETTIE: I just was curious about advantageous application into the eye. The iris coloration. I kind of wondered how that could be avoided at least.

DR. BAILEY: Again, this is addressed in the submissions and the ones that are commenting to provide a level of confidence that's not getting into the eye. And some of the data talks about the IOP versus when you're using this product and that there's no decrease in the IOP during application.

DR. RETTIE: So that would speak to a limited --

DR. BAILEY: Very limited exposure. I think you can almost view this as a de minimis exposure, but a de minimis exposure with controls. And those controls are the directions for use and the applicator to make sure that the product is applied in a controlled way.

DR. BELSITO: So perhaps we need that kind of information as well into this report as some more information about application and consumer instructions and what the applicator looks like.

DR. BAILEY: And again, the two PGAs that people I'm working with are not drug active ingredients, they're not used in drugs. And I think that's because they're not as powerful as bimatoprost and some of the others. But again, I think it's a de minimis use of something that is a class of chemicals that some of which are drug active ingredients and approved by FDA.

DR. RETTIE: So, in terms of the relative potency, I was curious about that, too. I did try to look into it and found a few numbers for KIs against clone receptors, but it doesn't help, the clone receptors, without knowing who they are. The KIs that I found were for ethyl cloprostenate. I think they were -- I heard like 0.4 nanomolar. I mean, that's incredibly biological.

DR. BELSITO: Allan, I'm having trouble hearing you.

DR. RETTIE: Yeah.

DR. BELSITO: The KIs that you found for?

DR. RETTIE: Let me just see which one it was. I was talking about the potency, trying to pick up on your comment about potency because I was curious about the potency of these non-drugs. And there's one here, I actually have it somewhere. So cloprostenol, which we're not talking about --

DR. BAILEY: We're not talking about that.

DR. RETTIE: -- that's the one where the KIs down about one to two nanomolar. Yeah, yeah. So, it gets a bit confusing to kind of compare because you've got a prodrug for cloprostenol and then you've got the biological data from the acids.

DR. BAILEY: Right.

DR. RETTIE: So, I just didn't have a good feel about the potency comparisons.

DR. BAILEY: Maybe that can be explained better for the purposes of the report.

DR. BELSITO: And perhaps we can get that information for these specific compounds.

DR. RETTIE: If it's out there.

DR. BELSITO: You know, their binding affinity for the PGF2 alpha receptor.

DR. BAILEY: Also, one other comment if I may. And that is the idea of read across. I know there's some question about read across, but I think there may be some valuable data that can be mined, if you will, from read across. I wouldn't rule it out. And I think when we do have the experts here, maybe they can explain that better than I can.

But I think read across may be applicable, for example, carcinogenicity or something like that where there's been a study for one of the analogues because they should be similar.

DR. KLAASSEN: So, is the purpose as a cosmetic, is it to increase the hair growth?

DR. BAILEY: No, it's not. And in fact, I think some of the studies have shown that there's not a hair growth. I'm just drawing from memory on it, but I think that's the case. Especially the IOP measurements, I think they were important to understand that this is not an exposure that is systemic and enough to cause pharmacological effects.

DR. KLAASSEN: So even -- it won't increase the hairs on the eyelid to grow?

DR. BAILEY: Not -- not --

DR. KLAASSEN: I guess my question is, why are they putting this on the eyelid?

DR. BAILEY: It's listed in the dictionary as a hair conditioner. And apparently the function is to -- in conjunction with the other ingredients of the product -- to condition the hair and the eyelashes and eyebrows.

DR. BELSITO: And, John, do you have any idea of what kind of data we would expect to receive in August or September, which would be too late for our September meeting, so this would be pushing it to December, I presume, at the very earliest?

DR. BAILEY: Yeah. It's listed in the --

MS. FIUME: PDF page 180 has a list.

DR. BAILEY: And that's just one list. There's also another sponsor who maybe providing more data as well, so.

DR. BELSITO: So, we'll get in vitro skin penetration, we'll get a DPRA, HRIPT, KeratinoSens, some genotox, bacterial and mammalian, EpiDerm irritation, absorption, UV. So, with the tox analysis, again, are we going to get KIs, binding affinities, that type of stuff? Do you know?

DR. BAILEY: I don't know. But I can take that back and ask them and see what they have.

DR. BELSITO: And I think that would be helpful to, you know, if they can provide that vis-a-vie something like the bimatoprost so we could compare a drug to a cosmetic, give us some idea of potency.

DR. SNYDER: And 28-day dermal. I mean, we just got to ask for it. If we get other data that says, yes, we don't need it, but I think we should ask for it.

DR. BELSITO: Okay.

DR. SNYDER: Don't you think?

DR. BELSITO: Yeah.

DR. SNYDER: I mean, and then if it's, you know, and then it's the litany afterwards if it shows, you know, potential issues then we need to have the full gamut. I think with this group we don't assume anything other than there's going to be biological activity.

DR. BELSITO: Could be.

DR. SNYDER: Could be.

DR. BELSITO: Curt, Allan, any other comments? So, what I have here is concentration of use, need information on the application and packaging and instructions to consumers to prevent skin exposure. 28-day dermal and if positive, other data, DART may be needed. Genotox we're expecting to get. You're saying there's going to be an AMES and a mammalian. Sensitization and irritation, we've got an HRIPT and irritation studies coming.

And if industry could provide some idea of relative potency through KIs comparing it to bimatoprost I think that would be very helpful for us. And hopefully we can get that by September at the latest so it can be incorporated in December rather than a data dump a week before our December meeting.

DR. RETTIE: It may be difficult to get the KI data. I'm just reading around this and bimatoprost is prost so it hits the prostamide receptor. We don't know what that is, so how can you get KI against something you can't clone? I suspect we won't get that data, but we can certainly ask, who knows what industry might have.

DR. BELSITO: I mean, I'm not a chemist, is there some way of comparing potencies across classes of prostaglandin analogues?

DR. BAILEY: I mean, I don't know. I'd have to take it back.

MS. FIUME: Bart may be able -- or our chemist might be able to answer it. Or Curt, can you answer?

DR. RETTIE: Curt can probably talk to this. They're all terribly potent. I mean, is there a very weak --

DR. KLAASSEN: Well, the other problem is that there's more than one type of prostaglandin receptor. You know, there's a dozen of them.

DR. RETTIE: Sure. But they've cloned most of those. It's this amide receptor that's out of there.

DR. KLAASSEN: Yeah. I would think that that data might even be available. I mean, people that are really working in that area. I mean, that's kind of the first thing you do, you clone the receptor and then you take a bunch and do a SAR on it and see which ones inhibit it. So, yeah, I think we definitely should ask for it and hopefully they even have it.

DR. BELSITO: Presumably this is the PGF2 alpha receptor, which is specific on the hair bulb.

DR. RETTIE: That's true, I believe for the cloprostenol. I think everybody agrees it's the PGF2 alpha receptor.

DR. BELSITO: But that's what's on the hair bulb that allows bimatoprost, I believe, to cause increase lash growth.

DR. RETTIE: My reading is that that's maybe a little controversial and it probably does interact with that particular receptor. But the amides interact because they have different properties on different cell types compared to the acids, supposedly at this unknown prostamide receptor which might be some splice variant that's going to be hard to get a recombinant preparation to test against. That's my reading of it at least.

DR. BELSITO: Okay.

DR. RETTIE: But we surely can ask for data that is out there.

DR. BELSITO: Other comments? Paul?

DR. SNYDER: Mm-uhm.

MS. FIUME: Don, typically with an IDA we tend to try and skip a meeting. So normally when we were scheduling this it would be scheduled for December, so is that acceptable? It gives industry time and Priya time to -- yeah.

DR. BELSITO: Yeah.

MS. FIUME: Okay. So, yeah.

DR. BELSITO: What I said is hopefully we get the data in September so we're not getting a data dump right after thanksgiving for our December meeting.

MS. FIUME: I think Priya would very much appreciate that.

MS. CHERIAN: Yeah.

MS. FIUME: I'll be nice to Priya. And I just want to clarify, you said irritation/sensitization appears to be okay or it's part of the IDA?

DR. BELSITO: No, I mean, the irritation/sensitization, there was some irritation but it's part of the IDA because at this point, I don't know the concentration of use. I mean, I'm basing all of this off of -- I mean, I'm presuming if people are doing these studies that's what is being marketed out there, but we don't have that data. But, I mean, yeah.

DR. BAILEY: We can clarify that.

DR. BELSITO: You know, I -- we're told that that's part of the data that's being developed to give us in August or September, so yes, I'd like to see that data.

MS. FIUME: I just wanted to clarify that.

DR. BELSITO: These weren't clinical studies, right, they were just reports.

MS. FIUME: They were use studies.

DR. BELSITO: Yeah.

MS. FIUME: Yeah.

DR. SNYDER: And there is a nail conditioning use. Don't forget. It's not just eye lash.

DR. BELSITO: It's reported in the dictionary.

DR. SNYDER: Oh, okay.

DR. BELSITO: I didn't see any products listed here that were specific for nail.

DR. SNYDER: And we do have the application data instructions on page 181 for what product, product A there? On page 181?

DR. BELSITO: Yeah, I saw that, but I think John was saying that the way it's packaged is also -- was that not the case?

DR. BAILEY: Well, it's formulated, number one, to be thick so it doesn't drip into the eyes. And number two, its instructions are very clear for how to use it and where to apply it and the package is designed to make sure that that all works together. And some of that was submitted in the one report, but we can cull that out and make it much clearer.

DR. SNYDER: That'd be kind of unique to have in our summary that the explicit instructions need to be included. Yeah, I mean, I'm not quite certain how we're going to handle that.

DR. BELSITO: I'm sorry?

DR. SNYDER: I'm not quite certain how we're going to handle that if there's a requirement that it be packaged a certain way to minimize ocular exposure or adjacent skin exposure. I mean, I guess safe as used when packaged with application or whatever.

DR. BELSITO: I think we say, you know, safe as used and we refer to the application method in the paper, right? We've done that with hydroxyethyl methacrylate and the acrylates for nail products, right?

MS. FIUME: Alpha hydroxy acids had a very long --

DR. BELSITO: Alpha hydroxy acids and use with a sunscreen and --

MS. FIUME: Yes.

DR. BELSITO: We've done this before.

DR. SNYDER: Okay.

MS. FIUME: It just gets complicated in deciding -- well, not complicated. The decision comes down to is it something that's purely in the discussion or is it in the discussion and the conclusion. Because I think it may have gone both ways in the past.

DR. BELSITO: Well, with alpha hydroxy acids, it was in the conclusion that it be used with a sunscreen or recommendations. With the acrylates, it was not, it was in the discussion.

MS. FIUME: So that would be a decision the panel would need to make at that time if that was the route they were going to take.

DR. SNYDER: Okay.

DR. BELSITO: I mean, I think it would be nice to see this, right, then we can decide how exactly we want to handle it.

MS. FIUME: So then, Don, if that information comes in -- so currently, it has been maybe in the in-use study where the directions to the participants of the study, they were told what to do. So for the request it's for what the actual instructions are as it would be packaged so that it wasn't just an in-use study, it would actually be actual cosmetic use and instructions. Is that what the panel's requesting?

DR. BELSITO: I mean, I would just like to see what the applicator looks like and what the instructions to the consumer are.

MS. FIUME: And all that should go under the Use section, or would you like to see it somewhere else?

DR. BELSITO: No, I think as part of the Use section is enough.

MS. FIUME: Okay. That's what I thought. Thank you.

DR. BELSITO: Anything else?

DR. KLAASSEN: There is a risk assessment here which I --

DR. BELSITO: What PDF are you on?

DR. KLAASSEN: On Page 23. And it's not explained very well but it says there's a margin of safety --

DR. SNYDER: Two and a half.

DR. KLAASSEN: -- of 2.5. I mean, we're usually -- I mean, if that's really true that's, well, bad news. I don't understand how they got it. I mean, we usually are looking for a hundred, right?

DR. BELSITO: I think this was the SCCS calculation and it was based upon -- I forget what their point of departure was, but it's reference 29, is that to the SCCS?

DR. KLAASSEN: What page is that?

DR. SNYDER: 23.

DR. BELSITO: It says for non-allowed pharmacological substances present in food of animal origin and (inaudible).

MS. CHERIAN: Jinqiu added that reference because he did this part because we confused about the MOS calculation too.

DR. KLAASSEN: It might not be relevant but if it is relevant, it's very bad. So that definitely needs to be looked into what's really going on there.

DR. BELSITO: I mean, the problem here is that we know none of what they're talking about, right? We don't know the point of departure. I mean, what is the endpoint that they're using?

DR. KLAASSEN: Right. That's what I said, we need a lot of information there.

DR. BELSITO: Yeah.

DR. KLAASSEN: But they even go on to say here that a margin of safety greater than 1 is considered to be protective. That's a pretty strange statement in itself. I mean, that might be true for cancer chemotherapeutic drug but nothing else.

DR. BELSITO: I think that we need to -- that paper needs to be fleshed out a little bit more.

DR. KLAASSEN: Yes.

MS. FIUME: So, the risk assessment did come from the SCCS, that was the additional information I believe that Jinqiu added. I just asked Bart if Jinqiu was available to step over here. I don't know if he'll see the message. But the risk assessment was in the SCCS paper.

MS. CHERIAN: And it's referenced here, the first reference.

DR. KLAASSEN: Yeah.

DR. BELSITO: But why is it referenced as a Knutson article that references 29? Because that's what I thought it was from, was the SCCS paper.

MS. FIUME: This was Jinqiu's explanation, he delved into it a little more. Hopefully, Jinqiu can step into the room and give an explanation.

DR. BELSITO: Okay. So, we just want to move on and come back to this one when Jinqiu --

DR. KLAASSEN: Yes, let's do that.

MS. FIUME: That's fine. Yes.

Full Panel – June 13, 2023

DR. BELSITO: This is the first time that we're seeing this report. And after reviewing the data and being told that there would be some data forthcoming in the fall, we thought that this was insufficient. First for use concentration, we only have imputed uses based upon some sensitization and irritation data.

We're told that the way that this product is packaged, it would prevent skin contact, so we wanted information on the packaging and the directions to consumers for use. We wanted a 28-day dermal and if absorbed other tox endpoints.

We wanted some type of information on KI or binding of these materials, particularly as it would compare to matoprost, which is a prescription medication that causes eyelash growth. And sensitization and irritation at concentration of use, if its reported concentration of use is higher than what we currently have data on.

DR. COHEN: Second. For discussion, we checked all the boxes together. Intraocular pressure for Isopropyl Cloprostenate, because it looked like we had it for ET, but in the eyelash prep, right.

DR. BELSITO: Yes.

DR. COHEN: And we're fine with your IDA. Our group had suggested that we table this report until all of that data on PDF 180 -- this is a wave of data coming at us. But, we went back and forth between an IDA and a table. And if we tabled it, we were still going to list the things anyways. So, we'll second your motion, and just, perhaps, add that intra-ocular pressure.

DR. BELSITO: Sure.

DR. BERGFELD: Any other discussion? David?

DR. ROSS: I just raise the point we did discuss -- the issue here, as you pointed out, was exposure, you know, what is going to be the exposure amount with the way it's applied. I think the clarification is a really good one.

We talked a fair bit about the DART data, and not being any developmental tables with either of these two compounds. We wanted your opinions on that, whether you had any discussion on it.

DR. BELSITO: Again, I think it's going to depend upon the 28-day dermal and what we see in terms of absorption.

DR. SNYDER: And I agree that in silico that it was not predicted to be a repro.

DR. BELSITO: Right.

DR. ROSS: I think they were both flagged in silico, were they not?

DR. BERGFELD: Can't hear you, David.

DR. ROSS: I thought they were both flagged as potential in silico, maybe I got that wrong.

DR. COHEN: It says SCCS flagged both as potential reproductive developmental toxicants.

DR. ROSS: Yeah, that's what I thought.

DR. COHEN: With a reasonable model certainty.

DR. SNYDER: We're also looking at .02 percent maximum concentration.

DR. ROSS: Yeah, that was the whole thing. It's a discussion about exposure.

DR. SNYDER: We just have to see when we get the rest of the data.

DR. COHEN: That's in the insufficiency with the absorption.

DR. ROSS: But let's not forget that one when we get the absorption.

DR. BERGFELD: All right, any other discussion? Because we can call the question to go insufficient on this ingredient. John Bailey, you want to come forward? Mic, please.

DR. BAILEY: I just wanted to add from yesterday, what I heard is to move forward thinking about tabling this until the December meeting. I just wanted to make sure that I heard it right.

DR. BELSITO: No, we weren't discussing tabling.

DR. COHEN: We discussed tabling at ours. Don's motion is for an IDA with all the insufficiencies. Our team talked about a table, listing the insufficiencies. I guess the question is if there are more insufficiencies we'd have to issue a new IDA, as opposed to tabling it, we would have a single IDA at the next go around.

DR. HELDRETH: I think in this particular case, since even with an IDA our plan is to wait until December to bring this back, it's a little bit semantics, IDA or table. It's going to achieve the same goal.

DR. BAILEY: Okay, that was semantics. Okay. That's fine. I just want to make sure I heard things right.

DR. BERGFELD: Thank you. So we'll call the question on this insufficient, all those in favor of going insufficient at this time? And Tom is yes? Unanimous then. All right, Dr. Cohen, you have the next big one, Yeast.

DECEMBER 2023 PANEL MEETING – DRAFT TENTATIVE REPORT

Belsito Team – December 4, 2023

DR. BELSITO: Okay, so we're starting with the Prostaglandins and sort of little bit overwhelming as to where to start. I guess we take each one individually and then look at where the data needs are there. So, the first one we'll do is ethyl tafluprostamide and whether we can use tafluprost as the read across, which I gathered from our earlier discussion that we're not going to allow. But I'll let you further comment Allan and Curt.

DR. RETTIE: Yeah. I wasn't a fan of tafluprost read across for reasons that I mentioned earlier and also on the presentation by Dr. Petry there.

DR. BELSITO: Curt, you're I believe also in concurrence with that?

DR. KLAASSEN: Yeah. I have concerns with this chemical in particular because it's been mentioned it has the amide on it and et cetera. But my bigger problem is this whole business of read across with chemicals that work through receptors and even in toxicology for the last 15 years, "when we try to regulate chemicals that work through a receptor, what the measure is, is the ability to activate a receptor."

And, Tom, you mentioned that you knew a lot about the PCB's and the PBBs, and the dioxins and so what we have done in the United States, and I think also in Europe, because there's hundreds of thousands of these chemicals -- we can't do toxicology studies on them. The structure activity relationship leads us to false answers and what we've come to conclude is we need to look at their ability to activate for those chemicals, the AH receptors.

(Audio skip) how to regulate them. And I guess as a big overall point I'd like to make, is shouldn't we be doing this for these cosmetics that might be acting through these receptors? So what I would like to see personally is the activation of the receptor. Now, here we might have a couple of prostaglandin receptors that one might need to look at and you compare it to your, "better known chemical," rather than doing this kind of traditional structure activity relationship. I think that's the better way (audio skip) than the established mechanism for doing risk assessment and toxicology.

Tom, have you thought about that? Do you think I'm way -- not talking about your chemicals here right now, but in the big picture is my suggestion okay or is a little crazy?

DR. RETTIE: I kind of go along with you, Curt, for the very potent things that we look at and this has happened before when we look at -- well, not prostaglandins, this is the only time I've seen any prostaglandins from my short time on the panel. But we've seen so many steroid-like molecules that are attached to various things and this same discussion comes up when we're looking at steroidal ingredients. And so, I guess the steroids and the prostaglandins are two classes where can I routinely -- I would like to see some of this and I was pleased to see Dr. Petry seemed to think that was something he could tackle for the prostaglandins.

I had another comment, though. It seems like it could be quite difficult, this prostamide receptor, I cannot find hardly anything new on it. It seems to be not sort of a discrete receptor but some kind of chimeric thing with -- it has not been cloned to my knowledge. So, in instances like that you've got to go back to the organ bath pharmacology and if you look into that you might find yourself trying to set up a cat iris sphincter or cat lung parenchymal cells, because those are the ones that worked to give you high affinity binding to bimatoprost.

Bimatoprost being an amide, too, was different when it first came out. It has nothing close to nanomolar binding for all the prostaglandin receptors that are known and have been cloned, including PGF2 alpha. So old organ bath pharmacology might be something that rears its ugly head -- well its ugly head -- rears its head again.

DR. KLAASSEN: Except they can't do those because it's animals, at least in Europe.

DR. RETTIE: That's right.

DR. BELSITO: Well, they can get data from the pharmaceutical use of it. I mean, that's the other thing since you brought bimatoprost up it's also used in some over the counter -- not over the counter -- it's also used in some cosmetic products out on the market, Bart, for promoting hair growth. So, I don't know if you want to throw that in. Anyway, the bottom line is we're not taking the argument for the read across which means that for the ethyl tafluprostamide we have the systemic endpoints, repeat dose, repro, and DART that still haven't been addressed.

And I think the other endpoint that hasn't been addressed is whether it actually grows the length of the eyelash because I mean the problem, as David and I both kept hinting on, is the application here, the recommended application is the same recommendation for an Rx drug called Latisse/bimatoprost. And I just don't understand how you can enhance the appearance of eyelashes by applying a cosmetic to the eyelid margin unless you're affecting the hair bulbs.

DR. BAILEY: This is John Bailey; can I inject something here?

DR. BELSITO: Yes.

DR. BAILEY: Yeah. I think read across is intended to address those toxicological endpoints, not pharmaceutical activity or (inaudible) activity or anything like that. We're really looking at filling those data gaps of carcinogenicity, mutagenicity, and so forth. So, I think in extending this to pharmaceutical activity I think we're getting maybe a little veered off somewhat.

And I think there's a good amount of data that addresses those endpoints in a very thorough way that I think should be useful in informing the decision that we're talking about. So, I would like to suggest that we kind of keep it in play and I know Dr. Petry might be able to help us with that and so I would keep that in mind.

The hair growth, I mean, this is not intended to grow hair. I think that it's a legitimate functional use as a hair conditioning agent applied to the base of the eyebrows and when you think about it, I think functionally you can certainly envision that it would have hair conditioning to improve the shine and the look and the overall health of the hair shaft. So, I think it's a legitimate function for a cosmetic purpose.

Also, it's my understanding that in looking at receptors, these receptor measurements are very limited value because it varies so much in terms of which receptors and what it all means. I think it's probably not unusual for cosmetic ingredients in a broader class to bind to receptors and so forth. And besides, those end points should be addressable in the toxicological assessments that are appropriate for a cosmetic. I know David Abramowitz knows a lot about the receptor issue and I don't know if he's on or not, but he could certainly help to address those -- that aspect of it -- because he's worked in that area quite a bit.

So, I just would like to say that rejecting this is maybe something we'd want to rethink because the data -- the toxicological data, carcinogenicity, reproductive toxicity, and so forth I think that data's very relevant to the assessment of these two analogues. I see Tom Petry has his hand raised too. Maybe he would weigh in as well.

DR. PETRY: Yeah, there was just one point I just wanted to say and I have to say while you were on lunch break, we just Googled a little bit and just looked a little bit in our data. For example, in what was striking us was a little bit this argument about whether that's a double receptor activity. What type of effect that might have on the toxicity, basically how each might be different to tafluprost.

And here we saw for example that for bimatoprost, which is probably also having this double receptor activity, we just quickly just check the NOAEL which was used in the German BFR assessment. And that NOAEL is for example higher than the NOAEL that we used for the assessment on the basis of the tafluprost data.

So at least -- and this is now -- I have to say we need a little bit more time to substantiate this, but my first reaction to that, a quick reaction would be that at least that one example does not, let's say, justify a higher toxicity in the case of this double receptor activity. But as I said it's just one example now.

We would need to have a little bit of additional time just to work this out a little bit better because we might find this in the literature.

DR. RETTIE: Yeah, I think you might find it in the literature and while I agree with a lot of what you said there, with regard to bimatoprost, which was the index drug for showing up this prostamide receptor occupation. From the get go, they did that with these organ bath techniques and the IC_{50}s that they got for the receptors present in the cat sphincter, iris sphincter, what-have-you, now they gave AC_{50}s around 30 nanomolar, right. That was very different from how bimatorprost acted at this standard prostanoid receptors. But 30 nanomolars, not one nanomolar, and we've sort of seen compounds like this that bind so tightly that you see single digit nanomolar affinities.

And that's the kind of thing I would -- I feel like I would want to know doesn't happen with ET. I'd feel more confident then. But what if ET has a 0.04 nanomolar affinity at the same receptors that bimatoprost has? What confidence do I have then that toxicities that might derive from that type of receptor activation are going to be the same?

DR. BAILEY: David are you on? David Abramowitz, are you available?

MR. ABRAMOWITZ: I am. I am. I can address a little bit about the receptor. I think we are going a bit far afield here. I mean, I do know that this Panel had referred this issue of drug versus cosmetic to FDA and even CDER reviewed this issue -- my friends at CDER -- and I referred it back to CIR that as long as there aren't being drug claims made about hair growth then this Panel shouldn't be assessing safety not worried about the hair growth issue. Regardless, as far as the receptor issue goes, I think this is a red herring to some extent.

Now, there are certainly issues where one receptor controls efficacy, safety, everything. But we're dealing with things sometimes that even if they do bind, they don't bind in a way that causes an effect and I'll give a good example. I know there are dermatologists on the call here. There are two drugs that are very similar chemically. They are a part of the same program, one is called Revlimid, one is called Otezla -- apremilast and lenalidomide. They both were developed by the same program.

As a matter of fact, they both bind TNF alpha and PDE4, but it turns out that the way Revlimid binds TNF alpha and PDE4, it's no good on psoriasis but it cures cancer. The way Otezla binds both of those receptors, it's no good on cancer but it alleviates psoriasis and psoriatic arthritis. So just because something binds a receptor doesn't mean that that's the same efficacy or AE as in both of those two drugs have very, very different AE profiles even though they both bind those two receptors.

So, I think the going down that route is a little misleading here, especially in prostaglandins where for example latanoprost binds both the prostanoid receptor and the traditional prostamide receptors in the pain circuit but no one's prescribing latanoprost to relieve inflammation like Tylenol. Even though acetaminophen works on that same prostamide system with respect to pain and redness. So, I think we need to be careful and there were some talks about receptors laying too much weight on those receptors as whether they're causing sort of a drug effect.

DR. BELSITO: Curt and Allan, I mean, ultimately, it's -- and Paul -- it's our choice here where are you with, first of all, doing the read across for ET to tafluprost?

DR. RETTIE: I haven't heard anything that would change my mind about not liking that for ET. Now, IC I think a case could be made there for sure or at least a much better case. It's the amide ester thing that I'm hung up on. I mean, it could be the situation that Dr. Abramowitz referred to, but we don't know that.

DR. BELSITO: Okay. Curt are you -- where are you now?

DR. KLAASSEN: Well, I'm in Kansas but I'm thinking about, you know, I do have problems with these chemicals that are so close to drugs and then doing read across from them. I think it's a little dangerous. I guess I was wondering what the companies might be able to do and suggest to convince us a little bit more that there's not a problem and is there or is there not hair growth and with this chemical and if, I guess, even if there is, how would we respond to that in response to what the FDA kind of told us? That this is a cosmetic.

So, the FDA said yes, but CIR (audio skip). But they didn't say anything about eyelash growth.

DR. BELSITO: Yeah. Bart, can you chime in on this? I mean, if this product actually increased the length of lashes, it would no longer be a cosmetic, right, it'd have a biological effect.

DR. HELDRETH: No, I don't think that's exactly true. The primary reason that FDA is not calling this a drug is because of packaging and press releases. They're not saying it makes your lashes longer. They're not saying that it's going to make any structure or function change and because of the lack of that literature stating that this -- cosmetic product does some drug effect the FDA buckets it in with cosmetics. We've seen the same thing with certain sunscreen actives that are out there that falls within the purview of the FDA as a drug even though it's on this outdated monograph pathway.

But you here, the Panel, have looked at those same chemicals as cosmetics when they're used as UV filters to protect the product instead of the consumer. So, it doesn't really come down to does it have a drug effect or not for FDA, in this case, to classify it as a drug. It comes down to how's it being sold. How's it being marketed.

DR. BELSITO: Okay. Because I always thought that if it had a biological effect, it was a drug.

DR. HELDRETH: It's not necessarily true. I mean, theoretically a manufacturer could take bimatoprost, put it in a product, not advertise that it does anything to make your hair longer and FDA could choose to call that a cosmetic product.

DR. BAILEY: Yeah, it's the intended use as determined by labeling. So, the claims on the product determine the product classification as a drug or a cosmetic and that's what FDA was affirming in their response when the prostaglandins first came up. So, in the absence of claims it's regulated as a cosmetic and the safety assessment should be as a cosmetic.

DR. BELSITO: Okay. That does not appear in our document, that FDA decision.

DR. HELDRETH: I mean, it was part of the communications between you and Linda Katz about how they looked at it internally and determined that these products that we're looking at right here are considered cosmetics by FDA. And it's based on the labeling.

DR. BELSITO: I would feel more comfortable having that incorporated into the report, then.

DR. HELDRETH: Let me see what we have.

DR. BAILEY: It's early on in the notes from the minutes but I don't think it's in the report at this point.

DR. BELSITO: It's not in the report.

DR. HELDRETH: Yeah. We could certainly beef up the communications references in the cosmetic use section to say that FDA has looked at these products and have declared them to be cosmetic products based on labeling and not on drug effects or safety.

DR. BELSITO: I think that would be helpful to me and it would take away one requirement.

DR. HELDRETH: Right. I think Sanghamitra Mishra may have a question or comment to contribute here. I see her hand up.

DR. MISHRA: Thank you, Bart. I just had two points to make here. One with regards to the read across and second with regard to the risk assessment which has been done for ethyl tafluprostamide.

So, first for the read across, just wanted to come back on the point which Thomas mentioned earlier and the concern which was raised here by the Panel with regard to it being an amide and the analogue tafluprost not being sufficient for being used as read across.

So, the two substances bimatoprost and tafluprost which actually compliment the structure of ethyl tafluprostamide were evaluated by the German authorities and basically comparing the data of both substances and it was clearly shown that the bimatoprost data or the repeated dose of the systemic endpoints there the effects or the doses showing

the responses were at higher doses. And because of this comparison the tafluprost as an analogue was considered to present the worst case. So that's just a point I wanted to make.

And the second is that the experimental data which we have on ethyl tafluprostamide which is through the dermal route despite being a slow metabolism it has shown 70 percent metabolism or hydrolysis to the expective effect. It's not insignificant.

So, this is just to make a point that we will not have a perfect analogue but are we being conservative in the risk assessment, and I would say so, yes. Because if you go onto compare with all the conservative exposure assumptions which was presented earlier, we were still able to show a margin of safety in the standard rate of greater than hundred.

And here in light of all the discussion of the pharmacological active property or the activity of these potential prostaglandins or the prostaglandin analogues, I would like to draw the Panel's attention to the other regulatory authorities, in this case the European Food Safety authority, who have come up with a way to kind of control or determine a reference point of action for such substances when they're unwanted and present in food as contaminants.

So there they use -- they have reviewed the data of different prostaglandins because they cannot use TTC as an alternative value for the risk assessment, they have come up with something called a toxicological screening values and those values, if you were even to use those for the risk assessment and compare the safety of it through tafluprostamide from its use in the cosmetic, we're able to demonstrate a safety or that the fact that the exposure from this formulation is below those extremely conservative toxicological screening values.

The same assessment approach was published in the SCCS opinion because we had submitted that as part of the data for prostaglandins. Of course, SCCS has not commented on it because there was insufficient data for the systemic toxicity, but this is just to show that the exposure from these formulations is negligible, and this aspect is not to be ignored. While it has pharmacological activity at that dose amounts or not, there's no evidence showing it but at least for the concerns like the interocular pressure which where these analogs have been shown to exert effects, at the concentration of use for ethyl tafluprostamide you do not see similar effects or parallel affects.

So, this is just I wanted to kind of bring up a few points about these (inaudible) for ethyl tafluprostamide. Thank you.

DR. BELSITO: Just a question for you. You mentioned TTC. TTC cannot be used for pharmaceutical agents, is that correct?

DR. MISHRA: Yeah. Yes.

DR. BELSITO: Right.

DR. MISHRA: It cannot be. That's why they had come up with these alternative values. That's simply because the TTC database which was based on the TTC levels were drawn did not include these kind of substances so that's why they specifically looked at the pharmacological active substances including prostaglandin which is known to be having effects on reproductive toxicity and they have separate values for these different pharmacological active class of compounds.

DR. BELSITO: Curt, Allan, you're our experts here -- Paul, too.

DR. RETTIE: I don't want to beat this to death with this group. We have definitely different views. But maybe just say one more thing about the structural aspects of compounds we're discussing. We've got ethyl tafluprostamide and as I look at the prostaglandin analogues out there, I sort of think driven by the receptor activation differences bimatoprost looks at least as good a candidate as tafluprost would be as a read across with one exception here. And that would be the substitution of the 15/15 difluoro substituent in ET relative to bimatoprost which has the conventional prostaglandin olefin hydroxy motif coming off the 13 and 15 positions.

So, my recollection from my medicinal chemistry was that the gem-difluoro introduction generally into the prostaglandins had a phenomenal effect on potency, kind of unexpected because you might imagine that it would have something to do with altering the catabolism of these things by blocking the position there with fluorenes. But in the literature, it seems that most people refer to the enhancement of biological potency by the gem-difluoro compounds.

So, looking at ethyl tafluprostamide it seems to me that together the structural motifs that we have, the ethyl amide and the difluoro substituent put us in a place where we really don't have anything that's close to it in terms of

biological -- distribution of biological activity at different receptors and potency. So, it's out there as a bit of an outlier and we can't get away from that. It's a complication I'm not helping here but I did want to look and see if you guys had considered bimatoprost as a read across.

We're mostly concerned, I think, in the dossier here -- several of us -- with the absence of repro and developmental toxicity and would bimatoprost have helped those questions. I mean, it's an improved FDA drug so there must be positive data out there for that. Again, there's no likely difference in potency and I don't know what that is.

DR. PETRY: I don't know if you want to trust it but all I can say is according to, let's say, the normal read across criteria and the local identification criteria, bimatoprost is not a good analogue. But if you were to choose it then I think that's what Mishra explained as well, we would still be at the safe side because toxicity -- our exposure doses would give us a substantially or high enough margin of safety if we were to use, let's say, the most sensitive POD study for bimatoprost.

DR. RETTIE: I just wanted to hear what you would say about that. Thanks.

DR. BELSITO: Okay, so having heard these arguments we really need to move on. Look through -- there's a lot else in this document that we need to discuss but of course the most important and critical thing is read across, so, Allan if I'm understanding you, you're still against using the read across?

DR. RETTIE: Yes. I think I must stay there.

DR. BELSITO: Okay. Curt? Dr. Klaassen? Paul?

DR. SNYDER: Yeah. I mean, I went to Priya's memo in page 3 there and we had insufficient data announcement and we asked for specific data and we kind of gotten sidetracked here onto this read across issue. And so, I think we should, like you said, go back and look at this data that we requested. To John Bailey's point, we're really looking at safety in relationship to the intended cosmetic use and it's not like we're worried about -- it's not a toxicity if it enhances hair growth. It's not a tox endpoint and these things appear to be relatively safe. The margin of safeties for all of them, they calculated the one was almost 400 and one was over a thousand.

And so, I just -- for me -- I mean, this is way out of my league for chemistry -- medicinal chemistry -- but I thought maybe going to Priya's memo we'd go through the data needs we asked for, which we got, and whether that's sufficient for the insufficient data and then we go to the -- I think we should include them.

She had a thing in her memo, should we include them in the document? And I think, yes. Even one was -- I can't remember which one it was -- one was in the dictionary, but it's not reported to be used. But again, it's all a part of this data set that we have on these analogues and then we need to make our system of that and in the discussion, we can discuss some of this stuff, you know what I mean? I think we're trying to get the cart ahead of the horse here a little bit because I don't think these things are very toxic, in my opinion.

Now, the issue whether we have data to support no developmental or repo toxicity, but I would be surprised if that would be present at these levels for these ingredients but that's just my two cents.

DR. BELSITO: Curt? Okay, so Paul you're saying we don't really need read across because you're not concerned about those tox endpoints, and they weren't what we originally asked for? Is that my understanding of what you just said?

DR. SNYDER: I'd like to go through that process first, Don, to see whether the data needs were met because like we've kind of gotten sidetracked here and I'd like to see where the data align. And maybe we need to look at this thing again -- I hate to say that -- and maybe give them a chance to provide us some additional data. I certainly understand the concern using a read across of a carboxylic versus an amide and I totally rely on the chemist on that. Like I said, that's, you know -- but margin of safeties it was 1,029 for isopropyl and for ethyl it was 481.

I mean, so, we do have data and so I just would like to step back and look at what --

DR. KLAASSEN: One of the positive things here is that we can (audio skip). We have a lot of human data which we usually don't have.

DR. BELSITO: Okay, so why don't we go through the document and look at the data and then come back to what our needs are? So, just going through the document for the writer, under composition that eyelash product doesn't belong there. This is PDF page 33 where they describe the composition of an ET eyelash product. That's not part of composition or impurities.

If any place it belongs in cosmetic use, but I don't even think it belongs there. And the question that I had on ET here was that the purity is no less than 99 percent given the low level of use in cosmetics, and we'd be concerned that we don't know what the other one percent is. Allan, Curt, Paul?

DR. RETTIE: I'm not that concerned. The synthesis of these things is well established. We don't have the MOM in the report, but I just sent some references to Bart and Priya to have those included. I mean, people have been making these things based on Corey's first effort more than a generation ago and I think one of them, the major impurity was the epimer for IC and that's what you would expect.

DR. BELSITO: Okay.

DR. RETTIE: But we don't have that stereochemistry for ET, so I don't know what to make of that.

DR. BELSITO: Okay. But you're okay with the fact that we don't know what the other one percent is?

DR. RETTIE: I think so.

DR. KLAASSEN: Yes.

DR. BELSITO: Okay. And then for the writer under non-cosmetic use you didn't mention the use of Latisse as a prescription eyelash growth. I would just add that in. When you were doing the absorption calculations on ET you had used the 6.51 plus or minus 2.16 as your absorption, but the highest reported dose that I saw was 0.02 and on PDF Page 34 of our document, the 0.02 absorbed at 9.12 plus or minus 7.23. Why didn't you use that absorption as your highest maximum?

DR. PETRY: Actually, I can you address that. It started with 0.018 so that's why we took that value. But if we were to use 0.2 percent obviously we would need to take the other one. I agree with you on this. Would not change the outcome of, let's say, the risk assessment in terms of the MOS might be slightly lower, but it does not impact it significantly.

DR. SNYDER: I think that was because of our original report it was lower. It was 0.18 and then we got new data that jumped it to 0.02 is that not right, Bart?

DR. HELDRETH: That sounds right off the top of my head.

DR. BELSITO: But I think that if we're going ahead with this that in the report the calculation should be done with the 9.12 absorption.

DR. SNYDER: I agree.

DR. BELSITO: And then for the IC, when you were looking at the applied per brush stroke you did it for 0.005 percent but the maximum concentration that you reported at 0.0075. So again why did you use a lower concentration? This is PDF Page 34 at the bottom of the page.

MR. ABRAMOWITZ: So, at least for us, that's not our product. Our product is maximum concentration of 0.005 percent. So that's not us. I don't know which manufacturer submitted that information.

DR. BELSITO: I've got here that the maximum concentration is 0.0075.

MS. CHERIAN: That's right. It was data sent to us later closer to the submission of the report. Oh, no, it was sent right before we sent off the original version of this report. It wasn't submitted as Wave 2. But it was an anonymous submission sent to us by Carol.

MR. ABRAMOWITZ: At least for our purposes our report is only substantiating safety after 0.005. So that submission was not part of our work.

DR. BELSITO: It's part of our work because we're being told it's used up to 0.0075. So we would have to redo the calculation for that. Again, I don't think it's going to make a huge difference, but I think we need to be conservative and use the highest values that we're being told are being used in cosmetics.

MS. CHERIAN: Just going back to the comment about Latisse since that's a brand name, that's why I didn't include that. I put bimatoprost in there which is the same thing as Latisse. Is that okay?

DR. BELSITO: Right. Yeah, I mean, you can put bimatoprost but you didn't put it for hair growth.

MS. CHERIAN: Oh, okay. Specifically for hair growth.

DR. BELSITO: Right.

MS. CHERIAN: Okay.

DR. BAILEY: Would it be possible for the 0.0075 and the 0.005, those are two different submissions, would that be reflected in the text of the document, or would you want to use the highest dose reported?

DR. BELSITO: I mean, I think we need to use the highest dose reported. That's what we usually use.

DR. BAILEY: Okay.

MS. CHERIAN: As of right now -- sorry, Bart, go ahead.

DR. HELDRETH: That's all right. I was just going to say, yeah, typically we evaluate the "Worst case scenario," the maximum concentration of use and then if all the data are available. And there's consensus that it paints a safe picture for consumers then the Panel's able to come out with a safe as used conclusion.

But if we base things on a lower concentration than the maximum of use then our conclusion will have to reflect that to say safe as used up to 0.005 percent. So, the attempt is always to go for the worst-case scenario as far as this reported and then if we have to check back because there's missing data to come in at a lower concentration then that's a fallback position.

DR. BAILEY: So those numbers would be recalculated based on the 0.0075?

DR. BELSITO: Yes, please. And then on page 35 of the document for IC up at the top where the eyelash product, the amount calculated for use at 0.02 micrograms, was that per eyelid or was that total for both? I thought it was per eyelid, no?

DR. BAILEY: Maybe Jennifer, are you on? She should be available later. Give her a chance to sign in.

DR. BELSITO: I think it needs to be recalculated from 0.0075 in our report. But I just wasn't clear if that was one eyelid application or if that was for both.

DR. BAILEY: I'm sorry, go ahead.

DR. BELSITO: David, your mouth is moving but can't hear you.

MR. ABRAMOWITZ: Sorry, but I believe the decision based up to the 0.005 for the margin of safety was both eyelids.

DR. BELSITO: So, 0.02 mics was the amount for both eyelids?

MR. ABRAMOWITZ: In the tox report that we submitted for the up to 0.005, yeah.

DR. BELSITO: And Priya, on Page 35 under the acute tox studies for, isopropyl cloprostenate, you said no adverse effects regarding clinical parameters. What clinical parameters were assessed in that study?

MS. CHERIAN: I'd have to go check. And if this was summary data it might have not told me specifically, but I can go check.

DR. BELSITO: And just a question. Since there was an eight-month study on isopropyl cloprostenate, would that in any way classify it as some type of chronic tox study since they did look at some endpoints during that eight months? And should that study be included under toxicology as well?

MR. ABRAMOWITZ: I think from our perspective it's subchronic, but it'd be accumulated.

DR. BELSITO: Subchronic? That's what I should've said. Correct. But, Bart, I mean, and team, do you think that it should be reflected under tox area?

DR. SNYDER: Yes. Yes, I agree.

DR. HELDRETH: That's a Panel purview to make that call so I can certainly move it if that's what you want.

DR. BELSITO: Yeah. I would've moved it to subchronic tox. And I'm just curious why the only DART studies were done on male testicles but, I mean, that doesn't really help us with the DART endpoint. Is that where it's thought that the tox is for these prostaglandins? Allan, Curt, do you know anything about that?

DR. RETTIE: Well, I always kind of associate reproductive toxicity with the risk of abortion. These compounds are used to induce labor and at least on one of them there is a cautionary note and not to be used in --

DR. BELSITO: Pregnant women.

DR. RETTIE: -- pregnant women and I think that's a major concern for me at any rate. The use of these things, topical or not, given their potency.

DR. KLAASSEN: I had the same question. Why did they select the testes and why did they not question the abortifacient activities of the chemical. That wasn't addressed at all that I recall.

DR. BELSITO: Yeah, and nothing was addressed other than male gonads here.

MR. ABRAMOWITZ: At least in our tox report there's both ovarian and testicular reviews and that's because there are versions of prostaglandins used in luteal phase issues and those are larger studies associated with those uses in animals from many, many, many, many, many years ago. I have no idea why DART was done on testes. I can only presume that has something to do with the fact that male and female reproductive cycles are different in terms of prostaglandins.

DR. ATOR: But we did look at all endpoints, all DART related endpoints in our assessment.

DR. BELSITO: We have nothing on ovary. Was that submitted to us?

MR. ABRAMOWITZ: It was. It was part of our toxicological safety report for IC.

DR. BELSITO: Didn't appear in our document.

MS. CHERIAN: Are you talking about the eight-month study?

UNKNOWN MALE: No, it's a data submission from ToxServices, LLC, entitled Additional Data for Consideration by CIR. And it goes through the exposure data in the safety assessment.

DR. BELSITO: I missed it then. Do you know what PDF page it is?

UNKNOWN MALE: No, I honestly don't know what PDF you guys are reading from. I only know what our data submission looked like.

DR. RETTIE: I think it's something on that back around page 790, that's not an exact number. I'm trying to find it.

DR. BELSITO: So it was on ovary, I mean, I should be able to find it quickly by searching ovary?

DR. RETTIE: Yeah.

DR. **ATOR:** Yes, we did a comprehensive dose response analysis of --

UNKNOWN MALE: Sorry, Jennifer. It's on page 774 of the PDF.

DR. **ATOR:** Yeah. So that included all endpoints. And I think to address the question about the effects on pregnancy, the goal of the dose response assessment was to identify that critical effect. The effects seen at the lowest dose level which was not an effect on pregnancy overall, it was more subtle than that. The point of departure that we wound up choosing, the most sensitive one was for reduced liter size rather than an effect on pregnancy.

DR. RETTIE: There's a Table 1, dose response evaluation in certain --

DR. **ATOR:** Exactly. Yes.

DR. RETTIE: -- on page 781, that gives information that's relevant to this discussion. Increased resorptions, what-have-you, reduced litter size.

DR. ATOR: That's on travoprost. If that's why, then that's why it was included in our report. But it's included in the data in the build.

DR. RETTIE: Right. So, if you're reading across to travoprost for the reproductive toxicology you would worry about that, right? You would want to see direct dart data on the actual prostaglandins we're looking at.

DR. ATOR: Not necessarily. The entire goal of the read across approach is to substantiate that substance specific data might not be needed because data are available on a close structural surrogate and in this case it's actually a very conservative surrogate. You'll see further data in Table 1. We also include data on cloprostenol which is the direct hydrolysis product of IPC should it be hydrolyzed in the body. And it is actually more conservative to include the travoprost as our point of departure for the calculations. We actually included quite a bit of DART data for appropriate structure and analogues in our assessment.

DR. HELDRETH: Right. So that's why you don't see it in the report, Don, because it's on a read across source instead of the two ingredients in the report.

DR. BELSITO: Right. So, this was on cloprostenol.

DR. ATOR: Yes, cloprostenol and travoprost, we both considered them appropriate surrogates.

DR. BELSITO: So that puts us back to the read across.

DR. ATOR: And I will say that the read across approach was influenced by the group assessing EC, we did essentially the exact same thing.

DR. BELSITO: Allan, Curt? You still not buying read across right?

DR. RETTIE: Well, for repro and tox. I know that's very conservative, but I'd just like to see direct DART on the compounds that were evaluated here.

DR. ATOR: Right. And that's the challenge is that animal testing bans preclude us from doing so. And read across is becoming ever-more established as a viable approach for this exact sort of exercise.

DR. KLAASSEN: I guess it's become an accepted because there's not an alternative.

DR. ATOR: There really isn't.

DR. KLAASSEN: It's not because it's right. Yeah.

DR. ATOR: That's the purpose of adhering to these frameworks for read across. These are based on the procedures that are used in terms of comparing metabolism, functional groups, structural elements. It actually has a quantitative nature to it. The ET folks use the dice coefficient, we use the MCS Tanimoto coefficient. Both are acceptable. Those rigorous types of frameworks really strengthen the overall approach as that we are able to accurately predict health effects for substances that happen to have less data.

DR. SNYDER: If we're not going to use the read across then we have data deficiencies and I'm hearing that pretty strongly from Curt and from Allan.

DR. RETTIE: Yep.

DR. BELSITO: Okay. So, let's continue to go through the reports and then we can come up with our needs if we don't find it sufficient. Which it looks like may be the case. Staying on that -- the male reproductive effects -- they're in several places on PDF 35 and 36. Priya, you say decreased spermatozoa and effected spermatogenesis, was that decreased number or decreased size and how was this spermatogenesis effected? Was it decreased/increased?

DR. SNYDER: It's decreased numbers. Decreased numbers of developing spermatocytes. That's what that is.

DR. BELSITO: And spermatogenesis was, therefore, decreased?

DR. SNYDER: Correct.

MS. CHERIAN: I can take care of the language a little bit.

DR. BELSITO: Okay. Okay, so then for genotox if we're not accepting the read across. We need in vitro and in vivo data, correct? Allan, Curt, Paul?

DR. BAILEY: I thought I heard that the report would be revised to add the read across to it and then it would be discussed later. Did I hear that correctly?

DR. HELDRETH: No, the read across would only be added to the report if the Panel agrees with the choice of source and target and endpoints.

DR. BAILEY: Okay.

DR. ATOR: If I could just jump in for a second. I think that you need to see the read across first before you make a determination about whether it's acceptable. It's hard to know if something's acceptable if you don't know the nuts and bolts and the details. We believe very strongly in the read across approach. It's very well substantiated scientifically. It meets criteria for OECD, ECHA, REACH, use of read across. And believe me, they are very particular with their grouping of chemicals for the purpose of REACH and to my mind there's really no scientific argument that could be made that would not support the use of appropriately performed read across.

DR. BELSITO: I mean, I'm familiar with read across from my role on the Expert Panel for Fragrance Safety. From what I'm hearing, and I don't -- I'm not the one choosing the read across materials because that's not my area of expertise. But what I'm hearing from my panel members that have that expertise is they're concerned about read across for drug effects as opposed to read across for cosmetic materials that don't act by receptor binding.

DR. ATOR: Right. The read across is the same.

DR. BELSITO: I don't deal in pharmaceuticals.

DR. KLAASSEN: I mean, you can't use read across for pharmaceutical drugs. The pharmaceutical industry doesn't use it.

DR. ATOR: Not for establishing safety, no. But, for assessing efficacy in early development process, absolutely they do use read across. That's how they decide which candidates to go forward with.

DR. KLAASSEN: They do binding assays for (audio skip). This is the way drugs are determined to figure out which drug of a hundred similar or analogues is going to be the best drug. They find out which one has the greatest affinity for that receptor. It's also the read across doesn't even work for a number of toxicology compounds. Look to see how we determine the risk assessment of compounds like dioxin, the polybrominated biphenyls, the chlorinated biphenyls. It's by activation of a receptor.

Now if you have a chemical --

DR. ATOR: And that's been worked out.

DR. KLAASSEN: Yes, it's been worked out, but it hasn't been worked out for your chemicals.

DR. ATOR: That requires the assumption.

DR. KLAASSEN: They don't use SAR techniques for determining how much of these environmental chlorinated and brominated compounds you should be exposed to. They use receptor binding assays.

DR. ATOR: They do.

DR. KLAASSEN: So, what I am saying is because we have receptors here that drugs do bind to and this might bind to, why don't we do receptor binding assays?

DR. ATOR: Right. So, just a couple of observations. The chemical classes that you name have no beneficial human health effects and we know that their adverse effects are mediated, if not totally, then at least in majority part by receptor activation. It's not quite as clear cut for the prostaglandin analogues. We know there are different prostaglandin receptors. You get into the question prostanoids and some of them bind to androgenous cannabinoid receptors and things like that.

We do know that cloprostenol is a fairly effective prostaglandin receptor binder. However, what we also have to remember is that this is de minimis exposure here and as we all remember from our pharmacology classes you have to reach a certain threshold of receptor occupancy in order to get that activation and in order to get the eventual functional or physiological or observable effect.

And in this case, we have the clinical data that demonstrate very clearly that we do not see any of these adverse effects. So really the question of receptor binding, it's not really germane because we don't see any of the downstream sequalae that we would expect from that receptor activation if it were occurring.

DR. KLAASSEN: I agree. (Inaudible) pretty good but how about in the uterus?

DR. ATOR: Right, and that's why we chose the margin of safety approach that we did that looked on very conservative systemic tox data for a conservative analogue, travoprost, that was given by subcutaneous injection. So that provides us even more assurance and we also assumed 50 percent dermal absorption in the margin of safety calculations.

Collectively, those -- it's the accumulative effect of all of those individual conservative steps that mean that our overall systemic risk assessment is very conservative in nature, and we have a more than adequate margin of safety.

DR. KLAASSEN: I suggest that we kind of move on from this aspect and see what the other group says tomorrow. But to realize which aspects we are most concerned about if it is decided that we can do read across.

DR. HELDRETH: Yes, Mishra, did you have a comment or question?

DR. MISHRA: Yes, I just wanted to make one additional point here because we're talking about the endpoint of concern that is reproductive toxicity. Well, since we're also talking about receptor binding and comparison of these different substances, we believe the assays which could be helpful or, let's say, the receptors apart from the pharmacological activity, you're not going into that because we're not claiming their drug use.

So, the relevant receptors could be the endocrine related receptors which one could look at and compare the different substances, and to kind of show that they have the same potential or non-potential to bind or not. So that can be looked at and what we have shown in our dossier is at least we have compared the (inaudible) receptor binding for the substance of interest that is ethyl tafluprostamide and the analogue tafluprost and have shown that they have similar, for example, estrogen receptor binding potential.

So, in the similar manner we could add additional data to kind of show that these kind of receptors, which is responsible for the toxicity eventually and the assessment here, are they behaving the same or not that can be complimented because we have now shown the endocrine assessment for our substance only based on in silico data. And we have done comparison only for few endpoints when we were doing the analogue evaluation but these receptors which is relevant for this endpoint could be looked at in addition to all the arguments which has been provided to support read across.

DR. BELSITO: You said that you looked at endocrine effects with IC because, again, I don't see that in the report. There are endocrine --

DR. MISHRA: I was talking ethyl tafluprostamide. ET. I was talking about ET, sorry.

DR. BELSITO: ET, yeah. And it said that the results were mixed and that it may have some endocrine disruption activity.

DR. MISHRA: Yeah, my point here was not what was the outcome, my point here was to relatively compare to support the read across. The endocrine assessment at the end has been positioned by looking at all the level self-evidence and in silico is just one level. And then we have complimented it with the in vivo data, which does not at the end show the adversity, even though you may have activity. It's not surprising that you see activity with these substances. They are bound to show.

So, they are showing but you at the end, even though you've taken conservative analogue-like where we have taken tafluprost, the in vivo studies do not show the ED-mediated adversity. So, you're specifically talking about the gonadal effects and the ones which are specific to ED assessment. Not the ones which are sensitive but not diagnostic of because they could be activated or affected by other mechanisms other than ED.

So that has been addressed in our tox assessment. So, but here I was just talking about relative (audio skip).

DR. BELSITO: It was addressed by use of a read across?

DR. MISHRA: Yes.

DR. BELSITO: And if you put your read across through those same in silico tools, would you get the same output?

DR. MISHRA: Yes. We have got it for at least estrogen receptor, we have shown it in our dossier; we can show it for the other receptors. This is what I was saying, is this would give you additional confidence if you could show that both (audio skip). There are some assays, in silico tools, which give you the comparison of whether it's a strong or a low. So, you do have potential indication apart from just the binding aspect. So, this can be complimentary and I think would be relevant here to additionally support the read across.

DR. BELSITO: I totally agree. I mean, when we're doing read across again for fragrances, we're getting all the in silico alerts and comparing them side by side for each of the endpoints. And I think that if you're going to tell us you can use one of these for read across it would be nice to see that the in silico predictions are the same for both of your compounds.

DR. MISHRA: Yeah. I've been able to show for two endpoints; we can add the others. Receptors, I mean, from the (inaudible) as pathways.

DR. BELSITO: Yeah. I mean, as much information as you can give us will, I think, make us more comfortable with the idea of using a read across. And that would go for the IC people as well. Okay, moving along on our document. So, the dermal irritation studies in vitro, it looks like DPRT had problems with lysine and precipitated. And the KeratinoSens -- this is for ET -- so above 250 mics you saw cytotoxicity so you couldn't really go higher in

your KeratinoSens but at least in one you were seeing 150 percent or greater activation of luciferase so why are you saying the KeratinoSens was negative?

I mean, there's no dose response but the problem is you couldn't go to a higher dose because of cytotoxicity.

DR. MISHRA: If I remember correctly, it was not meeting the criteria for interpreting it to be positive because you need to have -- there was a fold requirement as well if I --

DR. BELSITO: Yeah, it's 1.5-fold increase and you did see it in one of your runs. So, I mean, I thought the KeratinoSens was -- I wouldn't have called it negative, I would call it inconclusive because in the second -- in one of your runs you didn't get up to 1.5, you got close. But the issue really was that (audio skip) 250 mics because (audio skip) cytotoxicity. I mean, it was very sharp as well. I mean, it went from above 70 percent at 250 mics to like 20 percent living cells when you went to 500.

DR. MISHRA: Yes. We believe that was kind of getting close with the cytotoxic concentration as well that's why we probably see that in one of the runs but not both because you need to see it also in both. Yes, it could be inconclusive, but we have a second assay as well which kind of was more clean and was negative, so that's why we had to kind of set up another one.

Because even if you would've gone for a third run it might have turned out to be inconclusive so we kind of discontinued that test there. And, yeah, the second assay was clean. It was not sensitizing.

DR. BELSITO: Okay. And I just am concerned, again, with the ET. So, you're above Henry's Law which suggests that there could be phyto effects. But you're saying that because you're absorbing in the UVB area only, not getting into UVA, you're not concerned about phototoxicity. But there are some agents that are photoallergic in the UVB rage where they absorb primarily in the UVB so I'm not sure that you can dismiss that absorption into UVB.

I mean, you're saying that the wavelengths are below 313 but UVB is 290 to 320. And you're above that magic cutoff that Henry made of a thousand liters per mol per centimeter.

DR. MISHRA: Well, according to the guidance we are not necessarily need to do any further in vitro testing because we are below the cutoff so that's the notes of guidance from SCCS which says it can be photoreactive but not necessarily phototoxic. This is what the only information we can draw from here. So, and plus there has been no reported evidence of phototoxicity with these kind of substances. So, this is what was our background information why we also did not conduct any further testing.

So, it's not that it's completely not reactive; it is because we see them on an extension coefficient to be about the cutoff. But the overall absorbance is below the limit beyond which you need to do more follow-up testing.

DR. BELSITO: According to the SCCS.

DR. MISHRA: Yes. Right. That's right.

DR. BELSITO: Okay. I think that's something that we need to discuss with the other team tomorrow as well. And on PDF 38 for IC that use in glaucoma patients, it says no other observed adverse effects were observed and there's no mention of measuring interocular pressure. Was IOP measured in that study? This is for IC. It's an eyewash with ICF at 0.01 percent.

MR. ABRAMOWITZ: We're taking a look. I don't recall IOP being measured in that study. It was a --

DR. ATOR: This one was cited by SCCS, the three-month study with the eyewash product.

DR. BELSITO: Right.

DR. ATOR: Yeah. So, there was no -- here's my summary of it -- daily instillation of an eyewash containing 0.01 percent IPC for three months did not affect interocular pressure or produce changes to the eyes upon ophthalmoscopic examination in 23 patients.

DR. BELSITO: That should be added. So those were the adverse effects that were looked for?

MR. ABRAMOWITZ: Correct. They were (inaudible) response.

DR. BELSITO: Okay. So, I mean, it shouldn't say no other adverse effects. It should say that these effects were not seen.

DR. HELDRETH: We'll make that change.

DR. BELSITO: So, I just have another question. For adverse event reports you're giving percentages for ET 0.018 percent over two years, 2011 to 2013 and it looks like there were 0.007 percent adverse reports for total number of units sold. But if there are only how many units were sold what level of confidence do I have in that low number of adverse reports? I always find percentages misleading when you don't know what the denominator is.

DR. MISHRA: Not sure I can comment on that, but we can see if we can get you the details on the number of units. So that's something we can check and get back.

DR. PETRY: Yeah, we don't have this information readily available. We need to check that with the --

DR. MISHRA: Company.

DR. PETRY: -- with our client.

DR. MISHRA: Yeah.

DR. BELSITO: Numbers I think are helpful here.

DR. PETRY: I know. We understand.

DR. MISHRA: Yeah.

DR. BELSITO: Okay. Okay, so I've finished my comments on the document. Curt, Allan, Paul, did you have other issues that I haven't brought up and then there's a Wave 2 from PCPC.

DR. SNYDER: I didn't have anything else that hasn't already been addressed now, Don.

DR. BELSITO: Okay.

DR. KLAASSEN: I'm fine.

DR. BELSITO: Okay, from PCPC have we figured out this ED_5 question that they had, Priya, about the interpretation of ED_5? They're saying for ED_5 other than the abbreviation, the definition is not correct. ED_{50} is a median effective dose of pupil constriction section says potency was expressed as an ED_5 value which represents the dose estimated to produce five-unit areas millimeter hours in a graph of distance. So, pupil diameter.

So, has that been addressed?

MS. CHERIAN: It will be addressed in the next iteration. I think there is just some confusion between the use of ED_5 and ED_{50}. I can go back and look at the reference and make sure I have it correct in the next iteration.

DR. BELSITO: Okay. Then I think everything else was really more just editorial, their comments. Okay, so where are we team? As far as we're concerned the DART and the genotox are not sufficient because we're not accepting --

DR. SNYDER: In the absence of read across.

DR. BELSITO: And also, that we're using read across for repeated dose. Do we feel the eight-month study on the isopropyl is sufficient for repeated dose as used and so the repeated dose need would only be for ET?

DR. RETTIE: We asked for a 20-day dermal, did we not, and don't we get a great deal more information from a 28-day dermal than we would get here from this study?

DR. BELSITO: From what study are you referring to, Allan?

DR. RETTIE: The clinical study that you brought up at the start of this segment. The eight-month study.

DR. BELSITO: Yeah, but the eight-month study was product as used, right?

DR. RETTIE: Yeah, I guess so. Yeah. I'm okay with that, then.

DR. KLAASSEN: And again, we run into the problem of doing the animal study.

DR. BELSITO: Well, if we don't accept read across they're going to have problems with doing the genotox and -- I mean --

DR. KLAASSEN: But the human study can take the place of the 28-day animal study. I'm okay with that.

DR. BELSITO: Okay. Okay, and the genotox? Are we okay using even though not validated, suggesting they do some in vivo micronucleus?

DR. RETTIE: I thought they did.

DR. BELSITO: Pardon?

DR. RETTIE: I thought they did.

DR. KLAASSEN: I think their genotox is (audio skip), isn't it?

DR. MISHRA: Yep. We have data --

DR. BELSITO: The in vivo genotox was on the THB.

DR. KLAASSEN: Yeah.

DR. RETTIE: Yeah. Genotox here was in silico, wasn't it?

DR. BELSITO: Yeah.

DR. PETRY: Can I just ask are you referring to genotox issues for ET?

DR. BELSITO: I'm referring to genotox issues for both.

DR. PETRY: Because we have, actually, a complete set of in vitro data for ET.

DR. MISHRA: Yes.

DR. RETTIE: My notes say no info on the amide.

DR. BELSITO: You have a complete set of genotox for ET?

DR. PETRY: Well, meaning bacterial mammalian mutation assays and other (audio skip) micronucleus test.

DR. MISHRA: Yes, it's part of the drop test, right.

DR. BELSITO: Yeah. You're right. I'm sorry. For IC I'm referring.

DR. ATOR: We have a whole suite of genotoxicity data for the direct hydrolysis product, cloprostenol, all of which are negative.

DR. BELSITO: Allan, Curt, it gets back to the read across for us, with the hydrolysis product here rather.

DR. KLAASSEN: If they have both the parent and the metabolites that should be sufficient. I guess the real question I have is the DART studies, the developmental and reproductive.

DR. ATOR: Right. And we do have a lot of data available on the DART properties of cloprostenol but if we'll go back to that Table 1 in the other document that we were referring to, travoprost was actually more conservative.

DR. BELSITO: Okay, well, I think we've been on this for an hour and a half so at this point our major concerns are the validity of the read across and the potential need for data on genotox and repro-toxicity and we can hammer this out with the other team tomorrow. Is that a fair summary of where we're at, at this point?

DR. KLAASSEN: Yes.

DR. RETTIE: I think so.

DR. SNYDER: I agree.

DR. BELSITO: Okay. Then, I think we're going to just move onto the yeasts, and we'll see what the other team says tomorrow and try and figure this out.

DR. PETRY: All right, thank you.

DR. MISHRA: Thank you.

DR. BELSITO: You're welcome. Thank you. Make a note here.

Cohen Team – December 4, 2023

DR. COHEN: Let's move on to Prostaglandins.

DR. BERGFELD: Oh, lucky us.

DR. COHEN: I thought it was very interesting, very well-done presentations today. And there's just a lot to contemplate here. So, for ethyl tafluprostamide and isopropyl cloprostenate, at the June 2023 meeting the panel issued an IDA for these ingredients and requested the following data: concentration of use, information on

packaging of products in direction for consumer use, 28-day dermal tox, dermal sensitization and irritation data at max concentration of use, intraocular pressure data, potency, inhibition binding affinity data for ET and IC as compared to bimatoprost.

Since the issuing of that IDA, we got a fair amount of data. We had conversations today about the read across. We had a discussion about corollary prescription products. I suppose I'll just open it up. There's just so much to discuss here. I suppose from the panel do you think that we've had all our data needs met and are there any new data needs?

DR. ROSS: I didn't think we had all of our data needs met and I think some of that comes from the discussion this morning on the read across. And I'm convinced on the read across for ethyl tafluprostamide and I'll hear Susan's then Allan's comments tomorrow, I guess. And Susan's comments right now but they're just different structures. You know, tafluprost is a carboxcylic acid ester and the ethyl tafluprostamides an amide and we had that discussion with Dr. Petry this morning and I -- fairness point of view, I hear that -- but certainly at this point I'm still unconvinced.

And that has downstream implications, David, because it was used as read across for quite a few endpoints and the DART endpoints, you know, as Curt said this morning, I'm a little uncomfortable with using read across anyways on DART endpoints on prostaglandins. But if you haven't got read across here you would need some sort of substitute read across, but you would need DART with ethyl tafluprostamide.

You know, my other issues were the cautionary statements. I mean, I feel pretty strongly about that. I think there has to be statements on there for use by pregnant women. I realize I can't mandate that, but I think it should be on these products and, indeed, it was on the isopropyl cloprostenate product, but I didn't see it on the ethyl tafluprostamide and I think that should be extended to the lactation.

And then I had issues with their margin of safety calculation. And I have a similar long list on isopropyl cloprostenate but I'm just sticking with ET right now.

DR. COHEN: What were your issues with the MOS for ET?

DR. ROSS: Well, I think, and I talked about them this morning. The mascaras in SCCS and all the eye products have retention factors of 1.0 and I understand why they used 50 percent because it was conservative. They didn't see a lot of migration in the skin. I'm just saying if you want to be consistent with SCCS they're using a 1.0 rather than a 0.5. So, it probably doesn't make that much difference because even if it did that it would still come in as more than a hundred. But any way I think to be consistent they should at least consider that.

And then I have a similar laundry list for IPC but let's deal with ET first.

DR. COHEN: Okay. You want to just comment -- Susan, you want to comment on ET?

DR. TILTON: I think my comments were pretty consistent with David's. So, we've talked previously about difficulty with prostaglandin's doing read across, especially just based on the potential for differences in biological activity and I think that holds true in this case too. And so that was used to fulfill a number of the data requests so we would still have insufficiencies.

DR. COHEN: I'm looking --

DR. BERGFELD: I'm just going to say something while you're thinking, David. I was concerned with the fact that it was probably acting like a drug and all these people have eyelash growth and it was never measured, never counted, never looked at. Probably photographed, you could probably go back and see if it actually did that. And then the comments that were made about if does attach to the receptor sites, it is a drug. If that is true then we have a drug activity.

DR. COHEN: Well, we got a comment about it being inert.

DR. BERGFELD: Yeah, that was a strange comment.

DR. COHEN: Right. I mean, inert was an absence of AE data that was not absent and just from being fairly comfortable around clinical trial data I don't think you demonstrate biologic activity by looking at rare events. And if they don't occur you can't conclude -- your substance doesn't have biologic effects.

Instead, you look at the most likely biologic effect to see biologic effect, not an uncommon AE. As a matter of fact, volume was so uncommon it didn't even make it into the original packaging.

So, I know there's a number of people that want to comment but we're going to hold for comments and questions until we just run the traps with the current group. Tom, your thoughts on ET?

DR. SLAGA: Yeah. I think that Curt brought up a very, very important point about the receptors and if something works through a receptor, I don't see how we could do read across. I mean, you know, it's either going to bind to the receptor or not at different degrees and I think that has to be clarified in terms of these analogues and their interaction with the receptor.

Overall, I agree with both what David and Susan stated already, too, that -- and there seems to be, as Wilma pointed out, too much of a drug effect that kind of bothers me and I don't know how to deal with that. It seems like we're right on the edge of either a drug or not a drug.

DR. COHEN: Okay. Let's move to isopropyl cloprostenate. David, I know you were chomping at the bit to say something about it.

DR. ROSS: Was I?

MR. ABRAMOWITZ: Thanks, Dr. Cohen I --

DR. BERGFELD: You had a list.

DR. COHEN: David Ross. David A. and John B. I promise we're going to call on you, I just want to get through our first round. David Ross, you said that you had a different list for IP.

DR. ROSS: Yeah, it's we had a different data set in there. I thought we received the interocular pressure study on the serum which was very nice. My list for IC is a little different. First of all, which is a little but off topic from the other ones, we had a new reported max use concentration of the IC, and this may be something that isn't easily addressed but it was 0.75 percent rather than the 0.05 percent max we had previously, and we have no data with that preparation.

And so, I wanted to know how relevant was that, how wide spread was that. Getting back to the more similar issues --

DR. COHEN: David, just point me to the PDF on that because we're going to have to have that discussion.

DR. ROSS: I mean, it was in our discussion in our -- it was flagged, I think, in our documents. Let me see if I can find it quickly.

MS. FIUME: Maybe I can help. PDF page 33.

DR. ROSS: Oh, thank you. Well, there you have it, David. So, on the other issues for read across for IC we had travoprost was a proposed read across and I think that's a better option than with tafluprost for the ethyl tafluprostamide. Travoprost is much more similar with respect to structure; they're both carboxylic acid esters. One is a 3 prim chloro substitution, one is a 3 prime trifluromethyl substitution. The PKA -- the Log Ps are actually quite similar. So, I was a little bit more comfortable for that read across.

The only issue there was there was some variation in endpoints between isopropyl cloprostenate and travoprost. For example, a six to ten-fold difference in concentrations that would produce conjunctival hyperemia in rabbits and that came from SCCS. In fairness, on other endpoints they were quite similar. So, there's that issue. You get at this whole issue -- as Tom has just pointed out -- of using read across on prostaglandin effects on DART and that's why they use the DART endpoints for margin of safety calculations. I understand that, but it's still a sensitivity.

And I had issues with the margin of safety, and I'll get to that in a second. The cautionary statements, again, that's -- this one I thought was better. It had a caution for pregnant individuals and other potentially sensitive groups. I think I said this morning I would add the lactation on there, but I don't make the final call on this. Other issues. There's no genotox that I could see with the isopropyl cloprostenate. I mean, I'm not expecting any, but I didn't see any in the dossier so one can maybe point me in the right direction there.

And yeah. And with respect to the margin of safety calculations, briefly they were taken from a three-generation development and reproductive tox study and their LOAEL was found and that was the 0.00012 milligrams/kilogram per day for travoprost. They're reading across from travoprost. The issue was, it was stated that a further correction from the LOAEL to the NOAEL wasn't needed since the study is a developmental study and that the citation there was SCCS guidelines.

Now, I may have missed this, and it's quite possible, I miss things all the time. But I didn't see that statement in the SCCS guidelines, so I think we need to look at that. If someone could clarify at some point, that would be useful. And then the other question I had on the MOS calculation was a POD question. And the statement was made that there was no adjustment necessary because the study providing the LOAEL use parental exposure, injection exposure. Now my understanding of that is that's true for IV but with subcut -- subcutaneous injection you still have an absorption phase and so I think there needs to be a little discussion around that as well.

So, I think those two issues on the margin of safety calculation, the LOAEL to the NOAEL and the subcutaneous dosing. And right now, that margin of safety's the right side of a thousand so I think it's looking good the way it is right now. But those things need to be taken into consideration. So, they were my laundry list. I'm sorry that went on a little while.

DR. COHEN: No. We're going to have to swing back to our old IDA and any new insufficiencies eventually. We have to go back to that. Susan? You're on mute, Susan.

DR. TILTON: Had to find the right screen. So, I had also noted that we received the data for interocular pressure on eyelash preparation and I made note -- and I was trying to go back and look for this -- but I had made note that it was tested at max use of 0.005 percent with no statistical difference. And then also margin of safety was also calculated at that max use concentration. So, I also had the question about the report -- the new report of max use up to 0.0075 percent.

I would also have just questions about margin of safety with the LOAEL but in this case, I felt like I was -- that we need more complete information for isopropyl cloprostenate compared to ET.

DR. COHEN: Okay. Tom?

DR. SLAGA: I really don't have any additional to add except the tafluprost, isn't that a cosmetic ingredient? Can't we just add it and add all the database with it?

DR. ROSS: I'm not sure we have the database, Tom. We can put the injection stuff in. For cosmetic endpoints we probably need to have some aspects of dermal there. We don't have that.

DR. SLAGA: Oh, okay.

DR. ROSS: If I just type to project you might find that because you didn't have those endpoints you might find it insufficient and that's a bit unfair on the ingredient if I can be clear on that, because it was never intended to be used that way.

DR. SLAGA: Okay. I don't have any additional comments.

DR. COHEN: Okay, so, you know what we can do. We can open up for comment and then we got to go back to our old IDA and check off what we have and don't have and then go through what we do need. There's so much stuff here. Okay. Let's see. I know there's two hands up. David A., you want to go?

MR. ABRAMOWITZ: Sure, Dr. Cohen. I have a few things and I also have Dr. Ator here to answer all the questions on the IC tox report which I think will be helpful. But first before we get to that, I'd like to remind the panel -- I hope all of you remember this, but IC was referred by this panel to the FDA to OCIC -- which check with CDER, my friends at CDER determined that use of IC is not a drug. So, we're talking a lot about drug effects but it's not a drug according to FDA and referred it back to this panel saying that unless claims are being made by the product of hair growth that it should be treated as a cosmetic and reviewed by this panel.

And I understand that people are concerned about hair growth, and I get that, that's not claims being made by this product. This product is here for a cosmetic review and while we can debate all along about whether this is growing hair or not, it doesn't matter because FDA has determined it's not to be a drug and no one's making claims that IC grows hair. So, we're here for a safety review and I'm going to let Dr. Ator talk a bit about the perceived issues with the tox report to explain stuff.

DR. ATOR: Hi everyone. So, yeah. We have a lot to discuss. So, which topic should we take first? Read across or margin of safety or all of the above? I know that there's some discomfort with the notion of using read across. So, I can walk you through our rationale and our process if you would find that helpful or you can just ask questions.

DR. COHEN: Well, you guys raised your hands to comment on the questions that we brought up.

DR. ATOR: All right.

DR. COHEN: So, comment on what we brought up. Please feel free.

DR. ATOR: Sure, sure. So, as David mentioned, the focus of our assessment was on potential adverse effects. We utilized the OECD/ECHA framework in the evaluation or the identification of suitable structural analogues for IPC. And using that approach we determined that travoprost is the best surrogate. We also considered cloprostenol which is the direct hydrolysis product of IPC as a secondary surrogate as well.

And we performed a detailed dose response analysis and determined that actually travoprost is more conservative than use of the direct hydrolysis product closprostenol, so that is what we selected in the margin of safety calculations. And I hear the point about including an additional safety factor for use of the LOAEL, that's certainly fine. And that leaves us with a margin of safety somewhere in the range of about 3 or 400. Yeah, 343.

The next question about the use of the subcutaneous route of exposure. Did you have a suggestion for what an appropriate safety factor would be in your mind in terms of the point of departure of (inaudible) in the margin of safety calculation?

DR. ROSS: I brought that up. I didn't have a number in mind. No, if that's your specific question.

DR. ATOR: Yeah.

DR. ROSS: I mean, it just seems to me that my understanding of that was it's IV rather than subcutaneous and your reference on that, I think, was FDA 2000 in your dossier and when I tried to link to that it didn't work. And I got Jinqiu on the CIR staff to work overtime for me this weekend on this study and it turned out it was the travoprost FDA approval document.

DR. ATOR: Yes.

DR. ROSS: And so, I think it should really be for an IV and I don't know what the adjustment would be for a sub cut, subcutaneous. But that's something we'd have to look into.

DR. ATOR: Yeah. SCCS does not differentiate between the different forms of parenteral exposure. In my experience the subcutaneous, intermuscular, intraperitoneal IV are all treated similarly in doing these types of assessments. But I would be happy to go to the literature and see if I can find an actual number, although I don't know if there will be one.

MR. ABRAMOWITZ: Honestly, I know that FDA for the purpose of pharmacokinetics does not distinguish between sub cut, IGA, or IV.

DR. ATOR: That was my understanding as well.

DR. COHEN: Any product or these?

MR. ABRAMOWITZ: Any product.

DR. ATOR: Right. Because the main --

DR. COHEN: So in vitro injection of a hormone, that's equivalent to an IV injection?

MR. ABRAMOWITZ: For the purpose of determining pharmacokinetics there's not a correction.

DR. ROSS: Well, there's certainly an additional absorption phase.

DR. ATOR: Well, its absorption rate. And really the purpose of this factor is to (audio skip) unique challenge posed by oral absorption and then first pass metabolism.

DR. ROSS: I don't want to get too hung up on the sub cut versus IV. It's a point that needs to be considered. I think a major one is the LOAEL to NOAEL.

DR. ATOR: Yep, yep. Yeah, and SCCS recommends a safety factor of three for that, so that's not a problem to do that. In terms of, let's see, looking at the -- there's been a lot of talk about looking at receptors and receptor binding. We (audio skip) the agents in this class are receptor binders.

But it's important to remember that everything has a dose response. And at such de minimis exposure levels even if theoretically there were to be some receptor binding, really the key thing to remember is that we do not see any of the downstream effects of concern in our clinical studies. And that provides us with a lot of confidence in terms of being able to conclude that the use of IC at this very, very low level in a very specific manner does not pose a concern for systemic toxicity.

And furthermore, for this class of ingredients, the argument that receptor binding somehow makes something a drug -- well in general even. The argument that receptor binding makes something a drug, I do not agree with that. There are plenty of things that bind receptors. I think about the retinol derivatives, for example, bind receptors. Not classified as drugs. So that's just one example there. What am I missing today?

MR. ABRAMOWITZ: The SSCS drug admission, the instruction.

DR. ATOR: Oh, yeah. In terms of a duration adjustment factor, it's common practice in risk assessment not to place a duration adjustment factor -- and I can get you references if you like -- when the effect is developmental in nature. Because it's recognized that due to the unique nature, the critical window theory for example, short term exposures reflect long-term effects. So that is the common risk assessment approach, not to adjust for study duration when the effect is developmental in nature.

DR. COHEN: Any other comments?

DR. BAILEY: Tom Petry or David go ahead and get Tom on.

MR. ABRAMOWITZ: Yeah, I think there was one more that John was following up on this receptor binding issue. I agree with Jennifer, Dr. Ator.

An example that this Panel has some dermatologists on it may be familiar with, I'll give you two compounds. One is called Otezla apremilast, one is called Revlimid lenalidomide. They're both in the same class, they're both (inaudible). They're part of the same development program at CelGene. They're structurally nearly identical. They both bind TNF alpha receptors and PDE 4 receptors. One works on PDE 4 and reduces psoriasis and psoriatic arthritis but does not work on cancer. The other is a cancer drug and doesn't help your skin.

So, while receptor binding certainly plays a role in everything, anything can bind even receptors and not have any physiological effect. I think focusing on the receptor binding is a red herring. We should be looking at whether there are real world AEs for the safety of the cosmetic products.

DR. COHEN: David, we use Revlimid in dermatologic and autoimmune diseases. We use it off label for dermatologic diseases.

MR. ABRAMOWITZ: As far as -- and I represent companies that make lenalidomide -- as far as we're concerned it doesn't not eliminate psoriatic arthritis or psoriasis.

DR. COHEN: I didn't say it eliminated psoriatic arthritis. You're using drug discussions when it's suitable and then using cosmetic discussions when it's suitable as well. So, I don't know why Revlimid has anything to do with this argument.

And I know you bring up the retinoids. The retinoids are something we're very familiar with. We use them all the time, both as cosmetic agents and drugs. And the adverse effects of concern from retinoids on the skin are irritation, largely irritation. If there's absorption we worry about retinoids when it comes to DART, right, because they're potent teratogens.

MR. ABRAMOWITZ: Correct.

DR. COHEN: But from a dermatologic standpoint, from a cosmetic standpoint, the impact of retinoids are entirely reversable, right?

DR. ATOR: That's exactly what we see here.

DR. COHEN: What you've presented to us were extensively the absence of drug adverse effects in a cosmetic product and even in your reports --

DR. ATOR: Yeah, your body doesn't care.

DR. COHEN: -- presented to us there's statistically significant differences in ocular -- in iris pigmentation that by one ophthalmologist who is performing the study suggested that it wasn't relevant. The problem is that for the most part ocular iris pigmentation from these types of drugs is irreversible, right, and so that's a concern of mine and one of the issues I'm going to bring up is bringing in an outside panel of ophthalmologists to review the data in depth because I think that's an important one.

I think the issues you bring up are very reasonable. And you're, of course, reminding us what our fiduciary responsibilities are to review these as cosmetic products but we're presented with a dossier that looks an awful lot like you were evaluating a drug product and then trying to --

DR. ATOR: That highlights the conservatism of the assessment.

MR. ABRAMOWITZ: That highlights the conservatism of our assessment as Dr. Ator was about to say, as well as those are what you asked for. The CIR put out a list of things that were missing in its last report. We looked at those things. That was IOP, that was ocular pigmentation. And so, we did studies for that purpose.

Now, it may sound like a drug dossier because that's what you guys wanted, but we did those studies and they're safety studies whether it's a cosmetic or whether it's a drug. It's the same thing. Now, I understand your concern about iris pigmentation, but there's simply no evidence.

I understand that a few pixels were enough to cause statistical significance, but in the reports in the ophthalmological area of the people who have darkening irises, it's visible. I mean, this was eight months of use. And as you pointed out iris pigmentation is a very low occurrence even with the glaucoma meds. So, we're talking about concentrations lower than that. We're talking about dermal or eyelash application. That seems to be a way of limiting risk.

And one more note just so people know. At least our clients' products include a pregnancy and lactation statements, so no nursing mothers, no pregnant people. So, we do our best to avoid that.

DR. COHEN: We did notice that.

DR. ROSS: We noticed the pregnancy, David, we didn't notice the lactation statement. That wasn't in our dossiers so we should add that, Monice, if that's the case.

MR. ABRAMOWITZ: I'll turn it over to John.

DR. BAILEY: Yeah, I think I'd like to turn it over to Dr. Petry now to talk about the DDDE and the read across that was performed.

DR. PETRY: I think we had a good discussion this morning, your time -- this afternoon, my time -- already. But I listened now to the discussion here as well where you don't really buy into the structural similarity of tafluprost to ET. Basically, one is an amide the other one is an ester. And, again, I would like to remind that we have motabolism data that actually show that there is hydrolysis down to the tafluprost acid for ET.

I think this is still kind of a good indication and then in comparison to tafluprost metabolism that we are dealing systemically with the certain type or very similar type of compounds. Okay, I made that comment already before, so I don't want to dwell on it much further. But we also looked a little bit now, if I would take that argument that, okay, because the amide may bind to a different receptor, the prostamide receptor for example, and that might increase the toxicity.

One can look now at the data for bimatoprost, for example, which is an amide and the German Risk Assessment Institute, they also looked at the data for bimatoprost and clearly the NOAEL is quite higher than the tafluprost NOAEL which we used in our risk assessment. So, I think now along with the other conservative exposure assessment assumptions, I think we have a relatively good degree of conservatism here as well.

And the last point and that's probably a little bit more, also, a question to you is whether, let's say, how could we improve the justification of the risk assessment considering that you're not entirely happy with what we've presented so far. Would, for example, would you say that comparative receptor binding assays would help or eventually related down-stream profiling assays where we could see if the biological activity coming via the receptor how they compare to each other and then we could benchmark somehow and let's say if we were to see some different potencies obviously that can be considered in the risk assessment as well.

So maybe that is a question back to the panel. But this is sort of just on how can we improve it. But I still think we have already a good case here because we really and, of course, we looked -- it was not all presented today. We looked in a lot of data that are available and used a lot of tools and looked in different -- also from a receptor binding standpoint -- and we didn't see significant differences there. But anyway, maybe just back to the panel here.

DR. COHEN: Any comments, David?

DR. ROSS: Yeah. Dr. Petry, thanks. I mean, those comments are certainly well-taken and I think your question about how to improve things, I mean, I think, is a really good one because it's a very tough question. And I do think what you're suggesting there is certainly along the right lines. If you look at receptor profiling of these different types of molecules and then interactions, I.E. and then, perhaps, downstream effects of these two different types of molecules, I think that would help.

We can hear the discussion tomorrow of both teams, but I think this really is a discussion for both teams together because it is a tough one, but I think those things would certainly help.

DR. BAILEY: Just one final comment. I mean, one of the challenges here, and I mean, I've heard the DART issue mentioned a couple of times, and that is in the absence of animal testing which apparently is where we are, the read across and the rational application of extracting from other data sources becomes very important and I think the discussion is very helpful in terms of understanding how that can be designed to make it as comfortable and relevant as possible. But I'm hearing it, over and over again, the same thing that this is particularly a problem and I want to end with we are talking about a very, very low exposure.

I do note also that the 0.0075 level for IC was a separate submission and if the panel conclusion can embrace that, then that's fine -- if it can't, then we'll need to see in what way it might not. So, just to put that into context as well.

DR. ROSS: Yeah. Thank you, John. This is Dave Ross, again. I think the sensitivity, if you like, around the DART. I mean, these are prostaglandins. They were flagged as potential repro-developmental toxins with reasonable certainty. That's the phrase that came up in the dossier via QSAR. You have the sensitivity of using the read across for DART endpoints for prostaglandins and whether that's right or wrong. You then have the actual question of read across and whether it is appropriate read across.

I did appreciate the comments I heard this morning about being willing to include cautionary statements. I thought that was a very good aspect of this discussion, but I think that's where all this comes from. Read across for prostaglandins and particularly for DART endpoints. I think we said this the last time, it is one of our concluding statements. It's difficult. So.

DR. COHEN: Okay. So, if the group could help me out as we go through this list. Concentration -- our last IDA. Concentration of use we have, but we also have some new, perhaps concentration of use for IP. Correct? We have information from the product and direction for use. Do we need any more data on dermal tox as far as you guys are concerned or is this 0.0075 an issue now?

DR. ROSS: I think the 0.0075 is an issue. We have to find out how widespread that is.

DR. COHEN: Well, what do you mean by that?

DR. ROSS: Well, I mean, I think, as John commented, it was an external submission. It wasn't this group but, if you go on and look at isopropyl cloprostenate use in eyelash lotions is it all at 0.048 percent or is how many are at 0.0075 percent? And I don't know the answer to that question. All I know is I've got no data at 0.0075 percent.

DR. COHEN: Well, I mean, if there was one product at 0.0075 percent, that's the report.

DR. ROSS: Yeah, if there's at least one we could've report but, you know, is that the common -- is that a common concentration that's used?

DR. COHEN: Well, again --

DR. ROSS: And if it is, we're going to need data at 0.0075 percent?

DR. COHEN: Are you talking about dermal tox -- 28-day dermal tox?

DR. BAILEY: Well, if I can add, if the existing review and margins of safety can incorporate the 0.0075 then it should. If there's some reason to exclude it, then that's a different issue. You'll have to decide that but that is a real use level.

DR. COHEN: Then we're going to need irritation and sensitization at that.

DR. ROSS: Local ocular effects as you pointed out were an issue for you, David. Presumably at 0.0075.

DR. COHEN: What other data do we need at that concentration?

DR. TILTON: I think it's primarily sensitization and irritation.

DR. COHEN: And the ocular effect.

DR. TILTON: Yeah.

DR. COHEN: Well, ocular effects are a pretty broad thing. Ocular pressure -- is it irritation, is it volume around the eyes? Periocular volume?

DR. ROSS: Wouldn't it be the case with all of the data you've got with the 0.048 percent on ocular you would need for the 0.0075 percent?

DR. COHEN: Yeah. Okay.

DR. ROSS: And that's --

MR. ABRAMOWITZ: If I could just interject. I mean, as sponsors we understand the 0.0075 issue but to the extent the panel can reach some decisions on 0.005 percent or less, we would prefer that considering that there seems to be no data apart from an anonymous submission of that concentration and we're not aware of how much or what other companies are doing.

DR. ROSS: That point is well taken, I think.

MS. FIUME: Can I add that most of our concentrations of use are anonymous so the fact that it's a submission different than was submitted by a previous group really shouldn't matter because it is data that have been submitted to CIR in response to the IDA.

DR. ROSS: But, David, your specific question, you'd need the whole suite --

DR. BERGFELD: Shebang. Yeah.

DR. ROSS: Yeah.

DR. COHEN: So, you want dermal tox and absorption?

DR. ROSS: Well, we haven't got dermal tox as a requirement on the other data insufficiencies we had.

DR. COHEN: We did. Well, we had 28-day dermal tox data if absorbed further systemic tox.

DR. ROSS: Oh, last time through. Yeah, we did.

DR. COHEN: On our last (audio skip).

DR. ROSS: Correct. We still have no dermal tox.

DR. COHEN: (Audio skip).

DR. BAILEY: That's largely because of the challenge of using animals to do the 28-day dermal.

DR. COHEN: Okay.

DR. ROSS: But you would certainly need irritation and sensitization. Every piece of data we have is on the 0.048 percent. It's not on the 0.0075 percent.

DR. COHEN: Okay. And for ET?

DR. ROSS: We need to sort out the read across issue.

DR. COHEN: Well, what is -- we have to articulate our data needs.

DR. ROSS: Well, it's the read across issue if it's the conclusion of the whole panel, the read across is not going to work then you are going to need additional data to fill in for the lack of read across.

DR. COHEN: So, can you outline that?

DR. ROSS: Well, it was beautifully outlined in Dr. Petry's presentation. I'll just go back to your dossier. What you were using for read across, I think there was some local tox, but DART as well for --

DR. COHEN: We may be able to go to the very nice chart that's in front of all of our reports, right?

DR. ROSS: I like the one that Dr. Petry -- that submission was -- even though, as I said, I disagreed with some of it -- it was a beautiful submission and really helped.

DR. PETRY: I can quickly try to get it out here.

DR. ROSS: I've got it --

DR. PETRY: Is this the chart you mean?

DR. COHEN: Is this it?

DR. ROSS: I don't see --

DR. COHEN: Acute dermal tox or 28-day dermal tox?

DR. ROSS: There's acute toxicity read across to tafluprost, repeat dose toxicities read across to tafluprost, and repro and developmental is read across to tafluprost.

DR. TILTON: And the alternative to that is additional data to support the read across.

DR. ROSS: Correct, Susan. Just going to go there, yeah.

DR. COHEN: So, Suan, these aren't the terms we'd necessarily use in our IDA's. Can we outline what we want in our IDA if we don't have additional information for the read across, what are our data needs? So, we need dermal --

DR. TILTON: It may be easier to request data to support a read across.

DR. COHEN: Well, we just may not get what we want. So, we need the whole acute tox profile, repeated tox profile. I mean, there's a lot of boxes there, guys.

DR. ROSS: There's a lot of boxes and --

DR. COHEN: I know. But I want to make sure we're asking for what we really need to help this along.

DR. ROSS: But I think if you approach it from the sense that you may need more data to decide on read across or not and if the data that might help support that read across would be -- I think, Dr. Petry made two additional suggestions this evening. Receptor interaction studies of amides and carboxylic acids and downstream profiling of effects of these two prostaglandin types (audio skip) and that could support the read across. If the read across is still not supported, then you need to fill up these gaps that the read across was previously filling.

DR. COHEN: Okay. I think I have --

DR. PETRY: If I may add. I think it depends a little bit if we, let's say, even if we have -- if the receptor interaction studies show there is receptor interaction I would still look if there's a downstream consequence to it.

DR. ROSS: That comment --

DR. COHEN: Yes. It's an and, not an or. Right?

DR. ROSS: Yeah.

DR. PETRY: Yep.

DR. COHEN: Any other comments on that? I think I have my work cut out trying to summarize this into an intelligible discussion tomorrow.

DR. ROSS: And David --

DR. BERGFELD: It needs it.

DR. ROSS: -- can we make sure we have something about the cautionary statements on both of these?

DR. COHEN: I believe I have that already written but I'll -- okay. Okay.

MS. FIUME: David, can I clarify that? And then I see that there is a hand up. So, the plan is to issue a second IDA even though some of the requests are the same as what you had issued before because there wasn't a concentration limit in those original requests. It was maximum concentration of use. So, some of them will be the same but the reason for the second IDA is because you do have additional data requests that weren't included in the original IDA. Is that correct?

DR. COHEN: Well, I think that's partially correct, right? I mean, the original IDA was predicated on a max concentration of use which has been updated, right? And then there's this discussion about a failure to read across or potential failure to read across which is creating additional insufficient data needs.

MS. FIUME: Okay. No, I was just curious because the original IDA didn't specify what that max concentration of use was, it just stated at max concentration of use.

DR. COHEN: Now, I don't remember, but did we know it was 0.075 in that original report?

DR. ROSS: No.

MS. FIUME: So, we did, but then as part of the IDA it had -- no we did not know that. That was the lower, I believe. But we said at max concentration of use. If max concentrations of use are higher than what's used already in the report. So, that dermal irritation and sensitization included a caveat in case there was a higher concentration.

DR. COHEN: What you're saying is that the original IDAs weren't met?

MS. FIUME: Part of them were, but the reason for the second IDA is because you have additional needs, correct, compared to what was in the original IDA? Okay.

DR. COHEN: Yes, yes. Yeah, I think I understand your question and I think you've articulated what we're asking for.

MS. FIUME: Okay. So, I guess my point was that if you knew that there was a higher concentration of use, but you didn't have those data, typically you would go forward then maybe stating it's okay at one concentration but not the other because of the data you've received. But now that there are additional needs, that predicates the second IDA and then anything from that first IDA can be updated. Does that make sense what I'm trying to say?

DR. COHEN: Yes, yes.

MS. FIUME: Okay.

DR. COHEN: Let me just come back. I don't know where I went here. Okay.

MS. FIUME: We can see you.

DR. COHEN: I can't see anybody. Let me just --

MS. FIUME: Oh.

DR. COHEN: -- Paul came in and then just knocked me off the meeting.

DR. ROSS: We can still see you. And hear you.

DR. COHEN: I'm sorry for that.

DR. ROSS: I think he's trying to say he's on vacation right now. Now he's frozen.

MS. FIUME: Now he's frozen. He may have been going out and coming back in.

DR. ROSS: He's frozen smiling.

DR. BERGFELD: Well, that's a nice freeze. David, are you with us or are you frozen in all ways?

MS. FIUME: I have a feeling he might be trying to come back in even though it doesn't show that he left the meeting.

DR. BERGFELD: Do you want to send him a chat that we can't see him or hear him?

MS. FIUME: I'll see if I can send a text because I think the chat is disabled.

DR. BERGFELD: Okay.

DR. ROSS: I'm just thinking how well we've done on the technology.

MS. CHERIAN: It looks like Dr. Cohen needs to be made a presenter. He's listed as --

MS. FIUME: Okay. So, he must've come back in. Thank you.

MS. CHERIAN: You're welcome.

DR. COHEN: Yes. I was waving my hands, but I could not get in.

MS. FIUME: Sorry.

DR. COHEN: So, Monice, I think I understand what you're saying but I'm not sure what I'm supposed to do with this statement, to be honest with you.

MS. FIUME: So, I guess it was just more for understanding procedure as just for the purpose of this meeting and future meetings that originally some of the list that was given were the same IDA requirements from the last meeting that didn't state at a concentration of 0.005. so even though you would've received a different concentration we still could've gone forward -- the panel still could've gone forward with the information that was in the report to decide if it covered that higher concentration or not.

But since there were additional data needs that predicates going forward with the second IDA because if you had -- if the data needs weren't any different and it was just based on a concentration typically the panel doesn't issue a

second insufficient data announcement especially since the first one said at maximum concentrations of use even if they're higher. Give us what you have. It was just a matter of procedure.

DR. COHEN: Yeah, yeah. No, no. I get that. So, I mean, one way to look at it is we don't have that data at max use.

MS. FIUME: Right. So typically, if that's the case, then you go insufficient as a conclusion not asking for a second IDA.

DR. COHEN: I get it. So, I und- --

MS. FIUME: But you have additional needs so then that does predicate a second IDA because they weren't included the first time around.

DR. COHEN: Well, what we have is, we have an IDA for ET, but we have insufficient data for ethyl. For isopropyl, I mean. Right?

MS. FIUME: Right. So, since it's going out anyway, then to combine it all and ask again makes total sense.

DR. COHEN: Right. It's not technically an IDA. It's insufficient to the original IDA.

MS. FIUME: Well, it would be a second. Right, you'd be issuing a second IDA. If you didn't have additional data needs, then you would probably -- according to procedure -- you would typically go forward with an insufficient conclusion which someone could comment on. But since you have additional needs then it's an insufficient data announcement.

DR. COHEN: Yes. I think for the purposes of giving everyone a chance, I think I'd prefer an IDA here because it gives people another go around with this as opposed to getting it closer to a final report that's not going to work.

MS. FIUME: Right. And, as I said, you have needs that weren't in the previous one so it would have to go IDA regardless because you can't go insufficient conclusion for something that you hadn't asked for.

DR. COHEN: Right, right. No, I get that. Okay. Any other business on the prostaglandins?

MR. ABRAMOWITZ: Just before we go, I'm going to try to understand. Is there no way that the panel can issue a safe for use up to 0.005 percent while issuing an IDA on 0.0075 percent since all the data has been submitted and that the panel has done that before where it's issued to a certain concentration when it has a full data set on that particular concentration? We're just trying to understand because, obviously, it's a product that's not represented here that's simply been an anonymous submission and we don't have a basis for understanding whether that data will ever come in.

DR. COHEN: Well, we don't know that but sometimes we'll get reports on lots of uses. I don't know who they are from. Most of the time, we don't have representatives advocating for a specific product, we have 80 uses and maybe a couple dozen concentration reports. So, this is routine for us to get different concentrations of use.

DR. BERGFELD: I think that maybe what you're really asking is to go forward faster than we would like. It doesn't mean at the next meeting we won't do exactly what you suggested.

MR. ABRAMOWITZ: Okay. I mean, I think that's our concern is that we share that concern, they'll never be any data on that particular concentration.

DR. COHEN: We get it.

DR. BERGFELD: I think that we're going to make another try and then we're going to make a decision. Yeah.

DR. ROSS: There were some insufficiencies even in the IC reports even though I'm not expecting any surprises there but there was no genotox in that report. For example, I pointed that out earlier. So that's probably needed. But yeah. I think we got the gist of it.

DR. BERGFELD: Okay.

DR. COHEN: One second. The genotox, David, was that in our original IDA?

DR. ROSS: No, I don't think so. And someone can correct me if I missed it. I don't think I missed it. It was there for ET, but I didn't see it for IC.

UNKNOWN MALE: Did we have any genotox?

MR. ABRAMOWITZ: We have read across data for genotox for IC. There's not a lot of direct IC genotox because of the animal testing ban.

DR. KOWCZ: They want the dermal tox.

MS. FIUME: Dr. Cohen, in response genotox was not in the original IDA so that would need to be added.

DR. ROSS: Well, I mean, yeah. The point is that you've got read across here. So, they're reading across from travoprost and if that read across is okay then --

MR. ABRAMOWITZ: And cloprostenol, both of them, genotox from travoprost and cloprostenol.

DR. ROSS: Yeah, we already looked at cloprostenol as not read across. But anyway, that's a different discussion.

DR. COHEN: Okay. I think we've had a good conversation here.

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DR. COHEN: That's a lot of time on this one. Yes, so there was quite a robust conversation both in the morning and in the afternoons about the Prostaglandins. This assessment is for Ethyl Tafluprostamide and Isopropyl Cloprostenate. At the June 2023 meeting the Panel issued an Insufficient Data Announcement for these ingredients and requested the following information: concentration of use, information on packaging of products and directions for consumer use, 28-day dermal tox data and if absorbed further systemic tox, dermal sensitization and irritation data at maximum concentration of use (if maximum concentration of use are higher than the concentration used in dermal irritation and sensitization studies already present in the report) -- that was a provision -- intraocular pressure data on eyelash preparation containing Isopropyl Cloprostenate, and potency/inhibition constant binding affinity data on Ethyl Tafluprostamide and Isopropyl Cloprostenate as compared to bimatoprost (an FDA-approved prostaglandin analog for ocular hypertension/glaucoma as well as eyelash lengthening)

Since the issuing of this IDA, new data has been received on both chemicals. Amongst the several discussions we had, our group was generally uncomfortable with read-across and this was pointedly expressed by Dr. Klaassen yesterday. We have new max use of Isopropyl Cloprostenate at 0.0075 percent. We had some issues in the calculations of margin of safety for Ethyl Tafluprostamide as it relates to retention factors in the use of LOAEL instead of NOAEL.

Our motion is as follows, since there's new max concentration of use we're issuing an IDA for Isopropyl Cloprostenate with the following needs recognizing that some of these are previously insufficient from the initial IDA, however, our motion is for a new IDA; our needs are irritation and sensitization at max use, local ocular effects including pressure and eye risk color change at max use, acute tox, repeated dose tox, DART, we still need genotox, and we're requesting an independent ophthalmology evaluation to assess color imagery data regarding iris color change data.

For Ethyl Tafluprostamide we have an IDA for the following needs; acute tox, repeat dose tox, DART or if there are confirmatory data on read-across that would suffice such as receptor interaction studies and downstream profiles of adverse effects. Lastly, we have a need for eyelash product information or consumer use instructions to include cautionary statements for pregnancy, lactation, pediatric use and those who are sensitive individuals such as those undergoing chemotherapy or treatment of eye disease. That's the completion of my motion.

DR. BELSITO: So for Ethyl you did not want genotox? You didn't mention that.

DR. COHEN: Let me look back, Don, once second.

DR. SNYDER: I wrote down that he mentioned it.

DR. BELSITO: No, for Isopropyl he mentioned genotox.

DR. BERGFELD: I didn't hear it either.

DR. COHEN: Genotox.

DR. BERGFELD: You're adding genotox?

DR. COHEN: Yeah.

DR. BERGFELD: Okay.

DR. BELSITO: I'll second it. You want way more than we asked for, but it would go out as an IDA anyway so we can just go out with all of your requests.

DR. COHEN: Okay.

DR. BELSITO: The only other thing, if they want, read-across would be to give us more robust information on all the possible targets and mechanism that would come out.

DR. BERGFELD: Any other comments for the IDA, the second one?

DR. COHEN: Priya?

MS. CHERIAN: We had genotoxicity data for Ethyl. That's probably why it wasn't in your IDA mention.

DR. ROSS: Was that read-across?

MS. CHERIAN: It was in vitro data.

DR. ROSS: Okay.

DR. BELSITO: But we need in vivo data.

MS. CHERIAN: Okay.

DR. BELSITO: It's on PDF Page 36. Oh, we do have a micronucleus assay as well.

DR. COHEN: Yeah.

DR. HELDRETH: We have a couple hands up from attendees, if this is a good time to address those.

DR. BERGFELD: Can you call them? I can't see them.

DR. HELDRETH: Yeah, I'm bringing them in. Okay, so we have Mr. Abramowitz and John Bailey.

MR. BAILEY: I'll let David go first and then I'll follow. And I think Tom Petry has also raised his hand to address the IDA element. David, won't you go ahead.

MR. ABRAMOWITZ: Thank you, John. And thank you to the Panel. After hearing the needs on ICN we understand the max use issue as opposed to the .005 percent concentration issue, which is where you've received most of your data. I do want to ask some questions about for example the genotox request. We are certainly -- we started our safety program long before this commission and Panel started looking at IC, and so we've been continuously working towards establishing the safety of IC at .005 percent or less. So, could you please, if you can tell me, what exact genotox studies you'd like to see, so we can move forward with that progress, we'd appreciate it. Genotox is a big category.

DR. BERGFELD: Curt, or Tom, or David?

DR. SLAGA: Well, actually we'd prefer to see several in vitro and in vivo tests.

MR. ABRAMOWITZ: Which in vitro test would you like to see, AMES?

DR. SLAGA: In vitro would be AMES, primarily.

MR. ABRAMOWITZ: And given the animal testing done, what in vivo test are you looking for?

DR. KLAASSEN: Micronucleus test, isn't that what you're asking for, Tom?

DR. SLAGA: Come again?

DR. ROSS: Yeah. It's usually micronucleus.

DR. KLAASSEN: The micronucleus test we would like.

DR. SLAGA: Yes, micronucleus test would be one.

MR. ABRAMOWITZ: Okay. And we're trying to outline here so we can make sure the Panel has everything they need on .005 percent or less. In addition, you mentioned DART. Now, obviously we can't go back in time and run DART studies on mice or rats, or rabbits. Is there an in vitro test you're looking for on DART, or other information? I know we have provided read-across both on travoprost and on the primary metabolite of IC, cloprostenol. We'd appreciate it, if you don't have any suggestions for DART, if the Panel would go back and reexamine that particular read-across considering that cloprostenol, for example, is the primary metabolite of IC in the body. So it should provide a robust system for looking at IC.

DR. BERGFELD: Comment? Curt?

DR. COHEN: I think we discussed the read-across quite a bit yesterday.

DR. BERGFELD: Yeah.

DR. KLAASSEN: There aren't a large number of in vitro DART studies unfortunately. So what we usually hope to see is some in vivo studies. It's about the only way we have for looking at teratogenicity and other reproductive problems.

MR. ABRAMOWITZ: Thank you, we appreciate that. And we would like the Panel to continue to consider that the read-across for IC. It's different than a read-across for ET. And our methodology is different since we're using the primary metabolite as a bases as well as a more similar chemical.

I don't know exactly how to deal with your DART request. In addition to that individual ophthalmology assessment, we have photos from our clinical study, if that's something that the panel would like us to submit. We can submit something like eight gigabytes worth of photos for the ophthalmology study, if the Panel wishes to seek an ophthalmologist to assist it.

DR. COHEN: I think that would be useful to get an independent person to look at it, and look at the colorimetric data.

DR. BERGFELD: I think it would be important if it's someone who's very knowledgeable of that kind of technique and interpretation. Any other comments?

MR. BAILEY: I just want to thank the Panel for your comprehensive deliberations. And I think the IDA that you're issuing is extremely helpful. We need the details to understand how we need to fill the data gaps between now and the next meeting. You know I keep emphasizing read across, but it's extremely important to be able to fill these data gaps. And what I think I heard is that you're willing to consider more in-depth justifications for applications for the IC and the ET. So, we'll certainly work to provide that.

And one final thing, yesterday there was a lot of discussion back and forth, and I just wanted to reemphasize that we're really talking about cosmetic uses here, not drug uses, not drug endpoints, but cosmetic safety. And this was affirmed by FDA early on. So I just want to make sure that we keep that in mind, we're looking at cosmetic safety endpoints, going forward. So, with that, I think we have a pretty good idea of what we're going to need. And, again, appreciate the Panel's deliberations.

DR. BERGFELD: Thank you, John. Dr. Petry, did you want to say something? I heard that your hand was up.

DR. PETRY: Yeah, first of all, again also to thank the Panel for the good discussions we had on the read-across justification. I certainly understood what you're specifically looking for. And I think we have some good ideas on what we can do and hopefully we can provide the data for the next review.

There was one question I do have with regard to genotoxicity for ET. Because you included now in the IDC, we have a base set of the (inaudible) tox in vitro data, which is the AMES test, the mammalian mutagenicity test and also the micronucleus test in vitro. Is there anything in addition that you're looking for?

DR. BELSITO: We usually ask -- yeah, I think you're fine. It was my mistake.

DR. BERGFELD: So, you're withdrawing the need for genotox?

DR. BELSITO: For ET.

DR. BERGFELD: For ET.

DR. KLAASSEN: In regard to the micronucleus, you said you did that but it was in vitro, or in vivo?

DR. PETRY: It was in vitro.

DR. BELSITO: It was in vitro.

DR. KLAASSEN: Yeah, well, we would much -- we usually look at in vivo micronucleus. That would make us much happier; let me put it that way.

DR. PETRY: Well, maybe we can bring you that next time again. Because if we can establish a good read-across, we know that for Tafluprost there is in vivo micronucleus data as well.

DR. KLAASSEN: Great.

DR. COHEN: So, Don, we could keep your suggestion in my undated motion.

DR. BELSITO: Thank you.

DR. BERGFELD: And that suggestion is keeping the genotox need in there?

DR. COHEN: Yes.

DR. BERGFELD: Okay.

DR. ROSS: Yeah, the weight of evidence usually goes down on the in vivo genotoxicity, so, Dr. Petry, that would be good to have.

DR. PETRY: Well, on ET it's obviously for animal testing reasons we cannot do it. But let's hope that we can provide an improvement of the read-across justification. And then we should have also the link to the in vivo data which we have for Tafluprost.

DR. BELSITO: And just a comment. In terms of using the major metabolite of IC, one of the issues as we have no information on absorption distribution of metabolism. So we don't really know how long IC sits un-metabolized on the skin, and to Curt's point about the uniqueness of chemical interaction with receptor.

DR. ROSS: Yeah, and Dr. Petry's point about Tafluprost. We had almost exhaustive and certainly exhausting discussions on read-across yesterday. And I think we came down that Tafluprost, certainly in our meeting, wasn't going to cut that. I don't want to reopen that issue, but you should be aware of that.

DR. BERGFELD: All right. We have any other discussion, because we're ready to vote on the second IDA.

DR. ATOR: Can I just ask a quick question regarding genotoxicity testing?

DR. BERGFELD: Okay. Yes.

DR. HELDRETH: Can you please state your name and affiliation on the record, please?

DR. ATOR: This is Jennifer Ator. I wrote the tox report for the IC.

DR. HELDRETH: Thank you.

DR. ATOR: We are concerned that doing an in vivo micronucleus assay is not feasible because of the animal test guideline band. With that in mind I want to ask the Panel if maybe a different approach would be acceptable. For example, if we perform a battery of in vitro tests that covers all the relevant assays. And they are all negative, would that address the Panel's concern. And secondarily, if the answer is no, would and in vitro micronucleus assay address the concern.

DR. BERGFELD: Curt, Tom, David?

DR. KLAASSEN: Well, I think I'll just start off and then let Tom follow up. I guess it kind of depends on the whole package. And to give you an absolute yes or an absolute no, I think is impossible at this time. I think this group is always willing to look at the whole package, and how you describe it to us, what you have, you know, which chemicals you're looking at, which read-across, is there any in vitro that you can kind of use like Dr. Petry was just mentioning. So, put your best package together. We realize that there is a problem in this day and age with getting the ideal information. So that's what I would say. Tom?

DR. SLAGA: I agree with Curt. I think we'd like to see whatever package with genotoxicity you bring forward. And, if possible, the read-across data it's acceptable for eliminating doing an in vivo genotox. We would like to look at that and make a decision from it.

DR. ATOR: Okay, great, thank you very much.

DR. BERGFELD: All right, are we ready to vote on this? So this is a vote on an IDA, seconded, on the Prostaglandins. All those opposed? Abstaining? Unanimously voted on to approve to go forward with the second IDA. All right, at last we're going on to Yeast, Dr. Belsito.

JUNE 2024 PANEL MEETING – DRAFT TENTATIVE REPORT

Belsito Team – June 3, 2024

DR. BELSITO: Which brings us to Prostaglandins. Okay. So, Allan, I obviously didn't get a chance to open your email. Can you tell us an update on what the Read-Across Committee thought about the read-across for the Prostaglandins before we even start this document?

DR. RETTIE: Sure. So, Paul, I just sent you a Word document. I think everybody on the team now has this Word document for the Group that you might want to open.

So, we met. Well, Curt couldn't make the day that we met, but Dave Ross, Susan Tilton, myself, Bart had a Teams discussion last week about read-across considerations, not just for the Prostaglandins, but also for the amphocarboxylates, which is the next topic after this one. We came up with some general considerations, and maybe I'll just read those general summary points from our discussion.

Goes without saying that read-across is complex. We felt it should be done in a compound-specific and endpoint-specific manner. We're particularly concerned about read-across for sensitive endpoints such as DART, particularly where positives have been detected with either the source or the target ingredient. And so, we thought that should be avoided. That was a point Dr. Klaassen has brought up, I think, on a number of occasions.

DR. BELSITO: I'm sorry, I don't follow why you thought it should be avoided.

DR. RETTIE: Particularly because when positives have been detected with either the source or the target ingredient, that's a complication for one of these reports.

DR. BELSITO: I'm still having difficulty following what you're saying.

DR. RETTIE: Okay. So, we're saying that read-across for particularly sensitive systemic endpoints like DART should avoided where in the report we can see positives for that toxicity with either the source or the target ingredient.

DR. BELSITO: You can see positives in the report for the target?

DR. RETTIE: I'm trying to pull up an example.

DR. BELSITO: For the read-across? Then you're already saying you're using read-across. That's what I'm not following.

DR. KLAASSEN: Think what he's saying is that, if we have a chemical that has some hints of teratogenicity or other developmental effects, we would need to be very careful in how we interpret that and how we should consider that in the whole read-across.

DR. RETTIE: Or whether we should even do it.

DR. KLAASSEN: Or whether we should even do it.

DR. BELSITO: But your sentence here -- I just opened it -- says, "Read-across for sensitive endpoints such as DART, particularly where positives have been detected with either the source or target ingredient, should be avoided." Obviously, if we have data on the target -- and what do you mean by source? What's the difference between source or target, the cosmetic ingredient -- the cosmetic product?

DR. HELDRETH: Source means the chemical you're planning to read-across from. And then target is, in all the cases here, the ingredient --

DR. BELSITO: Okay. So, with either the read-across or the target. But then you've already included read-across. So, why would you avoid? It says, "Read-across for sensitive endpoints such as a DART, particularly when positives were detected with either the read-across source," but you're already read-acrossing. You're already doing read-across if source is read-across, right.

DR. HELDRETH: But you don't feel confident about making that conclusion by comparing the source and the ingredient.

DR. BELSITO: Okay. So, when you're using the word source here, you're talking about the read-across material.

DR. HELDRETH: Yes.

DR. BELSITO: Okay.

DR. HELDRETH: The chemical that's being used to fill the gap.

DR. BELSITO: So, if we had no data on a target but we had perfectly legitimate reasons for doing the read-across on that endpoint -- because when you look at -- to me, you're doing read-across on endpoints, but you're using endpoint-specific targets for your read-across, right. You're using targets that have structural activity relationships pertinent to genotox or pertinent to DART. You're running them through all of these in silico predictive tools. And you're coming up with, okay, the metabolites are going to be the same. Why not use them as read-across?

DR. RETTIE: I think you're perhaps assuming that we've decided to do read-across on this, and maybe read-across should be prefaced with potential read-across for sensitive endpoints because, a message that's come out of looking at these two ingredients with the Read-Across Working Group, we just didn't feel confident applying any read-across for much of what we've been asked to look at.

I'm sort of hesitating on this a little bit since I got this statement from Dr. Ross this morning just before I came down here. So, I think we can maybe revisit that one at least in the full team meeting or even tonight.

So, we had concerns there for particularly sensitive endpoints. We also had concerns for potential read-across for potent pharmacological compounds, and this is the thing that Curt has brought up in the past, particularly challenging when we're looking at ingredients that act through receptors. And prostaglandins and steroids are right in there. We feel that that was very challenging, and we need to approach it with extreme caution. So, that was very instructive for the two ingredients we were considering for read-across here.

The other complication we felt we had in this group of two was that read-across A is extremely difficult for a mixture. And for the amphocarboxylates, we have increasingly well-defined ingredients, but there's still, in my mind, a little bit of doubt about exactly what we're looking at. And so, mixtures beyond potent pharmacological compounds were another group where felt a little worried about using read-across. We come back to that with the amphocarboxylates.

DR. BELSITO: Yeah, we can do that with the amphocarboxylates. But I just want to point out the mixture of amphocarboxylates are a relatively pure chemical with water and other individual ingredients, not a mixture of different actives.

DR. RETTIE: As -- excuse me -- as it's stated in the report. And I just have questions about that relative to the methods used to make them, and then cross-referencing to the data that we see from Europe. Major consideration there, at least for me, was not at the level of the different acyl chain groups. You have all seen them a number of times, and I think there's more of a degree of comfort around these different chain acyl compounds. But they are heterogenous, these compounds, even though it's not written like that in our report.

DR. BELSITO: Well, let's do that when we get to the amphocarboxylates. We have a hand raised back there.

DR. ATOR: I'm Jennifer Ator.

DR. BELSITO: Identify -- yeah.

DR. ATOR: Do you want me to stand up, speak louder?

DR. BELSITO: Yeah, go to the mic and identify yourself.

DR. ATOR: Come to the mic? Great. Great. Good afternoon. I'm Jennifer Ator, and I just wanted to ask a question of the Read-Across Working Group.

Will those minutes be made available because I'm particularly interested to see the sources, the literature that were consulted? I know a lot of people consider DART to be really sensitive, and we certainly understand that. But I would also respectfully remind everyone that dose response is a thing. The exposure levels we're talking about here are de minimis. And really one of the strengths of the read-across approach that we leveraged in this case was the knowledge that these are potent pharmacological agents and that they do have reproductive and developmental effects. It seems to me that there's a tendency to shy away from those kind of effects.

And I get it; nobody wants to make a mistake as far as that's concerned. But the very fact that we have leveraged the most sensitive data on those reproductive and developmental endpoints is not only good science; it's also established risk assessment procedure and confers and adequate margin of safety.

So, I would just ask the Working Group to consider some of the available frameworks that are out there. To my knowledge, there's no endpoint for which read-across has been demonstrably unsuitable. It is endpoint-specific. It

is model-specific. It must be carefully done, absolutely. But we would just appreciate the opportunity to really have a very specific discussion about the nuts and bolts of doing it to go about it in the most scientifically acceptable way.

DR. RETTIE: So --

DR. HELDRETH: To your first question, yes, the minutes will be available. Just like tomorrow, the full panel will vote on the approval of last meeting's minutes. At a future meeting when the full panel is voting on the minutes from this meeting, they'll also have an opportunity to vote on the minutes of the Read-Across Working Group.

DR. RETTIE: Yes, for sure you'll see that.

DR. ATOR: Great.

DR. RETTIE: I don't mean to be dismissive of all the information provided, but really as we got into it, the Working Group, it was sort of a first level look at this for us. And it was our first --

DR. BELSITO: Who's on the working?

DR. RETTIE: Curt, myself, Susan, and Dave Ross.

DR. BELSITO: Okay. But Curt was not at this meeting.

DR. RETTIE: He was not part of it, yeah.

DR. BELSITO: But Susan and Dave were.

DR. RETTIE: Susan and Dave were there, yeah.

DR. BELSITO: And these were unanimous decisions, split decisions?

DR. RETTIE: Well, yes, they were. Everyone agreed.

DR. BELSITO: Okay.

DR. RETTIE: There was some minor disagreement on some points of detail that I included in my write-up for the report, and Dr. Ross will probably talk about those when we get together tomorrow. But we were looking more at high-level issues here for our first meeting to establish working methods really. I wouldn't say they were rules, but working guidances for us. We felt a lot of what we were presented with kind of fell out for us because all of the team members, including Curt, when we had this discussion before felt that the betaines were not a suitable read-across, and I have some detailed examples for why we felt that.

DR. BELSITO: You're on the amphos. We're on prostaglandin.

DR. RETTIE: Yeah, okay. So, that was an issue for us for the amphos. The main issue I think, at least for me, with regard to the prostaglandins was the ethyl tafluprostamide. We have mentioned several times that it has a distinct pharmacological target that we can't molecularly get our hands around, or at least from the literature. I don't know what it is. It's a prostamide receptor. That gave us pause.

So, we didn't go very far with read-across to ethyl tafluprostamide. There were a number of questions and some nice proposals and examples provided to the isopropyl cloprostenate, possible read-across to two or three different compounds that structurally look pretty similar. I would say the arguments in general there we accepted, and we focused in on maybe one specific chemical, the travoprost, as perhaps being -- well, as being the best-looking structural surrogate for isopropyl cloprostenate. So, that was an area where we felt we could make progress, but we're kind of stymied with ethyl tafluprostamide.

DR. ATOR: I'll just jump back in here. I appreciate the distinction between the different chemicals. And I think it's absolutely appropriate, scientifically substantiated to look at it on a case-by-case, chemical-specific basis. I've done a lot of work in this space, and if anyone here would find it helpful, I would be glad to answer any questions, provide any resources that I'm aware of to help, not only the Read-Across Working Group, but the Panel in general understand what can a pretty novel topic for people. It can be a little daunting because, like I said, people, we don't want to make a mistake. We understand that. But we believe we've based our assessment on the best scientific practices. So, thanks for listening. Appreciate it.

DR. RETTIE: Well, I can go through some of the other specifics for the prostaglandins --

DR. BELSITO: No, please do for us.

DR. RETTIE: -- that came out of the report, the Working Group.

So, to back up, at our earlier meeting here with this group, we agreed the first time around that it was not appropriate for us to use a prostaglandin prodrug formulation as read-across for the active pharmacological agent because those are carboxylic acids, and so the point was made numerous times about differences in distribution and dermal penetration. I think that's a reasonable, very reasonable, point to make.

DR. ATOR: Yeah, I'm nodding over here. But, yes, indeed. And that is the exact purpose of us doing the dermal metabolism penetration study, to address that.

DR. RETTIE: Very good. But beyond that -- and we covered that before -- the group, primarily me I think, had concerns that read-across for any prostamide needs itself to be an amide because of the different receptor targets. Also, I was struck by the fact that there was extensive metabolism over 24 hours of the amide. It was hydrolyzed to the active -- well, who knows what the active is for that. So, technically, we have two active agents floating around when considering amide application. That was a bit of a surprise. But I think it didn't help the argument for read-across.

What we more specifically came to was that we considered using travoprost as maybe the best read-across for isopropyl cloprostenate. The reasoning there was that the (inaudible) -- just on structural considerations, the (inaudible) is there. The aromatics, halogenated aromatics, get our attention, get my attention. So, the read-across possibilities for that seemed pretty good to us, although, maybe not for DART if their biological potencies of travoprost, the thing we want to read-across to, are highly different with regard to the reproductive system. We're more or less happy with tafluprost as a read-across. It is structurally -- looks pretty structurally close to the trafluprostamide, but it's isopropyl ester, not a prostamide. So, that can overrode other considerations for our group.

Couple of points to note from Dr. Ross. He wanted to include travoprost as a read-across for isopropyl cloprostenate. SCCS compared an ocular endpoint, conjunctival hyperemia, and found up to a tenfold variation with doses required for a cloprostenate, latanoprost and travoprost. And so, he was -- and I agree with him -- he was questioning the use of either latanoprost or travoprost as accurate read-across when we have this tenfold variation to maybe be concerned about. So, I hope that made some sense in terms of the specific compounds that came across from you.

DR. ATOR: Yeah, thank you very much. Just with respect to the ocular hyperemia, we do have clinical data, substance-specific, that we've included. Hopefully, that will be reassuring to the panel in terms of the ability to irritate the eyes. And also, to go back to your point about it not being appropriate to use in ester as a surrogate for the pharmacologically active acid, of course, and that is what we are hoping that the dermal penetration and metabolism study will demonstrate and further support the suitability of using the hydrolysis product as an additional surrogate.

DR. BELSITO: Your data on ocular safety though was on an N of 56, is that not correct? Just pulling out of my head.

DR. ATOR: Right. We actually have several studies. We have several studies. We have several clinical studies. And we also have HET-CAM assays.

DR. RETTIE: Can you just remind me when we will likely see these data?

DR. ATOR: The HET-CAM assays have been submitted. The summaries of the clinical studies should have been available to you, the data, end of April, that were submitted.

DR. BELSITO: Yeah. Just a question when you're asking us to read-across. Why did you not pick brimatoprost, which has been approved for the same endpoint that you're looking at?

DR. ATOR: Right. Well, using the available read-across framework such as the ECHA guidance, the EU's Read-Across Assessment Framework for example, they are very specific in their approaches and the endpoints that they consider when assessing structural and mechanistic similarity.

The first place that we started was structural similarity. We really on the Tanimoto coefficient, as many people do. It had a lower Tanimoto coefficient. It's also different. It's an amide. It's not a carboxylic acid. It's receptor binding profile is also different from those agents that are the carboxylic acid esters. So, that's why we felt that it was not the most suitable surrogate.

DR. RETTIE: But there are new data coming, correct?

DR. ATOR: Pardon?

DR. RETTIE: There's new data coming that's not ready yet for us?

DR. ATOR: Yes, that is the dermal metabolism and penetration study. We are thrilled that it's in progress. We have two out of four donors completed. We hope that the remaining two will be completed in short order, so those data are in progress. We also have the genotoxicity studies that we submitted and the independent ophthalmological review of our colorimetric data as well.

DR. RETTIE: Those would all be very useful.

DR. KLAASSEN: So, there at least a half a dozen prostaglandin receptors, and we know that various chemicals can interact with these various receptors quite differently. What do we know about these two chemicals in regard to their activation of these half a dozen receptors?

DR. ATOR: There are no data on IC itself in terms of PGF2 alpha receptor binding. That's thought to be the receptor subunit that is responsible for the inner ocular pressure, also the iris color change, a couple of other effects.

A lot of the receptor binding studies are done on an academic basis, and it is not feasible in terms of commercial assays that we could easily put in a lab, other than F2 alpha, which is commercially available. But I think it's important to recognize that what we do have are the downstream clinical data that show that these effects that are thought to be mediated by these various types of prostaglandin receptors do not occur with a cosmetic use of at least the isopropyl cloprostenate. So, I think that is important to keep in mind, that even if we don't have all the pieces of all the receptors that are potentially in play, what we do have is outcome data.

DR. KLAASSEN: How about the reproductive properties?

DR. ATOR: Yeah. That's a great question, and that is why we are working hard to substantiate the use of cloprostenol, the hydrlolysis product, as the surrogate. We know that cloprostenol is reproductively active. There's a robust database in terms of reproductive and developmental effects. That is why we are intentionally pursuing this line as a conservative approach. We based our original margin of safety calculations on reproductive and developmental effects. It was actually travoprost because that was more conservative than cloprostenol.

DR. BELSITO: That bothers me because if you read the package label on brimatoprost, it's not to be used during pregnancy, and it's at 0.003 percent as well. I just feel -- I don't know. If you read the ads for the products that are marketed, they are talking about what I see as drug effects, not as cosmetic effects.

DR. ATOR: Which drug effects would those be?

DR. BELSITO: Enhance the growth of eyelashes. It's a change in structure and function, right.

DR. ATOR: Right. That has only been demonstrated for brimatoprost, which for the reasons that I enumerated a couple of minutes ago we did not feel was an appropriate surrogate.

DR. BELSITO: But if you go on the way they're being marketed, that's not the way they're being marketed. Just go online and look at any of the ads for you what you can buy, and they're showing before and after pictures similar to what the type of data we saw on brimatoprost was going before the FDA. The whole area just sort of bothers me, you know, the eye color change, the interocular pressure. Also, we had previously asked for the ophthalmologic data to be reviewed by an outside consultant. That wasn't done.

DR. ATOR: That is in progress.

MR. BAILEY: Which kind of brings up sort of an overarching point here, and that is --

DR. BELSITO: John, you just need to introduce yourself.

MR. BAILEY: I'm John Bailey.

DR. BELSITO: Okay.

MR. BAILEY: Consulting firm that's representing the three sponsors for the prostaglandins. The overarching point is that there are three sponsors. They are in the process of doing studies to answer these questions. They are either going to be started soon, or they're under way. In the information that was submitted, roadmaps were provided to describe those studies and the completion dates. So, what has been requested is that the prostaglandins be tabled to the December meeting to allow these to be completed and submitted in plenty of time for Panel to review the data and be ready in December to look at the final revies.

So, as Jen has explained, there's a lot of work going on to answer these questions. The minutes from the Working Group meeting will be very helpful in helping the sponsors to understand what sorts of questions you have. So,

again, what we're requesting is for it to be tabled so that these can be done. The clinical studies, that's under progress now, trying to respond to that request for an independent assessment, however that might work out.

DR. KLAASSEN: To me, the thing that bothers me the most is potential reproductive problems. And how you can ever provide sufficient information to eliminate that concern, I'm having difficulties seeing to put two and two together.

DR. ATOR: Understandable. That is why we have put so much effort into the read-across approach. For example, our dermal metabolism and absorption study, we anticipate will show the majority conversion to the active acid form. We know what that does in terms of its adverse reproductive and developmental effects. If there's little to no systemic exposure to the intact ester, then I think it would be reasonable to conclude that that is not a hazard for this particular ingredient.

DR. BELSITO: Well, it's a hazard. It's not a risk.

DR. ATOR: It's not a risk, yes. Thank you.

DR. BELSITO: So, let's go through the current document we have, decide if we're going to table it, but also get some idea where we're at. But, Allan, I'm still not certain. When we get this back, what is the read-across? So, you've said for the -- there's a potential read-across that you would allow for isopropyl cloprostenate, and that was tafluprost, is that correct?

DR. RETTIE: That was travoprost.

DR. ATOR: Travoprost.

DR. BELSITO: Travoprost, rather, I mean. So, are we going to allow travoprost? Is Priya going to take in all the information on travoprost? We need these decisions or at least the recommendations from our team.

DR. RETTIE: Our group, Read-Across Group, has not sat down and decided if this is a unanimous thing to do. My recommendation would be that travoprost should be used -- or should be considered.

DR. BELSITO: Okay.

DR. RETTIE: And we would --

DR. BELSITO: But I think before we decide to proceed, Priya needs to know what she's bringing into the document. So, we need some firm decision from the Read-Across Group whether we can use any read-across or not.

DR. RETTIE: And I think we'll get in the team's meeting.

DR. BELSITO: Okay. So, you're thinking that travoprost may be okay for isopropyl cloprostenate, but the other suggested read-acrosses for that particular material were not good.

DR. RETTIE: Correct.

DR. BELSITO: Is that correct? Okay. And then for the tafluprostamide, you thought there were no available read-acrosses.

DR. RETTIE: At our meeting, we left that alone. We did not feel there were any appropriate read-acrosses. What I'm hearing from you is that the acid is now under consideration. You're going to provide some data for us on the acid.

DR. ATOR: John, could you comment on that. I do not myself work on the ethyl tafluprostamide.

DR. RETTIE: Maybe I misheard. John.

MR. BAILEY: There are studies that are either started or underway that would address such things as receptor activity and to support the appropriate surrogates for potential read-across.

DR. BELSITO: Tafluprostamide was absorbed at ten percent. It was the only one we had some dermal absorption on.

DR. RETTIE: Twenty percent estimate for tafluprostamide, but that was an estimate.

DR. BELSITO: Yeah. No, this is data on dermal.

DR. RETTIE: Let me see, 8.67 dermal absorption. Yes. But you have new data coming to us for that?

DR. BELSITO: Yeah, the 8.67 was used in the risk assessment, but go to PDF Page 63, bottom of the line. So, this was looking at different concentrations. At 0.024 percent, 10.68 plus-or-minus 7.18 percent of the applied dose was absorbed. So, I think if you're being conservative, you need to use the higher amount, no?

DR. RETTIE: Agreed.

DR. BELSITO: So, it's not eight percent. Okay. So, your own personal feeling that we'll hash out tomorrow, Allan, is that there is for isopropyl cloprostenate, tafluprost would be a reasonable read-across for the DART endpoint.

DR. RETTIE: Travoprost.

DR. BELSITO: Travoprost, rather.

DR. RETTIE: Yeah.

DR. BELSITO: I'm sorry. Would be a reasonable read-across.

DR. RETTIE: We had questions about whether we should be doing any read-across for DART.

DR. BELSITO: Okay.

DR. RETTIE: So, I can't say that for DART.

DR. BELSITO: Okay. So, DART is where we're going to get stuck with this, so let's discuss read-across for DART. So, your own personal recommendation is no read-across for DART.

DR. RETTIE: I think that was actually the recommendation of the group as a whole, that we should not read-across.

DR. BELSITO: But --

DR. RETTIE: And I would agree with that.

DR. BELSITO: Okay. And, Curt, you --

DR. ATOR: Can I ask what is the basis of that?

DR. KLAASSEN: Well, I wasn't at the meeting.

DR. BELSITO: That's why I going to ask you next.

DR. KLAASSEN: When you're talking about biologically active compounds, we know they interact with different receptors. What is pharmacology all about? Pharmacology is about how you switch something from making it nonactive to active. Basically, pharmacology says there's no structure (inaudible) the relationship when you're interacting with these receptors. And if you change one little carbon here or a hydroxyl here, it can interact with a different receptor.

So, with something as important as reproduction, I think one needs to be conservative there. If it's a slight drop in blood pressure, it's one thing. But we know that this class of compounds can do bad reproductive things. It's doing it not from a structure activity relationship, but from a pharmacological (inaudible) have completely different effects, et cetera, et cetera, et cetera. To go to that extreme, I think I just don't feel happy about it.

I mean, if it's a drug and you say that, hey, children shouldn't take it, that's maybe one thing. But now we have something over the counter that people will be using, and young girls will be using it without their parents even knowing about it and getting pregnant without their parents knowing it. We know that's happened with the retinols over the years. Is this going to be another retinoic acid-type phenomena? I just think this is very scary to me, so that's where I'm at.

DR. ATOR: Understandable.

DR. BELSITO: So, Paul, we haven't heard from you. Paul, are you with us?

DR. SNYDER: Yeah, I'm largely in support of the discussion. I think these are highly specific. And, for me, not understanding the basis for the finding, whether it's a malformation or whether it's a defect, or whatever, really limits us for read-across. Because as been clearly stated here, if we don’t know the mechanism by which it's operating through, how can we know which read-across is appropriate? Because as Curt said, these are very diverse ingredients. And for me, I think we have to exert extreme caution on these.

DR. BELSITO: Extreme caution means we can't use read-across --

DR. SNYDER: On the read-across. On the use of read-across, yes.

DR. BELSITO: But extreme caution means that at this point you don't feel read-across for DART would be appropriate?

DR. SNYDER: I'm very concerned if I don't understand the underlying mechanism for the pathogenesis of the defect.

DR. BELSITO: So, very concerned translates to you would not be willing to rule on safety based upon a read-across for DART?

DR. SNYDER: Not if there is a related or similar ingredient target, or whatever you're calling these things, surrogates, that shows positive DART effects. To me, I think it's very dangerous. Clearly, Curt and Allan and Ross -- they know a lot more about this area than I do. So, I'm going to largely defect to them. But what I'm hearing here is that they're not very comfortable.

DR. ATOR: If I could just jump in for a minute. The presence of reproductive and developmental effects has not necessarily precluded the safe use of certain ingredients in cosmetics. I'm thinking of the ethylene glycol acyl ethers that are known to produce developmental effects at certain exposure levels. So, that's just one example of a chemical class that presents a hazard but not a risk, and that is the entire purpose of performing this exercise in identifying read-across. Now, I completely understand Dr. Klaassen's statement about seemingly small structural differences conferring differences in receptor binding ability, either qualitatively or quantitatively, and potentially resulting in a different spectrum of effects.

Now, to respond to that, I would again bring up the idea of using the direct hydrolysis product structurally identical, toxicologically robust dataset for the endpoints of concern. If supported by the dermal absorption and metabolism study that we're currently proceeding, that to me seems like a very sensible way to address a lot of the concerns that the panel members are raising.

DR. BELSITO: But in the example you gave us where we had reproductive effects, we had target data. We weren't dependent upon read-across for that.

DR. ATOR: Well, but I don't think that the ethylene glycol ethers were dependent on target data, but they are known to produce developmental effects generally as a class.

DR. RETTIE: Again, I go back to the points we were trying to make about pharmacological activity and receptor binding and receptor targets. At nanomolar concentrations, if you sort of survey the literature, think you mentioned earlier a potentially mitigating factor in some of this would be the extremely low concentrations. But I lay against that the fact that the targets that they're binding to have KI values that, they're so far down now, they might be less than nanomolar. So, I rather feel that falls out a little bit, that argument, based on concentration.

DR. ATOR: Yeah. And that is absolutely something that I considered, is what are the range of concentrations that we're looking at, consumer exposure concentrations, versus what we know about receptor binding potency. So, yes, that is something that we looked at. But again, I would suggest that cloprostenol is -- to me, it's like the perfect read-across substance because it is the direct hydrolysis product, and we know what kind of effects it causes. We have experimental NOAELs and LOAELs in multiple species, the full regulatory package that would've been submitted to FDA.

DR. RETTIE: For ester hydrolysis, you're in a different place than amide hydrolysis.

DR. ATOR: Yes.

DR. RETTIE: And so, I have sympathy for what you've just said because prodrug reactivation of ester can be in many circumstances a hundred percent, nearly a hundred percent.

DR. ATOR: Yes.

DR. RETTIE: And the flux is all there. You have no residual prodrug.

DR. ATOR: Exactly.

DR. RETTIE: And that could well be the case here.

DR. BELSITO: When applied topically. You're thinking systemic. I mean, got to look at metabolism.

DR. RETTIE: Well, that's true systemically. I guess we're waiting for some ADME data.

DR. ATOR: Yes. And that's what we are --

DR. RETTIE: Or for dermal metabolism, right.

DR. ATOR: -- hoping that our study will demonstrate, that, yes, that is the case.

DR. RETTIE: And some data's already been provided for the amide hydrolysis in skin, isn't that correct?

DR. ATOR: Again, I'm only working on isopropyl cloprostenate. I'd ask John to comment.

MR. BAILEY: I think so.

DR. RETTIE: But it's very much a flux that's much less than it is for the ester. So, again, I'd come back to a thought that that Working Group had, which was that almost all the time in between application you have two active species, one you know quite a bit about in terms of biological outcomes or biological consequences, and then the other one you're kind of flailing around in the dark for the amides. And I have sympathy for you flailing around in the dark with that because I think the whole field is. But they're substantially different, in my view at least.

DR. ATOR: Right. To that exact point, it seems appropriate that they should be treated as such on a case-by-case basis. We would love the opportunity to present our dermal absorption data to strengthen our read-across approach. There are other things that we can do in terms of in silico modeling of receptor binding potential, things like that.

DR. RETTIE: Well, you're not going to be able to do anything for receptor binding to this prostamide receptor.

DR. ATOR: Not for prostamide.

DR. RETTIE: It's a big (inaudible).

DR. ATOR: Right.

DR. RETTIE: But for PGF2 alpha, yeah. Well, there may actually even be experimental data from academic labs --

DR. ATOR: Indeed.

DR. RETTIE: -- for receptor binding of these compounds or very similar compounds to PGF2 alpha.

DR. ATOR: Right.

DR. RETTIE: I'm quite interested to survey that to find out what the range is, right. I mean, is it all very tight, or are they all (inaudible) to (inaudible)?

DR. ATOR: Indeed. And I actually -- yeah -- I actually did look into that, and the only one that is commercially available to my knowledge that's available in the kit is PGF2 alpha. The rest of them are mostly done by academic labs.

DR. RETTIE: Well, I was thinking that some of that data might already be in the literature. You just have to dig very deeply for it.

DR. ATOR: Yeah.

DR. RETTIE: I haven't done that myself yet, but I'm looking forward to it.

DR. ATOR: Yeah. Yeah. Yeah, like I said, I'm entirely at your disposal to provide whatever supporting anything.

MR. BAILEY: I think the sponsor for the tafluprostamide is similarly available and ready to answer your questions. But I'll again come back to I think it's important to have this discussion but also table this to the December meeting so all of this data can come together and help make an informed decision.

DR. HELDRETH: So, I have a proposal. Since it's sounding like this team is going to suggest tabling the report, why not table it until this data comes in, and then we have an open meeting of the Read-Across Working Group where sponsors can participate; Science and Support Committee and industry can participate; and we have this over Teams or something like that so that everybody can easily access it and have that discussion there? If there's suitable time between that meeting and the December full-panel meeting, then, sure, bring it to December, but if there's not, then maybe the full-panel meeting in March.

DR. BELSITO: Well, I think whatever is done, regardless of whether we table or not -- we're not going to be going sufficient today -- I think that it is foolish for us to bring this to a full-panel meeting until it's been agreed upon

whether there are suitable read-across and that material has been brought into the report. Because I think don't want to spend -- I think I spent over three hours on this report, and it's going to get table. And then I'm going to probably have to spend another three hours to remember what I did at this meeting.

DR. HELDRETH: Right. That's the idea. We wouldn't even put it in the first read-across source that we were talking about that Dr. Rettie feels rather comfortable about until after that next open meeting of the Read-Across Working Group, and then before it came back to the full panel, it may get delayed until March so that we can incorporate their recommendations into the report and simplify it for the full panel to see what's agree upon and what's not. What makes it difficult is we're trying to shoehorn in the heavy discussions into a small timeframe. Maybe if we just take a step back and let it stretch out a little bit further, maybe we can make it easier on everybody.

DR. KLAASSEN: The other piece of data I would like you people to see if you can find in the literature or even do if it's not too -- which is probably difficult for you, but to take the various prostaglandin analogues, including the ones that you're interested in, and then from the literature what is the binding to the various receptors. I'd like to see a table. What does that look like? I think that would give me a bigger ballpark of knowing where we're at, or where I'm at at least. It'll take a little literature work to do it, but I think that would be well worth the time, at least for me, to look at that. I think I could make maybe more intelligent decisions, or less bad decisions.

DR. RETTIE: I agree with that. I started down that path. It was just so deep and complex.

DR. KLAASSEN: Yeah.

DR. ATOR: Yeah, it's a lot.

DR. RETTIE: It wasn't that complex; it was just a huge amount, and I didn't know for sure whether I was overlooking things. But if something like that comes back to us and everything is subnanomolar to the receptors that have been cloned. Five of them, still going to miss that one. I don't know that it's really going to help us move forward with a decision. But I still would like to see it.

DR. ATOR: Okay.

DR. RETTIE: I mean, it's part of all the data. And if you're willing to do that, I'm sure it'll save me a lot of time.

DR. ATOR: Absolutely. Yeah, yeah. I think that's a really great idea, put everything together, comparative for receptor binding profiles, and see what patterns we can glean. There have been some papers published on different structural elements that affect binding as well.

DR. KLAASSEN: Okay, fine.

DR. RETTIE: Yeah, I talked to some people at IUPHAR who keep up with all these receptor tables, and they just threw up their hands and said, we don't know anything.

DR. ATOR: No, that's all right. I mean, we appreciate the opportunity to do anything that would be informative and the ability to participate. I love the idea of an open Read-Across Working Group meeting. That would be great.

DR. RETTIE: The more expertise, the better.

DR. BELSITO: Curt, I just want to go back to something I thought I heard you say, that in the absence of knowing the mechanism of action, you have difficulty accepting a read-across. Did I hear you correctly?

DR. KLAASSEN: No, I didn't say that. Paul said that.

DR. BELSITO: Oh, okay.

DR. SNYDER: Yeah, that was me saying that. For me, it's at more of a biological level of trying to translate what does it mean. And if you don't understand what is the mechanism by which it occurs, I think it's a little bit problematic because you're making a broad assumption.

DR. ATOR: Yeah, point well taken. That's certainly something that we could provide as well, is mechanistic data.

DR. RETTIE: I mean, there's a nice, straight line from prostaglandin pharmacology to some things that would be adverse on a uterine lining.

DR. ATOR: Exactly.

DR. RETTIE: And that's a good start. Then there's all this other kind of malformation thing that we just don't know about.

DR. ATOR: Right.

MR. BAILEY: So, this will be defined out of the meeting tomorrow after the two teams come back together and described, and then we'll move forward for their tabling it till December, both the possibility of the Read-Across Working Group and the interim. Then use that to sort of guide us further toward the final review. Did I hear that right, I guess is the question?

DR. HELDRETH: Yeah. I mean, if the full panel agrees and votes tomorrow to table it, absolutely. We can for that information to come in. I think it makes the most sense to give the Read-Across Working Group time to really evaluate it and have a meeting that allows everybody to participate in it, whether it be FDA or industry members or anybody else, because it's a huge thing to wrap you mind around. It's making my head hurt trying to think about it. I would imagine that it'd better if we can take time on it.

Then following the results of that, there's time for Priya to bring that into a report so that, when it lands in the full panel's lap, it's not another headache to wrap around here as well. So, maybe depending on the timing of the meeting and when the data comes in, maybe it wouldn't even be until March before we would have a full panel meeting, again, depending on all the factors.

DR. RETTIE: My first thoughts when we saw in the read-across docket with our (inaudible) read-across group was this is great. There's only two ingredients here. Maybe this will set the scene for us as we go down the line.

What I've come to the realization of now is we'd have much preferred something that didn't have unusual outcomes where we could apply chemical principles to read-across, which many of our groups beyond ours have done successfully over time. So, it's complex.

DR. BELSITO: Okay. So, basically, where I started this was to hear about what the Read-Across Working Group has done. And so, what I'm hearing is there hasn't been a final decision, but we'll hash it a little bit more tomorrow. But the recommendations coming from Bart is that that group re-meet in an open meeting with any interested parties and come across with a decision as to which, if any, materials can be used to read-across for isopropyl cloprostenate. And right now we, at least from the Read-Across Working Group, have nothing for ethyl tafluprostamide, but there may be.

MR. BAILEY: To be fair, they're not represented here by a toxicologist. So, I would just point that out.

DR. BELSITO: Right.

MR. BAILEY: They will want --

DR. BELSITO: But they could be. They can join that open meeting if that's what the Panel decides on.

MR. BAILEY: Absolutely. Yeah.

DR. BELSITO: What I'd like to do now is go into the actual report that we received just so that as this moves forward, if there are any corrections, questions we have for Priya, they can be included in the next iteration that we see. I just want to point out that we have no manufacturing data. But are we okay with a lack of manufacturing data given the impurities?

DR. RETTIE: I was okay with it.

DR. BELSITO: Okay. Under the Cosmetic Use section and table, I think the PCPC commented on this. You had information like, corresponding to so many nanograms of isopropyl cloprostenate per usage of each serum. I just didn't think that was appropriate there, perhaps in a consumer use or whatever section. When we're looking at a margin of safety, we would want to know that kind of information. But I just didn't think it belonged in that section.

Then on PDF Page 63, the second paragraph, the ingredients in the report, it says, "The SCCS is not able to conclude on the safety of these when used in intended concentrations." I thought that was a little misleading because my understanding is it's still under investigation by the SCCS. So, it's not that they haven't been able to determine; they haven't determined at this point. It's still under investigation.

MR. BAILEY: They agreed to reopen (inaudible).

DR. BELSITO: But they agreed to reopen at different concentrations of use, right? I thought at lower.

MR. BAILEY: (Inaudible).

DR. BELSITO: I thought they agreed to reopen at 0.006 percent. Well, maybe -- I guess they're the same concentration, sorry, essentially --

MR. BAILEY: I think they are.

DR. BELSITO: -- 0.007 instead of 0.0075. But anyway, it was just misleading because they have not decided that it's under investigation. The 10.6 percent absorption, we already discussed when doing margin of safety for the ethyl tafluprostamide.

MS. RAJ: I think -- sorry -- I think the eight was used because the concentration for the product is usually 0.018. And so, the dermal absorption corresponding to the product for 0.018 was eight.

DR. BELSITO: Okay. Okay, the genotoxicity data. We've been focused on DART, but we don't really have genotoxicity data. Did you discuss the read-across for that endpoint, David -- Allan?

DR. RETTIE: Not specifically. I think that one also kind of got umbrellaed in under sensitive endpoints, and we were not terribly keen to go with read-across for those.

DR. ATOR: If I could interject just for a second. We did perform an Ames assay and an in vitro micronucleus assay and provide those results.

DR. RETTIE: Both of those are clear.

DR. ATOR: Pardon?

DR. RETTIE: And both of those are clear.

DR. ATOR: Both were clear, yes.

DR. KLAASSEN: If they're included, provide it, please.

DR. RETTIE: Yes, my note says you have in silico isopropyl cloprostenate, clear, as well as Ames, negative, and in vitro lymphocyte micro, also clear. But then we get to potential for non-genotoxic carcinogenicity, which is not my (inaudible), but both were flagged for potential carcinogenicity with a reasonable model certainty. So, that draws one's attention.

DR. ATOR: Yes, of course it does. And for that, we would submit the same read-across approach or similar. We can modify it to be endpoint-specific, absolutely, relying on one of the same potential analogues that we have previously identified, all of which have two-year carcinogenicity studies available, travoprost in particular.

DR. RETTIE: But can arguments you had developed for that be predicated on the mechanism of action being a little uncertain? Non-genotoxic (inaudible), what do you use there?

DR. ATOR: Right. That can be any number of mechanisms, and it's actually more often the case that something is a non-genotoxic carcinogen than a genotoxic carcinogen.

DR. RETTIE: Okay.

DR. ATOR: I think we have to make the best use of the available data of sufficient quality together with perhaps additional in silico modeling to provide a response to that endpoint.

DR. RETTIE: I think that's something we might want to engage Dr. Slaga in for his ideas tomorrow.

DR. BELSITO: He's not here.

DR. RETTIE: Oh, damn, that's right.

DR. KLAASSEN: Well, you usually think about cell proliferation when you think about a non-genotoxic effect.

DR. RETTIE: Okay.

DR. KLAASSEN: So, I don't know if there's any information on the eye. Is there any in regard to the eyelid, et cetera? Is there any difference in the histopathology in the laboratory animals? Has that been looked at? I don't recall.

DR. ATOR: There are data from animal studies' histology with respect to hyperemia, for example. There are also histologic data for the analogues with respect to the iris and whether some of the color changes in the iris could be potentially indicative of that kind of proliferative effect, and there was no evidence of that.

DR. KLAASSEN: Did they look at the eyelid per se?

DR. ATOR: I don't think in this particular study that I'm recalling they looked at the eyelid, but I would be glad to look and see if that's available.

DR. KLAASSEN: Okay.

DR. BELSITO: And, Priya, previously, I'd asked for clarification on this ED5, whether that's supposed to be ED50. This is PDF Page 66 under People Constriction. Do we know?

MS. RAJ: I think ED5's correct.

DR. BELSITO: Okay. Then the one thing that bothered me beyond the genotox and the DART is PDF Page 66 on the isopropyl cloprostenate, an eyelash serum at 0.0044 percent, looking at ocular pigmentation and periorbital changes. And there were effects seen. Sometimes, it was seen on the left eyelid but not the right -- or the right eyelid but not the left, is that right? I can't remember which eyelid. But, again, these seemed almost like drug effects to me. We're seeing changes in statistically significant increases in redness value, statistically significant increases in yellowness value in the left iris. Yeah, it was mostly the left. And then I'm thinking, well, most right-handed people, maybe they put more pressure on one eyelid than the other. I don't know. But there were just changes here that I don't really want to see in a cosmetic product, and this was at the concentrations that you're using.

DR. ATOR: Yeah, understandable. And we do have some outside additional experts that we are bringing in for a more extensive analysis of the colorimetric data.

DR. BELSITO: But you also had some volume loss, right?

DR. ATOR: Not consistent over time and not clinically meaningful, according to our study ophthalmologist, and that goes into one of the other requests from the team for an independent ophthalmological review of the data. So, yes, we are pursuing those things.

DR. BELSITO: Yeah, I mean, there was statistically changes in right orbital volume at two and eight months of the test material, and this is the material at less than concentration of use. It's 0.0044, and the max concentration we're looking at is 0.0075. I mean, it's 114 subjects. If this were going in as a drug, they would require a lot more subjects than 114.

DR. ATOR: Yeah. And that's what we've discovered using this particular methodology. It is extremely sensitive, and that's really the purpose of having the independent ophthalmologist evaluate the photographs. Do these statistically significant changes in some of the colorimetric parameters translate to a clinically apparent effect?

When we talk about the effect of iris change, periocular color change, things like that, it is most commonly evaluated by direct observation, a panel of either trained evaluators or ophthalmologists directly inspecting the appearance of subjects. Even in those cases, it is not often obvious just using standard techniques for evaluating this endpoint. So, we think that some of these statistically significant effects are more a function of the sensitivity of the equipment, rather than something that is a clinically obvious issue. And again, we have independent experts assisting us in a more detailed analysis.

DR. BELSITO: But weren't there also some consumer issues that were reported with that that resolved after 12 months but still were seen in use?

DR. ATOR: There was one case report of somebody using an eyelash serum. It did resolve after several months, and it was only periocular skin discoloration. I don't say only, but that is not a permanent change. Whereas, something like iris color change would be more worrisome because that is thought to be permanent.

DR. BELSITO: Okay. So, I've gone through all the points that I had and had come to: insufficient for DART, genotox, long-term ocular safety, and a greater number of volunteers. Allan, Curt, Paul, other issues with the document or other data needs that you see would need to be addressed?

DR. SNYDER: I think we pretty well captured them all in my opinion, and I was under the same conclusion as you, insufficient for those needs.

DR. ATOR: Even for the genotoxicity?

DR. BELSITO: Yes.

DR. ATOR: Can I ask what additional studies or information you would want for genotoxicity? We did cement the Ames and the in vitro micronucleus.

DR. BELSITO: It may have simply been for ethyl tafluprostamide. Let me just check. They're in Table 4, right?

DR. KLAASSEN: We have the data for the cloprostenate. But do we have any data for the amide?

DR. BELSITO: I don't think so, but let me see.

DR. KLAASSEN: Oh, yes, it does. It says, "The substance was considered to be non-mutagenic." And micronucleus.

DR. HELDRETH: Yeah.

DR. KLAASSEN: So, we're okay on genotox on both chemicals, I think.

DR. RETTIE: Well, are we?

DR. KLAASSEN: Aren't we?

DR. RETTIE: I agree with isopropyl cloprostenate. Just have a note on the amide that we don't have any info, is that incorrect?

DR. BELSITO: We have Ames. We have human lymphocyte, non-mutagenic with and without metabolic activation.

DR. RETTIE: Oh.

DR. BELSITO: That's all we have for the amide, one Ames and one human lymphocyte.

DR. RETTIE: So, are we okay on the amide for that?

DR. KLAASSEN: I'm satisfied.

DR. BELSITO: And for isopropyl --

DR. KLAASSEN: We have even more, I think, or about the same.

DR. BELSITO: -- we have an Ames, and we have a Chinese hamster ovary.

DR. KLAASSEN: Yeah. We always like to see more, but it does turn out that the Ames is so good that, if we have Ames and they're negative, I don't need more myself.

DR. RETTIE: It's rather --

DR. KLAASSEN: Not everybody will agree with me, but most people do nowadays.

DR. RETTIE: I think it's nice that the in silico agrees with the others that you note here.

DR. KLAASSEN: It was nice to see.

DR. BELSITO: Paul? So, what I'm hearing --

DR. SNYDER: Yeah, I'm fine for that. My notes, I didn't have it right. I just had isopropyl as the one that had Ames and the micronucleus. But I do see the other ones, so we're okay.

DR. BELSITO: So, we don't need genotox. Okay. So, we're going to tomorrow go -- who's reporting on this? Prostaglandin.

DR. SNYDER: You got the short straw.

DR. BELSITO: All right. I got it. So, we're going to go with the table, is that right?

DR. KLAASSEN: Yes.

DR. BELSITO: And then tables get no discussion, so how do we work in our idea of a Working Group open meeting?

DR. HELDRETH: I think it could be mentioned that his is -- oh, sorry. I think it could just be mentioned as part of your motion that this is the plan that once the date is received a Read-Across Working Group meeting will be planned and announced, and they will have the opportunity to go over that. Once that's concluded and we have all of the evaluation in hand, we'll begin the process to bring back in the report to the Panel, whether that be December, if the timing allows, or in March if that's what's required to be able to put it together into the report and make it organized and easy for everybody to read.

DR. BELSITO: Okay. So, we're tabling it with the expectation there will be an open meeting of the Read-Across Working Group and a decision as to what materials can be used for Read-Across for DART, if any, is that correct?

DR. KLAASSEN: Correct.

DR. BELSITO: And then, we're expecting from industry. You said you have a study on ADME.

DR. ATOR: Yes, dermal absorption and metabolism.

DR. KLAASSEN: In humans?

DR. ATOR: Yes, freshly excised humans skin samples.

DR. BELSITO: But that was only for isopropyl cloprostenate. We don't know anything about the ethyl tafluprostamide, is that correct, John?

MR. BAILEY: The roadmap that was submitted the sponsor's going to obtain confirmatory data for receptor inaction studies and downstream profiles of adverse effects to support read-across. So, that's to bridge the receptor issue. They're also going to be submitting acute toxicity, development toxicity, and a ToxProfiler assay with ethyl tafluprostamide. Again, this should be in your materials. There's a question about what else? And they're going to combine all this data to submit a supplemental report summarizing it.

DR. KLAASSEN: When do you expect that?

MR. BAILEY: That's going to be in September or October.

DR. KLAASSEN: Okay.

DR. RETTIE: The dermal absorption, is that radio C-14?

DR. ATOR: Pardon?

DR. RETTIE: Is there radioactivity, the dermal absorption study?

DR. ATOR: Oh, no, mass spec-based.

DR. RETTIE: Okay.

DR. BELSITO: And then industry was going to provide with a table of the prostaglandin receptors, comparing the different IC50s. What did you want in that table, Curt?

DR. KLAASSEN: Well, yeah, it depends how they do it. I just say receptor binding studies exactly how they do it. Leave it up to them.

DR. RETTIE: And I suppose we'd prefer Kis.

DR. KLAASSEN: Yeah.

DR. RETTIE: But we'll get an IC50. It seems more pragmatic.

DR. ATOR: I'll report whatever data are available.

DR. KLAASSEN: Right.

DR. BELSITO: Okay. So, let me see. Read-Across Working Group for DART materials. Dermal absorption metabolism on human skin for isopropyl cloprostenate. Tafluprostamide is going to be providing a bunch of additional data endpoints. We're going to get a table of prostaglandin receptors binding studies and KIs available in the literature from industry. Anything else we're expecting to get back during this table that I'm missing?

DR. RETTIE: I got nothing to add other than experience from during the first Read-Across Working Group. We really need to build in as much time as we can for a bit of back and forth on something as complex as this. I think it'll take, yeah --

DR. BELSITO: Additional clinical data and outside ophtho valid existing data.

DR. ATOR: Yes, additional analysis of the clinical data we've already submitted.

DR. BELSITO: But no additional clinical data, just additional analysis?

DR. ATOR: Correct.

DR. HELDRETH: That's why I was thinking probably March, so we don't have to rush everything back to the Panel.

DR. RETTIE: I mean, we only probably need one open reading group meeting, but I'm just thinking downstream of that in order to mold the report and come up with things that are as definitive as possible. We'll need a few iterations just within the working group itself.

DR. BELSITO: I guess I'm just concerned with the clinical data because it's still -- I mean, even if you get an analysis, outside analysis, there were tweaky little effects there, and it was only a group of 56. I mean, an HRIPT, we want a hundred. And we're looking at a sensitization endpoint, not endpoints like potential for interocular pressure changes or iris color changes, things that may be permanent, as opposed to, okay, you became sensitized. We identified the allergen. You read a label. You stay away from it. This is quite different. So, I'm just concerned about your end there in a clinical study. I don't know if anyone else is, but 56 seems terribly small to me.

DR. KLAASSEN: Don, someone has their arm up.

DR. BELSITO: Yeah.

MR. NICOLAI: Hello, I'm Jeff Nicolai, and I --

MR. BAILEY: Sit.

MR. NICOLAI: Oh, thank you. I'm a general counsel for a number of companies in the space. On the clinical, I just wanted to point out that it was actually run on 120 individuals, not 56, and it was an eight-month clinical. And I think your suggestion about getting an additional set of eyes from an ophthalmologist on the photographs is a good one.

The way that we designed this study was to, one, have an ophthalmologist, as you noted, do physical examinations and look for these issues at different time periods and then also get the actual photographs. And to my knowledge, there has not been a study looking at the potential for eye color change based on these very sophisticated cameras, and the good news we have the pictures. So, I think, looking back on it, the final step in that study should be, like you suggested, to not only have the colorimetric data, but to also have an ophthalmologist come in and compare the photos to ensure that the data is correct. And we're happy to do that.

I also have some of the photos showing the, quote-unquote, largest colorimetric data change that I'm happy to bring in tomorrow if you wanted to see what these are looking like. Like I said, this is the first time these have been used. And my hunch is that any kind of data that's showing change is probably because of light reflections or other kind of issues with the camera. Because when you look at the photographs of the individuals exhibiting that colorimetric data change, to a lay person, you don't see eye color change. That doesn't mean we shouldn't get an ophthalmologist to confirm that, but if it would be helpful for you, I'd be happy to bring in a couple example pictures for you tomorrow if it would ease some of your concerns.

DR. RETTIE: I'm trying to think back to the case reports. Were they mostly case reports of iris color change in our original report? If so, how many people would you need to look at to pick up statistically a very rare or a rare occurrence? Something gets noted, of course, because it's --

DR. BELSITO: Not 114. That's for sure. Or 50 --

DR. RETTIE: I mean, it gets noted --

DR. BELSITO: -- 56, I'm sorry, was in the Wave 2 study that we received on isopropyl cloprostenate.

DR. RETTIE: It gets noted because it's very distressing to the subject doing it, of course. But I didn't have a clear sense of incidents in the general population.

DR. ATOR: The incidence is actually quite low.

DR. RETTIE: That's what I would expect.

DR. ATOR: Yeah, yeah. And even in most cases, the subject experiencing such effect either didn't notice it, or it didn't bother them.

DR. RETTIE: But it's irreversible, is it not?

DR. BELSITO: Yep.

DR. ATOR: It is. It is. And that's why we think that, as Jeff suggested, showing some of the photographs of what exactly we're talking about here could be really helpful.

DR. RETTIE: Well, I applaud you for doing it. I just think you're going to have a hard time finding something that will help us move forward if it's -- so I don't want to say idiosyncratic, but rare.

DR. ATOR: Yeah, rare.

DR. RETTIE: But, yes, more data is good.

DR. ATOR: Okay.

MR. BAILEY: Yeah. This really has risen above just what this panel determines. I mean, this is obviously important work to do. If I had any concern whatsoever that our product could cause eye color change under the conditions of use, we wouldn't be selling it. And like I said, the good thing is we have the photography, so even though the clinical is closed, there's still an opportunity to go back and add that extra layer that I think you guys rightfully suggested.

DR. RETTIE: Good.

DR. SNYDER: Bart, if I could just make one comment to you point about whether we do this in December or March. This was quite a large data dump on Wave 2 for this meeting, and let's not let it be a large data dump in Wave 2. Let's try to get it out to us with the initial review if we can. If we need to push it to March, I'd rather do that because, as Don stated, I'd rather have some substantive data to look at.

DR. HELDRETH: Yeah, I agree. That's why I was thinking of March. I was thinking not only would the data be part of the initial report package, but also the Read-Across Working Group's public meeting results would be incorporated there and if they had a solid consensus one way or the other. And, yeah, it should shrink the overwhelming data package that each member of the full panel would have to review.

DR. KLAASSEN: What's our next in-person committee?

DR. BELSITO: September.

DR. KLAASSEN: September.

DR. HELDRETH: Yeah, end of September into October.

DR. BELSITO: And then after that, are we going to continue the virtuals -- hopefully not -- indefinitely?

DR. HELDRETH: At current, yes, we're planning to do two and two, and next year we're looking to possibly make a change on that. But nothing's set in stone yet. But, yeah, next meeting, in person. Then December meeting is currently scheduled for virtual. And then nothing is set in stone yet for 2025.

DR. RETTIE: I mean, I think an argument can be made for this extended discussion being in person. I think it'd be more valuable in person.

DR. BELSITO: Think an argument could be made for every panel meeting to be in person.

DR. KLAASSEN: Yeah.

DR. BELSITO: These are not webinars where there are lectures and Q-and-As. They're back-and-forths, and it's very difficult to do that virtually.

DR. RETTIE: But if we're in a two-and-two right now and we wanted an in-person meeting, then you're talking about pushing it again further down to June.

DR. HELDRETH: Yeah, I mean, but nothing is set yet for next year's schedule.

DR. BELSITO: We could theoretically do in-person in March.

DR. HELDRETH: We could March in person.

DR. RETTIE: Doesn't that conflict with the dermatology meetings?

DR. BELSITO: If it conflicted, it wouldn't be on our schedule as a virtual meeting either.

DR. RETTIE: True.

DR. KLAASSEN: I agree very much with that. I would like to see all of these meetings in person. But when we have something that needs this much discussion, to do over the computer just doesn't get to it. I mean, there's a margin of safety that we don't get when we do it on the computer.

DR. RETTIE: Perfect.

DR. ATOR: Well played.

DR. HELDRETH: Curt's (inaudible).

DR. BELSITO: Okay. Before we leave prostaglandins again for Priya, we did get a large Wave 2 data dump, and we've not discussed any of that. Of course, we're going to bring in the data that we got on the HET-CAM, the

EpiOcular Eye Irritation, HRIPT, the 56 single-center study. Again, we don't know about what the read-across is going to be. We also got data from two companies that manufacture a serum with 0.0044 percent and 0.005 percent and their comments, and we can get to those. We had comments from PCPC, and I was okay with the comments that they made and questions that they raised. Allan, Curt, Paul, did you agree with the PCPC comments?

DR. RETTIE: I did.

DR. SNYDER: I did.

DR. KLAASSEN: Yes.

DR. BELSITO: Okay. Then we got an extensive editorial rewrite from Company 1 and Company 2 that market the two different concentrations of isopropyl cloprostenate. Again, a lot of it was just concerning correcting certain concentrations of use, I think, as I read it. Yeah. They had a lot of comments on the study that I was mentioning about the 0.0044 percent safety and ocular irritation. But, again, I think we said we want that -- we didn't say. Is this study going to be re-reviewed by an outside ophthalmologist as well?

DR. ATOR: It went for clinical, yes.

DR. BELSITO: Yeah, okay. So, then I think we can hold any of their editorial comments until we've seen because I -- the thing I objected to most were the changes were deemed not clinically relevant, and I would like to see what an outside ophthalmologist thought of that. And then the Read-Across Working Group justifications that I went through are irrelevant at this point. Any other comments on the Wave 2 data that we received, Allan, Curt, Paul?

DR. KLAASSEN: No.

DR. RETTIE: I don't have any.

DR. BELSITO: Okay. Any other issues on prostaglandins that I haven't addressed? Okay.

Cohen Team – June 3, 2024

DR. COHEN: We're moving onto the prostaglandin analogues. So, this is a revised draft tentative report on the safety of ethyl tafluprostamide and isopropyl cloprostinate. And at the December 2023 meeting the panel issued a second IDA for these ingredients and requested the following for ethyl tafluprostamide: acute tox, repeated dose tox, developmental and reproductive tox, in vivo, genotox, further clarification on targets and mechanisms. And for isopropyl cloprostinate: dermal irritation and sensitization at the current max use concentration which was updated at that meeting. Data on local ocular effects, concentration of use.

We wanted an independent ophthalmologist to assess the colorimetric data. DART, genotox, information on targets. Also, at the December meeting, data were provided on the potential read across ingredients including tafluprost, which is Zioptan an FDA approved drug; travoprost which is Travatan Z, an FDA approved drug, and cloprostenol. And the read across team was meeting on this and I think it's important to hear what they have to say because there's a lot of discussion we have to have about this dossier.

DR. BERGFELD: There's also an added Wave 2.

DR. COHEN: There's a large Wave 2. And the Wave 2 had data on sensitization at max use. So, we had some data needs met. So, can we get some comments about the read across first?

DR. ROSS: Yeah. Should I start? I set this up this morning, I suppose I can read that. General comments on the read across. Okay, so I think there'll be a full minutes of that meeting and full conclusions will be coming out and I thought they've already come out of it. But just general sort of 40,000-foot things. First of all, on read across, and then we'll get to prostaglandins specifically, okay?

So generally, on read across, it's complex topic because you know we -- Susan, as I go through this, correct me when I'm misspeaking here -- but we should do in a compound specific and endpoint specific way. So that's the first point. We felt, in general -- this is the group -- in general, felt that read across for DART endpoints particularly were on the positive endpoints of being detected with source and target chemicals was not optimal and it would be stronger terminology that Alan used on that in a couple -- call.

Third point, we should be very cautious with read across for compounds that function through receptors, and I think Curt made this point last time. Such as steroids, prostaglandins, and these are potent pharmacological molecules that work through receptors. And fourthly, it's just extremely difficult to read across when you're using mixtures of varying composition. Whether it's compound, whether it's source, or target. And these different materials in the mixtures have very different chemical properties such as log Ps or solubility.

So, you're trying to read across something that's a mixture of chemicals in a log P and one of them might be three and the log P of another one might be negative three and you can't really do that very accurately. So that was an example that we had this time with amphocarboxylates. And so there were, I think, the four points. Compound specific and endpoint specific manner, read across for DART endpoints is particularly difficult and not optimal. We should be very cautious with read across when you're working through compounds of functions via receptors.

And finally, mixtures are a whole different topic. And so that's where we ended up, I think. Susan, you have anything to add to that that I --

DR. TILTON: No. I think that summarizes our conclusions. I mean, there were some more specific discussions when it came down to whether we were talking about the prostaglandins or the amphocarboxylates but, in this case overall, I mean we generally felt like the read across was very limited for these two particular examples. This was a hard set of examples to start with, we decided, for read across.

DR. ROSS: It's really tough, yeah. I mean, you couldn't have come up with two more difficult groups of chemicals. But, specifically, on the prostaglandins you want to go to there, you want more discussion about --

DR. BERGFELD: Can I ask you a question about your conclusions? So, any of these items that you've called out, if the ingredient chemical falls into this, we don't read across? Is that correct?

DR. COHEN: It doesn't sound like we're going to read across on them.

DR. BERGFELD: In prostaglandins it doesn't.

DR. COHEN: On the prostaglandins.

DR. BERGFELD: But this is the general concept.

DR. ROSS: Very general concept. I think I was fairly cautious in some of the descriptors I used. They're not optimal. I mean, I'm happy -- I mean, we have to have that discussion a little bit more today about whether that's not optimal or not acceptable. But I think that's what we need to talk about as a panel.

DR. BERGFELD: So, it's case-by-case.

DR. ROSS: Definitely case-by-case.

DR. TILTON: Yeah. It's endpoint specific and, I mean, I think the example where you talked about whether the target or source has positive results for certain sensitive endpoints makes it difficult. So, it is going to be case-by-case. But I think these are helpful general set of guidelines. They're -- I mean, they are the guidelines we have because these are the specific two groups that we've worked with so far so the guidelines could expand as we move into other examples.

DR. ROSS: You know, I just to step back one pace here. I mean, we thought it'd be relatively simple. We'd look at some of the chemical properties. We'd look at ten model coefficients for chemical similarity and then we just make yes, no, yes, no. And these two groups of chemicals brought us back to reality and we had broader issues to consider. There may be issues -- in the future -- there may be groups of compounds we could just do on their chemistry. And that's great. And that's fine. But that's not what these were.

DR. BERGFELD: Those are called no-brainers.

DR. COHEN: Yeah. I think these two were a difficult start and probably not emblematic of what the read across panel's going to come across on a regular basis. I must say, I struggled for a long time with this dossier and this application really appears to me to be a drug and not a cosmetic chemical, right? I think we're being asked to extrapolate toxicity in read across with API, active pharmaceutical ingredients.

So, we're asked to look at the toxicity portfolio from FDA approved drugs, but at the same time ignore the biologic effect of those drugs. Let's just look at the toxicity, read across, pull this through on read across, and then ignore biologic effects. I think these are very potent drugs. I think the dossier looks like it could be an over-the-counter drug, but not a cosmetic ingredient.

As I investigated this more and looked at the online presence of these, I see the wording is cautious. However, the influencers, the video, even the online presentations of before and after photos, which I have, I see drug like effects here.

I see 97 percent saw more visible lashes. That, I think, is a statement of having more hair. Eyebrows, where I'm looking at this as a dermatologist, as a clinician, and I'm seeing what looks like a drug effect to me. Even though

the verbiage is cautious. So, the predicate comparators that we're asked to look at are all drugs. The application of these is to the base of the eyelash line, and when you look at the videos it's to the skin. When I think of a hair conditioning ingredient, I expect it to go on the hair, not necessarily on the skin.

And there's a fine line here, but this seems like it's an attempt to do a 505(b)(2) drug application by drawing in prescription drug data on toxicity and using careful verbiage for it to be a cosmetic agent. So, I think my recommendation might be to send this back to the FDA for consideration and not further adjudicate this as a cosmetic agent.

DR. BERGFELD: John?

DR. BAILEY: Yes. I appreciate your comments. I think this is one that's a little more difficult than maybe some others, but I think what the panel is here to do is to define the conditions of safe use for an ingredient that is used in a cosmetic product and I think the effort that's going into this by the sponsors -- and we do have three sponsors -- and responding to the panel's comments and request for information is, in fact, intended to bridge that gap to show that these are ingredients that can be safely used in cosmetics, not drugs, cosmetics in the hands of consumers when, in fact, at very low concentrations, very careful application, and so forth.

And they have a long history of safe use in cosmetics, so I think there's -- I would hate to see us jump ahead when, in fact, we do have a significant number of studies that are ongoing now. We submitted road maps for studies going forward and some of these studies are underway. Some are very close to starting, and I think that our recommendation is that this be tabled until December meeting to allow these studies to be done. Also, we do have read across experts that are going to be available that are experienced in this area, both domestically and in the European assessment process.

The SCCS has agreed to open this up for review and a lot of this data is going to serve both purposes. So, I think we are looking at some aspects of information from drug reviews. We're very fortunate to have this data, it's human data. It's relevant. But I don't think we're talking about a drug use, we're defining endpoints such as eye pigmentation, interocular pressure, other endpoints that are associated with the drugs -- drug uses -- to show that, in fact, these are not prevalent in these particular uses.

I would also say that the Panel has the ability in reaching a conclusion and I think that's really a very valuable in the process here in terms of defining the conditions of use to make sure that, in fact, it's functioning in a cosmetic way. This can be a number of different things. I would keep an open slate on it in order to -- when you do a final assessment, to be able to consider those in your conclusion. And this has happened before. I think the one that's most relevant are the alpha hydroxy acids where there were conditions of use that define in detail.

So, I think I understand the concerns. I think the sponsors are working very hard doing some very extensive and expensive studies, too, in order to answer your questions and to provide comfort in the safety review process. And I think Dr. Ator's here if you have any questions about some of the toxicology that has been presented. And so, I would just encourage you to consider tabling this until December when all these studies can be completed, and that data can be provided to you in advance of the meetings so that you have plenty of time.

DR. BERGFELD: Is that what you're proposing different than what was asked for? It would be nice to have what is expected to be coming in December.

DR. BAILEY: There are two submissions that were made that lay out studies that are planned, the questions they are answering and the estimated dates of completion which, I think the furthest one is in September with the data being presented in October or November.

DR. ROSS: It's a pretty comprehensive memo. And I think two major issues were the dermal metabolism and penetration study.

DR. BAILEY: Yes. Right.

DR. ROSS: 0.1 and 0.2 was the receptor binding studies -- 0.2.

DR. BAILEY: Correct.

DR. ROSS: And so, I think they will get at some of these. You could read across, and I did note your request for deferral until December and that was before I had the move to send this back to the FDA. But I'm going to -- David -- respond to your comments, John. You got to respond to David's comments.

DR. BAILEY: Thank you.

DR. COHEN: I think the comments are fair. I think the alpha hydroxy acid analogy where you might suggest there's change to structure and function, those have been on the market a very long time. The downside risk of alpha hydroxy acids, which we have tremendous experience with, is minor transient irritation. That's what the alpha hydroxy acids do.

DR. BERGFELD: They build collagen.

DR. COHEN: Yeah, yeah. But we're talking about even in the over counter cosmetic products, identical warnings to the prescription products. Identical warnings. The warnings on the videos, the warnings online are identical to the package inserts of the FDA approved prostaglandin drugs. So, there's clearly a connection there. There's a bridge built that you're suggesting there's a low enough concentration -- and parenthetically, I think this is an over-the-counter drug actually makes sense.

I think it needs a drug facts label, not a cosmetic label. That's what it seems to me. So, I think -- I'm not sure what I'm going to hear in December's going to change that, right? These are very potent drugs engaging receptors that have an absolute change in structure and function. They are changing structure and function. I'm not sure the alpha hydroxy acid analogy resonates much from a risk standpoint. I do think that iris color change is usually irreversible. I think periorbital fat and volume tends to be irreversible and I don't have clarity on use in pregnant women and young kids using it, right?

Now, that could be in the label, but in the adjudication of drugs, FDA requires maximum use studies well in excess of the intendent use of the topical drug and are we going to have a use study on this where it's used on upper and lower eyelids and eyebrows because the same companies are selling both. They're selling eyebrow and eye lash products at the same time.

DR. BAILEY: I'm not sure that the sponsors for the review we're doing promote it that way. In other words, I wouldn't take something from the internet like this and extrapolate that necessarily to what the sponsors are doing. What they're trying to do is define the safe conditions of safe use for a cosmetic and I think that's -- I mean, I understand to be honest -- this is from a group of substances that do have drug approvals and we're looking at the use for cleanse, beautiful eye, promote attractiveness and alter the appearance without direct claims and whether that's a safe use or not or safe with conditions.

So, I think -- I mean, again, back in the alpha hydroxy acids, we didn't know those aspects because obviously they knew the FDA chair at that time until all these studies were done, and these were extensive studies, phototoxicity testing and whatnot. So, yeah, I'd like to give us the opportunity to present the safety information with a more robust data set. So, I mean, tabling this until December I think would sort or provide that opportunity.

And I don't think this panel can conclude whether or not a product is a drug or cosmetic. I think the task here is, is this ingredient under the conditions described in the safety package, is this is a safe or unsafe, or safe with qualifications application in a cosmetic product? I think we kind of started with the idea at the beginning that this should be handed over to the Center of Drug Evaluation and Research and FDA came back and said, no, this is a cosmetic and the CIR should review it.

So, it's, I think, a bit back to where we started.

DR. ROSS: So, David has given a summary of why he thinks it's a drug and should be handed back to the FDA. Could you just give us a summary of why you, perhaps, think it's not a drug and why it shouldn't be handed back to the FDA?

DR. BAILEY: I think that there are many steps that are taken to make sure that these are not functioning as drugs. I mean, the one is the pigmentation of the iris. The other is the interocular pressure/the periorbital fat build up and showing that these are not present under these conditions of use. When they're used according to the descriptions that were proposed by the sponsors, I think that that's -- again, you're looking to condition the hair. You want to make the eyebrows look better. You're not trying to make them grow.

In other words, they shouldn't be growing. This is not a drug application. We're also talking about some different chemicals here in the sense that this is not Bimatoprost or some of the others that are specifically approved for that purpose and just in terms of the functionality and potency, I guess, you could call it, it's not really the same thing.

DR. ROSS: But they're pretty potent compounds, John, right? I mean, we talked about it last time --

DR. BAILEY: They can be under certain conditions of use. We're avoiding those conditions of use, that's what this is.

DR. BERGFELD: Is there not a big concentration difference between the cosmetic and the therapeutic?

DR. BAILEY: Yes. We started off the discussion to try to describe this as de minimis exposure and if you look at the concentrations of exposure that are estimated, it's quite a bit lower than what you would get in the pharmaceutical applications, OTC or otherwise.

DR. COHEN: I think the interocular -- sorry.

DR. ATOR: Hi. Sorry. I'm Jennifer Ator for those who don't know me. And I just want to point out that really the critical factor here is the exposure amount. Those of us who are toxicologists by training --

DR. BERGFELD: We can't hear you so well.

DR. ATOR: -- those of us who are toxicologists by training consider dose response when we are evaluating the safety of an ingredient and we have performed robust dose response analyses of these ingredients to calculate acceptable margins of safety. Even for something that has signals for DART effects -- and we know that about this class of ingredients -- it is still appropriate to use a dose response margin of safety approach to evaluate safety.

So, I know, that consumers included tend to get a little bit nervous when they hear DART effects, but it is a threshold-based phenomenon like so many other toxicological phenomenon and as long as we are performing the margin of safety calculation appropriately, that can be used to provide assurance that there is low risk of actual effects when used as intended.

DR. BAILEY: And they calculated margins of safety?

DR. ATOR: We did. And to address the point about concurrent use of an eyelash product and an eyebrow product, we can certainly do that exercise as well to calculate a margin of safety.

DR. COHEN: So, your discussion of calculating a non-drug effect is on an adverse effect. Is the presence or absence of an adverse effect one, the predicate comparators are instilled into the conjunctival sulcus and you're not doing that, right?

DR. ATOR: Correct.

DR. COHEN: So, I don't consider that necessarily so assuaging to my concerns because you don't know at all, at that point, if there's an effect on eye pressure because it's not an apples-to-apples comparison. You're additionally saying well, we're going to look at iris color and we might look at periorbital volume but some of these products are approved for hair growth, yet you don't look at that endpoint as a biologic effect proving it is not a drug.

So that's question A that really requires some answer. And two, explain how a prostaglandin conditions a hair follicle, a hair shaft, a cuticle. How does it do that? How is it a hair conditioning agent?

DR. ATOR: I think that your main question that you asked is about the drug effects. The drug effects that we're concerned about here are intraocular pressure, periorbital color change, periorbital fat loss, iris color change. We have robust clinical data that demonstrate that actual use of these products does not result in any of those drug effects of concern. A hair growth question is tangential to this.

DR. COHEN: Well, it's the cosmetic use. I don't think it's tangential. I think I'm asking how prostaglandin is a hair conditioning agent. How does that work?

DR. ATOR: You know, I don't know if we know that for a lot of cosmetic ingredients in terms of what exactly they do to the hair other than smoothing, for example, increases in volume of the hair follicle. Things like that. But again, I would really focus on the issue of adversity. I don't think anyone here would argue that a change in appearance of eye lashes is adverse. So, we're talking about adverse versus non-adverse and that is really the thing to focus on when you're evaluating safety.

The FDA has already opined that these are cosmetic ingredients and there are no drug claims being made about these ingredients. So, what we really need to focus on are the safety data. What are the established paradigms for evaluating the safety of cosmetic ingredients and how can we leverage those in this case?

DR. BAILEY: And that data's been provided. The studies have been done and the data's in the package that you've been provided. So, I mean --

DR. ROSS: We understand that.

DR. BAILEY: -- I'm sorry, go ahead.

DR. ROSS: We understand that. But I guess the issue on the table is whether we ask -- and I'm getting at the comments that we just made. Sorry, I can't see you but -- hi.

DR. ATOR: Hi, how are you?

DR. ROSS: Is whether we ask the FDA to reconsider that. So that was the issue that was brought up. So, I know the FDA handed it down to us, John, so I mean the question is, do we ask them to reconsider that? And I note that you've got a request in the dossier to move this out to December anyway for those additional pieces of data to come in. Could we do both things in the same time?

Could we ask them to reconsider that, and can we get that -- I mean, that data's approaching completion anyway, September/October.

DR. BAILEY: It's either underway or it's very close to starting.

DR. ROSS: Exactly. So, maybe both things can be done at the same time. But I had some specific comments actually on the prostaglandin dossier, but I don't know if I'm -- do you want me to go through those as well while we consider the question.

DR. COHEN: Yeah, we absolutely can.

DR. ROSS: First of all, there's a request for deferral. And this, the read across group was still a negative on the read across to tafluprost, which I think we commented on last time, so they didn't change their opinion on that. There were concerns with the safety statements for pregnant women and lactating women, which we covered last time and that was part of the use for us. And we didn't see that with every preparation.

There was a new read across suggestion going for isopropyl cloprostinate going to travoprost and to latanoprost on the read across group -- Susan, if I've got this wrong, correct me -- didn't agree with the latanoprost, we felt that was too far away from isopropyl cloprostinate.

Travoprost was -- I think we said this last time -- travoprost was a better example. But there was some items of concern there, that the -- for example -- the SCCS in the 2022 publication that compared isopropyl cloprostinate, latanoprost, and travoprost, the three compounds on an ocular endpoint and I think it was conjunctival hyperemia, and they found a six to ten-fold difference in response concentrations which were a critical read across endpoint.

You know, a ten-fold difference can be an issue. So, I think that was the first issue there. And then the second issue was Curt's point previously from last time -- Curt Klaassen's point -- whether or not we do read across with these agents that work through receptors. These potent pharmacological agents that work through receptors. And I take the comments from, sorry, the lady I couldn't see around the corner there from -- thank you -- with respect to the margin of safety calculations.

I got those three (inaudible) with read across. That's where we get to this. I mean, I think you use a three-generation study for the isopropyl cloprostinate if I recall correctly.

DR. ATOR: We did.

DR. ROSS: Yeah.

DR. ATOR: Yeah.

DR. ROSS: So, they were the general issues. So, if this does go to the December meeting, I would make those comments, which maybe should be noted before the December issue.

DR. BAILEY: Right. I mean, I think, capturing the thoughts and requests from the Panel is going to be very important going forward, so we're very interested in capturing those and being able to answer them. So, I think -- again, I think a lot of what's going on now is designed to answer those read across and we do have experts -- Jen is one -- and others who can help to explain that or defend it, if you will, to the Panel. So, I look forward to doing that.

DR. COHEN: So, neither of you feel there is a drug effect from these prostaglandins in cosmetic products. You believe there's no drug effect?

DR. BAILEY: I believe the studies that are being done helped to define a non-pharmaceutical effect in a finished cosmetic product and these studies have been extensive in terms of looking for those markers, if you will, that would suggest that maybe something is going on and we're not seeing that. We're not very comfortable with that body of data and the directions for safe use that have been proposed.

And the panel looking at this in an in-depth way that this can, in fact, be defined for cosmetic uses, non-pharmaceutical effects. That's I think what we're looking for.

DR. ATOR: And just to add to what John said with respect to the question of, yes, there is one analogue that is approved for drug use as a hair growth agent and that's the Bimatoprost which is structurally different. It's an amide versus a carboxylic acid ester. It also has a different receptor binding profile compared to isopropyl cloprostinate, for example, and the analogues that we used in our read across approach and in that approach, we specifically excluded Bimatoprost as a suitable read across substance because of those differences.

DR. COHEN: Again, I think it sounds like the conclusion of non-drug effect is the absence of selected adverse effects rather than a biologic effect -- an effect on structure and function.

DR. ATOR: Right. What structure and function -- biological effect can you maybe clarify?

DR. COHEN: Hair growth. Because that's how they're marketed. I've been doing patient care for 34 years. I know how the patients are interpreting these products. They're clear to me and it's the only drug effect that's not measured, right?

DR. BAILEY: If I could just interject. I think it's within the Panel's purview in developing a conclusion -- if this is the way it goes -- that these ingredients are safe under conditions of use when formulated to not result in hair growth. I mean, things like that as a way to communicate that. So just think about that.

DR. COHEN: That's interesting.

DR. BAILEY: Well, I think the Panel does this with irritation. When formulated to be non-irritating.

DR. COHEN: But that's never an endpoint.

DR. BAILEY: No, no. I understand.

DR. BAILEY: That's never a desired endpoint, right?

DR. BAILEY: No, but --

DR. TILTON: But you're suggesting it's not desired in a cosmetic.

DR. COHEN: No, it's a desirable effect. Please don't misinterpret that I don't think that these couldn't be excellent over the counter drugs. It's just that fine line between a cosmetic and an over-the-counter drug, right? I think they do work in growing hair. I also just want to comment from our panel here on PDF 68, it says when compared to baseline there was a statistically significant increase in overall color change of the left and right iris at all time points.

DR. ROSS: Yeah.

DR. COHEN: Similarly, when compared to baseline values for the right orbital side, there was a statistically significant difference in right orbital volume after two months and eight months of test material use. There's a comment about clinical significance in the report. I'm unaware of a MCID, a minimally clinically important difference, that's ever been established for this, and it was the reason I asked for an independent ophthalmologist to opine on this.

I understand that you had ophthalmologists in the studies, but I never saw an independent ophthalmologist coming from either the PCPC or the CIR side of this, which I think we need. I don't have an objection to the suggestion to table. It gives everyone a little more time to think about this. Does the group here have a concern about tabling? It doesn't preclude us from discussing this tomorrow or other issues of asking the FDA to look at this. We haven't made a decision on that.

DR. BERGFELD: If I could just make a comment about all of the ingredients we've looked at over the years -- now 6,000 plus -- frequently we come to this, not specifically around the eye, but the fact that the claim is moisturizing or conditioning and we see other things. Like, reduce wrinkles, depigmentation -- we see other things and then we look at to a concentration effect/duration effect and decide whether it's meaningful or not.

So, the FDA does allow these claims even though they may have some mild biological activity. It's -- in my mind -- they have done that over the years. They've allowed to be renamed as conditioning or some other feature. Improvement.

DR. ROSS: For me, I guess it boils down to -- I mean, I know what the claims are -- the label claims. I mean, are the users -- the end users of this -- using this as an eyelash conditioner or as an eyelash growth agent? And I would

suggest it's an eyelash growth agent that's why they're using it -- at least the people I've asked that use these things. That's why they're using it. Not to condition their eyelashes.

DR. BERGFELD: No, but that is a common trait that they use to rename the activity, so it isn't a drug activity. It sits on the margin of being drug versus cosmetic and it's been my impression over the years that the Department of Justice cannot take up all these issues and neither the enforcer of what they claim. Is that correct?

DR. BAILEY: It's a little different than that. In the cosmetics area you've got an expectation, like skin creams will make your skin look better, you know, including plumping a little bit, right? Okay, that's cosmetic. If you claim that the wrinkles are somehow reversed or eliminated or prevented, then you're making a drug claim for that product and FDA is the gatekeeper for taking such actions. And they have in the past many times. Recently it's not been so much but, in many incidences, campaigns I used to call them, where FDA would go out and do things.

DR. ATOR: With that in mind, we do take very seriously the points that you brought up about those statistically significant effects in some of the colorimetric endpoints and we are engaging independent experts for additional data analysis and independent opinions of some of those findings. So, yes, we understand the concern about independent review, and we are addressing that.

DR. COHEN: Thank you. That's important. I just don't know if there's any validated tools to measure clinically important differences.

DR. ATOR: Yeah, and that's something that we considered quite a bit when designing this clinical study because if you look at the body of literature on the prostaglandin analogues in general and how these effects were first noticed it was by simple observations or digital photography perhaps using the split lens of ophthalmoscope to obtain a magnified image. There are very few reports in the literature of using anything that is remotely validated.

One group used a special microscopic lens; one group used a categorical color change scale. But in general, there are no validated methods it's been direct observation.

DR. ROSS: So, I guess we've got the two things on the table. Going back to the FDA or tabling this until December --

DR. COHEN: Or both.

DR. ROSS: -- or both. Three things.

DR. COHEN: Well, I think we'll bring that tomorrow. We don't have to make a final decision right now.

DR. ROSS: Okay.

DR. BERGFELD: I think Don is presenting this, is he not?

DR. COHEN: Don is presenting this.

DR. BERGFELD: Yeah.

DR. TILTON: Yeah, with regard to evaluating safety and addressing the insufficiencies, I mean, tabling makes the most sense. The data that's coming forward -- David mentioned some of this that we discussed at the read across working group, regards of dermal metabolism and penetration studies to help improve comparisons to travoprost and --

DR. ROSS: It was the receptor bindings.

DR. TILTON: -- and the receptor binding studies. And then, also the insufficiencies with regard to ethyl tafluprostamide and some of the data that's coming forward to that in addition to the analogs. But we'll help fill some of those insufficiencies/some of those data gaps or help us in interpreting a potential for read across. So, I mean, tabling from that perspective makes sense.

DR. COHEN: I agree.

DR. ROSS: Yeah. I mean, yeah.

DR. BERGFELD: John it might be, well, I don't know who's here from the FDA. Is anyone here from the FDA? Thank you. That you could address the question of sending it back to the FDA for reevaluation of its category.

DR. MANGA: We'll take it back. I mean, I think we're saying the same thing as before. It's only intended use and the label claim, so that still holds. You all know that I did go back and talk to the CEDR side of things, and we

went back and forth, and it did come back it must be a cosmetic and recommended CIR to look at it. So, I will take it back with having said that it still depends on the intended use and the label claims.

DR. COHEN: I'm not saying you ought to take it back because we haven't decided on it, right? It's the mechanism, right, on the FDA's website you define drugs two different ways. One is by claims, and one is by structure and function. My thought is if I decided to bring a dihydrotestosterone inhibitor that's a prescription right now onto the market as a topical and make no claims other than hair conditioning agent, I'm pretty sure I would garner some attention, right, even though my claims would be carefully scripted never to make that.

Or if I brought a GLP-1 drug over the counter as a cosmetic to improve my physical appearance in clothing but not to change my hemoglobin A1C or my weight, I'm sure I would hear, right? So, this is my quandary. We have FDA approved drugs in this category that work on eye pathophysiology, and we have them growing hair and this is a bridge that's rather direct about hair conditioning. And I still don't know how prostaglandins are conditioning hair and it's true we don't always know the mechanism of how conditioning agents work, but we know a lot of them.

We know how a lot of them condition hair; how the cuticle is affected by hair conditioners. How skin is affected by skin conditioning agents. We do understand that, and since this is so novel, it would be interesting to know how this chemical is a hair conditioning agent, notwithstanding the fact that the formulation may be a conditioning agent and often doesn't ever wind up on the hair shaft. So, this is our dilemma.

I think from a verbiage standpoint, from a marketing standpoint, you've sent it to us but from a structure and function I'm not so sure that was adjudicated the same way. I may be wrong about that so I could stand correction at any point.

DR. BAILEY: Can I just inject here --

DR. COHEN: Of course.

DR. BAILEY: -- because I wrestled with this when I was at FDA because there are pharmaceutical active ingredients that are used in cosmetic formulations and the example, I would use is tetracycline in a cosmetic. Maybe that's not relevant 30 years later but that's what I would use. And I would say that in a drug it's very clearly defined and the conditions and everything. In a cosmetic it becomes a different matter if the cosmetic is properly labeled as a cosmetic, you know, cleanse, beautify, look, attractiveness, alter the appearance, but no pharmaceutical claims, then it becomes a question is this safe in this cosmetic.

And I think that's where FDA was coming from earlier in this process. So, I think what we're defining here are the conditions of safety used in a cosmetic. In some cases, the pharmaceutical active ingredient and I think we can do that. That's what we're trying to do by presenting data to support that safety. And there's other data that is available that is in market data. What kinds of in market experiences have taken place. So that's a possibility to include.

DR. COHEN: What was the purpose of the tetracycline in the cosmetic?

DR. BAILEY: I just made it up.

DR. COHEN: Oh. I might imagine it as an antimicrobial agent but a skin conditioning agent, but okay. I think there's more to come on this and I think the conversation was useful and tabling would prevent us from line iteming the IDA tomorrow. And perhaps by the September meeting we can have a conversation about what you might --

DR. BAILEY: Well, we'd like to table it until December.

DR. BERGFELD: They're going to be finished but they have to review the information; the statistics.

DR. COHEN: No, I meant in September I could hear what the operational issues would be for FDA to look at it again. I don't know. I don't know if we're going there, so we'll see. That was a very --

DR. ROSS: A short discussion.

DR. COHEN: No, it was a very necessary discussion. Any further comments from anybody? Okay. Perhaps yeast is next and may not have as long a conversation.

DR. BAILEY: Thank you.

DR. COHEN: Oh, thank you, guys. Thank you very much.

DR. ATOR: We greatly appreciate your patience. Thank you.

DR. BELSITO: At the December 2023 meeting the Panel issued a second Insufficient Data Announcement for these ingredients and requested a number of data. And we're also provided with the potential to use some read-across ingredients including tafluprost, travoprost, and cloprostenol for Isopropyl Cloprostenate. And then I guess it was travoprost for Ethyl Tafluprostamide. That went to the working group and I think there are still issues with what we're going to accept for read-across.

We did get a large number of new data. However, we felt that the data that we needed were still insufficient. We were told that there is some data coming along including some outside ophthalmologic evaluation of the clinical studies, some additional data looking at DART endpoints which are a particular concern to us. And also, a determination of whether we can or cannot use read-across.

We're promised a table looking at various Prostaglandins, and their receptor binding capabilities as well as KIs. So based upon all of this we thought perhaps we should table it at this point, bring in all of the new data we have, make a decision as to whether there are any potential read-across materials for this. So the motion is to table.

DR. COHEN: Second.

DR. BERGFELD: There's no discussion on a tabled; the ingredient is tabled then.

DR. COHEN: Wait, so, there's no discussion after a table.

DR. BERGFELD: We can, but basically it's no discussion. But you may go ahead.

DR. COHEN: So if you make a motion I don't have to second it until -- so I can withdraw my second. I just wanted to discuss with the other team some issues that came up in our discussion. I think you already brought up, Don, that Curt pointed out the read-across, particularly with engaging with drugs that engage with ceptors. We thought that the dossier that was provided was more akin to an over-the-counter drug than a cosmetic agent.

We were asked to extrapolate toxicity in this read-across attempt using API, active pharmaceutical ingredients, to look at adverse effects, but specifically not to look at biologic effects on structure and function. So this is going to be adjudicated as a cosmetic because of the absence of adverse effects that we know occurs in the drugs that are approved for eye use, and not further look at structure and function changes. Further to that, the online presence of these products, which are already on the market, while using cautious wording, have photo and innuendo that appear to be growing hair.

So when we asked a little bit further about the mechanism by which Prostaglandins are hair conditioning agents, we have no response. Right, we don't know how a Prostaglandin is a hair conditioning agent other than it growing hair. On PDF 68, when we're looking at this as a cosmetic agent not as a drug, because of the absence of known drug side effects, it says when compared to baseline there was a statistically significant increase in overall color change of the left and right iris at all time points. In another part, similarly, when compared to baseline values for the right orbital side there were statistically significant difference in right orbital volume after two and eight months of test use material.

In the dossier that we received it was indicated that these were non-clinically significant, although, I'm unaware of any validated tool for clinical significance of eye color change or volume change.

DR. BELSITO: Yeah, I had problems with that as well.

DR. COHEN: Right, so, in fact, in the report those side effects from a drug were seen. And we asked for an independent ophthalmologist, but I was unable to find any minimally clinically important difference in these changes, the permanency of those changes, and we in our group had considered a referral back to FDA for their consideration as an over-the-counter drug.

DR. BELSITO: I would not be adverse to that.

DR. BERGFELD: Please.

DR. SRINIVASAN: Jannavi for FDA. We are happy to take it back to the FDA to be considered. Thank you.

DR. BERGFELD: John?

MR. BAILEY: I think the discussion kind of is veering off. I mean, I think FDA's general requirements for determination of product classifications are very specially set. And, it's the intended use that addresses that issue.

And this comes up all the time when you're looking at ingredients, because there are a lot of ingredients that have pharmaceutical uses but are also used in cosmetics. So I think that needs to be kept in mind. And I think FDA is obligated to look at this based on the labeling that was present on the product. What is the intended use?

And the reason why these ingredients are here before the Panel, and I think they're appropriately here before the Panel, is because they're being marketed as cosmetics. And, I think, again, that's what we're here all about is to define safe conditions of use for cosmetics. And there's been a tremendous amount of data developed and presented. That data has been presented to the Panel. You've given us back feedback in terms of the insufficient data elements. And the sponsors are currently working to answer those questions. And so I think this just puts us back in a circle and does not help address the safety determination.

The question about the clinical study, we are working to answer that. We've submitted roadmaps for the two ingredients on times for submission. And I think that those times network should proceed so that we bring to the Panel the information necessary in order to make a science-base, regulatory-based decision about how these ingredients should be used. And it's certainly within the Panel's purview to define that. You do that in other situations as well.

So I would recommend the tabling, we talked yesterday about tabling this until March when we could have another in-person meeting. The data can be presented. The Read-Across Working Group can be presented with experts in order to support the read-across, because I think it's a valid thing to do. The endpoints that we're looking at, the pigmentation, and IOP, and so forth, are valid endpoints in order to determine; number one, are there any adverse effects under cosmetic uses. And are there any drug effects taking place.

The material that's on the internet I don't think it's appropriate to use, because it doesn't necessarily represent the sponsors of these ingredients. The internet is a very imperfect vehicle for going out and finding out information about products. These are promotional claims made for products, and we don't even know if they fall within the purview of what's being presented here, which is very low use level, levels of use in products, de minimis exposure, controlled formulation, controlled application, directions for safe use to make sure that they are properly used by the consumer. So, again, I think a lot of work has gone into this, to move this back to a question to FDA knowing that these products are being marketed legally as cosmetics because of intended use, would be (inaudible).

DR. BERGFELD: Courtney?

MS. GRIFFIN: Yeah, if I could just make a comment; for the record, Courtney Griffin, from Consumer Federation of America. So, intended use can be established in a number of ways. One of those ways is consumer perception. And the central question there would be what is the reputation, why are consumers purchasing this product, what are their expectations when they do so? I think it clear that consumers are purchasing lash serums for lengthening effects. And I would support the conversation that's been had between Dr. Belsito and Dr. Cohen.

DR. BELSITO: I guess, also, I have a hard time figuring out how these functions as a hair conditioning agent, unless they are affecting the PGF2 alpha receptor in the hair bulb.

DR. COHEN: Well, they're generally not applied to the hair shaft. Right, they're applied to the base.

DR. BELSITO: Right, they're applied to the line, right.

DR. COHEN: And my general impression of hair conditioning agents is that they go on the hair shaft. Right, they're conditioning the hair. And, I don't think this conversation digresses too much from the one that we had with *p*-Phenylenediamine, right. *p*-Phenylenediamine is being used in a way that it shouldn't be used, right. And, it's on the internet and there are case reports, right, there are case reports and it's on the internet. And just as you have seen and I have seen adverse effects to PPD, what I would say is the intent of the patients who are using this is to grow more lashes, or brows, and longer brows. That seems to be their intent when they're using it.

And the photos are not just random photos. They're from the manufacturers' website. Right, it's from the groups who have Prostaglandin containing lash serums. Seems like this needs -- they're very good products, I'm not suggesting otherwise in that regard, but we do need a drug facts label on them, not a cosmetics label on them.

DR. BERGFELD: Yes.

DR. BELSITO: Identification please.

MS. ATOR: For the record, Jennifer Ator, Tox Services. By that same reasoning one could look at before and after photos of crow's feet, or redness, or effects on melasma spots, things like that. And make a similar claim that based on those photos, those are in fact drugs effects, because they are also purportedly affecting structure or function.

Yet that would require labeling retinyl, hyaluronic acid, dimethecone, glycerin as drugs, all of which do have drug uses and are also appropriate for use in cosmetics. So I would caution against that line of reasoning as saying that a change in appearance is a drug effect.

DR. COHEN: The retinyl dossier of the 1980's indicates that retinyl and retinyl palmitate are GRAS. And nowhere in that dossier does it use prescription items as part of its toxicology dossier at all. Okay. So, no one's bringing in Tretinoin, no one's bringing in isotretinoin or etretinate to justify the safety of the product. In the 80's it was retinyl and retinyl palmitate on its own as a GRAS product. And we do have to weigh the potential side effects of these things.

So, if you're commenting on the appearance of fine lines and wrinkles that may be temporary, or permanent, that's a very different side effect as to permanent iris pigmentation or loss of periorbital volume. Right, just as you're skating that fine line, and that was very well articulated before, and in yesterday's panel, we have a fine line as well on what is a reasonable expectation for someone who may be using this what they may be at risk for and what the permanency of that might be. So, your point's well taken. I just don't I think it's a perfect parallel.

MS. ATOR: Right, well to support and further expand upon those things. We had two potential concerns here. The potential for a drug-like effect, which to my mind cannot be identified based on before and after photographs of unknown origin. And we also have adverse effects. Now, we would, if the Panel would allow us, like to show you some photographs. Those effects that you mentioned in the colorimetric data, those statistically significant effects, we'd like to show you the pictures that are associated with those effects. And I think that any reasonable trained individual would be able to determine that there's nothing there.

DR. COHEN: I would say reasonable trained ophthalmologist with specialty in this would.

MS. ATOR: Absolutely.

DR. COHEN: And I would also expect there to be some validated tools to measure what is clinically meaningful.

MS. ATOR: Right, there aren't.

DR. COHEN: They can be developed. Every single time a new product comes on the market there are new tools that are developed and validated. I see it almost every half a year, new validated tools on measuring patient reported outcomes and clinical reported outcomes. So the fact that they don't exist is no reason why they can't exist. There are new ones all the time.

MS. ATOR: Right, and that's certainly true, but the literature does not have anything that is validated. The standard approach for evaluating iris color change and changes in orbital volume is direct inspection of photographs by ophthalmologists. That is the standard.

DR. BERGFELD: Well, thank you, very much.

MS. ATOR: Thank you.

DR. BERGFELD: I did not accept your second your second that was thrown on the table.

DR. COHEN: That's okay. It stands anyway.

DR. BERGFELD: But we did have a long discussion, and I think that we have a promise from the industry that more data will be coming forward, and we'll look at this. What, Don?

DR. BELSITO: Can I rescind my table? It hasn't been voted on.

DR. BERGFELD: No, it hasn't been voted on.

DR. BELSITO: Because the FDA has said they'd gladly take it back.

DR. BERGFELD: You want to -- excuse me, go ahead.

DR. MANGA: Yes, sorry, I wanted to add to that the fact that there is data coming forward. I would wait for the Panel to tell us what you would like from FDA when you would want us to take it back, if there is data that you want to review. We are happy to take it back is what I'm saying. But, if there is data, and the Panel is tabling it, perhaps you can tell us. Because we know a product can be a cosmetic or a drug or both, so perhaps there's value to seeing the data.

DR. BERGFELD: So we have FDA taking it back and we'll see a --

DR. BELSITO: Okay.

DR. BERGFELD: -- Wave of that. Is that correct?

DR. BELSITO: Right.

DR. BERGFELD: And you are correct we did not vote on it. I would like to call a vote now, though. Because we had a first and second table until this information comes in, I was asking Bart if this is for December or this is March it's on the agenda?

DR. HELDRETH: So the initial information that was received from John and his colleagues was that we would get the data in this fall. And then the initial idea was, okay, well, that might be in time for the December meeting. But, if we're going to take the time to let the Read-Across Working Group look at this thoroughly, have an open meeting about it, and then get that incorporated neatly and succinctly into a report, so that the Panel won't have to spend arduous amounts of time on it, we were thinking maybe it would be better if the report date for that until March.

DR. BERGFELD: Okay.

DR. SNYDER: My only comment. Mark was to follow up with our team's recommendation. I think Curt made it and we strongly supported it. It should be an in-person meeting, because of the complexity of the issue.

DR. HELDRETH: Yeah, I don't see any problem with making the March meeting in-person.

DR. BERGFELD: Yes, if you would identify yourself.

MR. NICOLAI: Jeffrey Nicolai, General Counsel for a number of companies in the industry. Before it close, I just wanted to talk about the clinical, and maybe talk about some of your expectations about the independent ophthalmological review, so we can be sure that we're kind of working towards what you guys are looking for.

As you know this is new technology. These are the Canfield highly sophisticated cameras. And I think you rightful point out that there should be an independent review of the colorimetric data as a final step in the process. The way we've seen it in other situations is a three-panel, an ophthalmologist panel. Essentially, I think there's slightly different ways that they're looking to measure. Some are just yes, no; others on a scale of 1 to 10. So, I wanted to make sure that we were forming that panel, if you guys have suggestions about the ophthalmologists you'd like to see on that panel, we're open. You have suggestions about the way you would like it performed, we're open. What I want to avoid is coming back next meeting and doing something that you guys don't feel is the way you would've liked to have seen it.

DR. BERGFELD: Well, Canfield, we're well acquainted with their expertise, and they have done many, many studies since the 80's. So they are in charge of these standardization photographs. And they have also put together panels to judge their work, so --

DR. COHEN: I'd still think we'd like to see the panel members first. Because you're right, you'll come back here and we might say how'd you come up with this group?

MR. NICOLAI: But it's slightly different too, because, you're right, I've been talking to Canfield and they obviously have a very good grasp on the colorimetric data itself. But that's slightly different than an independent ophthalmological review that I believe you guys are requesting.

And, so, if it's three ophthalmologists, the panel, looking at colorimetric data, I don't think they would be doing as much as reviewing the photographs visually to see if there are any visible eye color change. Which, again, there's not. I reviewed every photo. So, is it an ophthalmological review you're looking for, or is it a review of the colorimetric data that has been produced by the camera technology?

DR. COHEN: I think it has to be both. We run into this exact issue with face pigmentation, and, determining whether a procedure improves face pigmentation. So, we use the same colorimetric scales, but they have to be correlated to clinical value. So as you guys are saying, it's possible you'll have statistically significant colorimetric changes that are not clinically important. I'm still at an impasse here because I don't know what that means. Right, what does clinically significant mean if patients are going to be on it for years, and were showing eight months, is that sufficient? I need that expertise from the panel.

DR. SNYDER: And that I would concur with that, with David's comments. I think it needs to be someone who has broad experience in translating the colorimetric data to clinical relevance. I think that's the key.

MR. NICOLAI: So that's not an ophthalmologist. Is that right?

DR. COHEN: No, I think it would be an ophthalmologist.

DR. BELSITO: Yeah.

DR. COHEN: But there are ophthalmologists who have expertise in iris, and those issues. And I'm sure we can find them, and you can find them, and we can come together on a panel that we would all agree to. Because I think we need to agree on the panel before you go ahead and do it, because then we'll all have to agree to accept the report at that point, right?

MR. NICOLAI: Yes, and that's fine. We're happy to work with you. Like I said, I'm very confident that our product cannot cause eye color change and this clinical supports that. So, happy to work with you guys and during the break in the meetings we could talk more about the panel that you're looking for. And I can send you some recommendations, maybe five ophthalmologists that we select.

I was looking at the previous study done, hopefully there's some ophthalmologist who've already conducted studies like this. And maybe they would be a good choice.

DR. BERGFELD: Thank you.

DR. BELSITO: Just to follow up, just to throw this out and see how other people feel. Given the findings in this study and our report on 114 patients, and then there's another study on 56 patients where there were some adverse effects of eye pain, hyperemia, erythema and irritation, but no statistically significant worsening cutaneous tolerance scores. Is that number of patients sufficient to identify issues? I mean, we're talking about essentially 170 patients who have been studied for this.

DR. COHEN: So, Don, this is the very issue that we're talking about. So, if it's a cosmetic, right, you'd use smaller numbers to prove the point. But I'm just less familiar with this concept of proving something's not a drug by looking at adverse effects, not looking at a biologic effect, right. We're not used to that, so to capture adverse effects you usually have a minimum number in a clinical trial. And there's usually an LTE, there's a long-term extension to look at that. So, that's my dilemma here. I don't have an answer for it. Because if it was a drug, we'd know that well. And if it's a cosmetic, that's what you guys have been doing for decades. This is a tweener, right. It's not a drug because it doesn't have drug adverse effects. So it's a little bit different.

DR. BELSITO: It's not a -- yeah, okay. It's not a drug because of the way it's marketed.

DR. BERGFELD: And the concentration.

DR. COHEN: Well, these are very potent substances.

DR. BELSITO: (Inaudible) is .0003.

DR. BERGFELD: Well, I think that we're come to the end of our discussion. We need to see the added information that's been promised. And, it looks like it would be in March.

DR. BELSITO: I guess it, you know, my point about numbers was added information. Do we need additional clinical studies? Do people feel that, because I sort of do. I'm not comfortable with the number that I've seen.

DR. ROSS: Could I just get a clarification? The proposal on the table is to defer, table, I should say until, now it's March, I believe. I think the discussion we had yesterday was for the FDA to reconsider concurrently while that was being tabled.

DR. COHEN: That's what I thought.

DR. ROSS: But now that has changed. So we're going to make a decision in March, and then maybe the FDA will get it at that point, or maybe they won't. So is that the process we're now following?

DR. BERGFELD: Yes, that's what I heard, yes.

DR. ROSS: And is that the optimum process? I would bring that up.

DR. BERGFELD: Well, we think that's the process we have at hand.

DR. COHEN: Well --

DR. BERGFELD: And, we have agreed to look at any additional data. And FDA has agreed to look at it with us, and, if appropriate, to turn it back to them.

DR. ROSS: And the question is whether that was going to be a concurrent process or whether it was going to be a --

DR. BERGFELD: I don't believe -- it's not concurrent. Is that correct? You're going to wait for the (inaudible)?

DR. MANGA: It's going to be a little difficult to make it concurrent, because the data is going to have to be reviewed, right. So we can call it concurrent, but I don't know if it's really going to be that way.

DR. ROSS: That sounds like a no.

DR. BERGFELD: It sounds like we have an open agreement that FDA is willing to look at it and decide if to take it back.

DR. ROSS: Yeah, no, I appreciate that, actually.

DR. BERGFELD: I think now we should call the question. All those in favor of tabling please raise your hand. Thank you, Paul?

DR. SNYDER: Aye.

DR. BERGFELD: Yes.

DR. SNYDER: I concur.

DR. BERGFELD: Okay. Thank you. Good discussion, now we're moving on to Dr. Cohen's report on Fatty Amphocarboxylates.

JUNE 2025 MEETING – RAWG DISCUSSION

RAWG – June 9, 2025

DR. HELDRETH: All right, so just as a reminder, this is a meeting of a working group of the Panel, not the full Panel itself. So no reports under discussion here will be proceeding to the next stage or next iteration thereof. This group is here to meet to look at the available data and make proposals to the full Panel at a future meeting.

So, no conclusions will be made herein per se, but the Read-Across Working Group will provide their proposals in writing to the full Expert Panel to be considered at a future meeting when those reports do actually advance. And so I'll turn it over to the chair of the Read-Across Working Group, Dr. Rettie.

DR. RETTIE: Thanks Dr. Heldreth. So just to remind people the Read-Across Working Group as convened by the CIR is myself, Dr. Ross, Dr. Klaassen and Dr. Tilton. And the four of us first met not very long ago, December 2023, I think it was. And we put down our initial thoughts about read-across and tried to establish some ground rules for what we would do moving forward. It was a new venture for most of us.

And I'd just like to read out our summary points from that meeting because in subsequent discussions with this small group, prior to this meeting and over the last couple of years, we haven't really moved off the salient points that have been guiding us about read-across, which is as we all know complex, and has to be done or we think it should be done in a compound specific manner -- endpoint specific manner.

Read-across is a very interesting discipline, and, yeah, I'd like to congratulate all the presenters this morning on the wealth of information that you pulled together on a lot of topics. Some of it was quite bewildering in its depth. I would say, though, to balance that, I kind of came down on seeing a lot of that information provided today as being rather preliminary. And I think the presenters acknowledge that too. And that certainly feeds into our evaluation of what's been put in front of us.

But to get back to these guidelines, these summary points beyond the complex nature of read-across. As a group we're very worried, since we're a safety group, about employing read-across for the most sensitive endpoints like DART. Especially when in some in silico or other modality positives have been detected rather source or the target. And that's almost always the case really somewhere along the line, so that we always have that caveat. That we feel that we need to employ read-across for sensitive endpoints with a great deal of caution. And perhaps they should even be avoided. That's where we came down on December 2023.

Now, beyond that, when we're looking at potent pharmacological compounds and active receptors we -- not commonly, but we do see steroids and other multifarious disguises come up. Those are obviously very potent pharmacological compounds and we've been very hesitant to apply read-across for those ingredients. Similarly, prostaglandins are in the same boat.

And we've been talking about nanomolar potencies in the in vitro assays that we saw some data for today. And that just hammers home the fact that these are indeed very, very potent pharmacological compounds.

And so with an abundance of caution, we hold the position that we just do not or should not use read-across for something like the prostaglandins that we've been seeing data for today. I don't think any of my group here have changed their minds, but I should be quiet now and give anybody else a chance to say something about those grounding principles.

DR. ROSS: I think that was a good summary and with respect to read-across for DART endpoints, I think you said whether it should be avoided or done with caution. There are caveats there, but, yeah, I mean, I haven't changed opinions on that aspect.

And I think Curt's original point was we have to be really careful with reading across with compounds like this that work through receptors. And small changes in bioavailability and concentration can lead to some significant effects. And then we haven't really dealt with how some of these molecules would change endogenous prostaglandin function. I didn't even consider that when we last met. But, I'll leave it at that.

DR. KLAASSEN: Yes, I'll just continue on to say that since day one I have said that it's very difficult to have read-across in pharmacology. And I'm a pharmacologist by training and we know the pharmaceutical companies spend a lot of time trying to find out the most specific and the most active compound for various receptors. And it takes in essence millions and billions of dollars to do that.

And if you slightly change the molecule of these things that work by some of these receptors, you tremendously alter either the effectiveness or which one of multiple receptors it interacts with. You know, here we are looking at one receptor and how about all the other prostaglandin receptors. Is it doing anything there, or not? I mean this is, I think, just scary business.

And, now, I don't know all about the laws between United States and Europe, but it's my understanding that in Europe you can't use laboratory animal studies to get cosmetics on the market. But in the United States, we can. We are encouraged to use alternate methodology. But if we don't have alternate methodology, there is nothing that hinders us from using whole animal science. And, in fact, maybe Europe should do it one way, and the United States should do it another way. And we'll find out in ten years if it's okay. In essence do an experiment to determine what kind of experiments you should use.

United States at the present time is being examined by how many chemicals Europe bans to how many are banned in the United States? And there are more chemicals banned in Europe than the United States. But that's not because of science, that's because of policy. And, I think, we should stand up for science and if we're not confident, I mean, we use science so much that we have many more chemicals that are not banned. And I think we should continue to use science and use that as the criteria.

And if we're not convinced that the read-across science is strong enough, I think the group today really has done a tremendous job of doing the best that they can, within reason, by trying to use read-across. I'm not disagreeing with the science that they presented and the approach that they took. But I think in the end, it just doesn't quite give me the confidence that I would like to have my three-week old great granddaughter using these compounds.

DR. TILTON: Well said. Actually, I think well said by all of my colleagues. I do appreciate all the information that's been provided. I think the incredibly detailed nature of the read-across that's been done really attest to the complexity of the situation. That we're seeing very structurally specific, endpoint specific, species specific responses with not a lot of information about toxicokinetics or the potential for other prostaglandin receptor effects, endocrine effects. It really is a very complex situation in terms of trying to read-across to these complicated endpoints like reproductive developmental endpoints, also similarly for carcinogenicity.

So I do appreciate, though, all of the information that has been provided to try to fill these gaps. But some of it leads to more questions than answers, is where I'm at right now.

DR. RETTIE: Quite often we wring our hands when we go through other reports about concentration of use, because maybe we don't have concentration of use or it's not clear to us from the data provided. And so we're flailing around in the dark. Here we do have, it seems to me, concentration of uses, which is helpful and perhaps maybe buttresses some of our concerns. Because if I'm reading it correctly for the amides, for ethyl tafluprost amide and bimatoprost, we're at either 0.02 percent for the drug use such as bimatoprost and 0.018 percent in the documents I've been looking at, so essentially the same thing.

So, we have, although it doesn't sound like very much, a relatively high concentration of use in that case for the proposed cosmetic ingredient, which doesn't help me feel that safe. And if we go to tafluprost and IPC, I think --

correct me if I'm wrong here -- we're around 0.05 percent for the cosmetic ingredient. And a quick look online told me that travoprost is used at 0.04 percent.

So, again, we're in the same ballpark there which makes it important that we really look closely at this in terms of adverse effects or potential adverse effects. So I threw that out there.

One of the biggest things in the data points that were provided today is, of course, around the receptor targeting profiles. And we have, if you believe IUPHAR, a European group, but a good European group, I used to be a member.

DR. KLAASSEN: IUPHAR?

DR. RETTIE: IUPHAR.

DR. KLAASSEN: IUPHAR stands for International Union of Pharmacology --

DR. RETTIE: There you go.

DR. KLAASSEN: -- which includes the United States and Europe, Canada, England, China.

DR. RETTIE: It is an august body. Yeah, it's an august body and I refer to them for my information on receptor, receptor affinities. And they still list the prostamide receptor in amongst all of the prostaglandin receptors and they have got very little information on it other than the point of Woodward's work in the 2000's and 2010's. So that appears to hold up as far as I can tell by the governing bodies for receptor pharmacology. And so I'm drawn back to that whenever we start talking about the amides.

And the prostamide receptor, I feel it's almost a dead end for anyone trying to put together a dossier, unless they're going to jump in there and co-express splice variants and hope that they had to re-dimerize the way that they apparently do in vivo. That seems like a big ask to me. And then nobody that I know of is doing it. But I would just say that if we are not going to get off this prostamide thing anytime soon, in my opinion, the best way to go about providing some data is old fashioned Oregon pharmacology.

You mentioned the availability of cats. I'm a cat lover, so I don't want to go down this route at all, but cat bath pharmacology at the level of the cat iris and the cat lung, if you are to believe the literature, will give you a population of prostamide receptors to work with. And lots of information and IC50's are in the literature using that from the old Woodward data.

I don't know to what extent you feel you need to connect that, but for me that would be a connection back to biological specimen that I think everybody agrees has some weird heterodimerized (phonetic) PGF2a receptor.

I'm just throwing that out in the hope that it's helpful for someone. I think it's a massive amount of work. I don't know that anybody's even doing it anymore, but that's what I see with regard to the receptor pharmacology intricacies. And maybe the only way to get beyond those for me would be to have some data that hopefully recapitulated the vast difference between the amide based PGF2a compounds and the esters.

And it was three orders of magnitude a few years ago, so I get stuck when I see the same levels of receptor potency. Although I appreciate that people have done varied concentrations in order to extract the IC50's from those response curves. I mean, this is a lot of work at any level, but there seems to be a big data gap or information gap for me there. And that's a hard thing for me to get beyond.

Any comments from the Panel on the receptor pharmacology stuff, which I feel is kind of key. Well, I'd invite comments from the presenters today on what you've heard with regard to the receptor pharmacology. What's your reaction to that? Because I realize it kind of put you in a bind. You have permission to speak.

DR. HELDRETH: And, please, just identify yourself.

DR. ATOR: This is Jennifer Ator. I gave one of the presentations earlier.

MS. MISHRA: Hi I'm Sanghamitra. Here, with regard to the prostamide receptor binding experiment, we attempted to actually go and conduct this study, but it was extremely challenging to find a test system and also to set up the experiment itself to conduct it, so it was not feasible for us to move ahead. But we made attempts, try to collect cat sphincter from the vet suppliers and to set up a robust experiment. It was too extremely challenging.

DR. ATOR: And just to add to that. There are some additional publications in the literature that have looked at receptor specificity for some of the PGF2a analogs in addition to Woodward. I can get you those citations. But the majority of the available data suggests that they are very specific for FP receptor.

And also just to address something you said earlier about testing on the animals, the state of California has prohibited animal testing on cosmetic ingredients since 2020. So I just want to make sure everyone is aware of that.

DR. RETTIE: Well, we got another 49 states I think where you're in better shape. And according to what I saw in the news for LA this morning, it's very depressing. So do we want to go into other specific data points that were raised today that we might flag so that the presenters know maybe a little better where we're coming from? Does anybody want to take a shot at that?

DR. KLAASSEN: I guess I think we should just go on to the next compound. I think they got the flavor of what we're saying. Go ahead, you want to say something, Dave?

DR. ROSS: Yeah. I guess I'd just make the comment that there were some great presentations this morning. And the presenters, as I said, did heroic work trying to do this read-across. And the read-across, I think we all realize read-across is not perfect. It doesn't get you to the perfect solution. It gets you to the "most appropriate." Okay.

And it gets you to the most appropriate PK, the most appropriate receptor interaction, the most appropriate for biological effects. And maybe for the prostaglandins, which you've pointed out, have such potent effects at relatively low concentrations, maybe most appropriate is not sufficient. That's where I was coming down for that, but, yeah

Allan, you wanted to go into specific data, or you wanted to just move on? Leave that to Bart and to Allen.

DR. RETTIE: I could maybe say one more thing about data insufficiencies or data that was presented that kind of got my attention today. And that was around dermal absorption we're always very, very interested in. And, if I remember correctly, we have a number of 8.67 percent from a radio label study for one of the compounds, which is the kind of data that when we see that you generally like that, right? That's a quantitative assessment of percutaneous absorption and systemic availability. I think at basically 9 percent doesn't sound like a lot, but 9 percent to me means it's systemically available to some degree.

So, there were in vitro data presented for the potential read-across compounds that said that less than 10 percent was what was predicted. And so, I just don't really know what to do with that. Is that one percent or is it 9.3 percent? Because at one percent maybe we would have a different interpretation than at 9.3 percent, maybe quite a drastically different interpretation in terms of systemic repercussions.

So what I wondered is, had you done an in silico analysis for the 8.67 percent data point, that's experimental data point, how does that match up? How does experiment and in silico match up for the known? Do you know? Do you have that data?

DR. ATOR: I do have that data, actually. We did an OECD Guidelines 428 Study, and it determined that the percutaneous absorption was indeed less than 10 percent. The number was approximately 5 percent. And the value that we used for the margin of safety calculation was means plus one standard deviation.

DR. RETTIE: So about five percent.

DR. ATOR: Yes.

DR. RETTIE: I don't know where my colleagues stand on this, but that sounds to me that you would have some significant systemic exposure after repeat applications every 24 hours. Is that fair?

DR. ATOR: Could you repeat that?

DR. RETTIE: At 5 percent, I would take from that that you would have some significant systemic absorption over repeated 24-hour applications. Would you agree with that?

DR. ATOR: The study that we did was a single 24-hour application. In terms of accumulation, I don't think there's any data suggesting that there's accumulation.

DR. RETTIE: But it's not been done.

DR. ATOR: No.

DR. RETTIE: And it doesn't mirror the use of these things, probably.

DR. ATOR: No.

DR. RETTIE: Just daily application, it's just the point I'm making.

DR. ATOR: Okay, point taken.

DR. HELDRETH: I think Ms. Mishra, one of our presenters, has something more to add, as Ms. Mishra I see has her hand up.

MS. MISHRA: I'm sorry, I could not hear Ms. Ator, probably, but, I can just tell for ethyl tafluprost amide, when we compared the in silico data, and did the same kind of assessment for the three substances, they belong to the same category for the dermal penetration potential. That means low dermal absorption potential with less than/equal to 10 percent as a readout if you compare them in silico level only. So this is just to answer your question about how did it compare in silico-wise.

That was one point, and second point I wanted to request is that the newly generated PGF2a receptor binding assay results for the three substances, which has been conducted using human cell lines expressing the receptors, we would really request you to review the data to see if there are any flaws in the study as such for us to not consider that data. Because the older data in the article were not conducted with the human receptors. They were conducted with, if I'm not wrong, it was bovine uterus tissues. And there were different protocols, not really head-to-head.

I don't expect that explains the entire difference, but maybe if you can give us a feedback on the assay itself, to see if there are any possibilities to refine it, that would be very helpful. Thank you.

DR. RETTIE: Well, we can certainly take a look at that, although, we'll definitely need a very qualified pharmacologist.

So I'm not sure what else I can add to this. I kind of feel like we are where we started with our concerns again at the levels these potent biological mediators being used at extensively the same concentration as ingredients in cosmetics as we see important pharmacological effects when we're using them for eye intraocular glaucoma reduction pressure.

DR. HELDRETH: All right, then, if that's the conclusion of the Read-Across Working Group discussion on this matter, then over the next few months the Read-Across Working Group will work together to take a closer look individually at all this data and these presentation they'll have before them and ultimately provide a proposal to the full Panel the next time that they look at this report as a full Panel.

Currently we're tentatively docketing that for December because of other things that we have on our schedule. So that will give everybody a great deal of time to thoroughly look at these very complex matters and many, many pages of information before we ultimately see the Panel make a decision on what is the next step for this report.

DR. RETTIE: Could I make one request on the report that we got? Not a real report because we're not considering it here.

DR. HELDRETH: Right.

DR. RETTIE: But I got kind of hung up on the differences in the concentrations reported. The first opening page there says the new concentration is 0.0075 for one of our prostaglandins. And I got different information today. I'd just like to have that resolved.

DR. HELDRETH: We'll take care of that for sure.

DR. RETTIE: Okay. Thanks.

Safety Assessment of Ethyl Tafluprostamide and Isopropyl Cloprostenate as Used in Cosmetics

Status: Draft Tentative Report for Panel Review
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The Expert Panel for Cosmetic Ingredient Safety members are: Chair, Wilma F. Bergfeld, M.D., F.A.C.P.; Donald V. Belsito, M.D.; Bruce A. Brod, M.D., M.H.C.I., F.A.A.D.; Samuel M. Cohen, M.D., Ph.D.; Curtis D. Klaassen, Ph.D.; Allan E. Rettie, Ph.D.; David Ross, Ph.D.; Paul W. Snyder, D.V.M., Ph.D.; and Susan C. Tilton, Ph.D. Previous Panel member involved in this assessment: David E. Cohen, M.D. The Cosmetic Ingredient Review (CIR) Executive Director is Bart Heldreth, Ph.D., and the Senior Director is Monice Fiume, M.B.A. This safety assessment was prepared by Priya Ferguson, M.S., Associate Toxicologist & Senior Scientific Analyst/Writer, CIR, and Jinqiu Zhu, Ph.D., DABT, ERT, DCST, CIR Toxicologist.

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ABBREVIATIONS

CA	chromosomal aberration
CAS	Chemical Abstracts Service
CIR	Cosmetic Ingredient Review
CLP	classification, labeling, and packaging
Council	Personal Care Products Council
DDDE	dechloro dihydroxy difluoro ethylcloprostenolamide
Dictionary	*International Cosmetic Ingredient Dictionary*
DMSO	dimethyl sulfoxide
DNA	deoxyribonucleic acid
DPRA	direct peptide reactivity assay
EC	European Commission
ECHA	European Chemicals Agency
$E_{product}$	estimated daily exposure to a cosmetic product per kg bw
ED_5	dose estimated to produce a 5 unit area (mm*h) in a graph of the different in pupil diameter in the dosed eye versus time
EP receptors	G-protein coupled receptors
EPA	US Environmental Protection Agency
EU	European Union
FDA	US Food and Drug Administration
FOU	frequency of use
HepG2	human liver cancer cells
HET-CAM	hen's egg test chorioallantoic membrane
HRIPT	human repeated-insult patch test
IC_{30}	30% inhibitory concentration
IC_{50}	half maximal inhibitory concentration
ISS	Istituto Superiore di Sanità
K_i	inhibitory constant
LD_{50}	median lethal dose
l.o.	leave-on
LOAEL	lowest-observed-adverse-effect-level
log K_{ow}	n-octanol/water partition coefficient
MNT	micronucleus test
MoCRA	Modernization of Cosmetic Regulations Act of 2022
MOE	margin of exposure
MOS	margin of safety
MW	molecular weight
NOAEL	no-observed-adverse-effect-level
NR	none reported
OASIS	Organization for Assessment of Structure–Activity Information System
OECD	Organisation for Economic Co-operation and Development
Panel	Expert Panel for Cosmetic Ingredient Safety
PGE_2	prostaglandin E2
$PGF_{2\alpha}$	prostaglandin $F_{2\alpha}$
POD	point of departure
QSAR	quantitative structure-activity relationship
RLD	Registration and Listing Data
SAR	structure-activity relationship
SCCS	EU Scientific Committee on Consumer Safety
SED	systemic exposure dosage
TG	test guideline
TMA	tear meniscus area
TSV	toxicological screening value
US	United States
UV	ultraviolet

DRAFT ABSTRACT

The Expert Panel for Cosmetic Ingredient Safety (Panel) assessed the safety of the synthetic prostaglandin analogues Ethyl Tafluprostamide and Isopropyl Cloprostenate, which are reported to be used as hair conditioning agents in cosmetics. Ethyl Tafluprostamide is also reported to function in cosmetics as a nail conditioning agent. The Panel reviewed all relevant data and concluded that Ethyl Tafluprostamide and Isopropyl Cloprostenate… [to be determined].

INTRODUCTION

This assessment reviews the safety of Ethyl Tafluprostamide and Isopropyl Cloprostenate as used in cosmetic formulations. According to the *International Cosmetic Ingredient Dictionary* (*Dictionary*), both Ethyl Tafluprostamide and Isopropyl Cloprostenate are reported to function in cosmetics as hair conditioning agents.[1] Additionally, Ethyl Tafluprostamide is reported to function in cosmetics as a nail conditioning agent (Table 1). Ethyl Tafluprostamide is also known as dechloro dihydroxy difluoro ethylcloprostenolamide (DDDE).

Some prostaglandin analogues (e.g., bimatoprost) are used as US Food and Drug Administration (FDA)-approved drugs.[2] However, the FDA has determined that at least one use of one of the prostaglandin analogues reviewed in this report (i.e., Ethyl Tafluprostamide) is considered to be a cosmetic use (jurisdictionally based on the observation that the product literature appears not to make drug claims).[3] Thus, this use is within the purview of the Panel and the safety of these cosmetic ingredients is reviewed herein in that light.

This safety assessment includes relevant published and unpublished data that are available for each endpoint that is evaluated. Published data are identified by conducting an extensive search of the world's literature; a search was last conducted August 2026. A listing of the search engines and websites that are used and the sources that are typically explored, as well as the endpoints that the Panel typically evaluates, is provided on the Cosmetic Ingredient Review (CIR) website (https://www.cir-safety.org/supplementaldoc/preliminary-search-engines-and-websites; https://www.cir-safety.org/supplementaldoc/cir-report-format-outline). Unpublished data are provided by the cosmetics industry, as well as by other interested parties.

Much of the data included in this safety assessment were found on the Scientific Committee on Consumer Safety (SCCS) website.[4,5] Please note that the SCCS website provides summaries of information generated by industry, and it is those summary data that are reported in this safety assessment when SCCS is cited.

CHEMISTRY

Definition and Structure

Ethyl Tafluprostamide (CAS No. 1185851-52-8; Figure 1) and Isopropyl Cloprostenate (CAS No. 157283-66-4; Figure 2) are structurally related as prostaglandin analogues. Prostaglandins are a ubiquitous group of physiologically active lipids (a.k.a. eicosanoids or autacoids) known to demonstrate diverse hormone-like effects. In humans and other animals, prostaglandins are derived enzymatically from the fatty acid arachidonic acid.[6] However, both of these ingredients are synthetic analogues. The definitions of these ingredients are provided in Table 1.

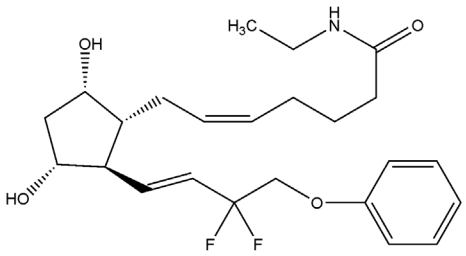


Figure 1. Ethyl Tafluprostamide

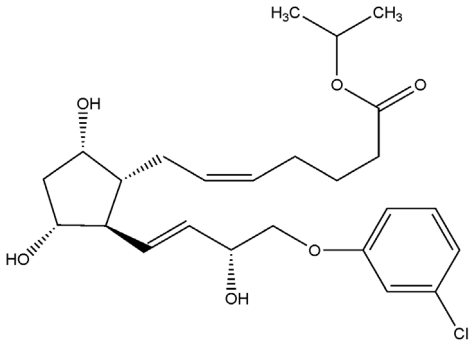


Figure 2. Isopropyl Cloprostenate

Chemical Properties

The ingredients reviewed in this report are hydrophobic, water-insoluble substances.[3] Ethyl Tafluprostamide is a colorless to pale yellow solution, with a molecular weight (MW) of 437.5 g/mol, reported water solubility of 1.05 g/l (at 20°C), and an octanol/water partition coefficient (log K_{ow}) of 2.74).[4,7] Isopropyl Cloprostenate is a liquid with a MW of 467 g/mol, reported water solubility of 99.1 mg/l (at 20° C), and a log K_{ow} of 3.86).[4] Other physical and chemical properties of Ethyl Tafluprostamide and Isopropyl Cloprostenate can be found in Table 2.

Method of Manufacture

Ethyl Tafluprostamide

The production of Ethyl Tafluprostamide follows a common synthetic pathway starting from Corey lactone acetate.[8] Corey lactone acetate is first converted into an alcohol and subsequently oxidized to the corresponding aldehyde, which then undergoes an olefination reaction to introduce the alkyl side chain. This reaction results in the formation of an enone which undergoes fluorination. The lactone ring is then opened through nucleophilic attack, allowing the formation of the second aliphatic chain. Protecting groups on hydroxyl functionalities are selectively removed or modified to facilitate subsequent transformations. The substance is then converted into an amide.

Composition and Impurities

Ethyl Tafluprostamide

According to an unpublished data submission, Ethyl Tafluprostamide has a purity of no less than 99%; the SCCS states purity levels of ≥ 98%.[4,9] In addition, according to the unpublished data submission, Ethyl Tafluprostamide should not contain more than 1% impurities. High-performance liquid chromatography regularly demonstrates that the C-5,6 *trans* double-bond isomer of the alpha chain is present at ≤ 2% relative to the predominant C-5,6 *cis* isomer.

Isopropyl Cloprostenate

The SCCS also reported that undiluted Isopropyl Cloprostenate has a purity level of ≥ 98%, and that the material is generally supplied as a 10% solution in ethanol.[4] Impurities and accompanying contaminants in this ingredient include 15-epimer (0.25%), ethyl acetate (0.2%), and water (0.15%).[5] In addition, high-performance liquid chromatography regularly demonstrates that the C-5,6 *trans* double-bond isomer of the alpha chain is present at ≤ 2% relative to the predominant C-5,6 *cis* isomer.

USE

Cosmetic

The safety of the cosmetic ingredients addressed in this assessment is evaluated based on data received from the US Food and Drug Administration (FDA) and the cosmetics industry on the expected use of these prostaglandin analogues in cosmetics. Registration and Listing Data (RLD) obtained from the FDA report frequency of use, and responses to a survey conducted by the Personal Care Products Council (Council) indicate maximum reported concentrations of use; it is these values that define the present practices of use and concentration that are assessed by the Panel. Since 2024, as a result of the Modernization of Cosmetics Regulation Act of 2022 (MoCRA), manufacturers and processors are required to register facilities and list their products (and ingredients therein) with the FDA (i.e., RLD). An exception is made for small businesses (average gross annual sales in the US of cosmetic products for the previous 3-yr period is less than $1,000,000, adjusted for inflation), which are exempt from MoCRA reporting for most cosmetic product categories. Eye area products, injected products, internal use products, or products that alter appearance for more than 24 h, and the facilities that manufacture these products, are not included in this exemption.[10] Another change resulting from MoCRA is the addition of tattoo preparations (permanent tattoo inks, temporary tattoo inks, and other tattoo products) to the product categories for which companies need to list their products with FDA. However, evaluating the safety of ingredients as used in tattoo preparations is not within the purview of the Panel; accordingly, such use is not included as part of the present practices of use that are assessed by the Panel.

According to RLD obtained from the FDA in 2025, Isopropyl Cloprostenate is reported to be used in 54 total formulations, most of which belong to the category "eyelash and eyebrow preparations (primers, conditioners, serums, and fortifiers);" however, uses were also reported for hair preparations and a leave-on face and neck product (Table 3).[11,12] Ethyl Tafluprostamide has been reported to be used in 6 total formulations, most of which belong to the category "eyelash and eyebrow preparations (primers, conditioners, serums, and fortifiers)." No concentrations of use were reported for either Ethyl Tafluprostamide or Isopropyl Cloprostenate in response to a survey initiated by the Council in 2022 (and for which results were submitted in 2023).[13] However, data submitted from industry as submissions separate from responses to the concentration of use survey indicate that Isopropyl Cloprostenate is used in eyelash serums ranging from 0.0044 – 0.0075%[14-16] and Ethyl Tafluprostamide is used in eyelash, eyebrow, or scalp hair products at concentrations ranging from 0.012 – 0.02%.[9,17] Information regarding the amount of these ingredients per use may be found in Table 4.

The amount of cosmetic eyelash product per brush stroke was determined by applying the product to commercially available mink samples via 2 different procedures.[8] In procedure 1, the same tube containing the test material was used with 10 different applicators. In procedure 2, the same applicator was used with 10 different tube containers. In both procedures, 2 different methods of inserting the applicator into the tube were used. In method A, the applicator tip was pushed to the bottom of the tube. In method B, the applicator tip was inserted just to the neck of the tube. The range of cosmetic eyelash product applied per brush stroke (for both procedures and application methods) was 0.64 – 3.72 mg. The average amount was 2.42 mg, and the highest maximum amount was 3.72 mg (for both procedures and application methods).

It is possible that some products containing Ethyl Tafluprostamide and Isopropyl Cloprostenate may be marketed for use with airbrush delivery systems. With the advent of MoCRA and the current product categories outlined therein, it is now mandatory that cosmetic products used in airbrush delivery systems be reported as such for some, but not all, product categories in the RLD. In other words, a reliable source of frequency of use data regarding the use of cosmetic ingredients in conjunction with airbrush delivery systems is now available, in some instances. None of the reported product categories for these ingredients as listed in the RLD include a designation using airbrush application, so it is possible that these ingredients are used with airbrush delivery systems, but not reported as such. Additionally, the concentration of use surveys are conducted based on product categories as stated in the RLD, but airbrush use was not reported in response to the survey. No consumer habits and practices data or particle size data are publicly available to evaluate the exposure associated with airbrush technology, thereby preempting the ability to evaluate risk or safety. Without information regarding the consumer habits and practices data or product particle size data (or other relevant particle data, e.g., diameter) related to this use technology, the data profile is incomplete, and the Panel is not able to determine safety for use in airbrush formulations. If these ingredients were to be used in airbrush formulations, the data are insufficient to evaluate the exposure resulting from cosmetics applied in such a manner.

The SCCS concluded that Ethyl Tafluprostamide (at a maximum concentration of 0.018%) and Isopropyl Cloprostenate (at a maximum concentration of 0.005%) are not considered safe for use in cosmetic products aimed at promoting eyelash and eyebrow growth.[4] This assessment is based on the potent pharmacological activity of these prostaglandin analogues, even at low concentrations, and the lack of sufficient data to rule out potential reproductive or developmental toxicity. This concern is particularly relevant given that such cosmetic products are likely to be used by women of childbearing age. Furthermore, in the absence of experimental carcinogenicity data, the SCCS considered that the possibility of non-genotoxic carcinogenicity could not be excluded.

Eyelash Product Information/Consumer Use Instructions

Ethyl Tafluprostamide

An eyelash product containing 0.018% Ethyl Tafluprostamide is reported to be a thickened solution provided in an aluminum, tube-like container.[9] A multi-use applicator wand is attached to the container's screw-on cap. The tip of the applicator consists of a very fine brush that is designed to optimize precise application of a small amount of the product to the eyelashes. The tube neck removes excess solution from the applicator when the applicator is removed from the container.

This product is to be used once per day, directly to the eyelashes, near the base, above the eyelash line, and should be dried completely prior to the application of other products. This product includes caution statements that inform consumers to avoid contact with the eye, rinse eyes if eye contact occurs, reduce and/or discontinue frequency of product use if irritation occurs, and to keep out of reach of children. The caution statement also informs users of potential skin discoloration of the eyelash base following use (predominantly excessive use) of this product, and to discontinue use of the product if this discoloration is of concern to the consumer.

Isopropyl Cloprostenate

Eyelash serums containing 0.004 and 0.005% Isopropyl Cloprostenate are to be applied once a day, as a thin line on the eyelid, just above the upper lash line.[18,19] These serums are reported to be packaged similarly to the eyelash product containing 0.018% Ethyl Tafluprostamide described above. Serums should be applied to a clean, dry lash line, using a single stroke (similar to application of liquid eyeliner). Users are instructed to use one dip into the bottle for both eyes, and to allow 1 - 2 min for the serum to dry. Applications should occur nightly for a duration of approximately 3 mo. After 3 mo, users should apply every other day or 2 - 3 times per week to maintain benefits. Caution statements on these products inform users to rinse eyes with cold water if eye contact occurs, and to discontinue use if irritation occurs. Statements also suggest certain

populations avoid use of the product (e.g., those who are pregnant, under the age of 18, undergoing chemotherapy, or with previous history of eye disorders or illnesses).

Non-Cosmetic

No FDA-approved prescription or over-the-counter drug uses for these ingredients were found in the literature. However, it should be noted that while these prostaglandin analogues are not reported to be used in FDA-approved drug formulations, other prostaglandin analogues are used in FDA-approved pharmaceuticals to treat glaucoma (e.g., bimatoprost, latanoprost, travoprost).[2] Bimatoprost is also used at a concentration of 0.03% to treat hypotrichosis of the eyelashes by increasing their length, thickness, and darkness.[20] Aside from cosmetics, no other types of industrial uses were found for Ethyl Tafluprostamide or Isopropyl Cloprostenate.

A 2026 qualitative market survey conducted by French investigators, based on declared ingredient lists, evaluated 79 products marketed for longer, thicker, and/or fuller eyelashes.[21] Approximately 30% of these products were reported to contain a prostaglandin analogue, with Ethyl Tafluprostamide and Isopropyl Cloprostenate being the most frequently identified prostaglandin analogues in the surveyed products. The geographic market represented by the survey was not clearly specified.

TOXICOKINETIC STUDIES

Dermal Absorption

In Vitro

Ethyl Tafluprostamide

A percutaneous absorption study performed according to Organisation for Economic Co-operation and Development (OECD) test guidelines (TG) 428, using human skin samples (n = 3 replicates/dose), was performed using different eyelash products containing radiolabeled Ethyl Tafluprostamide ([^{3}H]Ethyl Tafluprostamide) at concentrations of 0.012, 0.018, 0.020, and 0.024%.[22] The test formulations were applied to skin samples (approximately 1 µCi at 10 µg/cell) for 24 h (receptor fluid consisted of 5% bovine serum albumin and 0.9% sodium chloride in water). Following application of test substances containing 0.012, 0.018, 0.020, and 0.024% Ethyl Tafluprostamide, the absorbed fraction was reported to be 6.44 ± 2.14, 6.51 ± 2.16. 9.12 ± 7.23, and 10.68 ± 7.18% of the applied dose, respectively.

Isopropyl Cloprostenate

A dermal absorption assay was performed according to OECD TG 428 using a cosmetic product containing 0.005% Isopropyl Cloprostenate.[23,24] Measurements were taken in the following samples: treated skin washing, stratum corneum (tape strips), epidermis, dermis, receptor fluid (0.01 M phosphate-buffered saline), and rinsing of donor compartment. The test substance (10 µl per diffusion cell) was applied to 3 dermatomed human skin samples (prepared from each of 4 donors; 12 samples total) for 24 h using flow-through diffusion cells. Receptor fluid samples were collected at 0.5, 1, 2, 4, 6 and 24 h. Isopropyl Cloprostenate was evaluated in each sample using a liquid chromatography-mass spectrometry method and quantified against an analyte-specific calibration curve. The majority of the applied amount of Isopropyl Cloprostenate remained on the surface of the skin and was recovered in the skin washing samples. Cloprostenol was not quantifiable in the skin layers or receptor fluid, suggesting minimal dermal conversion of Isopropyl Cloprostenate to cloprostenol. An average of approximately 6.34% of the applied amount of Isopropyl Cloprostenate was absorbed (measured in the epidermis, dermis, and receptor fluid). The percentage of Isopropyl Cloprostenate recovered in the epidermis, dermis, and receptor fluid, individually, was 1.67, 0.05, and 4.61%, respectively. The amount of compound in the epidermis, dermis, and receptor fluid (combined) was estimated to be 0.04 ± 0.01 µg/cm^2. Concentrations of Isopropyl Cloprostenate in the receptor fluid were below the lower limit of quantification in 11 of 12 samples; therefore, the lower limit of quantification was used to estimate receptor fluid concentrations, resulting in a conservative upper-bound estimate of dermal absorption. For systemic exposure estimate, a dermal absorption value of 7.54% was applied, corresponding to the mean absorbed fraction recovered in the epidermis, dermis, and receptor fluid (6.34%) plus one standard deviation (1.20%). A similar in vitro dermal absorption assay, using the same protocol, was performed using 0.005% radiolabelled [^{3}H]Isopropyl Cloprostenate (vehicle not specified).[4] Following application, the bioavailable portion—including receptor fluid (3% polyethylene glycol 20 oleyl ether in 0.01 M phosphate-buffered saline), rinse receptor compartment, epidermis, and dermis—was 3.87 ± 1.74% of the applied dose (10 µl/cm^2; 24-h exposure)

Computational

Ethyl Tafluprostamide

According to unpublished data, the estimated maximum amount of Ethyl Tafluprostamide that would be dermally absorbed from an eyelash product containing 0.018% Ethyl Tafluprostamide was determined to be 0.144 µg per use.[9] This calculation was based on a conservative dermal absorption of 20% and maximum single brush stroke application of the product (corresponding to maximum amount of 0.72 µg Ethyl Tafluprostamide, per brush stroke). The dermal absorption value of 20% is based on a quantitative structure-activity relationship (QSAR) model that estimated a 10% dermal absorption rate for Isopropyl Cloprostenate, taking into account the similarity in lipophilicity between Isopropyl Cloprostenate and Ethyl Tafluprostamide. To maintain a conservative estimate, the dermal absorption value for Ethyl Tafluprostamide was set at twice that of Isopropyl Cloprostenate, resulting in a 20% estimate.

Absorption, Distribution, Metabolism, and Excretion

In Vitro

Ethyl Tafluprostamide

An in vitro percutaneous metabolism study was performed according to OECD TG 428 using human skin samples (n = 3 replicates/dose; 0.5 cm^2 skin area).[25] Ethyl Tafluprostamide (6.0 µg/cm^2 diluted in 50% ethanol) was applied to skin samples for up to 24 h on a static transwell system. Identification and quantification of Ethyl Tafluprostamide, its metabolite tafluprost (free acid), and the reference substance (caffeine) were evaluated using an ultra-high-performance liquid chromatography system. Ethyl Tafluprostamide was found to be extensively metabolized into the free acid (tafluprost; 68.5 ± 2.7%) after 24 h. Bioavailability of Ethyl Tafluprostamide and its metabolite, tafluprost free acid, across all skin compartments (stratum corneum + epidermis + dermis), including receptor fluid in the skin model, was 12.3 ± 2.2 and 42.2 ± 23.1% after 4 and 24 h, respectively. Penetration of the marker compound (caffeine) was comparable with existing data from the literature.

In a different study performed according to OECD TG 428, Ethyl Tafluprostamide (0.015%; 98.5% purity; dose of 5 mg/cm^2; using [^{3}H]Ethyl Tafluprostamide as tracer) was applied to human skin samples (3 replicates/dose; 1 cm^2 skin area).[4] Receptor fluid was evaluated at intervals from 30 min – 24 h after application. The mean total recovery was 92.6 ± 8.9% and the bioavailable portion as measured for skin absorption was 16.2 ± 9.6%. The SCCS noted substantial differences in the dermal absorption values proposed by the applicants, as well as high variability in this study.[4] Given these uncertainties, the SCCS considered a conservative default dermal absorption value of 50% to be more appropriate.

Human

Oral

Ethyl Tafluprostamide

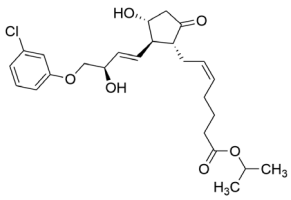
No oral toxicokinetic studies on Ethyl Tafluprostamide were found in the literature; however, based on the molecular weight (437.5 g/mol) and the log K_{ow} value (2.74), Ethyl Tafluprostamide is expected to have oral bioavailability potential ranging from ≥ 1% and < 50%.[8] Ethyl Tafluprostamide is a highly lipophilic substance; therefore, it is expected to undergo extensive distribution to various organs, once absorption occurs. Ethyl Tafluprostamide mainly undergoes hydrolysis, giving rise to tafluprost acid and ethylamine, and is expected to be mainly excreted in the urine.

Computational

Isopropyl Cloprostenate

Skin metabolites were predicted for Isopropyl Cloprostenate using the OECD QSAR Toolbox (module simulates non-enzymatic reactions as well as both Phase I and Phase II enzymatic reactions).[23] These metabolites (formed via oxidation, hydrolysis, or ether cleavage) are depicted in the images given in Figures 3 - 5. (CIR Staff have redrawn these images and provided corresponding names and other chemical identifiers, where available):

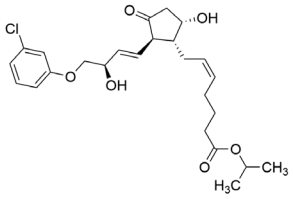
Oxidation Products



isopropyl (*Z*)-7-((1*R*,2*R*,3*R*)-2-((*R*,*E*)-4-(3-chlorophenoxy)-3-hydroxybut-1-en-1-yl)-3-hydroxy-5-oxocyclopentyl)hept-5-enoate

No CAS No.

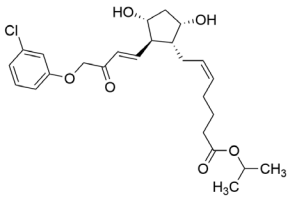
PubChem link



isopropyl (*Z*)-7-((1*R*,2*R*,5*S*)-2-((*R*,*E*)-4-(3-chlorophenoxy)-3-hydroxybut-1-en-1-yl)-5-hydroxy-3-oxocyclopentyl)hept-5-enoate

No CAS No.

PubChem link



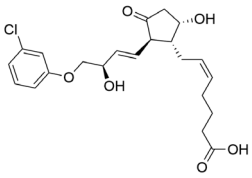
isopropyl (*Z*)-7-((1*R*,2*R*,3*R*,5*S*)-2-((*E*)-4-(3-chlorophenoxy)-3-oxobut-1-en-1-yl)-3,5-dihydroxycyclopentyl)hept-5-enoate

No CAS No.

No PubChem entry

Figure 3. Predicted dermal metabolites of Isopropyl Cloprostenate[23]

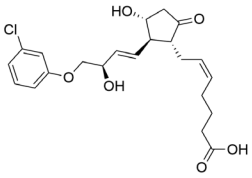
Hydrolysis Products (with or without additional oxidation)



(*Z*)-7-((1*R*,2*R*,5*S*)-2-((*R*,*E*)-4-(3-chlorophenoxy)-3-hydroxybut-1-en-1-yl)-5-hydroxy-3-oxocyclopentyl)hept-5-enoic acid

No CAS No.

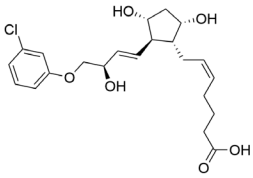
PubChem link



(*Z*)-7-((1*R*,2*R*,3*R*)-2-((*R*,*E*)-4-(3-chlorophenoxy)-3-hydroxybut-1-en-1-yl)-3-hydroxy-5-oxocyclopentyl)hept-5-enoic acid

No CAS No.

PubChem link



(*Z*)-7-((1*R*,2*R*,3*R*,5*S*)-2-((*R*,*E*)-4-(3-chlorophenoxy)-3-hydroxybut-1-en-1-yl)-3,5-dihydroxycyclopentyl)hept-5-enoic acid

CAS No. 57968-81-7

PubChem link



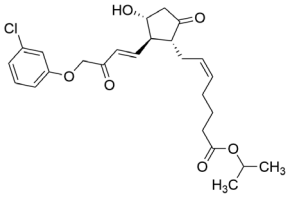
isopropanol

CAS No. 67-63-0

PubChem link

Figure 3. Predicted dermal metabolites of Isopropyl Cloprostenate[23]

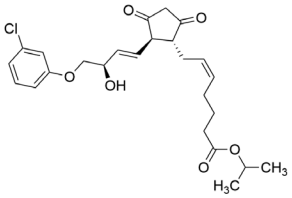
Oxidation Products



isopropyl (*Z*)-7-((1*R*,2*R*,3*R*)-2-((*E*)-4-(3-chlorophenoxy)-3-oxobut-1-en-1-yl)-3-hydroxy-5-oxocyclopentyl)hept-5-enoate

No CAS No.

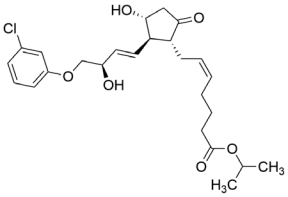
No PubChem entry



isopropyl (*Z*)-7-((1*R*,2*R*)-2-((*R*,*E*)-4-(3-chlorophenoxy)-3-hydroxybut-1-en-1-yl)-3,5-dioxocyclopentyl)hept-5-enoate

No CAS No.

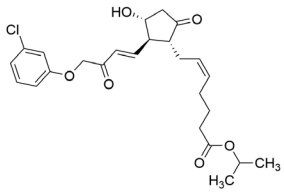
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isopropyl (*Z*)-7-((1*R*,2*R*,3*R*)-2-((*R*,*E*)-4-(3-chlorophenoxy)-3-hydroxybut-1-en-1-yl)-3-hydroxy-5-oxocyclopentyl)hept-5-enoate

No CAS No.

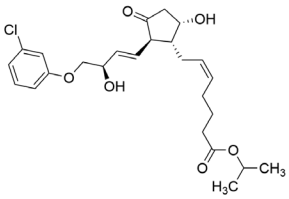
PubChem link



isopropyl (*Z*)-7-((1*R*,2*R*,3*R*)-2-((*E*)-4-(3-chlorophenoxy)-3-oxobut-1-en-1-yl)-3-hydroxy-5-oxocyclopentyl)hept-5-enoate

No CAS No.

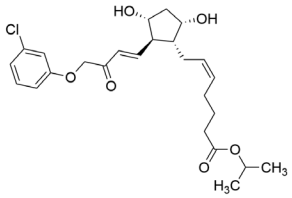
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isopropyl (*Z*)-7-((1*R*,2*R*,5*S*)-2-((*R*,*E*)-4-(3-chlorophenoxy)-3-hydroxybut-1-en-1-yl)-5-hydroxy-3-oxocyclopentyl)hept-5-enoate

No CAS No.

PubChem link

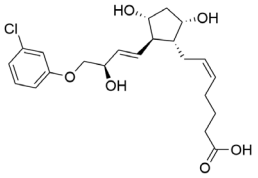


isopropyl (*Z*)-7-((1*R*,2*R*,3*R*,5*S*)-2-((*E*)-4-(3-chlorophenoxy)-3-oxobut-1-en-1-yl)-3,5-dihydroxycyclopentyl)hept-5-enoate

No CAS No.

No PubChem entry

Hydrolysis Products (with or without additional oxidation)



(*Z*)-7-((1*R*,2*R*,3*R*,5*S*)-2-((*R*,*E*)-4-(3-chlorophenoxy)-3-hydroxybut-1-en-1-yl)-3,5-dihydroxycyclopentyl)hept-5-enoic acid

CAS No. 57968-81-7

PubChem link



isopropanol

CAS No. 67-63-0

PubChem link



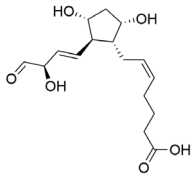
acetone

CAS No. 67-64-1

PubChem link

Figure 4. Predicted rat liver S9 metabolites of Isopropyl Cloprostenate[23]

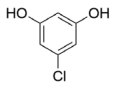
Ether Cleavage Products



(*Z*)-7-((1*R*,2*R*,3*R*,5*S*)-3,5-dihydroxy-2-((*R*,*E*)-3-hydroxy-4-oxobut-1-en-1-yl)cyclopentyl)hept-5-enoic acid

No CAS No.

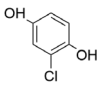
No PubChem entry



5-chlorobenzene-1,3-diol

No CAS No.

No PubChem entry



2-chlorobenzene-1,4-diol

CAS No. 52780-23-1

PubChem link



3-chlorophenol

CAS No. 615-67-8

PubChem link



3-chlorobenzene-1,2-diol

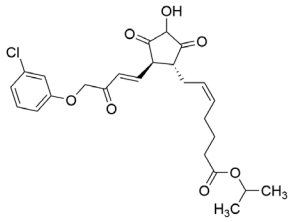
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PubChem link

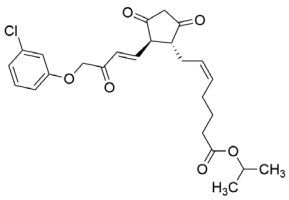
Figure 4. Predicted rat liver S9 metabolites of Isopropyl Cloprostenate[23]



isopropyl (*Z*)-7-((1*R*,2*R*)-2-((*E*)-4-(3-chlorophenoxy)-3-oxobut-1-en-1-yl)-4-hydroxy-3,5-dioxocyclopentyl)hept-5-enoate

No CAS No.

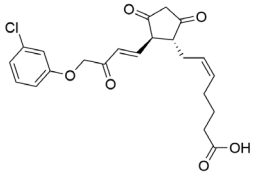
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isopropyl (*Z*)-7-((1*R*,2*R*)-2-((*E*)-4-(3-chlorophenoxy)-3-oxobut-1-en-1-yl)-3,5-dioxocyclopentyl)hept-5-enoate

No CAS No.

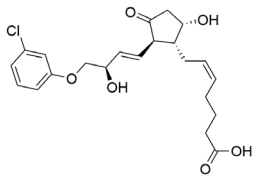
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(*Z*)-7-((1*R*,2*R*)-2-((*E*)-4-(3-chlorophenoxy)-3-oxobut-1-en-1-yl)-3,5-dioxocyclopentyl)hept-5-enoic acid

No CAS No.

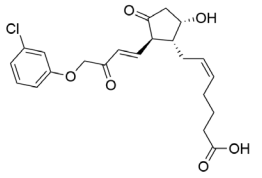
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(*Z*)-7-((1*R*,2*R*,5*S*)-2-((*R*,*E*)-4-(3-chlorophenoxy)-3-hydroxybut-1-en-1-yl)-5-hydroxy-3-oxocyclopentyl)hept-5-enoic acid

No CAS No

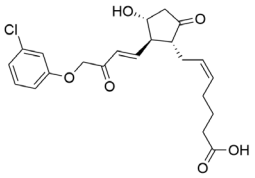
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(*Z*)-7-((1*R*,2*R*,5*S*)-2-((*E*)-4-(3-chlorophenoxy)-3-oxobut-1-en-1-yl)-5-hydroxy-3-oxocyclopentyl)hept-5-enoic acid

No CAS No.

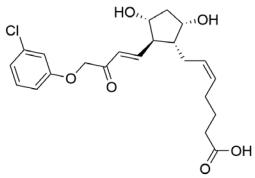
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(*Z*)-7-((1*R*,2*R*,3*R*)-2-((*E*)-4-(3-chlorophenoxy)-3-oxobut-1-en-1-yl)-3-hydroxy-5-oxocyclopentyl)hept-5-enoic acid

No CAS No.

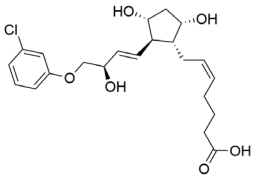
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(*Z*)-7-((1*R*,2*R*,3*R*,5*S*)-2-((*E*)-4-(3-chlorophenoxy)-3-oxobut-1-en-1-yl)-3,5-dihydroxycyclopentyl)hept-5-enoic acid

No CAS No.

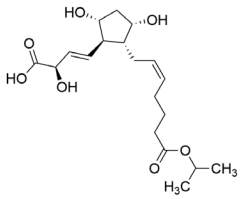
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(*Z*)-7-((1*R*,2*R*,3*R*,5*S*)-2-((*R*,*E*)-4-(3-chlorophenoxy)-3-hydroxybut-1-en-1-yl)-3,5-dihydroxycyclopentyl)hept-5-enoic acid

CAS No. 57968-81-7

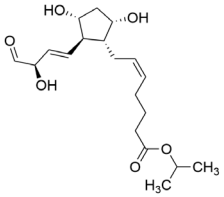
PubChem link



(*R*,*E*)-4-((1*R*,2*R*,3*S*,5*R*)-3,5-dihydroxy-2-((*Z*)-7-isopropoxy-7-oxohept-2-en-1-yl)cyclopentyl)-2-hydroxybut-3-enoic acid

No CAS No.

No PubChem entry



isopropyl (*Z*)-7-((1*R*,2*R*,3*R*,5*S*)-3,5-dihydroxy-2-((*R*,*E*)-3-hydroxy-4-oxobut-1-en-1-yl)cyclopentyl)hept-5-enoate

No CAS No.

No PubChem entry



acetic acid

CAS No. 64-19-7

PubChem link



2-hydroxypropanal

CAS No. 598-35-6

PubChem link



propane-1,2-diol

CAS No. 57-55-6

PubChem link

Figure 5. Additional predicted in vivo rat metabolites of Isopropyl Cloprostenate[23]

TOXICOLOGICAL STUDIES

Acute Toxicity Studies

Oral

Ethyl Tafluprostamide

An in vitro 3T3 neutral red reuptake assay was performed to evaluate the acute oral toxicity of Ethyl Tafluprostamide (99.78% purity; in 1% dimethyl sulfoxide (DMSO); up to 2 mg/ml; 48-h exposure) using Balb/c 3T3 fibroblasts.[8] The mean half maximal inhibitory concentration (IC_{50}) value was determined to be 0.083 mg/ml, corresponding to a median lethal dose (LD_{50}) of 880 mg/kg bw.

Parenteral

Isopropyl Cloprostenate

White albino Swiss mice (20/group; sex not stated) were administered a single dose of Isopropyl Cloprostenate (50, 75, or 100 mg/kg bw; dissolved in 1:19 DMSO and water) via intraperitoneal injection, and observed for 14 d.[26] Two control groups were treated with physiological solution or DMSO and water. No adverse effects regarding mortality, body weight, or hematological parameters were observed.

Short-Term Toxicity Studies

Isopropyl Cloprostenate

Hematological evaluations were performed on white Wistar rats (10/group; sex not stated) treated with Isopropyl Cloprostenate (15 mg/kg bw/d) for 7 d via intraperitoneal injection.[26] Control groups received a solution of DMSO and water. Parameters evaluated include red blood cell count, hemoglobin, hematocrit, and red/white cell indices. Two hours after the last administration, animals were killed, and blood was examined. Results were similar among control and treated groups.

DEVELOPMENTAL AND REPRODUCTIVE TOXICITY STUDIES

In Silico

Ethyl Tafluprostamide and Isopropyl Cloprostenate

The SCCS flagged both Ethyl Tafluprostamide and Isopropyl Cloprostenate as potential reproductive/developmental toxicants with a reasonable model certainty, based on an in silico assessment.[5] The systems used included QSAR-based systems (VEGA-QSAR and US EPA-TEST) and read-across (TOXREAD). No other details were provided. Structural profiling of Ethyl Tafluprostamide and Isopropyl Cloprostenate using OECD QSAR Toolbox (v4.6) identified a structural alert for estrogen receptor binding associated with the presence of a hydroxyl group.[4,8]

In Vitro

Ethyl Tafluprostamide

An in vitro ReproTracker® assay was performed to evaluate the potential of Ethyl Tafluprostamide (99% purity; up to 62.5 µg/ml; in DMSO) to interfere with human stem cell differentiation into cardiomyocytes, hepatocytes, and neural rosettes using human induced pluripotent stem cells.[8] Exposure to Ethyl Tafluprostamide caused a concentration-dependent increase in the mesodermal marker, bone morphogenetic protein 4, and a decrease in cardiomyocyte-specific myosin heavy chain 6 at concentrations ≥ 3.9 µg/ml, indicating cell arrest at the mesodermal stage. Ethyl Tafluprostamide also inhibited cardiac contraction at 62.5 µg/ml, decreased hepatocyte markers (forkhead box protein A2 and alpha-fetoprotein), and reduced neuroectodermal marker (paired box protein 6) expression at ≥ 15.6 µg/ml. Under study conditions, Ethyl Tafluprostamide altered differentiation marker expression at ≥ 3.9 µg/ml, while cytotoxicity and morphological disruption occurred at higher concentrations.

Animal

Parenteral

Isopropyl Cloprostenate

The effect of Isopropyl Cloprostenate 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.[27] 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 (after 7, 14, and 28 d 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 100 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 d 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 numbers, decreased spermatogenesis, and nuclear condensation of the testicular cells. Macrophages, decreased spermatozoa numbers, and decreased spermatogenesis were observed in treated mice.

A similar study was performed in male mice (12 mice/group; strain of mice not specified).[28] Mice were treated with Isopropyl Cloprostenate (25 µg/kg bw/d) for 28 d via intraperitoneal injection. A control group of mice was left untreated. After 7, 14, or 28 d, 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.

GENOTOXICITY STUDIES

Details on the genotoxicity assays summarized below can be found in Table 5.

Regarding gene mutation, Ethyl Tafluprostamide (purity 98.5 – 99.78%) was determined to be non-mutagenic in Ames assays (at concentrations up to 5000 µg/plate; with and without metabolic activation; using *Salmonella typhimurium* and *Escherichia coli* strains).[4,29] Isopropyl Cloprostenate was also non-mutagenic in Ames assays at concentrations up to 5000 µg/plate, with and without metabolic activation (using *S. typhimurium* and *E. coli* strains).[18] Regarding chromosomal damage, Ethyl Tafluprostamide (98.5% purity) was negative in a mammalian cell micronucleus assay using mouse lymphoma cells at concentrations up to 140 µg/ml, with and without metabolic activation, and no clastogenicity was observed in a micronucleus assay performed in human lymphocytes using Ethyl Tafluprostamide at concentrations up to 350 µg/ml, with and without metabolic activation.[30] Isopropyl Cloprostenate was negative in micronucleus assays using Chinese hamster ovary cells exposed to a test material containing 10% Isopropyl Cloprostenate at concentrations up to 750 µg/ml and human lymphocytes exposed to undiluted Isopropyl Cloprostenate at concentrations up to 2041 µg/ml, with and without metabolic activation.[18] Multiple structural alert profilers for Ethyl Tafluprostamide identified no alerts for DNA binding, chromosomal aberration, or in vitro/in vivo mutagenicity, with only one structural alert related to a hydroxyl-containing motif was identified.[8] A QSAR model and a statistical-based model of an Ames test on Isopropyl Cloprostenate predicted no genotoxicity.[5,31]

CARCINOGENICITY STUDIES

In Silico

Ethyl Tafluprostamide and Isopropyl Cloprostenate

According to a structure-activity relationship (SAR) analysis conducting using OECD QSAR Toolbox v.4.6 and Derek Nexus v.6.2.1, no structural alerts were found on Ethyl Tafluprostamide indicating a potential for carcinogenicity.[17] However, according to an in silico analysis of Ethyl Tafluprostamide and Isopropyl Cloprostenate performed by the SCCS, both Ethyl Tafluprostamide and Isopropyl Cloprostenate were flagged for potential carcinogenicity with a reasonable model certainty, raising the concern that these ingredients may be non-genotoxic carcinogens.[5] Ethyl Tafluprostamide and Isopropyl Cloprostenate are prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) analogs, not prostaglandin E2 (PGE_2) analogs, and are not known to bind to G-protein coupled receptors (EP; which are the receptors through which PGE_2 promotes tumorigenesis and/or enhances tumor progression in hepatocellular carcinoma via multiple pathways).[8,32] QSAR analysis conducted using the VEGA v.1.2 and liver specific cancer (rat/mouse in vivo) Danish QSAR model platforms gave mixed results (which included negative, positive, and inconclusive predictions of carcinogenicity for Ethyl Tafluprostamide).[17] However, predictions by these platforms were outside the applicability domain.

OTHER RELEVANT STUDIES

Characterization of $PGF_{2\alpha}$ Receptors in Human Eyelids

The distribution and presence of $PGF_{2\alpha}$ receptors in human hair follicles was evaluated in excised lower eyelid specimens.[33] Analysis was performed on 37 samples examining 17 eyes using 15 patients. Samples were stained with hematoxylin and eosin prior to analysis. All specimens contained hair follicles in the anagen phase, while only 4 samples had specimens in the catagen phase, and staining was only present in hair follicles on the anagen stage. Among the four parts of the hair follicle (bulb, stem/suprabulbar, isthmus, and infundibulum), only the bulb and stem/suprabulbar areas displayed positive staining for $PGF_{2\alpha}$ receptors. In the bulb, the strongest staining occurred in the matricular cells and in the inner sheath layer. Within the inner sheath of the bulb (consisting of Henley, Huxley, and cuticle layers), the presence of $PGF_{2\alpha}$ receptors was observed mainly in the Huxley layer. Generally, when staining was apparent, it occurred predominantly in the cytoplasm of cells with slight membranous staining.

In Vitro Mode of Action

Ethyl Tafluprostamide

An in vitro ToxProfiler assay using human liver cancer (HepG2) cells evaluated the toxicological mode of action of Ethyl Tafluprostamide.[8] The assay measured activation of 7 stress pathways – oxidative stress, cell cycle stress, endoplasmic reticulum stress, autophagy, ion stress, protein stress, and inflammation – using green fluorescent protein reporter cell lines. Ethyl Tafluprostamide primarily induced oxidative stress at a point of departure (POD) of 30 µg/ml and endoplasmic reticulum stress at concentrations ≥ 60 µg/ml. Mild autophagy was observed at 166 µg/ml.

PGF_{2a} Receptor Binding Potency

Ethyl Tafluprostamide

The binding affinity of Ethyl Tafluprostamide for $PGF_{2\alpha}$ receptors was assessed using isolated human recombinant receptors expressed in HEK-293 cells.[8] The test substance was evaluated at concentrations ranging from 0.3 nM to 0.1 mM. Ethyl Tafluprostamide demonstrated binding to the $PGF_{2\alpha}$ receptor. The IC_{50} and inhibitory constant (K_i) was determined to be 53 and 35 nM, respectively (results showing inhibition or stimulation higher than 50% were considered to represent significant effects of the test compound).

Evaluation of Conjunctival Hyperemia

Isopropyl Cloprostenate

Conjunctival hyperemia was evaluated in New Zealand albino rabbits.[34] The dose of Isopropyl Cloprostenate 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.

Pupil Constriction

Isopropyl Cloprostenate

The effect of Isopropyl Cloprostenate on the constriction of pupils was evaluated in cats.[34] Potency was expressed as an ED_5 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. The ED_5 for Isopropyl Cloprostenate was determined to be 0.013 µg. No other details were provided in this study.

Tear Film Stability and Ocular Surface Effects

Ethyl Tafluprostamide

The effects of an eyelash serum containing Ethyl Tafluprostamide (concentration not reported) on the ocular surface were evaluated in 19 healthy women.[35] The serum was applied once daily at night to the upper eyelid margin at the base of the eyelashes for 6 wk. Ocular assessments were performed before treatment and at the end of the 6-wk treatment period. A significant increase in ocular surface disease index score, from 2.68 ± 3.32 to 10.56 ± 15.74, was observed ($p = 0.006$), indicating an increase in dry eye symptoms. Tear film stability was significantly reduced, as demonstrated by decreases in first non-invasive tear break-up time (11.92 ± 6.61 to 7.49 ± 5.85 s; $p = 0.003$) and average non-invasive tear break-up time (13.50 ± 5.79 to 9.11 ± 5.49 s; $p < 0.001$). Significant decreases in tear meniscus height (0.18 ± 0.05 to 0.16 ± 0.03 mm; $p = 0.049$) and tear meniscus area (TMA; 2.08 ± 0.66 to 1.74 ± 0.54 mm^2; $p = 0.019$) were also reported. Lipid layer grade decreased significantly from 3.76 ± 0.43 to 2.69 ± 1.00 ($p < 0.001$), consistent with thinning of the tear film lipid layer. Ocular hyperemia increased significantly in all evaluated regions; the greatest change was observed in total conjunctival ocular hyperemia, which increased from 11.75 ± 3.13% to 14.12 ± 4.59% ($p = 0.001$). At the end of treatment, conjunctival hyperemia differed significantly between the temporal and nasal regions ($p = 0.009$), while no corresponding regional difference was observed for ciliary hyperemia ($p = 0.272$). No significant changes were observed in upper or lower meibomian gland loss ($p = 0.424$ and 0.439, respectively), upper or lower meibomian gland dysfunction ($p = 0.313$ and 0.600, respectively), or ocular surface staining ($p = 0.626$). Results regarding intraocular pressure observed in this study may be found in the Intraocular Pressure section of this report.

Intraocular Pressure

Ethyl Tafluprostamide

The effect of an eyelash serum containing Ethyl Tafluprostamide (concentration not reported) on intraocular pressure was evaluated in 19 healthy women.[35] The serum was applied once nightly to the upper eyelid margin at the base of the eyelashes for 6 wk. At the end of the treatment period, mean IOP was significantly decreased from 14 ± 2.11 mm Hg at baseline to 12.86 ± 2.38 mm Hg ($p = 0.001$). Results regarding tear film stability and ocular surface inflammation and damage are described above in the Tear Film Stability and Ocular Surface Effects section of this report.

The effect of an eyelash product containing 0.018% Ethyl Tafluprostamide on intraocular pressure was evaluated in 19 subjects.[9] Subjects were instructed to use the product for 28 d, and were evaluated at baseline and on day 28. No changes in intraocular pressure were observed in subjects after 28 d of product use. The within-eye differences in intraocular pressure from the beginning to the end of the study were not statistically significant ($t > 0.05$). A similar assay was performed in 19 subjects using an eyelash product containing 0.025% Ethyl Tafluprostamide.[17,36] Subjects applied the product to eyelashes, once per day for 28 d, with evaluations occurring at baseline and day 28. No statistically significant reduction in intraocular pressure was observed over the 28-d study. The results of the ocular irritation evaluation performed during these studies can be found in the Ocular Irritation section of this report.

Isopropyl Cloprostenate

The intraocular pressure lowering efficacy of Isopropyl Cloprostenate was evaluated in conscious ocular-hypertensive cynomolgus monkeys.[34] A 39% reduction in intraocular pressure was observed following application of Isopropyl Cloprostenate (1 µg) to lasered right eyes. No other details were provided for this study.

The potential for an eyelash serum containing 0.005% Isopropyl Cloprostenate to affect intraocular pressure was evaluated in a 28-d study on 21 subjects.[37] Subjects were instructed to apply the serum to the eyelashes of both eyes, nightly. Intraocular pressure measurements were taken at baseline and at day 28. No statistically significant differences in intraocular pressure was observed in either the left or right eyes after 28 d of use.

Ocular Pigmentation, Periorbital Volume, and Adverse Effects

Isopropyl Cloprostenate

The effect of an eyelash serum containing 0.0044% Isopropyl Cloprostenate on ocular pigmentation and periorbital volume was evaluated in 114 subjects.[18,38] 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 (evaluations performed by board-certified ophthalmologist investigator). 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). When compared to baseline, there was a statistically-significant change in overall color of the left and right iris at all time points (this effect was not considered to be clinically relevant according to an independent panel of board-certified ophthalmologists).[39] 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 not considered 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 statistically-significant difference noted after 2, 4, and 8 mo of use). Similarly, when compared to baseline values for the right orbital side, there was a statistically-significant decrease in the right orbital volume after 1 and 4 mo of test material use (no statistically-significant difference noted after 2 and 8 mo of use). Adverse events were observed as follows: 1 instance of 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 probably conjunctivitis with punctuate epitheliopathy following sand exposure (subject continued study, all symptoms resolved), and 1 instance of unilateral conjunctivitis (subject removed from study with all symptoms resolving). Ocular irritation evaluated in this study can be viewed in the Ocular Irritation section of this report.

Endocrine Effects

Ethyl Tafluprostamide

The endocrine activity potential of Ethyl Tafluprostamide was evaluated using several in silico tools (OECD QSAR Toolbox v.4.6, Derek Nexus version 6.2.1, Danish QSAR models, VEGA v.1.2.3, Endocrine Disruptome).[17] Mixed results were obtained, indicating that Ethyl Tafluprostamide may have some endocrine effects, with potential effects on the estrogen receptor, androgen receptor, thyroid, and steroidogenesis/glucocorticoid pathways.

DERMAL IRRITATION AND SENSITIZATION STUDIES

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

Ethyl Tafluprostamide (98.5% purity; tested neat) was determined to be non-irritating in one EpiDerm™ assay (1-h exposure);[40] however, Ethyl Tafluprostamide (99.78% purity; tested neat) was determined to be irritating in a different EpiDerm™ assay (15-min exposure).[41] Similarly, Isopropyl Cloprostenate (98% purity; tested neat) was determined to be irritating in an EpiDerm™ assay (1-h exposure).[42]

A negative prediction for sensitization was determined in a direct peptide reactivity assay (DPRA) using Ethyl Tafluprostamide (98.5% purity) in acetonitrile (100 mM; cysteine peptides only used in assay).[43] In a DPRA performed using Ethyl Tafluprostamide (99.78% purity) in acetonitrile (100 mM; cysteine and lysine peptides used in assay), a negative prediction for sensitization was determined according to the cysteine 1:10/lysine 1:50 prediction model; however, precipitation was observed in the lysine-peptide assay (conclusion of lack of reactivity could not be drawn with sufficient confidence).[44] Ethyl Tafluprostamide (98.5% purity; up to 2000 µM) in DMSO was not predicted to induce sensitization in a KeratinoSens™ assay.[45] However, inconclusive results were obtained in a KeratinoSensTM assay with Ethyl Tafluprostamide (99.78% purity; luciferase induction evaluation only performed at concentrations ≤ 250 µM) due to no clear dose-dependent results (increase in luciferase induction at 250 µM only; all lower test concentrations showed induction values in range of solvent control).[46] An eyelash product containing 0.018% Ethyl Tafluprostamide (n = 51; tested neat),[9,46] an eyelash conditioner containing 0.025% Ethyl Tafluprostamide (n = 51; tested neat),[47] and 7.5% Ethyl Tafluprostamide in phenoxyethanol (n = 54; final test concentration of 0.267% Ethyl Tafluprostamide) were considered to be non-sensitizing in human repeated-insult patch tests (HRIPTs).[9,47,48]

Isopropyl Cloprostenate (98% purity; 100 mM; cysteine peptides only used in assay) yielded inconclusive results in a DPRA.[42] A positive prediction for sensitization was observed in a KeratinoSens™ assay using Isopropyl Cloprostenate (98% purity; up to 2000 µM). HRIPTs were performed using eyelash serums containing Isopropyl Cloprostenate (0.0044% and

0.005%; tested neat; n = 50 - 56).[49-52] Three of the four assays (on Isopropyl Cloprostenate) were performed under semi-occlusive conditions. The serums tested were considered to be non-irritating and non-sensitizing in all assays. Isopropyl Cloprostenate (0.0075%; tested neat) was considered to be non-irritating and non-sensitizing in 3 HRIPTs (n = 54 - 102) performed under occlusive conditions.[53-55]

Phototoxicity

Ethyl Tafluprostamide

Although no photo-induced toxicity studies were available in the literature on these ingredients, an ultraviolet (UV)-visible study with Ethyl Tafluprostamide (neat oil) performed in accordance with OECD TG 101 revealed an absorption band in the range of 210 - 240 nm, with maximum absorption at 226 nm, and an absorption band in the range of 250 - 285 nm, with three maxima at 265, 258, and 276 nm.[56] Molar extinction coefficients for these three maxima were within the range of 1046.2 – 1306.1 l/(mol * cm). Because these maxima are above the cut-off limit (> 1000 l/(mol * cm), photoreactivity cannot be ruled out. According to SCCS, there was no trigger to investigate this endpoint as Ethyl Tafluprostamide showed 3 consistent absorption maxima in the UV-visible light spectrum at 220, 270, and 276 nm. (SCCS guidelines state that no phototoxicity testing is performed at wavelengths lower than 313 nm.)[42]

Isopropyl Cloprostenate

No phototoxicity studies were available in the literature on Isopropyl Cloprostenate; however, according to the SCCS, there is no trigger to investigate this endpoint as this ingredient has showed 2 consistent absorption maxima in the UV-visible light spectrum at 275 and 282 nm.[42] (SCCS guidelines state that no phototoxicity testing is performed when a test material only absorbs at wavelengths lower than 313 nm.)

OCULAR IRRITATION STUDIES

Details on the in vitro and human ocular irritation studies summarized below can be found in Table 7.

An eyelash product containing 0.018% Ethyl Tafluprostamide (tested neat),[9] an eyelash product containing 0.025% Ethyl Tafluprostamide (tested neat),[57] and Ethyl Tafluprostamide (99.78% purity; tested neat)[58] were not predicted to be an ocular irritant in a hen's egg chorioallantoic membrane (HET-CAM) and EpiOcular™ assays. Ethyl Tafluprostamide (98.5% purity; tested neat) resulted in minimal irritation in an isolated chicken eye assay.[42] Eyelash serums containing Isopropyl Cloprostenate (0.0044[59,60] and 0.005%[61-63]) were evaluated in HET-CAM assays (tested at 10 - 50% dilutions resulting in actual test concentrations of 0.00044% - 0.0025% Isopropyl Cloprostenate). All test substances were predicted to be slightly or non-irritating. Isopropyl Cloprostenate (0.0075%; tested neat) was non-irritating in an EpiOcular assay™.[64] However, Isopropyl Cloprostenate (98% purity; tested neat) was determined to be an irritant in an EpiOcular™ assay.[42] Isopropyl Cloprostenate (0.0075 and 0.1%) was predicted to be non-irritating in HET-CAM assays (tested at a 50% dilution, resulting in actual test concentrations of 0.0038 and 0.05%, respectively).[65,66]

Several use studies were performed with eyelash products. With an eyelash product containing 0.018% Ethyl Tafluprostamide, the majority of subjects displayed no signs of ocular irritation when the product was applied to the eyelashes of 19 subjects for 28 d (4 subjects reported minor allergic reactions).[9] Similar results were observed in a use study performed in 19 subjects using an eyelash product containing 0.025% Ethyl Tafluprostamide for 28 d.[17,36] No ocular irritation was observed in 29 subjects after use of an eyelash serum containing 0.0044% Isopropyl Cloprostenate for 6 wk and of an eyebrow serum containing 0.0044% Isopropyl Cloprostenate for 7 wk.[67] Reversible ocular irritation was observed in 2 subjects in a 12-wk assay in which 32 subjects applied an eyelash serum containing 0.0044% Isopropyl Cloprostenate. Slight, transient ocular irritation was observed in an 8-mo use study performed in 114 subjects using an eyelash serum containing 0.0044% Isopropyl Cloprostenate.[38] No ocular irritation, other than slight bulbar conjunctival irritation in one assay, was observed in two ocular irritation assays performed in humans (n = 30; 32) using eyelash and eyebrow serums containing 0.005% Isopropyl Cloprostenate.[68,69] No statistically-significant worsening slit-lamp evaluation scores or cutaneous tolerance scores were observed in a use study in which 56 subjects applied an eyelash conditioning serum containing 0.0075% Isopropyl Cloprostenate to the eyelashes and eyebrows, nightly, for 8 wk. However, adverse effects attributable to the test substance were observed (e.g., eyelid erythema, mild ocular hyperemia, and moderate eye irritation in one subject.[70] No ocular irritation was observed in a 4-wk assay in which an eyelash formulation containing 10% Isopropyl Cloprostenate was applied near the eyes of 27 subjects.[5]

CLINICAL STUDIES

Clinical Trial

Isopropyl Cloprostenate

The effect of an eyewash containing Isopropyl Cloprostenate (0.01%) in a phosphate-buffered saline was evaluated in 23 patients with glaucoma.[5] The eye wash was applied to the eyes once daily for 3 mo. Mild hyperemia of the bulbar conjunctiva was observed; however, this was reported to disappear after 2 - 3 d of treatment. No reactions relating to appearance of the optic disc, visual acuity, visual field, appearance of papilla, or intraocular pressure were observed.

Case Report

Isopropyl Cloprostenate

A 32-yr-old woman presented to an outpatient department due to periocular discoloration for 4 mo.[71] The patient denied the use of medications other than a Chinese tea mixture for acne treatment. The patient reported the use of an eyelash serum containing Isopropyl Cloprostenate (at an unknown concentration) which resulted in irritated periorbital skin after a month of treatment. Approximately 1 yr later, greenish discoloration appeared, which worsened over time; however, the patient continued use of the product. No pathological changes were found, and no ocular abnormalities were observed other than hyperemia of the eyelids, upon assessment. Confocal laser canning microscopy revealed small white spots in the perifollicular dermis and in the surrounding dilated vessels. A significant reduction of the discoloration was observed at a follow-up appointment at 17 mo later. (The study does not clearly state if serum use was discontinued prior to follow-up appointment.)

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.[72] During use period, the patient reported hollowing, thinning, wrinkling, and darkening of the skin of the periorbital region. Six months after discontinued use, the patient reported extensive improvement of symptoms.

Adverse Event Reports

Ethyl Tafluprostamide

According to an unpublished data submission, a company evaluated undesirable effects that were reported by consumers of an eyelash product containing 0.018% Ethyl Tafluprostamide over the course of 2 yr (2011 – 2013).[9] The number of reported undesirable effects for this product, during this time period, was 0.00717% of the number of sold units (number of sold units not stated). The reported adverse effects were described as typical in nature to those associated with cosmetic products near the eyes, specifically mascara and eyeliner.

RISK ASSESSMENT

The margin of exposure (MOE) is a quantitative ratio calculated for cosmetic ingredients by dividing experimentally determined POD for an ingredient by the estimated systemic exposure dose (SED) for the ingredient in humans, generally according to US Environmental Protection Agency (EPA) and European Commission (EC) SCCS guidelines. An MOE value greater than 100 has traditionally been considered an indication of safety. The basis for this MOE value of 100 comes from two multiplication factors: a 10-fold factor for extrapolating from experimental data to human beings (interspecies extrapolation), and an additional 10-fold for differences among the human population (intraspecies extrapolation). Notably, the MOE value is sometimes referred to as the margin of safety (MOS) despite the parameters being definitionally different.

No POD derived from either Ethyl Tafluprostamide or Isopropyl Cloprostenate was found in the publicly available literature or submitted as unpublished data. Accordingly, the following MOE calculations each utilize a read-across source material for the POD. Specifically, these calculations have been performed using a systemic POD derived from other prostaglandins proposed as read-across sources to Ethyl Tafluprostamide and Isopropyl Cloprostenate (tafluprost and travoprost, respectively). In a report of the Panel's Read-Across Working-Group (RAWG), members of the RAWG maintained their previous recommendation. They proposed that the suggested read-across molecules (tafluprost and travoprost) are not appropriate sources for the two target ingredients in this report.

The MOE for an eyelash product containing 0.018% Ethyl Tafluprostamide was calculated to be 481 when the average amount of eyelash product is applied, and 288 for when the maximum amount of product is applied.[17] This calculation was based on a dermal absorption rate at 8.67%, a dermal retention factor of 1, and an NOAEL at 0.0003 mg/kg bw/d for tafluprost (proposed read-across source to target, Ethyl Tafluprostamide) from a prenatal developmental toxicity study in rats following intravenous administration.[8,17,73] An MOE of an eyelash serum containing 0.005% Isopropyl Cloprostenate was calculated to be 2849.[23] This calculation was based on a dermal absorption rate of 7.54%, a daily exposure amount to lash serum at 0.26 mg/d, a dermal retention factor of 1, a body weight of 70 kg, and a lowest-observed-adverse-effect-level (LOAEL) at 0.00012 mg/kg bw/d for travoprost (proposed read-across source to target, Isopropyl Cloprostenate) from a 3-generation study in rats by subcutaneous injection, with an assessment factor of 3 for extrapolation from LOAEL to NOAEL.[24,74,75] Each of these MOE values is considered to be protective. Explanations of the parameters used for these calculations can be found in Table 8. Using a more conservative estimate of exposure to the eyelash formula, specifically, a median daily use of 7 mg/d (within a reported range of 4 – 10 mg/d), an SED was calculated to be 8.4×10^{-7} mg/kg bw/d, based on a use concentration of 0.0075% and dermal absorption rate at 10%.[32,76]

It should be noted that the SCCS considered that the proposed read-across between Isopropyl Cloprostenate and travoprost, as well as between Ethyl Tafluprostamide and tafluprost, to be not supported by the WoE as a sufficient read-across approach. The SCCS further noted that, in the absence of a suitable POD for Isopropyl Cloprostenate or Ethyl Tafluprostamide, a MOS cannot be established.

An alternative POD, i.e., a corrected toxicological screening value (TSV) of 2.1×10^{-6} mg/kg/d for substances acting as agonists or antagonists on $PGF_{2\alpha}$ receptors, was also used for MOS calculations.[8,32,77] Comparison of this conservative TSV

with the estimated SEDs yielded MOS values of greater than 1 Ethyl Tafluprostamide at 0.018% and for Isopropyl Cloprostenate at its maximum reported use concentration of 0.0075%. As the TSV already incorporates uncertainty factors to represent a safe dose, an MOS of ≥ 1 is considered protective.

SUMMARY

The safety of 2 prostaglandin analogues, Ethyl Tafluprostamide and Isopropyl Cloprostenate, is reviewed in this safety assessment. According to the *Dictionary*, these ingredients are reported to function as hair conditioning agents in cosmetics. Ethyl Tafluprostamide is also reported to function in cosmetics as a nail conditioning agent.

According to RLD obtained from the FDA in 2025, Isopropyl Cloprostenate and Ethyl Tafluprostamide were reported to be used in 54 and 6 total formulations, respectively. No concentrations of use were reported for either Ethyl Tafluprostamide or Isopropyl Cloprostenate in response to a survey initiated by the Council in 2022. However, unpublished data submitted separately from the survey state that Isopropyl Cloprostenate is used at up to 0.0075% in eyelash serums. In addition, an unpublished data submission indicated products used on eyelashes, eyebrows, or scalp hair contain Ethyl Tafluprostamide in concentrations ranging from 0.012% - 0.020%.

User instructions on an eyelash product containing 0.018% Ethyl Tafluprostamide state that the product is to be applied once per day, directly to the eyelashes, near the base, above the eyelash line, and should be dried completely prior to the use of other products. Eyelash serums containing 0.004 and 0.005% Isopropyl Cloprostenate are also to be applied once per day; however, these products are applied in a thin line on the eyelash line (similar to application of liquid eyeliner). Caution statements are provided on these products informing users to rinse eyes and discontinue use if irritation occurs.

In an in vitro percutaneous absorption study, the absorbed fraction of an eyelash product containing 0.018% Ethyl Tafluprostamide and an eyelash product containing 0.024% Ethyl Tafluprostamide was determined to be 6.51 ± 2.16 and 10.68 ± 7.18% of the applied dose, respectively (after a 24 h exposure period). In a dermal absorption assay performed using a cosmetic product containing 0.005% Isopropyl Cloprostenate, an average amount of approximately 6.3% of the applied amount of Isopropyl Cloprostenate was absorbed (as measured in the epidermis, dermis, and receptor fluid). In an in vitro study, 0.005% radiolabeled [^{3}H]Isopropyl Cloprostenate was applied to human skin at 10 µl/cm², with 3.87% of the dose absorbed over 24 h. According to unpublished data, the estimated maximum amount of Ethyl Tafluprostamide that would be dermally absorbed was calculated to be 0.144 µg (based on maximum use of a product containing 0.018% Ethyl Tafluprostamide and dermal absorption rate of 20%). The estimated maximum amount of Isopropyl Cloprostenate that would be dermally absorbed was calculated to be 0.02 µg (based on estimated maximum use of a product containing 0.005% Isopropyl Cloprostenate and a dermal absorption rate of 10%). In an in vitro percutaneous metabolism study, Ethyl Tafluprostamide (50% in ethanol) was found to be extensively metabolized into tafluprost (i.e., free acid) after 24 h. In an in vitro percutaneous study, the mean total recovery was 92.6 ± 8.9% after application of 0.015% Ethyl Tafluprostamide. Ethyl Tafluprostamide was estimated to have a low oral bioavailability potential based on the reported physical and chemical properties of this ingredient. Skin metabolites as estimated using the OECD QSAR toolbox of Isopropyl Cloprostenate were predicted to be isopropyl alcohol, cloprostenol, and both hydrolyzed and non-hydrolyzed oxidation products.

An IC_{50} of 0.083 mg/ml was determined in an in vitro 3T3 neutral red reuptake assay performed using Ethyl Tafluprostamide. An acute toxicity assay was performed in rats given Isopropyl Cloprostenate in DMSO 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 Ethyl Tafluprostamide and Isopropyl Cloprostenate as potential reproductive/developmental toxicants. An in vitro ReproTracker® assay showed that Ethyl Tafluprostamide altered stem cell differentiation markers, including bone morphogenetic protein 4, myosin heavy chain 6, forkhead box protein A2, alpha, and paired box protein 6, at concentrations ≥ 3.9 µg/ml. The effect of Isopropyl Cloprostenate (25 or 100 µg/kg bw/d) on gonads and testicular cells was evaluated in male mice and rats. In these assays, animals were treated for 28 d, and killed 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.

Ethyl Tafluprostamide and Isopropyl Cloprostenate were non-mutagenic in Ames assays at concentrations up to 5000 µg/plate, with and without metabolic activation. Ethyl Tafluprostamide was negative in in vitro micronucleus assays using mouse lymphoma cells at concentrations up to 140 µg/ml and human lymphocytes at concentrations up to 350 µg/ml, with and without metabolic activation. Isopropyl Cloprostenate was negative in in vitro micronucleus assays using Chinese hamster ovary cells at concentrations up to 750 µg/ml and human lymphocytes at concentrations up to 2041 µg/ml, with and without metabolic activation. In silico analyses of Ethyl Tafluprostamide and Isopropyl Cloprostenate generally supported the absence of genotoxic potential.

No structural alerts were observed for Ethyl Tafluprostamide for carcinogenicity according to SAR analyses performed using OECD QSAR Toolbox v.4.6 and Derek Nexus v.6.2.1. However, both Ethyl Tafluprostamide and Isopropyl

Cloprostenate were flagged for potential carcinogenicity by the SCCS due to in silico analyses. QSAR analysis conducted using the Vega v.1.2 and liver specific cancer (rat/mouse in vivo) Danish QSAR model platforms gave mixed results (negative, positive or inconclusive predictions of carcinogenicity for Ethyl Tafluprostamide). In the additional models examined by SCCS, Ethyl Tafluprostamide and Isopropyl Cloprostenate were "outside the applicability domain".

The distribution and presence of $PGF_{2\alpha}$ receptors in human hair follicles was evaluated using excised lower eyelid samples. Receptors were found in hair follicles in the anagen stage and were primarily present in the matricular cells of the bulb and inner sheath layer of the hair follicle.

In a ToxProfiler assay using HepG2 cells, Ethyl Tafluprostamide primarily induced oxidative stress at 30 µg/ml and endoplasmic reticulum stress at ≥ 60 µg/ml. Mild autophagy was observed at 166 µg/ml.

The binding affinity of Ethyl Tafluprostamide to $PGF_{2\alpha}$ receptors was evaluated using isolated human recombinant receptors derived from human recombinant cells. The IC_{50} and K_i were determined to be 53 and 35 nM, respectively.

The dose estimated to produce conjunctival hyperemia in 15% of test rabbits over a 4 h period was determined to be 0.3 µg Isopropyl Cloprostenate. The ED_5 for Isopropyl Cloprostenate was determined to be 0.013 µg in an assay performed in cats evaluating pupil constriction potential.

In a study of 19 healthy women, nightly application of an eyelash serum containing Ethyl Tafluprostamide (concentration not reported) for 6 wk resulted in increased dry eye symptoms, decreased tear film stability and volume, thinning of the tear film lipid layer, and increased ocular hyperemia. No significant changes in meibomian gland parameters or ocular surface staining were observed. Intraocular pressure significantly decreased from 14 ± 2.11 to 12.86 ± 2.38 mmHg.

No statistically-significant changes in intraocular pressure were observed in 19 subjects after a 28-d use period of an eyelash product containing 0.018% Ethyl Tafluprostamide or an eyelash product containing 0.025% Ethyl Tafluprostamide. A 39% reduction in intraocular pressure was observed in ocular hypertensive monkeys treated with 1 µg Isopropyl Cloprostenate (in lasered right eyes). No statistically-significant changes in intraocular pressure were observed in a 28-d study in which 21 subjects applied an eyelash serum containing 0.005% Isopropyl Cloprostenate nightly.

The potential for an eyelash serum containing 0.0044% Isopropyl Cloprostenate to cause changes in ocular pigmentation and periorbital volume was evaluated in an 8-mo study involving 114 subjects. Statistically-significant increases in overall ocular pigmentation were observed in both left and right iris at all observed time points (1, 2, 4, and 8 mo), compared to baseline. In addition, statistically-significantly decreased periorbital volume was observed 1 mo after test substance use in the left orbital side, and after 1 and 4 mo of use in the right orbital side (compared to baseline values).

Mixed results were observed in several in silico models evaluating the potential endocrine activity of Ethyl Tafluprostamide. This suggests that this ingredient may have an effect on endocrine activity.

Ethyl Tafluprostamide (98.5% purity; tested neat) was determined to be non-irritating in one EpiDerm™ assay; however, Ethyl Tafluprostamide (99.78%) was determined to be non-irritating in a different EpiDerm™ assay. Similarly, Isopropyl Cloprostenate (98% purity; tested neat) was determined to be irritating in an EpiDerm™ assay. A negative prediction for sensitization was determined in a DPRA using Ethyl Tafluprostamide (98.5% purity) in acetonitrile (100 mM); however, precipitation was observed in the lysine-peptide assay of a different DPRA using Ethyl Tafluprostamide (99.78% purity) in acetonitrile (100 mM). Ethyl Tafluprostamide (98.5% purity; up to 2000 µM) in DMSO was not predicted to induce sensitization in a KeratinoSens™ assay. However, inconclusive results were obtained in a KeratinoSens™ assay with Ethyl Tafluprostamide (99.78% purity; luciferase induction evaluation only performed at concentrations ≤ 250 µM) due to no clear dose-dependent results (increase in luciferase induction at 250 µM only; all lower test concentrations showed induction values in range of solvent control). Isopropyl Cloprostenate (98% purity; 100 mM; cysteine peptides only used in assay) yielded inconclusive results in a DPRA. A positive prediction for sensitization was observed in a KeratinoSens™ assay using Isopropyl Cloprostenate (98% purity; up to 2000 µM). An eyelash product containing 0.018% Ethyl Tafluprostamide (tested neat), an eyelash conditioner containing 0.025% Ethyl Tafluprostamide (tested neat), and 7.5% Ethyl Tafluprostamide in phenoxyethanol (final test concentration of 0.267% Ethyl Tafluprostamide) were considered to be non-sensitizing in HRIPTs. HRIPTs were performed using serums containing Isopropyl Cloprostenate (0.0044% and 0.005%; tested neat). The serums tested were considered to be non-irritating and non-sensitizing in all assays. Isopropyl Cloprostenate (0.0075%; tested neat) was considered to be non-irritating and non-sensitizing in 3 HRIPTs (n = 54 – 102) performed under occlusive conditions.

No phototoxicity studies on these ingredients were found in the literature; however, according to an ultraviolet-visible study with Ethyl Tafluprostamide, photoreactivity could not be ruled out as calculated molar extinction coefficients were above the cut-off limit. Because the maximum absorbance wavelength was below 313 nm, no further in vitro toxicity testing is required, according to SCCS notes of guidance.

An eyelash product containing 0.018% Ethyl Tafluprostamide (tested neat), an eyelash product containing 0.025% Ethyl Tafluprostamide, and Ethyl Tafluprostamide (99.78% purity; tested neat), were not predicted to be ocular irritants in HET-CAM and EpiOcular™ assays. Ethyl Tafluprostamide (98.5% purity; tested neat) resulted in minimal irritation in an isolated chicken eye assay. Eyelash serums containing Isopropyl Cloprostenate (0.0044 and 0.005%) were evaluated in HET-CAM

assays (tested at 10 - 50% dilutions resulting in actual test concentrations of 0.00044 - 0.0025% Isopropyl Cloprostenate). All test substances were predicted to be slightly or non-irritating. Isopropyl Cloprostenate (0.0075%; tested neat) was considered non-irritating in an EpiOcular™ assay. However, Isopropyl Cloprostenate (98% purity; tested neat) was determined to be an irritant in an EpiOcular™ assay. Isopropyl Cloprostenate (0.1%; tested at a 50% dilution, resulting in an actual test concentration of 0.05%) and Isopropyl Cloprostenate (0.0075%; tested at a 50% dilution, resulting in an actual test concentration of 0.0038%) were predicted to be non-irritating in HET-CAM assays.

The majority of subjects displayed no signs of irritation in two use assays in which eyelash products containing either 0.018% Ethyl Tafluprostamide or 0.025% Ethyl Tafluprostamide were applied daily for 28 d (n =19 in both assays). No ocular irritation was observed in 29 subjects after use of an eyelash serum containing 0.0044% Isopropyl Cloprostenate for 6 wk and of an eyebrow serum containing 0.0044% Isopropyl Cloprostenate for 7 wk. Slight ocular irritation was observed in an 8-mo use study in which 120 subjects used an eyelash serum containing 0.0044% Isopropyl Cloprostenate. Reversible ocular irritation was observed in 2 subjects in a 12-wk assay in which 32 subjects applied an eyelash serum containing 0.0044% Isopropyl Cloprostenate. No ocular irritation, other than slight bulbar conjunctival irritation in one assay, was observed in ocular irritation assays performed in humans (n = 30; 32) using eyelash and eyebrow serums containing 0.005% Isopropyl Cloprostenate. No statistically-significant worsening slit-lamp evaluation scores or cutaneous tolerance scores were observed in a use study in which 56 subjects applied an eyelash conditioning serum containing 0.0075% Isopropyl Cloprostenate to the eyelashes and eyebrows, nightly, for 8 wk. However, adverse effects attributable to the test substance were observed (e.g., eyelid erythema, mild ocular hyperemia, and moderate eye irritation in one subject. No ocular irritation was observed in a 4-wk assay in which an eyelash formulation containing 10% Isopropyl Cloprostenate was applied near the eyes of 27 subjects.

The effect of an eyewash containing Isopropyl Cloprostenate (0.01%) was evaluated in 23 glaucoma patients (treatment once daily for 3 mo.). No adverse effects other than reversible mild hyperemia of the bulbar conjunctiva were observed.

A 32-yr-old woman experienced periocular discoloration following the use of an eyelash serum containing Isopropyl Cloprostenate. The patient reported that discoloration began after 1 mo of treatment, which continued to worsen over time. Discoloration was significantly reduced at a 17-mo. follow-up appointment. A 35-yr-old woman reported hollowing, thinning, wrinkling, and darkening of the skin around the periorbital region following the use of an eyelash product containing Isopropyl Cloprostenate. Symptoms were significantly improved 6 mo after discontinued use.

A company evaluated undesirable effects that were reported by consumers of an eyelash product containing 0.018% Ethyl Tafluprostamide over the course of 2 yr (2011 – 2013). The number of reported undesirable effects for this product, during this time period, was 0.00717% of the number of units sold.

MOEs were calculated using proposed read-across PODs from tafluprost and travoprost for Ethyl Tafluprostamide and Isopropyl Cloprostenate, respectively. MOEs of 481 and 288 were calculated for a 0.018% Ethyl Tafluprostamide eyelash product based on average and maximum product use, respectively, and an MOE of 2849 was calculated for a 0.005% Isopropyl Cloprostenate eyelash serum. Using a corrected PGF2α receptor-based TSV of 2.1×10^{-6} mg/kg/d as an alternative POD, MOS values greater than 1 were calculated for Ethyl Tafluprostamide at 0.018% and Isopropyl Cloprostenate at its maximum reported use concentration of 0.0075%.

DRAFT DISCUSSION

[Note: This Discussion is in the draft form, and changes will be made following the Panel meeting.]

This assessment reviews the safety of 2 prostaglandin analogues, Ethyl Tafluprostamide and Isopropyl Cloprostenate, as used in cosmetic formulations. The Panel concluded [TBD].

The Panel's respiratory exposure resource document (https://www.cir-safety.org/cir-findings) notes that airbrush technology presents a potential safety concern. Although frequency and concentration of use data are now available (and in some cases mandated) for ingredients marketed for use with airbrush delivery systems in certain product categories, no data are available for consumer habits and practices thereof, product particle size, or other relevant particle data (e.g., diameter). As a result of deficiencies in these critical data needs, the data profile is incomplete, and the safety of cosmetic ingredients applied by airbrush delivery systems cannot be determined by the Panel. Accordingly, the Panel has concluded the data are insufficient to support the safe use of cosmetic ingredients applied via an airbrush delivery system.

CONCLUSION

To be determined.

TABLES

Table 1. Definitions, structures, and reported functions[1,CIR STAFF]

Ingredient (CAS No.)	Definition	Function
Ethyl Tafluprostamide (1185851-52-8)	Ethyl Tafluprostamide is a synthetic analogue of a prostaglandin. It conforms to the structure in Figure 1.	hair conditioning agents; nail conditioning agent
Isopropyl Cloprostenate (157283-66-4)	Isopropyl Cloprostenate is a synthetic analogue of a prostaglandin. It conforms to the structure in Figure 2.	hair conditioning agent

Table 2. Chemical properties

Property	Value	Reference
Ethyl Tafluprostamide		
Physical Form	liquid	4
Color	colorless to pale yellow	4
Molecular Weight (g/mol)	437.5	4
Density (g/ml)	1.21	17
Vapor Pressure (Pa at 25 ºC)	1.25×10^{-13}	17
Melting Point (°C)	95.08	17
Boiling Point (°C)	503.76	17
Water Solubility (g/l @ 20 ºC)	1.05	4,7
log K_{ow} (@ 25 °C)	2.74 ± < 0.01	4
UV Absorption (λ; nm; symmetric peak)	226 - 276	7,56
Isopropyl Cloprostenate		
Physical Form	liquid	4
Molecular Weight (g/mol)	467	4
Vapor Pressure (Pa at 25 ºC)	4.83×10^{-14} (estimated)	4
Water Solubility (mg/l @ 20 ºC)	99.1	4
log K_{ow}	3.86 ± < 0.01	4
UV Absorption (λ; nm)	275, 282	4

Table 3. Frequency and concentration of use according to likely duration and exposure and by product category[9,11-14,16]

	Ethyl Tafluprostamide		Isopropyl Cloprostenate	
	# of Uses	Max Conc of Use	# of Uses	Max Conc of Use
	RLD (2025)	% (2023)	RLD (2025)	% (2023)
Totals*	**6**	**0.012 – 0.02**	**54**	**0.0044 – 0.0075**
as reported by product category				
Eye Makeup Preparations (not children's)				
Eyeliner			2	NR
Mascara			2	NR
Eyelash and Eyebrow Preparations (primers, conditioners, serums, fortifiers)	4	NR	41	NR
Other Eye Makeup Preparations	1	0.012 – 0.02[a]	1	0.0044 – 0.0048[b]; 0.0075[c]
Hair Preparations (non-coloring)				
Tonics, Dressings, Other Hair Grooming Aids			5	NR
Other Hair Preparations	1 (l.o.)	0.012 – 0.02[a]	3 (l.o.)	NR
Skin Care Preparations				
Face and Neck (excluding shaving preps)			1 (l.o.)	NR

l.o. = leave-on; NR = not reported

*The sum of the counts given for duration of use and by exposure type, and the sum of the frequency reported by product category, may not equal the sum of total uses because each ingredient may be used in cosmetic formulations that are reported under more than one product category.

[a]concentration of Ethyl Tafluprostamide in products used on eyelashes, eyebrows, and scalp hair, according to an unpublished data submission[7]

[b]average concentration of Isopropyl Cloprostenate in eyelash serums according to unpublished data sources[10]

[c]concentration of Isopropyl Cloprostenate in eyelash serum according to an unpublished data submission[11]

Table 4. Application amounts of prostaglandin analogues

Product	Amount of Product Applied	Amount of Active Ingredient Per Use	Application Details	Reference
eyelash serum containing 0.0044% Isopropyl Cloprostenate	0.19 mg	8.4 ng	-	14
eyelash serum containing 0.005% Isopropyl Cloprostenate	0.58 ± 0.34 mg	30 ± 18 ng	once daily application of a thin layer of product to the base of upper eyelashes of both eyes; product intended to avoid direct eye contact	4
eyebrow serum containing 0.005% Isopropyl Cloprostenate	4.0 ± 1.7 mg	100 ± 40 ng	-	4
eyelash serum containing 0.015% Ethyl Tafluprostamide	1.5 ± 0.6 mg	240 ± 100 ng	once daily application of a thin layer of product to the base of upper eyelashes of both eyes; product intended to avoid direct eye contact	4
eyebrow serum containing 0.015% Ethyl Tafluprostamide	5.2 ± 1.3 mg	800 ± 200 ng	-	4
eyelash serum containing 0.005% Isopropyl Cloprostenate	0.26 mg	13 ng	-	14
eyelash serum containing 0.0075% Isopropyl Cloprostenate	-	19.5 ng	each use consists of one application to the upper lash line of both eyes	15,16, CIR STAFF
eyelash product containing 0.018% Ethyl Tafluprostamide	2.4 mg (average per brush stroke); 4 mg (maximum per brush stroke)	0.432 µg (average per brush stroke); 0.72 µg (maximum per brush stroke)	-	17

Table 5. Genotoxicity studies

Test Article	Vehicle	Concentration/Dose	Test System	Procedure	Results	Reference
IN VITRO						
Gene Mutation						
Ethyl Tafluprostamide (purity: 98.5%)	DMSO	0, 5, 16, 50, 160, 500, 1600, and 5000 µg/plate	*S. typhimurium* strains TA98, TA100, TA1535, TA1537, and *E. coli* WP2 uvrA	Ames assay; OECD TG 471; performed with and without metabolic activation; negative control: DMSO; positive controls: sodium azide, 4-nitro-*o*-phenylenediamine; 9-aminoacridine; methyl methanesulfonate	Non-mutagenic; controls gave expected results	42

Table 5. Genotoxicity studies

Test Article	Vehicle	Concentration/Dose	Test System	Procedure	Results	Reference
Ethyl Tafluprostamide (purity: 99.78%)	DMSO	Experiment 1: 3.16, 10.0, 31.6, 100, 316, 1000, 2500 and 5000 µg/plate (TA100) 31.6, 100, 316, 1000, 2500, and 5000 µg/plate (TA98, TA1535, TA1537, and E. coli WP2 uvrA (PKM101)) Experiment 2: 3.16, 10.0, 31.6, 100, 316, 1000, 2500 and 5000 µg/plate (TA100, TA1535) 31.6, 100, 316, 1000, 2500, and 5000 µg/plate (TA98, TA1535, TA1537, and E. coli WP2 uvrA (PKM101))	*S. typhimurium* strains TA98, TA100, TA1535, TA1537, and *E. coli* WP2 uvrA (pKM101)	2-part Ames assay; OECD TG 471; both parts performed with and without metabolic activation; negative controls: distilled water; positive controls: 4-nitro-o-phenylene-diamine, methylmethanesulfonate; 2-aminoanthracene	Non-mutagenic; controls gave expected results	29
Isopropyl Cloprostenate	sterile deionized water	50, 100, 500, 1000, and 5000 µg/plate	*S. typhimurium* strains TA 97a, TA98, TA100, TA 102, and TA 1535	Ames assay; performed with and without metabolic activation; negative control: sterile deionized water; positive controls: sodium azide, daunomycin, mitomycin C, ICR 191 acridine, 2-aminoanthracene	Non-mutagenic; controls gave expected results	18
Isopropyl Cloprostenate (100% purity)	DMSO	Experiment 1: 0, 3, 10, 33, 100, 333, 1000, 2500, and 5000 µg/plate Experiment 2: 0, 33, 100, 333, 1000, 2500, and 5000 µg/plate	*S. typhimurium* strains TA98, TA 100, TA 1535, TA 1537 and *E. coli* WP2 uvrA	2-part Ames assay performed with and without metabolic activation; OECD TG 471; negative control: DMSO; positive controls: sodium azide, 4-nitro-*o*-phenylenediamine; methyl methanesulfonate	Non-mutagenic; controls gave expected results	4
Chromosomal Damage						
Ethyl Tafluprostamide (purity: 98.5%)	DMSO	4-h exposure without metabolic activation: 4.4, 8.8, 17.6, 35, 70, 140, 150*, and 160* µg/ml 4-h exposure with metabolic activation: 17.6, 35, 70, 130, 140, and 150 µg/ml 24-h exposure without metabolic activation: 2.2, 4.4, 8.8, 17.6, and 35* µg/ml *due to high cytotoxicity at indicated concentration, evaluation not performed	L5178Y tk +/- mouse lymphoma cell line	mammalian cell micronucleus assay; OECD TG 487, performed with and without metabolic activation; 4 or 24-h exposure; negative control: DMSO: positive controls: 4-nitroquinoline N-oxide, colchicine, cyclophosphamide monohydrate	Non-clastogenic	42

Table 5. Genotoxicity studies

Test Article	Vehicle	Concentration/Dose	Test System	Procedure	Results	Reference
Ethyl Tafluprostamide (purity: 99.78%)	DMSO	Experiment 1: without metabolic activation: 250, 325, and 350 µg/ml with metabolic activation: 100, 250, and 300 µg/ml Experiment 2: without metabolic activation: 25, 50, and 100 µg/ml	human lymphocytes	2-part micronucleus assay; OECD TG 487; experiment 1 performed with and without metabolic activation for 4 h; experiment 2 performed without metabolic activation for 44 h; negative controls: culture medium, DMSO; positive controls: methylmethanesulfonate; colchicine, cyclophosphamide	Non-clastogenic; controls gave expected results	30
10% Isopropyl Cloprostenate	ethanol	Experiment 1: 2.9, 5.9, 12, 23, 47, 94, 190, 380, and 750 µg/ml Experiment 2: 0.23, 0.47, 0.94, 1.9, 3.8, 7.5, 15, and 30 µg/ml	Chinese hamster ovary cells	2-part mammalian cell micronucleus assay; OECD TG 487; in experiment 1, test item tested for 4 h in the absence and presence of metabolic activation and for 23 h in the absence of metabolic activation; in experiment 2, test item tested for 23 h in absence of metabolic activation; negative control: ethanol; positive controls: colchicine, cyclophosphamide, mitomycin C	Non-clastogenic; controls gave expected results	18
Isopropyl Cloprostenate (purity: 100%)	DMSO	Experiment 1: 0, 13.3, 23.2, 40.6, 71.1, 124, 218, 381, 666, 1166, 2041 µg/ml Experiment 2: 0, 3.7, 6.5, 11.4, 14.4, 19.9, 25.2, 34.8, 44.1, 60.9, 77.2, 88.8, 102, 107, 117, 135, 155, 179, 187, 327 µg/ml	human lymphocytes	2-part mammalian cell micronucleus assay; OECD TG 487; in experiment 1, test item tested for 3 and 28 h in the absence and presence of metabolic activation; in experiment 2, test item tested for 28 h in absence of metabolic activation; negative control: DMSO; positive controls: mitomycin C, vinblastine, cyclophosphamide	Non-genotoxic; no evidence of clastogenic or aneugenic effects. Controls gave expected results	42
COMPUTATIONAL						
Ethyl Tafluprostamide	-	-	-	QSAR Toolbox (v.4.7)	No alerts identified in DNA binding, protein binding, or in vitro (Ames)/in vivo mutagenicity (micronucleus) models; one structural alert noted in OASIS AMES/CA/MNT profiler (strong binder, OH group); Toxic hazard classification by Cramer: Class III	8
Isopropyl Cloprostenate	-	-	NR	QSAR model ToxTree v. 3.1.0-1851 and US EPA T.E.S.T v. 4.2.1	No prediction of genotoxicity; Cramer class III toxicity (substance with chemical structure that permits no strong initial impression of safety or may even suggest a significant toxicity)	5,31

CA = chromosomal aberration; DMSO: dimethyl sulfoxide; DNA = deoxyribonucleic acid; ISS = Istituto Superiore di Sanità; MNT = micronucleus test; NR = not reported; OASIS = Organization for Assessment of Structure–Activity Information System; OECD = Organisation for Economic Co-Operation and Development TG = test guideline

Table 6. Dermal irritation/sensitization

Test Article	Vehicle	Concentration/Dose	Test Population	Procedure	Results	Reference
IRRITATION						
In Vitro						
Ethyl Tafluprostamide (purity: 98.5%)	NR	100%; 30 µl	3 samples	EpiDerm™ assay; reconstructed human epidermis; OECD TG 439; 1h exposure period; negative control: phosphate-buffered saline; positive control: 5% sodium dodecyl sulfate	Non-irritating Tissue viability in 3 replicates was 100, 106, and 82% at end of test Control substances gave expected results	40
Ethyl Tafluprostamide (purity: 99.78%)	NR	100%; 30 µl	3 samples	EpiDerm™ assay; reconstructed human epidermis; OECD TG 439; 15-min exposure period; negative control: phosphate-buffered saline; positive control: 5% sodium dodecyl sulfate	Irritating Tissue viability in 3 replicates determined to be 3, 17.2 and 27.7% at end of test. The mean value of relative tissue viability was 16.0% after the treatment. This value is below the threshold for skin irritation (50 %). Thus, the test item is considered to be an irritant to skin. Control substances gave expected results.	41
Isopropyl Cloprostenate (purity: 98%)	none	100%; 30 µl	3 samples	EpiDerm™ assay; reconstructed human epidermis; OECD TG 439; 1-h exposure period; negative control: phosphate-buffered saline; positive control: 5% sodium dodecyl sulfate	Irritating The mean optical density of the test item, negative, and positive control were 0.5, 17, and 0.5%, respectively. The mean tissue viability of the test item and positive control were 2.5 and 1.8%, respectively. The mean value of relative tissue viability of the test item was reduced to 2.5 % after treatment. Controls gave expected results. Since the value of tissue viability for the test substance was below the threshold for skin irritation (50%), it was considered an irritant.	42
SENSITIZATION						
In Chemico/In Vitro						
Ethyl Tafluprostamide (purity: 98.5%)	acetonitrile	100 mM; 50 µl	cysteine peptides	DPRA; OECD TG 442C; cys- peptides assay; solvent used as negative control; positive control: cinnamic aldehyde	Negative prediction for sensitization Negative and positive controls gave expected results	43
Ethyl Tafluprostamide (purity: 99.78%)	acetonitrile	100 mM; 50 µl	lysine and cysteine peptides	DPRA; OECD TG 442C; cys- and lys- peptides assay; solvent used as negative control; positive control: cinnamic aldehyde	Negative prediction for sensitization according to cysteine 1:10/lysine 1:50 prediction model; however, observed precipitation in lys-peptide assay; conclusion on lack of reactivity could not be drawn from conditions of this study Mean peptide depletion in the cys-peptide assay, which showed no precipitation for test item was 3.2%, suggesting no or minimal reactivity Control substances gave expected results	41

Table 6. Dermal irritation/sensitization

Test Article	Vehicle	Concentration/Dose	Test Population	Procedure	Results	Reference
Ethyl Tafluprostamide (purity: 98.5%)	DMSO	Test 1: 0.98, 1.95, 3.91, 7.81, 15.63, 31.25, 62.5, 125, 250, 500, 1000, and 2000 µM Test 2: 55.80, 72.54, 94.30, 122.59, 159.37, 207.18, 269.33, 350.13, 455.17, 591.72, 769.23, and 1000 µM Test 3: 67.29, 80.75, 96.90, 116.28, 139.54, 167.45, 200.94, 241.13, 289.35, 347.22, 416.67, and 500 µM All concentrations tested at a dose volume of 50 µl	KeratinoSens™ cell line	KeratinoSens™ assay; OECD TG 442D; solvent used as negative control; positive control: trans-cinnamaldehyde; test 2 and 3 performed to determine IC_{30} and IC_{50} values more precisely since strong cytotoxicity was observed at high concentrations	Negative prediction for sensitization Control substances gave expected results	45
Ethyl Tafluprostamide (purity: 99.78%)	DMSO	0.98, 1.95, 3.91, 7.81, 15.63, 31.25, 62.5, 125, 250, 500, 1000, and 2000 µM; 50 µl In both repetitions, a cytotoxic effect was observed at concentrations above 500 µM; therefore, the three highest test item concentrations were excluded from the evaluation of the luciferase induction in both repetitions	KeratinoSens™ cell line	KeratinoSens™ assay; OECD TG 442D; solvent used as negative control; positive control: cinnamic aldehyde; experiment repeated due to lack of dose-response in first experiment	Inconclusive results In experiment 1, a statistically significant increase in luciferase induction >1.5-fold was observed at 250 Mm; all lower concentrations showed induction values in the range of the solvent control In experiment 2, a statistically significant increase in luciferase induction to exactly 1.5-fold was observed at 250 µM; induction values at lower concentrations were all in range of solvent control No clear dose-dependent results were observed – result was considered inconclusive Precipitation of test article not visible in any of the repetitions. Control substances gave expected results	46
Isopropyl Cloprostenate (98% purity)	acetonitrile	100 mM	cysteine peptides	DPRA; OECD TG 442C; cys- peptides assay; solvent used as negative control; positive control: cinnamic aldehyde	Inconclusive The cysteine 1:10 prediction model suggested a negative prediction, since showing no or minimal reactivity under the experimental conditions. However, following incubation, precipitation was observed in the case of cysteine peptides; therefore, a final conclusion on the lack of reactivity could not be drawn.	42
Isopropyl Cloprostenate (98% purity)	DMSO	1st test: 0.98 – 2000 µM 2nd and 3rd test: 5.58 – 100 µM	KeratinoSens™ cell line	KeratinoSens™ assay; OECD TG 442D; solvent used as negative control; positive control: trans-cinnamaldehyde	Positive prediction for sensitization The overall IC_{30} was determined to be 27.39 µM. Controls gave expected results.	42

Table 6. Dermal irritation/sensitization

Test Article	Vehicle	Concentration/Dose	Test Population	Procedure	Results	Reference
			Human			
eyelash product containing 0.018% Ethyl Tafluprostamide	NR	100%; dose not stated	51	HRIPT; level of occlusion not stated; 9, 24-h applications to the upper back over a 3-wk period for induction; 2 test challenge patches after a 10 - 14 d rest period; challenge patches were applied to a previously untreated site adjacent to the test site (48- and 96-h exposures)	Two of 561 total evaluations were scored "1" (indicating erythema throughout at least ¾ of patch area; unknown which stage of study these effects were seen); study reported no adverse effects or signs or symptoms of sensitization throughout study	9
eyelash conditioner containing 0.025% Ethyl Tafluprostamide	NR	100%; 0.02 – 0.05 ml	51	HRIPT; occlusive conditions; 9 applications to the upper back 9 a 3-wk period for induction (1st patch 24-h exposure; remaining patches 48-h exposures); test challenge patch after a 10 - 14 d rest period; challenge patch applied to a previously untreated site adjacent to the test site (48- and 96-h exposures)	Non-irritating; non-sensitizing	47
7.5% Ethyl Tafluprostamide in phenoxyethanol (final test concentration of 0.267% Ethyl Tafluprostamide)	deionized water	3.55%; 0.02 – 0.05 ml	54	HRIPT; semi-occlusive conditions; 8-9 applications to the upper back over a 3-wk period for induction (1st patch 24-h exposure; remaining patches 48-h exposures); test challenge patch after a 10 - 14 d rest period; challenge patch applied to a previously untreated site adjacent to the test site (48- and 96-h exposures)	Non-irritating; non-sensitizing	9,48
eyelash serum containing 0.0044% Isopropyl Cloprostenate	NR	100%; 0.2 ml	53	HRIPT; semi-occlusive conditions; 9, 24-h applications to the upper back over a 3-wk period for induction; challenge phase after a minimal 10-d rest period; challenge patches were applied to a previously untreated site adjacent to the test site, and the site was evaluated immediately after removal and 72 h after patch removal	Non-irritating; non-sensitizing	49
eyelash serum containing 0.0044% Isopropyl Cloprostenate	NR	100%; dose volume not stated	56	HRIPT; semi-occlusive conditions; 9, 24-h applications to the upper back over a 3-wk period for induction; challenge phase after a 10 - 21-d rest period; 24-h challenge patches were applied, and the site was evaluated immediately and 24 and 48 h after patch removal	Non-irritating; non-sensitizing	50
eyelash serum containing 0.005% Isopropyl Cloprostenate	NR	100%; 0.2 ml	50	HRIPT; occlusive conditions to the infrascapular region of the back; 9, 24-h applications over a 3-wk period for induction; challenge phase after a 10 - 14-d rest period; challenge patches were applied to a previously untreated site for 24 h, and the site was evaluated immediately and 48 h after patch removal	Non-irritating; non-sensitizing	51
eyelash serum containing 0.005% Isopropyl Cloprostenate	NR	100%; dose not stated	53	HRIPT; semi-occlusive conditions; 9 applications to the upper back over a 3-wk period for induction; challenge phase after a 10 - 21-d rest period; challenge patches were applied to the lower back and the site was evaluated immediately, 24, and 48 h after patch removal	Non-irritating, non-sensitizing	52

Table 6. Dermal irritation/sensitization

Test Article	Vehicle	Concentration/Dose	Test Population	Procedure	Results	Reference
0.0075% Isopropyl Cloprostenate	NR	100%; 0.02 – 0.05 ml	54	HRIPT; occlusive conditions; 9 applications to the intrascapular regions of the back over a 3-wk period for induction (1st patch 24-h exposure; remaining patches 48-h exposures); test challenge patch after a 10 - 14 d rest period; challenge patch applied to a previously untreated site adjacent to the test site (48- and 96-h exposures)	Non-irritating, non-sensitizing	53
0.0075% Isopropyl Cloprostenate	NR	100%; 20 – 50 mg	58	HRIPT; occlusive conditions; 9 applications to the intrascapular regions of the back over a 3-wk period for induction (1st patch 24-h exposure; remaining patches 48-h exposures); test challenge patch after a 10 - 14 d rest period; challenge patch applied to a previously untreated site adjacent to the test site (48- and 96-h exposures)	Non-irritating, non-sensitizing	54
0.0075% Isopropyl Cloprostenate	NR	100%; 20 – 50 mg	102	HRIPT; occlusive conditions; 9 applications to the intrascapular regions of the back over a 3-wk period for induction (1st patch 24-h exposure; remaining patches 48-h exposures); test challenge patch after a 10 - 14 d rest period; challenge patch applied to a previously untreated site adjacent to the test site (48- and 96-h exposures); positive control: 2% sodium lauryl sulfate solution; negative control: deionized water	Non-irritating,and non-sensitizing; when treated with the test substance, 5 subjects displayed a grade 1 reaction during the induction period; and 1 subject displayed a positive reaction during an induction period and during challenge (this subject was re-challenged and had no reactions) 1 subject displayed a grade 4 reaction, 6 subjects displayed a grade 2 reaction, and 65 subjects displayed grade 1 reaction to positive control No positive reactions observed to negative control Grade 0: no reaction Grade 1: erythema throughout at least ¾ of patch area Grade 2: erythema and induration throughout at least ¾ of patch area Grade 3: erythema, induration, and vesicles Grade 4: erythema, induration, and bullae	55

DMSO = dimethyl sulfoxide; DPRA = direct peptide reactivity assay; HRIPT = human repeated insult patch test; IC_{30} = 30% inhibitory concentration; IC_{50} = half maximal inhibitory concentration; OECD = Organisation for Economic Co-Operation and Development TG = test guideline

Table 7. Ocular irritation studies

Test Article	Vehicle	Concentration/Dose	Test Population	Procedure	Results	Reference
IN VITRO						
Eyelash product containing 0.018% Ethyl Tafluprostamide	NR	100%	hen's egg chorioallantoic membranes (n = 4)	HET-CAM assay; reference test articles include a one-coat mascara and waterproof eyeliner (details regarding these substances not stated); evaluations performed 0.5, 2, and 5 min after test article exposure	Irritation potential score: 0.0 (mean scores of 0.0 - 4.9 indicate minimal irritation potential) Reference test articles have historically been shown to be minimally irritating. Study author concluded the test substance would have minimal ocular irritation potential in vivo	9
Eyelash product containing 0.025% Ethyl Tafluprostamide	NR	100%; 0.3 ml	hen's egg chorioallantoic membranes (n = 4)	HET-CAM assay; reference test articles include a one-coat mascara and waterproof eyeliner (details regarding these substances not stated); evaluations performed 0.5, 2, and 5 min after test article exposure	Non-irritating Mean irritation score of 0.0 at all test points Reference test articles have historically been shown to be minimally irritating.	57
Ethyl Tafluprostamide (purity: 98.5%)	none	100%; 0.03 g	isolated chicken eyes (n = 1-3/group)	2-part isolated chicken eye test method; eyes evaluated pre-treatment and at approximately 30, 75, 120, 180, and 240 min post-treatment rinsing; negative control: sodium chloride; positive control: imidazole	Non-irritating The test substance showed minimal corneal swelling (1%), faint opacity (≤ 0.5), and negligible fluorescein retention (≤ 0.5) in both experiments. Based on these results, the substance was classified as "No Category" under United Nations Globally Harmonized Systems criteria, indicating no requirement for eye irritation or serious eye damage labeling. Controls gave expected results.	42
Ethyl Tafluprostamide (purity: 99.78%)	NR	100%; 0.03 ml	reconstructed human corneal epithelium (n = 2)	EpiOcular™ assay; OECD TG 492: negative control: phosphate-buffered saline; positive control: sodium dodecyl sulfate	Non-irritating Control substances gave expected results	58
Eyelash serum containing 0.0044% Isopropyl Cloprostenate	saline	10%: 0.3 ml	hen's egg chorioallantoic membranes (n = 6)	HET-CAM assay; vehicle control: saline; positive controls: sodium hydroxide and sodium dodecyl sulfate	Irritation potential score: 0.0 Threshold concentration (lowest concentration at which slight reactions occur) for this test substance was greater than 10% Control substances gave expected results Study author concluded that the irritation potential of the test substance was determined to be none to slight.	59
Eyelash serum containing 0.0044% Isopropyl Cloprostenate	NR	50%*; 0.3 ml	hen's egg chorioallantoic membranes (n = 4)	HET-CAM assay; reference test articles include a one-coat mascara and waterproof eyeliner (details regarding these substances not stated); evaluations performed 0.5, 2, and 5 min after test article exposure	Irritation potential score for eyelash serum: 1.25 (mean scores of 0.0 - 4.9 indicate minimal ocular irritation) Reference test articles have historically been shown to be minimally irritating, Study author concluded that the test substance, at 100%, would have minimal ocular irritation in vivo.	60
Eyelash serum containing 0.005% Isopropyl Cloprostenate	NR	50%*; 0.3 ml	hen's egg chorioallantoic membranes (n = 4)	HET-CAM assay; reference test articles include a one coat mascara and waterproof eyeliner (details regarding these substances not stated); evaluations performed 0.5, 2, and 5 min after test article exposure	Irritation potential score for eyelash serum: 2.50 (mean scores of 0.0 - 4.9 indicate minimal irritation potential) Reference test articles have historically been shown to be minimally irritating. Study author concluded that the test substance, at 100%, would have minimally irritating in vivo.	61

Table 7. Ocular irritation studies

Test Article	Vehicle	Concentration/Dose	Test Population	Procedure	Results	Reference
Eyelash serum containing 0.005% Isopropyl Cloprostenate	saline	10%: 0.3 ml	hen's egg chorioallantoic membranes (n = 6)	HET-CAM assay; vehicle control: saline; positive controls: sodium hydroxide and sodium dodecyl sulfate	Irritation potential score: 0.0 Threshold concentration (lowest concentration at which slight reactions occur) for this test substance was greater than 10% Control substances gave expected results Study author concluded that the irritation potential of the test substance was determined to be none to slight	62
Eyelash serum containing 0.005% Isopropyl Cloprostenate	saline	10%; 0.3 ml	hen's egg chorioallantoic membranes (n = 6)	HET-CAM assay; vehicle control: saline; positive controls: sodium hydroxide and sodium dodecyl sulfate	Irritation potential score: 2.6 Threshold concentration (lowest concentration at which slight reactions occur) for this test substance was greater than 10% Control substances gave expected results Study author concluded that the irritation potential of the test substance was determined to be none to slight	63
0.0075% Isopropyl Cloprostenate	NR	100%; 50 µl	reconstructed human corneal epithelium (n = 2)	EpiOcular™ assay; OECD TG 492: negative control: tissue culture water; positive control: methyl acetate	Non-irritating Control substances gave expected results	64
Isopropyl Cloprostenate (purity: 98%)	none	100%; 50 µl	reconstructed human corneal epithelium (n = 2)	EpiOcular™ assay; OECD TG 492: negative control: water; positive control: methyl acetate	Irritating The mean optical density scores of the test substance, negative, and positive control were 1.4, 2.5, and 8%, respectively. The mean tissue viability of the test substance and positive control were 49.1 and 17.7%, respectively. Controls gave expected results. As the mean tissue viability of the test substance was above the threshold for eye irritation potential (≤ 60%), the test substance was considered to be an eye irritant. It should be noted; however, that the SCCS did not agree with this conclusion, as the prediction model states that if the mean tissue viability is ≤ 60%, no prediction can be made for classification purposes.	42
0.0075% Isopropyl Cloprostenate	NR	50%*; 0.3 ml	hen's egg chorioallantoic membranes (n = 4)	HET-CAM assay; reference test articles include a one coat mascara and waterproof eyeliner (details regarding these substances not stated); evaluations performed 0.5, 2, and 5 min after test article exposure	Irritation potential score for eyelash serum: 1.75 (mean scores of 0.0 - 4.9 indicate minimal irritation potential) Reference test articles have historically been shown to be minimally irritating. Study author concluded that the test substance, at 100%, would have practically no ocular irritation potential.	66
0.1% Isopropyl Cloprostenate	NR	50%*; 0.3 ml	hen's egg chorioallantoic membranes (n = 6)	HET-CAM assay; reference test articles include a one coat mascara and waterproof eyeliner (details regarding these substances not stated); evaluations performed 0.5, 2, and 5 min after test article exposure	Irritation potential score for eyelash serum: 1.50 (mean scores of 0.0 - 4.9 indicate minimal irritation potential) Reference test articles have historically been shown to be minimally irritating, Study author concluded that 0.1% Isopropyl Cloprostenate would have minimal ocular irritation potential in vivo	65

Table 7. Ocular irritation studies

Test Article	Vehicle	Concentration/Dose	Test Population	Procedure	Results	Reference
HUMAN						
Eyelash product containing 0.018% Ethyl Tafluprostamide	NR	100%	19 subjects	Home use study. Subjects applied product to eyelashes for 28 d. Eyes were assessed by ophthalmologist at baseline and on day 28 (slit-lamp examinations)	The majority of subjects displayed no signs of irritation; however, one patient was scored a "2" (moderate intolerance to product). Four subjects reported minor adverse reactions consistent with allergic reactions.	9
Eyelash product containing 0.025% Ethyl Tafluprostamide	NR	100%	19 subjects	Home use study. Subjects applied product to eyelashes for 28 d. Intraocular pressure was measured in each eye of each subject at beginning and end of study. Eyes were assessed by ophthalmologist at baseline and on day 28 (slit-lamp examinations).	Minor ocular effects self-reported by 4/19 volunteers (slight dryness, slight itching, slight stinging, slight watering and redness, moderate to high burning) Study authors determined that the formulation did not produce an eye irritation or hypersensitivity of clinical magnitude.	17,36
Eyelash serum containing 0.0044% Isopropyl Cloprostenate and eyebrow serum containing 0.0044% Isopropyl Cloprostenate	NR	100%	29 subjects	Home use study. Subjects applied eyelash serum to the top eyelash line once daily for 6 wk; questionnaires completed after 2, 4 and 5 wk of eyelash serum use; photos taken at baseline, and after 4 wk of serum use. Subjects also instructed to apply the eyebrow serum for 7 wk; questionnaires completed after 6 and 7 wk of eyebrow serum use; photos taken at baseline and after 6 wk of serum use	No adverse effects observed relating to product use	67
Eyelash serum containing 0.0044% Isopropyl Cloprostenate	NR	100%	32 subjects	Home use study. Subjects applied eyelash serum daily for 12 wk; subjects completed questionnaires after 6 and 12 wk of use; subjects evaluated at testing facility at baseline and after 12 wk of serum use	Overall, the eyelash serum was considered to be well-tolerated, with at most, mild effects that are short-term and reversible One subject reported slight stinging in both eyes if product was applied too close to the corner of the eye One subject reported ocular pruritis 20 min after application for 2 wk after an unspecified number of applications; at the end of the 2-wk period, itching stopped and did not recur for the remainder of the study	67
Eyelash serum containing 0.0044% Isopropyl Cloprostenate	NR	100%	114 subjects	Home use study. Subjects applied eyelash serum daily for 8 mo. Slit-lamp evaluations occurred at baseline, 1 mo, 2 mo, 4 mo, and 8 mo intervals.	Slight transient ophthalmological irritation observed. The serum was determined to be safe for use by both contact lens and non-contact lens wearers.	37
Eyelash serum containing 0.005% Isopropyl Cloprostenate	NR	100%	32 subjects	Serum applied to eyelid, above upper lash line (lash root area), on both eyes, once per day, each evening; eyes evaluated for irritation from baseline to 3 mo of product use	Non-irritating Subjective evaluations by the test population were favorable	68
Eyelash serum containing 0.005% Isopropyl Cloprostenate and eyebrow serum containing 0.005% Isopropyl Cloprostenate	NR	100%	30 subjects	In- use study. Subjects applied eyelash serum to left eye lashes and eyebrow serum to right eyebrow; evaluations performed at baseline and 8 h after application; slit-lamp examination of bulbar conjunctival irritation, palpebral conjunctival irritation, and lid disease	Eyelash serum results: mean irritation score: 0.0 (non-irritating) at baseline; slight bulbar conjunctival irritation observed at 8 h observation (mean irritation score of 0.4/3) Eyebrow serum results: Mean irritation score of 0.0 (non-irritating) at baseline and at 8 h observation	69

Table 7. Ocular irritation studies

Test Article	Vehicle	Concentration/Dose	Test Population	Procedure	Results	Reference
Eyelash conditioning serum containing 0.0075% Isopropyl Cloprostenate	NR	100%	56	Home use study; subjects applied test material to upper eyelashes and eyebrows 1x/d, at night, for 8 wk; clinical evaluations performed at baseline, at each study wk, and 1 wk after study completion; slit-lamp ophthalmologist evaluations (observing bulbar irritation, lacrimation, tarsal conjunctival irritation, contact lens deposits, subjective sensations, fluorescein staining), cutaneous tolerance evaluations (evaluating erythema, dryness, edema, burning, stinging, itching, tightness, or tingling), and imaging procedures were performed	The test substance did not cause any statistically significant worsening slit-lamp evaluation scores, or cutaneous tolerance scores. Four subjects experienced adverse effects: 2 subjects experienced mild eye pain, dry eye, and/or ocular hyperemia possibly related to the test material; 1 subject with self-perceived sensitive eyes experienced moderate eye pain and eye irritation (likely attributed to test material); and 1 subject experienced mild ocular hyperemia and erythema of the eyelids, and moderate eye irritation (definitely related to the test material). Hordeolums were observed in 2 subjects but were considered to be unlikely attributed to the test substance, according to study authors. There was a statistically significant worsening in fluorescein staining (level of brightness and the percent of the eye that the stain covers) at wk 1 for the right eye, compared to baseline. The worsening was transient, and not considered to be clinically relevant (as the increase was very small and only seen in the right eye).	70
Eyelash formulation containing 10% Isopropyl Cloprostenate	NR	100%	27 subjects	Application of test substance for 4 wk; applications in both contact lens users and non-contact lens users; no details were provided	non-irritating	5

HET-CAM = hen's egg test chorioallantoic membrane; NR = not reported; OECD TG = Organisation for Economic Co-Operation and Development Test Guidelines
*study author states that a 50% dilution of the test and reference articles may be used to approximate in vivo irritation potential at 100%, as the hen's egg is more sensitive to liquid irritants than the rabbit eye

Table 8. MOE calculation parameters

	Ethyl Tafluprostamide[8,17]		Isopropyl Cloprostenate	
estimated daily exposure to eyelash products	2.4 mg/d (or 0.04 mg/kg bw/d on average) 4 mg/d (or 0.0667 mg/kg bw/d at maximum) *		0.26 mg/d (or 0.0037 mg/kg bw/d, for lash serum only) #[23]	7 mg/d (median estimated daily application of eyelash formula, based on a range of 4-10 mg/d)[32,76]
concentration of ingredient	0.018%		0.005%	0.0075%
dermal absorption	8.67% (based on in vitro percutaneous study provided in this report[22]; the mean absorbed mean absorbed fraction (6.51 ± 2.16%) plus 1 standard deviation was used)		7.54% (mean % in dermis + epidermis + Receptor Fluid) + 1 SD (dermis + epidermis + Receptor Fluid) = 6.34% + 1.20% = 7.54%)[24,74]	10% (Using a QSAR model (calculated by EpiSuite™ 1.0), the dermal absorption of Isopropyl Cloprostenate was estimated at 10%.)[5,32]
dermal retention factor	1		1	
body weight	60 kg		70 kg	60 kg
systemic exposure dose	6.24 x 10^{-7} mg/kg/d (average) 1.04 x 10^{-6} mg/kg/d (max)		1.4 x 10^{-8} mg/kg/d	8.4 x 10^{-7} mg/kg/d
systemic point of departure (POD)	NOAEL: 3 x 10^{-4} mg/kg bw/d (derived from a prenatal developmental toxicity study on tafluprost (rats, intravenous))[78]	Corrected TSV of 2.1 x 10^{-6} mg/kg/d for substances acting as agonists or antagonists on PGF2α receptors.[8,77] **	NOAEL: 4 x 10^{-5} mg/kg/d (derived from a systemic toxicity assay on travoprost: an LOAEL at 0.00012 mg/kg/d from a 3-generation study (rats, subcutaneous) with an assessment factor of 3 for extrapolation from LOAEL to NOAEL)[74,75]	Corrected TSV of 2.1 x 10^{-6} mg/kg/d for substances acting as agonists or antagonists on PGF2α receptors.[8,77] **
margin of exposure/safety	481 (based on average daily exposure) 288 (based on maximum daily exposure)	3.4 (based on average daily exposure) 2 (based on maximum daily exposure) ***	2849 ##	2.5 ***

LOAEL = lowest-observed-adverse-effect-level; NOAEL = no-observed-adverse-effect-level; PGF2α = prostaglandin $F_{2\alpha}$; TSV = toxicological screening value

* On average, 2.4 mg of cosmetic eyelash product was applied to the upper eyelashes with each brush stroke. Assuming once-daily application to both eyes, an average daily amount of 4.8 mg and a maximum of 8 mg were used in the MOE calculations.[17] Under a worst-case assumption that up to 50% of the cosmetic product could migrate to the eyelid and become available for dermal exposure, even though the product is applied as a thin line above the lash line and a viscosity agent is present to minimize migration, the daily exposure to eyelash products was estimated accordingly (i.e., 50% of the average amount of 4.8 mg/d= 2.4 mg/d and 50% of the maximum amount of 8 mg/d = 4 mg/d).

** The corrected TSV value of 0.0021 µg/kg/d is derived by applying a default oral absorption adjustment factor of 50% to the original TSV of 4.2 x 10^{-6} mg/kg/d, which is the value recommended by EFSA for substances that act as agonists or antagonists on PGF2α receptors.[8,77]

*** Given the TSV includes built-in uncertainty to represent a safe dose, an MOS ≥1 is considered protective.

Exposure amount was provided by unpublished reports based on two weight usage tests, (15 simulated applications per formulation); the higher mean value of 0.26 mg/application was used.[23]

Calculation of the Margin of Exposure (MOE), using a default body weight of 60 kg, yields an MOE of 2453.

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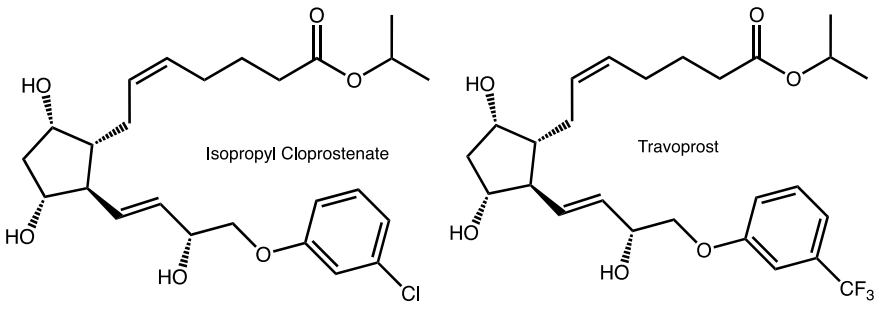
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Report of the Read-Across Working-Group (RAWG) at the 173rd meeting of the Expert Panel for Cosmetic Ingredient Safety - June 9, 2025

Section 43 (a) of the *Cosmetic Ingredient Review Procedures & Support to the Expert Panel for Cosmetic Ingredient Safety* (Procedures) provides that the Chair of the Expert Panel may appoint working teams (e.g., the RAWG) comprised of from two to four members of the Expert Panel to review information about designated ingredients, to prepare draft documents for consideration by the entire Expert Panel, or to perform other specific assignments for the Expert Panel. This recommendation report follows the June 9, 2025, meeting of the Read Across Working Group (RAWG). Present were Drs. Allan Rettie, David Ross, Susan Tilton, and Curtis Klaassen. In accordance with Section 43(a), this draft document is now presented for consideration by the entire Expert Panel.

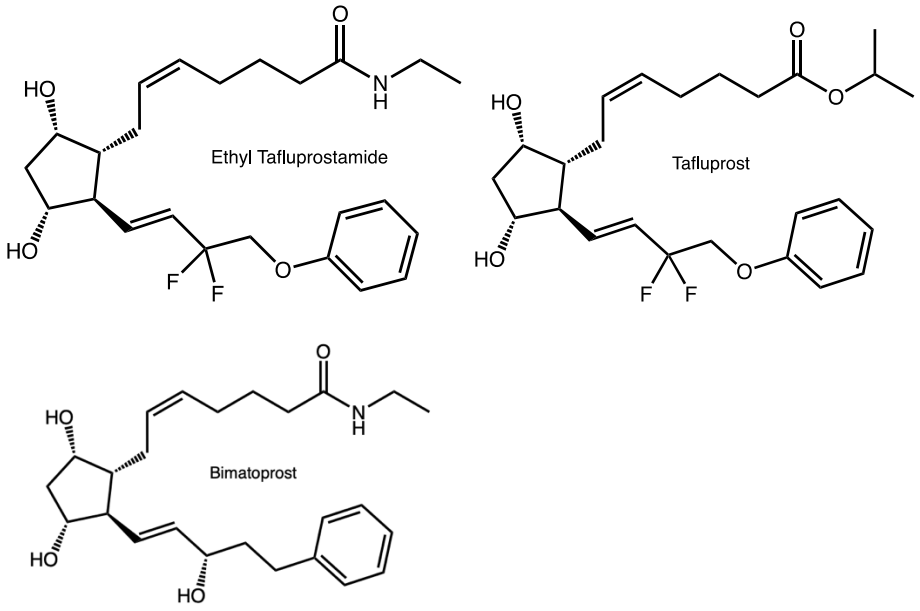
Previously, at the 169th meeting of the Expert Panel, the RAWG did not recommend the use of the proposed read-across strategy to clear endpoints for these biologically potent ingredients. The 173rd meeting opened with two Consultant presentations relevant to the Prostaglandins Report: i.e., for Isopropyl Cloprostenate (IPC) and Ethyl Tafluprostamide (DDDE). The presentations from Dr. Ator (ToxSciences) and Ms. Mishra (ToxMinds) sought to provide new justifications for reading across to these ingredients from Travoprost (for IPC) and from Tafluprost and/or Bimatoprost (for DDDE). The RAWG group appreciated the efforts of the applicants to generate new in-silico and biological data pertaining to potential read across.

However, at the conclusion of the presentations and subsequent discussions, the RAWG were left with (i) chemical concerns regarding the suitability of the source compounds for read across and (ii) potential toxicity concerns particularly with respect to ocular and developmental/reproductive (DART) endpoints.



From a **structural perspective**, both Travoprost and IPC are isopropyl esters, containing an omega chain 13,14-dehydro,15-hydroxy motif and an ether linkage to the terminal phenyl group. The major difference between the two structures is that Travoprost has a CF_3

substitution on the aromatic ring in place of a Cl in the case of IPC. While both are electron-withdrawing, the CF_3 substituent is more hydrophobic than the Cl group. Additionally, the predicted aqueous/lipid solubilities of the source and target are very different, which seems likely to impact distribution and the toxicokinetic profiles of the two compounds.



Regarding read-across to Ethyl Tafluprostamide, Tafluprost contains both the geminal difluoro group and terminal ether on the omega chain but notably has an ester instead of an amide functionality on the alpha-chain that introduces legitimate questions regarding receptor interactions that have not been answered. Bimatoprost possesses the requisite amide moiety for appropriate receptor interactions but has a geminal difluoro substituent and no ether on the omega chain, both of which will impact metabolism.

Receptor binding affinities that were reported were not consistent with previous literature, no values for receptor binding of IPC were provided, and new data presented showed a potential 10-fold difference in one measure of the in-vivo bioactivity of Travoprost and IPC.

All of the potential source compounds proposed, Tafluprost, Bimatoprost, and Travoprost have **DART toxicities** which render reliable read across for safety purposes difficult. Species differences in DART endpoints have also been demonstrated (e.g., Tafluprost).

The potential **ocular toxicity** of prostaglandins in eye preparations is concerning in that they have been associated with adverse periorbital and pigmentation effects.

Importantly, the **prostaglandins are potent compounds** with affinity values for their appropriate receptors in the nanomolar range so variations in dose and toxicokinetics can make a large difference in the eventual biological effect.

Finally, large variations in amounts of these materials applied in test systems have been reported (in our own dossier, up to 27-fold), which makes comparison difficult.

In summary, the RAWG maintained their previous recommendation - that the proposed read-across molecules were not appropriate sources for the two target ingredients (IPC and DDDE) under consideration due to:

- Differences in chemical structure of target and source compounds
- Potency of prostaglandin analog function and potential differences in receptor binding
- Difficult prediction of toxicokinetics and resultant biological activity
- Presence of DART effects for all potential source compounds.

In summary, the RAWG does not recommend the use of the proposed read-across strategies to clear endpoints for these biologically potent ingredients.

From: Katz, Linda
To: dvb2108@cumc.columbia.edu; Bart Heldreth
Cc: Manga, Prashiela
Subject: FW: [EXTERNAL] Revitalash
Date: Thursday, March 31, 2022 12:52:56 PM
Attachments: image001.png

Don and Bart

I'm aware of the discussion at the last CIR meeting regarding prostaglandin analogs in eyelash products. The response from FDA at the time of the meeting was that we had requested input from CDER regarding the jurisdiction of these products. CDER recently responded to our request for a review. Of note, we have seen this product and several other similar products over the years, and a warning letter was issued by CDER over a decade ago to Lifetech for its RapidLash and NeuLash products. The jurisdictional issue (drug vs. cosmetic) is based on the claims, such that if growth (or other drug) claims are made, it would be considered a drug, and the manufacturers would be required to follow drug requirements to market the product. In the absence of such claims, we would not be able to establish the intended use of such products as drugs, regardless of ingredients, and they would be cosmetics. We (OCAC and CDER) have reviewed the Revitalash website and it appears not to make drug claims. As a result, they would not be expected to register as an OTC drug. They could, however, register in the VCRP if they so desire.

Hope this helps. Let me know if you have any additional questions.

Linda

Linda M. Katz, M.D., M.P.H.
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FDA U.S. FOOD & DRUG ADMINISTRATION

From: Belsito, Donald V. <dvb2108@cumc.columbia.edu>
Sent: Tuesday, December 14, 2021 9:23 AM
To: Bart Heldreth (heldrethb@cir-safety.org) <heldrethb@cir-safety.org>; Katz, Linda <Linda.Katz@fda.hhs.gov>
Subject: [EXTERNAL] Revitalash

CAUTION: This email originated from outside of the organization. Do not click links or open attachments unless you recognize the sender and know the content is safe.

Hi Bart & Linda:
I went by Nordstrom's on my way home last night and checked out the RevitaLash product. It appears to be marketed as a cosmetic and does indeed contain DDDE.
Bart, is it possible to move these prostaglandin analogs up for cause – or, Linda, will the FDA be stepping in to make them register as OTC dugs.
Here is the website for the SCCS prelim opinion:

https://ec.europa.eu/health/sites/default/files/scientific_committees/consumer_safety/docs/sccs_o_258.pdf

Regards and happy holidays,
Don

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Additional Data Demonstrating the Safe Use of Ethyl Tafluprostamide (0.018%) in Cosmetics

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Submitted On Behalf Of:
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April 30, 2025

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1. BACKGROUND

In June 2022, the Cosmetic Ingredient Review (CIR), initiated a safety assessment of the use of two prostaglandin analogues, ethyl tafluprostamide (i.e., dechloro dihydroxy difluoro ethylcloprostenolamide, in the following abbreviated as 'DDDE') and isopropyl cloprostenate ('ICP') as cosmetic ingredients. In May 2023, a manufacturer of cosmetic products containing DDDE (the '**DDDE Sponsor'**) submitted an extensive report of product-based data demonstrating the safe use of DDDE in the DDDE Sponsor's eyelash product. The May 2023 report was considered by the CIR Expert Panel along with reports submitted by two sponsors of products containing ICP at CIR's June 2023 meeting. The Expert Panel requested ingredient-based data and the CIR issued an insufficient data announcement (IDA) specifying ingredient-based safety data requested for DDDE and ICP.

The DDDE Sponsor engaged ToxMinds to obtain the ingredient-based data requested in the June 2023 IDA. In October 2023, ToxMinds submitted an extensive dossier containing the data specified in the June 2023 IDA and a comprehensive safety analysis based on the new ingredient-based data and the product-based data submitted in the May 2023 report. ToxMinds' safety analysis incorporated established read across (RA) analyses because animal toxicological data are not available for DDDE and cannot be obtained due to recent bans on animal testing of cosmetic ingredients. Reports submitted by the ICP sponsors for the December 2023 CIR meeting also incorporated RA analyses.

At CIR's meeting in December 2023, the Panel members expressed discomfort with relying on RA as an alternative to *in vivo* toxicology data in the safety assessment of prostaglandin analogue cosmetic ingredients, in general, and DDDE and ICP, specifically. For both DDDE and ICP, the Panel expressed concern that it did not have the data to feel confident that the analogues used in the RA-based safety assessment were sufficiently predictive of the biological effects of DDDE or ICP. The Panel requested 'bridging data' (e.g., studies of receptor interaction and downstream systemic effects) to determine if the analogues are mechanistically and biologically similar to the target ingredients, DDDE and ICP.

To fill the data gaps identified by the Panel, the CIR issued an IDA at the December 2023 meeting (the December 2023 IDA) for additional data for both DDDE and ICP, which included:

1) Systemic toxicity endpoints, including:
    - acute toxicity data
    - repeated dose toxicity data
    - developmental and reproductive toxicity data
    - *in vivo* genotoxicity data
2) Confirmatory 'bridging' data for both DDDE and ICP to determine if the proposed RA analogues are appropriate for the target ingredients, DDDE and ICP, including studies of;
    - receptor interaction
    - downstream profiles of adverse effects
3) Mechanistic data on possible targets of DDDE and ICP

For DDDE, the Panel questioned if the analogue, tafluprost, was an appropriate analogue because of structural differences between DDDE and tafluprost. Unlike DDDE, tafluprost does not contain an amide functional group, which may interact with prostamide receptors in addition to the canonical prostanoid receptors. The Panel suggested that bimatoprost, which does have a functional amide group, may be a more appropriate analogue for DDDE. However, there are also structural differences between DDDE and bimatoprost. Both DDDE and tafluprost have a 'geminal fluorine' functional group, whereas bimatoprost does not.

In March 2024, the DDDE Sponsor submitted a roadmap (the 'DDDE roadmap') to CIR outlining a portfolio of additional literature research, *in silico* analysis and experimental testing to obtain the data for DDDE specified in the December 2023 IDA. This portfolio of testing and analysis is presented in

detail in **Section 2** of this report. Notably, the experimental plan included testing of both tafluprost ***and*** bimatoprost to determine the similarity in their biological and chemical activity with DDDE. These comparative tests were used to obtain mechanistic and bridging data to assess whether tafluprost and/or bimatoprost are suitable analogues for use in RA in the safety assessment of DDDE.

This report presents the following NEW data and analyses:

- Comparative data on the analogues, tafluprost *and bimatoprost* with DDDE.
- Results of *in vitro* testing of systemic mechanism of toxicity and endpoints for DDDE, tafluprost and bimatoprost.
- Results of PGF2α receptor binding assay for DDDE, tafluprost and bimatoprost.
- An additional comprehensive literature search to identify receptor binding/activation data on DDDE and analogues tafluprost and bimatoprost.
- An *in silico* analysis using accepted (Q)SAR models to assess the similarity of DDDE, tafluprost and bimatoprost with respect to receptor binding and activation potential.
- A comprehensive literature review of available *in vivo* studies for tafluprost and bimatoprost to assess their suitability for the assessment of DDDE systemic toxicity.
- A detailed analysis of the suitability of tafluprost and/or bimatoprost as analogues for use in RA to assess the safety of DDDE as a cosmetic ingredient.
- A detailed analysis of the validity of using the presented RA analyses to assess the safety of DDDE as a cosmetic ingredient.
- Analysis of differences in metabolism due to the presence of the 'geminal fluorines' functional group in the frame of the read across approach.
- Read across justification report in accordance with the ECHA Read Across Assessment Framework (RAAF) (ECHA, 2017).
- An updated calculation of the Margin of Safety (MoS) informed by the comparative analysis of new data collected and analysed on DDDE, tafluprost and bimatoprost.
- Calculation of a toxicological screening value (TSV), which was developed by the European Food Safety Authority (EFSA) Panel on Contaminants in the Food Chain (CONTAM) specifically to screen substances acting on ***PGF2α receptors*** (EFSA, 2018). EFSA sets a very conservative TSV. An MoS based on the TSV for DDDE was calculated and is included in this report.

The DDDE Sponsor has diligently collected the data and information requested by the CIR in the December 2023 IDA with the goal of providing the Expert Panel with the data it needs to conduct a safety assessment of DDDE as a cosmetic ingredient based on extensive product-based and ingredient-based scientific evidence, relevant scientific literature and contemporary analytic tools, including RA.

Collectively, the data and analyses presented in this and other reports submitted to the CIR by the DDDE Sponsor a) support the use of RA using the selected analogues in the safety assessment of DDDE, and b) provide strong scientific evidence that use of DDDE as a cosmetic ingredient at a concentration of 0.018% in the DDDE Sponsor's eyelash product is safe according to two established standards of calculating MoS, one specifically for prostaglandin molecules that bind to PGF2α receptors.

2. EXPERIMENTAL PLAN TO COLLECT DATA TO RESPOND TO CIR'S DECEMBER 2023 IDA

2.1. DATA REQUESTED BY CIR IN THE DECEMBER 2023 IDA FOR DDDE ASSESSMENT

Primarily to provide the Expert Panel with additional data to support a) the use of RA in the safety assessment of DDDE and b) the suitability of tafluprost and/or bimatoprost as analogues for DDDE in RA, the December 2023 IDA included:

1) Systemic toxicity endpoints, including:
    - acute toxicity data
    - repeated dose toxicity data
    - developmental and reproductive toxicity data
    - *in vivo* genotoxicity data
2) Confirmatory 'bridging' data for both DDDE to determine if the proposed RA surrogates are appropriate for the target ingredients, DDDE, including;
    - receptor interaction studies
    - downstream profiles of adverse effects
3) Mechanistic data on possible targets of DDDE

2.2. *READ ACROSS QUESTIONS ADDRESSED IN THIS REPORT*

At CIR's December 2023 meeting, the Panel raised concerns regarding the validity of using RA as an alternative to traditional animal toxicology data in the safety assessment of DDDE. These concerns were mainly related to: (1) concern that tafluprost was not a suitable analogue for DDDE because of structural differences between the two substances (i.e., ester versus amide group), and (2) the need to have sufficient bridging data (e.g., receptor binding studies and downstream profiles of adverse effects), to support the use of the RA approach.

(1) Evaluation of Suitable RA Analogues for DDDE

In its October 2023 report ToxMinds set out its scientific rationale for selecting tafluprost as the most suitable analogue to use in its RA analysis for the safety assessment of DDDE. However, at CIR's December 2023 meeting, the Panel questioned whether bimatoprost might be a more appropriate analogue than tafluprost for DDDE. As shown in the chart below, while DDDE, tafluprost and bimatoprost all share a 'prostaglandin core', there are structural differences between DDDE and both tafluprost and bimatoprost. DDDE and tafluprost have a geminal fluorine functional group but tafluprost does not contain an amide functional group, which may interact with prostamide receptors in addition to the canonical prostanoid receptors. DDDE and bimatoprost both have a functional amide group but bimatoprost does not have a geminal fluorine functional group.

	DDDE	Tafluprost	Bimatoprost
Prostaglandin core	✓	✓	✓
Amide functional group	✓		✓
Geminal fluorine functional group	✓	✓	

The underlying issue is whether tafluprost and/or bimatoprost have a sufficiently similar biological effect to be used as an analogue for DDDE by means of RA. To address this issue, as presented in the next section, the experimental plan to collect additional bridging data included DDDE and *both* candidate analogues, tafluprost and bimatoprost.

(2) Additional Bridging and Mechanistic Data to Support Use of Tafluprost and/or Bimatoprost in RA for DDDE

To validate the use of RA in the safety assessment of DDDE, the Panel requested data demonstrating structural, mechanistic, and toxicological similarity between DDDE and its analogues. These data included data on receptor binding and downstream biological response profiling to evaluate the appropriateness of the proposed analogues for RA. The tests and *in silico* analyses described in the Experimental Plan (below) were conducted on DDDE, tafluprost and bimatoprost.

2.3. EXPERIMENTAL PLAN

The roadmap provided to CIR in October 2023 by the DDDE Sponsor listed a portfolio of tests and analyses designed to obtain the data and information requested by the CIR Expert Panel. A targeted strategy was proposed in the roadmap to provide scientific evidence to support use of RA in the safety assessment of DDDE as a cosmetic ingredient. To ensure a robust RA justification and to address the Panel's stated concerns, each test in the roadmap was carefully selected to address specific uncertainties and to demonstrate structural, mechanistic, and toxicological similarity between DDDE and its analogues.

(1) Systemic Toxicity

The potential for systemic toxicity was evaluated for DDDE, tafluprost and bimatoprost in four *in vitro* assays evaluating: 1) acute oral toxicity (3T3 neutral red uptake assay), 2) receptor binding potency (PGF2alpha receptor binding assay), 3) biological activity (Toxprofiler assay) and 4) downstream adversity specifically with regard to developmental effects (ReproTracker assay) – as well as a detailed justification of the suitability of the proposed analogues for the safety assessment of DDDE by means of RA. The table below provides more detail about the *in vitro* studies conducted to obtain toxicological data for bridging purposes:

Additional NAMs testing	Rationale/justification
***In vitro* 3T3 neutral red uptake assay (according to OECD GD 129)**	This test was proposed to be conducted on DDDE, tafluprost and bimatoprost in order to generate bridging data for the **acute oral toxicity apical endpoint** and to demonstrate similarity from a **quantitative** point of view, thereby strengthening the overall read across approach.
Receptor binding potency assay	These tests were initially planned for both prostanoid and prostamide receptors. However, as the prostamide receptor has not yet been isolated, a commercial assay is not available. The possibility to set up an *in vitro* assay based on the use of feline iris sphincter or another tissue expressing the receptor was considered to entail a high technical risk and pose ethical issues with respect to animal welfare. Instead, the PGF2α receptor was selected as a representative target for DDDE, tafluprost and bimatoprost. The results from this assay contribute to support the overall read across justification by comparing the receptor binding potency across the three substances and providing mechanistic bridging data on a key molecular initiating event.
ToxProfiler® assay	The ToxProfiler assay was proposed to generate mechanistic data on DDDE and its analogues by evaluating their effects on key cellular stress pathways. This helps identify similarities with respect to biological activities, thereby supporting the relevance of the analogues in the read across approach.

Additional NAMs testing	Rationale/justification
ReproTracker® assay	The ReproTracker assay was proposed to assess potential effects on developmental endpoints by monitoring key biomarkers of stem cell differentiation. By comparing DDDE with its analogues, this assay provides additional downstream evidence to support the RA approach for this sensitive apical endpoint.

(2) Receptor Binding Analyses

- *In silico* predictions for Receptor Binding and Activation with focus on Developmental and Reproductive Toxicity/Endocrine endpoints

To further support the read-across justification, an *in silico* analysis was conducted using a range of well-established (Q)SAR models. The analysis focused on endocrine-related receptors, including ERα and ERβ (estrogen receptors), AR (androgen receptor), GR (glucocorticoid receptor), and TRα and TRβ (thyroid hormone receptors). These receptors are critical components of the endocrine system, involved in regulating various hormonal pathways. ERα and ERβ are responsible for mediating the effects of estrogen, influencing reproductive tissues and gene expression. AR responds to male sex hormones like testosterone, GR binds to cortisol, affecting metabolism and immune responses, while TRα and TRβ mediate the effects of thyroid hormones on growth and development.

The results from the *in silico* analysis, combined with the test data, help to establish the structural and toxicological similarities between DDDE and its analogues, tafluprost and bimatoprost.

- Literature Research of Endocrine Receptor Activation by Analogues

(3) Analysis of the Impact of Geminal Fluorine Moiety on Metabolism and Toxicology Profile

(4) Comprehensive Analysis of Scientific Support for Using RA in the Safety Assessment of DDDE

2.4. SUMMARY

The additional data collected and presented in this report directly respond to the information requested by the CIR Panel in the December 2023 IDA. The integrated assessment demonstrates that the weight of evidence supports the scientific validity of using RA to evaluate DDDE's safety as a cosmetic ingredient.

3. EXECUTIVE SUMMARY OF NEW DATA, ANALYSES AND CONCLUSIONS

3.1. SUMMARY OF DATA AND ANALYSES

Below is an executive summary of the data and analyses presented in this report.

- The **receptor binding potency assay** demonstrated that all three compounds bind to the PGF2α receptor within a comparable potency, further supporting ***similarity*** across the three substances and providing mechanistic bridging data on a key molecular initiating event.
- The ***in silico*** **analysis** using widely recognised (Q)SAR models indicates that DDDE presents an overall ***similar*** profile to tafluprost and bimatoprost with respect to receptor binding and activity potential.
- **Literature research of endocrine receptor activation** by the analogues: no relevant *in vitro* endocrine receptor binding or activation assays as defined in the OECD Conceptual Framework Level 2 (OECD, 2018) could be identified for DDDE or its analogues tafluprost and bimatoprost.
- The **ToxProfiler® assay** showed that DDDE and bimatoprost share a similar biological activity profile across the different cellular stress pathways, suggesting that bimatoprost is more similar than the profile of DDDE *versus* tafluprost, when considering the biological activity.
- The **ReproTracker® assay** also indicates that bimatoprost seems to represent a worst-case as compared to DDDE and tafluprost, when considering the effects on development in *in vitro* conditions.
- The ***in vitro*** **3T3 NRU assay** showed comparable cytotoxicity profiles for DDDE, tafluprost, and bimatoprost, indicating ***similar*** acute oral toxicity potential and supporting the read across at the apical level.
- A comprehensive review of available *in vivo* studies for the two analogues confirmed their suitability for the assessment of systemic toxicity. Overall, the available information on composition, physico-chemistry and toxicity suggests that the target and source substances share sufficient common properties, so that RA may be used for the toxicological endpoints listed in **Table 12**, which can be used in the safety assessment of DDDE.
- A literature review of *in vivo* repeated dose, reproductive and developmental toxicity studies *via* oral, intravenous and/or subcutaneous routes for the analogues tafluprost and bimatoprost showed that, in general, tafluprost showed a more severe toxicity and lower NOAELs compared to bimatoprost irrespective of the route of exposure (see **Table 13**).
- Considering all available information and in line with the approach adopted by the German BfR for the risk assessment of DDDE in cosmetic eye product formulations (BfR, 2017), the **NOAEL of 0.3 µg/kg bw/day** derived from a prenatal developmental toxicity study of tafluprost in rats following **i.v.** administration was selected as the most appropriate PoD. This NOAEL represents a very ***conservative*** and worst-case PoD when compared with the exposure route (dermal) for the intended application of DDDE present in cosmetic eye product formulations.
- The Margin of Safety (MoS) for DDDE at a concentration up to 0.018% in the DDDE Sponsor's cosmetic eyelash product exceeds the threshold of 100, exceeding the generally accepted criteria for a safe cosmetic ingredient.
- The safety of DDDE is also indicated by a screening tool developed by the European Food Safety Authority (EFSA) Panel on Contaminants in the Food Chain for substances that act on

PGF2α receptors. EFSA sets acceptable thresholds for toxicological screening values (TSVs). The threshold for substances acting on the PGF2α receptors is TSV of 0.0042 µg/kg bw/day. Comparison of this very ***conservative*** TSV value with the maximum SED value for DDDE results in a MoS of >1, suggesting a low risk.

CONCLUSIONS

This report presents new data and analyses that were specifically requested by the CIR panel to support the justification for using RA in the safety assessment of DDDE. The weight of evidence supports the scientific validity of using RA to evaluate DDDE's safety as a cosmetic ingredient.

Based on the available *in vivo* and newly generated NAMs data, the present safety assessment considering conservative exposure calculations reveals a calculated MoS greater than 100 (as per SCCS approach) or 1 (as per EFSA TSV approach). It thereby supports the safe use of DDDE at a concentration up to 0.018% in cosmetic eyelash products under the conditions presented in this evaluation.

4. PRESENTATION OF NEW DATA

The data presented below were generated in line with the DDDE roadmap and are intended to strengthen RA approach and the original safety assessment. Together, these data provide further scientific support for the use of RA in the safety assessment and the safety of DDDE at the proposed use level.

4.1. *IN VITRO* NEUTRAL RED UPTAKE (NRU) TEST

The acute oral toxicity of DDDE and its analogues (tafluprost and bimatoprost) was assessed based on a recently conducted *in vitro* 3T3 NRU assay. The results of this assay have been summarised below:

Guideline:	OECD Guidance Document 129 (Guidance document on using cytotoxicity tests to estimate starting doses for acute oral systemic toxicity tests)
Test system:	Balb/c 3T3 fibroblasts (clone 31) (for all three substances)
Replicates:	6 (for each substance)
Test substances:	DDDE, tafluprost and bimatoprost
Vehicle:	1% Dimethyl sulphoxide (DMSO)
Batch:	TAF-10-1122-01, TAF-IPE-0524-01 and BIM-0414-01 for DDDE, tafluprost and bimatoprost, respectively
Purity:	99.78, 99.48 and 99.8% for DDDE, tafluprost and bimatoprost, respectively
Dose levels:	DDDE: Dose range-finding (DRF): 0.0000002 to 2 mg/mL (10-fold dilution series) Main experiment 1&2: 0.00063 to 2 mg/mL (10-fold dilution series) Tafluprost: DRF: 0.0000002 – 2 mg/mL (10-fold dilution series) Main experiment 1: 0.00063 – 2 mg/mL (√10-fold dilution series) Main experiment 10.0016 – 0.2 mg/mL (2-fold dilution series) Bimatoprost: DRF: 0.0000002 to 2 mg/mL (10-fold dilution series) Main experiment 1 &2: 0.016 to 2 mg/mL (2-fold dilution series)
Duration:	48 hours
Positive control:	Sodium dodecyl sulphate
GLP:	Yes
Study period/year:	2024

An *in vitro* study was conducted to predict the acute oral toxicity of the test substance, DDDE (99.78% purity) and the analogues, tafluprost (99.48% purity) and bimatoprost (99.8% purity), in Balb/c 3T3 fibroblasts using the 3T3 NRU test. The study was performed in accordance with the OECD Guidance document 129 and in compliance with GLP. The data generated from this study can be used to determine starting doses for rodent acute oral toxicity tests.

DDDE, tafluprost and bimatoprost were dissolved in DMSO and assessed for cytotoxicity in a 3T3 NRU assay, which included a DRF study followed by two main experiments. In the DRF study, fibroblasts were exposed to DDDE, tafluprost and bimatoprost at concentrations ranging from 0.0000002 to 2 mg/mL in a 10-fold dilution series. In the main study, fibroblasts were exposed to DDDE at concentrations ranging from 0.00063 to 2 mg/mL in a √10-fold dilution series in both experiment 1 and 2; tafluprost at concentrations ranging from 0.000063 to 0.2 mg/mL in a √10-fold dilution series in the main experiment 1 and 0.0016 to 0.2 mg/mL in a 2-fold dilution series in the main experiment 2; and bimatoprost at concentrations ranging from 0.016 to 2 mg/mL in a √10-fold dilution series in experiments 1 and 2. Plates were also visually inspected for signs of cytotoxicity. Sodium dodecyl sulphate was used as a positive control in both studies. IC50 values obtained with this study type can

be used to determine LD50 values for acute oral systemic toxicity tests by using the algorithm: Log LD50 (mmol/kg) = 0.439 log IC50 (mM) + 0.62.

Results

DDDE exhibited dose-related toxicity in the DRF with a calculated IC50 value of 0.15 mg/mL. It also showed precipitation at 2 mg/mL. In the main experiments, DDDE exhibited precipitation at 2 and 0.63 mg/mL; therefore, cytotoxicity could not be evaluated at these doses. The calculated IC50 value for DDDE was determined to be 0.083 mg/mL in both experiments, resulting in a mean IC50 value of 0.083 mg/mL. The corresponding oral LD50 value was estimated to be 2.01 mmol/kg, i.e., equivalent to 880 mg/kg bw. (See **Table 1**, below, for a summary of data on the three substances.)

Tafluprost exhibited dose-related cytotoxicity in the DRF, with a calculated IC50 value of 0.0092 mg/mL. It showed precipitation at 0.2 and 2 mg/mL in the DRF. In the main experiments, tafluprost exhibited precipitation at 0.2 mg/mL in experiment 1 and at 0.2 and 0.1 mg/mL in experiment 2. A dose-related toxicity was observed in both experiments. In experiment 1, toxicity was observed at 0.063 and 0.020 mg/mL. Due to precipitation, cytotoxicity could not be evaluated at 0.2 mg/mL. In experiment 2, toxicity was observed at 0.05 and 0.025 mg/mL. Due to precipitation, cytotoxicity could not be evaluated at 0.2 and 0.1 mg/mL. The calculated IC50 values for the tafluprost after exposure in main experiments 1 and 2 were 0.0095 and 0.012 mg/mL, respectively. This results in a mean IC50 value of 0.011 mg/mL and an estimated oral LD50 value of 0.811 mmol/kg, equivalent to 367 mg/kg bw.

Bimatoprost exhibited dose-related toxicity in the DRF, with a calculated IC50 value of 0.33 mg/mL, and it showed precipitation at 2 mg/mL. In the main experiments, bimatoprost exhibited precipitation at 1 and 2 mg/mL and demonstrated dose-related toxicity in both experiments. Plates were also visually inspected for signs of cytotoxicity. In experiment 1, cytotoxicity was observed at 2, 1 and 0.5 mg/mL. In experiment 2, toxicity was observed at 1 and 0.5 mg/mL. Due to precipitation, cytotoxicity could not be evaluated at 2 mg/mL. The calculated IC50 values for the bimatoprost after exposure in main experiment 1 and main experiment 2 were 0.17 and 0.20 mg/mL, respectively. This results in a mean IC50 value of 0.181 mg/mL and an estimated oral LD50 value of 2.90 mmol/kg, equivalent to 1204 mg/kg bw.

Table 1: Summary of Mean IC50 values and estimated Oral LD50 values for DDDE, tafluprost, and bimatoprost

	DDDE	Tafluprost	Bimatoprost
Mean IC50 (mg/mL)	0.083	0.011	0.181
Estimated oral LD50 (mg/kg bw/day)	880	367	1204

The studies for all three substances met all acceptance criteria: 1) the mean OD_{540} of vehicle controls was >1.1 (>0.30 required), with variance ≤5.2% (≤15% required); 2) the positive control SDS had IC50 values of 52, 37 and 44 µg/mL in the DRF and main experiments (<100 µg/mL required), within ±2.5 SD of the historical mean; 3) the Hill function R^2 for SDS was ≥0.98 (≥0.85 required); 4) the test material showed toxicity, and at least one calculated cytotoxicity value was >0% and ≤50% viability, while at least one other calculated cytotoxicity value was >50% and <100% viability in the main experiments, fulfilling the acceptance criteria. All criteria for a valid test were met, and the study was considered valid.

Conclusion

Under the study conditions, all three substances showed cytotoxicity with mean IC50 values for DDDE, tafluprost, and bimatoprost determined to be 0.083, 0.011, and 0.181 mg/mL, respectively. The

corresponding estimated LD50 values were 880 mg/kg bw for DDDE, 0367 mg/kg bw for tafluprost, and 1204 mg/kg bw for bimatoprost, indicating a ***similar*** **acute toxicity potential** for the three substances (Westerink, 2024a; b; c).

4.2. PGF2α RECEPTOR BINDING POTENCY ASSAY

The binding affinity of DDDE (purity: 99.78%), tafluprost (purity: 99.48%) and bimatoprost (purity: 99.8%) to prostaglandin F2α (PGF2α) receptors was assessed using isolated FP receptors derived from human recombinant (HEK-293) cells. The compounds were tested at 8 concentrations ranging from 1E-04 – 3E-10 M and the binding was calculated as a percentage inhibition of the binding of a ligand specific for each target. Results showing an inhibition or stimulation higher than 50% were considered to represent significant effects of the test compounds.

All three compounds demonstrated binding to the PGF2α receptor. The IC50 (half-maximal inhibitory concentration) and Ki (inhibitory constant) values for DDDE, tafluprost, and bimatoprost were calculated as 53 nM, 130 nM, and 160 nM, and 35 nM, 89 nM, and 110 nM, respectively (see **Table 2**).

Table 2: Binding affinity of the substances to the PGF2α receptor

	DDDE	Tafluprost	Bimatoprost
IC50	53 nM	130 nM	160 nM
Ki	35 nM	89 nM	110 nM

Under the study conditions, DDDE, tafluprost and bimatoprost showed ***similar*** **potencies of binding to the PGF2α receptor**. For perspective, the 2- to 3-fold differences observed in IC_{50} and K_i values are within the accepted experimental variability of ±0.5 log units, which is commonly observed even with standard reference compounds. As such, these differences are not considered biologically relevant and support the conclusion that DDDE, tafluprost, and bimatoprost exhibit similar receptor binding properties, thereby reinforcing the mechanistic plausibility of the RA approach used in this report (Dupuis, 2025).

4.3. TOXPROFILER® ASSAY

The toxic properties and toxicological mode of action of DDDE, tafluprost and bimatoprost were assessed with and without the addition of liver metabolism (rat S9) in the ToxProfiler assay using the HepG2 cell lines. The ToxProfiler assay consists of seven human reporter cell lines that were used to study chemically induced activation of seven distinct stress pathways: oxidative stress (SRXN1-GFP), cell cycle stress (p21-GFP), ER stress (CHOP-GFP), autophagy (LC3-GFP), ion stress (MT1X-GFP), protein stress (HSPA1B-GFP) and inflammation (ICAM1-GFP). The differential induction of the GFP reporters as well as cytotoxicity of the tested compounds were determined by live cell confocal microscopy. The stress biomarker induction was accurately quantified with a single cell resolution using automated image segmentation and quantification pipelines. A custom R-package “modelpod” was used to determine the lowest concentration of the test compounds that resulted in a significant induction of the stress biomarker.

The PoD plotter software was applied to determine if a stress pathway was significantly affected in a concentration-dependent manner. The same PoD approach was applied on the propidium iodide cytotoxicity data. **Table 3** summarises the ToxProfiler results.

Table 3. Summary of ToxProfiler results with their respective PoDs expressed in µg/mL

Compound name	Oxidative stress SRXN1	Cell cycle stress p21	ER stress CHOP	Autophagy LC3	Ion stress MT1X	Prot. Stress HSPA1B	Inflammation ICAM1	Cytotoxicity PI
DDDE - S9	**30**		60	166	80			125
DDDE + S9	**30**		120		120			153
Tafluprost - S9					90			90
Tafluprost + S9								
Bimatoprost - S9	**50**		259		160			330
Bimatoprost + S9	**50**		357	200				

Red colour: significant induction of the corresponding readout;
Green colour: no significant induction of the corresponding readout at the tested concentrations

Cytotoxicity was observed for all compounds in the absence of the liver enzyme fraction, S9, but in the presence of S9, it was only observed for DDDE in the tested concentration range. This suggests that S9-modified metabolism influenced the cellular response differently for each substance, potentially reducing the cytotoxic effects of bimatoprost and tafluprost. Such findings highlight the importance of including metabolic activation in *in vitro* assays to better reflect *in vivo* conditions and identify substance-specific differences in toxicity profiles Oxidative stress was found to be the primary toxicological mode of action for DDDE and bimatoprost with a PoD of 30 and 50 µg/mL, respectively. The effect was reduced for DDDE upon the addition of rat S9 while it was relatively less pronounced for bimatoprost. ER stress was also induced by DDDE at ≥60 µg/mL and bimatoprost at ≥259 µg/mL, but these effects were observed at higher concentration as compared to the oxidative stress response. In addition, mild autophagy was observed in the absence of S9 extracts for DDDE and in the presence of S9 for tafluprost. The corresponding PoDs were determined to be 166 µg/mL and 200 µg/mL. Further, tafluprost induced a mild ion stress response at the same concentration (90 µg/mL) at which a cytotoxic response was observed.

Conclusion

Overall, clustering of the ToxProfiler results revealed that bimatoprost and DDDE have a ***similar* toxicological profile**, predominantly characterised by oxidative stress in a similar potency range as primary mode of action (ter Braak, 2025).

4.4. REPROTRACKER® ASSAY

The three compounds were tested for their ability to interfere with human stem cell differentiation and formation of cardiomyocyte, hepatocyte and neural rosette cell types. The ReproTracker® assay consists of 3 steps which were followed for the assessment: (1) cytotoxicity testing and dose-range selection, (2) differentiation assays and (3) morphology and biomarker expression analysis.

1) Cytotoxicity testing and dose selection

Test:	Alamar Blue cell viability assay
Test system:	Human induced pluripotent stem cells (hiPSC)
Test substance:	DDDE, tafluprost and bimatoprost
Stock concentration:	1000 mg/mL for DDDE and analogues
Maximum concentration:	1000 µg/mL
Batch:	TAF-10-1122-01 (DDDE), TAF-IPE-0524-01 (tafluprost) and BIM-024-001 (bimatoprost)

Purity:	99% for the three test substances
Stock concentration:	1000 mg/mL for DDDE and analogues
Duration:	7 days
Number of dilutions:	20
Dilution steps:	2
Positive control:	5-fluorouracil, 5mM in DMSO
Negative control:	Saccharin
Study period:	2024-25

The cellular toxicity of each test substance was assessed using the Alamar Blue cell viability assay in undifferentiated hiPSCs after 7 days of exposure. Cells were seeded in a 96-well plate, incubated at 37°C for 24 hours, and then exposed to 20 two-fold serial dilutions of the test substance, with a maximum concentration of 1 mg/mL. Test substances were administered twice: at Day 0 and 3. After exposure, cells were washed with PBS and incubated with resazurin solution (0.01 mg/mL) for 2 hours. Fluorescence was measured using a FluoroSkan Microplate Fluorometer (excitation: 530 nm; emission: 590 nm) and normalised against solvent controls (complete mTeSR1 medium). 5-Fluorouracil and saccharin were used as positive and negative controls, respectively, in each experiment.

2) Differentiation assay

Test:	Differentiation assay
Test system:	hiPS cells
Test substance:	DDDE, tafluprost and bimatoprost
Purity:	99%
Test concentrations:	DDDE: 0, 3.9, 7.8, 15.6, 31.3 and 62.5 mg/mL, Tafluprost: 0, 2, 3.9, 7.8, 15.7 and 31.3 mg/mL Bimatoprost: 0, 7.8, 15.6, 31.3, 62.5 and 125 mg/mL
Number of concentrations:	5
Number of dilutions:	20
Dilution steps:	2
Batch:	TAF-10-1122-01 (DDDE), TAF-IPE-0524-01 (tafluprost) and BIM-024-001 (bimatoprost)
Purity:	99% for the three test substances
Stock concentration:	1000 mg/mL for DDDE and analogues
Duration:	7 days
Positive control:	5-fluorouracil (5mM in DMSO)
Negative control:	Saccharin
Study period:	2024-25

hiPSCs were differentiated into cardiomyocytes, hepatocytes, and neural rosettes. During differentiation, cells were exposed to five concentrations of the test substances, typically four non-cytotoxic and one cytotoxic concentration (≤20% cytotoxicity) to confirm exposure. Stock solutions were prepared in 2-fold dilutions using DMSO as solvent. Thalidomide and saccharin were used as positive and negative controls, respectively, for cardiomyocyte and hepatocyte differentiation, while retinoic acid and saccharin were used as positive and negative controls, respectively, for neural rosette differentiation. The controls were treated in the same manner as the to test substances.

Cardiomyocyte differentiation

hiPSCs were seeded in a 24-well plate one day before differentiation. At D0, fresh compound dilutions (0.1% v/v solvent) were added to the differentiation medium. Medium was refreshed on D2, D6 and D10, with the test substances being added at each exchange. Morphology was monitored on D7 and

D14 for unexpected cytotoxicity or possible disruption of proper stem cell differentiation. RNA samples were collected on D0, D7 and D14 for downstream biomarker expression analysis, using quantitative Reverse Transcription Polymerase Chain Reaction (qRT-PCR). Cardiomyocyte contraction was assessed on D14 using an Operetta CLS instrument.

Hepatocyte differentiation

hiPSCs were seeded in a 24-well plate four days before differentiation. At D0, fresh test substance dilutions (0.1% v/v solvent) were added to the differentiation medium. Medium was refreshed on D3, D7, D10, D14, and D17, with the test substance being added at each exchange. Morphology was monitored on D7 and D21 for cytotoxicity or differentiation disruption. RNA samples were collected on D0, D7 and D21 for biomarker expression analysis using qRT-PCR.

Neural rosette differentiation

hiPSCs were seeded in a 24-well plate one day before differentiation. At D0, fresh test substance dilutions (0.1% v/v solvent) were added to the differentiation medium. Medium was refreshed on D3, D6, and D10, with the test substance being added at each exchange. Morphology was monitored on D7 and D13 for cytotoxicity or differentiation disruption. RNA samples were collected on D0, D7, and D13 for biomarker expression analysis using qRT-PCR.

3) Morphology and biomarker expression analysis

Throughout differentiation, cells were microscopically evaluated for successful commitment to hepatocytes, cardiomyocytes and neural rosettes. Solvent-exposed cells displayed appropriate morphology without cytotoxicity. Hepatocytes at Day 21 should exhibit a polygonal shape with well-defined boundaries. Cardiomyocytes at day 14 should show diverse morphologies, including pacemaker-like (round to branched), atrial-like, and ventricular-like cells (elongated with oval nuclei and tension-oriented myofibrils), progressing to spontaneously contracting cells. Neural rosettes at Day 13 should display well-defined boundaries and multi-layered structures.

Biomarker expression was assessed using qRT-PCR to confirm proper cell-fate transition. RNA was isolated using the Maxwell RSC 48 instrument (Promega), and one-step qRT-PCR was performed with PrimeTime mastermix (IDT) and specific primer-probes on a QuantStudio 5 instrument (Applied Biosystems). Relative gene expression was calculated using the LogΔCt method, normalised to GAPDH. Acceptance criteria included Ct values <35 for both housekeeping and target genes, with minimal variation between technical replicates.

Results

Exposure to DDDE increased the expression of the mesodermal marker *BMP4* and decreased the expression of the cardiomyocyte-specific marker *MYH6* at concentrations ≥3.9 µg/mL in a concentration-dependent manner, which indicates cell arrest at the mesodermal stage. It also stopped the cardiac contraction ability of cardiomyocytes at 62.5 µg/mL. In addition, DDDE induced a decrease in the expression of the hepatocyte-specific biomarkers *FOXA2* and *AFP.* However, it induced the disruption of the morphology of hepatocytes in both biological replicates only at the highest tested concentration (HTC) of 62.5 µg/mL. The expression of the neuroectodermal marker *PAX6* was decreased at ≥15.6 µg/mL with no effect on the neural rosette-specific biomarker *NESTIN*. DDDE showed cytotoxicity at the HTC (62.5 mg/mL) and disrupted the morphology of neural rosettes in both biological replicates only at 31.3 µg/mL. (See **Table 4**, below, for a summary of results for all three substances.)

Tafluprost induced an increase in the expression of the mesodermal marker *BMP4* and a decrease in the expression of the cardiomyocyte-specific biomarker *MYH6* at 31.3 µg/mL (HTC), while it did not affect the cardiomyocytes contraction. It showed no significant effect on the endodermal marker *FOXA2.* However, it increased expression of the hepatocyte-specific biomarker AFP with no dose response and did not affect the hepatocyte morphology. Tafluprost had no significant effect on the

expression pattern of the neuroectodermal marker *PAX6* or the neural rosette-specific biomarker *NESTIN,* nor did it disrupt the morphology of neural rosettes.

Bimatoprost had no significant effect on the expression pattern of the cardiomyocyte-specific biomarkers *BMP4* and *MYH6*, nor on the contraction ability of cardiomyocytes. For the hepatocyte differentiation, it showed no significant effect on the endodermal marker *FOXA2* but led to a significant decrease in the expression of the hepatocyte-specific biomarker *AFP* at 125 µg/mL (HTC) and disrupted hepatocyte morphology at ≥62.5 µg/mL. Bimatoprost caused a concentration-dependent decrease in the expression of the neuroectodermal marker *PAX6* and the neural rosette-specific biomarker *NESTIN*, starting from 7.8 µg/mL. It also disrupted the morphology of neural rosettes at the two highest tested non-cytotoxic concentrations ≥31.3 µg/mL.

The results of the ReproTracker® are presented below.

Table 4. Summary of the ReproTracker results

Compound	Reduction of biomarker expression			Morphological/functional changes			NOAEL	LOAEL
	Cardiomyocytes	Hepatocytes	Neural rosettes	Decreased cardiomyocyte contraction	Altered hepatocyte morphology	Altered neural rosette morphology		
Dechloro Dihydorxy Difluoro Ethylcloprostenolamide	Yes	No	Yes	No	No	No	**n.d.**	**3.9 µg/mL**
Tafluprost Isopropyl Ester	No	No	No	No	No	No	**15.7 µg/mL**	**31.3 µg/mL**
Bimatoprost (15S-Bimatoprost)	No	No	Yes	No	Yes	Yes	**n.d.**	**7.8 µg/mL**
Assay controls								
Thalidomide	Yes	Yes	-	Yes	Yes	-	**n.d.**	**0.1 µM**
Retinoic acid	-	-	Yes	-	-	Yes	**n.d.**	**0.3 µM**
Saccharin	No	No	No	No	No	No	**250 µM**	**n.d.**

Conclusion

Under the study conditions, DDDE decreased the biomarker expression for cardiomyocytes and neural rosettes at two consecutive concentrations; however, it did not induce any changes in the contraction of cardiomyocytes and in the morphology of hepatocytes and neural rosettes. Tafluprost increased the expression (not significant) of the mesodermal marker *BMP4* and decreased the expression of the cardiomyocyte-specific biomarker *MYH6* (not significant) at the highest tested concentration; however, it did not affect the contraction ability of cardiomyocytes. Bimatoprost significantly decreased the neuroectodermal marker expression and induced morphological changes in hepatocytes and neural rosettes. Under the study conditions, the lowest concentration at which changes in either the biomarker expression levels and/or cell morphology/function was determined to be 3.9 µg/mL for DDDE, 31.3 µg/mL for tafluprost and 7.8 µg/mL for bimatoprost.

Overall, under the *in vitro* testing conditions, bimatoprost was more toxic than tafluprost and DDDE. However, it should be noted that the *in vitro* test results for tafluprost do not reflect the *in vivo* results (see **Section 3 of Annex II**) and this could be due to the fact that the *in vivo* test conditions represent a more biological relevant test system for evaluating this complex endpoint.

(Hartvelt, 2025)

4.5. RECEPTOR BINDING AND ACTIVATION ANALYSES

4.5.1. *In silico* predictions for receptor binding and activation with focus on developmental and reproductive toxicity/endocrine endpoints

The *in silico* receptor binding and activation predictions with a focus on developmental and reproductive toxicity (DART)/endocrine endpoints was evaluated using different *in silico* tools (see **Table 5**, below).

Table 5. *In silico* predictions for DDDE, tafluprost and bimatoprost*

	(Q)SAR models	DDDE	Tafluprost	Bimatoprost
DART	OECD QSAR Toolbox v.4.7	No alerts	No alerts	No alerts
	Derek Nexus version 6.4.1	No alerts	No alerts	Teratogenicity in mammal is EQUIVOCAL** (Extrapolation from Glucocorticoid receptor agonism)
Oestrogen	OECD QSAR Toolbox v.4.7	Strong binder, OH group	Strong binder, OH group	Strong binder, OH group
	Derek Nexus version 6.4.1	No alerts	No alerts	No alerts
	Danish QSAR models	ERα binding: Positive, within the applicability domain (ID) ERα activation: Negative, out of the applicability domain (OD)* Erβ activation at max. 20µM (*in vitro*): Negative (ID) Erβ activation at max. 50µM (*in vitro*)* Erβ inhibition at max. 10µM (*in vitro*): Positive (OD)* Erβ inhibition at max. 20µM (*in vitro*): Positive (ID) ER activation: Negative (ID)	ERα binding: Positive, within the applicability domain (ID) ERα activation: Negative, out of the applicability domain (OD)* ERα activation: Negative (OD)* Erβ activation at max. 20µM (*in vitro*)* Erβ activation at max. 50µM (*in vitro*): Positive (OD)* Erβ inhibition at max. 10µM (*in vitro*): Positive (OD)* Erβ inhibition at max. 20µM (*in vitro*): Positive (ID) ER activation: Negative (ID)	ERα binding: Negative, within the applicability domain (ID) ERα activation: Negative (ID) Erβ activation at max. 20µM (*in vitro*): Negative (ID) Erβ activation at max. 50µM (*in vitro*): Negative (ID) Erβ inhibition at max. 10µM (*in vitro*): Positive (ID) Erβ inhibition at max. 20µM (*in vitro*): Positive (ID) ER activation: Negative (OD)*
	VEGA v.1.2.3	ER relative binding affinity: Inactive (OD)* ER mediated effects: Inactive (ID)	ER relative binding affinity: Inactive (OD)* ER mediated effects: Inactive (ID)	ER relative binding affinity: Inactive, out of the applicability domain (OD)* ER mediated effects: Inactive (ID)
	Endocrine disruptome	Medium binding to ERα as agonist/antagonist	No binding to ERα as agonist/antagonist	No binding to ERα as agonist/antagonist
Androgen	Derek Nexus version 6.4.1	No alerts	No alerts	No alerts

	(Q)SAR models	DDDE	Tafluprost	Bimatoprost
	Danish QSAR models	AR binding: Negative (OD)* AR activation: Negative (ID) AR inhibition: Negative (ID)	AR binding: Negative (ID) AR activation* AR inhibition: Negative (ID)	AR binding* AR activation* AR inhibition: Negative (OD)*
	VEGA v.1.2.3	AR binding activity: Active (OD)*	AR binding activity: Active (OD)*	AR binding activity: Active (OD)*
	Endocrine disruptome	Potential binding to AR as antagonist	Potential binding to AR as antagonist	Potential binding to AR as antagonist
Thyroid modalities	Derek Nexus version 6.4.1	No alerts	No alerts	No alerts
	Danish QSAR models	-	-	-
	VEGA v.1.2.3	TR_{α} and TR_{β} binding: Inactive (ID)	TRα and TRβ binding: Inactive (ID)	TRα and TRβ binding: Inactive (ID)
	Endocrine disruptome	Medium probability to bind TR_{α}	Medium probability to bind TR_{α}	Medium probability to bind TR_{α}
Steroidogenesis	Derek Nexus version 6.4.1	No alerts	No alerts	No alerts
	Endocrine disruptome	Medium probability to bind to GR as agonist	Medium probability to bind to GR as agonist	Medium probability to bind to GR as agonist
Other receptors	Danish QSAR models	TSHR inhibition at any concentration (in vitro)* PPARγ activation at max. 20 µM (*in vitro*): Positive (ID) PPARγ activation at max. 50 µM (*in vitro*): Negative (ID) PPARγ inhibition at max. 10 µM (*in vitro*): Positive (ID) PPARγ inhibition at any concentration (*in vitro*): Positive (ID) RAR inhibition at max. 10 µM (in vitro)* RAR inhibition at any concentration (*in vitro*): Positive (ID) PXR binding (human in vitro)* PXR binding, new model (human *in vitro*): Negative (OD)*	TSHR inhibition at any concentration (*in vitro*)* PPARγ activation at max. 20 µM (*in vitro*): Positive (OD)* PPARγ activation at max. 50 µM (*in vitro*)* PPARγ inhibition at max. 10 µM (*in vitro*): Positive (ID) PPARγ inhibition at any concentration (*in vitro*): Positive (ID) RAR inhibition at max. 10 µM (*in vitro*): Negative (ID) RAR inhibition at any concentration (*in vitro*): Positive (OD) PXR binding (human *in vitro*): Positive (ID)	TSHR inhibition at any concentration (*in vitro*): Positive (ID) PPARγ activation at max. 20 µM (*in vitro*): Positive (OD)* PPARγ activation at max. 50 µM (*in vitro*)* PPARγ inhibition at max. 10 µM (*in vitro*)* PPARγ inhibition at any concentration (*in vitro*)* RAR inhibition at max. 10 µM (*in vitro*): Positive (ID) RAR inhibition at any concentration (*in vitro*): Positive (OD)* PXR binding (human *in vitro*): Positive (OD)*

	(Q)SAR models	DDDE	Tafluprost	Bimatoprost
		PXR activation (human *in vitro*): Negative (OD)* PXR activation (rat *in vitro*): Negative (OD)* AhR agonism, rational sampling (human *in vitro*): Negative (ID) AhR agonism, random sampling (human *in vitro*): Negative (ID) CAR activation at max. 20 µM (*in vitro*): Negative (OD)* CAR activation at max. 50 µM (*in vitro*): Negative (ID) CAR inhibition at max. 20 µM (*in vitro*): Negative (OD)* CAR inhibition at max. 50 µM (in vitro)*	PXR binding, new model (human *in vitro*): Positive (OD)* PXR activation (human *in vitro*): Positive (ID) PXR activation (rat *in vitro*)* AhR agonism, rational sampling (human *in vitro*): Negative (ID) AhR agonism, random sampling (human *in vitro*): Negative (ID) CAR activation at max. 20 µM (*in vitro*): Negative (OD)* CAR activation at max. 50 µM (*in vitro*): Positive (ID) CAR inhibition at max. 20 µM (*in vitro*): Negative (ID) CAR inhibition at max. 50 µM (*in vitro*)*	PXR binding, new model (human *in vitro*): Negative (ID) PXR activation (human *in vitro*): Negative (OD)* PXR activation (rat *in vitro*): Negative (ID) AhR agonism, rational sampling (human *in vitro*): Negative (ID) AhR agonism, random sampling (human *in vitro*): Negative (ID) CAR activation at max. 20 µM (*in vitro*): Negative (ID) CAR activation at max. 50 µM (*in vitro*): Negative (ID) CAR inhibition at max. 20 µM (*in vitro*)* CAR inhibition at max. 50 µM (*in vitro*)*
	Endocrine disruptome	Low probability to bind to PPARα and PPARβ as agonist Low probability to bind to RXRα as agonist Low probability to bind to PR as agonist	Low probability to bind to PPARα and PPARβ as agonist Low probability to bind to RXRα as agonist Low probability to bind to PR as agonist	Low probability to bind to PPARα and PPARβ as agonist Low probability to bind to RXRα as agonist Low probability to bind to PR as agonist

ER: estrogen receptor; AR: androgen receptor; TR: thyroid hormone receptor; GR: glucocorticoid receptor ; TSHR: thyroid stimulating hormone receptor; PPAR: peroxisome proliferator-activated receptor ; RAR: retinoic acid receptor; PXR: pregnane X receptor ; AhR: aryl hydrocarbon receptor; CAR: chimeric antigen receptors; RXR: retinoic X receptor; PR: progesterone receptor.

*The predictions which were 'inconclusive' and outside the applicability domain were not taken into consideration.

**There is an equal weight of evidence for and against the proposition

Conclusion: Based on the *in silico* analysis using widely recognised (Q)SAR models, **DDDE presents an overall *similar* profile to tafluprost and bimatoprost with respect to receptor binding and activation potentia**l.

4.5.2. Literature research of endocrine receptor activation by analogues

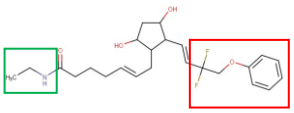
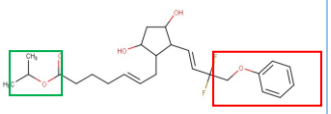
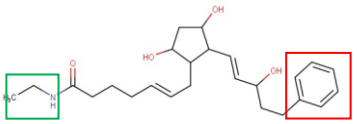
No endocrine disruption (ED) relevant *in vitro* receptor binding or activation assays as defined in the OECD Conceptual Framework Level 2 (OECD, 2018) could be identified for DDDE or its analogues tafluprost and bimatoprost.

4.6. IMPACT OF THE PRESENCE OF GEMINAL FLUORINE MOIETY ON THE TOXICOLGICAL PROFILE

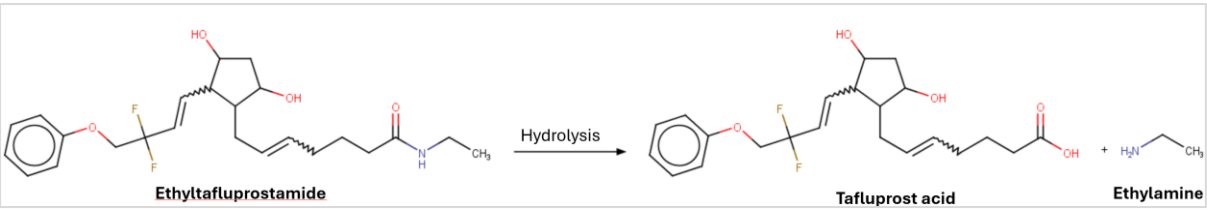
The three substances are prostaglandin derivatives, in which the 'prostaglandin core' is bound on one side to a benzene group - via an ether bond in the case of DDDE and tafluprost and via a non-ether bond in the case of bimatoprost (red square in **Table 6**) - and, on the other side, to an ester group in the case of tafluprost and to an amide group in the case of DDDE and bimatoprost (green square in **Table 6**).

In summary, DDDE and the analogues, tafluprost and bimatoprost share the key functional groups, such as 'cycloalkane' and 'aryl' groups, with tafluprost lacking the amide functional group and bimatoprost lacking the 'geminal fluorine' moiety.

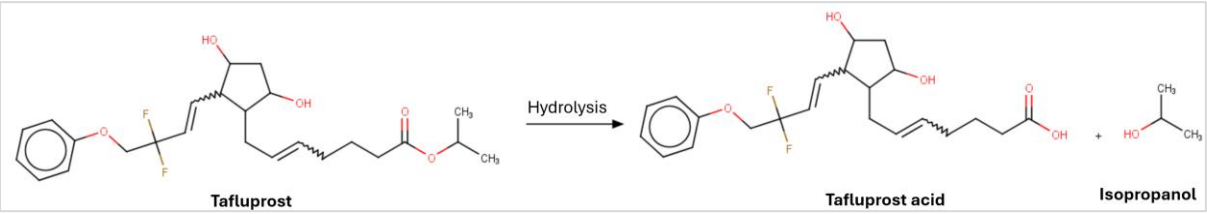
Table 6: Structures of DDDE, Tafluprost and bimatprost

	DDDE	Tafluprost	Bimatoprost
2D Structure			

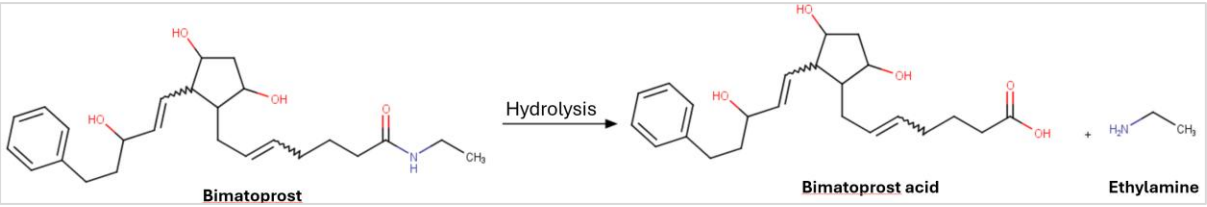
Regarding their metabolism differences, the first metabolic reaction is hydrolysis for the target (DDDE) and source (tafluprost and bimatoprost) substances. Specifically, for DDDE about 65.8 - 71.2% of the absorbed fraction was found to be transformed into its main metabolite, tafluprost acid after 24 hours (Weghuber, 2022).



Tafluprost has been shown to be hydrolysed in the eye to give rise to its main metabolite, tafluprost acid, which is further metabolised via fatty acid β-oxidation and phase II conjugation (CDER, 2011a).



Similarly, bimatoprost has been described to undergo amide hydrolysis with the formation of bimatoprost acid, and to a lesser extent oxidation, N-deethylation and glucuronidation (Al-Ghananeem and Crooks, 2007; HRES, 2018).



Therefore, in the case of DDDE and tafluprost, the test organism is mainly exposed to tafluprost acid, which is a common metabolite, still containing the fluorine geminal group and representing the driver of toxicity.

Conclusion: The potential differences due to the presence of the germinal fluorine group are addressed by considering the toxicological data of tafluprost whose metabolite, tafluprost acid, is common with DDDE and still contains the geminal fluorine group.

5. READ ACROSS ANALYSES AND JUSTIFICATION

The following RA analysis and justification have been put together to support the safety of DDDE by means of RA, using available *in vivo* information for the identified analogues, in accordance with the structured workflow presented in ECHA's Read Across Assessment Framework (RAAF) (ECHA, 2017). It intends to 1) identify and select potential analogues of DDDE with toxicological data, and 2) demonstrate that the selected analogues share common properties with the target substance (i.e., DDDE) as required for the purpose of RA.

5.1 IDENTIFICATION AND SELECTION OF POTENTIAL ANALOGUES OF DDDE

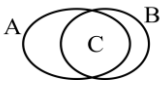
Based on the recommendation of the SCCS note of guidance, several *in silico* tools can be used to identify 'closely related analogues' with a similarity index[1] ≥0.7 (SCCS, 2023). In this frame, GenRA (Patlewicz and Shah, 2023) v.3.3.1, ToxEraser cosmetics GUI (VEGA HUB, 2025b) v.0.8.5, LRI AMBIT2 Read Across tool (AMBIT, 2024) and ToxRead (VEGA HUB, 2025a) v.0.25, which are recommended by SCCS (SCCS, 2023), have been used to identify potential analogues with a similarity index ≥0.7[2]. The identified analogues were screened in order to determine whether they could be taken into further consideration for read across purposes: the similarity index - calculated in the same manner for the different analogues using the Dice index[3] of the OECD QSAR Toolbox v.4.7 (OECD, 2024) -, common functional groups and common structural alerts were used as selection criteria (**Part A of Annex I**).

As shown in **Table 7**, GenRA v.3.3.1 and LRI AMBIT2 Read Across tool did not identify analogues with a similarity index ≥0.7. The analogue identified by ToxEraser cosmetics v.0.8.5, sodium D-pantothenate, was not taken into further consideration, as it did not present key functional groups present in DDDE (e.g., 'aryl', 'alkyl halide' and 'cycloalkane' functional groups) and showed differences in structural alerts (see **Part A of Annex I** for further details). ToxRead v.0.25 identified several analogues, with only tafluprost (CAS No. 209860-87-7) fulfilling the three selection criteria. In fact, tafluprost presents a relatively high Dice index (0.86), common functional groups (e.g., 'aryl', 'alkyl halide' (geminal fluorine), 'cycloalkane' and 'ether moieity') and the same structural alerts when compared to DDDE.

[1] The similarity index quantifies the structural resemblance between molecules based on shared features, with higher values indicating greater similarity. It calculates the overlap of structural features, often represented as binary fingerprints.

[2] The selection was carried out based on the similarity index of each tool.

[3] The Dice index measures the similarity between two molecules on the basis of structure fingerprints, by counting the number of bits 'on' in both molecules (C) and the number of bits 'on' in each molecule separately (A, B).



The Dice index is calculated with the following formula: 2C/A+B.

Table 7: Potential analogues of DDDE identified using different *in silico* tools

***In silico* tool**	**Analogue**	**Conclusion**
GenRA v.3.3.1	No analogue identified with the similarity index of the tool ≥0.7	-
ToxEraser cosmetics GUI v.0.8.5	Sodium D-pantothenate (CAS No. 867-81-2)	The analogue has not been taken into further consideration due to the low Dice index (0.17) and the differences in the key functional groups and structural alerts (see **Annex I**)
LRI AMBIT2 Read Across tool	No analogue identified	-
ToxRead v.0.25	<u>Module for the reproductive toxicity endpoint</u> - Tafluprost (CAS No. 209860-87-7) - 7-(3,5-dihydroxy-2-{2-[2-(phenoxymethyl)-1,3-dioxolan-2-yl]ethenyl}cyclopentyl)hept-5-enoic acid (CAS No. 77698-96-5) - Cloprostenol (CAS No. 40665-92-7) <u>Module for the carcinogenicity endpoint</u> - Misoprostol (CAS No. 59122-46-2) - Timodine (CAS No. 60103-17-5) - Triamcinolone acetonide (CAS No. 76-25-5)	**Tafluprost** has been selected for further analysis because of the Dice index (0.86), common functional groups (cycloalkane, ether moiety and difluoro functional groups) and common structural alerts. The other analogues did not fulfil the selection criteria (see **Annex I** for further details).

Tafluprost shares the 'prostaglandin core' as well as the 'geminal fluorine' functional group with DDDE; however, it does not contain the amide functional group present in DDDE, which is considered a 'prostamide' type of molecule. Prostamides have been described to bind to a novel prostamide receptor (Matias *et al.*, 2004; Woodward *et al.*, 2013), in addition to the canonical prostanoid receptors. This prostamide receptor is expressed in few cell types, including feline iris, feline lung parenchyma and rabbit uterus (Woodward *et al.*, 2008).

The Federal Institute for Risk Assessment of Germany (BfR) considered DDDE, tafluprost and bimatoprost (CAS No. 155206-00-1) as analogues with 'high structure similarity' (BfR, 2017). The grouping of these three substances has been proposed by an opinion released by the SCCS on prostaglandins and prostaglandin-analogues used in cosmetic products in February 2022 (**Figure 1**) (SCCS, 2022). In consideration of these factors, **<u>bimatoprost</u> has been selected as a second analogue in the RA analysis of DDDE**.

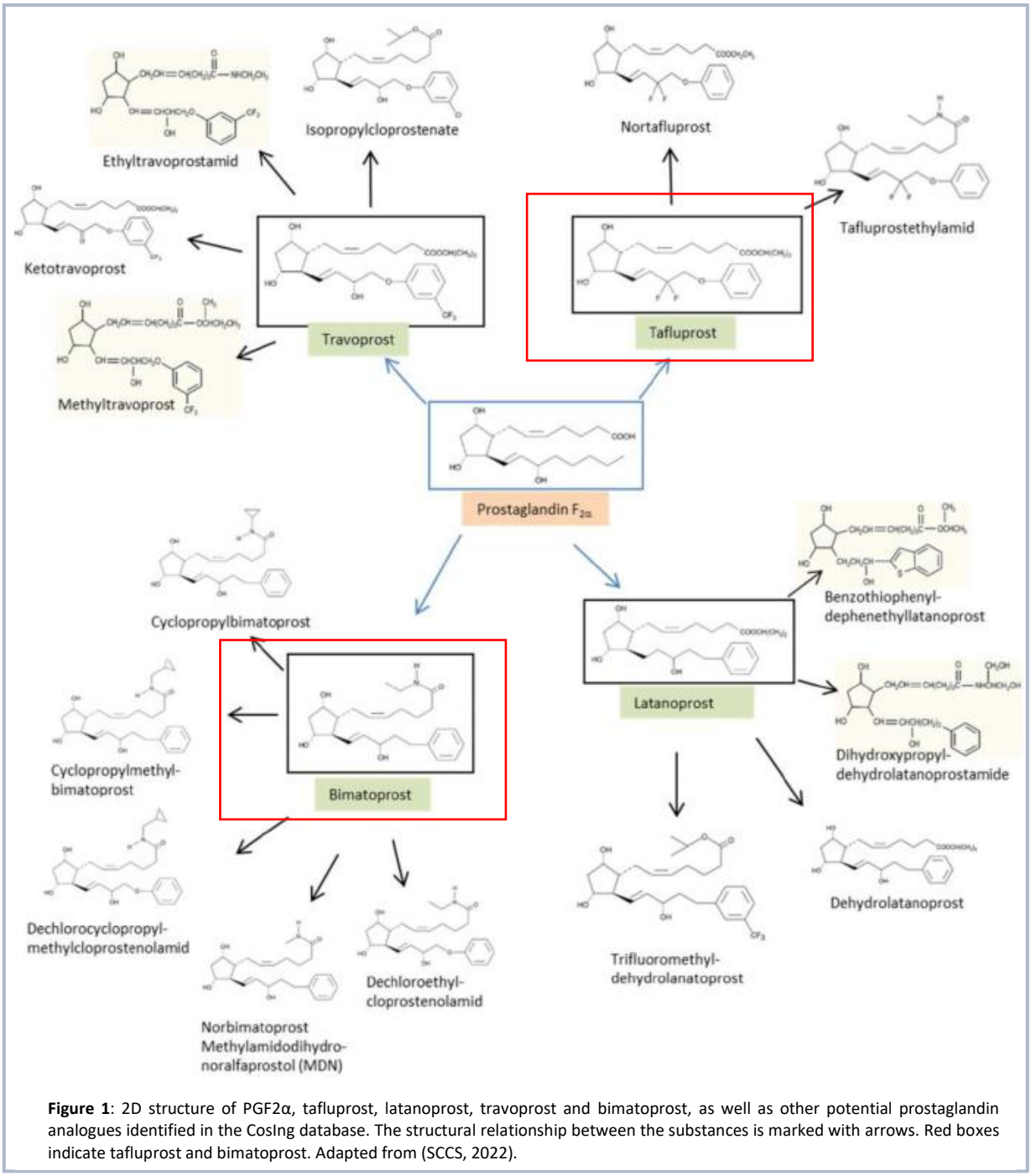


Figure 1: 2D structure of PGF2α, tafluprost, latanoprost, travoprost and bimatoprost, as well as other potential prostaglandin analogues identified in the CosIng database. The structural relationship between the substances is marked with arrows. Red boxes indicate tafluprost and bimatoprost. Adapted from (SCCS, 2022).

5.2 PREPARATORY ASSESSMENT

Tafluprost (source substance 1) and bimatoprost (source substance 2), on which toxicological tests have been conducted, have been selected as potential RA source substances.

The studies from the source substances will be used under an analogue approach for the following endpoints:

- Repeated dose toxicity
- *In vivo* genotoxicity
- Carcinogenicity
- Reproductive toxicity
- Developmental toxicity

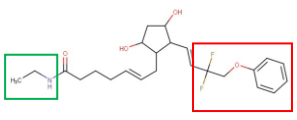
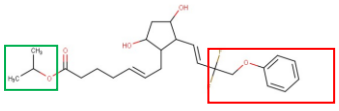
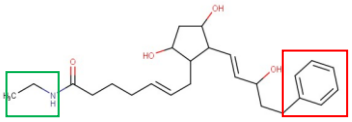
The following assessment is intended to demonstrate that DDDE (target substance) and the two source substances have common properties and present a comparable toxicological behaviour, so that RA between them is justified.

5.3 CHARACTERISATION OF THE TARGET AND SOURCE SUBSTANCES

5.3.1 Overview of the target and source substances

DDDE, tafluprost and bimatoprost are all mono-constituents with a degree of purity of at least 98% (**Table 8**). The three substances are prostaglandin derivatives, in which the 'prostaglandin core' is bound on one side to a benzene group - via an ether bond in the case of DDDE and tafluprost and via a non-ether bond in the case of bimatoprost (red square in **Table 8**) - and, on the other side, to an ester group in the case of tafluprost and to an amide group in the case of DDDE and bimatoprost (green square in **Table 8**).

Table 8: Overview of the target and source substances

	Target substance	Source substance 1	Source substance 2
Name	DDDE	Tafluprost	Bimatoprost
CAS No.	1185851-52-8	209860-87-7	155206-00-1
EC No.	867-521-0	682-458-7	642-890-9
2D Structure			
Molecular weight (g/mol)	437.53	452.5	415.6
Purity	99%	99%	98%

5.3.2 Manufacturing process

The production of the target and source substances follows a common synthetic pathway starting from corey lactone acetate (CAS No. 57930-46-8) to produce prostaglandin derivatives via 10 intermediate reaction steps (**Figure 2**). Specifically, corey lactone acetate is first converted into an alcohol and subsequently oxidised to the corresponding aldehyde, which then undergoes an olefination reaction, in order to introduce the alkyl side chain. This reaction results in the formation

of an enone, which undergoes either fluorination in the case of DDDE and tafluprost, or reduction to an alcohol in the case of bimatoprost. The lactone ring is then opened through nucleophilic attack, enabling the formation of the second aliphatic chain. Protecting groups on hydroxyl functionalities are selectively removed or modified to facilitate subsequent transformations. DDE and bimatoprost are finally converted into an amide, while tafluprost undergoes isopropyl ester formation.

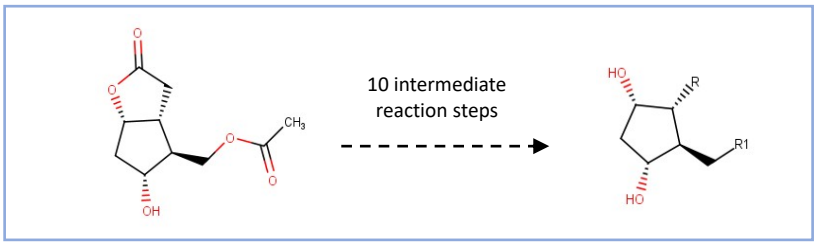


Figure 2: Corey lactone acetate general transformation to prostaglandins

5.4 READ ACROSS CONSIDERATIONS

In accordance with the EU REACH Regulation (EU, 2008) and with the ECHA read across assessment framework (RAAF) (ECHA, 2017), RA of environmental fate, toxicological or ecotoxicological data from an analogue may be justified on the basis of:

- Common functional groups and structure
- Common structural alerts or reactivity
- Similar physico-chemical properties
- Likelihood of common breakdown products via biological/degradation processes

The following sections review the target and source substances against these aspects to support the hypothesis that the three substances show qualitatively and quantitatively a comparable toxicological behaviour.

5.4.1 Common functional groups and structure

The target and the source substances present a 'prostaglandin core' sharing key functional groups, such as 'cycloalkane' and 'aryl' groups. In addition, the target substance and source substance 1 share the 'alkyl halide' group and more specifically the 'geminal fluorine' group[4]. The target and source substance 2 share the amide functional group. This chemical similarity was confirmed by a relatively high similarity Dice index of the source substances to the target substance (**Table 9**). This index was calculated using the OECD QSAR Toolbox v.4.7 (OECD, 2024).

Table 9: Dice index of the source substances compared to the target substance

	Tafluprost *versus* DDDE	**Bimatoprost *versus* DDDE**
Dice index	0.86	0.79

5.4.2 Common structural alerts and reactivity

Because of the presence of the same key functional groups in the target and the source substances, it is not expected to find significantly different structural alerts. Indeed, (Q)SAR analysis, as provided by the OECD QSAR Toolbox v.4.7 (OECD, 2024), showed that **the target and source substances share *similar* structural alerts** (see **Part B of Annex II**). The following summarises the similarities in structure

[4] Geminal fluorine corresponds to a substituent with two fluorine atoms attached to the same carbon.

and where available, positions the (Q)SAR information with existing toxicological data for the target substance and the source substances (see also **Section 5.6**):

Systemic toxicity: According to the OECD QSAR Toolbox toxicity classification, the target and source substances are classified as Cramer Class III (high toxicity) chemical by the 'toxic hazard classification by Cramer' profiler. Cramer Class III chemicals refer to substances with chemical structures that permit no strong initial presumption of safety or may even suggest significant toxicity or have reactive functional groups. This analysis is in agreement with *in vitro* acute oral toxicity studies in rat fibroblasts available for the target and source substances, showing that they all have moderate acute oral toxicity with LD50 value of 880 mg/kg bw for the target substance, 367 mg/kg bw for source substance 1 and 1204 mg/kg bw for source substance 2. In addition, *in vivo* sub-acute, sub-chronic and chronic studies available for tafluprost (via the parenteral and ocular routes) and bimatoprost (via the oral, parenteral and ocular routes) revealed critical toxicity, such as effects on the reproductive, haematological, and skeletal systems at doses ranging from ≥3 to 16000 µg/kg bw/day, depending on the route, duration, and substance tested.

Overall, tafluprost revealed a more severe toxicity profile and lower NOAELs compared to bimatoprost irrespective of the route of exposure. Specifically, haematological and bone marrow-related changes were observed following intravenous administration of tafluprost at doses ≥10 µg/kg bw/day in rats. Additionally, bone changes including hyperostosis and increased splenic extramedullary haematopoiesis were seen with subcutaneous administration at ≥3 µg/kg bw/day. The NOAELs across all studies ranged from 1 to 100 µg/kg bw/day.

For bimatoprost, oral administration caused testicular degeneration and ovarian changes at concentrations ranging from 0.3 to 16 mg/kg bw/day, while intravenous dosing led to effects on corpora lutea at a concentration of 1 mg/kg bw/day. The NOAEL across all studies ranged from 0.1 to 16 mg/kg bw/day.

Ocular administration of both substances in monkeys, rabbits and/or dogs did not result in systemic toxicity at doses up to 10 µg/kg bw/day for tafluprost and up to 127.27 µg/kg bw/day for bimatoprost. The observed effects were limited to local changes related to this type of molecule and considered non-adverse (**Section 5.4.6.2**).

Irritation/corrosion: ***The target and source substances do not present any alerts for skin irritation or eye irritation***. *In vitro* and *in vivo* skin and eye irritation studies available for DDDE and *in vivo* eye irritation studies available for tafluprost and bimatoprost showed that the three substances are not irritating to skin and eye at their respective use concentrations (**Section 5.6**).

Skin sensitisation: ***The target and source substances do not present structural alerts for skin sensitisation***. *In vitro and in vivo* studies (target substance) and *in vivo* studies (target and source substances) did not reveal any signs indicative of skin sensitisation, confirming similarity of the tree substances for this endpoint (**Section 5.6**).

Genotoxicity and carcinogenicity: Across the genotoxicity and carcinogenicity profilers used by the OECD QSAR Toolbox, DDDE, tafluprost and bimatoprost show a similar pattern of predicted structure activity for genotoxicity and carcinogenicity. Specifically, DDDE and tafluprost do not present any alerts related to genotoxicity, while bimatoprost presents only an alert identified by the 'DNA binding alert by OECD v.2.3' profiler. This analysis is in agreement with *in vitro* (target and source substances) and *in vivo* studies (source substances 1 and 2), showing that ***the three substances are not genotoxic*** (**Section 5.6**). In addition, carcinogenicity studies in mice and rats available for tafluprost and bimatoprost showed that both substances do not present a potential for carcinogenicity.

Reproductive and developmental toxicity: ***The target and source substances do not present any alerts related to reproductive and developmental toxicity***. The available studies on reproductive toxicity - via the intravenous route for tafluprost and the oral route for bimatoprost - did not indicate any significant effects on fertility and on reproductive parameters in rats at doses up to 100 µg/kg bw/day

for tafluprost and up to 600 µg/kg bw/day for bimatoprost. Pre-natal and post-natal toxicity studies are available for tafluprost via the intravenous route in rats and rabbits, and for bimatoprost via the oral route in rats and mice. Both substances showed maternal and developmental effects: tafluprost caused intrauterine death, teratogenicity and delayed development, while bimatoprost induced maternal toxicity and increased pup mortality without significant teratogenicity. The developmental NOAEL for tafluprost was set in the range between 0.3 µg/kg bw/day and 3 µg/kg bw/day in rats and at 0.01 µg/kg bw/day in rabbits, while for bimatoprost it was set at 600 µg/kg bw/day in mice and in the range between 100 and 600 µg/kg bw/day in rats (see **Section 5.6**).

5.4.3 Similar physico-chemical properties

The target substance is in the liquid form, while both source substances are in the solid form. The target and the source substances have comparable physico-chemical properties with similar estimated values with respect to melting point, boiling point and vapour pressure. They show some differences with respect to log Kow value, with the target substance and tafluprost having estimated values in the same range and bimatoprost presenting the lowest value. As to water solubility, the target substance and tafluprost present very low solubility, while bimatoprost presents a higher value (see **Section 5.6**). ***This suggests that in relative terms bimatoprost is more bioavailable as compared to DDDE and tafluprost***.

5.4.4 Likelihood of common breakdown products via degradation/biological processes

The skin metabolism of the target substance was investigated in an OECD Test Guideline 428-compliant *in vitro* study. Following topical application of DDDE to human skin *in vitro*, about 65.8 - 71.2% of the absorbed fraction of the target substance was found to be transformed into its main metabolites, tafluprost acid and ethylamine (CAS No. 75-04-7) (Weghuber, 2022). Tafluprost has been shown to be hydrolysed in the eye to give rise to its main metabolite, tafluprost acid, which is further metabolised via fatty acid β-oxidation and phase II conjugation (CDER, 2011a). Similarly, bimatoprost has been described to undergo amide hydrolysis with the formation of bimatoprost acid, and to a lesser extent oxidation, N-deethylation and glucuronidation (Al-Ghananeem and Crooks, 2007; HRES, 2018).

Conclusion

Overall, the results of the studies on metabolism of the target and source substances indicate that DDDE, tafluprost and bimatoprost undergo hydrolysis, with ***DDDE and tafluprost sharing the main metabolite, tafluprost acid***.

5.4.5 Similar biological activities

In order to gain mechanistic insights on the mode of action (MoA), biological assays were performed for DDDE, tafluprost and bimatoprost. Specifically, the target and source substances were analysed to determine whether they present 1) a similar binding affinity to the PGF2α receptor, and 2) the same toxicological profile when analysed in two biological assays, ToxProfiler® and ReproTracker®.

5.4.5.1 Binding assay to the PGF2α receptor

The target and source substances were studied in a binding assay using isolated FP receptors derived from human recombinant (HEK-293) cells, in order to determine their affinity to the PGF2α receptor. Specifically, the binding of radiolabelled PGF2α was assessed in the presence of each substance (agonist radioligand). As shown in **Table 2**, the target substance presented an IC50 value of 53 nM (half-maximal inhibitory concentration) and a Ki value (inhibitory constant) of 35 nM, while source substance 1 showed an IC50 value of 130 nM and a Ki value of 89 nM and source substance 2 presented an IC50 value of 160 nM and a Ki value of 110 nM. ***Overall, DDDE, tafluprost and bimatoprost showed binding to the PGF2α receptor and are more or less within the same range of potency***.

5.4.5.2 ToxProfiler assay

The target and source substances were analysed by using the ToxProfiler® platform (Niemeijer *et al.*, 2018; Wink *et al.*, 2017; Wink *et al.*, 2018). This platform consists of a panel of HepG2 reporter cell lines cultured as a 2D monolayer, in which biomarkers of cellular stress response pathways - oxidative stress (SRXN1), cell cycle stress (p21), endoplasmic reticulum (ER) stress (CHOP), autophagy (LC3), ion stress MT1X), protein stress HSPA1B) and inflammation (ICAM1) - have been tagged with a fluorescent fluorophore, green fluorescent protein (GFP).

As shown in **Table 3**, DDDE induced oxidative stress as the primary MoA in a concentration-dependent manner, with the Point of Departure (PoD) being set at 30 µg/mL. ER, ion stress and autophagy responses were also induced at higher concentrations (≥60 µg/mL). The addition of liver metabolism (rat S9) showed that metabolism has a detoxifying effect on DDDE.

Tafluprost induced ion stress and cytotoxicity at concentrations ≥90 µg/mL, with the addition of S9 liver metabolism extract having a detoxifying effect on the substance (**Table 3**).

Bimatoprost induced oxidative stress as the primary MoA in a concentration-dependent manner with the PoD being set at 50 µg/mL. ER, ion stress and autophagy responses were also induced at higher concentrations (≥160 µg/mL). The addition of liver metabolism (rat S9) showed that metabolism has a small detoxifying effect on bimatoprost.

These results suggest that the profile of DDDE versus bimatoprost is more similar than the profile of DDDE versus tafluprost, when considering the biological activity.

5.4.5.3 ReproTracker assay

The effect of the target and source substances on the early phases of the developmental processes was studied using the ReproTracker® assay. This assay is a human induced pluripotent stem cells (hiPSCs)-based biomarker assay, that assesses various key events during early embryonic development (Jamalpoor *et al.*, 2022). As shown in **Table 4**, DDDE induced a decrease of the expression levels of the cardiomyocyte (*MYH6*) and neural rosette (*PAX6*) specific biomarker genes, while it did not affect cardiac contraction ability nor the morphology of hepatocytes and neural rosettes. Tafluprost induced a decrease of the expression of the cardiomyocyte-specific biomarker *MYH6* at the highest tested concentration and, therefore, this change was not considered significant; it did not affect the contraction ability of cardiomyocytes. Bimatoprost induced a decrease of the expression levels of the neural rosette-specific biomarker genes (*PAX6* and *NESTIN*); it also caused a decrease in cardiomyocyte contraction and altered neural rosette morphology.

Overall, bimatoprost seems to represent a worst-case as compared to DDDE and tafluprost, when considering the effects on development in *in vitro* conditions.

5.4.6 Similar endpoint-specific properties

Available toxicological data on the target and source substances is summarised in the data matrix presented in **Section 5.6**.

5.4.6.1 Toxicokinetics

In the absence of experimental data, the oral bioavailability of the target substance is assessed taking into consideration the physico-chemical properties (ECHA, 2023; Masuda-Herrera *et al.*, 2023). Based on the molecular weight and the log Kow value, DDDE is expected to have low oral bioavailability potential (≥1% and <50%). An *In vitro* dermal penetration study using human skin (OECD 428) is available for the target substance, showing that the absorbed fraction following topical application of 0.018% DDDE in a representative cosmetic eyelash product formulation is 8.67% of the applied dose after 24 hours of exposure. Due to its high lipophilicity, it is expected to undergo extensive distribution to various organs, once absorbed. As described in **Section 5.4.4**, DDDE mainly undergoes hydrolysis giving rise to tafluprost acid and ethylamine, and it is expected to be mainly excreted via urine.

In the case of source substance 1, it is expected to have low oral bioavailability potential (≥1% and <50%) (Masuda-Herrera *et al.*, 2023) as well as low dermal absorption (≤10%) (Kroes *et al.*, 2007), when considering the physico-chemical parameters. Studies on absorption, distribution, metabolism and excretion (ADME) available for tafluprost showed that the substance presents an extensive tissue distribution with the exception of the central nervous system and is able to cross the placental barrier in pregnant rats. It undergoes ester hydrolysis with the formation of tafluprost acid and isopropanol (CAS No. 67-63-0) (see also **Section 5.4.4**) and is excreted via urine, bile and faeces (CDER, 2011b).

As to source substance 2, it is expected to have moderate oral bioavailability potential (≥50% and <90%)(Masuda-Herrera *et al.*, 2023) as well as low dermal absorption (≤ 10%) (Kroes *et al.*, 2007), when considering the physico-chemical parameters. Studies on ADME available for bimatoprost showed that has extensive tissue distribution, it is hydrolysed to give rise to bimatoprost acid and ethylamine (see also **Section 5.4.4**) and is excreted via urine, bile and faeces (CDER, 2007; HRES, 2018).

Overall, the target substance presents a profile which is more similar to source substance 1 than source substance 2, when comparing absorption and metabolism.

5.4.6.2. Toxicological properties

Acute toxicity: *In vitro* 3T3 Neutral Red Uptake (NRU) study in rat fibroblasts available for both the target and source substances showed that they all have moderate acute oral toxicity with LD50 value of 880 mg/kg bw for the target substance, 367 mg/kg bw for source substance 1 and 1204 mg/kg bw for source substance 2.

Local endpoints: *In vitro* and *in vivo* studies available for DDDE and *in vivo* studies available for tafluprost and bimatoprost showed that the three substances are not irritating to skin and eye at their respective use concentrations.

Skin sensitisation: *In vitro* and *in vivo* studies available for DDDE and *in vivo* studies available for tafluprost and bimatoprost showed that the three substances are not skin sensitisers.

Repeated dose toxicity: Repeated dose oral toxicity studies are available for bimatoprost in rats and mice. A review of the critical effects and reported NOAELs of repeated dose toxicity studies indicates that tafluprost showed a more severe toxicity and lower NOAELs compared to bimatoprost irrespective of the route of exposure.

In 28-day oral sub-acute studies available for bimatoprost, no effects were observed in mice, while rats showed testicular degeneration and abnormal germ cells at a concentration of 16 mg/kg bw/day. In 13-week oral sub-chronic studies available for bimatoprost, mice exhibited vaginal inflammation at a concentration of 16 mg/kg bw/day, while rats showed reversible ovarian changes at doses ≥0.3 mg/kg bw/day. In a 52-week oral chronic study available for bimatoprost, rats also displayed reduced body weight gain and ovarian changes at doses ≥0.3 mg/kg bw/day. The NOAEL was established at: (1) 16 mg/kg bw/day in mice and 8 mg/kg bw/day in rats for sub-acute studies; (2) 8 mg/kg bw/day in mice and between 0.1 and 16 mg/kg bw/day in rats for sub-chronic studies; and (3) between 0.1 and 2 mg/kg bw/day in rats for chronic studies. Given the similarity in biological activities between DDDE and bimatoprost and in the absence of experimental data of tafluprost for repeated dose oral toxicity, read across to source substance 2 is proposed for this endpoint.

Repeated dose parenteral (intravenous route) sub-acute and sub-chronic toxicity studies are available for tafluprost and bimatoprost in rats, dogs and monkeys. In the case of tafluprost, systemic effects included increased respiratory rate, altered cardiac parameters, hyperostosis and myelofibrosis in femoral and sternum bone marrow, increased haematopoiesis in the spleen, liver, and male femoral bone marrow, and corticomedullary mineralisation in female kidneys at doses ≥10 µg/kg bw/day. The NOAEL was established at 1) 100 µg/kg bw/day in rats and 1 µg/kg bw/day in dogs for sub-acute studies, and 2) 1 µg/kg bw/day in dogs for sub-chronic studies. For bimatoprost, systemic effects included decreased regression of corpora lutea in female rats at 1 mg/kg bw/day and reversible

palpebral fissure widening in monkeys (a known effect of prostaglandin derivatives) at ≥0.1 mg/kg bw/day. The NOAELs were set at 1) 1 mg/kg bw/day in monkeys and 1 mg/kg bw/day (males) and 0.3 mg/kg bw/day (females) in rats for sub-acute studies, and 2) 1 mg/kg bw/day in monkeys for sub-chronic studies. Given the structural similarity between DDDE and tafluprost and the similarity in biological activities between DDDE and bimatoprost, read across to source substances 1 and 2 is proposed for this endpoint.

Repeated dose sub-cutaneous sub-chronic studies are available for tafluprost in rats and mice. In sub-chronic studies, only minor, non-significant histopathological changes in the spleen and kidney were observed in rats at a concentration of 30 µg/kg bw/day, and no adverse effects were observed in mice up to a concentration of 100 µg/kg bw/day. However, in a 24-month chronic carcinogenicity study in rats, systemic effects including reduced body weight, hyperostosis of the sternum and femur, and increased extramedullary haematopoiesis in the spleen were observed at doses ≥3 µg/kg bw/day. No adverse effects were reported in mice in a 78-week carcinogenicity study at doses up to 100 µg/kg bw/day. The NOAELs were set at 1) 100 µg/kg bw/day in mice and 30 µg/kg bw/day in rats for sub-chronic studies, and 2) 100 µg/kg bw/day in mice for chronic studies. Given the structural similarity between DDDE and tafluprost and in the absence of experimental data for bimatoprost, read across to source substance 1 is proposed for this endpoint.

Repeated dose parenteral (ocular route) studies are available in monkeys for tafluprost, and in rabbits, dogs, and monkeys for bimatoprost. Both substances did not cause systemic toxicity up to the highest tested doses (10 µg/kg bw/day for tafluprost and 127.27 µg/kg bw/day for bimatoprost). Observed effects were limited to mild, reversible ocular changes such as iridial pigmentation, sunken eyelids, and palpebral fissure widening without a functional impact. In the case of tafluprost, a study in monkeys established the NOAEL in the range from 0.067 to 10 µg/kg bw/day; for bimatoprost, a study in rabbits set the NOAEL in the range from 12.4 to 127.27 µg/kg bw/day, and a study in dogs established the NOAEL in the range from 0.933 to 20 µg/kg bw/day. Given the structural similarity between DDDE and tafluprost and the similarity in biological activities between DDDE and bimatoprost, read across to source substances 1 and 2 is proposed for this endpoint.

Genotoxicity: The target and source substances have a similar behaviour with respect to the genotoxicity endpoint. In fact, DDDE was found to be non-genotoxic in Ames test and in an *in vitro* mammalian cell micronucleus test. Tafluprost was also shown to be non-genotoxic in 1) Ames test, 2) an *in vitro* mammalian chromosomal aberration test and 3) *in vivo* micronucleus test in mice. Similarly, bimatoprost was demonstrated to be non-genotoxic in 1) Ames test, 2) an *in vitro* mammalian cell gene mutation test using the *thymidine kinase* gene and 3) *in vivo* micronucleus test in mice. Given the structural similarity between DDDE and tafluprost and the similarity in biological activities between DDDE and bimatoprost, read across to source substances 1 and 2 is proposed for the *in vivo* genotoxicity endpoint.

Carcinogenicity: Carcinogenicity studies in mice and rats available for tafluprost and bimatoprost showed that both substances do not present a carcinogenic potential. Given the structural similarity between DDDE and tafluprost and the similarity in biological activities between DDDE and bimatoprost, read across to source substances 1 and 2 is proposed for this endpoint.

Reproductive toxicity: Studies on reproductive toxicity in rats are available for tafluprost and bimatoprost. No significant treatment-related effects on fertility or reproductive parameters were observed following intravenous administration of tafluprost at doses up to 100 µg/kg bw/day, or oral administration of bimatoprost at doses up to 600 µg/kg bw/day. Given the structural similarity between DDDE and tafluprost and the similarity in biological activities between DDDE and bimatoprost, read across to source substances 1 and 2 is proposed for this endpoint.

Developmental toxicity: Pre-natal and post-natal toxicity studies are available for tafluprost via the intravenous route in rats and rabbits, and for bimatoprost via the oral route in rats and mice. Both substances revealed maternal and developmental effects. Tafluprost caused intrauterine death,

decreased viability, teratogenicity (e.g., vertebral defects), and delayed pinna unfolding at doses ≥10 in rats and ≥0.1 µg/kg bw/day in rabbits. In rats, maternal NOAEL ranged from 10 to 30 µg/kg bw/day, and developmental NOAEL ranged from 0.3 to 3 µg/kg bw/day; in rabbits, both maternal and developmental NOAELs were set at 0.01 µg/kg bw/day. For bimatoprost, maternal toxicity - with effects including abortions, alopecia, and reduced gestation duration - and developmental effects (such as increased peri- and postnatal pup mortality) were observed in mice and rats at doses of 300 and 600 µg/kg bw/day, respectively. The maternal NOAEL ranged from 100 to 600 µg/kg bw/day in mice and from 100 to 300 µg/kg bw/day in rats, while developmental NOAEL was established at 600 µg/kg bw/day in mice and at in the range between 100 and 600 µg/kg bw/day in rats. The observed differences between tafluprost and bimatoprost are likely due to the differences in routes of exposure. Given the structural similarity between DDDE and tafluprost and the similarity in biological activities between DDDE and bimatoprost, read across to source substances 1 and 2 is proposed for this endpoint.

5.5 SCENARIO-BASED READ ACROSS JUSTIFICATION

5.5.1 Human health endpoints

The toxicological studies from the source substances are proposed to be used for the following endpoints in the SCCS dossier of DDDE (target substance):

- Repeated dose toxicity
- *In vivo* genotoxicity
- Carcinogenicity
- Reproductive toxicity
- Developmental toxicity

5.5.1.1 Selection of the scenario(s) for human health assessment

The most appropriate scenario described in the ECHA guidance document (ECHA, 2017) to address this particular read across situation is **Scenario 2**, which covers the analogue approach for different compounds with the same type of effects.

5.5.1.2 Common assessment elements ('Scenario 2')

5.5.1.2.1 Identity and characterisation of the source substance (AE C.1)

The identity and characterisation of 'tafluprost' (source substance 1), bimatoprost (source substance 2) and the target substance 'dechloro dihydroxy difluoro ethylcloprostenolamide' are clear and unambiguous for read across purposes.

The identity and characterisation of the source and target substances is **acceptable with high confidence**.

5.5.1.2.1.2 Link of structural similarities and differences with the proposed prediction (AE C.2)

The target and source substances are structurally similar. The target and the source substances share key functional groups, such as 'cycloalkane', and 'aryl' groups. In addition, the target substance and source substance 1 share the 'alkyl halide' group and more specifically the 'geminal fluorine' group. The target and source substance 2 share the amide functional group. This chemical similarity was confirmed by a relatively high Dice index of the source substances to the target substance (**Table 9**).

Also, the target and source substances show similar physico-chemical properties, with the source substance 2 being more bioavailable due to the differences in log Kow and water solubility values.

Based on the above elements, the link of structural similarity and differences with the proposed prediction is considered to be **acceptable with high confidence** for tafluprost and **acceptable with**

medium confidence for bimatoprost. Indeed, there is a minor reservation for bimatoprost due to the fact that bimatoprost does not present the 'geminal fluorine' functional group.

5.5.1.2.3 Reliability and adequacy of the source study (AE C.3)

All studies intended for use as read across were conducted according to nationally or internationally agreed guidelines (OECD). The study results are adequate for the purpose of classification and labelling and/or risk assessment. The conclusions made are consistent with reported results.

The source studies are therefore considered to be **reliable and adequate with high confidence** for read across.

5.5.1.2.4 Bias that influences the prediction (AE C.4)

Source substances 1 and 2 have been identified and selected, according to the process described by Wu *et al*. (Wu *et al.*, 2010) and Blackburn and Stuard (Blackburn and Stuard, 2014) and taking into consideration the recommendations of the SCCS note of guidance (SCCS, 2023) (see **Section 5.1)**.

This parameter is therefore considered as **acceptable with high confidence** for both source substances**.**

5.5.1.3 Specific assessment elements ('Scenario 2')

5.5.1.3.1 Compounds the test organism is exposed to **(2.1)**

As discussed in **Section 5.4.4**, the first metabolic reaction is hydrolysis for the target and source substances. Specifically, about 65.8 - 71.2% of the absorbed fraction of the target substance was found to be transformed into its main metabolite, tafluprost free acid after 24 hours (Weghuber, 2022). Tafluprost has been shown to be hydrolysed in the eye to give rise to its main metabolite, tafluprost acid, which is further metabolised via fatty acid β-oxidation and phase II conjugation (CDER, 2011a). Similarly, bimatoprost has been described to undergo amide hydrolysis with the formation of bimatoprost acid, and to a lesser extent oxidation, N-deethylation and glucuronidation (Al-Ghananeem and Crooks, 2007; HRES, 2018). Therefore, in the case of DDDE and tafluprost, the test organism is mainly exposed to tafluprost acid, which is a common metabolite still containing the 'geminal fluorine' group, and to ethylamine for DDDE and isopropanol for tafluprost. In the case of bimatoprost, the test organism is mainly exposed to bimatoprost acid and to ethylamine.

Based on the above elements, information on the compounds that the test organism is exposed to is considered to be **acceptable with high confidence**.

5.5.1.3.2 Common underlying mechanism, qualitative aspects (2.2)

Based on (a) common structures, (b) similarities in the first metabolic reactions, (c) similarities in predicted chemical reactivity, as described in **Section 5.4.2** (mechanistic profilers), (d) similarities in biological activities (**Section 5.4.5**), and (d) the presence of bridging data for a number of endpoints (skin and eye irritation, sensitisation and *in vitro* mutagenicity in mammalian cells), which indicates overall comparable toxicological properties (**Section 5.4.6.2**), the source and the target substances are not expected to exert toxicity due to different underlying mechanisms.

However, due to the slight differences with respect to biological activity for tafluprost and with respect to chemical reactivity for bimatoprost, this point is considered **acceptable with medium confidence (minor reservations)** for both source substances.

5.5.1.3.3 Common underlying mechanism, quantitative aspects (2.3)

As described above, no additional underlying mechanisms are anticipated for the target substance, which could potentially cause a higher toxicity as compared to the source substances. This is supported by the presence of bridging mechanistic and toxicological data for the quantitative endpoints. Indeed, the three substances showed more or less similar binding potency to PGF2alpha receptor when tested in an *in vitro* binding assay using isolated FP receptors derived from human

recombinant (HEK-293) cells. When tested in the ToxProfiler® platform, DDDE and bimatoprost induced the oxidative stress pathway as the primary MoA and showed similar PoD values (30 µg/mL for the target substance and 50 µg/mL for source substance 2). Furthermore, testing of the three substances using the *in vitro* 3T3 NRU assay in rat fibroblasts indicated a similar moderate acute oral toxicity potential for the target and source substances, with LD_{50} values of 880 mg/kg bw for DDDE, 367 mg/kg bw for tafluprost, and 1204 mg/kg bw for bimatoprost.

Therefore, based on the above data as well as (a) structural similarity (b) similarities in the first predicted metabolic steps, (c) similarities in predicted chemical reactivity, as described in **Section 5.4.2** (mechanistic profilers), (d) similarities in biological activities (**Section 5.4.5**), the source and target substances are not expected to exert toxicity due to different underlying mechanisms.

However, because of the limited bridging data for systemic endpoints, this point is considered **acceptable with medium confidence (minor reservations)**.

5.5.1.3.4 Exposure to other compounds than to those linked to the prediction (2.4)

As discussed in **Section 5.4.4**, the target and the source substances have been shown to undergo metabolism via similar pathways. The exposure to the uncommon metabolites - ethylamine for DDDE and bimatoprost, and isopropanol for tafluprost - does not have any impact on the read across strategy. As shown in **Part C of Annex I**, ethylamine does not show critical systemic adverse effects, while isopropanol is not a developmental toxicant and presents significantly higher NOAELs in systemic toxicity studies when compared to tafluprost.

Based on the above elements, information on exposure to other compounds than those linked to the prediction is considered to be **acceptable with high confidence**.

5.5.1.3.4 Occurrence of other effects than covered by the hypothesis and justification (2.5)

There is no evidence that additional mechanisms than those identified in the hypothesis may be acting, either:

- On the basis of mechanistic insights in relation to the structural alerts (e.g., there are no differences of toxicity properties between the source and the target substances that could clearly be linked to differences in underlying mechanisms) and biological properties, or
- Derived from information in the data matrix (e.g., there are no inconsistencies in the data matrix which are indicative of qualitative differences of toxicity between the source and the target for the predicted property)

In addition, considering that tafluprost and bimatoprost complement each other with respect to structural activity and biological properties when compared to DDDE, a weight-of-evidence approach has been used for this read across hypothesis.

Based on the above elements, the information on other effects than covered by the hypothesis is considered to be **acceptable with medium confidence (minor reservations)**.

5.5.1.4 Overall conclusion on 'Scenario 2' read across hypothesis for human health

The summary of all assessment elements and subsequent assessment option outcome have been summarised in the below table:

Table 10: Summary of assessment elements and assessment options of Scenario 2

AE #	Assessment element	Score (Assessment option) Tafluprost	Score (Assessment option) Bimatoprost	Remarks
Common assessment elements				
AE C.1	Identity and characterisation of the source substance	5 (acceptable with high confidence)	5 (acceptable with high confidence)	-
AE C.2	Link of structural similarities and differences with the proposed prediction	5 (acceptable with high confidence)	4 (acceptable with medium confidence)	-
AE C.3	Reliability and adequacy of the source study	5 (acceptable with high confidence)	5 (acceptable with high confidence)	-
AE C.4	Bias that influences the prediction	5 (acceptable with high confidence)	5 (acceptable with high confidence)	-
Specific assessment elements				
AE 2.1	Compounds the test organism is exposed to	4 (acceptable with medium confidence)	4 (acceptable with medium confidence)	-
AE 2.2	Common underlying mechanism, qualitative aspects	4 (acceptable with medium confidence)	4 (acceptable with medium confidence)	-
AE 2.3	Common underlying mechanism, quantitative aspects	4 (acceptable with medium confidence)	4 (acceptable with medium confidence)	-
AE 2.4	Exposure to other compounds than to those linked to the prediction	5 (acceptable with high confidence)	5 (acceptable with high confidence)	-
AE 2.5	Occurrence of other effects than covered by the hypothesis and justification	4 (acceptable with medium confidence)	4 (acceptable with medium confidence)	-

Overall, detailed scientific assessment of the different assessment elements (AEs) laid down for Scenario 2 of the analogue approach as per the RAAF indicates that the read across hypothesis for endpoints related to human health is considered acceptable with medium to high confidence for both source substances.

5.6 DATA MATRICES

Table 11: Comparative data matrix for the target and the source substances: physico-chemical endpoints

Name	**DDDE [Target substance]**	**Tafluprost [Source substance 1]**	**Bimatoprost [Source substance 2]**
CAS No.	**1185851-52-8**	**209860-87-7**	**155206-00-1**
EC No.	**867-521-0**	**682-458-7**	**642-890-9**
Reference	**SCCS dossier**	**Estimation using QSAR tools**	**Estimation using QSAR tools**
Molecular weight (g/mol)	437.52	452.54	415.6
State of the substance at 20°C and 101.3 kPa	Liquid	Solid	Solid
Melting/freezing point	95 °C (Estimated using US EPA T.E.S.T. v.5.1.2)	78 °C (Estimated using US EPA T.E.S.T. v.5.1.2)	101 °C* (Estimated using US EPA T.E.S.T. v.5.1.2)
Boiling point	504 °C (Estimated using US EPA T.E.S.T. v.5.1.2)	474 °C (Estimated using US EPA T.E.S.T. v.5.1.2)	525 °C* (Estimated using US EPA T.E.S.T. v.5.1.2)
Relative density	1.21 g/cm3 (Estimated using US EPA T.E.S.T. v.5.1.2)	1.16 g/cm3 (Estimated using US EPA T.E.S.T. v.5.1.2)	1.14 g/cm3 (Estimated using US EPA T.E.S.T. v.5.1.2)
Vapour pressure (25 °C)	1.2E-013 Pa (Estimated using MPBPWIN of the Epi Suite™ platform v.4.11)	5.06E-011 Pa (Estimated using MPBPWIN of the Epi Suite™ platform v.4.11)	7.03E-16 Pa (Estimated using MPBPWIN of the Epi Suite™ platform v.4.11)
Partition coefficient n-octanol/water (25 °C)	Log Kow: 5$ (Estimated using KOWWIN of the Epi Suite™ platform v.4.11)	Log Kow: 6.5 (Estimated using KOWWIN of the Epi Suite™ platform v.4.11)	Log Kow: 3.97 (Estimated using KOWWIN of the Epi Suite™ platform v.4.11)
Water solubility (25 °C)	0.091 mg/L$ (Estimated using WSKOW of the Epi Suite™ platform v.4.11)	0.0039 mg/L (Estimated using WSKOW of the Epi Suite™ platform v.4.11)	0.9948 mg/L (Estimated using WSKOW of the Epi Suite™ platform v.4.11)

* Experimental values are available but for comparative purposes only estimated values have been reported due to the fact that experimental values are missing for the target substance and the source substance 1.
$ Experimental values are available but for comparative purposes only estimated values have been reported due to the fact that experimental values are missing for source substances 1 and 2.

Table 12: Comparative data matrix for the target and the source substances: toxicological endpoints

Name **CAS No.** **EC No.** **Main references**	**DDDE** **[Target substance]** **1185851-52-8** **867-521-0** **SCCS dossier**	**Tafluprost** **[Source substance 1]** **209860-87-7** **682-458-7** **(CDER, 2011a; b)**	**Bimatoprost** **[Source substance 2]** **155206-00-1** **642-890-9** **(CDER, 2001a; b)**
Toxicokinetics	**Absorption:** Low oral bioavailability potential (≥1% and <50%) based on MW and estimated log Kow (Masuda-Herrera *et al.*, 2023) **Distribution:** Due to its high lipophilicity, it is expected to undergo extensive distribution to various organs once absorbed **Metabolism:** Extensively hydrolysed (68.5 ± 2.7%) giving rise to tafluprost acid after 24 hours (OECD TG 428) **Excretion:** Considering the metabolism, it is expected to be mainly excreted via urine	**Absorption:** Low oral bioavailability potential (≥1% and <50%) based on MW and estimated log Kow (Masuda-Herrera *et al.*, 2023); rapid absorption with high bioavailability upon post ocular administration **Distribution:** Extensive tissue distribution; limited distribution in the central nervous system; crossed the placental barrier in pregnant rats (CDER, 2011b) **Metabolism:** Hydrolysed by carboxylesterase into tafluprost acid followed by glucuronidation or beta-oxidation (*In vitro* studies using hepatocytes and corneal tissue) (CDER, 2011a) **Excretion:** Excreted *via* urine (25-41%), bile (33-50%) and faeces (3-5%); transferred into milk in lactating rats (CDER, 2011b)	**Absorption:** Moderate oral bioavailability potential (≥50% and <90%) based on MW and estimated log Kow (Masuda-Herrera *et al.*, 2023); rapid absorption upon post ocular administration **Distribution:** Extensive tissue distribution **Metabolism:** Hydrolysed to give rise to bimatoprost acid followed by oxidation, N-deethylation and glucuronidation (CDER, 2007; HRES, 2018) **Excretion:** Excreted *via* urine (67%), bile (33-50%) and faeces (25%) (CDER, 2007; HRES, 2018)
Dermal absorption (*in vitro*)	8.67% after 24 h (OECD TG 428)	Low dermal absorption potential (≤10%) based on MW and estimated log Kow (Kroes *et al.*, 2007)	Low dermal absorption potential (≤10%) based on MW and estimated log Kow (Kroes *et al.*, 2007)
Acute toxicity: oral (*in vitro/ in vivo*)	IC50 (Balb/c 3T3 fibroblasts): 0.083 mg/mL LD50 (Balb/c 3T3 fibroblasts): 880 mg/kg bw (OECD GD 129)	IC50 (Balb/c 3T3 fibroblasts): 0.011 mg/mL LD50 (Balb/c 3T3 fibroblasts): 367 mg/kg bw (OECD GD 129) (Westerink, 2024b) ---- LD50 (rat): ≥ 100 mg/kg bw (guideline not specified) (CDER, 2011b)	IC50 (Balb/c 3T3 fibroblasts): 0.181 mg/mL LD50 (Balb/c 3T3 fibroblasts): 1204 mg/kg bw (OECD GD 129) (Westerink, 2024a)

Name CAS No. EC No. Main references	DDDE [Target substance] 1185851-52-8 867-521-0 SCCS dossier	Tafluprost [Source substance 1] 209860-87-7 682-458-7 (CDER, 2011a; b)	Bimatoprost [Source substance 2] 155206-00-1 642-890-9 (CDER, 2001a; b)
Skin irritation *(in vitro)*	At least irritating (undiluted) (OECD TG 439) --- Not irritating (undiluted) (OECD TG 439)	-	-
Skin irritation (*in vivo*)	Not irritating (0.025%) (Human repeat insult patch test)	-	-
Eye irritation (*in vitro*)	Not irritating (undiluted) (OECD TG 492) ---- Not irritating (0.025%) (Hen's egg test - chorioallantoic membrane assay; HET-CAM assay)	-	-
Eye irritation (*in vivo*)	Not irritating (0.025%)	Not irritating (0.005% and 0.05% of ophthalmic solution) (rabbits) (Guideline not specified) (CDER, 2011b) ---- Slightly irritating[5] (0.0015% and 0.0045% of ophthalmic solution) (monkeys) (Guideline not specified) (CDER, 2011b) ---- Not irritating (0.0005 – 0.05% of ophthalmic solution) (monkeys; twice a day for 28 days) (Guideline not specified)	Not irritating[7] (0.03% of ophthalmic solution) (rabbits) (Guideline not specified) (CDER, 2001a) ---- Not irritating (0.03% of ophthalmic solution) (rabbits) (Guideline not specified) (CDER, 2001a) ---- Not irritating (0.001 – 0.1% of ophthalmic solution) (rabbits; exposure: four times a day for 1 month)

[5] The tested solutions contained benzalkonium chloride as a preservative, which is known to have irritant properties. Therefore, the observed irritant effect may be attributed to the excipient (TGA, 2012).
[7] Slight transient conjunctival was noted on Day 1, but not on Days 2 to 4.

Name CAS No. EC No. Main references	DDDE [Target substance] 1185851-52-8 867-521-0 SCCS dossier	Tafluprost [Source substance 1] 209860-87-7 682-458-7 (CDER, 2011a; b)	Bimatoprost [Source substance 2] 155206-00-1 642-890-9 (CDER, 2001a; b)
		(CDER, 2011b) ---- Not irritating (0.0005 – 0.05% of ophthalmic solution) (monkeys) (Guideline not specified) (CDER, 2011b) ---- Not irritating (0.0045% ophthalmic solution) (monkeys) (Guideline not specified) (CDER, 2011b) ---- Slightly irritating[6] (0.0005 – 0.05% of ophthalmic solution) (monkeys) (Guideline not specified) (CDER, 2011b)	(Guideline not specified) (CDER, 2001a) ---- Not irritating (0.001 - 0.1% of ophthalmic solution) (dogs) (Guideline not specified) (CDER, 2001a) ---- Not irritating (0.03% and 0.1% of ophthalmic solution) (rabbits) (Guideline not specified) (CDER, 2001a) ---- Not irritating (0.03% and 0.1% of ophthalmic solution) (monkeys) (Guideline not specified) (CDER, 2001a)
Skin sensitisation (*In chemico* and *in vitro*)	Not sensitising Direct Peptide Reactivity Assay (DPRA) (OECD TG 442C) ---- Not sensitising DPRA (OECD TG 442C) ---- Inconclusive (lack of a clear dose-response) KeratinoSens™ assay (OECD TG 442D) ----	-	-

[6] Iris colour darkening, blue-grey discoloration of the lower eyelid and minimal focal inflammation were observed.

Name CAS No. EC No. Main references	DDDE [Target substance] 1185851-52-8 867-521-0 SCCS dossier	Tafluprost [Source substance 1] 209860-87-7 682-458-7 (CDER, 2011a; b)	Bimatoprost [Source substance 2] 155206-00-1 642-890-9 (CDER, 2001a; b)
	Negative KeratinoSens™ assay (OECD TG 442D)		
Skin sensitisation (*In vivo*)	Not sensitising (7.5%) (Human repeat insult patch test; HRIPT) ---- Not sensitising (0.025%) (HRIPT)	Not sensitising (up to 0.05%) (Guinea pig) (Guinea Pig Maximization Test; GPMT) (Guideline not specified) (CDER, 2011b)	Not sensitising (0.2%; 0.1 - 2 mg/mL) (Guinea pig) (GPMT) (Guideline not specified) (CDER, 2001a) ---- Not sensitising (0.2%; 0.7 - 7 µg/kg) (Guinea pig) (systemic and passive cutaneous anaphylaxis (PCA) study) (Guideline not specified) (CDER, 2001a) ---- Not sensitising (0.2%; 0.7 – 7 µg/kg) (mice) (PCA assay) (Guideline not specified) (CDER, 2001a)
Repeated dose toxicity: oral (sub-acute)	Read across to source substance 1	-	28-day NOAEL (mice): 16 mg/kg bw/day (gavage) (Guideline not specified) (CDER, 2001b) ---- 28-day NOAEL (rats): 8 mg/kg bw/day (gavage) (Guideline not specified) (CDER, 2001b)
Repeated dose toxicity: oral (sub-chronic)	Read across to source substance 1	-	13-week NOAEL (mice): 8 mg/kg bw/day (gavage) (Guidance not specified) (CDER, 2001b)

Name CAS No. EC No. Main references	DDDE [Target substance] 1185851-52-8 867-521-0 SCCS dossier	Tafluprost [Source substance 1] 209860-87-7 682-458-7 (CDER, 2011a; b)	Bimatoprost [Source substance 2] 155206-00-1 642-890-9 (CDER, 2001a; b)
			---- 13-week NOAEL (rats): 1 and 0.1 mg/kg bw/day for males and females, respectively (gavage) (Guidance not specified) (CDER, 2001b) ---- 13-week NOAEL (rats): 16 mg/kg bw/day (gavage) (Guideline not specified) (CDER, 2001b)
Repeated dose toxicity: oral (chronic)	Read across to source substance 1	-	52-week NOAEL (rats): 2 and 0.1 mg/kg bw/day for males and females, respectively (gavage) (Guideline not specified) (CDER, 2001b)
Repeated dose toxicity: parenteral (intravenous - sub-acute)	Read across to source substances 1 and 2	28-day NOAEL (rats): 100 µg/kg bw/day (Guideline not specified) (CDER, 2011b) ---- 28-day NOAEL (dogs): 1 µg/kg bw/day (Guideline not specified) (CDER, 2011b)	28-day NOAEL (rats): 1 and 0.3 mg/kg bw/day for males and females, respectively (Guideline not specified) (CDER, 2001a) ---- 28-day NOAEL (monkeys): 1 mg/kg bw/day (Guideline not specified) (CDER, 2001a)
Repeated dose toxicity: parenteral (intravenous - sub-chronic)	Read across to source substances 1 and 2	26-week LOAEL (rats): 10 µg/kg bw/day (Guideline not specified) (CDER, 2011b) ---- 39-week NOAEL (dogs): 1 µg/kg bw/day (Guideline not specified) (CDER, 2011b)	17-week NOAEL (monkeys): 1 mg/kg bw/day (Guideline not specified) (CDER, 2001a)
Repeated dose toxicity: parenteral (sub-cutaneous - sub-chronic and chronic)	Read across to source substance 1	13-week NOAEL (mice): 100 µg/kg bw/day (Guideline not specified) (CDER, 2011b) ---- 13-week NOAEL (rats): 30 µg/kg bw/day	-

Name CAS No. EC No. Main references	DDDE [Target substance] 1185851-52-8 867-521-0 SCCS dossier	Tafluprost [Source substance 1] 209860-87-7 682-458-7 (CDER, 2011a; b)	Bimatoprost [Source substance 2] 155206-00-1 642-890-9 (CDER, 2001a; b)
		(Guideline not specified) (CDER, 2011b) ---- 78-week NOAEL (mice): 100 µg/kg bw/day (Guideline not specified) (CDER, 2011b) ---- 24-month LOAEL (rats): 3 µg/kg bw/day (Guideline not specified) (CDER, 2011b)	
Repeated dose toxicity (ocular)	Read across to source substances 1 and 2	28-day - 52-week NOAELs (monkeys): 0.067 to 10 µg/kg bw/day (Guideline not specified) (CDER, 2011b)	28-day - 6-month NOAELs (rabbits): 12.4 - 127.27 µg/kg bw/day (Guideline not specified) (CDER, 2001a) ---- 1-month NOAEL (dogs): 0.933 - 20 µg/kg bw/day (Guideline not specified) (CDER, 2001a) ---- 29 – 52-week NOAEL (monkeys): 25.93 – 36.84 µg/kg bw/day (Guideline not specified) (CDER, 2001a)
Genetic toxicity (*in vitro*)	Negative (Ames assay; OECD TG 471) --- Negative (Micronucleus Test, MNT; OECD TG 487)	Negative (Ames assay; similar to OECD TG 471) (CDER, 2011b) ---- Negative (chromosomal aberration test: guideline not specified) (CDER, 2011b)	Negative (Ames assay; Guideline not specified) (CDER, 2001a) ---- Negative (L5178Y/Tk+/- mouse lymphoma mutagenesis assay; guideline not specified) (CDER, 2001a)
Genetic toxicity (*in vivo*)	Read across to source substance 1 and 2	Negative (mice) (MNT; guideline not specified) (CDER, 2011b)	Negative (mice) (MNT; Guideline not specified) (CDER, 2001a)

Name CAS No. EC No. Main references	DDDE [Target substance] 1185851-52-8 867-521-0 SCCS dossier	Tafluprost [Source substance 1] 209860-87-7 682-458-7 (CDER, 2011a; b)	Bimatoprost [Source substance 2] 155206-00-1 642-890-9 (CDER, 2001a; b)
Carcinogenicity	Read across to source substance 1 and 2	Non carcinogenic in mice (78-week sub-cutaneous carcinogenicity study; guideline not specified) (CDER, 2011b) ---- Non carcinogenic in rats (24-month sub-cutaneous carcinogenicity study; guideline not specified) (CDER, 2011b)	Not carcinogenic in mice (104-week oral gavage carcinogenicity study; guideline not specified) (Apotex Inc., 2022) ---- Non carcinogenic in rats (104-week oral gavage carcinogenicity study; guideline not specified) (Apotex Inc., 2022)
Reproductive toxicity	Read across to source substance 1 and 2	10-week reproductive and parental NOAEL (rats): 100 µg/kg bw/day (intravenous) (Guideline not specified) (CDER, 2011b)	10-week reproductive and parental NOAEL (rats): 600 µg/kg bw/day (gavage) (Guideline not specified) (CDER, 2001a)
Pre-natal developmental toxicity	Read across to source substance 1 and 2	Maternal NOAEL (rats): 30 µg/kg bw/day Developmental NOAEL: 3 µg/kg bw/day (intravenous) (Guideline not specified) (CDER, 2011b) ---- Maternal NOAEL (rabbits): 0.01 µg/kg bw/day Developmental NOAEL: 0.01 µg/kg bw/day (intravenous) (Guideline not specified) (CDER, 2011b)	Maternal NOAEL (mice): 600 µg/kg bw/day Developmental NOAEL: 600 µg/kg bw/day (gavage) (Guideline not specified) (Roshdy, 2005) ---- Maternal NOAEL (mice): 100 µg/kg bw/day Developmental NOAEL: 600 µg/kg bw/day (gavage) (Guideline not specified) (CDER, 2001a) ---- Maternal NOAEL (rats): 300 µg/kg bw/day Developmental NOAEL: 600 µg/kg bw/day (gavage) (Guideline not specified) (CDER, 2001a)
Pre- and post-natal developmental toxicity	Read across to source substance 1 and 2	Maternal NOAEL (rats): 10 µg/kg bw/day Developmental NOAEL: 0.3 µg/kg bw/day (intravenous) (Guideline not specified)	Maternal NOAEL (rats): 100 µg/kg bw/day Developmental NOAEL: 100 µg/kg bw/day (gavage) (Guideline not specified)

Name CAS No. EC No. Main references	DDDE [Target substance] 1185851-52-8 867-521-0 SCCS dossier	Tafluprost [Source substance 1] 209860-87-7 682-458-7 (CDER, 2011a; b)	Bimatoprost [Source substance 2] 155206-00-1 642-890-9 (CDER, 2001a; b)
		(CDER, 2011b)	(CDER, 2001a)

Abbreviations: TG: test guideline; GD: guidance document

5.7 OVERALL CONCLUSION

Overall, the available information on composition, physico-chemistry and toxicity suggests that the target and source substances share sufficient common properties to justify the read across for the toxicological endpoints specified above.

Therefore, the following IDA requirements can be fulfilled using a read across approach:

Data requirements as part of CIR IDA	**Assessment strategy**
Acute toxicity	Assessed based on a weight of evidence approach considering: • *In vitro* NRU assay available for DDDE • *In vivo* studies available for read across substances (tafluprost and bimatoprost)
Repeated dose toxicity	Assessed based on read across to *in vivo* studies available for read across substances (tafluprost and bimatoprost)
Reproductive toxicity	Assessed based on read across to the *in vivo* studies available for read across substances (tafluprost and bimatoprost)
Developmental toxicity	Assessed using a weight of evidence approach considering: • *In vitro* ReproTracker® assay available for DDDE *In vivo* studies available for read across substances (tafluprost and bimatoprost)
Genotoxicity (*in vivo*)	Assessed based on a weight of evidence approach considering: • *In vitro* genotoxicity data on DDDE • *In vivo* studies available for read across substances (tafluprost and bimatoprost) Considering the available data for DDDE and the read across substances (tafluprost and bimatoprost), additional *in vivo* genotoxicity testing for DDDE is not considered considering the weight of evidence of the existing information and animal welfare considerations.

6. SAFETY EVALUATION (INCLUDING CALCULATION OF THE MoS)

In the absence of repeated dose, reproductive, and developmental toxicity studies for DDDE, and given its structural similarity to tafluprost and biological activity similarity to bimatoprost, a read across to both analogues is proposed to assess systemic toxicity.

6.1 SELECTION OF THE POD (UPDATED)

A range of repeated dose, reproductive and developmental toxicity studies *via* oral, intravenous and/or subcutaneous routes are available for the analogues tafluprost and bimatoprost, to establish a critical NOAEL to be used as PoD for the risk assessment of DDDE used in cosmetic applications. A review of the critical effects and reported NOAELs from RDT and DART studies indicates that tafluprost in general showed a more severe toxicity and lower NOAELs compared to bimatoprost irrespective of the route of exposure (see **Table 13**).

Table 13: Overview of the NOAELs from the RDT and DART studies

Endpoint	Route	Tafluprost	Bimatoprost
RDT	Oral	-	100–16000 µg/kg bw/day
	SC	100 µg/kg bw/day (mice) <3 µg/kg bw/day (rats)	-
	IV	1–100 µg/kg bw/day	300–1000 µg/kg bw/day
Reproductive toxicity	Oral	-	≥600 µg/kg bw/day
	IV	≥100 µg/kg bw/day	-
Developmental toxicity	Oral	-	600 µg/kg bw/day (mice) 100–600 µg/kg bw/day (rats)
	IV	0.3–3 µg/kg bw/day(rats) 0.01 µg/kg bw/day (rabbits)	-

'-' no study available

Considering all available information and in line with the approach adopted by the German BfR for the risk assessment of DDDE in cosmetic eye product formulations (BfR, 2017), the **NOAEL of 0.3 µg/kg bw/day** derived from the prenatal developmental toxicity study of tafluprost in rats following intravenous administration is considered as the most appropriate PoD. As the NOAEL is based on critical effects i.e., developmental/teratogenic effects following intravenous dosing, it represents a very conservative and worst-case PoD when compared with the exposure route (dermal) for the intended application of DDDE present in cosmetic eye product formulations. Further, the study is considered to be of good quality with appropriate dose spacing and it corresponds to the lowest NOAEL established in the available set of studies for tafluprost.

Further, no bioavailability correction is required for deriving a systemic dose **(PoD_{sys})** as the PoD has been derived from an intravenous study, resulting in a systemic PoD (PoD_{sys}) of **0.3 µg/kg bw/day**.

6.2 EXPOSURE ASSESSMENT (SAME AS PREVIOUS SUBMISSION)

As described in **Section 6.2**, DDDE is used at a concentration of 0.018% in the cosmetic eyelash product [REDACTED]. It is intended to condition and strengthen the eyelashes by protecting against breakage and brittleness, while enhancing flexibility, moisture, and shine—resulting in more dramatic-looking eyelashes. It is not marketed for eyelash growth (DDDE Sponsor., 2020).

6.2.1 Instructions for use of the eyelash cosmetic formulation containing DDDE

The cosmetic eyelash product is applied with a multi-use fine brush applicator as a thin line directly to eyelashes, primarily the upper eyelashes, above the lash line. It is formulated with a thickener (xanthan gum) to ensure that the product remains on the eyelashes and does not migrate toward the eye fluid or surrounding membranes. As such, the eyelash product is designed to act on the appearance of eyelashes and not intended to come into contact with the eye itself.

Further, the below 'Directions for Use' and 'Caution Statements' are provided in the packaging for cosmetic eyelash product in Europe.

Directions For Use	Caution Statements
Step 1. Remove makeup and residue. Keep in mind, oil-based makeup removers and cleansers can leave behind a film which may create a barrier between your lashes and the lash conditioner. It's important to wash off any residue. **Step 2.** Once a day, apply one thin line of [REDACTED] directly to clean, dry upper eyelashes only, above the lash line. Dip applicator brush into the bottle one time for both eyes. Use sparingly as dripping into the eye may cause irritation or other unwanted effects. Let dry completely (2-3 minutes) before applying additional beauty products or going to sleep.	Do not get in eye. Rinse immediately with water if eye contact occurs. Do not use on damaged or irritated skin, if pregnant or nursing or undergoing treatment for glaucoma or cancer. Not recommended if you have dry eyes, eye infections, styes or any other eye-related disorder. If redness, irritation or other unwanted effects occur, stop using product immediately. If symptoms persist, seek medical attention. This cosmetic product contains a small amount of an ingredient classified as a prostaglandin analogue. When applied as directed, [REDACTED] should not get into the eyes, and therefore, should not have any effect on your eyes. A very small percentage of users reported sensitivity including temporary redness, swelling, itching, and burning sensation in the eyes. Even fewer users reported dry eyes, styes, darkening of the skin at the lash line, periorbital fat loss, and eyelash loss. It is not known whether [REDACTED] or some other factor caused the reported reactions. If this is a concern, do not use. Keep out of reach of children.

6.2.2 Concentration of DDDE in the cosmetic eyelash product

The concentration of DDDE in the cosmetic eyelash product is 0.018%.

6.2.3 Amount of product applied per application

A very small amount of DDDE is applied per application to the eyelashes. The amount of the cosmetic eyelash product applied per brushstroke to the upper eyelashes was determined by weighing the brush applicator that is part of the cosmetic eyelash product container after the applicator was removed from the tube container (pre-application) and again immediately after it was used to apply the cosmetic eyelash product to the upper eyelashes, above the lash line (i.e., before and after a single brushstroke to the eyelashes). Ten different applicator brushes were used in the study with the same person applying the cosmetic eyelash product. The range of the cosmetic eyelash product applied per brush stroke was 1 – 4 mg. On **average, 2.4 mg** of cosmetic eyelash product was applied to the upper eyelashes with each brush stroke (DDDE Sponsor, 2013) (see **Annex III** for details).

In another recently conducted in-house test, the amount of cosmetic eyelash product per brush stroke was determined by applying it to commercially available mink hair samples. The results are presented in **Annex III.** The tests used different combinations of tube containers and applicators. In Procedure 1, the same tube container was used with 10 different applicators. In Procedure 2, the same applicator was used with different tube containers. In both procedures, two different methods of inserting the applicator into the tube were used. In Insertion Method A, the applicator tip was pushed to the bottom of the tube. In Insertion Method B, the applicator tip was inserted just to the neck of the tube. The amount of cosmetic eyelash product applied per brushstroke was calculated as the difference between the amount on the applicator brush before and after application of the product across the mink hair samples. There were 10 replications of each procedure/insertion method. The range of the cosmetic eyelash product applied per brush stroke was 0.64 – 3.72 mg. The highest '**average amount'** from both the procedures (**2.42 mg**) is almost identical to the average amount of cosmetic eyelash product applied (2.4 mg) per brushstroke to human upper eyelashes. However, the highest **'maximum amount'** is lower (i.e., **3.72 mg)** than the maximum amount of cosmetic eyelash product applied (4

mg) per brushstroke to human upper eyelashes. Calculations of the average and maximum amounts of DDDE used per application to the upper eyelashes from both the methods are presented in **Annex III**.

Based on the above measurements and as a conservative approach, both, the 'average amount' and the 'maximum amount' per brush stroke, were taken into consideration for the purpose of risk assessment. Therefore, considering once daily application to both eyes, the 'average amount' of **4.8 mg/day** and the 'maximum amount' of **8 mg/day** were used for the MoS calculations.

6.2.4 Systemic exposure dose calculations (SED)

DDDE is present at concentrations up to **0.018%** in the cosmetic eyelash product, which has to be applied as a thin line to the eyelashes, above the lash line. Although not completely identical to the product types defined in the SCCS NoG, in application terms, it is close to the eyeliner and mascara cosmetic products for which the dermal, and to lesser extent, the ocular routes are the potential routes of exposure.

However, unlike eyeliners, the cosmetic eyelash product is applied directly to eyelashes (similar to mascara), which decreases the risk of dermal exposure. Further, the risk of its ocular exposure is also low, as a relatively small amount (see **Section 6.2.3**) of the cosmetic eyelash product is applied as a fine line across the eyelashes above the lash line. This is in contrast to mascara, where a significantly large amount (i.e., 25 mg as per the SCCS NoG) is applied along the full length of eyelashes.

In addition, unlike the other cosmetic products used near the eyes, the cosmetic eyelash product includes cellulose gum which acts as a 'viscosity increasing agent 'or 'thickener' that will minimise the migration of the product to the eye lid skin or eye. Therefore, the quantity of DDDE that is likely to migrate to the eyelid and be available for skin penetration is negligible (Bailey, 2023). Nevertheless, for a worst-case calculation, 50% of the applied amount (i.e., an average amount of 2.4 mg and a maximum amount of 4 mg) was used for the MoS calculations.

Therefore, when compared to other cosmetics applied near eyes, there is less potential for dermal or ocular exposure with the cosmetic eyelash product. Accidental unintended exposure to eyes may occur but it is not expected to present a significant risk. DDDE was found to be non-irritating to the eyes, when tested neat in an EpiOcular^{TM} RhCE test and did not reduce IOP following exposure to 0.025% DDDE in a cosmetic eyelash product formulation in female volunteers for 28 days. Therefore, 0.018% concentration of DDDE in the cosmetic product is not considered to alter the overall eye irritation profile or to have a physiological effect on the eye. This is further supported by data on consumer experience with the cosmetic eyelash product, which shows that the rate of adverse event reports is very low (0.154%, April 2022 – April 2023) (Bailey, 2023). This is further supported by the absence of systemic effects following subchronic/chronic repeated administration of ophthalmic formulations containing the analogue tafluprost in monkeys (CDER, 2011b).

Oral ingestion is considered an unlikely route of exposure under normal and reasonably foreseeable conditions of use.

Taking all the above information into consideration, only a potential **systemic exposure dose (SED)** *via* the dermal route was estimated in the present assessment.

The estimated **'SED'** from the dermal route can be calculated as follows:

$$\mathbf{SED = E_{product} \times C/100 \times DAp/100}$$

With
- **SED** = Systemic Exposure Dosage from dermal route (mg/kg bw/day)
- $\mathbf{E_{product}}$= Estimated daily exposure to a cosmetic product per kg bw
- **C** = Concentration of the ingredient under study in the finished cosmetic product (%)
- **DAp** = Dermal Absorption expressed as a percentage of the test dose assumed to be applied in real life conditions (%)

The dermal SED calculation is based on:

- **$E_{product}$** – Considering 50% of the measured 'average amount' or 'maximum amount' of the cosmetic product per kg bw is 0.04-0.067 mg/kg bw/day for a 60 kg adult (i.e., 50% x 4.8 mg/day ÷ 60 kg = 0.04 mg/kg bw/day; 50% x 8 mg/day ÷ 60 kg bw = 0.067 mg/kg bw/day) (see **Section 6.2.1**)
- **C** – The concentration of the substance in the cosmetic product (C) (i.e., **0.018%**; see **Section 6.2.2)**
- **DAp** – dermal absorption = **8.67%**

The estimated systemic exposure doses SEDs resulting from the daily application of a cosmetic eyelash product containing 0.018% DDDE are calculated as follows:

- SED (based on average amount of 4.8 mg the cosmetic eyelash product) = **0.000624 µg/kg bw/day**, calculated as 0.04 mg/kg bw x 0.018% x 8.67%.
- SED (based on maximum amount of 8 mg the cosmetic eyelash product) = **0.00104 µg/kg bw/day**, calculated as 0.0667 mg/kg bw x 0.018% x 8.67%.

It is important to note that the SED calculations are based on conservative worst-case assumptions. The underlying assumption that a maximum of 50% of the cosmetic eyelash product[8] could migrate to the eyelid and be available for dermal exposure is made even though the product is applied as a thin line to the eyelashes above the lash line and an added viscosity agent further minimises product migration to the eyelid.

6.3 MARGIN OF SAFETY CALCULATION (MOS) (UPDATED)

The MoS, which represents the ratio between the systemic PoD (PoD_{sys}) and the estimated SED, was calculated according to the following algorithm:

$$\mathbf{MoS = PoD_{sys}/SED}$$

With:
- PoD_{sys} = Systemic Point of Departure (mg/kg bw/day)
- SED = Systemic Exposure Dosage (mg/kg bw/day)

The results are presented in **Table 14**. In accordance with SCCS NoG, to consider a substance to be safe for use or an acceptable risk assessment, the MoS for systemic toxicity should be ≥100.

Table 14: SED and MoS calculations

Product daily amount (mg)	SED (µg/kg bw/d)	PoD_{sys} (µg/kg bw/d)	MoS
Based on an average daily amount of 4.8 mg of the cosmetic eyelash product	0. 000624	0.3	481
Based on maximum daily amount of 8 mg of the cosmetic eyelash product	0.00104	0.3	288

Furthermore, the risk assessment of DDDE is further supported by comparing the exposure with the toxicological screening values (TSVs), used by the European Food Safety Authority (EFSA) Panel on Contaminants in the Food Chain (CONTAM) for establishing Reference Points for Action (RPAs) for

[8] 50% of the average amount of 4.8 mg = 2.4 mg and 50% pf the maximum amount of 8 mg = 4 mg

non-allowed pharmacologically active substances in food of animal origin (EFSA, 2018). The EFSA panel recommends a **TSV of 0.0042 µg/kg bw/day** for substances acting on **PGF2α receptors** either as agonists or antagonists. The comparison of this very conservative TSV value with the maximum SED value results in a MoS of >1[9], suggesting a low risk.

6.4 CONCLUSION (UPDATED)

Based on the available data and the conservative SED calculations, the present safety assessment reveals a calculated MoS greater than 100 (as per SCCS approach) or 1 (as per EFSA TSV approach) and thereby supports the safe use of DDDE at a concentration up to 0.018% in cosmetic eyelash products under the conditions presented in this evaluation.

[9] Comparing the corrected TSV value of 0.0021 µg/kg bw/day following a correction for default oral absorption correction of 50% with the maximum SED of 0.00104 µg/kg bw/day, results in MoS of 2.

7 SUMMARY AND CONCLUSIONS

To address the CIR Expert Panel's request for additional data and to reinforce the read across approach proposed for DDDE, a targeted testing strategy was implemented, focusing on the generation of confirmatory data using NAMs. These tests were carefully selected to address the uncertainties raised with respect to structural, mechanistic, and toxicological similarities between DDDE and the selected analogues, tafluprost and bimatoprost.

- The **receptor binding potency assay** demonstrated that all three compounds bind to the PGF2α receptor within a comparable potency, further supporting similarity across the three substances and providing mechanistic bridging data on a key molecular initiating event.
- The *in silico* analysis using widely recognised (Q)SAR models indicates that DDDE presents an overall similar profile to tafluprost and bimatoprost with respect to receptor binding and activity potential.
- The **ToxProfiler® assay** showed that DDDE and bimatoprost share a similar biological activity profile across the different cellular stress pathways, suggesting that bimatoprost is more similar than the profile of DDDE *versus* tafluprost, when considering the biological activity.
- The **ReproTracker® assay** also indicates that bimatoprost seems to represent a worst-case as compared to DDDE and tafluprost, when considering the effects on development in *in vitro* conditions.
- The ***in vitro* 3T3 NRU assay** showed comparable cytotoxicity profiles for DDDE, tafluprost, and bimatoprost, indicating similar acute oral toxicity potential and supporting the read across at the apical level.

In parallel, a comprehensive literature review of available *in vivo* studies for the two analogues confirmed their suitability for the assessment of systemic toxicity. A range of repeated dose, reproductive and developmental toxicity studies are available for tafluprost and bimatoprost. Among these, tafluprost generally showed greater toxicity and lower NOAELs regardless of the exposure route. Following the approach adopted by the BfR (BfR, 2017), the NOAEL of **0.3 µg/kg bw/day** from an intravenous prenatal developmental study on tafluprost in rats was identified as the most appropriate point of departure (PoD) for DDDE. As this NOAEL is based on critical developmental effects via a systemic route, it is considered conservative and protective, particularly when compared to the intended dermal exposure for cosmetic use. Since the study used intravenous dosing, no bioavailability correction is required, and the systemic PoD (PoDsys) remains **0.3 µg/kg bw/day**.

The present safety assessment, based on conservative systemic exposure dose (SED) calculations for DDDE at 0.018% in cosmetic eyelash products, results in a **MoS >100**, thus meeting the standard safety criteria. In addition, the MoS calculated by comparing the SED to the **EFSA CONTAM Panel's TSV of 0.0042 µg/kg bw/day** for PGF2α receptor-acting substances also exceeds a MoS of 1, suggesting low risk even under the most conservative regulatory assumptions.

Conclusion

The newly generated NAMs data, the available *in vivo* toxicological data for tafluprost and bimatoprost, and the resulting favourable margins of safety support the safe use of DDDE at a concentration up to 0.018% in cosmetic eyelash products under the conditions presented in this evaluation.

8 REFERENCES

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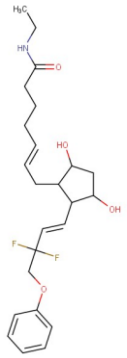
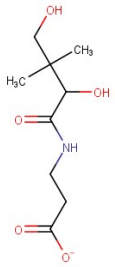
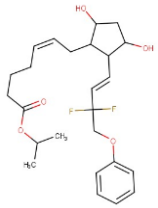
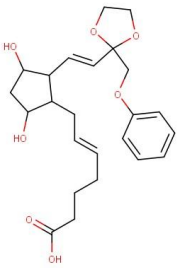
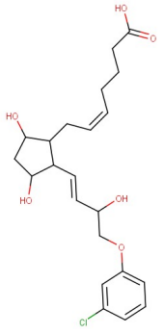
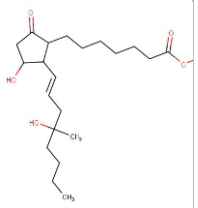
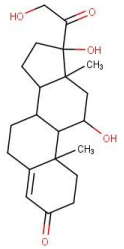
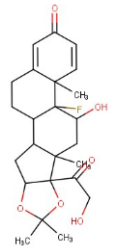
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ANNEX I – SUPPORTING DATA FOR READ ACROSS JUSTIFICATION

PART A: Identification of potential analogues of DDDE*

	Target substance	**Analogue 1**	**Analogue 2**	**Analogue 3**	**Analogue 4**	**Analogue 5**	**Analogue 6**	**Analogue 7**
Chemical name	Dechloro dihydroxy difluoro ethylcloprostenolamide	Sodium D-pantothenate	Tafluprost	7-(3,5-dihydroxy-2-{2-[2-(phenoxymethyl)-1,3-dioxolan-2-yl]ethenyl}cyclopentyl) hept-5-enoic acid	Cloprostenol	Misoprostol	Timodine	Triamcinolone
2D structure								
CAS number	1185851-52-8	867-81-2	209860-87-7	77698-96-5/ 59619-81-7[10]	40665-92-7	59122-46-2	60103-17-5	76-25-5
DICE index from the OECD QSAR Toolbox v.4.7	-	0.17	0.86	0.74	0.63	0.31	0.17	0.19
Organic functional groups from the OECD QSAR	Alcohol Alkene moiety Alkyl halide Allyl Aryl Cycloalkane Dihydroxyl derivatives	Alcohol Alkane, branched with quaternary carbon Carboxylic acid/ Carboxylic acid salts	Alcohol Alkane, branched with secondary carbon Alkene moiety Alkyl halide	Alcohol Alkene moiety Allyl Aryl Carboxylic acid/ Carboxylic acid salts Cycloalkane	Alcohol Alkene moiety Allyl Aryl Aryl halide Carboxylic acid/ Carboxylic acid salts	Alcohol Alkane, branched with tertiary carbon Alkene moiety Allyl Carboxylic acid ester Cycloketone	Acyloin Alcohol Alkene moiety Allyl Cycloalkane Cycloalkene moiety Cycloketone	Alcohol Alkene moiety Alkyl halide Allyl Cycloalkane Cycloalkene moiety Cycloketone

[10] Based on the 2D structure provide by the QSAR tool, the correct CAS number corresponds to 59619-81-7.

	Target substance	Analogue 1	Analogue 2	Analogue 3	Analogue 4	Analogue 5	Analogue 6	Analogue 7
Chemical name	Dechloro dihydroxy difluoro ethylcloprostenolamide	Sodium D-pantothenate	Tafluprost	7-(3,5-dihydroxy-2-{2-[2-(phenoxymethyl)-1,3-dioxolan-2-yl]ethenyl}cyclopentyl) hept-5-enoic acid	Cloprostenol	Misoprostol	Timodine	Triamcinolone
Toolbox v.4.7	Ether moiety Organic amide and thioamide	Dihydroxyl derivatives Organic amide and thioamide	Allyl Aryl Carboxylic acid ester Cycloalkane Dihydroxyl derivatives Ether moiety Isopropyl	Dihydroxyl derivatives Dioxolane Ether moiety Ketal Saturated heterocyclic fragment	Cycloalkane Ether moiety	Dihydroxyl derivatives Ketone Terpenes	Diketone Fused saturated carbocycles Fused unsaturated carbocycles Ketone Steroids Terpenes Unsaturated carbocyclic fragment	Dihydroxyl derivatives Diketone Dioxolane Ether moiety Fused saturated carbocycles Fused unsaturated carbocycles Ketal Ketone Saturated heterocyclic fragment Steroids Terpenes Unsaturated carbocyclic fragment
Structural alerts from the OECD QSAR Toolbox v.4.7	**Estrogen Receptor Binding**: Strong binder, OH group **Toxic hazard classification by Cramer:** High (Class III)	**Toxic hazard classification by Cramer:** Low (Class I) **Carcinogenicity (genotox and nongenotox) alerts by ISS:** Substituted n-alkylcarboxylic acids (Nongenotox) ***In vivo* mutagenicity (Micronucleus) alerts by ISS**: H-acceptor-path3-H-acceptor	**Estrogen Receptor Binding:** Strong binder, OH group **Toxic hazard classification by Cramer:** High (Class III)	**Estrogen Receptor Binding:** Strong binder, OH group **Toxic hazard classification by Cramer:** High (Class III) ***In vivo* mutagenicity (Micronucleus) alerts by ISS**: H-acceptor-path3-H-acceptor	**Estrogen Receptor Binding**: Strong binder, OH group **Toxic hazard classification by Cramer:** High (Class III) **Carcinogenicity (genotox and nongenotox) alerts by ISS:** Halogenated benzene (Nongenotox) ***In vivo* mutagenicity (Micronucleus) alerts by ISS:** H-acceptor-path3-H-acceptor **Oncologic Primary Classification:** Halogenated Aromatic Hydrocarbon Type Compounds	**Estrogen Receptor Binding:** Strong binder, OH group **Protein binding by OASIS:** Nucleophilic addition >> Addition to carbon-hetero double bonds >> Ketones **Toxic hazard classification by Cramer:** Intermediate (Class II) **DART scheme**: Prostaglandin receptor agonists (3c) ***In vivo* mutagenicity (Micronucleus)**	**Estrogen Receptor Binding:** Strong binder, OH group **Protein binding by OASIS:** Nucleophilic addition >> Addition to carbon-hetero double bonds >> Ketones **Protein binding potency Cys (DPRA 13%):** DPRA above 21% (DPRA 13%) >> 1,2- and 1,3-Diketones (reactive) **Toxic hazard classification by Cramer:** High (Class III) **Carcinogenicity (genotox and**	**DNA binding by OECD:** Michael addition >> Polarised Alkenes-Michael addition >> Alpha, beta- unsaturated ketones **Estrogen Receptor Binding**: Strong binder, OH group **Protein binding by OASIS:** Michael addition >> Michael addition on conjugated systems with electron withdrawing group >> alpha,beta-Carbonyl compounds with polarized

	Target substance	Analogue 1	Analogue 2	Analogue 3	Analogue 4	Analogue 5	Analogue 6	Analogue 7
Chemical name	Dechloro dihydroxy difluoro ethylcloprostenolamide	Sodium D-pantothenate	Tafluprost	7-(3,5-dihydroxy-2-{2-[2-(phenoxymethyl)-1,3-dioxolan-2-yl]ethenyl}cyclopentyl) hept-5-enoic acid	Cloprostenol	Misoprostol	Timodine	Triamcinolone
					Protein Binding Potency h-CLAT: Monohaloarenes	**alerts by ISS**: H-acceptor-path3-H-acceptor **Protein binding alerts for skin sensitization by OASIS:** Nucleophilic addition >> Addition to carbon-hetero double bonds >> Ketones Skin irritation/ **corrosion Inclusion rules by BfR:** Ketones	**nongenotox) alerts by ISS:** alpha,beta-unsaturated carbonyls (Genotox) **DART scheme:** Steroid derivatives; Steroid nucleus derived ER and AR binders >> Glucocorticoid and mineralcorticoid receptor binders (2a-2); Steroid nucleus derived ER and AR binders >> Progesterones, anti-androgens (2a-3) ***In vitro* mutagenicity (Ames test) alerts by ISS:** alpha,beta-unsaturated carbonyls ***In vivo* mutagenicity (Micronucleus) alerts by ISS:** alpha,beta-unsaturated carbonyls;H-acceptor-path3-H-acceptor **Keratinocyte gene expression**: Very high gene expression >> alpha, beta-Unsaturated carbonyl compounds **Protein binding alerts for skin sensitization by OASIS:** Nucleophilic addition >> Addition to carbon-hetero double bonds >>	double bonds; Nucleophilic addition >> Addition to carbon-hetero double bonds >> Ketones **Protein binding by OECD:** Michael addition >> Polarised Alkenes >> Polarised alkene – ketones **Toxic hazard classification by Cramer:** High (Class III) **Carcinogenicity (genotox and nongenotox) alerts by ISS:** alpha,beta-unsaturated carbonyls (Genotox) DART scheme: Steroid derivatives; Steroid nucleus derived ER and AR binders >> Glucocorticoid and mineralcorticoid receptor binders (2a-2) ***In vitro* mutagenicity (Ames test) alerts by ISS:** alpha,beta-unsaturated carbonyls ***In vivo* mutagenicity (Micronucleus) alerts by ISS:**

	Target substance	Analogue 1	Analogue 2	Analogue 3	Analogue 4	Analogue 5	Analogue 6	Analogue 7
Chemical name	Dechloro dihydroxy difluoro ethylcloprostenolamide	Sodium D-pantothenate	Tafluprost	7-(3,5-dihydroxy-2-{2-[2-(phenoxymethyl)-1,3-dioxolan-2-yl]ethenyl}cyclopentyl) hept-5-enoic acid	Cloprostenol	Misoprostol	Timodine	Triamcinolone
							Ketones **Protein Binding Potency h-CLAT:** alpha, beta-Unsaturated ketones **Skin irritation/ corrosion Inclusion rules by BfR:** Ketones **Repeated dose (HESS):** Aldosterone (Renal Toxicity) Alert; Anabolic-androgenic steroids (AAS) (hepatotoxicity) Rank B; Cyproterone Acetate (Hepatotoxicity) Alert; Danazol (Hepatotoxicity) Alert; Potassium canrenoate (Renal toxicity) Alert; Spironolactone (Renal toxicity) Alert	alpha,beta-unsaturated carbonyls; H-acceptor-path3-H-acceptor **Keratinocyte gene expression**: Very high gene expression >> alpha, beta-Unsaturated carbonyl compounds **Protein binding alerts for skin sensitization according to GHS**: Skin sensitization Category 1B >> alpha, beta-carbonyl compounds with polarized double bond_low reactivity Protein binding **alerts for skin sensitization by OASIS**: Michael Addition >> Michael addition on conjugated systems with electron withdrawing group >> alpha,beta-Carbonyl compounds with polarized double bonds; Nucleophilic addition >> Addition to carbon-hetero double bonds >>

	Target substance	Analogue 1	Analogue 2	Analogue 3	Analogue 4	Analogue 5	Analogue 6	Analogue 7
Chemical name	Dechloro dihydroxy difluoro ethylcloprostenolamide	Sodium D-pantothenate	Tafluprost	7-(3,5-dihydroxy-2-{2-[2-(phenoxymethyl)-1,3-dioxolan-2-yl]ethenyl}cyclopentyl) hept-5-enoic acid	Cloprostenol	Misoprostol	Timodine	Triamcinolone
								Ketones **Protein Binding Potency h-CLAT:** alpha, beta-Unsaturated ketones **Skin irritation/ corrosion Inclusion rules by BfR:** Ketones
Conclusion on the analogue selection based on three criteria and expert judgement	-	**Not selected due to:** - Similarity index <0.7 - Absence of key functional groups including the geminal fluorine - Differences in structural alerts related to genotoxicity and carcinogenicity	**Selected due to:** - Relatively, high similarity index (0.86) - Common functional groups (cycloalkane, ether moiety and difluoro functional groups) and structural alerts	**Not selected due to:** - Absence of key functional groups, including the geminal fluorine group, and presence of different functional groups including dioxolane - Differences in structural alerts related to genotoxicity	**Not selected due to:** - Similarity index <0.7 - Absence of key functional groups, including the geminal fluorine group - Differences in structural alerts related to genotoxicity and carcinogenicity	**Not selected due to:** - Similarity index <0.7 - Absence of key functional groups including the geminal fluorine group - Differences in structural alerts related to sensitisation, genotoxicity and developmental toxicity	**Not selected due to:** - Similarity index <0.7 - Absence of key functional groups, including the geminal fluorine group - Differences in structural alerts related to sensitisation, carcinogenicity, genotoxicity and developmental toxicity	**Not selected due to:** - Similarity index <0.7 - Absence of key functional groups, including the geminal fluorine group - Differences in structural alerts related to sensitisation, carcinogenicity, genotoxicity and developmental toxicity

*The text in **blue colour** highlights common key functional groups (selection based on expert judgement), while the text in **red colour** highlights the different functional groups structural alerts when compared to the target substance.

PART B: QSAR profiling

Method: Structural alerts related to human health toxicity endpoints were identified by using the OECD QSAR Toolbox v.4.7

Human Health Hazard

Systemic toxicity

Profilers	Background information	Result		
		Target substance	Source substance 1	Source substance 2
Toxic hazard classification by Cramer (v.2.7)	Toxic hazard classification by Cramer profiler is built on the basis of the original paper of Cramer. Categorization rules classifying chemicals into different levels of toxicological concern (when administered orally) are organized in a decision tree scheme. The profiler includes 33 original Cramer rules and places the compound in one of the three classes: 1) Low (Class I), 2) Intermediate (Class II) and 3) High (Class III).	High (Class III)[11]		
Acute Oral Toxicity (v.2.2)	The profiler is considered relevant to the endpoint associated with acute oral toxicity of chemicals. It is based on *in vivo* oral rat LD50 data. It is based on a category approach. Chemicals are grouped in the same category based on their known or hypothetical interaction mechanism and toxicological mode of action. Each category has a local training set of chemicals associated with similar functional groups and same toxicological and chemical mechanism (molecular initiating event, MIE).	Not categorized[12]		
Repeated dose (HESS) (v.4.13)	The profiler contains category boundaries expected to induce similar toxicological effects in **repeated dose oral toxicity**. These category boundaries were developed based on repeated dose toxicity test data in the database of Hazard Evaluation Support System (HESS).	Not categorized[10]		

[11] High (Class III): Substances with chemical structures that permit no strong initial presumption of safety or may even suggest significant toxicity or have reactive functional groups.
[12] The target chemical does not match any of the criteria specified in the profiling scheme.

Skin irritation/corrosion

Profilers	Background information	Result		
		Target substance	Source substance 1	Source substance 2
Skin irritation/corrosion Inclusion rules by BfR[13] (v.3.0)	The profiler contains 40 structural alerts which can be used for positive classification of chemicals causing **irritation, corrosion** or the combination irritation/corrosion depending on their mechanisms.	Inclusion rules not met[10]		

Eye irritation/corrosion

Profilers	Background information	Result		
		Target substance	Source substance 1	Source substance 2
Eye irritation/corrosion inclusion rules by BfR[11] (v.2.1)	The profiler includes 17 structural alerts and is based on empirically derived structural inclusion rules. It identifies chemicals that show potential for **eye irritation or corrosion**. The profiler is also based on known mechanisms of action (biochemical reaction within the eye and/or conjunctival tissues). The model is applicable to organic substances with at least 95% purity and contain hetero atoms not other O, N, S, P, Si and halogen.	Inclusion rules not met[10]		

Sensitisation

Profilers	Background information	Result		
		Target substance	Source substance 1	Source substance 2
Protein binding by OASIS (v.3.9)	The scope of the profiler is to investigate the presence of alerts within target molecules responsible for interaction with proteins. The list of 133 structural alerts has been separated into 11 mechanistic domains. Each of the mechanistic domains has been separated into more than 2 mechanistic alerts.	No alert found		
Protein binding by OECD (v.2.3)	This profiler is based on the analysis of direct acting structural alerts based on theoretical organic chemistry (the profiler does not contain metabolically / abiotically activated structural alerts). The alert compilations were analysed in order to place the information contained within the literature into a mechanistic chemistry framework. The protein binding by OECD profiler contains 18 mechanistic alerts covering 102 structural alerts.	No alert found		

[13] BfR: German Federal Institute for Risk Assessment.

Profilers	Background information	Result		
		Target substance	Source substance 1	Source substance 2
Protein binding potency GSH (v.3.5)	This profiler is developed based on empirical data for thiol reactivity expressed by the *in chemico* RC50 value. Data are obtained by measuring the target chemical covalent binding to the thiol group of glutathione (GSH). The profiler contains 137 structural alerts which are separated into five potency categories: Extremely, Highly, Moderately, Slightly reactive and Suspect. Classification of potency categories is as follows: extremely reactive (RC50 < 0.099 mmol/L); highly reactive (RC50 = 0.100– 0.990 mmol/L); moderately reactive (RC50 = 1.000 – 15.000 mmol/L); slightly reactive (RC50 = 16.000 – 70.000 mmol/L); suspect (RC50 = 71.000 – 135.000 mmol/L).	Not possible to classify according to these rules (GSH)[10]		
Protein binding potency Cys (DPRA 13%) (v.1.0)	The profiler has been built in relation to the implementation of the adverse outcome pathway (AOP) for **skin sensitisation**. It is developed on the basis of data derived from Direct Peptide Reactivity Assay (DPRA). The DPRA is a reactivity assay which evaluates the ability of chemicals to react with proteins. Reduced glutathione and two synthetic peptides – lysine and cysteine – are used as model peptides. The profiler contains 77 structural alerts extracted from about 229 chemicals with experimentally measured cysteine depletion values. The set of 77 structural alerts are separated into three potency categories: DPRA above 21% (DPRA 13%), DPRA less than 9% (DPRA 13%) and Grey zone 9-21% (DPRA 13%).	DPRA less than 9% (DPRA 13%)		
Protein binding potency Lys (DPRA 13%) (v.1.0)	The profiler has been built in relation to the implementation of the AOP for **skin sensitisation**. It is developed on the basis of data derived from DPRA. It contains 73 structural alerts extracted from about 228 chemicals with experimentally measured lysine depletion values. The set of 73 structural alerts are separated into three potency categories: DPRA above 21% (DPRA 13%), DPRA less than 9% (DPRA 13%) and Grey zone 9-21% (DPRA 13%).	DPRA less than 9% (DPRA 13%)		

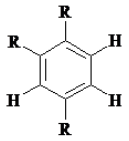
Profilers	Background information	Result		
		Target substance	Source substance 1	Source substance 2
Keratinocyte gene expression (v.2.0)	The profiler is built in relation to the implementation of the AOP for skin sensitisation. It is developed based on the data from the KeratinoSens assay. The profile contains 18 structural alerts extracted from about 300 chemicals comprised within EC1.5 data. The set of 18 structural alerts are separated into four categories: very high gene expression, high gene expression, moderate gene expression and low gene expression. The classification of categories depends on the EC1.5 values and is as follows: chemicals having very high gene expression (EC1.5< = 15 µM); high gene expression (EC1.5 = 15 – 50 µM); moderate gene expression (EC1.5 = 50 – 100 µM); low gene expression (EC1.5 = 100 – 1999 µM).	Not possible to classify according to these rules[10]		
Protein Binding Potency h-CLAT (v.1.0)	This profiler is built in relation to the implementation of the AOP for **skin sensitisation** and developed on the basis of data derived from the human cell line activation (h-CLAT) assay. The profiler contains 28 structural alerts extracted from 223 chemicals with positive/negative data values.	No alert found		
Protein binding alerts for skin sensitisation according to GHS (v.1.4)	The profiler is based on the recently developed OASIS TIMES model for predicting **skin sensitisation** according to GHS criteria. The protein binding alerts are extracted from 1167 chemicals used as a training set for the model. In the current version of the profiler 129 alerts are available, classifying chemicals into GHS categories 1A and 1B.	No alert found		
Protein binding alerts for skin sensitisation by OASIS (v.2.9)	This profiler aims to investigate the presence of alerts within the target molecules responsible for the interaction with proteins and especially with skin proteins. This profiler accounts for the incapability of some chemicals having an alert to interact with skin due to electronic and steric factors. This is explicitly defined by inhibition masks associated with some alerts. The list of 113 structural alerts has been separated into 11 mechanistic domains. Each of the mechanistic domains has been separated into more than 2 mechanistic alerts.	No alert found		
Respiratory sensitization (v.1.1)	This profiler is intended to be used for the assessment of respiratory sensitisation potential of low molecular weight chemicals. It has been developed from mechanistic knowledge of the elicitation phase of respiratory sensitization and, therefore, identifies chemicals able to covalently bind to proteins in the lung.	No alert found		

Profilers	Background information	Result		
		Target substance	Source substance 1	Source substance 2
	This mechanistic hypothesis makes the profiler suitable for the identification of chemicals capable of inducing respiratory sensitisation *via* both the skin and lung.			

Genotoxicity

Profilers	Background information	Result		
		Target substance	Source substance 1	Source substance 2
DNA Binding by OASIS (v.2.10)	This profiler is a general mechanistic profiler, including minimum structural requirements for interaction of chemicals with DNA. The profiler is based on 244 chemical categories having minimum structural requirements for interaction with DNA. The 244 chemical categories have been separated into nine mechanistic domains.	No alert found		
DNA Binding by OECD (v.2.3)	The profiler is based on the mapping of existing structural alerts for mutagenicity and carcinogenicity. The mapping was performed to achieve maximum overlap and usability whilst restricting redundancy in the alerts, and to ensure that the alerts related to the molecular initiating event of covalent DNA binding by OECD. A total of 60 new or re-defined alerts have been created; of these all but two are supported by mechanistic information and meta data.	No alert found		Michael addition >> P450 Mediated Activation to Quinones and Quinone-type Chemicals >> Arenes[14]
DNA alerts for AMES, CA and MNT by OASIS (v.3.10)	The profiler is based on Ames Mutagenicity TIMES model. It is based on structural alerts responsible for the interaction of chemicals with DNA. The structural alerts are distributed in 244 categories, separated into nine mechanistic domains. The scope of this profiler is to investigate the presence of alerts within the target molecules responsible for the interaction with DNA. This profiler accounts for the incapability of some chemicals	Strong binder, OH group		

[14] The chemical class includes the following structure:



R = alkyl carbon, hydrogen

A P450 mediated epoxidation followed by conversion to a reactive quinone has been postulated as the primary cause of benzene derivatives ability to bind to biological nucleophiles (via a Michael addition mechanism).

Profilers	Background information	Result		
		Target substance	Source substance 1	Source substance 2
	having an alert to interact with DNA due to electronic and steric factors. This is explicitly defined by specific structural requirements associated with the alerts.			
Protein binding alerts for chromosomal aberration by OASIS (v.1.9)	The profiler is based on 66 categories alerts accounting for the interactions of chemicals with specific proteins, such as topoisomerases, cellular protein adducts, etc. The scope of this profiler is to investigate the ability of target molecules to elicit clastogenicity. Functionalities related to steric (or electronic) hindrance in molecules that can impede the interactions with proteins are explicitly defined and associated with some of the alerts as "inhibition" masks.	No alert found		
***in vitro* mutagenicity (Ames test) alerts by ISS (v.2.7)**	The profiler is based on the mutagenicity/carcinogenicity module of the software Toxtree. It works as a decision tree for estimating *in vitro* (Ames test) mutagenicity, based on a list of 46 structural alerts.	No alert found		
***in vivo* mutagenicity (micronucleus) alerts by ISS (v.2.7)**	The profiler is based on the ToxMic rule base of the software Toxtree which provides a list of 35 structural alerts for a preliminary screening of potential ***in vivo* mutagens**. The compilation of structural alerts for the *in vivo* micronucleus assay in rodents provided here, is based on both the existing knowledge on the mechanisms of toxic action and on a structural analysis of the chemicals tested in the assay.	No alert found		

Carcinogenicity

Profilers	Background information	Result	
		Target substance	Source substance
Carcinogenicity (genotox and non-genotox) alerts by ISS (v.2.7)	This profiler is an expanded and updated version of the correspondent module of the software Toxtree. It works as a decision tree for estimating carcinogenicity, based on a list of 55 structural alerts of which 35 derive from the Toxtree module and 20 are newly derived. Most of the new structural alerts are relative to nongenotoxic carcinogenicity, whereas the structural alerts in the initial list mainly coded genotoxic carcinogenicity.	No alert found	
Oncologic Primary Classification (v.4.3)	The OncoLogic Primary Classifier consists of molecular definitions developed by LMC and OECD to mimic the	Not classified	

Profilers	Background information	Result	
		Target substance	Source substance
	structural criteria of chemical classes of potential carcinogens covered by the U.S. Environmental Protection Agency's OncoLogic™ Cancer Expert System for Predicting the Carcinogenicity Potential.		

Reproductive and developmental toxicity

Profilers	Background information	Result		
		Target substance	Source substance 1	Source substance 2
Estrogen Receptor Binding (v.2.2)	Estrogen receptor (ER) binding is a molecular initiating event related to protein binding. The ER-binding profiler classifies chemicals as non-binders or binders depending on the molecular weight and structural characteristics of the chemicals.	Strong binder, OH group[15]		
rtER Expert System – USEPA (v.1.0)	The profiler identifies the potential to bind the rainbow trout Estrogen Receptor by using criteria covered by the US EPA Estrogen Receptor Expert System (ERES). This system was built upon a training set of chemicals to cover the defined regulatory inventories using *in vitro* assays specifically optimized to pick up any indication of binding by testing up to chemical solubility or cytotoxicity within the assays to increase confidence that a chemical predicted negative is unlikely to bind ER.	No alert found		
Retinoic Acid Receptor Binding (v.1.1)	Retinoic acid receptor (RAR) binding is a molecular initiating event. *In vivo* retinol is oxidized to **retinoic acid (RA)** and is involved in the control of cell proliferation and differentiation. In the embryo, RA regulates germ layer formation, and the organogenesis of the heart, pancreas, digits, ears, eyes and lungs. The influence of retinoids in over activation of the RAR signalling can lead to a range of developmental defects including cardiovascular, central nervous system, limb, ear, mandible and urogenital defects.	Not possible to classify according to these rules[10]		
DART scheme (v.1.6)	The profiler is based on a decision tree to identify chemicals with structural features associated with the potential to act as reproductive or developmental toxicants. It was developed on the basis of the combination of known modes of action (MOA) and associated structural features, as well as an empirical association of structural fragments within	Not known precedent reproductive and developmental toxic potential		

[15] Strong binder, OH - MW > 200 and MW =< 500 and with a non-impaired OH group attached to 5 or 6 C-atoms ring

Profilers	Background information	Result		
		Target substance	Source substance 1	Source substance 2
	molecules of **reproductive or developmental toxic** (DART) chemicals when MOA information was lacking. The design of this tool is based on a detailed review of 716 chemicals (664 positive, 16 negative, and 36 with insufficient data) that have been evaluated for their DART potential. These chemicals were grouped into 25 different categories, and 129 sub-categories, based on defined receptor binding and chemical properties, and when known, their MOA.			

PART C: Toxicological data of uncommon metabolites

Name	**Ethylamine**	**Isopropanol**
CAS No.	**75-04-7**	**67-63-0**
Acute toxicity: oral	LD_{50} (rat): 390-400 mg/kg bw (ECHA, 2025)	LD_{50} (rat): 4710 - 5840 mg/kg bw; LD_{50} (mouse): 4475 mg/kg bw; LD_{50} (rabbit): 5030 mg/kg bw; LD_{50} (dog): 4830 mg/kg bw (OECD SIDS, 1997)
Acute toxicity: dermal	LD_{50} (rabbit): 265 - 360 mg/kg bw (ECHA, 2025)	LD_{50} (rabbit): 12,870 mg/kg bw (OECD SIDS, 1997)
Repeated dose toxicity: oral	**Read across to methylamine (CAS No.74-89-5)** 28-day NOAEL (systemic): 500 mg/kg bw/day based on reductions in parental body weights and food consumption (gavage) (OECD 422) (OECD SIDS, 2011)	27-day NOAEL (rats): 600-1000 mg/ kg bw/day based decreased body weight (drinking water) (Guideline not specified) ---- 12-week NOAEL (male rats): 870 mg/ kg bw/day based on increased relative organ weights of liver, kidneys, and adrenals (drinking water) (Guideline not specified) (OECD SIDS, 1997)
Genetic toxicity *in vitro* (bacteria)	Negative (Ames assay; similar to OECD TG 471) (No guideline followed) (ECHA, 2025)	Negative (Ames test; similar to OECD 471) (ECHA, 2022; OECD SIDS, 1997) ---- Negative (Ames test; similar to OECD 471) (Guideline not specified) (ECHA, 2022)
Genetic toxicity *in vitro* (cytogenicity study in mammalian cells)	-	Negative (Sister chromatid exchange assay; similar to OECD 479) (Guideline not specified) (OECD SIDS, 1997)
Genetic toxicity *in vitro* (mutagenicity study in mammalian cells)	-	Negative (*in vitro* mammalian cell gene mutation; similar to OECD 476) (Guideline not specified) (OECD SIDS, 1997)
Genetic toxicity *in vivo* cytogenicity study in mammalian cells	Negative (*in vivo* DNA damage and/or repair) (No guideline followed) (ECHA, 2025) ---- **Read across to methylamine (CAS No.74-89-5)** Negative	Negative (*in vivo* mammalian erythrocyte MNT; similar to OECD 474) (ECHA, 2022)

Name CAS No.	Ethylamine 75-04-7	Isopropanol 67-63-0
	(*in vivo* mammalian erythrocyte MNT; similar to OECD 474) (Guideline not specified) (OECD SIDS, 2011)	
Toxicity to reproduction	**Read across to methylamine (CAS No.74-89-5)** 28-day systemic and reproductive NOAEL (rats): 500 mg/kg bw/day based on reduced corpora lutea and subsequent reductions in implantations and litter size (gavage) (OECD 422) ---- 24-week NOAEC (rats): 922 mg/m^3 based on the absence of adverse effects on gonads (inhalation) (OECD SIDS, 2011)	2-generation parental NOAEL (rats): 500 mg/kg bw/day based on increases in absolute and/or relative liver and/or kidney weights 2-generation reproductive NOAEL (rats): >1000 mg/kg bw/day 2-generation offsprings NOAEL (rats): 100 mg/kg bw/day based on increased mortality and/or, reduced body weights (gavage) (similar to OECD 416) (ECHA, 2022) ---- 10-week reproductive NOAEL (rat): 625-825 mg/kg bw/day based on reduced pup weight gain and decreased survival (drinking water) (similar to OECD 415) (OECD SIDS, 1997)
Developmental toxicity	**Read across to methylamine (CAS No.74-89-5)** 28-day developmental NOAEL (rat): 1000 mg/kg bw/day based on the absence of adverse effects (gavage) (OECD 422) (OECD SIDS, 2011)	Maternal NOAEL (rats): 400 mg/kg bw/day based on reduced body weight gain and food consumption; Developmental NOAEL (rats): 400 mg/kg bw/day based on reduced foetal body weights/litter (pre-natal developmental toxicity study; similar to OECD 414) Maternal NOAEL (rabbits): 240 mg/kg bw/day based on reduced body weight and clinical signs of toxicity Developmental NOAEL (rabbits): 480 mg/kg bw/day (pre-natal developmental toxicity study; similar to OECD 414) Maternal NOAEL (rats): 596 mg/kg bw/day based on decreased food consumption, water consumption, and body weight Developmental NOAEL (rats): 596 mg/kg bw/day based on decreased mean foetal body weight (pre-natal developmental toxicity study; similar to OECD 414) (ECHA, 2022) ---- Maternal and developmental neurotoxicity NOAEL (rats): 1200 mg/kg bw/day (pre-natal developmental toxicity study; similar to OECD 414) (OECD SIDS, 1997)

** The NOAEL/LOAELs were not established in the source documents but was concluded based on the available details on the adverse effects in the underlying studies*

ANNEX II – TOXICOLOGICAL DATA ON THE ANALOGUES

1. Acute toxicity

1.1 Acute oral toxicity *(PREVIOUSLY SUBMITTED)*

The *in vitro* acute oral toxicity study available for DDDE is further supported by the acute toxicity data available for the structural analogue tafluprost.

Guideline:	Not specified
Species/strain:	Rats/Sprague-Dawley
Number of animals:	5/sex/group
Test substance:	Tafluprost
Product name:	AFP-168
Vehicle:	Not specified
Batch:	Not specified
Purity:	Not specified
Dose levels:	0, 10, 30 and 100 mg/kg bw
Dose volume:	Not specified
Route:	Oral
Administration:	Not specified
Observation	14 days
GLP:	Not specified
Study period/year:	Not specified (pre-2009)

The test substance tafluprost (purity not specified) was investigated for acute oral toxicity in rats. Sprague-Dawley rats (5/sex/group) were administered single doses of 0, 10, 30 and 100 mg/kg bw. After dosing, the animals were observed daily for clinical signs of toxicity and mortality for 14 days. Body weights, food intake and water intake were determined at defined intervals.

Results

No mortalities and no marked changes in body weight, food intake, water intake or gross pathology were observed. The hunched posture and wasted appearance beginning one day after dosing and chest sores or loss of chest fur at Day 7 were observed in one animal of the 10 mg/kg bw dose group and one animal of the 100 mg/kg dose group. The chest sores or loss of chest fur was reversed by Day 14.

Conclusion

Under the conditions of the study, the LD_{50} of test substance was considered to be ≥100 mg/kg bw in rats.

(CDER, 2011b; TGA, 2012)

1.2 Acute intravenous (IV) toxicity

Two acute intravenous studies are available for the analogue tafluprost and one for the analogue bimatoprost. The acute parenteral studies on the analogues have been included to provide perspective about the doses inducing lethality, since the repeated dose and reproductive and developmental toxicity endpoints have been addressed based on read across studies of the analogue *via* parenteral routes.

Tafluprost *(PREVIOUSLY SUBMITTED)*

1st study: Acute intravenous toxicity study in rats

Guideline:	Not specified
Species/strain:	Rats/Sprague-Dawley
Number of animals:	5/sex/group
Test substance:	Tafluprost
Product name:	AFP-168
Vehicle:	Not specified
Batch:	Not specified
Purity:	Not specified
Dose levels:	0, 1, and 3 mg/kg bw
Dose volume:	Not specified
Route:	Intravenous
Observation	14 days
GLP:	Not specified
Study period/year:	Not specified (pre-2009)

Tafluprost (purity not specified) was investigated for acute intravenous toxicity in rats. In this study, Sprague-Dawley rats (5/sex/group rats) were administered single intravenous doses of the test substance at 0, 1 and 3 mg/kg bw. Following exposure, the animals were observed daily for clinical signs of toxicity, and mortality for 14 days. Body weights, food intake and water intake were determined at defined intervals.

Results

Neither mortality, clinical signs nor marked changes in body weight, food intake, water intake or gross pathology were observed by the study investigators.

Conclusion

Under the conditions of the study, the LD_{50} of test substance was determined to be >3 mg/kg bw in rats.

(CDER, 2011b; TGA, 2012)

2nd study: Acute intravenous toxicity study in dogs

Guideline:	Not specified
Species/strain:	Beagle dogs
Number of animals:	2 males/group
Test substance:	Tafluprost
Product name:	AFP-168
Vehicle:	Not specified
Batch:	Not specified
Purity:	Not specified
Dose levels:	0, 0.3, 3, and 30 µg/kg bw (i.e., 0.0003, 0.003 and 0.03 mg/kg bw)
Dose volume:	Not specified
Route:	Intravenous
Observation	14 days
GLP:	Not specified
Study period/year:	Not specified (pre-2009)

Tafluprost (purity not specified) was investigated for acute intravenous toxicity in dogs. In this study, Beagle dogs (2 males/group) were administered single intravenous doses of the test substance at 0,

0.3, 3, and 30 µg/kg bw. Following exposure, the animals were observed daily for clinical signs of toxicity and mortality for 14 days. Body weights and food intake were determined at defined intervals. Haematology, urinalysis, body temperature, blood pressure or ophthalmologic findings were also evaluated.

Results

No mortalities or marked changes in body weight, haematology parameters, urinalysis, body temperature, or ophthalmologic findings were observed.

Clinical signs including salivation, vomiting, moderate miosis, irregular respiration and increased heart rate were observed at doses ≥0.003 mg/kg bw. The miosis was severe, and elevated blood pressure was also observed at 0.03 mg/kg bw. No adverse effects were noted at 0.0003 mg/kg.

Conclusion

Under the conditions of the study, the LD_{50} of test substance was determined to be >0.03 mg/kg bw in dogs.

(CDER, 2011b; TGA, 2012)

Two acute intravenous studies are available for the analogue tafluprost and one for the analogue bimatoprost. The acute parenteral studies on the analogues have been included to provide perspective about the doses inducing lethality, since the repeated dose and reproductive and developmental toxicity endpoints have been addressed based on read across studies of the analogue *via* parenteral routes.

Bimatoprost (NEW)

1st study: Acute intravenous toxicity study in rats

Guideline:	Not specified
Species/strain:	Sprague Dawley rats
Number of animals:	6/sex/dose
Test substance:	Bimatoprost
Product name:	AGN 192024 ophthalmic solution
Vehicle:	Not specified
Batch:	Not specified
Purity:	Not specified
Dose levels:	0.03, 0.3 and 3.0 mg/kg
Dose volume:	3 mL/kg
Route:	Intravenous
Observation	14 days
GLP:	Yes
Study period/year:	1995

Bimatoprost (AGN 192024; purity not specified) was investigated for acute intravenous toxicity in rats. In this study, Sprague Dawley rats (6/sex/group) were administered a single intravenous dose of the test substance at 0.03, 0.3 and 3 mg/kg bw. Animals were observed for 14 days post-treatment for clinical signs of toxicity and mortality.

Results

No mortalities or marked changes in body weight and gross pathology and clinical signs were observed in any dose group.

Conclusion

Under the study conditions, the LD_{50} of the analogue bimatoprost can be considered as >3 mg/kg bw in rats.

(Apotex Inc., 2022; CDER, 2001b)

Overall conclusion on acute toxicity potential

Based on the oral LD50 value of 880 mg/kg bw determined in the *in vitro* NRU tests in 3T3 fibroblasts together with the analogue data supports a moderate acute oral toxicity potential for DDDE. The acute toxicity studies on the analogues *via* the parenteral route did not induce mortality at a dose of 3 mg/kg bw in rats and 0.03 mg/kg bw in dogs.

2. Repeated dose toxicity

No repeated dose toxicity studies could be identified for DDDE. Therefore, data available for the analogues tafluprost and bimatoprost is used for assessing the systemic toxicity potential of DDDE by means of read across. The scientific justification for the read across is provided in **Section 5**. with more details provided in **Annex I**.

The repeated dose toxicity dataset for the analogue tafluprost includes 4 intravenous (2 subacute, 2 subchronic), 2 subcutaneous (subchronic) and 4 ocular studies (1 subacute, 2 subchronic and 1 chronic). Similarly, the dataset for the analogue bimatoprost consists of 11 oral (7 subacute, 2 subchronic and 1 chronic), 5 intravenous (4 subacute and 1 subchronic) and 6 ocular (4 subacute and 2 chronic). The overview of the repeated dose studies with the analogues tafluprost and bimatoprost has been summarised in **Table 1 of Annex II**.

Table 1. Overview of repeated dose toxicity studies with the analogues tafluprost and bimatoprost

Study type, Species	Doses	Key findings	NOAEL/LOAEL	Reference
Oral route				
No oral RDT studies are available for tafluprost				
Bimatoprost (NEW)				
2 weeks, gavage study, CD1 mice, (18/sex/dose), no guideline specified[#] Study to obtain PK information	0.1, 0.3, 1 and 2 mg/kg bw/day	No significant adverse effects at any dose level	NOAEL = 2 mg/kg bw/day (2000 µg/kg bw/day)*	(CDER, 2001b)
2 weeks, gavage study, Swiss-Webster mice, (3/sex/dose), no guideline specified[#]	0, 10, 50 and 100 mg/kg bw/day	No significant adverse effects at any dose level	NOAEL = 100 mg/kg bw/day (100000 µg /kg bw/day)	(CDER, 2001b)
2 weeks, gavage study, Swiss-Webster mice, (3/sex/dose), no guideline specified[#]	0, 250 and 500 mg/kg bw/day	At 500 mg/kg bw/day, treatment-related lesions in males with a minimal, transient increase in the number of abnormal germ cells in the epididymides mostly in the caudal region	NOAEL = 250 mg/kg bw/day (250000 µg/kg bw/day)	(CDER, 2001b)
2 weeks, gavage study, Sprague Dawley rats, (3/sex/dose), no guideline specified[#]	0, 10, 50 and 100 mg/kg bw/day	At 50 and 100 mg/kg bw/day, treatment-related lesions in one male in each group, with minimal to mild degeneration	NOAEL = 10 mg/kg bw/day (10000 µg/kg bw/day)	(CDER, 2001b)

Study type, Species	Doses	Key findings	NOAEL/LOAEL	Reference
		in 1 testis and abnormal germ cells in the epididymides		
2 weeks, gavage study, Sprague Dawley rats, (3/sex/dose), no guideline specified[#]	0, 250 and 500 mg/kg bw/day	At all doses, decreased body weight gain and increased white blood cell counts and serum BUN levels. Without corresponding histopathological findings, the toxicological significance of these changes was not determined	LOAEL = 250 mg/kg bw/day (250000 µg/kg bw/day) No NOAEL could be established	(CDER, 2001b)
28 days, gavage study, Swiss-Webster mice, (10/sex/dose), no guideline specified	0, 4, 8 and 16 mg/kg bw/day	No significant adverse effects at any dose level	NOAEL = 16 mg/kg bw/day (16000 µg/kg bw/day)	(CDER, 2001b)
28 days, gavage study, Sprague Dawley rats, (8/sex/dose), no guideline specified	0, 4, 8 and 16 mg/kg bw/day	At 16 mg/kg bw/day, bilateral degeneration of the testis and increased abnormal germ cells in the tubular lumen of the epididymis	NOAEL = 8 mg/kg bw/day (8000 µg/kg bw/day)	(CDER, 2001b)
13 weeks (+4 weeks recovery period), gavage study, CD1 mice, (10/sex/dose), no guideline specified Range finding study for carcinogenicity study.	0, 4, 8 or 16 mg/kg bw/day	At 16 mg/kg bw/day, increased incidence of acute inflammatory cells in the superficial layers of the vagina for females and extensive lymphoma deposits across multiple organs in one female	NOAEL = 8 mg/kg bw/day (8000 µg/kg bw/day)*	(CDER, 2001b)
13 weeks (+4 weeks recovery period), gavage study, CD rats, (10/sex/dose), no guideline specified	0, 0.1, 0.3 and 1 mg/kg bw/day	At 0.3 and 1.0 mg/kg bw/day, an increased total number of corpora lutea and ovary weights in females; no effects in males	NOAEL = 1 and 0.1 mg/kg bw/day (1000 and 100 µg /kg bw/day for males and females, respectively)*	(CDER, 2001b)
13 weeks (+4 weeks recovery period), gavage study, CD BR rats, (10/sex/dose), no guideline specified	0, 4, 8 or 16 mg/kg bw/day	At all doses, decrease in terminal body weights and body weight gain in males and females and increase in ALT and AST activities (2- to 5-fold) in male rats. Prominent corpora lutea (total of more than 70 in both ovaries), vacuolated corpora lutea and increased ovary weights in females**.** All treatment-related changes showed reversibility during the recovery period. *Note: As per the report, the mechanisms of the ovarian changes were likely related to pharmacologic*	NOAEL = 16 mg/kg bw/day (16000 µg/kg bw/day)*	(CDER, 2001b)

Study type, Species	Doses	Key findings	NOAEL/LOAEL	Reference
		effects of luteolysis by PGF2a and were considered species-specific.		
52 weeks (+8 weeks recovery period), gavage study, CD rats, (15/sex/dose), no guideline specified	0, 0.1, 0.3 and 2.0 mg/kg bw/day	At 0.3 and 2 mg/kg bw/day, cellular vacuolation in corpora lutea and decreased terminal body weights and body weight gain in females. All treatment-related changes except decrease in body weight gain showed reversibility during the recovery period.	NOAEL = 2 and 0.1 mg/kg bw/day (2000 and 100 µg/kg bw/day for males and females, respectively)	(CDER, 2001b)
Intravenous route				
Tafluprost (PREVIOUSLY SUBMITTED)				
28 days, intravenous study, rats (strain not specified) (12/sex/dose)	0, 10, 30 and 100 µg/kg bw/day	No significant adverse effects at any dose level	NOAEL = 100 µg/kg bw/day	(CDER, 2011b)
28 days, intravenous study, dogs (strain not specified) (4/sex/dose)	0, 0.1, 1 and 10 µg/kg bw/day	At 10 µg/kg bw/day, salivation, vomiting, miosis, increased respiratory rate, increased heart rate, and prolonged corrected QT interval (QTc) interval in both sexes, increased diastolic pressure only in males, and reduced urinary chloride concentration only in females	NOAEL = 1 µg/kg bw/day	(CDER, 2011b)
26 weeks, intravenous study, rats (Crl:CD®(SD)IGSBR) (15/sex/dose)	0, 10, 30 and 100 µg/kg bw/day	At all dose levels, histopathology findings in bone marrow in the femoral and sternum bone, spleen, liver, and kidney	LOAEL = 10 µg/kg bw/day *	(CDER, 2011b)
39 weeks, intravenous study, dogs (strain not specified) (4/sex/dose)	0, 0.1, 1 and 10 µg/kg bw/day	At 10 µg/kg bw/day, transient clinical signs of nausea, transient miosis, transient slight elevations in heart rate, blood pressure, and respiratory rate, minor adrenal cortical eosinophilia in 3 out of 4 dogs and acinar cell hypertrophy in salivary glands in all dogs	NOAEL = 1 µg/kg bw/day	(CDER, 2011b)
Bimatoprost (NEW)				
1-week, intravenous study, Sprague Dawley rats, (3/sex/group), no guideline specified[#]	0, 5, 10, 50 and 100 mg/kg bw/day	At 50 and 100 mg/kg bw/day, decreased body weight gain in male rats and decreased motor activity, dyspnoea, cyanotic tail, soft stool, ataxia, gasping, ptosis, lethargy and piloerection	NOAEL = 10 mg/kg bw/day (10000 µg/kg bw/day)*	(CDER, 2001a; b)
2 weeks, intravenous study, Sprague Dawley	0, 0.03, 0.3 and 1 mg/kg bw/day	No significant adverse effects at any dose level	NOAEL = 1 mg/kg bw/day (1000 µg /kg bw/day)	(CDER, 2001a)

Study type, Species	Doses	Key findings	NOAEL/LOAEL	Reference
rats, (10/sex/group), no guideline specified[#]				
1-month, intravenous study, Sprague Dawley rats, (12/sex/group), no guideline specified	0, 0.1, 0.3 and 1 mg/kg bw/day	At 1 mg/kg/day, decreased regression of corpora lutea, with increased corpora lutea in the ovary of female rats	NOAEL = 1 and 0.3 mg/kg bw/day (1000 and 300 µg/kg bw/day for males and females, respectively)	(CDER, 2001a)
1-month, intravenous study, cynomolgus monkeys, (3/sex/group), no guideline specified	0, 0.1, 0.3 and 1 mg/kg bw/day	No significant adverse effects at any dose level	NOAEL = 1 mg/kg bw/day (1000 µg/kg bw/day)	(CDER, 2001a)
17 weeks, intravenous study, cynomolgus monkeys, (6/sex/dose), no guideline specified	0, 0.01, 0.1, and 1 mg/kg bw/day	No significant adverse effects at any dose level	NOAEL = 1 mg/kg bw/day (1000 µg/kg bw/day)*	(CDER, 2001a)
Subcutaneous route (SC)				
Tafluprost (PREVIOUSLY SUBMITTED)				
13-week, subcutaneous study, mice (Crl:CD-1(ICR)BR) (12/sex/dose) Range finding studies for carcinogenicity study.	0, 3, 10, 30 and 100 µg/kg bw/day	No significant adverse effects at any dose level	NOAEL = 100 µg/kg bw/day*	(CDER, 2011b)
13-week, subcutaneous study, rats (Crl:CD®(SD) IGSBR) (10/sex/dose) Range finding studies for carcinogenicity study.	0, 3, 10, and 30 µg/kg bw/day	At 30 µg/kg bw/day, minor histopathological changes in spleen and kidney	NOAEL = 30 µg/kg bw/day*	(CDER, 2011b)
78 weeks, subcutaneous study, mice (Crl:CD-1(ICR)BR) (51/sex/dose)	0, 10, 30 and 100 µg/kg bw/day	No significant adverse effects at any dose level	Systemic and carcinogenicity NOAEL = 100 µg/kg bw/day*	(CDER, 2011b)
24-month, subcutaneous study, rats (Crj:CD(SD) IGSBR) (60/sex/dose)	0, 3, 9 and 30 µg/kg bw/day	<u>Systemic</u> At 3 µg/kg bw/day, significant reduction in body weight in both sexes. <u>Non-neoplastic</u> Histopathology changes	Systemic LOAEL = 3 µg/kg bw/day* Carcinogenicity NOAEL = 30 µg/kg bw/day	(CDER, 2011b)

Study type, Species	Doses	Key findings	NOAEL/LOAEL	Reference
		including hyperostosis of the sternum and femur in some animals and increased incidence of extramedullary haematopoiesis in the spleen in males at all dose levels No carcinogenicity at any dose level		
No subcutaneous RDT studies are available for bimatoprost				
Topical ocular				
Tafluprost[16] (NEW)				
28 days, ocular study, monkeys (strain not specified) (3/sex/dose)	0.0005, 0.005% and 0.05% of the ophthalmic solution (i.e., equivalent to 0.067–0.1, 0.67–1 and 6.7–10 µg/kg bw/day)	Systemic and local No significant toxicity at any dose level	Systemic NOAEL = 6.7 and10 µg/kg bw/day for males and females, respectively	(CDER, 2011b; TGA, 2012)
13-week, ocular study, monkeys (strain not specified) (4/sex/dose)	0.0005, 0.005% and 0.05% of the ophthalmic solution (i.e., equivalent to 0.075–0.1, 0.75–1 and 7.5–10 µg/kg bw/day)	Systemic No systemic toxicity at any dose level Local At all dose levels, irreversible darkening in iris colour in left treated eye[17]	Systemic NOAEL = 7.5 and 10 µg/kg bw/day for males and females, respectively	(CDER, 2011b; TGA, 2012)
13-week, ocular study, monkeys (strain not specified) (3/sex/dose)[18]	0.0045% of the ophthalmic solution (i.e., equivalent to 0.75 – 0.9 µg/kg bw/day)	Systemic No systemic toxicity at any dose level Local At all dose levels, changes in iris colour in 2 animals[19]	Systemic NOAEL = 0.75 and 0.9 µg/kg bw/day for males and females, respectively	(CDER, 2011b; TGA, 2012)
52-weeks, ocular study, monkeys (strain not specified) (4/sex/dose)	0.0005, 0.005% and 0.05% of the ophthalmic solution (i.e.,	Systemic No systemic toxicity at any dose level Local	Systemic NOAEL = 7.5 and 10 µg/kg bw/day for males and females, respectively	(CDER, 2011b; TGA, 2012)

[16]Doses in µg/kg bw/day are reported in the Australian public assessment report (TGA, 2012).
[17] These effects are considered to be mainly cosmetic, not associated with loss of function, and not toxicologically significant (CDER, 2011).
[18] The study was conducted in multiple test groups, including a combination of tafluprost with timolol. Only the test group with a standalone administration of tafluprost was considered for the assessment (CDER, 2011).
[19] These effects are considered to be mainly cosmetic, not associated with loss of function, and not toxicologically significant (CDER, 2011).

Study type, Species	Doses	Key findings	NOAEL/LOAEL	Reference
	equivalent to 0.075–0.1, 0.75–1 and 7.5–10 µg/kg bw/day)	At all dose levels, sunken, dark iris colour, blue-grey discolouration in 3/4 animals of both the genders throughout study period		
Bimatoprost[20] (NEW)				
3-day, ocular study, New Zealand white and Dutch Belted rabbits, (4/sex/group), no guideline specified[#]	0.03% ophthalmic solution (35 µL volume) in left eye only, 4 times a day x 3 days (2 hr intervals) = 21 µg/day	Systemic No systemic toxicity at any dose level Local Slight transient conjunctival hyperaemia in one group *Note: statistical significance not specified*	Systemic NOAEL = 0.03%*	(CDER, 2001a)
1-month, ocular study, Dutch Belted rabbits, (10/sex/group), no guideline specified	0.03% ophthalmic solution (35 µL volume) in left eye only, two times a day, every day, at a 6 hr interval	Systemic and local No significant toxicity at any dose level	Systemic NOAEL = 0.03% equivalent to 12.4 and 19.1 µg/kg bw/day for males and females, respectively*	(CDER, 2001a)
1-month (+14 days recovery period), ocular study, New Zealand rabbits, (10/sex/dose), no guideline specified	0.001, 0.01 and 0.1% ophthalmic solution (35 µL volume) in left eye only, 4 times a day, every day, for 1 month	Systemic and local No significant toxicity at any dose level	Systemic NOAEL = 0.1% equivalent to 82.35 and 127.27 µg/kg bw/day for males and females, respectively *	(CDER, 2001a)
1-month, ocular study, Beagle dogs, (3/sex/dose), no guideline specified	0.001, 0.01 and 0.1% ophthalmic solution (35 µL volume) in left eye only, 4 times a day, every day, for 1 month	Systemic and local No significant toxicity at any dose level	Systemic NOAEL = 0.1% equivalent to 9.33 and 10 µg/kg bw/day for males and females, respectively*	(CDER, 2001a)
6-month (+1-month recovery period), ocular study, Dutch Belted rabbits, (15/sex/dose), no guideline specified	0.03 and 0.1% ophthalmic solution (35 µL volume) in left eye only, one or two times a day, every day for 6 months	Systemic and local No significant toxicity at any dose level	Systemic NOAEL = 0.1% equivalent to 25 – 41.18 µg/kg bw/day*	(CDER, 2001a)

[20] Doses are converted in µg/kg bw/day using the animal weights provided in the source document.

Study type, Species	Doses	Key findings	NOAEL/LOAEL	Reference
29 or 52-weeks, ocular study, cynomolgus monkeys, (6/sex/dose), no guideline specified	0.03% and 0.1% (bid) or 0.03% (qid) ophthalmic solution (35 µL volume) in right eye only, one or two times a day	Systemic and local No significant toxicity at any dose level	Systemic NOAEL = 0.1% equivalent to 25.93 – 36.84 µg/kg bw/day*	(CDER, 2001a)

** The NOAEL/LOAELs were not established in the source documents but were determined based on the available details regarding adverse effects in the underlying studies.*

#Dose range finding studies and short-term studies conducted to obtain information like pharmacokinetics, maximum tolerated dose and systemic exposure are not taken up for summarisation.

2.1 Oral route

2.1.1 Subacute toxicity

Bimatoprost (NEW)

1st study: Subacute toxicity study in mice *via* the oral route

Guideline:	Not specified
Species/strain:	Mice/Swiss-Webster
Number of animals:	10/sex/group
Test substance:	Bimatoprost
Product name:	AGN 192024 (0.2% solution)
Vehicle:	Not specified
Batch:	80597-02B
Purity:	Not specified
Route:	Oral
Administration:	Gavage
Dose levels:	0, 4, 8 and 6 mg/kg bw/day (equivalent to 0, 4000, 8000, 16000 µg/kg bw/day
Duration:	28 days
GLP:	No
Study period/year:	1997

The subacute repeated dose toxicity of the analogue bimatoprost (purity not specified) was investigated in mice following oral administration of 0, 4000, 8000, 16000 µg/kg bw/day of AGN 192024 (0.2% solution) or vehicle control, daily for 28 days. During the treatment period, animals were observed for clinical signs, mortality, body weight and food consumption at defined intervals. Haematological and clinical chemistry parameters were examined. All animals were sacrificed on Days 29 and 30 and macroscopically examined, organs were weighed, and comprehensive histopathology was performed.

Results

No treatment-related mortality, clinical signs, or changes in body weight, food consumption, clinical chemistry and gross necropsy were noted.

During the histopathological examinations, minimal mononuclear cell infiltration in the liver and kidneys, tubular degeneration and regeneration of the kidneys, and hypoplasia of the testis with the associated reduced germ cell in the epididymides were noted in both control and treated animals. These changes were not considered toxicologically significant.

Conclusion

Under the conditions of the study, the NOAEL for bimatoprost was established at 16000 µg/kg bw/day (highest tested dose) in mice.

(CDER, 2001b)

2nd study: Subacute toxicity study in rats *via* the oral route

Guideline:	Not specified
Species/strain:	Rat/Sprague Dawley
Number of animals:	8/sex/group
Test substance:	Bimatoprost
Product name:	AGN 192024
Vehicle:	Not specified
Batch:	80597-02A
Purity:	Not specified
Route:	Oral
Administration:	Gavage
Dose levels:	0, 4, 8 and 6 mg/kg bw/day (equivalent to 0, 4000, 8000, 16000 µg/kg bw/day (dosing volume: 8, 2, 4 and 8 mL/kg, respectively)
Duration:	28 days
GLP:	No
Study period/year:	1997

The subacute repeated dose toxicity of bimatoprost (purity not specified) was investigated in rats following oral administration of 0, 4, 8 and 6 mg/kg bw/day (equivalent to 0, 4000, 8000, 16000 µg/kg bw/day (dosing volume: 8, 2, 4 and 8 mL/kg, respectively) of AGN 192024 (0.2% solution) or vehicle control, daily for 28 days. During the treatment period, animals were observed for clinical signs, mortality, body weight and food consumption at defined intervals. Haematological parameters, clinical chemistry parameters and urinalysis were examined. All animals were sacrificed on Days 29 and 30 and macroscopically examined, organs were weighed, and comprehensive histopathology was performed.

Results

No treatment-related mortality, clinical signs, or changes in food consumption, clinical chemistry, urinalysis and gross necropsy were noted. A decrease in body weight gain was seen in male animals; however, there were not significant differences in body weights among different groups. In females, no differences in body weight and body weight gain were noted. Therefore, the decrease in body weight gain in males might not be toxicologically significant.

During histopathological examinations, statistically significant, treatment-related bilateral degeneration of the testis and increased abnormal germ cells in the tubular lumen of the epididymis were observed in 3 of 8 male animals at 16000 µg/kg bw/day. A higher incidence of vacuolization of the cortical cells in the adrenal glands was seen in treated males; however, the statistical significance of this adrenal change is not clear.

Conclusion

Under the study conditions, the NOAEL for the analogue bimatoprost was established at 8000 µg/kg bw/day based on treatment-related bilateral degeneration of the testis and increased abnormal germ cells in the tubular lumen of the epididymis in rats.

(CDER, 2001b)

2.1.2 Subchronic toxicity

Bimatoprost (NEW)

1st study: Subchronic toxicity study in mice *via* the oral route

Guideline:	Not specified
Species/strain:	Mice/Crl:CD-1 (JCR) BR
Number of animals:	10/sex/group (main study); 5/sex/group (recovery group)
Test substance:	Bimatoprost
Product name:	AGN 192024 (0.2% solution)
Batch/Lot:	11220 and 11233
Purity:	Not specified
Route:	Oral
Administration:	Gavage
Dose levels:	0, 4, 8 and 6 mg/kg bw/day (equivalent to 0, 4000, 8000, 16000 µg/kg bw/day
Dose volume:	8, 2, 4 and 8 mL, respectively
Vehicle:	Not specified
Satellite group	No
Duration:	13 weeks
Recovery:	4 weeks
GLP:	Yes
Study deviation:	Yes, study deviations were not considered to have altered the outcome or integrity of the study.
Study year:	Not specified (before 2013)

The subchronic repeated dose toxicity of bimatoprost (purity not specified) was investigated in mice following oral administration of 0, 4000, 8000, 16000 µg/kg bw/day (dosing volume: 8, 2, 4 and 8 mL/kg, respectively) of AGN 192024 (0.2% solution) or vehicle control, daily for 13 weeks followed by 4 weeks recovery period. The data from this study has been used in the dosage selection for the carcinogenicity study. During the treatment period, animals were observed for mortality, changes in clinical signs, body weight and food consumption at defined intervals. Haematological, biochemical, ophthalmological examination and urinalysis were performed. All animals were sacrificed and macroscopically examined, organs were weighed, and comprehensive histopathology was performed.

Results

One female from the 16000 µg/kg bw/day group was found dead on Day 4, and one male from 4000 µg/kg bw/day was sacrificed on Day 35. The reasons for these deaths were unknown. No treatment-related clinical signs in surviving animals, changes in body weight, water consumption, clinical chemistry, urinalysis, ophthalmic parameters, gross pathology, or organ weights were noted.

A decrease in total white blood cell count was noted across all treatment groups. However, this reduction was not statistically significant and was not associated with any pathological findings; thus, it was not considered toxicologically relevant.

Histopathological examination revealed an increased incidence of medullary lymphoid proliferation in the thymus of females at 8000 and 16000 µg/kg bw/day, as well as an increased presence of acute inflammatory cells in the superficial layers of the vagina in females at 16000 µg/kg bw/day. The thymus changes fully reversed, while vaginal changes showed partial recovery during the 4-week recovery period. Additionally, one female from the 16000 µg/kg bw/day group exhibited extensive lymphoma deposits across multiple organs. After the recovery period, a pulmonary adenoma was detected in one male from the same dose group. No other toxicologically significant findings were observed.

Conclusion

Under the study conditions, the NOAEL for the analogue bimatoprost can be considered to be 4000 µg/kg bw/day based on increased incidence of acute inflammatory cells in the superficial layers of the vagina for females at 16000 µg/kg bw/day.

(CDER, 2001b)

2nd study: Sub chronic toxicity study in rats *via* the oral route

Guideline:	Not specified
Species/strain:	Rats/Crl:CD BR
Number of animals:	10/sex/group (main study); 5/sex/group (recovery group)
Test substance:	AGN 192024 (0.2% solution)
Product name:	AGN 192024
Batch/Lot:	11246
Purity:	99.8%
Route:	Oral
Administration:	Gavage
Dose levels:	0, 0.1, 0.3 and 1 mg/kg bw/day (equivalent to 100, 300, 1000 µg/kg bw/day)
Dose-volume:	5, 0.5, 1.5 and 5 mL, respectively
Vehicle:	Not specified
Satellite Group	No
Duration:	13 weeks
Recovery:	4 weeks
GLP:	Yes
Study deviation:	Yes, study deviations were not considered to have altered the outcome or integrity of the study.
Study year:	1998

The sub-chronic repeated dose toxicity of bimatoprost (99.8% purity) was investigated in rats following oral (gavage) administration of 100, 300, 1000 µg/kg bw/day (dosing volume: 5, 0.5, 1.5 and 5 mL, respectively) of AGN 192024 (0.2% solution) or vehicle control, daily for 13 weeks followed by 4-week- recovery period. During the treatment period, animals were observed for mortality, changes in clinical signs, body weight and food consumption at defined intervals. Haematological, biochemical, ophthalmological examination and urinalysis were performed. At the end of the treatment or recovery period, all the rats of the main study were euthanised and necropsied, changes in organ weights and histopathology were evaluated.

Results

No treatment-related clinical signs, mortality, changes in body weight, water consumption, clinical chemistry, urinalysis, ophthalmic parameters and gross pathology were noted.

Histopathological evaluations revealed prominent corpora lutea (total of more than 70 for both ovaries) in 1 female at 300 µg/kg bw/day. An increase in the number of vacuolated corpora lutea in females at ≥300 µg/kg bw/day was noted, which was completely reversible after the recovery period. An increase in ovary weights was noted in treated females; however, the statistical significance of this effect is not specified.

Conclusion

Under the study conditions, the NOAEL for the analogue bimatoprost was considered to be 1000 µg/kg bw/day for males and 100 µg/kg bw/day for females, based on the increased number of corpora lutea in females and the absence of dose-related effects in males.

(CDER, 2001b)

3rd study: Sub-chronic toxicity study in rats *via* the oral route

Guideline:	Not specified
Species/strain:	Rats/Crl:CD BR
Number of animals:	10/sex/group (main study); 5/sex/group (recovery group)
Test substance:	AGN 192024 (0.2% solution)
Product name:	AGN 192024
Batch/Lot:	11220 and 11233
Purity:	99.1% and 98.7
Route:	Oral
Administration:	Gavage
Dose levels:	0, 4, 8 and 6 mg/kg bw/day (equivalent to 0, 4000, 8000, 16000 µg/kg bw/day
Dose-volume:	8, 2, 4 and 8 mL, respectively
Vehicle:	Not specified
Satellite Group	No
Duration:	13 weeks
Recovery:	4 weeks
GLP:	Yes
Study deviation:	Yes, study deviations were not considered to have altered the outcome or integrity of the study.
Study year:	1998

The sub chronic repeated dose toxicity of bimatoprost (98.7-99.1% purity) was investigated in rats following gavage administration of 0, 4000, 8000, 16000 µg/kg bw/day (dosing volume: 8, 2, 4 and 8 mL, respectively) of AGN 192024 (0.2% solution) or vehicle control, daily for 13 weeks followed by a 4-week recovery period. During the treatment period, animals were observed for mortality, changes in clinical signs, body weight and food consumption at defined intervals. Haematological, biochemical, ophthalmological examination and urinalysis were performed. At the end of the treatment or recovery periods, all the rats of the main study were euthanised and necropsied, and changes in organ weights and histopathology were evaluated.

Result

No treatment-related mortality, changes in clinical signs, haematology, urinalysis, ophthalmoscopy and gross pathology were observed. A decrease in body weight gain was noted in all treated animal groups; however, during the recovery period, there were no differences in body weight gains among different groups. Animals receiving 16000 µg/kg bw/day, showed a slight decrease in food consumption, which was reversed during the recovery period.

At all doses, treatment-related increases in GOT (aspartate aminotransferase (AST)) and GPT (alanine aminotransferase (ALT)) levels were observed in male rats, which returned to the control level after the 4-week- recovery period. These changes were not corroborated by histopathological changes. No other toxicologically significant differences were noted in the clinical chemistry. Additionally, prominent corpora lutea (total of more than 70 for both ovaries) as well as a dose dependent increase in the number of vacuolated corpora lutea was found in all treated groups. However, these changes were partially reversible after a 4- week recovery period. At all doses, increased ovary weights were noted. As per the CDER report, the mechanisms of the ovarian changes were likely to be related to the pharmacological effects of luteolysis by PGF2a, which were considered species-specific.

Conclusion

Under the study conditions, the NOAEL for the analogue bimatoprost was considered to be 16000 µg/kg bw/day based on the reversibility of all the observed changes.

(CDER, 2001b)

2.1.3 Chronic toxicity

Bimatoprost (NEW)

1st study: Chronic toxicity study in rats *via* the oral route

Guideline:	Not specified
Species/strain:	Rats/Crl:CD (SD) IGS BR
Number of animals:	15/sex/group (main study); 5/sex/group (recovery group)
Test substance:	AGN 192024 (0.2% solution)
Product name:	AGN 192024
Batch/Lot:	11455, 11482 and 11483
Purity:	96 – 100%
Route:	Oral
Administration:	Gavage
Dose levels:	0, 0.1, 0.3 and 2 mg/kg bw/day (equivalent to 0, 100, 300, 2000 µg/kg bw/day)
Dose volume:	2 mL
Vehicle:	Not specified
Satellite group	No
Duration:	52 weeks
Recovery:	8 weeks
GLP:	Yes
Study deviation:	Yes, study deviations were not considered to have altered the outcome or integrity of the study.
Study year:	1999

The chronic repeated dose toxicity of bimatoprost (96-100% purity) was investigated in rats following gavage administration of 0, 100, 300, 2000 µg/kg bw/day (dosing volume: 2 mL) of AGN 192024 (0.2% solution) or vehicle control, daily for 52 weeks followed by a 8-week recovery period. During the treatment period, animals were observed for mortality, changes in clinical signs, body weight, and food consumption at defined intervals. Haematological, biochemical, ophthalmological examination and urinalysis were performed. At the end of the treatment or recovery periods, all of the main study rats were euthanised and necropsied, changes in organ weights and histopathology were evaluated.

Results

No treatment-related mortality, changes in clinical signs, organ weights, haematology, urinalysis, ophthalmoscopy and gross pathology were observed. No treatment-related changes in body weight or body weight gain were noted in males. However, reduced body weight gain was observed in females at 300 and 2000 µg/kg bw/day. A slight decrease in food consumption was also noted in females at this dose; however, this change was not statistically significant.

An increase in ALT and AST activities was observed in all dose groups; however, since the increase was small, reversible and was not associated with histopathological changes, these changes might not be toxicologically significant.

Histopathological evaluation revealed cellular vacuolation in a proportion of corpora lutea, observed in one rat at 300 µg/kg bw/day and three rats at 2000 µg/kg bw/day. In the recovery animals,

vacuolated corpora lutea were detected in only one female at 2000 µg/kg bw/day. The proportion of affected corpora lutea in this rat was lower than in the main study animals, suggesting that these changes were reversible after the 8-week recovery period.

Conclusion

Under the study conditions, the NOAEL for analogue bimatoprost was established at 100 and 2000 µg/kg bw/day for females and males, respectively, based on dose-related body weight changes in females and no adverse effects in male rats.

(CDER, 2001b)

Overall conclusion for repeated dose toxicity potential via the oral route

Several subacute, sub-chronic, and chronic oral toxicity studies of bimatoprost (AGN 192024) were conducted in mice and rats. The 28-day subacute studies displayed no treatment-related mortality or significant toxicological effects in mice, whereas, in rats, testicular degeneration and abnormal germ cells in the epididymis were noted at 16000 µg/kg bw/day. In 13-week studies, mice exhibited vaginal inflammation at 16000 µg/kg bw/day, while rats showed ovarian changes at ≥300 µg/kg bw/day, which were reversible in another study up to 16000 µg/kg bw/day and were considered species-specific and consistent with the pharmacological effects of prostaglandin analogues. In a 52-week chronic study, rats showed treatment-related reduced body weight gain and reversible ovarian changes in females at doses ≥300 µg/kg bw/day. The NOAELs across all studies ranged from 1000 to 16000 µg/kg bw/day for males and from 100 to 16000 µg/kg bw/day for females, based on the species and duration. Given the similarity in biological activities between DDDE and bimatoprost, similar NOAELs can be established for DDDE.

2.2 Intravenous route

2.2.1 Subacute toxicity

Tafluprost (PREVIOUSLY SUBMITTED)

1st study: Subacute toxicity study in rats *via* the IV route

Guideline:	Not specified
Species/strain:	Rats/ Not specified
Number of animals:	12/sex/group
Test substance:	Tafluprost
Product name:	AFP-168
Vehicle:	Not specified
Batch:	Not specified
Purity:	Not specified
Route:	Intravenous
Dose levels:	0, 10, 30 and 100 µg/kg bw/day
Duration:	28 days
Recovery:	14 days
GLP:	Yes
Study period/year:	Not specified (pre-2013)

The subacute repeated dose toxicity of tafluprost (purity not specified) was investigated in rats following intravenous administration of 0, 10, 30 and 100 µg/kg bw/day daily for 28 days, followed by a 14-day recovery period. During the treatment period, animals were observed for clinical signs, mortality, body weight and food consumption at defined intervals. Haematological parameters, clinical chemistry parameters, urinalysis, ophthalmic and electrocardiographic parameters were examined. At the termination of treatment, all animals were sacrificed and macroscopically examined, organs were weighed, and comprehensive histopathology was performed.

Results

No treatment-related mortality, clinical signs, or changes in body weight, water consumption, clinical chemistry, urinalysis parameters, ophthalmic parameters, electrocardiograph traces, organ weights, gross pathology or histopathology were noted. Slightly lower haemoglobin concentration, erythrocyte numbers, and packed cell volume occurred in males at 100 µg/kg bw/day and erythrocyte numbers were marginally reduced in males at 30 and 10 µg/kg bw/day. Also, platelet numbers were marginally low in all treated females and males at 30 and 100 µg/kg bw/day. However, significant differences did not occur, and these parameters returned to normal levels during the recovery period.

Conclusion

Under the conditions of the study, the NOAEL for the analogue tafluprost was established at 100 µg/kg bw/day (highest tested dose) in rats.

(CDER, 2011b)

2nd study: Subacute toxicity study in dogs *via* IV route

Guideline:	Not specified
Species/strain:	Dogs/ Not specified
Number of animals:	4/sex/group
Test substance:	Tafluprost
Product name:	AFP-168
Vehicle:	Not specified
Batch:	Not specified
Purity:	Not specified
Route:	Intravenous
Dose levels:	0, 0.1, 1 and 10 µg/kg bw/day
Duration:	28 days
Recovery:	14 days
GLP:	Yes
Study period/year:	Not specified (pre-2013)

The subacute repeated dose toxicity of tafluprost (purity not specified) was investigated in dogs following intravenous administration of 0, 0.1, 1 and 10 µg/kg bw/day daily for 28 days, followed by a 14- day recovery period. During the treatment period, animals were observed for clinical signs, mortality, body weight and food consumption at defined intervals. Haematological parameters, clinical chemistry parameters, urinalysis, ophthalmic parameters, and electrocardiograph were assessed. All animals were sacrificed on Days 29 and 30 and macroscopically examined, organs were weighed, and comprehensive histopathology was performed.

Results

No treatment-related mortality, clinical signs, or changes in body weight, food consumption, haematology, body temperature, ophthalmology, gross pathology, organ weights or histopathology were noted. Slight miosis, sporadic salivation and vomiting were observed in both sexes at 1 µg/kg bw/day. Salivation, vomiting, miosis, increased respiratory rate, increased heart rate, and prolonged QTc interval were observed in both sexes at 10 µg/kg bw/day. Increased ALT activity, increased urine volume and decreased urinary potassium concentration were observed in both sexes during the dosing period, but these effects disappeared after the recovery period. Increased diastolic pressure was found only in males and reduced urinary chloride concentration occurred only in females.

Conclusion

Under the conditions of the study, the NOAEL for the analogue tafluprost was established at 1 µg/kg bw/day in dogs.

(CDER, 2011b)

Bimatoprost (NEW)

1st study: Subacute toxicity study in rats *via* the IV route

Guideline:	Not specified
Species/strain:	Rats/Sprague Dawley
Number of animals:	12/sex/group
Test substance:	Bimatoprost
Product:	AGN 192020 (0.2% solution)
Vehicle:	Not specified
Batch:	Not specified
Purity:	Not specified
Route:	Intravenous
Dose levels:	0, 0.1, 0.3 and 1 mg/kg bw/day (equivalent to 0, 100, 300 and 1000 µg/kg bw/day)
Dosing volume:	0.5 mL
Duration:	1 month
GLP:	Yes
Study period/year:	1998

The subacute repeated dose toxicity of bimatoprost (purity not specified) was investigated in Sprague Dawley rats following intravenous administration of to 0, 100, 300 and 1000 µg/kg bw/day (dosing volume: 0.5 mL) of AGN 192024 (0.2% solution) or vehicle control, daily for 1 month. During the treatment period, animals were observed for clinical signs, mortality, body weight and food consumption at defined intervals. Haematological parameters, clinical chemistry parameters, were evaluated and ophthalmoscopy and urinalysis were conducted. All animals were sacrificed and macroscopically examined, organs were weighed, and comprehensive histopathology was performed.

Result

No treatment-related mortality, clinical signs, or changes in body weight and body weight gain, food consumption, organ weights, clinical chemistry, urinalysis, ophthalmoscopy and gross necropsy were noted.

In histopathological examination, no dose-related adverse effects were noted in males at any dose level; however, decreased regression of corpora lutea, with increased corpora lutea in the ovary was observed at 1000 µg/kg bw/day.

Conclusion

Under the study conditions, the NOAEL for the analogue bimatoprost was established at 1000 µg/kg bw/day for males based on the absence of adverse effects in males and 300 µg/kg bw/day for females, based on decreased regression of corpora lutea.

(CDER, 2001a)

2nd study: Subacute toxicity study in monkeys *via* the IV route

Guideline:	Not specified
Species/strain:	Monkeys/Cynomolgus
Number of animals:	3/sex/group
Test substance:	Bimatoprost
Product:	AGN 192020 (0.2% solution)
Vehicle:	Not specified
Batch:	9123X-11268
Purity:	100%

Route:	Intravenous
Dose levels:	0, 0.1, 0.3 and 1 mg/kg bw/day (equivalent to 0, 100, 300 and 1000 µg/kg bw/day)
Dosing volume:	0.5 mL
Duration:	4 weeks
GLP:	Yes
Study period/year:	1998

The subacute repeated dose toxicity of bimatoprost (100% purity) was investigated in cynomolgus monkeys following intravenous administration of 0, 100, 300 and 1000 µg/kg bw/day (dosing volume: 0.5, 0.05, 0.15 and 0.5 mL/kg bw/day, respectively) of AGN 192024 (0.2% solution) or vehicle control, daily for 4 weeks. During the treatment period, animals were observed for clinical signs, mortality, changes in body weight, food consumption, Electrocardiogram (ECG), heart rate, respiratory rate, temperature and blood pressure at defined intervals. Haematological parameters, clinical chemistry parameters, were evaluated and ophthalmoscopy and urinalysis were conducted. All animals were terminated at the end of the treatment period and received a complete necropsy followed by histopathological examination of selected organs at selected doses.

Result

No treatment-related mortality, clinical signs, or changes in body weight and body weight gain, food consumption, ECG, heart rate, respiratory rate, temperature, blood pressure, organ weights, clinical chemistry, urinalysis, ophthalmoscopy, gross necropsy and histopathological examination were noted.

Conclusion

Under the study conditions, the NOAEL for the analogue bimatoprost was established at 1000 µg/kg bw/day based on no dose-related adverse effects at any of the tested dose levels.

(CDER, 2001a)

2.2.2 Subchronic toxicity

Tafluprost (PREVIOUSLY SUBMITTED)

1st Study: Subchronic toxicity study in rats *via* the IV route

Guideline:	EMA note for guidance on repeated dose toxicity (CPMP/SWP/1042/99)
Species/strain:	Rats/Crl:CD®(SD)IGSBR
Number of animals:	15/sex/group
Test substance:	Tafluprost
Product name:	AFP-168
Batch/Lot:	FP-0002
Purity:	98.6%
Route:	Intravenous
Dose levels:	0, 10, 30 and 100 µg/kg bw/day
Dose volume:	10 mL/kg
Vehicle:	Sterile saline (0.9% NaCl)
Satellite group	8/sex/group for toxicokinetic analysis
Duration:	26-weeks
GLP:	Yes
Study deviation:	Yes, study deviations were not considered to have altered the outcome or integrity of the study.
Study year:	2001

The subchronic toxicity of tafluprost (98.6% purity) was investigated for repeated dose toxicity according to the EMA note for guidance on repeated dose toxicity (CPMP/SWP/1042/99) (TGA, 2012). Crl:CD®(SD)IGSBR rats (15/sex/group) were administered intravenously at doses of 0, 10, 30 and 100 µg/kg bw/day for 26-weeks. The stability of the dosing solution was confirmed by analysis. During the treatment period, animals were observed for clinical signs, mortality, body weight, food consumption and water consumption at defined intervals. Haematological, biochemical, ophthalmological examination and urinalysis were performed. ECG measurements were performed pre-treatment and in Week 26 before dosing. At the termination of treatment, all animals were sacrificed after overnight fasting and macroscopically examined, organs were weighed, and comprehensive histopathology was performed. The study report noted multiple minor deviations from the study protocol. However, these deviations were not considered to have altered the outcome or integrity of the study.

Results

Fifteen animals (eleven males and four females) died or were sacrificed during the treatment period. The apparent cause of death of one male in the low dose group and two males in the intermediate dose group was due to glomerulonephropathy, but the cause of death for the other animals (all intermediate and high doses) was not apparent.

No treatment-related clinical signs in surviving animals, changes in body weight, water consumption, clinical chemistry or ophthalmic parameters were noted. Few ECG findings were of uncertain relationship to the test substance administration. In haematological examinations, some effects were evident in males and females. In Week 26, haemoglobin concentration and the number of erythrocytes were slightly decreased in females in all dose groups. Reticulocyte numbers, mean cell volume, mean cell haemoglobin and red cell distribution widths were slightly higher in females at 30 and 100 µg/kg bw/day. Similar patterns were observed in males at 100 µg/kg bw/day but generally to a lesser extent for most parameters. Platelet numbers and plateletcrit were slightly low at all dose levels in males, and the mean platelet volume and platelet distribution width were slightly high in males at 0.03 and 100 µg/kg bw/day. For these parameters, similar patterns were observed in females at 100 µg/kg bw/day but generally, to a lesser extent for most parameters. Neutrophils were also slightly increased in high-dose males, but not in females.

In urinalysis, slightly smaller volumes of urine with slightly greater specific gravity were noted in males. The total output of sodium, potassium, and calcium was similar to controls. These changes were considered to be toxicological insignificant. No changes were observed in females.

Analysis of bone marrow smears revealed a reduced number of early erythropoietic and myelopoietic cell types and increased late erythropoietic and myelopoietic cell types at 100 µg/kg bw/day. A slight but significant increase in mean spleen weights was recorded in males at 100 µg/kg bw/day and in females at 30 µg/kg bw/day and 100 µg/kg bw/day.

Histopathology findings were noted in bone marrow in femoral and sternum bone, spleen, liver and kidney in all dose groups. The findings included dose-related hyperostosis and myelofibrosis in the femoral and sternum bone marrow. Also, increased haematopoiesis was noted in the spleen, liver, and male femoral bone marrow. Generally, minor liver haematopoiesis occurred as foci in liver parenchyma in all dose groups without a clear dose-dependent trend for severity. A dose-dependent increase in the incidence and severity of femoral bone marrow haematopoiesis was only apparent in males. A dose-dependent increase in the incidence and severity of corticomedullary mineralization was observed only in females. The histopathology was consistent with the known pharmacological activity of prostaglandin F_{2a} analogues.

Conclusion

Under the conditions of the study, the LOAEL for the analogue tafluprost was considered to be 10 µg/kg bw/day in rats based on histopathology findings in bone marrow in femoral and sternum bone, spleen, liver and kidney in all dose groups. A NOAEL value could not be established.

(CDER, 2011b; TGA, 2012)

2nd study: Subchronic toxicity study in dogs *via* the IV route

Guideline:	EMA note for guidance on repeated dose toxicity (CPMP/SWP/1042/99)
Species/strain:	Beagle dogs
Number of animals:	4/sex/group
Test substance:	Tafluprost
Product name:	AFP-168
Batch/Lot:	FP-0002
Purity:	98.6%
Route:	Intravenous
Dose levels:	0, 0.1, 1, and 10 µg/kg bw/day
Dose volume:	1 mL/kg
Vehicle:	0.9% sodium chloride
Satellite group	None
Duration:	39 weeks
GLP:	Yes
Study deviation:	Yes, study deviations were not considered to have altered the outcome or integrity of the study.
Study year:	2000

The subchronic toxicity of tafluprost (98.6% purity) was investigated for repeated dose toxicity according to the EMA note for guidance on repeated dose toxicity (CPMP/SWP/1042/99) (TGA, 2012). Beagle dogs (4/sex/group) were dosed daily *via* the intravenous route at doses of 0, 0.1, 1, and 10 µg/kg bw/day for 39 weeks. The stability of dosing solution was confirmed by analysis. During the treatment period, animals were observed for clinical signs, mortality, body weight and food consumption at defined intervals. Ophthalmological examinations were performed in all animals' pre-treatment and in Weeks 13, 26, and 39. An indirect binocular ophthalmoscope was used to examine the eyelids, optic disc, tapedal and non-tapedal fundus, and retinal blood vessels. In addition, a pupillary light response was observed in Weeks 14, 27, 38/39 of the study for all animals. Electrocardiograms were performed on all animals. In addition, blood pressure, body temperature, respiratory rate measurements were made pre-treatment and before dosing and 5, 30 and 120 minutes after dosing in Weeks 4, 8, 13, 26 and 39. Blood samples for haematological and clinical-chemical examination were collected from all animals before treatment, and in Weeks 13, 26 and 39. For urinalysis, urine samples were collected overnight from all animals pre-treatment and in Weeks 12, 26, and 38. At the termination of treatment, all animals were sacrificed and macroscopically examined, organs were weighed, and comprehensive histopathology was performed.

Results

One high-dose male displaying multiple indications of hepatic failure was killed *in extremis* during Week 19 of treatment of the experiment. Several clinical signs were observed on an almost daily basis including salivation, emesis/retching, and pacing at 10 µg/kg bw/day. Emesis/retching and loose faeces were less frequently observed at 1 µg/kg bw/day. Other less frequent clinical signs included panting, subdued mood, vocalization, aggression, loose faeces, poor mobility and vasodilation were observed at 10 µg/kg bw/day. Several clinical signs were observed in high-dose animals on an almost daily basis including salivation, emesis/retching and pacing. Emesis/retching and loose faeces were less frequently observed in dogs receiving 1 µg/kg/day. Clinical signs in single high-dose male killed *in extremis* included, inappetence, progressive body weight loss, thin appearance, sluggishness and yellow coloration of the whole body.

No treatment-related changes in body weight, food consumption, body temperature, or urine parameters were noted.

No ocular changes were considered to be treatment-related other than dose-dependent changes in pupillary light response. Marked miosis was observed shortly after dosing during Weeks 14, 27, and 39 at 10 µg/kg bw/day. Miosis was slight to moderate two hours after dosing in weeks 27 and 39 for most high-dose animals. Slight to moderate miosis occurred at 1 µg/kg bw/day after dosing in Weeks 14, 27 and 39. No changes were observed at a low dose level of 0.1 µg/kg bw/day.

In ECG examination, increased heart rate was observed in a dose-dependent manner at 5 and/or 30 minutes after dosing. As a consequence of increased heart rate, RR-intervals were slightly shortened. The QT values corrected for heart rate (QTc) were normal.

Slight but significant increased mean arterial blood pressure was observed 30 minutes after dosing in Weeks 4, 26, and 39 in females at 10 µg/kg bw/day. Also, mean arterial blood pressure was significantly increased at a single time-point, 5 minutes after dosing in Week 26 in males at 1 and 10 µg/kg bw/day.

Respiratory rates were slightly but significantly elevated in animals 30 minutes after dosing at 10 µg/kg bw/day. However, the rates were less elevated or similar to control, two hours after dosing indicating a transient effect.

No treatment-related changes in haematology and clinical chemistry parameters were observed in any of the animals except the high-dose male killed *in extremis*. For this animal, prolonged prothrombin, activated partial thromboplastin times and increased AST, ALT, gamma glutamyl transferase (GGT) and alkaline phosphatase (ALP) activities and total bilirubin concentration were noted.

The primary gross pathology finding was reddening at injection site but not in a dose-dependent manner. No other treatment-related findings were observed except the high-dose male killed *in extremis*. For this animal, a small, firm, mottled liver, a small thymus, a slightly enlarged kidney and a discoloured intestinal tract were observed. The relative salivary gland weights were increased by 28 and 37% in males and females, respectively, compared to controls. No other treatment-related changes were observed.

In histopathology examination, minor adrenal cortical eosinophilia and acinar cell hypertrophy in salivary glands were observed primarily in males and females at 10 µg/kg bw/day. For the affected animals, the salivary gland acini tended to be larger due to an increased amount of normal appearing cytoplasm. No other treatment-related findings were observed except the high-dose male killed in extremis. For this animal, extensive histopathology occurred in the liver. The centrilobular cords were atrophic and consisted mainly of collapsed sinusoids with pigmented cells and few normal hepatocytes. The periportal region consisted of proliferating bile ducts variably surrounded by basophilic hypertrophic hepatocytes interspersed with necrotic cells and mitosis.

Conclusion

Under the conditions of the study, the NOAEL for the analogue tafluprost was established at 1 µg/kg bw/day in dogs based on transient clinical signs of nausea, transient miosis, transient slight elevations in heart rate, blood pressure, and respiratory rate, enlarged salivary glands, and adrenal and salivary gland histopathology at 10 µg/kg bw/day.

(CDER, 2011b; TGA, 2012)

Bimatoprost (NEW)

1st study: Subchronic toxicity study in monkeys *via* IV route

Guideline:	Not specified
Species/strain:	Monkeys/Cynomolgus
Number of animals:	6/sex/group
Test substance:	Bimatoprost
Product:	AGN 192020 (0.2% solution)
Vehicle:	Not specified
Batch:	Not specified
Purity:	Not specified
Route:	Intravenous
Dose levels:	0, 0.01, 0.1 and 1 mg/kg bw/day (equivalent to 0, 10, 100 and 1000 µg/kg bw/day)
Dosing volume:	0.5 mL
Duration:	17 weeks
Recover:	12 weeks
GLP:	Yes
Study period/year:	1998

The subchronic repeated dose toxicity of bimatoprost (purity not specified) was investigated in cynomolgus monkeys following intravenous administration of 0, 10, 100 and 1000 µg/kg bw/day of AGN 192024 (0.2% solution) or vehicle control (dosing volume: 0.5 mL/kg), daily for 17 weeks followed by a 12-week recovery period. During the treatment and recovery period, animals were observed for clinical signs, mortality, changes in body weight, food consumption, ECG, heart rate, respiratory rate, temperature and blood pressure at defined intervals. Haematological parameters, clinical chemistry parameters, were evaluated and ophthalmoscopy and urinalysis were conducted. At the end of the treatment period (Days 121 and 122) or recovery period (Day 205), all animals were sacrificed and macroscopically examined followed by histopathological examination of selected organs.

Result

A female in the 10 µg/kg bw/day dose group was sacrificed *in extremis* on Day 99 after experiencing severe diarrhoea for 16 days. Histology indicated gastroenteritis and renal tubular dilatation with cast formation, which likely contributed to its condition but may not be treatment related.

A test substance-related widening of the palpebral fissure was observed in all treated animals at the end of treatment, with severity decreasing during the recovery period. By Week 27, this effect was observed in one male at 100 µg/kg bw/day and one male and one female at 1000 µg/kg bw/day. However, these changes were considered consistent with the pharmacological effects of prostaglandin analogues.

No treatment-related mortality, clinical signs, or changes in body weight and body weight gain, food consumption, ECG, heart rate, respiratory rate, temperature, blood pressure, organ weights, clinical chemistry, urinalysis, gross necropsy and histopathological examination were observed.

Conclusion

Under the study conditions, the NOAEL for the analogue bimatoprost can be considered as 1000 µg/kg bw/day based on absence of dose-related adverse effects at any of the tested dose levels.

(CDER, 2001a)

Overall conclusion for repeated dose toxicity potential via the IV route

Several subacute and sub-chronic toxicity studies *via* the intravenous route are available for the structural analogues tafluprost and bimatoprost. Studies with tafluprost revealed increased

respiratory rate, altered cardiac parameters, hyperostosis and myelofibrosis in femoral and sternum bone marrow as well as haematopoiesis in spleen, liver and male femoral bone marrow, and increased corticomedullary mineralization of the kidney of females at a dose ≥10 µg/kg bw/day in rats and dogs. The studies with bimatoprost in rats and monkeys showed decreased regression of corpora lutea in female rats at 1000 µg/kg bw/day and reversible palpebral fissure widening in monkeys, a consistent with the pharmacological effect of prostaglandin analogue at ≥100 µg/kg bw/day. The NOAELs ranged from 1 to 100 µg/kg bw/day for tafluprost and 300 to 1000 µg/kg bw/day for bimatoprost. Given the structural similarity between DDDE and tafluprost and the similarity in biological activities between DDDE and bimatoprost, similar NOAELs can be established for DDDE.

2.3 Subcutaneous route

2.3.1 Subchronic toxicity

Tafluprost (PREVIOUSLY SUBMITTED)

1st study: Sub-chronic dose range finding study for carcinogenicity study in mouse *via* the SC route

Guideline:	Not specified
Species/strain:	Mice/Crl:CD-1(ICR)BR
Number of animals:	12/sex/group
Test substance:	Tafluprost
Product name:	AFP-168
Vehicle:	Not specified
Batch/Lot:	F01X010
Purity:	99.5%
Route:	Subcutaneous
Dose levels:	0, 3, 10, 30 and 100 µg/kg bw/day
Duration:	90 days
GLP:	Yes
Study year:	2003

The sub-chronic repeated dose toxicity of tafluprost (99.5% purity) was investigated in Crl:CD-1(ICR)BR mice following subcutaneous administration of 0, 3, 10, 30 and 100 µg/kg bw/day daily for 90 days. During the treatment period, animals were observed for clinical signs, mortality, body weight and food consumption at defined intervals. Haematological and clinical chemistry parameters were examined. At the termination of treatment, all animals were sacrificed and macroscopically examined, organs were weighed, and comprehensive histopathology was performed.

Results

No treatment-related mortality, clinical signs, or changes in body weight, food consumption, haematology parameters, clinical chemistry parameters, gross pathology, organ weight or histopathology were observed at any dose levels.

Conclusion

Under the conditions of the study, the NOAEL for the analogue tafluprost can be set at 100 µg/kg bw/day in mice.

(CDER, 2011b; TGA, 2012)

2nd study: Sub-chronic dose range finding study for carcinogenicity study in rats *via* the SC route

Guideline:	Not specified
Species/strain:	Rats/Crl:CD®(SD)IGSBR
Number of animals:	10/sex/group
Test substance:	Tafluprost
Product name:	AFP-168
Vehicle:	Not specified
Batch/Lot:	F01X010
Purity:	99.5%
Route:	Subcutaneous
Dose levels:	0, 3, 10, and 30 µg/kg bw/day
Duration:	90 days
GLP:	Yes
Study year:	2003

The sub-chronic repeated dose toxicity of tafluprost (99.5% purity) was investigated in Crl:CD®(SD)IGSBR rats following subcutaneous administration of 0, 3, 10 and 30 µg/kg bw/day daily for 90 days. During the treatment period, animals were observed for clinical signs, mortality, body weight and food consumption at defined intervals. Haematological parameters, and clinical chemistry were assessed. At the termination of treatment, all animals were sacrificed and macroscopically examined, organs were weighed, and comprehensive histopathology was performed.

Results

Only minor and/or dose-independent changes were noted for mortality. No treatment-related effects on clinical signs, gross pathology, or changes in body weight, food consumption, haematology, clotting parameters, clinical chemistry, or organ weights were observed.

Histopathology in the spleen and kidney was considered to be treatment related. In the spleen, a minor increase in the incidence and severity of haematopoiesis in high-dose males and females compared to vehicle control animals was noted. In the kidney, a minor increase in the incidence and severity of corticomedullary mineralization in high-dose females was observed. However, the histopathological changes in spleen and kidney were not considered to be statistically significant.

Conclusion

Under the conditions of the study, the NOAEL for the analogue tafluprost was considered to be 30 µg/kg bw/day in rats.

(CDER, 2011b; TGA, 2012)

Overall conclusion for repeated dose toxicity potential via the SC route

The repeated **subcutaneous** administration of the analogue tafluprost for 13 weeks in rats produced only minor non-significant histopathological changes in spleen and kidney at 30 µg/kg bw/day. However, the chronic administration for 24 months in the carcinogenicity study induced reduced body weight and histopathology changes including hyperostosis of the sternum and femur in some animals, and increased incidence of extramedullary haematopoiesis in the spleen at ≥3 µg/kg bw/day. No significant adverse effects were observed at any dose level in the dose range finder as well as chronic carcinogenicity studies in mice at doses up to 100 µg/kg bw/day. Based on the chronic studies, a NOAEL of <3 µg/kg bw/day is derived in rats and 100 µg/kg bw/day is derived in mice. Given the structural similarity between DDDE and tafluprost, similar NOAELs can be established for DDDE.

2.4. Ocular route

2.4.1 Subacute toxicity

Tafluprost (PREVIOUSLY SUBMITTED)

1st study: Subacute toxicity study in monkeys *via* the ocular route

Guideline:	Not specified
Species/strain:	Monkey/Not specified
Number of animals:	3/sex/group
Test substance:	Ophthalmic solution containing 0.0005-0.05% tafluprost
Product name:	AFP-168
Test concentrations:	0, 0.0005%, 0.005%, 0.05%
Batch:	Not specified
Purity:	Not specified
Route:	Topical ocular
Dose levels:	0, 0.15, 1.5 and 15 µg/left eye/time, twice daily (i.e., 0, 0.3, 3 and 30 µg/eye/day) Equivalent to 0, 0.067–0.1, 0.67–1 and 6.7–10 µg/kg bw/day[21]
Formulation/Vehicle:	Not specified
Duration:	28 days
GLP:	Yes
Study period/year:	Not specified (pre-2013)

The ocular toxicity of an ophthalmic solution of tafluprost (purity not specified) was investigated in a subacute toxicity study in monkeys. Monkeys (3/sex/group) were exposed to ophthalmic solutions containing 0, 0.0005%, 0.005% and 0.05% of tafluprost (equivalent to 0, 0.067–0.1, 0.67–1 and 6.7–10 µg/kg bw/day) *via* the ocular route for 28 days. During the treatment period, animals were observed for clinical signs, mortality, body weight and food consumption at defined intervals. Haematological parameters, clinical chemistry parameters, ECG parameters, blood pressure, electroretinogram parameters, macroscopic ocular findings and intraocular pressure were examined. At the termination of treatment, all animals were sacrificed and macroscopically examined, organs were weighed, and comprehensive histopathology was performed.

Results

No treatment-related mortality, clinical signs, changes in body weight, food consumption, macroscopic ocular findings, lens or vitreous body changes, or changes in intraocular pressure, electroretinogram parameters, or eye lash colour, ECG parameters, blood pressure, haematology parameters, clinical chemistry, gross pathology, organ weights or histopathology were noted.

Treatment-related changes were restricted to local changes in iris colour in two animals at 3 µg/eye/day (i.e., 0.67–1 µg/kg bw/day). In addition, transient corneal precipitates, anterior chamber cells, superficial corneal opacity and erosion, positive epithelial topical ocular fluorescein staining and conjunctiva redness were occasionally observed at the two highest tested doses. The erosion, positive epithelial topical ocular fluorescein staining and conjunctiva redness were also observed in vehicle control animals and untreated eyes and may have been related to corneal surface drying due to the anaesthesia.

[21] Reported in the Australian public assessment report (TGA, 2012).

Conclusion

Under the conditions of the study, the NOAEL for local (ocular) and systemic toxicity was established at 30 µg/eye/day (i.e., 6.7–10 µg/kg bw/day) due to the lack of any local and systemic effects of tafluprost in monkeys.

(CDER, 2011b; TGA, 2012)

Bimatoprost[22] (NEW)

1st study: Subacute toxicity study in rabbits *via* the ocular route

Guideline:	Not specified
Species/strain:	Rabbits/Dutch belted
Number of animals:	10/sex/group
Test substance:	Ophthalmic solution containing 0.03% bimatoprost
Product name:	AGN 192024
Test concentrations:	0 and 0.03%
Batch:	9106X-11179
Purity:	99.7%
Route:	Topical ocular
Vehicle:	Not specified
Dose levels:	21 µg/day (Equivalent to 12.4 and 19.1 µg/kg bw/day, for males and females, respectively)
Treatment volume:	35 µL
Duration:	1 month
GLP:	Yes
Study period/year:	1997

The ocular toxicity of an ophthalmic solution of bimatoprost (99.7% purity) was investigated in a subacute toxicity study in rabbits. Dutch belted rabbits (10/sex/dose) were exposed to ophthalmic solutions containing 0 or 0.03% of bimatoprost or vehicle to the left eye twice daily for 1 month, while the right eye remained untreated as a control. During the treatment period, animals were observed for clinical signs, mortality, changes in body weights, and gross ocular, biomicroscopic and direct ophthalmoscopic examination. All animals were sacrificed and macroscopically examined, organs were weighed, and comprehensive histopathology was performed.

Result

The treatment produced no ocular irritation in rabbit eyes. No treatment-related mortality, clinical signs, or changes in body weights, gross ocular, biomicroscopic and direct ophthalmoscopic examination, gross pathology, organ weight or histopathology were observed at any dose levels. Overall, no toxicologically significant systemic or ocular toxicities were observed at the tested dose levels.

Conclusion

Under the study conditions, the local (ocular) and systemic NOAEL for the analogue bimatoprost can be considered as 0.03% equivalent to 12.4 and 19.1 µg/kg bw/day for males and females, respectively, based on the absence of dose-related adverse effects.

(CDER, 2001a)

[22] Doses are converted in µg/kg bw/day using the animal weight mentioned in the source document.

2nd study: Subacute toxicity study in rabbits *via* the ocular route

Guideline:	Not specified
Species/strain:	Rabbits/New Zealand white rabbits
Number of animals:	10/sex/group
Test substance:	Ophthalmic solution containing 0.001, 0.01 and 0.1% of bimatoprost
Product name:	AGN 192024
Test concentrations:	0, 0.001, 0.01 and 0.1%
Batch:	Not specified
Purity:	99.99%
Route:	Topical ocular
Vehicle:	Not specified
Dose levels:	35 µL left eye/time, four times daily (i.e., 0, 1.4, 14, or 140 µg/day Equivalent to 0, 0.82 – 1.27, 8.24 – 12.73 or 82.35 – 127.27 µg/kg/day for males and females, respectively)
Duration:	1 month
Recovery:	14 days
GLP:	Yes
Study period/year:	1995

The ocular toxicity of an ophthalmic solution of bimatoprost (99.99% purity) was investigated in a subacute toxicity study in rabbits. New Zealand white rabbits (10/sex/dose) were exposed to ophthalmic solutions containing 0 0.001, 0.01 or 0.1% (equivalent to 0, 0.82 – 1.27, 8.24 – 12.73 or 82.35 – 127.27 µg/kg/day for males and females, respectively) of bimatoprost or vehicle to the left eye four times daily for 1 month followed by a 14-day recovery period, while the right eye remained untreated as a control. During the treatment period, animals were observed for clinical signs, mortality, changes in body weights, and gross ocular, biomicroscopic and direct ophthalmoscopic examination. All animals were sacrificed and macroscopically examined, organs were weighed, and comprehensive histopathology was performed.

Result

No treatment-related mortality, clinical signs, changes in body weights, ophthalmoscopy, slit lamp examination, clinical pathology, necropsy, organ weights and changes in histopathology were noted. Slight ocular discomfort (≤30 sec) was observed in 23–35% of instillations across all groups. Mild conjunctival hyperaemia occurred in 2–14% of instillations. However, the average Draize score was <0.3, indicating that bimatoprost was non-irritating at the tested concentrations.

Conclusion

Under the study conditions, the local (ocular) and systemic NOAEL for the analogue bimatoprost can be considered as 0.1%, equivalent to 82.35 and 127.27 µg/kg bw/day for males and females, respectively, based on the absence of dose-related adverse effects at any of the tested dose levels.

(CDER, 2001a)

3rd study: Subacute toxicity study in dogs *via* the ocular route

Guideline:	Not specified
Species/strain:	Dogs/Beagle
Number of animals:	3/sex/group
Test substance:	Ophthalmic solution containing 0.001, 0.01 and 0.1% of bimatoprost
Product name:	AGN 192024

Test concentrations:	0, 0.001, 0.01 and 0.1%
Batch:	Not specified
Purity:	99.99%
Route:	Topical ocular
Vehicle:	Not specified
Dose levels:	35 µL left eye/time, four times daily (i.e., 0, 1.4, 14, or 140 µg/day Equivalent to 0, 0.09 – 0.1, 0.93 – 1 and 9.33 – 10 µg/kg bw/day, for males and females, respectively)
Duration:	1 month
GLP:	Yes
Study period/year:	1995

The ocular toxicity of an ophthalmic solution of bimatoprost (99.99% purity) was investigated in a subacute toxicity study in dogs. Beagle dogs (3/sex/dose) were exposed to ophthalmic solutions containing 0 0.001, 0.01 or 0.1% (equivalent to 0, 0.09 – 0.1, 0.93 – 1 or 9.33 – 10 µg/kg/day for males and females, respectively) of bimatoprost or vehicle to the left eye four times daily for 1 month, while the right eye remained untreated as a control. During the treatment period, animals were observed for clinical signs, mortality, changes in body weights, food consumption, ECG and blood pressure. Haematological, clinical chemistry, urinalysis, gross ocular, biomicroscopic and direct ophthalmoscopic examination was also conducted. All animals were sacrificed and macroscopically examined, organs were weighed, and comprehensive histopathology was performed.

Result

No treatment-related mortality, clinical signs, changes in body weights, ophthalmoscopy, slit lamp examination, clinical pathology, urinalysis, ECG, blood pressure, necropsy, organ weights and histopathology were noted. Slight ocular discomfort (<30 sec) and mild conjunctival hyperaemia were observed in animals from all dose groups. However, the average Draize score (<0.85) fell within the practically non-irritating range (0.5–2.5). Miosis was also noted in all substance-treated left eyes; however, this effect was consistent with an expected canine-specific pharmacological effect and hence not considered relevant. Further, the average Draize score was <0.85, indicating that bimatoprost was practically non-irritating potential at the tested concentrations

Conclusion

Under the study conditions, the local (ocular) and systemic NOAEL for the analogue bimatoprost can be considered as 0.1% equivalent to 9.33 and 10 µg/kg/day for males and females, respectively, based on the absence of any dose-related adverse effects.

(CDER, 2001a)

2.4.2 Subchronic toxicity

Tafluprost (PREVIOUSLY SUBMITTED)

1st study: Subchronic toxicity study in monkeys *via* the ocular route

Guideline:	Not specified
Species/strain:	Cynomolgus monkey
Number of animals:	4/sex/group
Test formulation:	Ophthalmic solution containing 0.0005-0.05% tafluprost
Test substance:	AFP-168
Test concentrations:	0, 0.0005%, 0.005%, 0.05%
Batch/Lot:	Not specified
Purity:	Not specified
Route:	Topical ocular

Dose levels:	0, 0.15, 1.5, 15 µg/left eye/time, twice daily (i.e., 0, 0.3, 3 and 30 µg/eye/day) Equivalent to 0, 0.075–0.1, 0.75–1 and 7.5–10 µg/kg bw/day[23]
Dose volume:	Not specified
Formulation/Vehicle:	The vehicle for the 0.05, 0.005% and 0.0005% AFP-168 ophthalmic solutions contained Tween 80, NaH_2PO_4 X H_2O, EDTA x 2Na, glycerine, 0.01 % benzalkonium chloride, and NaCl
Duration:	13 weeks
Recovery:	28 days
GLP:	Yes
Study period/year:	Not specified (pre-2013)

The ocular toxicity of an ophthalmic solution of analogue tafluprost (purity not specified) was investigated in a subchronic toxicity study in monkeys following dosing for 13 weeks. Monkeys (4/sex/group) were exposed to ophthalmic solutions containing 0, 0.0005%, 0.005% and 0.05% tafluprost (i.e., equivalent to 0, 0.075–0.1, 0.75–1 and 7.5–10 µg/kg bw/day) *via* the ocular route for 13 weeks, followed by a 4-week recovery. The right eyes remained untreated. During the treatment period, animals were observed for clinical signs, mortality, body weight and food consumption at defined intervals. Macroscopic ocular examination as well as fundus examination, slit lamp examination and fluorescein staining of the cornea, and IOP measurements were performed. Similarly, iridial colour, eyelash colour examinations, electroretinography (ERG), ECG and blood pressure measurement were performed. Blood samples for haematological and clinical-chemical examination were collected from all animals. For urinalysis, urine samples were collected overnight from all animals. At the termination of treatment, all animals were sacrificed and macroscopically examined, organs were weighed, and comprehensive histopathology was performed.

Results

No treatment-related mortality, changes in body weight, food consumption, gross pathology, or histopathology, or changes in ECG, blood pressure, haematology parameters, clinical chemistry parameters, urinalysis, or organ weights were observed. No treatment-related effects in fundus and slit lamp examination or no changes in ERG parameters, or eyelash colour and ocular histopathology were observed.

Treatment-related findings in the treated eye, including a reversible finding of sunken eyelids, slight punctuate fluorescein staining of the cornea, a tendency to reduce intraocular pressure, and irreversible iris colour darkening, were observed at all dose levels. Changes in iris colour in animals were not reversed following a 4-week treatment-free period. The ocular effects were considered to be mainly cosmetic, not associated with loss of function, and not toxicologically significant. These effects were, however, noted in the warnings and precautions of the product labels.

Conclusion

Under the conditions of the study, the local (ocular) and systemic NOAEL for the test substance tafluprost was established at 30 µg/eye/day (7.5 and 10 µg/kg bw/day for males and females, respectively).

(CDER, 2011b; TGA, 2012)

[23] Reported in the Australian public assessment report (TGA, 2012)

2nd study: Subchronic toxicity study in monkeys *via* ocular route

Guideline:	Not specified
Species/strain:	Monkey/not specified
Number of animals:	3/sex/group
Test substance:	Ophthalmic solution containing 0.0045% tafluprost
Test concentrations:	0, 0.0045%
Batch/Lot:	100002-04
Purity:	102.4%
Route:	Ocular
Dose levels:	0, 1.35 µg/left eye/time, twice daily (i.e., 0, 2.7 µg/eye/day) Equivalent to 0, 0.75 and 0.9 µg/kg bw/day for males and females, respectively
Dose volume:	30 µL/eye/dose
Formulation/Vehicle:	Vehicle for DE-111 ophthalmic solution contained benzalkonium chloride with a pH of approximately 7.0, and an osmolar ratio of approximately 1)
Satellite group	None
Duration:	13 weeks
GLP:	Yes
Study deviation:	Yes, study deviations were not considered to have altered the results or the validity and integrity of the study
Study year:	2010

The ocular toxicity of an ophthalmic solution of tafluprost (102.4% purity) was investigated in a subchronic toxicity study in monkeys. Monkeys (3/sex/group) were exposed to ophthalmic solutions containing 0 and 0.0045% of tafluprost (i.e., equivalent to 0, 0.67 and 0.9 µg/kg bw/day for males and females, respectively) *via* the ocular route for 13 weeks. The right eyes remained untreated. The stability of the dosing solutions was analytically confirmed. During the treatment period, animals were observed for clinical signs, mortality and body weight at defined intervals. Macroscopic ocular examination as well as fundus examination, slit lamp examination and fluorescein staining of the cornea and IOP measurements were performed once before dosing, and 2 to 6 hours after dosing in Weeks 4, 8 and 13. Similarly, iridial and eyelash colour examinations were performed before dosing, and 2 hours 26 minutes to 5 hours 13 minutes after dosing in weeks 4, 8, and 13. Electroretinography (ERG) was performed before dosing and during Week 13. Scotopic ERG, oscillatory potentials, 30 Hz flicker, and photopic ERG were measured. At the termination of treatment, all animals were sacrificed and macroscopically examined, organs were weighed, and comprehensive histopathology was performed.

Results

No treatment-related mortality, changes in body weight, macroscopic ocular findings, fundus, or slit lamp examinations or changes in electroretinogram parameters, gross pathology, organ weights or histopathology were noted. Treatment-related changes in eye colour were observed in 2 animals.

A slight tendency for decreased IOP was observed compared to the untreated eyes; however, the reduced IOP in the treated eyes fell within the normal variations. The iridial colour was found to be darkened relative to pre-dose colours in two male animals. The ocular effects were considered to be mainly cosmetic, not associated with loss of function, and not toxicologically significant. These effects were, however, noted in the warnings and precautions of the product labels.

Conclusion

Under the conditions of the study, the local (ocular) or systemic NOAEL for the analogue tafluprost

was established at 2.7 µg/eye/day (0.67 and 0.9 µg/kg bw/day for males and females, respectively).

(CDER, 2011b)

2.4.3 Chronic toxicity

Tafluprost (PREVIOUSLY SUBMITTED)

1st study: Chronic toxicity study in monkeys *via* the ocular route

Guideline:	EMA note for guidance on repeated dose toxicity (CPMP/SWP/1042/99)
Species/strain:	Monkey/not specified
Number of animals:	4/sex/group
Test substance:	Ophthalmic solution containing 0.0005-0.05% tafluprost
Product name:	AFP-168
Test concentrations:	0, 0.0005, 0.005 and 0.05%
Batch/Lot:	D01114, D01115, D01116
Purity:	98, 100, 102% respectively
Route:	Topical ocular
Dose levels:	0, 0.15, 1.5, 15 µg/left eye/time, twice daily (i.e., 0, 0.3, 3 and 30 µg/eye/day) Equivalent to 0, 0.075–0.1, 0.75–1 and 7.5–10 µg/kg bw/day[24]
Dose volume:	30 µL/eye/dose
Formulation/Vehicle:	The vehicle for the 0.05 (30 µg/eye/day), 0.005% (3 µg/eye/day) and 0.0005% (0.3 µg/eye/day) AFP-168 ophthalmic solutions contained Tween 80, NaH_2PO_4 X H_2O, EDTA x 2Na, glycerine, 0.01 % benzalkonium chloride, and NaCl.
Satellite group	None
Duration:	52 weeks
GLP:	Yes
Study deviation	Yes, study deviations were not considered to have altered the results and integrity of the study
Study year:	2001

The ocular toxicity of an ophthalmic solution of tafluprost (98-102% purity) was investigated in a chronic toxicity study in monkeys, according to the EMA note for guidance on repeated dose toxicity (CPMP/SWP/1042/99) (TGA, 2012). Monkeys (4/sex/group) were exposed to ophthalmic solutions containing 0, 0.0005%, 0.005% and 0.05% tafluprost (equivalent to 0, 0.075–0.1, 0.75–1 and 7.5–10 µg/kg bw/day) *via* the ocular route for 52 weeks. The right eyes remained untreated. The stability of dosing solutions was analysed but no stability information was provided. During the treatment period, animals were observed for clinical signs, mortality body weight and food consumption at defined intervals. Macroscopic ocular examination as well as fundus examination, slit lamp examination and fluorescein staining of the cornea, and IOP measurements were performed once before dosing, and 2 to 6 hours after the first dose in Weeks 13, 26, 39 and 52. Similarly, iridial and eyelash colour examinations were performed once before dosing, and once after dosing in Weeks 13, 26, 39 and 52. ERG was performed once before dosing and during Weeks 25 and 26, 29 or 30 , 51 or 52. Scotopic ERG, oscillatory potentials, 30 Hz flicker, and photopic ERG were measured. ECG were performed on all animals (unanaesthetised, temporarily restrained) once before treatment and in Weeks 13, 26 and 52 (prior to and 15 minutes after the first dosing). Heart rates (beats/minute), ECG intervals, RR, P, PR, QRS and QT intervals (QTc and Qt dispersion), as well as voltage measurement of P, R, S, and T were measured. Blood samples for haematological and clinical-chemical examination were collected from

[24] Reported in the Australian public assessment report (TGA, 2012)

all animals once before treatment, and once in Weeks 13, 26 and 52. For urinalysis, urine samples were collected overnight from all animals once pre-treatment and once in Weeks 13, 26, and 52. At the termination of treatment, all animals were sacrificed and macroscopically examined, organs were weighed, and comprehensive histopathology was performed.

Results

No treatment-related mortality, changes in body weight, food consumption, fundus examination, pupil size, irritation score, corneal examination or changes in electroretinography, blood pressure, haematology, clinical chemistry, or urine parameters, were observed.

Only eye-related clinical signs were considered to be treatment-related. A sunken eye was noted at the two highest doses and darker iris colour was noted in all doses in at least 3 out of 4 animals of each gender in the beginning as early as Day 23, and this change was permanent throughout the experiment. Four animals (three males and one female) demonstrated sunken eyes on single occasions. Many of the same animals demonstrated blue-grey discolouration of the lower eyelid. A statistically significant decrease in IOP occurred in males and females in the high-dose group during Week 26. The IOP of the treated eye remained lower compared to the untreated eye for the rest of the study, but not significant. Slight decreases in IOP also occurred in low and intermediate-dose groups during Week 26. The lower eyelid darkening was observed in the intermediate and high dose group.

In gross pathology, no treatment-related changes were noted other than iris darkening, sunken eye. No treatment-related changes in organ weights were noted. Significant increase in the mean thymus/brain weight ratios relative to control animals were observed in two males of intermediate and high dose groups. However, for all the other animals of these groups, no statistically significant increase was observed in similar thymus/brain weight ratios and absolute thymus and thymus weight/body weight ratios.

No treatment-related changes in the systemic organ histopathology were observed. Most of the animals in all dose groups demonstrated increased melanocyte pigment in the iris stroma of the left eye, which is in line with the observed iris darkening. In addition, the treated eyes showed increased pigment around the hair follicles and minimal focal inflammation in the epithelium predominantly in high-dose males and females.

All the ocular effects were considered to be mainly cosmetic, not associated with loss of function, and not toxicologically significant.

Conclusion

Under the conditions of the study, the local (ocular) and systemic NOAEL for the analogue tafluprost was established at 30 µg/eye/day (7.5 and 10 µg/kg bw/day for males and females, respectively).

(CDER, 2011b; TGA, 2012)

Bimatoprost[25] (NEW)

1st study: Chronic toxicity study in rabbits *via* the ocular route

Guideline:	Not specified
Species/strain:	Rabbits/Dutch belted rabbits
Number of animals:	15/sex/group
Test substance:	Ophthalmic solution containing 0.03 and 0.1% of bimatoprost
Product name:	AGN 192024
Test concentrations:	0, 0.03 and 0.1%

[25] Doses are converted in µg/kg bw/day using the animal weights provided in the source document.

Batch:	9131X-11242
Purity:	Not specified
Route:	Topical ocular
Vehicle:	Not specified
Dose levels:	35 µL left eye/time, two (0.03%) and four (0.03 and 0.1%) times daily (i.e., 0, 10.5, 21, and 70 µg/day Equivalent to 0 (Group 1), 3.75 – 6.18 (Group 2), 7.5 – 12.35 (Group 3) and 25 – 41.18 µg/kg bw/day (Group 4))
Duration:	6 months
Recovery:	1 month
GLP:	Yes
Study period/year:	1995

The ocular toxicity of an ophthalmic solution of analogue bimatoprost (purity not specified) was investigated in a subacute toxicity study in rabbits. Dutch belted rabbits (15/sex/dose) were exposed to ophthalmic solutions containing 0 0.03 or 0.1% twice or four times daily (equivalent to 0, 3.75 – 6.18, 7.5 – 12.35 or 25 – 41.18 µg/kg bw/day) of bimatoprost or vehicle to the left eye for 6 months while the right eye remained untreated as a control followed by a 1-month recovery period. During the treatment and recovery period, animals were observed for clinical signs, mortality, changes in body weights, and gross ocular, biomicroscopic and direct ophthalmoscopic examination. All animals were terminated at the end of the treatment/recovery period and received a complete necropsy examination followed by evaluating organ weights and histopathological examination of selected organs/tissues.

Result

No treatment-related mortality, clinical signs, changes in body weights, ophthalmoscopy, slit lamp examination, clinical pathology, necropsy, organ weights and changes in histopathology were noted. No ocular irritation and clinically significant discomfort occurred during the 6-month treatment period. Following dosing, occasional transient slight ocular discomfort (≤30 seconds) was observed in 3 males and 5 females in Group 4, and in 1 male each in Groups 1, 2 and 3. Tearing was observed in 1 female in Group 4 prior to and following instillation, which was not considered toxicologically significant because it was observed in only 1 rabbit.

Conclusion

Under the study conditions, the local (ocular) and systemic NOAEL for the analogue bimatoprost can be considered as 0.1%, equivalent to 25 and 41.18 µg/kg/day for males and females, respectively, based on the absence of dose-related adverse effects at any of the tested dose levels.

(CDER, 2001a)

2nd study: Chronic toxicity study in monkeys *via* the ocular route

Guideline:	Not specified
Species/strain:	Monkey/Cynomolgus
Number of animals:	6/sex/group
Test substance:	Ophthalmic solution containing 0.03 and 0.1% of AGN 192024
Product name:	AGN 192024
Test concentrations:	0, 0.03 and 0.1%
Batch:	9106X-11240B and 9131X-11242
Purity:	Not specified
Route:	Topical ocular

Vehicle:	Not specified
Dose levels:	35 µL left eye/time, once (0.03%) and two (0.03 and 0.1%) times daily (i.e., 0, 10.5, 21, and 70 µg/day Equivalent to 0, 3.89 – 5.53, 7.78 – 11.05 and 25.93 – 36.84 µg/kg bw/day)
Duration:	29 or 52 weeks
Recovery:	24 or 20 weeks
GLP:	Yes
Study period/year:	1997 – 2000

The ocular toxicity of an ophthalmic solution of bimatoprost (purity not specified) was investigated in a subacute toxicity study in monkeys. Cynomolgus monkeys (6/sex/dose) were exposed to ophthalmic solutions containing 0 0.03 or 0.1% once or two times daily (equivalent to 0, 3.89 – 5.53, 7.78 – 11.05 and 25.93 – 36.84 µg/kg bw/day) of bimatoprost or vehicle to the right eye for 29 or 52 weeks followed by a 24 or 20-week recovery period, while the left eye remained untreated as a control. During the treatment and recovery period, animals were observed for clinical signs, mortality, changes in body weights, ECG, heart rate, respiratory rate, blood pressure, gross ocular, biomicroscopic and direct ophthalmoscopic examination. At the termination of treatment, all animals were sacrificed and macroscopically examined, organs were weighed, and comprehensive histopathology was performed.

Result

No treatment-related mortality, clinical signs, or changes in body weight, ECG, heart rate, respiratory rate, blood pressure, clinical chemistry, haematology, urinalysis, organ weights, gross pathology, or microscopic examination were observed. In bimatoprost-treated eyes, increased pigmentation in iris was observed from Day 87 onwards and periocular findings like widening of the palpebral fissure/prominent sulci appeared from Day 178 onwards; however, these effects were consistent with the pharmacological effects of prostaglandin analogues. The periocular findings resolved during recovery, while iridal pigmentation remained unchanged. No functional or anatomical ocular abnormalities were detected.

Conclusion

Under the study conditions, the local (ocular) and systemic NOAEL for the analogue bimatoprost can be considered as 0.1%, equivalent to 25.93 and 36.84 µg/kg/day for males and females, respectively, based on the absence of any dose-related adverse effects.

(CDER, 2001a)

Overall conclusion for repeated dose toxicity potential via the ocular route

Subacute, subchronic and chronic ocular dose toxicity studies have been conducted with ophthalmic solutions containing either tafluprost or bimatoprost. Studies in monkeys with 0.0005-0.05% of tafluprost (equivalent to 0.067 to 10 µg/kg bw/day) and in rabbits, dogs and monkeys with 0.0001 – 0.1% of bimatoprost (equivalent to 0.09 – 127.27 µg/kg bw/day) did not produce systemic toxicity up to the highest concentration. As per the FDA report on tafluprost, *"these data strongly suggest that clinical administration of tafluprost by the topical ocular route is unlikely to cause systemic toxicity."* (CDER, 2011b; TGA, 2012). Given the structural similarity between DDDE and tafluprost and the similarity in biological activities between DDDE and bimatoprost, similar NOAELs can be established for DDDE.

3. Reproductive and developmental toxicity

The reproductive and developmental toxicity of DDDE was assessed based on *in vivo* studies available for the analogues, tafluprost and bimatoprost. The results of the recently conducted ReproTracker® assay on DDDE and the analogues were used for comparison purposes (See **section 5.4.5.3**).

3.1 *In vivo* reproductive and developmental toxicity data

No *in vivo* reproductive and developmental toxicity studies could be identified for DDDE. Therefore, data available on the analogues tafluprost and bimatoprost is used for assessing this endpoint. The dataset for assessing reproductive and developmental toxicity includes studies conducted in rats and rabbits by the intravenous route.

The overview of the reproductive and developmental toxicity studies with the analogues tafluprost and bimatoprost are summarised in **Table 2 of Annex II**.

Table 2. Overview of reproductive and developmental toxicity studies with tafluprost and bimatoprost

Study type, Species	Doses	Key findings	NOAEL	Reference
Reproductive toxicity				
Tafluprost (PREVIOUSLY SUBMITTED)				
Reproductive toxicity, intravenous study, Crl:CD(SD)IGSBR rats (24/sex/dose)	0, 10, 30 and 100 µg/kg bw/day (duration not specified)	No significant adverse effects at any dose level	NOAEL = 100 µg/kg bw/day	(CDER, 2011b)
Bimatoprost (NEW)				
Fertility and reproductive toxicity study, gavage, Sprague-Dawley rats (25/sex/dose)	0, 0.1, 0.3 and 0.6 mg/kg bw/day Males: 10 weeks prior to cohabitation until copulation Females: 15 days prior to cohabitation through Gestation Day (GD) 7	No significant adverse effects at any dose level	NOAEL = 0.6 mg/kg bw/day (600 µg/kg bw/day)*	(CDER, 2001a)
Developmental toxicity				
Dose range finding studies				
Tafluprost (PREVIOUSLY SUBMITTED)				
Prenatal developmental toxicity, intravenous study, rats (strain not specified) (7 females/dose)#	0, 10, 30 and 100 µg/kg bw/day GD Not specified	At 30 and 100 µg/kg bw/day, pale extremities immediately after dosing A dose-dependent increase in the incidence of post-implantation loss at 30	NOAEL for maternal toxicity = 10 µg/kg bw/day* NOAEL for developmental	(CDER, 2011b)

Study type, Species	Doses	Key findings	NOAEL	Reference
		(12.1%) and 100 µg/kg bw/day (49.6%) compared to controls (6.0%) with two high-dose animals having litters with no live foetuses	toxicity = 10 µg/kg bw/day*	
Prenatal and postnatal development toxicity, intravenous study, rats (strain not specified) (7 females/dose) #	0, 0.3, 1, 3 and 10 µg/kg bw/day GD Not specified.	At ≥3 µg/kg bw/day, decreased maternal body weight gains At 3 and 10 µg/kg bw/day, increased number of dead foetuses and reduced birth index and viability index on Day 4 of lactation	NOAEL for maternal toxicity = 1 µg/kg bw/day* NOAEL for developmental toxicity = 1 µg/kg bw/day*	(CDER, 2011b)
Prenatal developmental toxicity, intravenous study, rabbits (strain not specified) (7 females/dose) #	0, 1, 3 and 10 µg/kg bw/day GD Not specified.	At 10 µg/kg bw/day, clinical signs including tremors, splayed legs and excessive licking immediately after dosing At 1 µg/kg bw/day, intrauterine deaths with no live foetuses in 2 females, and at 3 and 10 µg/kg bw/day, no live foetuses in all the females	LOAEL for maternal toxicity = 1 µg/kg bw/day* LOAEL for developmental toxicity = 1 µg/kg bw/day*	(CDER, 2011b)
Bimatoprost (NEW)				
Prenatal developmental toxicity, gavage study, Crl:CD-1 (ICR)BR mice (8 females/dose) #	0, 0.3, 1, 4 and 16 mg/kg bw/day GD 6-15	At ≥0.3 mg/kg bw/day, maternal toxicities (deaths, abortions and decreased body weights) At 0.3 mg/kg bw/day, increased resorptions At 1 mg/kg bw/day, decreased foetal body weight At 4 and 16 mg/kg bw/day, total resorptions in all animals	LOAEL for maternal and developmental toxicity = 0.3 mg/kg bw/day (300 µg/kg bw/day)*	(CDER, 2001a)
Prenatal developmental toxicity, gavage, Sprague Dawley rats, (8 females/group) #	0, 0.1, 0.3, 1 and 3 mg/kg bw/day GD 7 – 17	At ≥1 mg/kg/day, maternal toxicity including abortions, premature delivery and decreased body weights and food consumption At ≥1 mg/kg/day, decrease in implantation, litter size and live foetuses per litter At 3 mg/kg bw/day total resorption	NOAEL for maternal = 0.3 mg/kg bw/day (300 µg/kg bw/day) NOAEL for developmental toxicity = 0.3 mg/kg bw/day (300 µg/kg bw/day)	(CDER, 2001a)

Study type, Species	Doses	Key findings	NOAEL	Reference
Main studies				
Tafluprost (PREVIOUSLY SUBMITTED)				
Prenatal developmental toxicity, intravenous study, Crl:CD(SD)IGSBR rats (24 females/dose)	0, 3, 10, and 30 µg/kg bw/day GD 6-17	At 10 and 30 µg/kg bw/day, increased number of intrauterine deaths, reduced foetal maturity, and an incidence of defects of the vertebral column	NOAEL for maternal toxicity = 30 µg/kg bw/day NOAEL for developmental toxicity = 3 µg/kg bw/day	(CDER, 2011b)
Prenatal and postnatal development toxicity, intravenous study, rats (Crl:CD(SD)IGSBR) (22 females/dose)	0, 0.3, 1, 3, and 10 µg/kg bw/day GD 6 to lactation Day (LD) 20	At ≥1 µg/kg bw/day, poor nursing behaviour in some dams. Poor nursing resulting in decreased F1 offspring viability At 10 µg/kg bw/day, delayed pinna unfolding, increased F1 newborn mortality and decreased body weight	NOAEL for maternal toxicity = 10 µg/kg bw/day NOAEL for developmental toxicity (F1 generation) = 0.3 µg/kg bw/day **# PoD study**	(CDER, 2011b)
Prenatal developmental toxicity, intravenous study, rabbits (Crl.NZW/Kbl BR rabbits) (24 females/dose)	0, 0.03, 0.1 and 3 µg/kg bw/day GD 1-19	At 0.1 and 3 µg/kg bw/day, abortions in eleven and three animals and significantly increased early post-implantation loss . . At 3 µg/kg bw/day, no live foetuses in animals. At 0.1 µg/kg bw/dayonly two litters contained live foetuses At 0.03 µg/kg bw/day, majority of litters viable but three foetuses in separate litters with abdominal wall malformations, and six foetuses in five litters with cranial and/or spinal malformations	No NOAELs were established (see the below summary for details)	(CDER, 2011b)
Prenatal developmental toxicity, intravenous study, rabbits (Crl.NZW/Kbl BR rabbits) (24 females/dose)	0, 0.001, 0.003 and 0.01 µg/kg bw/day GD 7-19	No significant adverse effects at any dose level	NOAEL for maternal and developmental toxicity = 0.01 µg/kg bw/day	(CDER, 2011b)
Bimatoprost (NEW)				
Prenatal developmental toxicity,	0, 0.05, 0.2, 0.3 and 0.6	No significant adverse effects at any dose level	NOAEL for maternal and developmental	(Roshdy, 2005)

Study type, Species	Doses	Key findings	NOAEL	Reference
gavage study, Swiss mice (20 females/dose)	mg/kg bw/day GD 1-18		toxicity = 0.6 mg/kg bw/day (600 µg/kg bw/day)	
Prenatal developmental toxicity, gavage study, Crl:CD-1 (ICR)BR mice (25 females/dose)	0, 0.1, 0.3 and 0.6 mg/kg bw/day GD 6-15	At 0.3 and 0.6 mg/kg bw/day, dose-related abortion and early delivery occurred No developmental toxicity at any dose level	NOAEL for maternal toxicity = 0.1 mg/kg bw/day (100 µg/kg bw/day) NOAEL for developmental toxicity = 0.6 mg/kg bw/day (600 µg/kg bw/day)	(CDER, 2001a)
Prenatal developmental toxicity, gavage study, Sprague Dawley rats (25 females/group)	0, 0.1, 0.3 and 0.6 mg/kg bw/day GD 7-17	At 0.6 mg/kg bw/day, increased incidence of alopecia and abortions. No developmental toxicity at any dose level	NOAEL for maternal toxicity = 0.3 mg/kg bw/day (300 µg/kg bw/day) NOAEL for developmental toxicity = 0.6 mg/kg bw/day (600 µg/kg bw/day)	(CDER, 2001a)
Prenatal and postnatal development toxicity, gavage study, Sprague Dawley rats (25 females/group)	0, 0.05, 0.1, 0.3 and 0.6 mg/kg bw/day GD 7 to LD 20	F0 generation: At ≥0.3 mg/kg bw/day, reduced duration of gestation and increased peri- and postnatal pup mortality; decrease of the number of pups per litter at birth, the number of live-born pups per litter, the percentage of pups surviving after 1 and 4 days, and pup weights during the preweaning period F1 generation: A slight increase in the number of days required for mating, and a slight decrease in the fertility index at 0.3 mg/kg bw/day	NOAEL for maternal toxicity = 0.1 mg/kg bw/day (100 µg/kg bw/day) NOAEL for developmental toxicity = 0.1 mg/kg bw/day (100 µg/kg bw/day)	(CDER, 2001a)

** The NOAEL/LOAELs were not established in the source documents but was concluded based on the available details on the adverse effects in the underlying studies.*
#Dose range finding studies are not selected for summarising.

3.1.1 Reproductive toxicity

The key reproductive toxicity studies for the analogues tafluprost and bimatoprost *via* IV and oral routes, respectively, are summarised below:

Tafluprost *(PREVIOUSLY SUBMITTED)*

Guideline:	Not specified
Species/strain:	Rats/Crl:CD(SD)IGSBR rats
Number of animals:	24/sex/group
Test substance:	Tafluprost
Product name:	AFP-168
Batch/Lot:	FP-0002
Purity:	98.6%
Route:	Intravenous
Dose levels:	0, 10, 30 and 100 µg/kg bw/day
Dose volume:	10 mL/kg
Formulation/Vehicle:	0.9% sodium chloride
Satellite groups:	No
Duration:	Two weeks before mating, throughout the mating period, and until Day 6 of gestation for the females or until necropsy in Week 9 of the treatment period for the males
GLP:	Yes
Study deviation:	Yes, study deviations were not considered to have altered the results or integrity of the study
Study year:	2001

The reproductive toxicity of tafluprost (98.6% purity) was investigated in rats *via* the IV route. Crl:CD(SD)IGSBR rats (24/sex/group) were dosed daily at 0, 10, 30 and 100 µg/kg bw/day *via* the intravenous route for two weeks before mating, throughout the mating period, and until Day 6 of gestation for the females or until necropsy in Week 9 of the treatment period for the males. During the treatment period, animals were observed for clinical signs, mortality, body weight and food consumption at defined intervals.

The stage of oestrous was recorded for each female from 15 days prior to treatment until mating confirmation. The stages of oestrous were assessed *via* daily vaginal washings. Rats were paired on a 1:1 basis within each treatment group during the mating period. A vaginal copulatory plug or the presence of sperm in a vaginal washing confirmed positive evidence of mating. The day on which mating was confirmed was designated Day 0 of gestation. On GD 13, mated females were sacrificed and examined macroscopically. The ovaries and uteri were removed and examined. The pregnancy status, number of corpora lutea, number and intrauterine position of implantations with recording of live embryos, early intrauterine deaths and late intrauterine deaths were recorded.

Rats were sacrificed and examined macroscopically for structural or pathological changes. The tissues included ovaries, uterus, cervix, vagina, pituitary, testes, epididymides, seminal vesicles, prostate, coagulation gland and lesions from all adult animals were retained. The histopathology of the reproductive organs from all control and high-dose animals was examined.

Results

No treatment-related changes in body weight, food consumption, fertility or histopathology were observed.

At 100 µg/kg bw/day, two males died on Day 10 and 15 of treatment. These animals were found dead within one hour of dosing. The animals appeared unremarkable at necropsy. Treatment-related clinical signs including pale extremities were observed in all dose groups immediately after treatment

and appeared to be of approximately equal severity in all groups. This effect was observed daily from the second day of treatment in the 100 µg/kg bw/day and from the third day of treatment for the 10 and 30 µg/kg bw/day groups. The effect was transient and disappeared after one hour.

No treatment-related effects were observed on the oestrous cycle except one control pairing, all males mated within the initial four days of mating. No treatment-related effects were observed on fertility parameters (mating/fertility index, corpora lutea, preimplantation loss, etc.). The mating index was found to be 100% for all treatment and control groups. The fertility and fecundity indexes were 100, 91.7, 91.7 and 100% for both males and females in the control group, low, intermediate and high dose group, respectively. On GD 13, no effects were observed on the outcome of the pregnancy and all pregnant females had live embryos. The mean number of corpora lutea and the mean number of implantations per female were similar to the controls. Similar or fewer losses in the mean pre-and post-implantation loss were observed in the treated animals when compared to the control animals.

In histopathology examination, no treatment-related changes in reproductive organs were observed. No treatment-related abnormalities were observed in any of the cell types present within the different stages of the spermatogenic cycle in testis staging.

Conclusion:

Under the conditions of the study, the NOAEL for reproduction/fertility of the analogue tafluprost was established at 100 µg/kg bw/day in rats based on the absence of toxicity.

(CDER, 2011b; TGA, 2012)

Bimatoprost *(NEW)*

Guideline:	Not specified
Species/strain:	Rats/Sprague Dawley
Number of animals:	25/sex/group
Test substance:	Bimatoprost (AL06221, 0.2% solution)
Product name:	AGN 192024
Batch/Lot:	9123X11246 and 9124X11268
Purity:	Not specified
Route:	Gavage
Dose levels:	0, 0.1, 0.3 and 0.6 mg/kg bw/day (equivalent to 0, 100, 300 and 600 µg/kg bw/day)
Dose volume:	Not specified
Formulation/Vehicle:	Not specified
Satellite groups:	No
Duration:	Males were dosed from 10 weeks prior to cohabitation until copulation and females were dosed 15 days prior to cohabitation until GD 17
GLP:	Yes
Study deviation:	Yes, study deviations were not considered to have altered the results or integrity of the study
Study year:	1998

The reproductive toxicity of bimatoprost (purity not specified) was investigated in rats *via* the gavage route. Four groups of male rats (25/group) were treated with 0, 0.1, 0.3 and 0.6 mg/kg bw/day (equivalent to 0, 100, 300 and 600 µg/kg bw/day) of AGN 192024 by gavage for 10 weeks, then cohabitated with female rats (25/group) given similar dose for 15 days. Cohabitation was on a 1:1 ratio and continued until evidence of mating (copulatory plug or sperm in the oestrus smear) was observed. The day on which evidence of copulation was observed was designated as Day 0 of gestation. Dosing was continued in males until evidence of copulation was observed, and in females until gestation Day 7. Males were sacrificed following cohabitation and females were sacrificed on gestation Day 10 for

caesarean-section. During the treatment period, animals were observed for clinical signs, mortality, body weight and food consumption at defined intervals.

On GD 10, all females were euthanised and examined macroscopically. The ovaries and uteri were removed and examined. The number of viable and nonviable embryos, early resorptions, total implantations and corpora lutea were recorded.

A necropsy examination was performed on all treated male and female animals. For the males euthanised at the study termination, the testes, seminal vesicles, prostate and epididymides were weighted and examined histopathologically. The concentration and motility of the sperm were also measured.

Results

No treatment-related mortalities, clinical signs, changes in body weight and food consumption were noted. No treatment-related changes were observed in copulation and fertility index, and in sperm and oestrus cycle analysis in male and female rats.

No toxicologically significant changes were observed in uterine and ovarian examinations. At 600 µg/kg bw/day, one animal had a litter of 3 embryos that did not survive. However, this was an isolated case, and the total incidence of nonviable embryos remained within the historical range; hence, it was not considered treatment related. No other treatment-related abnormal findings were observed in histopathology examinations.

Conclusion:

Under the study conditions, the NOAEL for reproduction/fertility of the analogue bimatoprost was established at 600 µg/kg bw/day in rats based on the absence of any dose-related adverse effects on fertility or general reproduction.

(CDER, 2001a)

Overall conclusion for reproductive toxicity potential

The available reproductive toxicity study with tafluprost *via* the intravenous route and bimatoprost *via* the oral routes do not indicate any significant treatment-related effects on fertility or reproductive parameters at doses up to 100 and 600 µg/kg bw/day in rats, respectively. Given the structural similarity between DDDE and tafluprost and the similarity in biological activities between DDDE and bimatoprost, similar NOAELs can be established for DDDE.

3.1.2 Developmental toxicity

Key developmental toxicity studies conducted with tafluprost *via* the intravenous route in rats and rabbits, as well as studies conducted with bimatoprost *via* the oral route in mice and rats, are summarized below:

Tafluprost *(PREVIOUSLY SUBMITTED)*

1st Study: Prenatal developmental toxicity study in rats *via* the IV route

Guideline:	Not specified
Species/strain:	Rats/Crl:CD(SD)IGSBR
Number of animals:	24 females/group
Test substance:	Tafluprost
Product name:	AFP-168
Batch/Lot:	FP-0002
Purity:	98.6%
Route:	Intravenous
Dos/kg levels:	0, 3, 10 and 30 µg/kg bw/day

Dose volume:	10 mL/kg
Formulation/Vehicle:	0.9% sodium chloride
Basis of dose selection	Range-finding study
Satellite groups:	No
Duration:	12 days (GD 6 to 17)
Termination:	GD 20
GLP:	Yes
Study deviation:	Yes, study deviations were not considered to have altered the results or integrity of the study
Study year:	2001

The prenatal developmental toxicity of tafluprost (98.6% purity) was investigated in rats. Based on the findings in the preceding dose range-finding study, Crl:CD(SD)IGSBR rats (24 females/group) were dosed daily *via* the intravenous route at doses of 0, 3, 10 and 30 µg/kg bw/day during GD 6 to GD 17. The stability of dosing solutions was analysed and proven to be stable. During the treatment period, animals were observed for clinical signs, mortality, body weight and food consumption at defined intervals. At sacrifice (GD 20), the ovaries and uterus of each female were removed, and the parameters including pregnancy status, gravid uterus weight and the number of corpora lutea were assessed. Also, the status of each implantation site was recorded (live, dead, early resorption or late resorption). Fully formed foetuses that appeared to have died shortly before necropsy were classified as dead foetuses. Each live foetus was removed, weighed, sexed, and examined for gross abnormalities. Approximately half of the foetuses in each litter were subjected to a dissection and examined for developmental abnormalities in internal organs and skeletal abnormalities.

Results

No treatment-related mortality, clinical signs, change in body weight and food consumption were noted in dams.

No maternal toxicity was observed at any dose level. The 20% mean post-implantation loss was greater in the high-dose group compared to the 8.6% loss in the vehicle control group. The mean number of corpora lutea and the mean number of implantations was similar to those of the controls in all dose groups. Total litter loss in two rats was observed at 30 µg/kg bw/day and one in the vehicle control group mainly due to late intrauterine deaths for all three females. An increased number of early and late intrauterine deaths increased post implantation loss and decreased mean numbers of foetuses were observed at 30 µg/kg bw/day. However, no statistically significant differences were observed except for the number of late intrauterine deaths.

Foetal weights were significantly decreased at 10 and 30 µg/kg bw/day compared to controls with mean foetal weights of 3.70, 3.59 and 3.89 g in 10, 30 µg/kg bw/day and control group respectively. Slight, but non-significant increased mean placental weight was observed. Visceral malformations included but were not limited to the additional structure in the eye, severe renal pelvic cavitation, severely distended left ureter, umbilical hernia, absent kidneys and ureters, and abnormal lung lobulation in all the dose groups. However, specific visceral malformations occurred in only one litter per dose group, different visceral malformations occurred with the different doses and the least number of malformations occurred in the high-dose group. The visceral malformations were not affected in terms of the number of foetuses or litters affected. These factors indicate that the visceral malformations were not treatment related.

Skeletal malformations occurred at 10 and 30 µg /kg bw/day. A greater number of variations of the lumbar centra, thoracic arches, and thoracic centra were observed at 30 µg/kg bw/day. Also, a dose-related significant increase in the numbers of litters with unossified 5th sternebrae was observed at 10 and 30 µg/kg bw/day. No effects occurred at the low dose (3 µg/kg bw/day).

Conclusion

Under the conditions of this study, the maternal NOAEL was established at 30 µg/kg bw/day based on the absence of significant toxicity. The developmental NOAEL was established at 3 µg/kg bw/day based on increased number of intrauterine deaths, reduced foetal maturity and an incidence of defects of the vertebral column at 10 and 30 µg/kg bw/day.

(CDER, 2011b; TGA, 2012)

2nd Study: Pre- and post-natal developmental toxicity study in rats *via* the IV route

Guideline:	Not specified
Species/strain:	Rats/Crj:CD(SD)IGS
Number of animals:	22 females/group
Test substance:	Tafluprost
Product name:	AFP-168
Batch/Lot:	037010
Purity:	101.5%
Route:	Intravenous
Dose levels:	0, 0.3, 1, 3, and 10 µg/kg bw/day
Dose volume:	3 mL/kg
Formulation/Vehicle:	0.9% sodium chloride
Basis of dose selection	Range-finding study
Satellite groups:	No
Duration:	35 days (GD 6 to lactation Day (LD) 20)
GLP:	Yes
Study deviation:	Yes, study deviations were not considered to have altered the results or integrity of the study
Study year:	2004

The pre and postnatal developmental toxicity of tafluprost (101.5% purity) was investigated in rats. Based on the findings in the preceding dose range finding study, Crj:CD(SD)IGS rats (22 females/group) were dosed daily *via* intravenous route at 0, 0.3, 1, 3 and 10 µg/kg bw/day from GD 6 to LD 20. The stability of dosing solutions was analysed and proven to be stable.

In F0 dams, mortality, clinical signs, body weight changes, food consumption, uterine content and gross pathology findings were assessed. Delivery, gestation and nursing parameters were also assessed.

In F1 generation, mortality, clinical signs, body weight changes, food consumption, physical development, gross pathology, findings were assessed. Neurological parameters and reproduction parameters were also evaluated.

In F2 generation, mortality, number of corpora lutea, implantations, live F2 embryos and preimplantation loss were evaluated. However, body weights, male/female ratio were not assessed, and F2 foetuses were not externally evaluated for malformations.

Minimal deviations from the study protocol were noted. These deviations were not considered to have altered the study results or compromised the integrity of the study.

Results

In F0 dams, no treatment-related mortality, clinical signs, changes in body weight and food consumption were observed. No significant difference was observed in the number of implantation sites, total number of newborns, delivery index, or proportion of male live newborns compared to control. No abnormal gross pathology findings were observed at necropsy. Delayed delivery was noted in one female at 3 µg/kg bw/day. However, no abnormal delivery was noted in any dam. No significant

differences were observed in the duration of gestation, gestation index, or delivery index between any of the treatment groups. Poor nursing behaviour was observed in one dam in the 0.3, 3 and 10 µg/kg bw/day dose groups and in 3 dams in 1 µg/kg bw/day dose group. Poor nursing behaviour resulted in mortality within 2 days after birth in the offspring from 2 dams in each of the 1, 3 and 10 µg/kg bw/day dose groups.

In F1 generation, at birth, the number of dead newborns and a lower birth index were not significantly increased in the 10 µg/kg bw/day dose group compared to the control group. No treatment-related effects were observed in the number of implantation sites, total number of newborns, delivery index, or male proportion of live newborns. During lactation, the viability index on LD 4 was lower in the 1, 3 and 10 µg/kg bw/day dose groups compared to the control group with a significant reduction for the high-dose group. No treatment-related effects were observed in the weaning index.

The absence of milk in the stomach was observed in newborns at 1, 3 and 10 µg/kg bw/day. All newborns from 2 dams in each of these groups died within 2 days of birth. During the lactation period, mortality was observed in a few offspring among 2 to 3 dams in control and 0.3, 1 and 3 µg/kg bw/day groups and in some of the offspring from 10 dams in the 10 µg/kg bw/day group. After weaning, no abnormal clinical signs were evident in any offspring.

The body weight was significantly decreased in male and female newborns at 10 µg/kg bw/day. However, similar body weights were observed in the offspring in all groups after ≥ 4 days of age. A significant reduction in body weight was observed in 28 days old female offspring at 10 µg/kg bw/day. However, this effect was transient and, therefore, was not considered to be treatment related.

Growth retardation was observed in the high-dose group. The incidence of pinna unfolding at 3 days of age was statistically significantly lower in the high-dose group (56.5%) compared to the control group (95.5%). This effect was associated with the low birth weights in the high-dose group. Approximately 100% incidence of pinna unfolding was observed at ≥ 4 days of age in all the groups.

No significant difference was observed for the incidence of back righting or negative geotaxis before weaning compared to the control group. At post-weaning, all F1 offspring exhibited positive visual placing responses, pupillary reflexes, Preyer's reflexes and pain responses. No treatment-related gross pathology findings were noted in any male or female offspring culled at four days of age or in offspring at 21 days of age. A few no dose-related incidences including dilation of the renal pelvis were noted at both ages. Gross pathology was not evident at 10 weeks of age.

For reproduction parameters, no treatment-related changes were observed in preputial separation or vaginal opening. Also, for mating or the fertility index, no significant differences were observed between any dose groups and the control group.

In F2 generation, no treatment-related effects were observed for embryonic mortality, a number of corpora lutea, implantations, live F2 embryos, or preimplantation loss.

Conclusion

Under the conditions of this study, the NOAEL for maternal toxicity was established at 10 µg/kg bw/day based on the absence of treatment related effects in dams. The NOAEL for developmental toxicity was established at 0.3 µg/kg bw/day, based on decreased F1 offspring viability at ≥ 1 µg/kg bw/day and delayed pinna unfolding, increased F1 newborn mortality and decreased body weight at 10 µg/kg bw/day.

(CDER, 2011b; TGA, 2012)

Note: The intravenous developmental NOAEL of 0.3 µg/kg bw/day from this study was considered in the present assessment as the PoD for MoS calculations.

3rd Study: Prenatal developmental toxicity study in rabbits *via* the IV route

Guideline:	Not specified
Species/strain:	Rabbits/Crl.NZW/Kbl BR
Number of animals:	24 females/group
Test substance:	Tafluprost
Product name:	AFP-168
Batch/Lot:	FP-0002
Purity:	98.6%
Route:	Intravenous
Dose levels:	0, 0.03, 0.1 and 3 µg/kg bw/day
Dose volume:	1 mL/kg
Formulation/Vehicle:	0.9% sodium chloride
Basis of dose selection	Range-finding study
Satellite groups:	No
Duration:	19 days (GD 1 to 19)
Termination:	GD 29
GLP:	Yes
Study deviation:	Yes, study deviations were not considered to have altered the results or integrity of the study
Study year:	2001

The prenatal developmental toxicity of tafluprost (98.6% purity) was investigated in rabbits. Based on the findings of a dose range-finding study, Crl.NZW/Kbl BR rabbits (24 females/group) were dosed at 0, 0.03, 0.1 and 3 µg/kg bw/day daily *via* intravenous rout, from GD 1 to 19. The stability of dosing solutions was analysed and proven to be stable. During the treatment period, animals were observed for clinical signs, mortality, body weight and food consumption at defined intervals. Surviving females were sacrificed on Day 29 after mating and examined for gross pathology. At sacrifice, the ovaries and uteri of each female were removed, and the parameters including pregnancy status, gravid uterus weight, number of corpora lutea, the number and intrauterine position of implantations subdivided into live foetuses, early intrauterine deaths, late intrauterine deaths and dead foetuses were assessed. Live foetuses were sacrificed, and individual foetal and placental weights were recorded. Each live foetus was sexed and examined for gross abnormalities. The heads and hearts of approximately one-half of the foetuses in each litter were fixed for subsequent examinations. All foetuses were subjected to dissection and examined for developmental abnormalities in internal organs and skeletal abnormalities.

Results

No treatment-related changes in food consumption were noted. Animals were not found dead during the experiment. However, single animals in the vehicle control and the low dose groups, and eleven and three mothers in 0.1 and 3 µg/kg bw/day groups, respectively, aborted their pregnancies between GD 17 and GD 23 and were euthanised prior to GD 29. The only clinical sign observed was red tissue and fluid under the cages of the mothers that aborted.

A significant weight loss was observed between GD 12 and GD 15 only at 0.1 µg/kg bw/day. A slight weight loss occurred in the intermediate and high-dose animals between GD 7 and GD 10 and between GD 10 and GD 15 relative to the control animals. The mean gravid uterus weight was significantly decreased in the two intermediate-dose females with viable foetuses.

Treatment-related effects on pregnancy outcome parameters occurred in all dose groups. The mean number of implantations was significantly decreased in 10 and 13 females of the intermediate and high dose group, respectively, with evidence of pregnancy on GD 29. The mean implantation values of 10.5, 7.9, and 7.8 were observed in the control, 0.1 and 3 µg/kg bw/day group, respectively. In

addition, in 8/10 intermediate-dose, and 13/13 high-dose females, all implantations died early in gestation without live foetuses. The mean incidence of post-implantation loss was significantly higher in the intermediate (91.6%) and high dose group (100%) compared to control females (13.1%). Also, the mean number of corpora lutea in the intermediate (3.6) and high dose (3.6) groups was significantly lower compared to control animals (11.5). The mean post-implantation loss in the low-dose group was 29.2% compared to 13.1% in control females, with three low-dose females and one control female having total intrauterine death with no live foetuses. In low-dose females with live foetuses, the percentage of post-implantation loss (18.0%) was slightly higher than that of control females (8.7%), with the majority of intrauterine deaths occurring late in gestation.

No live foetuses were observed in the 3 µg/kg bw/day group. The mean foetal weight in the two litters of the intermediate-dose group was similar to controls. A slight but not significant increase in placental weight was observed. Also, the mean litter weight was reduced compared to controls due to the reduced number of live births and one litter contained only females and one litter was approximately equal for each gender.

The mean foetal and placental weights, and the litter gender proportionality in the low dose group were similar to those of the controls. The reduced number of live foetuses was reflected by the mean litter weight. Foetal malformations were observed in nine foetuses from six mothers. Among the affected foetuses, defects of the skull, brain, and/or spine were noted in six cases, three of which also exhibited abdominal wall defects.

Overall, in the low-dose group, the number of foetal variations was similar to that of the controls, but the skeletal variations were more heterogenous than in the control group and often associated with abnormalities. Visceral and/or skeletal variations were similar in incidence and type compared to the control litters in the two intermediate-dose litters with surviving foetuses.

Conclusion

Under the conditions of the study, abortions, reduced body weight and/or uterus weight were observed in dams at ≥0.1 µg/kg bw/day. However, due to high post implantation loss observed at mid and high doses and the lack of details on statistical significance, the abdominal wall malformations and cranial and/or spinal malformations observed at the low dose (0.03 µg/kg bw/day) could not be assessed further (see the below note).

(CDER, 2011b; TGA, 2012)

Note: The study is considered to be of questionable reliability due to the following reasons:

- Dosing started from GD1, which is not as per the standard guidelines from EMA or US-CDER/CBER, and this is very likely the reason for the high implantation loss observed in the study[26];
- High implantation loss led to a low number of foetuses, and this did not allow any further evaluation of the developmental parameters or the establishment of a dose-response relationship;
- No mention of the statistical significance of the effects observed at the lowest tested dose;
- No mention of historical control data.

Due to these limitations, the above study was considered to be of questionable reliability and was, therefore, not considered further for risk assessment purposes.

[26] Dosing during the initial days post fertilisation is to be avoided as this corresponds to a very sensitive stage involving the transport of the zygote and the implantation of the embryo in the endometrium of the uterus. Any disturbance by handling, dose application or toxic effects by substances during this period may lead to a very high rate of pre-implantation loss up to total loss.

4th Study: Prenatal developmental toxicity study in rabbits *via* the IV route

Guideline:	Not specified
Species/strain:	Rabbits/Crl.NZW/Kbl BR
Number of animals:	24 females/group
Test substance:	Tafluprost
Product name:	AFP-168
Batch/Lot:	FP-0002
Purity:	98.6%
Route:	Intravenous
Dose levels:	0, 0.001, 0.003 and 0.01 µg/kg bw/day
Dose volume:	Control: 0.1 mL/kg Low dose: 0.01 mL/kg Intermediate dose: 0.03 mL/kg High dose: 0.1 mL/kg
Formulation/Vehicle:	0.9% sodium chloride
Basis of dose selection	Range-finding study
Satellite groups:	3 pregnant females/group for low, intermediate, and high dose group
Duration:	13 days (GD 7 to 19)
Termination:	GD 29
GLP:	Yes
Study deviation:	Yes, study deviations were not considered to have altered the results or integrity of the study
Study year:	2002

A second intravenous prenatal developmental toxicity study with tafluprost (98.6% purity) was conducted in rabbits. Based on the findings from the first rabbit study, 24 females/group were dosed at 0, 0.001, 0.003 and 0.01 µg/kg bw/day *via* the intravenous route, from GD 7 to 19. The stability of dosing solutions was analysed and proven to be stable. During the treatment period, animals were observed for clinical signs, mortality, body weight and food consumption at defined intervals. Surviving females were sacrificed on Day 29 after mating and examined for gross pathology. At sacrifice, the ovaries and uteri of each female were removed, and the parameters including pregnancy status, gravid uterus weight, number of corpora lutea, the number and intrauterine position of implantations, early intrauterine deaths, late intrauterine deaths and dead foetuses were assessed. Live foetuses were sacrificed, and individual foetal and placental weights were recorded. Each live foetus was sexed and examined for gross abnormalities. The heads and hearts of approximately one-half of the foetuses in each litter were fixed for subsequent examinations. All foetuses were subjected to dissection and examined for developmental abnormalities in internal organs and skeletal abnormalities.

Multiple deviations from the study protocol were noted but they were not considered to have altered the study results or compromised the integrity of the study.

Results

No treatment-related mortality, changes in body weight and mean gravid uterus weight were observed. Clinical signs included distended urinary bladders which were noted at euthanasia and necropsy in all animals that were euthanised due to very low food consumption and appeared thin. Among these animals were one control female and three intermediate-dose females. In the surviving animals, only one high-dose female appeared thin between GD 25 and GD 29, and this was due to weight loss and reduced food intake during this period. Also, pale and mottled liver was observed at the necropsy of this animal. The food intake was found to be very low from GD 4 in three control and

four intermediate-dose animals and these animals were euthanised. However, this effect was not considered related to be treatment related.

No treatment-related effects were observed in a mean number of foetuses, group mean pre-and post-implantation loss, mean foetal weight, litter weight and placental weights. Individual malformation was not significantly increased in any of the dose groups compared to the control group. Generally, a similar number of foetuses with external, visceral and skeletal variations was observed in all dose groups compared to the control group. A statistically non-significant increased number of foetuses with abnormally pale contents in the gall bladder and/or non-eruption of the incisors was observed in all dose groups.

Conclusion

Under the conditions of this study, the maternal and developmental NOAEL was established at 0.01 µg/kg bw/day in rabbits.

(CDER, 2011b; TGA, 2012)

Bimatoprost *(NEW)*

1st Study: Prenatal developmental toxicity study in mice *via* the oral route

Guideline:	Not specified
Species/strain:	Mice/Swiss
Number of animals:	20 females/group
Test substance:	Bimatoprost
Product name:	Lumigan (0.03% solution)
Batch/Lot:	Not specified
Purity:	Not specified
Route:	Oral
Dose levels:	0, 0.05, 0.2, 0.3, and 0.6 mg/kg bw/day (equivalent to 0, 50, 200, 300 and 600 µg/kg bw/day)
Dose volume:	1 mL
Formulation/Vehicle:	Ethyl alcohol
Basis of dose selection	Multiples of recommended human dose
Satellite groups:	No
Duration:	18 days (GD 1 to 18)
Termination:	GD 18
GLP:	Not specified
Study deviation:	Yes, study deviations were not considered to have altered the results or integrity of the study
Study year:	2004-2005

The prenatal developmental toxicity of bimatoprost (purity not specified) as an 0.03% ophthalmic solution was investigated in Swiss mice *via* the oral route. Pregnant mice (20/group) were dosed with 0, 50, 200, 300 and 600 µg/kg bw/day bimatoprost or vehicle control ethyl alcohol (dosing volume = 1mL), daily *via* gavage administration from GD 1 to 18. No information is available regarding the analytical evaluation of substance stability. During the treatment period, animals were observed for clinical signs, mortality and body weight at defined intervals. The day on which evidence of copulation was observed was designated as Day 0 of gestation. On GD 18, all animals were euthanised and caesarean sectioned. The uterus of each female was removed, weighed and the parameters, like the number of implantation sites, early and late resorptions, and live and dead foetuses were recorded. Foetuses were weighed, sexed and examined for morphological, visceral and skeletal malformations and variations.

Results

No treatment-related significant changes were observed in the body weight and clinical signs of the dams.

All pregnant females had viable foetuses. There was no significant treatment-related change in the total number of implantations, implantations/female and mean foetal and maternal weight gain.

While morphological examination, cleft palate, club foot and facial cleft were observed in each dose group; however, the incidence of anomalies was not statistically significant compared to controls. The visceral observations displayed anomalies such as abnormal ureter, abnormal testis and abnormal liver in all treated groups and in the control group. The skeletal observations revealed minor anomalies such as wavy, fused, or extra ribs and vertebral anomalies. However, these anomalies were not statistically significant as compared to the control.

Conclusion

Under the study conditions, the maternal and developmental toxicity NOAEL was established at 600 µg/kg bw/day based on the absence of treatment-related toxicity.

(Roshdy, 2005)

2nd Study: Prenatal developmental toxicity study in mice *via* the oral route

Guideline:	Not specified
Species/strain:	Mice/Crl:CD-1 (ICR)BR
Number of animals:	25 females/group
Test substance:	Bimatoprost (AL06221, 0.2% solution)
Product name:	AGN 192024
Batch/Lot:	11482
Purity:	Not specified
Route:	Oral
Dose levels:	0, 0.1, 0.3 and 0.6 mg/kg bw/day (equivalent to 0, 100, 300 and 600 µg/kg bw/day)
Dose volume:	5 mL/kg
Formulation/Vehicle:	Not specified
Basis of dose selection	Range-finding study
Satellite groups:	No
Duration:	10 days (GD 6 to 15)
Termination:	GD 18
GLP:	Yes
Study deviation:	Yes, study deviations were not considered to have altered the results or integrity of the study
Study year:	1999

The prenatal developmental toxicity of bimatoprost (purity not specified) was investigated in mice *via* the oral route using a 0.2% solution. Pregnant mice (25/group) were dosed with 0, 100, 300 and 600 µg/kg bw/day of bimatoprost or vehicle control (dosing volume 5 mL) daily *via* gavage administration from GD 6 to 15. No information is available regarding the analytical evaluation of substance stability. During the treatment period, animals were observed for clinical signs, mortality and body weight at defined intervals. The day on which evidence of copulation was observed was designated as Day 0 of gestation. On GD 18, all animals were euthanised and caesarean sectioned. A gross necropsy of the thoracic, abdominal and pelvic viscera was performed. The ovaries and uterus of each female were removed, and the parameters like number of implantation sites and corpora lutea, early and late

resorptions, and live and dead foetuses were recorded. Foetuses were weighed, sexed and examined for external malformations and variations. One-half of the foetuses were examined for soft tissue defects. The other half of the foetuses were examined for skeletal anomalies.

Results

No treatment related changes were observed in the body weight of the dams.

Abortion occurred in 1 mouse (GD 17) and 3 mice (GD 16 and 17) in the 300 and 600 µg/kg bw/day groups, respectively. In addition, at 600 µg/kg bw/day, premature deliveries were observed in 3 mice on GD 18. The abortion and premature deliveries were considered treatment related. Necropsy examinations revealed no abnormal findings in dams or foetuses.

During the uterine and ovarian examinations, there were one and two dead foetuses in the 300 and 600 µg/kg bw/day groups, respectively; this was within the historical control ranges and was not considered treatment related. External, visceral and skeletal examinations in foetuses revealed malformations; however, these findings were common in this species and strain, were within the historical control ranges, were not dose-dependent, and, hence, were not considered drug-related. No other developmental effects were observed at any dose levels.

Conclusion

Under the study conditions, the maternal NOAEL was established at 100 µg/kg bw/day based on dose-related abortions and premature deliveries. The developmental NOAEL was established at 600 µg/kg bw/day based on the absence of treatment-related developmental toxicity.

(CDER, 2001a)

3rd study: Prenatal developmental toxicity study in rats *via* the oral route

Guideline:	Not specified
Species/strain:	Rats/Sprague Dawley
Number of animals:	25 females/group
Test substance:	Bimatoprost (AL06221, 0.2% solution)
Product name:	AGN 192024
Batch/Lot:	9124X-11246
Purity:	Not specified
Route:	Oral
Dose levels:	0, 0.1, 0.3 and 0.6 mg/kg bw/day (equivalent to 0, 100, 300 and 600 µg/kg bw/day)
Dose volume:	1.5 mL/kg
Formulation/Vehicle:	Not specified
Basis of dose selection	Range-finding study
Satellite groups:	No
Duration:	11 days (GD 7 to 17)
Termination:	GD 20
GLP:	Yes
Study deviation:	Yes, study deviations were not considered to have altered the results or integrity of the study
Study year:	1998

The prenatal developmental toxicity of bimatoprost (purity not specified) was investigated in rats *via* the oral route. Pregnant rats (25/group) were given 0, 100, 300 and 600 µg/kg bw/day bimatoprost or vehicle control (dosing volume = 1.5 mL) daily *via* gavage from GD 7 to 17. No information is available regarding the analytical evaluation of substance stability. During the treatment period, animals were

observed for clinical signs, mortality, body weight and food consumption at defined intervals. The day on which evidence of copulation was observed was designated as Day 0 of gestation. On GD 20, each female was euthanised and caesarean sectioned. A gross necropsy of the thoracic, abdominal and pelvic viscera was performed. The ovaries and uterus of each female were removed, and the parameters like number of implantation sites and corpora lutea, early and late resorptions, and live and dead foetuses were recorded. For teratological evaluations, foetuses were weighed, sexed and examined for external malformations and variations. One-half of the foetuses were examined for soft tissue defects. The other half of the foetuses were examined for skeletal anomalies.

Results

No treatment-related changes were observed in the body weights, food consumption and necropsy examination of the dams.

Abortion occurred in 2 rats at 600 µg/kg bw/day on GD 19. However, no gross lesions were observed during the necropsy of these rats. Localised alopecia was noted in 8 dams in the 600 µg/kg bw/day group *versus* 4 in the control group. Five of the 8 dams had alopecia on the ventral surface, while none in the control group. This effect was considered treatment related. No other toxicologically significant abnormal findings were noted in clinical observations and uterine and ovarian examinations. External, visceral and skeletal examinations in foetuses revealed malformations; however, these findings were common in this species and strain, were within the historical control ranges, were not dose-dependent, and, hence, were not considered drug-related. No other developmental effects were observed at any dose levels.

Conclusion

Under the study conditions, the maternal NOAEL was established at 300 µg/kg bw/day based on dose-related alopecia on the ventral surface. The developmental NOAEL was established at 600 µg/kg bw/day based on the absence of treatment-related developmental toxicity.

(CDER, 2001a)

4th study: Pre- and post-natal developmental toxicity study in rats *via* the oral route

Guideline:	Not specified
Species/strain:	Rats/Sprague Dawley
Number of animals:	25 females/group
Test substance:	Bimatoprost (AL06221, 0.2% solution)
Product name:	AGN 192024
Batch/Lot:	9124X-11482
Purity:	Not specified
Route:	Oral
Dose levels:	0, 0.05, 0.1, 0.3 and 0.6 mg/kg bw/day (equivalent to 0, 50, 100, 300 and 600 µg/kg bw/day)
Dose volume:	Not specified
Formulation/Vehicle:	Not specified
Basis of dose selection	Not specified
Satellite groups:	No
Duration:	34 days (GD 7 to LD 20)
Termination:	21-day postpartum period
GLP:	Yes
Study deviation:	Yes, study deviations were not considered to have altered the results or integrity of the study

Study year: 1999

The pre and postnatal developmental toxicity of bimatoprost (purity not specified) was investigated in rats *via* oral route. Pregnant rats (25/group) were administered 0, 50, 100, 300 and 600 µg/kg bw/day bimatoprost (AGN192024) or vehicle control daily *via* gavage from Day 7 of gestation to Day 20 of lactation. The day on which evidence of mating was observed was designated as Day 0 of gestation and the day of birth was defined as Day 1 of lactation.

In F0 dams, mortality, clinical signs of toxicity, body weight changes, food consumption, uterine content, necropsy findings, delivery and gestation duration and nursing parameters were evaluated. After the 21-day postpartum period, female rats were euthanised and a gross necropsy of the thoracic, abdominal and pelvic viscera was performed. The number and distribution of implantation sites were recorded.

In selected F1 generation rats, assessments included mortality, clinical signs of toxicity, body weight, food consumption, physical development, and findings from gross pathology or necropsy. Neurological and reproductive parameters were also evaluated. Male F1 rats chosen for reproductive assessment were euthanized after the cohabitation period, followed by a gross necropsy of the thoracic, abdominal, and pelvic organs. The female rats were examined for the number of corpora lutea, implantation sites, live and dead foetuses and early and late resorptions.

In F2 generation, each foetus was weighed and examined for sex and internal alterations.

Results

In F0 dams, no treatment-related mortality, changes in food consumption, clinical signs and necropsy findings were observed. The maternal body weight gain was comparable among different groups prior to GD 18. However, the average body weight from GD 18-20 was reduced at 600 µg/kg bw/day. The decrease was attributed to the litter loss (dead or resorbed conceptuses) in this group. During the lactation period, maternal body weights were comparable among the different groups. At 600 µg/kg bw/day, 3 dams were observed not to have delivered a litter by gestation Day 25. Postmortem examination showed that 2 of these rats had early resorptions, while the third delivered normally with no abnormalities. In the 300 and 600 µg/kg bw/day groups, the duration of gestation was reduced, and peri- and postnatal pup mortality was increased.

In the F1 generation, the number of pups per litter at birth, the number of live born pups per litter, the percentage of pups surviving after 1 and 4 days, and pup weights during the preweaning period were decreased at 300 and 600 µg/kg bw/day. No treatment-related mortality and clinical signs were noted during the premating period and during the gestation period. No treatment-related effects were observed on body weights, food consumption, sexual maturation, behaviour, or in necropsy. The number of days in cohabitation was increased and the fertility index was slightly decreased at 300 µg/kg bw/day.

In the F2 generation, no treatment-related abnormal findings were noted.

Conclusion

Under the study conditions, the NOAEL for maternal toxicity was established at 100 µg/kg bw/day, based on reduced duration of gestation and increased peri- and postnatal pup mortality. The developmental NOAEL was also established at 100 µg/kg bw/day, based on a slight increase in the number of days required for mating and a slight decrease in the fertility index in the F1 generation.

(CDER, 2001a)

Overall conclusion for developmental toxicity potential

The available developmental toxicity studies with the analogues tafluprost and bimatoprost *via* intravenous and oral routes, respectively, showed developmental and maternal effects. Tafluprost caused developmental effects such as intrauterine death, decreased viability and teratogenicity

including vertebral defects, delayed pinna unfolding in rats and rabbits at ≥10 and ≥0.1 µg/kg bw/day, respectively. The maternal NOAELs in rats ranged from 10 to 30 µg/kg bw/day and developmental NOAELs ranged from 0.3 to 3 µg/kg bw/day. The maternal and developmental NOAEL in rabbits was established at 0.01 µg/kg bw/day. Bimatoprost induced maternal toxicity including abortions, alopecia, reduced gestation duration and developmental effects, such as increased peri- and postnatal pup mortality but no significant teratogenicity in mice and rats at 300 and 600 µg/kg bw/day, respectively. The maternal NOAELs ranged from 100 to 600 µg/kg bw/day in mice and from 100 to 300 µg/kg bw/day in rats, while the developmental NOAEL was 600 µg/kg bw/day in mice and ranged from 100 to 600 µg/kg bw/day in rats. The difference in the results between tafluprost and bimatoprost can be mainly attributed to differences in routes of exposure.

Based on the above information, the potential interaction of DDDE with prostamide receptors is not expected to result in additional or more severe developmental effects compared to tafluprost. Therefore, the lower intravenous NOAELs based on studies with tafluprost would represent a worst-case approach.

4. Genotoxicity

One guideline-compliant *in vitro* Ames test as well as an *in vitro* micronucleus test (MNT) is available for DDDE.

4.1 *Mutagenicity / Genotoxicity in vitro (PREVIOUSLY SUBMITTED)*

1st study: *In vitro* Bacterial Reverse Mutation Test (Ames Test)

Guideline:	OECD Guideline 471
Test system:	*Salmonella typhimurium* strains TA 1535, TA 1537, TA 98 and TA 100, *Escherichia coli* WP2 uvrA (pKM101)
Replicates:	Triplicate
Test substance:	DDDE (neat oil)
Vehicle:	DMSO
Batch:	TAF-10-1122-01
Purity:	99.78%
Test concentrations:	**Experiment I:** 3.16, 10.0, 31.6, 100, 316, 1000, 2500 and 5000 µg/plate (TA100) 31.6, 100, 316, 1000, 2500 and 5000 µg/plate (TA98, TA1535, TA1537, E. coli WP2 uvrA (pKM101)) **Experiment II:** 3.16, 10.0, 31.6, 100, 316, 1000, 2500 and 5000 µg/plate (TA100, TA1535 [without S9-mix]) 31.6, 100, 316, 1000, 2500 and 5000 µg/plate (TA98, TA1535 [with S9-mix], TA1537, E. coli WP2 uvrA (pKM101))
Negative control:	Purified water
Positive control:	4-nitro-o-phenylene-diamine, methylmethanesulfonate sodium azide (without S9-mix); 2-aminoanthracene (with S9-mix)
GLP:	Yes
Study period:	2023

The mutagenicity potential was evaluated for DDDE in an *in vitro* bacterial reverse mutation assay, according to OECD Guideline 471 in compliance with GLP. In this assay, DDDE was tested according to the plate incorporation method (experiment I) and the pre-incubation method (experiment II), using *Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537 and tester strain *E. coli* WP2 uvrA (pKM101). The test concentrations ranged from 3.16 to 5000 µg/plate in experiments I and II.

Results

No precipitation of the test substance was observed in any tester strain used in experiment I and II (with and without S9-mix).

In experiment I, toxic effects of the test substance were observed in tester strains TA98, TA100 and TA1535 (with and without metabolic activation) and in tester strain *E. coli* WP2 uvrA (pKM101) (without metabolic activation) at concentrations of 2500 µg/plate and higher. In experiment II, toxic effects of the test substance were observed at concentrations of ≥316 µg/plate (with and without S9-mix), depending on the particular tester strain (i.e., at ≥1000 µg/plate in the strain TA 98; ≥316 µg/plate in the strain TA 100 and TA1535; ≥2500 µg/plate in the strains TA 1537 and *E. coli* WP2 uvrA)

No biologically relevant increases in revertant colony numbers of any of the five tester strains were observed following treatment with the substance at any concentration, neither in the presence nor absence of metabolic activation in experiments I and II.

Overall, under the experimental conditions reported, the test substance did not cause gene mutations by base pair changes or frameshifts in the genome of the tester strains used.

Conclusion

Under the conditions of the study, DDDE was not mutagenic in the bacterial reverse mutation assay (Ames test), neither in the presence nor absence of metabolic activation.

(Klock, 2023a)

2st study: *In vitro* Micronucleus Test (MNT)

Guideline:	OECD Guideline 487
Test system:	Human lymphocytes
Replicates:	Duplicate
Test substance:	DDDE (neat oil)
Vehicle:	DMSO
Batch:	TAF-10-1122-01
Purity:	99.78%
Test concentrations:	**Pre-Experiment:** 7.8, 15.6, 31.3, 62.5, 125, 250, 500, 1000, 1500, 2000 µg/mL (with and without S9-mix) **Experiment I:** 100, 250, 300, 325, 350, 375, 400, 425, 450 and 500 µg/mL (with and without S9-mix) **Experiment II:** 25, 50, 100, 150, 200, 250, 300 and 350 µg/mL (without S9-mix)
Main experiment (microscopic analysis)	Experiment I (short-term exposure- 4 hours): 250, 325 and 350 µg/mL (without S9-mix) 100, 250 and 300 µg/mL (with S9-mix) Experiment II (long-term exposure- 44 hours): 25, 50 and 100 µg/mL (without S9-mix)
Negative control:	Cell culture medium
Positive control:	Methylmethanesulfonate and colchicine (without S9-mix) Cyclophosphamide (with S9-mix)
GLP:	Yes
Study period:	2023

The genotoxicity of DDDE was evaluated in an OECD Guideline 487 and GLP-compliant *in vitro* MNT using human lymphocytes. In this assay, DDDE, dissolved in DMSO, was tested in the presence and absence of metabolic activation (i.e., phenobarbital/β- naphthoflavone-induced rats' liver S9-mix).

The selection of the concentrations was based on the data from the pre-experiment. In the main experiment, the test concentrations in experiment I with 4 h short-term exposure were 250, 325 and 350 µg/mL, without S9 mix and 100, 250 and 300 µg/mL, with S9 mix. In experiment II, with 44 h long-term exposure, the test concentrations were 25, 50 and 100 µg/mL, without S9 mix.

Methylmethanesulfonate (50 and 65 µg/mL) and cyclophosphamide (15 µg/mL) were used as controls for clastogenicity. Colchicine (0.02 and 0.4 µg/mL) was used as control for aneugenicity. A solvent control (DMSO) was also included in the test.

Results

No precipitation of the test substance was observed up to the highest concentration used in experiments I (with and without S9-mix) and II (without S9-mix).

In experiment I (without S9-mix), no increase of the cytostasis above 30% was observed up to 250 µg/mL. At 325 and 350 µg/mL, a cytostasis of 37 and 55%, respectively, was observed. In experiment I (with S9-mix), no increase of the cytostasis above 30% was observed up to 100 µg/mL. At 250 and 300 µg/mL, a cytostasis of 44 and 59%, respectively, was observed.

In experiment II (without S9-mix), no increase of the cytostasis above 30% was recorded up to 50 µg/mL. At 100 µg/mL, a cytostasis of 66% was observed.

The numbers of micronucleated cells were within the historical control limits of the solvent control and did not show a biologically relevant increase compared to the concurrent solvent control in both experiments I and II.

The micronucleated cell frequency of the negative and solvent control was also within the historical control limits. Clastogenic (methylmethanesulfonate, cyclophosphamide) and aneugenic positive controls (colchicine) induced distinct and statistically significant increases in the micronucleus frequency, demonstrating the validity of the assay.

Overall, the treatment with DDDE did not result in a statistically significant increase in the number of cells with micronuclei compared to concurrent solvent controls in experiments I and II, both in the presence and absence of an S9-mix.

Conclusion

Under the conditions of the study, DDDE did not induce structural and/or numerical chromosomal damage in human lymphocytes.

(Klock, 2023b)

4.2 Mutagenicity / Genotoxicity *in vivo* *(PREVIOUSLY SUBMITTED)*

No *in vivo* genotoxicity studies could be identified for DDDE. Therefore, the endpoint can be addressed based on the available *in vitro* data for DDDE (see **Section 4.1 of Annex II**) as well as the *in vivo* data for the analogues tafluprost and bimatoprost.

The overview of the *in vivo* genotoxicity studies with the analogues tafluprost and bimatoprost are summarised in the below table.

Table 3: Overview of *in vivo* genotoxicity studies with the analogues tafluprost and bimatoprost

Study type	Study details	Exposure/procedure	Result	Reference
Tafluprost				
In vivo micronuclei formation assay; No guideline specified	Test system: Outbred CD-1 mice Group size: 6 males/group Neg. control: Corn oil (vehicle) Pos. control: Cyclophosphamide (40 mg/kg in saline)	Dosing: Definitive study: 0, 175, 350 and 700 mg/kg bw Main study: 0, 700, 1000, 1400, and 2000 mg/kg bw/day Route: Intraperitoneal Duration: 24 and 48 hours	Negative	(CDER, 2011b)
Bimatoprost				
In vivo DRF micronuclei formation assay; No guideline specified	Test system: Outbred CD-1 mice Group size: 3 mice/sex/group Neg. control: Corn oil (vehicle) Pos. control: Cyclophosphamide (80 mg/kg in saline)	Dosing: 0, 5, 10, and 20 mg/kg bw Route: Intravenous Duration: 48 hours	Negative	(CDER, 2001b)

Overall conclusion on the genotoxicity potential of DDDE

Based on the absence of mutagenic response in the Ames test and clastogenic response in the *in vitro* MNT assays conducted with neat DDDE, there is no genotoxic concern for DDDE. This is further supported by the absence of genotoxicity observed for the analogue tafluprost in (1) *in vitro* Ames test, (2) *in vitro* chromosomal aberration assay and (3) in *in vivo* MNT assay. Similarly, bimatoprost was not genotoxic in (1) *in vitro* Ames test, (2) *in vitro* mouse lymphoma assay and (3) *in vivo* MNT assay (CDER, 2011b).

ANNEX III – THE COSMETIC EYELASH PRODUCT APPLICATION AMOUNT IN HUMAN EYELASHES AND MINK HAIR

[redacted]

[redacted] – ***Eyelash Application Amount***

Purpose: To determine the average amount of [redacted] that is applied to the upper eyelashes per application.

Study Date: January 11, 2013

Study Coordinator: [redacted]

Study Assistant: [redacted]

Study Description: The average amount of [redacted] that is applied per brushstroke to the upper eyelashes was determined by weighing the applicator brush used to apply [redacted] to the eyelashes in 3 conditions: 1) before being inserted into a standard vial of [redacted] ("**Dry**"); 2) after being inserted into a standard vial of [redacted] ("**Wet Pre-Application**"); and 3) after application of [redacted] by the study volunteer to the upper eyelashes in accordance with the package directions for use ("**Wet Post-Application**").

The average amount of [redacted] that is applied to the applicator ***brush*** = [Wet Pre-Application – Dry].

The average amount of [redacted] that is applied to the ***eyelashes*** = [Wet Pre-Application – Wet Post-Application].

Study Equipment: Mettler Toledo Analytical Balance Model Number MS303S Serial Number B201596128 ("**Mettler Balance**").

Study Materials: 20 of the following components from inventory:

- Empty (no [redacted] 3.5mL tubes with brush applicator (sku #641).
- Unopened 3.5mL vials filled with [redacted] (sku #1128).

Study Volunteers: Five employees at the [redacted]

Study Procedures:

1. Remove the dry applicator brush from the tube and record its weight on the Mettler Balance.
2. Insert the dry applicator brush into the vial filled with [redacted] (= **Dry weight**).
3. Slightly shake the filled vial with inserted brush.
4. Standing near the Mettler Balance, remove the applicator brush from the tube, place on Mettler Balance and record its weight (= **Wet Pre-Application weight**).
5. Remove the wet applicator brush from the Mettler Balance and give to study volunteer, standing near Mettler Balance. Study volunteer used applicator brush to apply [redacted] to upper eyelashes of one eye in accordance with the package directions for use (one continuous brush stroke to upper eyelashes above the skin).
6. Gently take the applicator brush from the study volunteer, place the applicator brush on the Mettler Balance and weigh the applicator brush (= **Wet Post-Application weight**).
7. Discard the applicator brush, tube and vial just used.
8. Repeat steps 1 – 7 with the same study volunteer; apply [redacted] to the opposite eye.
9. Repeat steps 1-8 with a total of 5 study volunteers (total of 10 eyes).

[redacted]

[redacted] [redacted]

[redacted] – *Eyelash Application Amount*

Study Results: On average, **2.4 mg** of [redacted] was applied to the upper eyelashes per normal brushstroke (see Table 1, below).

Table 1. Weights of applicator brush with no [redacted] ("Dry"), with [redacted] before application (Wet Pre-Application") and after application (Wet Post-Application"). Weights measured using MettlerBalance. All weights are in mg.

Sample	Brush Applicator: Dry	Brush Applicator: Wet Pre-Application	Amount of [redacted] on Applicator *Brush*	Brush Applicator: Wet Post-Application	Amount of [redacted] Applied to *Eyelashes*
1	3814	3823	9	3819	4
2	3850	3855	5	3854	1
3	3860	3866	6	3863	3
4	3827	3834	7	3833	1
5	3812	3818	6	3815	3
6	3855	3861	6	3857	4
7	3846	3855	9	3852	3
8	3876	3881	5	3880	1
9	3814	3821	7	3818	3
10	3828	3831	3	3830	1
Average	**3838.2mg**	**3844. 5mg**	**6.3 mg**	**3842.1 mg**	**2.4 mg**

Report Prepared By: [redacted] Date: 1-11-13

Approved By: [signature] Date: 1/11/2013

[redacted]

Product Testing Mink Lashes Summary Current Component

Three different "swipe" testing protocols were performed on the componentry to determine the amount of product expressed by the current cosmetic eyelash product applicator.

Procedure 1:

Procedure Summary:

This procedure keeps the same tube and the applicator changes. Each applicator is used for 2 applications.

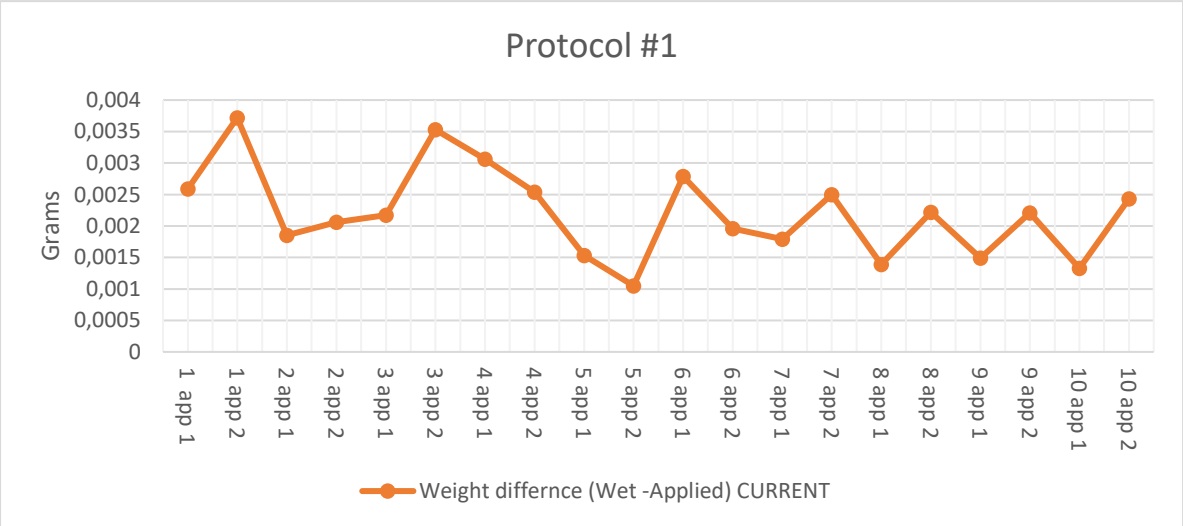
Application 1: Applicator was inserted into the tube, applicator was twisted on all the way, removed, weighed, swiped across measured length of mink eyelash (see photo reference), and then reweighed.

Application 2: Applicator was inserted into the tube, applicator was only pushed down to contact the neck of the tube, applicator was removed, weighed, swiped across a measured length of mink eyelashes, and then reweighed. Then lashes were then discarded, and the applicator set aside. The tube was then set for a rest period. This was done for 10 current applicators.

Current:

Total	0.02410
Average (Total /20)	0.001205
Total App 1	0.01999
Average App 1 (total app 1/10)	0.001999
Total App 2	0.02422
Average App 2 (total app 1/10)	0.002422

Graphical Data:



In terms of molecule consumption per average:

Calculation: $Average_{applied}$ X [DDDE]= $Amount_{DDDE}$ in grams

[DDDE] = 0.00018

1. Average Overall Current: 0.001205 x 0.00018 = 2.169 x 10^{-7} = 0.0000002169 g
2. Average App 1 Current: 0.001999 x 0.00018 = 3.5982 x 10^{-7}= 0.00000035982 g
3. Average App 2 Current: 0.002422 x 0.00018 = 4.3596 x 10^{-7} = 0.00000043596 g

Summary:

All values are less than the 3.4 x 10^{-6} grams of prostaglandin that is mentioned in the Donzis report. Donzis stated that [redacted] drop contained .0034 mg of the prostaglandin which converts to 3.4 x 10^{-6} grams.

Procedure 2:

Procedure Summary:

This procedure keeps the same applicator and the tube changes. Each tube is used for 2 applications.

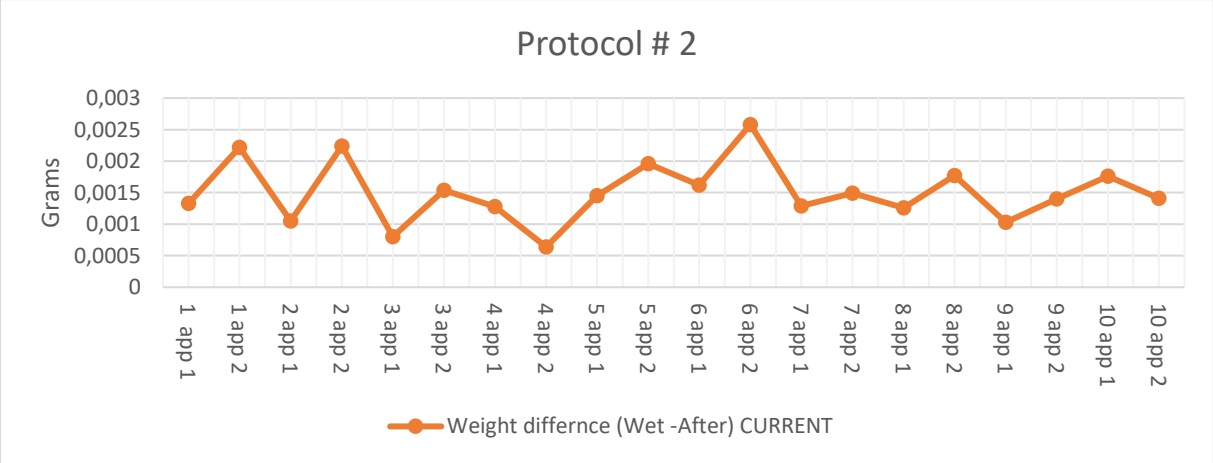
Application 1: Applicator was inserted into the tube, applicator was twisted on all the way, removed, weighed, swiped across measure eyelash (see photo reference), and then reweighed.

Application 2: The applicator was inserted into the tube, applicator was only pushed down to contact the neck of the tube, applicator was removed, weighed, swiped across a measured length of mink eyelashes, and then reweighed. The lashes were then discarded, and the applicator set aside. The applicator was then wiped down with a soft towel and set aside for a drying period. This was done for 10 current tubes.

Current:

Total	0.03012
Average (Total /20)	0.001506
Total App 1	0.01287
Average App 1 (total app 1/10)	0.001287
Total App 2	0.01725
Average App 2 (total app 1/10)	0.001725

Graphical Data:



In terms of molecule consumption per average:

Calculation: $Average_{applied}$ X [DDDE]= $Amount_{DDDE}$ in grams

[DDDE] = 0.00018

1. Average Overall Current: .001506 x .00018 = 2.7108 x 10^{-7} g = 0.00000027108 g
2. Average App 1 Current: .001287 x .00018 = 2.3166 x 10^{-7} g = 0.00000023166 g
3. Average App 2 Current: .001725 x .00018 = 3.105 x 10^{-7} g = 0.0000003105 g

Summary:

All values are less than the 3.4 x 10^{-6} grams of prostaglandin that is mentioned in the Donzis report. Donzis stated that [redacted] drop contained .0034 mg of the prostaglandin which converts to 3.4 x 10^{-6} grams.

ANNEX IV – ABBREVIATIONS

AE	Adverse Event
AFP	Alpha-Fetoprotein
ALT	Alanine Aminotransferase
AMBIT	Assessment and Management of Bioaccumulation Information Tool
AOP	Adverse Outcome Pathway
AR	Androgen Receptor
AST	Aspartate Aminotransferase
BUN	Blood Urea Nitrogen
CAS	Chemical Abstracts Service
CBER	Center for Biologics Evaluation and Research
CDER	Center for Drug Evaluation and Research
CFR	Code of Federal Regulations (US)
CPMP	Committee for Proprietary Medicinal Products
DDDE	Dechloro Dihydroxy Difluoro Ethylcloprostenolamide
DMSO	Dimethyl Sulfoxide
DPBS	Dulbecco's Phosphate-Buffered Saline
DPRA	Direct Peptide Reactivity Assay (skin sensitisation test)
DRF	Dose Range Finding
EATS	Estrogen, Androgen, Thyroid, and Steroidogenesis (endocrine pathways)
EC	Effective Concentration
EC1.5	Effective concentration at 1.5% level
ECG	Electrocardiogram
ECHA	European Chemicals Agency
EDTA	Ethylenediaminetetraacetic Acid
EFSA	European Food Safety Authority
EMA	European Medicines Agency
EPA	Environmental Protection Agency (US)
ER	Estrogen Receptor
ERG	Electroretinogram
EU	European Union
FDA	Food and Drug Administration (US)
GC	Gas Chromatography
GD	Gestation Day
GLP	Good Laboratory Practice
GPMT	Guinea Pig Maximization Test
GR	Glucocorticoid Receptor
GUI	Graphical User Interface
GW	Gestational Week
HD	High Dose
HEK	Human Embryonic Kidney (cells)
HET CAM	Hen's Egg Test-Chorio Allantoic Membrane
HPLC	High-Performance Liquid Chromatography

HRIPT	Human Repeated Insult Patch Test
HTC	Highest Tested Concentration
IC50	Inhibitory Concentration at 30% / 50%
ICH	International Council for Harmonisation
INCI	International Nomenclature of Cosmetic Ingredients
IOP	Intraocular Pressure
IPCS	International Programme on Chemical Safety
IUPAC	International Union of Pure and Applied Chemistry
IV	Intravenous
KOW	Octanol-Water Partition Coefficient
LD50	Lethal Dose 50%
LOAEL	Lowest Observed Adverse Effect Level
MCE	Molar Extinction Coefficients
MNT	Micronucleus Test
MoS	Margin of Safety
MS	Mass Spectrometry
MTD	Maximum Tolerated Dose
MTT	Methylthiazol Tetrazolium (MTT assay)
NIS	Sodium/Iodide Symporter
NOAEC	No Observed Adverse Effect Concentration
NOAEL	No Observed Adverse Effect Level
NOEC	No Observed Effect Concentration
NOEL	No Observed Effect Level
NRU	Neutral Red Uptake assay
OECD	Organisation for Economic Co-operation and Development
OHT	Ocular Hypertension
PBS	Phosphate-Buffered Saline
PCR	Polymerase Chain Reaction
PoD	Point of Departure
PR	Progesterone Receptor
QSAR	Quantitative Structure–Activity Relationship
RDT	Repeated Dose Toxicity
RHE	Reconstructed Human Epidermis
RNA	Ribonucleic Acid
SAR	Structure-Activity Relationship
SCCS	Scientific Committee on Consumer Safety
SD	Standard Deviation
SED	Systemic Exposure Dose
SIDS	Screening Information Data Set
SMILES	Simplified Molecular Input Line Entry System
TEWL	Transepidermal Water Loss
TGA	Therapeutic Goods Administration (Australia)

Supplemental Toxicological Safety Assessment of Isopropyl Cloprostenate (up to 0.005%) in Eyelash Serums

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Submitted on Behalf of:

Company 1
(markets cosmetic lash serum containing 0.0044% isopropyl cloprostenate)

&

Company 2
(markets cosmetic lash serum containing 0.005% isopropyl cloprostenate)

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INTRODUCTION

This submission provides additional data supporting the safe use of isopropyl cloprostenate ("IC") in cosmetic lash serums containing up to 0.005% IC. Previously-provided data are summarized first, with new data summarized after. An updated margin of safety (MOS) calculation is also provided, based on the results of an OECD Guideline 428 dermal absorption study. The last component of this submission comprises a detailed read-across assessment that expands upon methodologies used in the original Toxicological Safety Assessment for IC.

SUMMARY OF PREVIOUSLY-PROVIDED DATA FOR IC

In response to the Cosmetic Ingredient Review (CIR) Expert Panel's Scientific Literature Notice to Proceed, dated March 17, 2023, the Companies submitted the results of 6 HET-CAMs, 4 HRIPTs, 3 Clinical Ocular Irritation Studies, 1 Clinical In-Use Eye Assessment, 1 In Vitro Ocular Irritation Assay, 2 IC Assays, and 2 Average Weight Usage Tests.

- The results of *in vitro* and clinical studies confirm that IC is not irritating to the eyes at up to 0.005%, nor is it irritating or sensitizing to the skin.
- Collectively, these studies fulfill the skin irritation, skin sensitization, and eye irritation endpoints.
- The results of the Average Weight Usage Tests have been integrated into the margin of safety (MOS) calculation for IC.

In response to the Expert Panel's Insufficient Data Announcement ("IDA"), dated June 15, 2023, the Companies submitted a report, dated October 27, 2023, entitled Additional Data Supporting the Safe Use of IC in Cosmetics (the "October 2023 Submission"), an 8-month Ophthalmological In-Use Safety Evaluation on 120 female subjects (the "8-Month Clinical Study"), a 28-day clinical trial on 24 female participants to evaluate potential impact on intraocular pressure (the "Intraocular Pressure Assay"), and a toxicological safety assessment prepared by ToxServices LLC (the "Toxicological Safety Assessment").

- The 8-Month Clinical Study confirms that there are no adverse systemic effects arising from the cosmetic use of up to 0.005% IC in eyelash serums; this study fulfills the repeated-dose toxicity endpoint.
- The 8-Month Clinical Study did not identify any evidence of iris pigmentation or periorbital fat loss associated with the intended cosmetic use of IC at levels up to 0.005%.
- The Intraocular Pressure Test confirms that the intended cosmetic use of IC at levels up to 0.005% does not affect IOP.
- In the original Toxicological Safety Assessment, a sufficient MOS of 343, obtained via read-across methodology, confirms that the intended cosmetic use of IC at levels up to 0.005% poses no significant risk of adverse systemic effects.

In response to the Expert Panel's IDA, dated December 8, 2023, the Companies submitted an OECD Guideline 471 Bacterial Reverse Mutation Test and an OECD Guideline 487 In Vitro Micronucleus Test (the "Micronucleus Test").

- The results of these studies confirm that IC is not mutagenic and does not cause structural or numerical chromosomal aberrations.

NEW ANALYSES/TESTING ON IC (UP TO 0.005%)

In a Post Meeting Announcement dated June 7, 2024, the Expert Panel tabled the Draft Tentative Report on prostaglandin analogues for the receipt of incoming data. In response to this, this Supplement Toxicological Safety Assessment provides supplemental information on the following responsive data on IC:

- Dermal metabolism and penetration assay (see Section I);
- Updated margin of safety (MOS) calculations (see Section II);
- Independent ophthalmological analysis of ocular parameters (e.g., colorimetry) observed in the 8-month clinical study (see Section III); and
- Enhanced analysis supporting the use of read across-across methodologies, including QSAR assessment (see Section IV)

I. Dermal Metabolism and Penetration Assay

An OECD Guideline 428 study evaluated the *in vitro* dermal absorption of IC, present in a formulated cosmetic product at 0.005%, through freshly-excised, healthy human skin and measured IC in the following samples: treated skin washing, *stratum corneum*, epidermis, dermis, receptor fluid, and rinsing of donor compartment [1]. The formulation containing 0.005% IC was applied to three dermatomed skin samples prepared from each of four donors (total of 12 skin samples) for 24 hours using flow-through diffusion cells. Receptor fluid samples were collected at 0.5, 1, 2, 4, 6, and 24 hours. Prior to application, the integrity of each skin sample was confirmed by measuring transepidermal water loss. IC was evaluated in each sample and in quality control samples using an LC-MS/MS method and quantified against an analyte-specific calibration curve [1].

The 24-hour data are summarized below in Table 1; mean values apply to 12 samples (i.e., 3 samples/donor * 4 donors). Values provided in bold, italicized font were below the laboratory's lower limit of quantification (BQL) and were derived from the appropriate matrix-specific quantification limits.

Table 1: Dermal Absorption Data for IC [1]

Parameter	Isopropyl Cloprostenate			Cloprostenol		
	Mean	SD	CV (%)	Mean	SD	CV (%)
Test substance applied (ng) *	560.42	24.63	4.39	560.42	24.63	4.39
Test substance applied (µg/cm²) *	0.56	0.02	4.39	0.56	0.02	4.39
Stratum Corneum (Tape Strips) (µg/cm²)	0.01	0.003	66.41	***0.002***	***0.001***	***36.35***

Stratum Corneum (Tape Strips) (%) **	0.91	0.61	66.92	***0.32***	***0.12***	***36.76***
Epidermis (µg/cm²)	0.01	0.01	67.03	***0.01***	***0.00***	***0.00***
Epidermis (%) **	1.67	1.12	67.13	***0.89***	***0.04***	***4.35***
Dermis (µg/cm²)	0.0003	0.0002	63.85	***0.01***	***0.00***	***0.00***
Dermis (%) **	0.05	0.03	63.87	***0.89***	***0.04***	***4.35***
Receptor Fluid Total (µg/cm²)	0.03	0.001	2.91	***0.03***	***0.00***	***2.90***
Receptor Fluid Total (%) **	4.61	0.30	6.46	***4.61***	***0.30***	***6.50***
Epidermis + Dermis + Receptor Fluid (µg/cm²)	0.04	0.01	18.61	***0.04***	***0.001***	***2.09***
Epidermis + Dermis + Receptor Fluid (%) **	6.34	1.20	18.95	***6.40***	***0.37***	***5.83***
SC + Epidermis + Dermis + Receptor Fluid (µg/cm²)	0.04	0.01	19.00	***0.04***	***0.00***	***2.76***
SC + Epidermis + Dermis + Receptor Fluid (%) **	7.25	1.40	19.27	***6.71***	***0.41***	***6.12***
Skin Washing (µg/cm²)	0.61	0.10	15.62	***0.02***	***0.00***	***0.00***
Skin Washing (%) **	109.27	15.72	14.38	***2.90***	***0.13***	***4.35***
Recovery (%) **	116.52	14.63	12.56	***9.62***	***0.53***	***5.49***

* As IC. The applied formulation theoretically contained no cloprostenol.
** Percentages for cloprostenol are calculated using the cloprostenol quantification limit relative to the applied amount of IC.

The data indicate that IC is poorly absorbed through the skin. The majority of the applied amount of IC remained on the surface of the skin and was measured in the Skin Washing samples; excess skin (i.e., non-application area) contributed a trace amount of IC to the Skin Washing values in 2/12 samples. An average of approximately 6.3% of the applied amount of IC was absorbed (i.e., measured in the epidermis, dermis, and receptor fluid) and was contributed mostly by the receptor fluid. Since IC levels in 11/12 receptor fluid samples were below the quantification limit (BQL), the lower limit of quantitation (LLOQ) is used in the estimation of total dermal absorption as a conservative upper bound.

Cloprostenol was not present at measurable levels in the skin layers or the receptor fluid; dermal metabolism of IC to cloprostenol therefore appears minimal. However, the LLOQ in the epidermis and dermis was 0.05 ng/mL for IC but 1 ng/mL for cloprostenol, and the LLOQ for both analytes in receptor fluid was 1 ng/mL. Cloprostenol could therefore be present at very low levels in these fractions.

II. Updated Margin of Safety Calculation

The Company formulates its eyelash serums to have a concentration of IC of 0.005% or less. The Company's applicator, wiper, and instructions for use are specifically designed to keep the eyelash serum on the upper lash line, reducing the chance of ocular exposure to IC. Further, consumers are instructed to immediately rinse with cool water if eyelash serum does contact the eye. Therefore, the dermal route will be the major route of exposure to IC. We incorporated the results of the OECD Guideline 428 dermal absorption study into the MOS calculation.

MOS calculations are based on procedures described in the EU's Scientific Committee on Consumer Safety (SCCS) Notes of Guidance [2]. SCCS defines daily systemic exposure

as the amount of substance which is absorbed upon use of the product.

Accordingly, the systemic exposure dose (SED) through the dermal route has been calculated as follows for eyelash serum products:

$$SED = E_{product} * C/100 * DA_p/100$$

Equation 1

Where

$$E_{product} = (q_x * f_{ret}) / BW$$

Equation 2

Values selected for each parameter in Equations 1 and 2 are provided in Table 2.

Table 2: Systemic Exposure Dose (SED) Calculation

Parameter	Explanation	Value	References
q_x	Amount of IC formulation (eyelash serum) that is applied per day (mg/day)	0.26	[3, 4]
f_{ret}	Dermal retention factor for eyelash serum (as decimal)	1	[2]
BW	Body weight (kg)	70	Default
$E_{product}$	Estimated daily exposure to eyelash serum (mg/kg/day)	0.00372	[2]
C	Concentration of IC in the finished eyelash serum (%)	0.005	Given
DA_P	Dermal absorption (%) = (mean % in dermis + epidermis + RF) + 1 SD (dermis + epidermis + RF) = 6.34% + 1.20% = 7.54% (real life conditions)	7.54	[1, 2]
SED	Systemic exposure dose through the dermal route (mg/kg/day)	1.40E-08	Calculated

The amount of eyelash serum used per application was estimated in two use simulation studies conducted on behalf of the Company, one performed with a formulation containing 0.0044% IC and the second performed with a formulation containing 0.005% IC [3, 4]. In the use simulation studies, investigators tested IC formulations using functionally identical instructions to the instructions for use that the Company provides to consumers with its eyelash serums *i.e.*, investigators dipped the applicator once into the formulation bottle, wiped excess product onto the rim of the container, swiped the applicator on a pre-weighed petri dish or slide for a length of approximately one inch, and swiped the applicator a second time on a different area of the petri dish/slide for another length of approximately one inch, not dipping the applicator into the bottle between the two swipes. The petri dish/slide was weighed again, and the amount applied calculated as the difference between

the post-application and pre-application weights. This process was repeated for a total of 15 application simulations per formulation, and averages for each formulation calculated. The average amount of the 0.005% IC formulation applied was slightly higher than the average amount of the 0.0044% IC formulation applied, 0.26 mg compared to 0.19 mg, respectively. For conservatism, the higher value for the 0.005% IC formulation was included in the SED calculation.

With respect to dermal absorption, SCCS specifies that the mean +1 standard deviation (SD) of the amounts of the ingredient measured in the dermis, epidermis (without stratum corneum), and receptor fluid (RF) of an appropriately performed dermal absorption study are typically used as the amount dermally absorbed in the SED calculation [2]; if the data are highly variable, value of mean +2 SDs is used [2]. For IC, the mean amount measured in the epidermis (without stratum corneum), dermis, and receptor fluid was 6.34% (see Table 1). As the calculated standard deviation was 1.20%, the value used for dermal absorption in the SED calculation is 7.54%.

The margin of safety (MOS) is calculated by dividing the highest NO(A)EL that is lower than the lowest LO(A)EL of the cosmetic substance (termed the point of departure) by its exposure through use of the cosmetic. SCCS identifies a target MOS of at least 100 as acceptable for demonstrating safety of cosmetic ingredients [2].

Although a NO(A)EL or LO(A)EL is not directly available for IC, through critical dose-response and read-across analyses, detailed below, we identified a LOAEL of 0.12 µg/kg/day for the read-across substance travoprost, based on reduced litter size in a 3-generation rat study [5], as the point of departure for IC. If the point of departure is a LOAEL, a safety factor of 3 is typically applied, resulting in an adjusted POD (POD_{adj}) of 0.04 µg/kg/day [2].

The MOS is usually calculated by dividing the internal (systemic) dose (designated N(L)OAELsys) by the SED, assuming that 50% of the administered dose will be bioavailable in an oral-route toxicity study [2]. In this case, however, since the point of departure was obtained from a study that utilized subcutaneous injection [5], systemic availability was 100% (i.e., external dose = internal dose), and adjustment of the point of departure for systemic absorption is not necessary.

As discussed above, two use simulation studies conducted on behalf of the Company indicated that a conservative estimate of the average amount of IC formulation applied by consumers when using cosmetic eyelash serums with an IC concentration of 0.005% is 0.26 mg/day. Based on this exposure, and the mean + 1SD dermal absorption of 7.54% for eyelash serums with an IC concentration of 0.005% calculated based on data provided in Table 2, the systemic exposure dose through the dermal route is estimated at 1.40E-08 mg/kg/day.

An updated MOS of 2,849 has therefore been calculated for eyelash serums with an IC concentration of 0.005% according to Equation 3, with the calculation shown thereafter in Table 3:

$$MOS = POD_{adj} / SED$$

Equation 3

Table 3: Margin of Safety Calculation			
Parameter	**Explanation**	**Value**	**References**
POD	Point of departure (mg/kg/day), a LOAEL from a 3-generation study with surrogate travoprost.	0.00012	[5]
POD_{adj}	A safety factor of 3 is applied to the POD because it is a LOAEL.	0.00004	[2]
POD_{sys}	No additional adjustment to the POD is needed because the study providing the LOAEL used parenteral exposure.	N/A	[2]
SED	Systemic exposure dose through the dermal route (mg/kg/day)	1.40E-08	Table 2
MOS	$MOS = POD_{sys} / SED$	2,849	Calculated

This calculated MOS of 2,849 is significantly larger than the target of 100 identified as acceptable by SCCS [2]. Therefore, this safety assessment concludes that IC at concentrations up to 0.005% in eyelash serum cosmetic products is safe for use.

III. Independent Ophthalmological Analysis of Data from 8-Month Clinical Study

This safety-in-use study was a baseline controlled clinical trial intended to evaluate the safety of an eyelash serum containing 0.0044% IC with respect to ocular irritation, change in ocular pigmentation, and periorbital fat loss over 1-, 2-, 4-, and 8-month periods [6]. The study methods and results are summarized, followed by a summary of the independent ophthalmological analysis.

One hundred and twenty female subjects ranging in age from 18 to 55 years were recruited and qualified for this study, including 11 subjects that wore contact lenses. At baseline (Day 0), subjects were distributed an 8-month supply of the product with the following instructions for use: "Apply once daily on clean, dry, upper lash line only using a single stroke on the eyelid just above the upper lash line with the provided product applicator. Do not apply to the lower lash line. Use one dip into the bottle for both eyes and allow 1-2 minutes for the serum to fully dry before applying other products to the eye. Use sparingly as dripping into the eye may cause irritation or other unwanted effects. If serum drips into the eye, immediately rinse thoroughly with cool water. Apply the serum every day for at least 3 months and every other day thereafter after desired improvement is achieved." The first application of the product on Day 0 was supervised [6].

Each subject was given a daily diary to document test article use and to record any observations pertaining to the test material. The subjects were instructed to report any adverse reactions or problems immediately to the testing facility staff. At the conclusion

of the study at 8 months, subjects returned any unused test material and completed daily diaries [6].

Subjects reported to the testing facility at qualification, at baseline (Day 0), and at 1 month, 2 months, 4 months, and 8 months for an evaluation by a board-certified ophthalmologist and capture of VISIA-CR® and Aeva^{3D}-HE images. Ophthalmological examinations comprised gross and/or slit-lamp inspection of each subject's eyelids, conjunctivae, corneas, anterior chambers, and pupillary reactions, in addition to measuring visual acuity. Observations also included evaluations of dryness, erythema, and edema [6] .

VISIA-CR® Digital Imaging

After each subject acclimated in an environmentally-controlled room maintained at a temperature of 70 ± 5°F (21.1 ± 2.8°C) and a relative humidity of 40 ± 5% for at least 15 minutes, a bioinstrumentation technician took a series of standardized, reproductible digital images of the eyes with VISIA-CR® instrumentation. Stool height and careful placement of the subject's chin and forehead on the imaging device were maintained, the subject's hair was off the face, jewelry was removed, and a black drape was used to standardize clothing. Each subject's front view was captured with their eyes open using general purpose white light, flat lighting, and cross-polarized lighting. Contact lenses were removed prior to image capture [6].

Images were catalogued using MIRROR PhotoTools Software (Canfield Scientific., Inc., Parsippany, NJ) and exported as a .dx2 file for color analysis using Canfield's VAESTRO™ Image Analysis Toolkit. Briefly, VAESTRO™ captures the following six color parameters:

- L* (lightness or luminosity) value from the L*a*b* (CIELAB) color space; ranges from 0 (black) to 100 (white);
- a* (red-green axis) value from the L*a*b* (CIELAB) color space; negative = green & positive = red;
- b* (blue-yellow axis) value from the L*a*b* (CIELAB) color space; negative = blue & positive = yellow;
- R is the red value from the RGB color space;
- G is the green value from the RGB color space; and
- B is the blue value from the RGB color space.

This system evaluates changes in any part of the captured image, which for this study includes the sclera as well as the iris [6]. Change in each individual CIELAB color parameter and in overall color were evaluated using Equation 4, below, with an increase in ΔE representing an increase in overall color from baseline:

$$\Delta E = \sqrt{[(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]}$$

Equation 4

Aeva^{3D}-HE Imaging

The Aeva^{3D}-HE system is a high resolution, 3D measurement system for face topography,

skin topography, and body morphological changes. Evaluations of periorbital fat volume occurred at baseline (Day 0), 1 month, 2 months, 4 months, and 8 months. After each subject acclimated in an environmentally-controlled room maintained at a temperature of 70 ± 5°F (21.1 ± 2.8°C) and a relative humidity of 40 ± 5% for at least 15 minutes, a bioinstrumentation technician took a series of front-view images using the 250 FOV Lens. Images captured each subject's left and right orbital regions and were used to assess periorbital volume [6].

Findings

General Safety and Ocular Irritation Potential

The board-certified ophthalmologist investigator determined that the test material is safe for contact lens and non-contact lens wearers.

Ophthalmologic evaluation results are presented in Table 4 and reveal a slight potential for transient eye irritation.

Table 4: Ophthalmologist's Observations [6]

Study Timepoint	Subject #	Observation
1 Month	83	Meibomian gland dysfunction in both eyes†
	113	Erythema right and left conjunctiva
2 Months	6	Cornea: Punctate epithelial erosion right
	8	Cornea: Punctate epithelial erosion right and left
	9	Conjunctiva: Mild conjunctival erythema both eyes
	10	Conjunctiva: Mild conjunctival erythema both eyes
	19	Cornea: Punctate epithelial erosion left inferior
	26	Started a new prescription for glasses that improved her visual acuity score from 50-1/50-1 to 25/20
	30	Plus 1 right eye tarsal erythema
	34	Plus 1 bilaterial tarsal erythema
	36	Left upper lid chalazion†
	50	Lids: Increase meibomian gland dysfunction right and left
	52	Cornea: Right and left punctate epithelial erosion
	93	Conjunctiva: Mild erythema right and left
	103	Lids: Meibomian gland dysfunction right and left
	113	Conjunctivitis: right eye†
4 Months	40	Conjunctiva: Mild conjunctival erythema
	45	Tarsal follicles bilaterial
	53	Conjunctiva: Mild erythema right and left
	64	Conjunctiva: Mild erythema right and left
	101	Conjunctiva: Conjunctival erythema and papillae
	111	Conjunctiva: Conjunctival erythema
	115	Conjunctiva: erythema right greater than left
	120	Conjunctiva: Mild erythema and papillae
8 Months	38	Conjunctiva: erythema right greater than left Cornea: Punctate epitheliopathy right eye†
	93	Lids: Meibomian gland dysfunction right and left
	101	Conjunctiva: erythema conjunctiva bilaterial

† Identified as an adverse event, as described below.

Four (4) adverse events occurred during the study: 1 instance of 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) [6].

The results of the ophthalmologist's examinations of the periorbital skin are presented in Table 5. Mild redness was observed in 15 subjects and mild dryness was observed in four subjects at baseline. Only one subject had mild redness after 1 and 2 months of product use, while there was no evidence of irritation in any subjects after 4 and 8 months of product use. Overall, the examinations remained within normal limits throughout the study, indicating that the treatment did not cause clinically meaningful periorbital irritation [6].

Table 5: Periorbital Skin Evaluations [6]

Study Timepoint	Subject #	Erythema		Edema		Dryness	
		Right	Left	Right	Left	Right	Left
Baseline (Day 0)	21	1	1	0	0	0	0
	26	1	1	0	0	1	1
	32	1	1	0	0	0	0
	35	0	0	0	0	1	1
	38	0	0	0	0	1	1
	46	0	0	0	0	1	1
	53	1	1	0	0	0	0
	57	1	1	0	0	0	0
	60	1	1	0	0	0	0
	62	1	1	0	0	0	0
	75	1	1	0	0	0	0
	78	1	1	0	0	0	0
	81	1	1	0	0	0	0
	88	1	1	0	0	0	0
	93	1	1	0	0	0	0
1 Month	17	1	1	0	0	0	0
2 Months	17	1	1	0	0	0	0
4 Months	-	-	-	-	-	-	-
8 Months	-	-	-	-	-	-	-

0 – None; 0.5 – Barely perceptible; 1 – Mild; 2- Moderate; 3 – Marked; 4- Severe.

Potential for Change in Periorbital Volume

The results for periorbital volume and statistical analysis of AEVA^{3D}-HE positive volume (mm^3) values are presented in Table 6.

Table 6: Aeva^{3D}-HE Periorbital Volumes [6]

Side	Parameter	Baseline	1 Month	2 Months	4 Months	8 Months
Left	Mean Volume (mm^3)	329.5	313.1	324.6	323.8	328.1

	Mean % Change from Baseline	--	-5.0%†	-1.5%	-1.7%	-0.4%
Right	Mean Volume (mm^3)	292.9	276.0	287.7	277.1	282.2
	Mean % Change from Baseline	--	-5.8%†	-1.8%	-5.4%†	-3.7%

† Statistically significant ($p < 0.050$).

When compared to baseline values, there was a slight but statistically significant decrease in volume after 1 month of the test material use for the left orbital side and after 1 and 4 months of the test material use for the right orbital side. However, the degree of the reduction was at most 6% at all time points, and is not readily observable in corresponding subject photographs. The board-certified ophthalmologist investigator reported no evidence of changes in periorbital volume associated with product use as part of the subjects' physical exams over the course of 8 months [6].

There were no statistically significant differences in the left orbital volume after 2, 4, and 8 months of test material use or in the right orbital volume after 2 and 8 months of test material use [6]. Stated another way, there was no statistically significant change in periorbital volume from baseline after 8 months of use.

Potential for Change in Ocular Pigmentation

The results of the ocular pigmentation analysis using VISIA-CR® imaging are presented in Tables 7 and 8.

Table 7: VISIA-CR® RBG Color Analysis [6]

Side	Color	Parameter	Baseline	Month 1	Month 2	Month 4	Month 8
Left	R/RGB	Mean	56.14	56.02	56.43	56.61	57.16
		Mean % Change from Baseline	--	-0.2%	0.5%	0.8%	1.8%
	G/RGB	Mean	45.86	46.04	46.37	45.65	46.25
		Mean % Change from Baseline	--	0.4%	1.1%	-0.4%	0.9%
	B/RGB	Mean	38.87	39.13	39.46	38.25	38.83
		Mean % Change from Baseline	--	0.7%	1.5%	-1.6%	-0.1%
Right	R/RGB	Mean	55.17	55.33	54.81	54.63	55.87
		Mean % Change from Baseline	--	0.3%	-0.7%	-1.0%	1.3%
	G/RGB	Mean	44.04	44.76	44.08	43.26	44.43
		Mean % Change from Baseline	--	1.6%	0.1%	-1.8%†	0.9%
	B/RGB	Mean	36.71	37.63	36.90	35.70	36.68

		Mean % Change from Baseline	--	2.5%	0.5%	-2.8%†	-0.1%

† Statistically significant ($p < 0.050$).
R/RGB – Mean red value from the RGB color space, for the detected features withing the masked region of interest (ROI)
G/RGB – Mean green value from the RGB color space, for the detected features withing the masked ROI
B/RGB – Mean blue value from the RGB color space, for the detected features withing the masked ROI

Table 8: VISIA-CR® Overall Color Analysis [6]

Side	Value	Parameter	Baseline	Month 1	Month 2	Month 4	Month 8
Left	L*	Mean	19.62	19.66	19.81	19.60	19.87
		Mean % Change from Baseline	--	0.2%	1.0%	-0.1%	1.3%
	a*	Mean	5.10	4.96	4.99	5.37	5.33
		Mean % Change from Baseline	--	-2.7%	-2.2%	5.3%	4.5%†
	b*	Mean	11.09	11.01	11.02	11.44	11.48
		Mean % Change from Baseline	--	-0.4%	-0.4%	1.8%†	2.0%†
	ΔE	Total color change	--	2.33†	2.56†	2.42†	2.52†
Right	L*	Mean	18.90	19.15	18.85	18.54	19.08
		Mean % Change from Baseline	--	1.3%	-0.3%	-1.9%	0.9%
	a*	Mean	5.44	5.21	5.28	5.55	5.55
		Mean % Change from Baseline	--	-4.2%	-3.0%	1.9%†	2.0%†
	b*	Mean	11.23	11.07	11.01	11.26	11.50
		Mean % Change from Baseline	--	-0.8%	-1.2%	0.1%	1.4%†
	ΔE	Total color change	--	2.70†	2.68†	2.55†	2.49†

† Statistically significant ($p < 0.050$).
L* – Luminosity of the test site. The higher the value, the greater the lightness of the test site.
a* – Redness of the test site. The higher the value, the greater the redness of the test site.
b* – Yellowness of the test site. The higher the value, the greater the yellowness of the test site.
ΔE – Total color change of the test site. The larger the value, the greater the change in color.

The board-certified ophthalmologist investigator reported no evidence of changes in iris color associated with product use as part of the subjects' physical exams over the course of 8 months, and differences in iris color were not observable on the corresponding subject photographs [6]. However, based on small yet statistically significant changes in some VISIA-CR® parameters (see Table 8), further analysis of the ocular pigmentation colorimetric data is necessary to determine whether product usage resulted in iris stromal pigmentation darkening that is characteristic of prostaglandin-induced iris color change.

This additional analysis entailed a critical assessment of the colorimetric data as well as an expert assessment of participant photographs by an independent panel of ophthalmologists.

In the CIELAB color space, a reduction in the L* value over time would be most indicative of darkening, while an increase in the L* value over time would indicate lightening. The a* and b* values do not indicate lightness or darkness, but rather indicate hue and chromaticity (i.e., saturation) (increase in a* = shift toward redness / increase in b* = shift toward yellowness). The ΔE parameter calculates total color change as a function of the changes in all three scales (see Equation 4).

Given that clinically relevant iris stromal color changes develop over time, the relevant time period to evaluate is baseline vs 8-month changes. After 8 months of usage there were no statistically significant changes in the RGB color space values or in L* (lightness or luminosity) of the left or right iris. After 8 months of usage there were small but statistically significant increases in the a* and b* values, indicating slightly more redness and slightly more yellowness (i.e., greater saturation[1] of these hues), respectively, resulting in a corresponding significant increase in overall color change (ΔE) from baseline. These changes were found not clinically relevant by the board-certified ophthalmologist investigator [6].

The increases in ΔE from baseline to 8 months can be attributed to the relatively greater increases in both a* and b*, compared to L*. That is, the statistically significant increases in ΔE over 8 months are not likely due to the much smaller (and non-significant) increases in L*. Furthermore, an increase in L* would indicate a lightening effect (i.e., reduction in pigmentation), which is not consistent with iris stromal pigmentation darkening that is characteristic of prostaglandin-induced iris color change.

Left and right iris a* and b* value tallies & percentages showed wide variation throughout the study. For instance, after 8 months of use, 77 participants (67.54%) showed increased a* values in the left iris, while 37 participants (35.09%) showed decreased a* values in left iris. Similarly, after 8 months of use, 74 participants (64.91%) showed increased b* values in the left iris, while 40 participants (35.09%) showed decreased b* values in left iris. Despite these variations, the calculated ΔE values are similar. This indicates that it is the magnitude of the change as opposed to the direction of the change (i.e., positive or negative) in a* and b* that impacts ΔE. For this reason, when determining the clinical relevance of potential iris color changes, where the *direction* of the change is a critical factor, it is not appropriate to rely on ΔE alone. L* is the key parameter: if iris pigmentation were darkening, we would have expected to see a decrease in L* over the course of the study. This may or may not be accompanied by an effect on ΔE, depending on the magnitude of the collective effects on L*, a*, and b*.

[1] Saturation is the brightness or purity of a hue (i.e., color). Saturation can be reduced by adding white, gray, or black. While a blacker or grayer version of the same hue may "look darker" than the pure (i.e., fully saturated) hue, these blacker/grayer versions arise from hue desaturation, which would likely correspond to a reduction in a* or b*, not an increase. Increases in a* or b* indicate an increase in saturation, which could be observable as increased brightness or vividness of the color, which is not characteristic of prostaglandin-induced iris color change. Please see: https://learn.leighcotnoir.com/artspeak/elements-color/hue-value-saturation/

Colorimetric data analysis is a new and relatively untested way to measure the potential for iris color change. Most clinical studies have relied on expert panel members to review the clinical photographs for evidence of iris stromal pigmentation darkening. In order to confirm the conclusion that the statistically significant changes in ΔE were not clinically relevant, a panel of three independent ophthalmologists (the "Panel Members") evaluated paired baseline and 8-month photographs ("image pairs") captured during the 8-Month Study to determine whether any image pair showed a pattern of iris stromal pigmentation darkening that is characteristic of prostaglandin-induced iris color change [7]. Each Panel Member independently reviewed 228 randomized image pairs comprising baseline and 8-month photographs of each subject's left eye and of each subject's right eye via the Canfield Scientific Inc. ("Canfield") Clinical Services Website using identical Canfield-provided computers. The Panel Members additionally assessed 60 randomly selected control image pairs (15 baseline vs. baseline pairs, 15 8-month vs. 8-month pairs, and 30 baseline vs. 8-month pairs).

Panel members unanimously found that 228 of the 228 image pairs were of sufficient quality to make an assessment and were of equal stromal iris pigmentation. This indicates that the statistically significant increase in a* and b* values and overall color change (ΔE) of the iris over the length of the 8-month study is not clinically relevant [7].

IV. Read-Across Methodology for IC

Read-across is applied globally as an effective tool for utilizing available data to ensure the safety of ingredients under use conditions in cosmetic products as defined and regulated under the Federal Food, Drug and Cosmetic Act and the Modernization of Cosmetics Regulation Act of 2022 (MoCRA). Published read-across frameworks are available, such as OECD's Guidance on the Grouping of Chemicals[2] and EHCA's Read-Across Assessment Framework [8, 9]. Reliance on read-across is even more important considering that bans on the testing of cosmetic ingredients in animals preclude performance of new *in vivo* toxicology assays to fill data gaps.

SCCS identifies read-across as one method that can be used to support cosmetic ingredient safety assessments in a weight-of-evidence, integrative approach [2]. In read-across, analogues are identified on the basis of similar functional groups, similar mechanism of action, similar physicochemical properties, or similar chemical class, with the goal of filling data gaps for the target substance [2, 8]. OECD's stepwise approach for analogue identification is provided below in Figure 1.

[2] https://one.oecd.org/document/env/jm/mono(2014)4/en/pdf

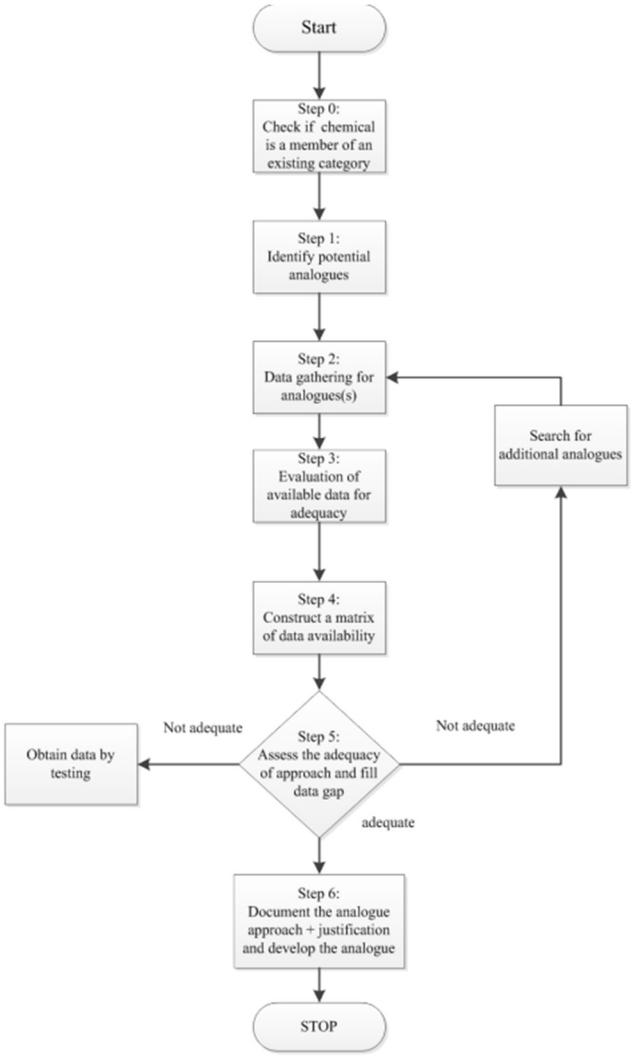


Figure 1: OECD's Stepwise Approach to Analogue Identification [8]

The principles of OECD's analogue identification approach have been leveraged for the safety assessment of cosmetic ingredients. Alexander-White et al. [10] have developed a 10-step, tiered framework for the use of read-across in the safety evaluation of cosmetic ingredients, depicted below in Figure 2.

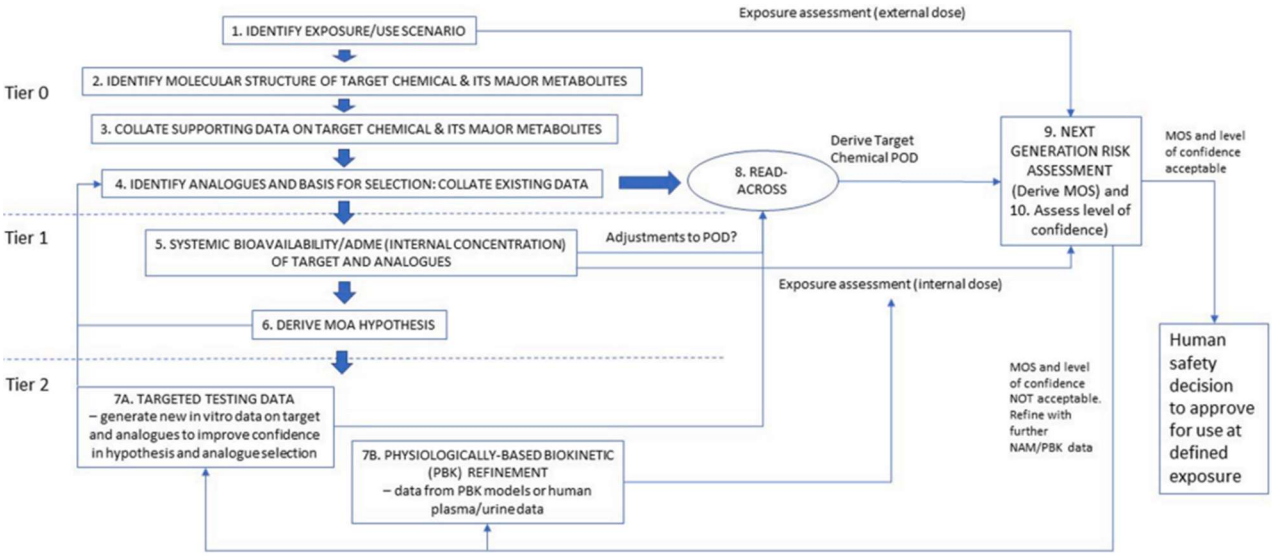


Figure 2: Framework for the Safety Evaluation of Cosmetics Ingredients [10]

Ouedraogo et al. [11] applied this framework to the safety evaluation of propylparaben for use in cosmetics, in the absence of substance-specific reproductive and developmental toxicity data. Factors considered in this framework include the target chemical's exposure level, structure, metabolites, physicochemical properties, and existing toxicity data. Analogues are selected on the basis of expert judgment and/or structural similarity scores, together with metabolic pathway similarity, physicochemical property similarity, and *in silico* profile similarity for the endpoint(s) to be read-across. If sufficient reliable data are available for the identified analogue(s), a point of departure can be identified. When multiple potential analogues are available, the most conservative effect level (i.e., NOAEL or LOAEL) is selected as the POD. If this approach results in an insufficient MOS, additional analysis of systemic bioavailability (i.e., absorption), metabolism, and bioactivity can be undertaken as part of a Tier 1 or Tier 2 assessment. For IC, surrogate candidates have sufficient health effects datasets, which supports the use of a Tier 0 read-across approach.

In their evaluation of prostaglandin analogues used in cosmetics, IC and ethyl tafluprostamide (DDDE) in particular, the SCCS identified data gaps for eye irritation, reproductive and developmental toxicity, and carcinogenicity. SCCS considered latanoprost, travoprost, bimatoprost, and cloprostenol as potential analogues to fill data gaps [12]. While not used as cosmetic ingredients, latanoprost, travoprost, and bimatoprost have a long history of human use as treatments for ocular hypertension and glaucoma. Cloprostenol is a luteolytic agent that is used in veterinary medicine. This assessment therefore considered these substances as potential analogues to fill the reproductive and developmental toxicity and carcinogenicity data gaps for the cosmetic use of IC.

To prevent bias in analogue selection and to increase the robustness of the read-across assessment, we searched for additional surrogate candidates by performing structural similarity searches in AMBIT [13] and OECD QSAR Toolbox [14]. As shown in Appendix A, AMBIT identified two substances at the 0.77 similarity level, cloprostenol

(CAS #54276-21-0/40665-92-7) and sodium cloprostenol (CAS #55028-72-3). OECD QSAR Toolbox identified these same surrogates at a similarity level of >70%.

We additionally used ChemMine's Similarity Workbench [15] and OECD QSAR Toolbox [14] for the purpose of calculating similarity metrics for all analogue candidates. For each pair-wise comparison, ChemMine calculated an atom pair Tanimoto similarity score (AP Tanimoto) and a maximum common substructure Tanimoto similarity score (MCS Tanimoto) [16, 17]. Similarly, we compared chemicals in a pairwise manner using OECD QSAR Toolbox to calculate an atom pair Tanimoto (Jaccard) similarity score [18].

The results of the analogue structural similarity analysis are shown in Table 9. A Tanimoto score of at least 0.7 is generally considered sufficient for read-across purposes [19].

Table 9: Structural Similarity Analysis Between IC and Analogue Candidates

Name	SMILES	ChemMine	OECD QSAR Toolbox
Isopropyl Cloprostenate	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	-	-
Cloprostenol	C1[C@@H]([C@@H]([C@H]([C@H]1O)/C=C/[C@H](COC2=CC(=CC=C2)Cl)O)C/C=C\CCCC(=O)O)O	AP Tanimoto: 0.725 MCS Tanimoto: 0.906 MCS Size: 29 MCS Min: 1.000 MCS Max: 0.906	Tanimoto (Jaccard): 0.743
Sodium cloprostenol	C1[C@@H]([C@@H]([C@H]([C@H]1O)/C=C/[C@H](COC2=CC(=CC=C2)Cl)O)C/C=C\CCCC(=O)[O-])O.[Na+]	AP Tanimoto: 0.725 MCS Tanimoto: 0.879 MCS Size: 29 MCS Min: 0.967 MCS Max: 0.906	Tanimoto (Jaccard): 0.771
Latanoprost	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	AP Tanimoto: 0.623 MCS Tanimoto: 0.465 MCS Size: 20 MCS Min: 0.645 MCS Max: 0.625	Tanimoto (Jaccard): 0.537
Travoprost	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	AP Tanimoto: 0.742 MCS Tanimoto: 0.861 MCS Size: 31 MCS Min: 0.969 MCS Max: 0.886	Tanimoto (Jaccard): 0.811
Bimatoprost	CCNC(=O)CCC\C=C/C[C@H]1[C@@H](O)C[C@@H](O)[C@@H]1\C=C\[C@@H](O)CCc2ccccc2	AP Tanimoto: 0.549 MCS Tanimoto: 0.476 MCS Size: 20 MCS Min: 0.667 MCS Max: 0.625	Tanimoto (Jaccard): 0.512

AP Tanimoto and Tanimoto (Jaccard)– similarity between corresponding atom pairs based on Tanimoto coefficient. MCS Tanimoto – similarity of the maximum common substructure (MCS) based on the Tanimoto coefficient. Structural similarity evaluation supported methods described in Dreyer et al. 2018 [20].

Based on Tanimoto scores from the two software programs, IC displays the greatest structural similarity with cloprostenol, sodium cloprostenol, and travoprost. Latanoprost and bimatoprost are not sufficiently similar, based solely on Tanimoto scores, to IC to serve as analogues. However, since structural similarity alone is not sufficient to support analogue selection [9], additional metrics were evaluated:

1. General structural features and physicochemical properties
2. Toxicokinetic properties
3. Mechanism of action
4. Potency

For developmental toxicity in particular, reliance on similarity metrics alone can lead to errors in the read-across predictions because currently-available models have not incorporated all potential mechanisms of developmental toxicity. Biosimilarity is as important a consideration as structural similarity. Ciallella et al. [21] combined developmental toxicity data, structural features, and the results of high-throughput screening assays to predict the developmental toxicity potential of new chemicals. Yan et al. [22] also recommend considering biosimilarity, metabolism/bioactivation, and mode of action, specifically, when performing read-across. As noted by these authors and others, even seemingly small differences in structure can have a disproportionally large effect on toxicity.

General structural features and physicochemical properties

The analogue candidates are all variations of the basic structure of endogenous prostaglandins, PGF2α, specifically. The prostaglandin backbone structure is shown below in Figure 3.

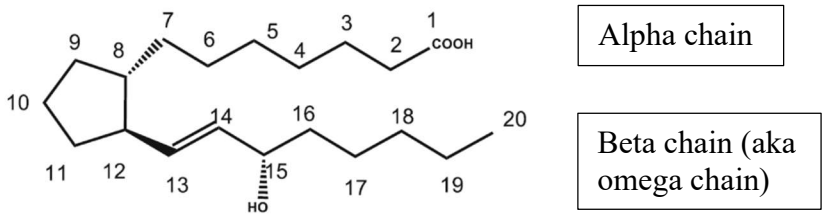
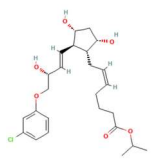
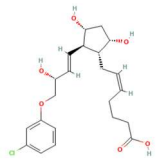
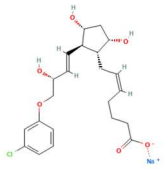
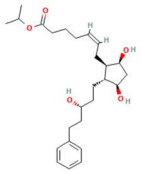
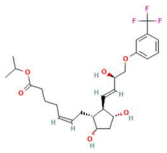
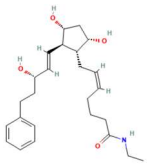


Figure 3: Basic Structure of Endogenous Prostaglandins [12]

Information on physicochemical properties and general structural features of IC and the analogue candidates is provided in Table 10.

Table 10: Physiochemical Properties and General Structural Features of IC and Analogue Candidates [12, 23, 24]

Property	Isopropyl Cloprostenate	Cloprostenol	Sodium Cloprostenol	Latanoprost	Travoprost	Bimatoprost
CAS	157283-66-4	40665-92-7 / 54276-21-0	55028-72-3 / 62561-03-9	130209-82-4	157283-68-6	155206-00-1
Molecular formula	$C_{25}H_{35}ClO_6$	$C_{22}H_{29}ClO_6$	$C_{22}H_{28}ClNaO_6$	$C_{26}H_{40}O_5$	$C_{26}H_{35}F_3O_6$	$C_{25}H_{37}NO_4$

Structure						
MW	467.0	424.9	446.9	432.6	500.6	415.6
Charges	0	0	0	0	0	0
Water solubility	0.047 mg/L @ 25°C (calculated)	1.60E-04 mol/L (calculated) *	2.24E-05 mol/L **	12.9 mg/L	7.59 mg/L	18.7 mg/L
Partition coefficient	5.15 (calculated)	2.50 (calculated)	0.715 (calculated)	3.98	4.6	3.2
Functional Groups						
RNH_2	0	0	0	0	0	0
R_2NH	0	0	0	0	0	0
R_3N	0	0	0	0	0	0
RCONHR’	0	0	0	0	0	1
$ROPO_3$	0	0	0	0	0	0
ROH	3	3	3	3	3	3
RCHO	0	0	0	0	0	0
RCOR	0	0	0	0	0	0
RCOOH	0	1	0	0	0	0
RCOOR	1	0	1	1	1	0
ROR	1	1	1	0	1	0
RCCH	0	0	0	0	0	0
RCN	0	0	0	0	0	0
Rings	2	2	2	2	2	2
Aromatic	1	1	1	1	1	1

* Equivalent to 68 mg/L (i.e., 1.60E-04 mol/L * 424.9 g/mol * 1,000 mg/g).
** Equivalent to 10 mg/L (i.e., 2.24E-05 mol/L * 446.9 g/mol * 1,000 mg/g)

These analogue candidates are similar to IC with respect to size, charge, and functional groups, with one notable exception: IC, latanoprost, and travoprost are esters (and cloprostenol/sodium cloprostenol can be formed via ester hydrolysis), while bimatoprost is an amide. Bimatoprost, therefore, is less similar to IC than the esters. In addition, IC is considerably less water soluble than any of the analogue candidates, and is the most lipophilic. This indicates that the ideal analogue for IC would also be lipophilic, with low water solubility. Of the evaluated surrogate candidates, travoprost is the most lipophilic and least water soluble. Furthermore, both IC and travoprost contain a meta-halogenated phenoxy group on the omega chain, while latanoprost and bimatoprost each have a non-halogenated phenyl ring.

Toxicokinetic properties

As a next step in the analogue identification process, we considered data on the toxicokinetic properties of IC and the analogue candidates. Available data are shown in Table 11.

Table 11: Toxicokinetic Properties of IC and Analogue Candidates					
	Property				
Substance	**Absorption**	**Distribution**	**Metabolism**	**Excretion**	**References**
Isopropyl cloprostenate	Displays low dermal absorption.	Data gap	Hydrolyzed to cloprostenol via esterases. OECD QSAR Toolbox predicted 11 hepatic metabolites, 7 skin metabolites, and 10 additional metabolites for IC, all in rats. Predicted skin metabolites include isopropyl alcohol, cloprostenol, and both hydrolyzed and non-hydrolyzed oxidation products (see Appendix B).	Data gap	[1, 12, 14]
Cloprostenol / Sodium cloprostenol	Data gap	In pigs and cows, the highest levels were measured in the kidneys and liver after a single i.m. injection.	Undergoes β-oxidation following i.m. injection. The main metabolite in most species (e.g., rat, pigs, cattle) is the tetranor acid. OECD QSAR Toolbox identified 11 hepatic metabolites, 3 skin metabolites (all of which were also identified for IC), and 5 additional metabolites, all in rats (see Appendix B).	Primarily urinary (60%) following subcutaneous injection. Terminal half-life of elimination is approx. 3 hours in cows and pigs after i.m. injection.	[12]
Latanoprost	C_{max} = 53 pg/mL and t_{max} = 5 min for the acid after a 2.3 µg ocular dose of the ester (humans).	V_d = 0.16 L/kg in humans; up to 90% plasma protein binding. No evidence of accumulation in plasma over one year.	Hydrolyzed by corneal esterases. Also completely hydrolyzed in plasma following i.v. injection. The acid undergoes rapid fatty acid β-oxidation in the liver to dinor and tetranor metabolites, which are likely glucuronidated.	Rapidly eliminated, primarily in the urine, after i.v. and topical administration. Plasma elimination half-life is approx. 16 minutes for both routes of exposure (humans).	[25]
Travoprost	Very low systemic exposure to the ester after repeated ocular	Approx. 80% plasma protein binding in human plasma. No evidence of	Rapidly hydrolyzed by corneal and plasma esterases. The acid is rapidly metabolized via β-oxidation, oxidation	Rapidly eliminated from plasma after ocular exposure in humans. In	[12, 26]

	administration. C_{max} = 25 pg/mL and t_{max} = 15 min for the acid after a single topical dose of the ester (2 µg) (all human data).	accumulation over 7 days. In pregnant and non-pregnant rats administered radiolabeled travoprost by s.c. injection, the highest levels were measured in the kidneys, liver, and lungs. Levels in fetal tissues, primarily liver and lung, are about 2-4% of maternal plasma levels.	of the 15-hydroxy group, and reduction of the 13,14 double bond to dinor and tetranor metabolites.	rats, excreted primarily in feces.	
Bimatoprost	Absorbed following ocular exposure. C_{max} = 0.07 ng/mL and t_{max} = 0.1 hr for bimatoprost following a single ocular dose in humans, with no evidence of accumulation over 14 days when given once per day.	Vd = 0.67 L/kg. The highest levels of bimatoprost were measured in the GI tract, liver, kidneys, and urinary bladder after a single i.v. dose in rats. Plasma protein binding in humans is about 88%.	Bimatoprost is not metabolized in human blood, and the acid metabolite was not detected in human blood after a single ocular dose of bimatoprost. The acid metabolite is detectable in human blood after a single i.v. injection. Undergoes deamidation and glucuronidation in human liver slices. Incubation of bimatoprost with human hepatic CYP3A4/5 yields hydroxylated metabolites.	Rapidly eliminated following i.v. injection, with an elimination half-life of 45 minutes. Primarily renal elimination.	[27, 28]

For the purpose of assessing systemic exposure to IC following its topical application, cloprostenol is not a suitable surrogate because of differential physicochemical properties [11]. Although information on the dermal absorption of the surrogate candidates is not available, data are available for IC; this endpoint therefore does not rely on read-across. Since the dermal absorption study indicates that low-level systemic exposure to intact (i.e., non-hydrolyzed) IC may occur, one of the ester compounds would likely be a better analogue for IC on the basis of tissue distribution. That said, cloprostenol and IC do display similar predicted metabolite profiles on a qualitative basis, however, and are predicted to undergo similar biotransformations - β-oxidation and oxidation of the 15-hydroxy group – as travoprost.

Mechanism of action

Prostaglandins play important roles in tissue homeostasis/normal physiology and are also activated during inflammation. PGF2α mediates female reproductive functions, cardiovascular function, and pain, and is upregulated during inflammation. Binding to its cognate receptor activates G-protein coupled intracellular signal transduction pathways, resulting in increased intracellular calcium [29].

The PGF2α analogues act as PGF2α receptor (also known as FP receptor) agonists. FP receptors are located on the corpus luteum as well as the ciliary body, ciliary muscle, and trabecular meshwork of the eye; they are also located in smooth muscle, female reproductive tissues (e.g., myometrium, endometrium), urinary bladder, and gall bladder [30]. A splice variant of the human FP receptor has been identified in the heart and placenta, but its functions have not been well characterized. The FP receptor has also been identified in the mouse uterus, corpus luteum, ovary, testis, kidney, liver, heart, stomach, lung, and skin [31]. In the female reproductive system, FP receptor activation causes luteolysis/regression of the corpus luteum, resulting in premature parturition, and mediates vasoconstriction and smooth muscle contraction in the uterus during menstruation [12, 32]. Specifically, FP-mediated increases in intracellular calcium promote myometrial contractions [33]. Together with the known role of PGF2α in female reproductive function, this suggests a role for FP receptor activation in the adverse reproductive effects that have been observed in some animal studies with PGF2α analogues [12].

Although data are limited, FP receptor activation may also play a role in carcinogenesis, as has been demonstrated in endometrial adenocarcinoma, via nuclear factor of activated T cells (NFAT)-mediated upregulation of IL-11, a cytokine involved in cellular growth and differentiation as well as tumor progression/invasiveness [34].

FP receptor activation → calcium mobilization + calcineurin activation → activation of NFAT transcription factor → increased IL-11 expression

The mechanism of action for FP-mediated IOP reduction has been characterized to a relatively greater degree, and can be summarized as follows [32]:

FP receptor activation → PLC activation → IP3 production → calcium mobilization + cAMP activation

In the eye, analogue-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 [35].

Available data indicate that FP activation is also responsible for side effects associated with drug uses of prostaglandin analogue eyedrops, namely iris and periorbital hyperpigmentation and periorbital fat loss. Iris pigment color changes observed following

direct ocular exposure to prostaglandin analogues for the purpose of glaucoma treatment have been attributed to analogue-mediated increases in melanin production in existing iris stromal melanocytes. Prostaglandin analogues exert a similar effect on melanocytes in eyelid skin, resulting in some patients in eyelid hyperpigmentation [36]. The mechanism of pigment change can be specifically summarized as follows [37]:

FP receptor activation → increased tyrosinase activity (not mediated via cAMP) → increased melanin production in melanosome organelles in iridial melanocytes

Periorbital volume loss that has been observed following drug uses of prostaglandin analogues is also likely to be a result of FP receptor activation [38, 39]:

FP receptor activation → MAPK activation → PPARγ inhibition → inhibition of adipocyte differentiation

Thus, for the safety evaluation of IC, information on FP receptor binding/activation can be used to inform analogue selection for the reproductive and developmental toxicity endpoint and also for carcinogenicity.

Structural determinants of FP receptor binding

Once activated through hydrolysis,[3] the analogues bind the PGF2α receptor with varying effectiveness (affinity). To inform surrogate selection, we searched for data on structural features that can impact binding to the FP receptor and FP receptor activation. Two types of data are available in the literature: receptor binding data (measures agonist-mediated displacement of endogenous ligand in cells that express FP receptor at high levels, such as the bovine corpus luteum) and receptor activation data (measures increase in IP production in cells expressing the cloned human FP receptor following incubation with test substance), as shown in Tables 12 and 13.

Table 12: Prostaglandin Analogue FP Receptor Binding [40, 41]

Molecule	Structural Change	K_i (nM) *
9-deoxy cloprostenol	Remove of one of the two hydroxy groups on the 5-membered ring	9.9
5,6-dihydrocloprostenol	Remove the 5,6 double bond on the alpha chain	28
Cloprostenol	Modify C17 with a halide-substituted phenoxy moiety	31
Travoprost acid [(+)-fluprostenol]	The non-ester form of travoprost	35
13,14-dihydro travoprost acid	Remove the 13,14 double bond on the omega chain.	60

[3] In contrast to the other analogue candidates, bimatoprost is pharmacologically active as the intact molecule [40].

Latanoprost acid	The non-ester form of latanoprost	98
Racemic fluprostenol	Mixture of + and - travoprost acid	98
PGF2α	Original structure (endogenous ligand)	130
5,6- and 13,14-dihydrocloprostenol	Remove both double bonds	220
5-Trans travoprost acid	Change conformation at the 5,6- double bond from cis to trans	260
1-hydroxy cloprostenol	Replace the C1 carboxyl group with an alcohol	2,000
Cloprostenol amide	Replace the C1 carboxyl group with an amide	2,600
Travoprost alcohol	Replace the C1 carboxyl group with an alcohol	10,000

*The Ki is the test substance concentration at which it occupies 50% of the receptor sites in the absence of a competing ligand.

Table 13: Determinants of FP Receptor Binding [42]

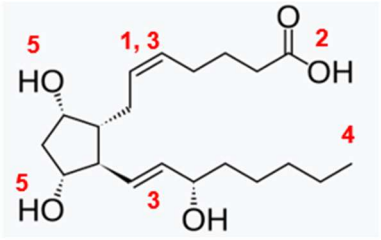
Molecule	Structural Change	IC_{50} (nM) *
Cloprostenol	Modify C17 with a halide-substituted phenoxy moiety	5
PGF2a	Original structure (endogenous ligand)	10
13,14-dihydro PGF2a	Remove PGF2a's 13,14- double bond	21
Fluprostenol	Modify C17 with a halide-substituted phenoxy moiety	34
11-deoxy PGF2a	Remove hydroxy group from C11 ring position	37
5-trans PGF2a	Change conformation at the 5,6-double bond from cis to trans	61
PGF1a	Remove trans-PGF2a's 5,6-double bond	153
PGF2a isopropyl ester	C1 ester	383
15-keto PGF2a	Oxidize the C15 hydroxy group to a carbonyl	667

*The IC_{50} is the concentration needed to inhibit binding of the endogenous ligand by 50%.

Consistent with the findings of Sharif et al. [30] and Hellberg et al. [40], Schuster et al. [43] demonstrated that the carboxylic acid functional group on the alpha chain is essential for FP receptor binding: modification of the carboxylic acid moiety to an amide reduced FP receptor binding affinity by more than 2 orders of magnitude. This suggests that bimatoprost would have lower affinity for the PGF2α receptor compared to IC, cloprosteol, latanoprost, and travoprost. While substitution of the hydroxy group at position 15 did not appreciably impact FP receptor binding affinity, introduction of a phenyl group on the omega chain enhanced FP receptor binding. Resul et al. [44] also demonstrated the increase in FP receptor binding affinity conferred by the introduction of the omega-phenyl group; these authors further showed that desaturation of the carbon-carbon bond at

positions 13-14 of the omega chain, creating a carbon-carbon double bond, resulted in increased FP binding activity.

Structural factors important for FP receptor binding and activation are summarized in Figure 4.



1. Conformation change from cis to trans on the alpha (top) chain reduces FP receptor binding.
2. Modification of the carboxylic acid to an alcohol, amide, or ester greatly reduces FP receptor binding.
3. Saturation of both double bonds reduces FP receptor binding.
4. Modification of C17 with a halide-substituted phenoxy moiety increases FP receptor binding relative to PGF2α.
5. The two ring hydroxy groups, the alpha chain carboxyl group, the alpha chain double bond, and the hydroxy group and benzene ring on the omega chain interact with specific residues on the FP receptor [45].

Figure 4: Structural Factors Impacting FP Receptor Binding

IC as well as the four analogue candidates each have two hydroxy groups on the 5-member ring and a phenyl group on the omega chain, although for IC, travoprost, and cloprostenol, the substituent is a halide-substituted phenoxy rather than simply phenyl. With the exception of latanoprost, the evaluated analogues also have a carbon-carbon double bond at C13-C14. Lastly, with the exception of bimatoprost, IC and the analogue candidates possess the alpha chain carboxyl group.

In terms of structural features important in FP receptor activation, cloprostenol and travoprost are most similar to IC.

The importance of these structural motifs is reflected in FP receptor activation data, shown in Table 14.

Table 14: Prostaglandin Analogue FP Receptor Activation [40, 46]

Analogue	FP Receptor EC_{50} (free acid) *	FP Receptor EC_{50} (prodrug/unhydrolyzed)
Cloprostenol	1.0 nM	Not tested
Latanoprost	54.6 nM	126 nM
Travoprost	3.2 nM	42.3 nM
Bimatoprost	5.8 nM	694 nM

*The EC_{50} is the test substance concentration needed to elicit half-maximal receptor function.

In these assays, a much higher concentration of bimatoprost prodrug is required to elicit half-maximal receptor activity (i.e., EC_{50}), compared to latanoprost and travoprost [46]. If hydrolyzed to its free amide, bimatoprost can effectively bind the PGF2α receptor; however, available data suggest that bimatoprost is not readily hydrolyzed in the human body [47, 48]. Additionally, bimatoprost acts on cannabinoid receptor CB1, while latanoprost and travoprost do not [49]. This may reflect the overall origin of prostamides from the endocannabin andanamide [50], which is different from the origin of endogenous prostanoids.

In summary, since IC and travoprost share structural features needed for FP receptor activation, it is likely that IC would exhibit an FP receptor activation potency similar to that of travoprost. This would be consistent with the observation that cloprostenol and travoprost free acid (i.e., the hydrolysis products) exhibit similar FP receptor activation potencies.

***In vivo* potency**

Prostaglandin F2α analogues, when instilled directly in the eye, have several FP receptor-mediated physiological effects, namely (i) conjunctival hyperemia, (ii) constriction of pupils, and (iii) intra-ocular pressure (IOP) lowering efficacy [51, 52]. The data are discussed in detail in the SCCS's 2022 opinion on prostaglandins and prostaglandin analogues [12] and are summarized below.

Substances that exhibit considerable differences in potency in these assays are not likely to be good surrogates, as this would suggest differences in FP receptor activation potency and/or differences in overall receptor selectivity that could result in differential effects on other FP-responsive endpoints.

IC and travoprost displayed similar potency in the cat pupil constriction assay, and were approximately 10-fold more potent in this assay compared to latanoprost. IC and travoprost were also more potent in terms of IOP-lowering ability in ocular hypertensive monkeys compared to latanoprost [51, 52]. Hyperemia data are less consistent, with IC showing the greatest potency in rabbits compared to travoprost and latanoprost, and IC and travoprost showing greater potency than latanoprost in guinea pigs [51, 52]. Bimatoprost was not tested in these assays.

These data indicate that travoprost is similar to IC and latanoprost dissimilar to IC in terms of FP-mediated *in vivo* effects.

Screening for non-FP effects

We performed predictive modeling to assess the potential for reproductive, developmental, and other endocrine-mediated effects arising from non-FP mechanisms [53] and to screen for secondary effects, as recommended by the SCCS [2].

Predictive modeling was performed using the Danish QSAR Database [54], VEGA version 1.2.4 [55], and OECD QSAR Toolbox, version 4.7 [14]. The use of more than one model/program increases the reliability of the predictions. In OECD QSAR Toolbox, modeling was performed in "account stereo" mode for consistency with the structure of IC, which contains cloprostenol in the "d" or "+" conformation. Modeling results served to predict specific effects relevant to IC and also supported surrogate identification.

Predictive modeling results for IC are shown in Tables 15, 16, and 17.

Table 15: Danish QSAR Database Predictions for IC*	
Model	**Prediction**
ER alpha binding (human)	Negative
ER alpha activation (human)	Negative
ER activation (CERAPP data)	Negative
ER beta activation (< 20 uM)	Negative
ER beta inhibition (< 10 uM)	Positive
ER beta inhibition (< 50 uM)	Positive
AR antagonism (human)	Negative
AR inhibition (CoMPARA data)	Negative
Aromatase inhibition (< 10 uM)	Positive
Aromatase inhibition (< 50 uM)	Positive
Retinoic acid receptor inhibition (< 10 uM)	Negative
Thyroperoxidase inhibition 1	Negative
Thyroperoxidase inhibition 2	Negative
DIO1 inhibition	Negative
DIO3 inhibition	Positive
TSHR inhibition	Negative
ER - estrogen receptor	
AR - androgen receptor	
DIO - iodothyronine deiodinase	
TSHR - thyroid stimulating hormone receptor	
*Results shown only for models for which IC was in domain.	

Table 16: VEGA Predictions for IC		
VEGA Model	**Result**	**Caveats**
CAESAR Developmental toxicity	Toxicant, reliable (ADI = 0.873)	Training set includes only moderately similar chemicals (0.75-0.77)
DART Library	Non-toxicant, not reliable (ADI = 0)	-
ER-mediated effects	Non-active, reliable (ADI = 0.903)	-
ER alpha relative binding affinity	Non-active, reliable (ADI = 0.894)	Training set includes only moderately similar chemicals (0.79-0.80)
AR-mediated effect	Active, not reliable (ADI = 0)	-
Thyroid receptor alpha effect	Non-active, reliable (ADI = 0.953)	-
Thyroid receptor beta effect	Non-active, reliable (ADI = 0.953)	-
Glucocorticoid receptor	Non-active, moderately reliable (ADI = 0.768)	Compound may be outside the model's applicability domain.
Thyroperoxidase inhibitory activity	Highly selective inhibitor, not reliable (ADI = 0)	-
Steroidogenesis	Active, reliable (ADI = 0.906)	Training set includes only moderately similar chemicals (0.78-0.84)
Endocrine disruptor activity screening	No prediction can be made	-

Aromatase activity (IRFMN)	Non-active, reliable (ADI = 0.956)	-
Aromatase activity (TOX21)	Agonist, not reliable (ADI = 0)	Compound is outside the model's applicability domain.

Table 17: OECD QSAR Toolbox Predictions for IC

Profilers	Results
General Mechanistic	
Estrogen Receptor Binding	Strong binder, OH group
Hydrolysis half-life (pH 6.5-7.4)	Very slow
Hydrolysis half-life (Ka, pH 7) (Hydrowin)	No value
Estrogen Receptor Binding, with Skin metabolism simulator	1 x Non binder, non-cyclic structure, 6 x Strong binder, OH group
Hydrolysis half-life (pH 6.5-7.4), with Skin metabolism simulator	3 x Very slow, 4 x No value
Hydrolysis half-life (Ka, pH 7) (Hydrowin), with Skin metabolism simulator	7 x No value
Estrogen Receptor Binding, with Hydrolysis simulator (neutral)	1 x Non binder, non-cyclic structure, 1 x Strong binder, OH group
Hydrolysis half-life (pH 6.5-7.4), with Hydrolysis simulator (neutral)	2 x No value
Hydrolysis half-life (Ka, pH 7) (Hydrowin), with Hydrolysis simulator (neutral)	2 x No value
Endpoint Specific	
Retinoic Acid Receptor Binding	Not possible to classify according to these rules
DART scheme	Not known precedent reproductive and developmental toxic potential
Retinoic Acid Receptor Binding, with Skin metabolism simulator	7 x Not possible to classify according to these rules
DART scheme, with Skin metabolism simulator	7 x Not known precedent reproductive and developmental toxic potential
Retinoic Acid Receptor Binding, with Hydrolysis simulator (neutral)	2 x Not possible to classify according to these rules
DART scheme, with Hydrolysis simulator (neutral)	2 x Not known precedent reproductive and developmental toxic potential

Collectively, the results of predictive modeling indicate that IC could exert non-FP effects through ER beta inhibition and aromatase inhibition and could also impact steroidogenesis. Developmental toxicity predictions were equivocal, with one reliable model identifying a hazard and a second model of unspecified reliability not identifying a hazard. An ideal read-across analogue would share these patterns.

To confirm the selection of travoprost as the surrogate for IC for the reproductive and developmental toxicity endpoints, we repeated the predictive modeling performed on IC. Results are shown in Tables 18, 19, and 20. Predictions that are the same for travoprost as for IC are indicated in **green** font.

Table 18: Danish QSAR Database Predictions for Travoprost*	
Model	**Prediction**
ER beta activation (< 20 uM)	**Negative**
ER beta inhibition (< 10 uM)	**Positive**
ER beta inhibition (< 50 uM)	**Positive**
ER activation (CERAPP data)	**Negative**
AR antagonism (human)	**Negative**
Aromatase inhibition (< 10 uM)	**Positive**
Aromatase inhibition (< 50 uM)	**Positive**
Retinoic acid receptor inhibition (< 10 uM)	**Negative**
Retinoic acid receptor inhibition (any concentration)	Positive
NIS higher sensitivity	Positive
NIS higher specificity	Positive
Thyroperoxidase inhibition 1	**Negative**
Thyroperoxidase inhibition 2	**Negative**
DIO1 inhibition	**Negative**
DIO 2 inhibition	Positive
DIO3 inhibition	**Positive**
ER - estrogen receptor	
AR - androgen receptor	
NIS - sodium iodide symporter	
DIO - iodothyronine deiodinase	
*Results shown only for models for which travoprost was in domain.	

Table 19: VEGA Predictions for Travoprost		
VEGA Model	**Result**	**Caveats**
CAESAR Developmental toxicity	Non-toxicant, not reliable (ADI = 0)	-
DART Library	Non-toxicant, not reliable (ADI = 0)	-
ER-mediated effects	**Non-active, reliable (ADI = 0.886)**	Training set includes only moderately similar chemicals (0.75-0.81)
ER alpha relative binding affinity	Active, not reliable (ADI = 0)	-
AR-mediated effect	Active, not reliable (ADI = 0)	-
Thyroid receptor alpha effect	**Non-active, reliable (ADI = 0.946)**	-
Thyroid receptor beta effect	**Non-active, reliable (ADI = 0.946)**	-
Glucocorticoid receptor	**Non-active, moderately reliable (ADI = 0.757)**	Compound may be outside the model's applicability domain.
Thyroperoxidase inhibitory activity	Non-active, moderately reliable (ADI = 0.802)	Training set includes only moderately similar chemicals (0.75-0.81)
Steroidogenesis	**Active, reliable (ADI = 0.89)**	Training set includes only moderately similar chemicals (0.75-0.81)
Endocrine disruptor activity screening	No prediction can be made	-
Aromatase activity (IRFMN)	**Non-active, reliable (ADI = 0.935)**	-
Aromatase activity (TOX21)	Agonist, not reliable (ADI = 0)	Compound is outside the model's applicability domain.

Table 20: OECD Toolbox Predictions for Travoprost	
Profilers	**Results**
General Mechanistic	
Estrogen Receptor Binding	Non binder, MW >500
Hydrolysis half-life (pH 6.5-7.4)	**Very slow**
Hydrolysis half-life (Ka, pH 7) (Hydrowin)	No value
Estrogen Receptor Binding, with Skin metabolism simulator	**1 x Non binder, non cyclic structure, 6 x Strong binder, OH group**
Hydrolysis half-life (pH 6.5-7.4), with Skin metabolism simulator	**3 x Very slow**
Hydrolysis half-life (Ka, pH 7) (Hydrowin), with Skin metabolism simulator	7 x No value
Estrogen Receptor Binding, with Hydrolysis simulator (neutral)	**1 x Non binder, non cyclic structure, 1 x Strong binder, OH group**
Hydrolysis half-life (pH 6.5-7.4), with Hydrolysis simulator (neutral)	2 x No value
Hydrolysis half-life (Ka, pH 7) (Hydrowin), with Hydrolysis simulator (neutral)	2 x No value
Endpoint Specific	
Retinoic Acid Receptor Binding	Not possible to classify according to these rules
DART scheme	**Not known precedent reproductive and developmental toxic potential**
Retinoic Acid Receptor Binding, with Skin metabolism simulator	7 x Not possible to classify according to these rules
DART scheme, with Skin metabolism simulator	**7 x Not known precedent reproductive and developmental toxic potential**
Retinoic Acid Receptor Binding, with Hydrolysis simulator (neutral)	2 x Not possible to classify according to these rules
DART scheme, with Hydrolysis simulator (neutral)	**2 x Not known precedent reproductive and developmental toxic potential**

Similar to IC, travoprost could exert non-FP reproductive or developmental effects through ER beta inhibition and aromatase inhibition and/or by impacting steroidogenesis. Predictions for other endpoints were either inconsistent or not reliable. This is probably because fluoridated compounds are not well represented in model training sets. Since hormone modulation is also a mechanism of carcinogenesis [56], these predictions are also relevant for analogue selection for the carcinogenicity endpoint.

Collectively, these predictions identified similar non-FP effects for IC and travoprost, which increases confidence in the use of travoprost as a surrogate for reproductive and developmental toxicity as well as for carcinogenicity.

To augment the analogue selection process for the carcinogenicity endpoint, predictive modeling was performed using VEGA and OECD QSAR Toolbox [14, 55]. Toolbox has two modules for prediction of carcinogenicity potential, a decision tree/rule-based module that identifies structural alerts and a data gap filling module that uses Danish QSAR Database statistical models based on *in vivo* data. OECD QSAR Toolbox modeling outputs are included in Appendix C.

Predictive modeling performed for IC yielded two reliable results (ADI > 0.7) in VEGA. The IRFMN-ISSCAN-CGX model predicted IC as positive for carcinogenicity with an

overall ADI of 0.882; however, the compounds in this model's training set are all only moderately similar to IC. A second model, IRFMN-Antares, predicted IC as a possible non-carcinogen with an ADI of 0.729, but IC could be out of the model's applicability domain and the compounds in this model's training set are all only moderately similar to IC. Collectively, an overall prediction of non-carcinogenicity is made, with low confidence, for the VEGA results, as only two models returned reliable results and their respective training set compounds were only moderately similar to IC. In addition, IC may have been out of domain for the model that predicted possible non-carcinogenicity.

OECD QSAR Toolbox identified the halogenated benzene structural alert for non-genotoxic carcinogenicity for IC. However, this structural alert has the lowest predictive value for *in vivo* carcinogenicity among all the structural alerts assessed by OECD QSAR Toolbox, 0.35 [57]. It is therefore not considered reliable. The OECD QSAR Toolbox data gap filling module did not return any results for IC for carcinogenicity. Collectively, the predictive modeling results for IC suggest a lack of carcinogenicity potential, although confidence in this conclusion is low.

Due to the uncertainties in the modeling results for IC, predictive modeling was performed for cloprostenol. It is unlikely that the presence of an isopropyl group would result in differential carcinogenic potential for IC compared to cloprostenol.

As for IC, VEGA's IRFMN-ISSCAN-CGX model predicted cloprostenol as positive for carcinogenicity with an overall ADI of 0.882, but the compounds in this model's training set are all only moderately similar to cloprostenol. None of the other VEGA models gave sufficiently reliable results for cloprostenol. However, OECD QSAR Toolbox's data gap filling module predicted that cloprostenol would be negative in 5/6 mouse carcinogenicity models (it was out of domain in the 6^{th} model) and negative in the rat and overall rodent carcinogenicity models.

OECD QSAR Toolbox also identified the halogenated benzene structural alert for cloprostenol. As noted above, this structural alert is not considered reliable because of its low positive predictive value. Collectively, an overall prediction of non-carcinogenicity is made for cloprostenol, with low-moderate confidence, primarily based upon the results of the statistical models in the OECD QSAR Toolbox data gap filling module.

To confirm the selection of travoprost as the surrogate for IC for the carcinogenicity endpoint, we repeated the predictive modeling performed on IC and cloprostenol.

Modeling performed using VEGA identified three reliable results (ADI > 0.7) for travoprost. The IRFMN-ISSCAN-CGX model predicted travoprost as positive for carcinogenicity with an overall ADI of 0.875. However, the compounds in this model's training set are all only moderately similar to travoprost. A second reliable model, IRFMN carcinogenicity oral classification, predicted that travoprost would not be carcinogenic (ADI = 0.86). As for the IRFMN-ISSCAN-CGX model, the compounds in this model's training set are all only moderately similar to travoprost. A third model, IRFMN carcinogenicity inhalation classification, also predicted that travoprost would not be

carcinogenic (ADI = 0.86), but noted that the compounds in the training set are all only moderately similar to travoprost. Collectively, an overall prediction of non-carcinogenicity is made, with moderate confidence due to less than ideal similarity between the molecules in the training sets and travoprost.

OECD QSAR Toolbox's data gap filling module predicted that travoprost would be negative for mouse, rat, and overall rodent carcinogenicity. No carcinogenicity structural alerts were identified for travoprost in Toolbox. The Toolbox predictions add weight to the conclusion that travoprost is not carcinogenic, as confirmed by the *in vivo* data discussed above.

Although not considered a strong analogue for IC, particularly on the basis of effects that may be mediated through the FP receptor, this assessment also considered latanoprost. Latanoprost may be a better indicator of potential non-FP carcinogenic effects than travoprost.

As for the other modeled compounds, VEGA's IRFMN-ISSCAN-CGX model predicted latanoprost as positive for carcinogenicity with an overall ADI of 0.893; the compounds in this model's training set are all only moderately similar to latanoprost. The IRFMN-Antares model yielded a possible non-carcinogen prediction with borderline reliability (ADI = 0.698), as predictions for similar compounds were not accurate, and latanoprost may be out of the model's applicability domain. The IRFMN oral classification model and IRFMN inhalation classification model yielded reliable predictions of non-carcinogenicity (ADI = 0.891 for both), although latanoprost is only moderately similar to molecules in the training sets. Collectively, an overall prediction of non-carcinogenicity is made, with moderate confidence due to less than ideal similarity between the molecules in the training sets and latanoprost.

OECD QSAR Toolbox's data gap filling module predicted that latanoprost would be negative for mouse, rat, and overall rodent carcinogenicity. No carcinogenicity structural alerts were identified for latanoprost in Toolbox. Thus, the overall prediction is that latanoprost is not carcinogenic, as confirmed by available *in vivo* data:

Latanoprost was not carcinogenic in GLP-compliant studies in Crl:CD(SD)BR rats (N=50/sex/group) and Crl:CD(SD)BR mice (N=51/sex/group). Animals were exposed to latanoprost in saline by oral gavage at 0, 2, 20, and 200 µg/kg/day for approximately 100 weeks (rats) or for approximately 80 weeks (mice). The highest dose was selected based on solubility in saline. There were no biologically significant differences in the incidences of neoplastic lesions in the treatment groups compared to the control group in either species. In addition, there were no biologically significant differences in weight gain, food consumption, terminal hematology, survival rate, and clinical signs in either species. The NOAEL was 200 µg/kg/day; however, the U.S. FDA reviewer noted that the maximum tolerated dose was not attained in either species [25].

The concordances between predictive modeling and *in vivo* data for travoprost and latanoprost suggest that predictive modeling for these compounds is accurate irrespective

of FP activation potential. That said, considering all of the evaluated similarity metrics, travoprost is the most suitable analogue for IC for the carcinogenicity endpoint.

Read-across summary

A summary of the read-across assessment is provided below in Table 21.

Table 21: Read-Across Summary Matrix

Metric	Analogue Candidate			
	Cloprostenol	**Latanoprost**	**Travoprost**	**Bimatoprost**
Structural similarity (Tanimoto score and visual structural comparison)	Highest	Moderate	Highest	Lowest
Physicochemical property similarity (MW, water solubility, and partition coefficient)	Lowest	Moderate	Highest	Moderate
Pharmacokinetic property similarity	Lowest	Moderate	Highest	Lowest
MOA similarity	Highest	Moderate	Highest	Lowest
Potency similarity (hyperemia, IOP reduction)	Not evaluated	Moderate	Highest	Not evaluated
In silico similarity – reproductive, developmental, and other endocrine effects	Not evaluated	Not evaluated	High	Not evaluated
In silico similarity – carcinogenicity	Moderate	Moderate	Moderate	Not evaluated

A structural comparison of IC to the analogue candidates indicates that cloprostenol/cloprostenol sodium and travoprost display the greatest structural similarity to IC. This report's assessment of structural and mechanistic properties of the surrogate candidates indicates that bimatoprost is different from latanoprost and travoprost in both aspects. Bimatoprost exerts different mechanism(s) of action than the other candidates and therefore is not a strong a surrogate for IC. For this reason, bimatoprost was not considered in the *in silico* evaluations.

The use of cloprostenol as a surrogate is supported by one toxicity study that evaluated both cloprostenol and IPC at comparable parenteral doses: this study identified similar effects of IC and cloprostenol on a sensitive target tissue, the testis [58]. The similarity of effects reported for cloprostenol and IC in the Indrei et al. (2001) study [58] suggests that either IC itself is an active species or that IC is rapidly and extensively hydrolyzed *in vivo* to cloprostenol. The latter is more plausible, as the ester forms of other prostaglandin analogues are extensively and rapidly hydrolyzed in the eye and in plasma to their

respective active acid forms. Although the results of the dermal absorption study with IC suggested little dermal metabolism to cloprostenol, metabolism in the plasma is likely. The results of the *in silico* analyses offer moderate additional support for the choice of cloprostenol as the primary analogue for IC.

The use of travoprost as a surrogate is supported by multiple factors: high structural similarity as assessed by multiple metrics, similar physicochemical properties (relative to the other candidates), similar *in vivo* potency for FP-mediated effects, and moderately similar *in silico* profiles. In addition, the results of the dermal absorption study with IC indicate that systemic exposure to low levels of the intact ester may occur, supporting the selection of another ester (i.e., travoprost) as a surrogate.

Point of departure identification

Multiple *in vivo* studies evaluating relevant reproductive and developmental endpoints are available for both cloprostenol and travoprost. As a U.S. FDA-approved drug, the full battery of standard reproductive and developmental toxicity tests was performed for travoprost, as were carcinogenicity studies in two species. Since these tests were sufficient to support travoprost's use as a drug in the U.S., they are also sufficient for this purpose, as tests considered acceptable by the U.S. FDA must be performed according to GLPs and must be of acceptable methodology and reporting. For cloprostenol, most data were obtained from a European Medicines Agency (EMEA) summary report. These data are also likely to be of sufficient quality, although the studies were not described in detail by SCCS. Since the data were described in greater detail for travoprost than for cloprostenol, this assessment has slightly greater confidence in the travoprost data.

Nonetheless, as a conservative approach, we identified a point of departure for MOS calculations by comparing available experimental NOAELs and LOAELs for both identified surrogates, as shown below in Table 22. Each study cited in Table 22 is briefly summarized in Appendix D. The point of departure (POD) is typically the highest NOAEL that is lower than the lowest LOAEL. The selected POD is shown in the shaded gray cell in Table 22. We selected the lowest LOAEL (reduced litter size from a 3-generation study with travoprost) from all available read-across reproductive and developmental toxicity studies as the basis for the MOS calculation. Since travoprost was not carcinogenic at a subcutaneous dose of 100 µg/kg/day [22], an exposure level considerably higher than doses causing reproductive and/or developmental effects, carcinogenicity is a less sensitive endpoint and is therefore not an appropriate basis for the POD.

Table 22: Dose-Response Evaluation of IC Analogues

Treatment	Study Design	Route of Exposure	NOAEL	LOAEL	Critical Effect	References
Travoprost	Developmental toxicity study in rats	s.c.	0.1 µg/kg/day	N/A	No evidence of adverse developmental effects.	[5]
Travoprost	3-Generation rat study	s.c.	N/A	**0.12 µg/kg/day**	Reduced litter size	[5]

Travoprost	Developmental toxicity study in mice	s.c.	0.3 µg/kg/day	1 µg/kg/day	Increased resorptions	[5]
Travoprost	Reproductive toxicity study in rats	s.c.	3 µg/kg/day	10 µg/kg/day	Increased resorptions	[5]
Travoprost	Developmental toxicity study in rats	i.v.	3 µg/kg/day	10 µg/kg/day	Increased resorptions	[5]
Travoprost	6-Month rat study	s.c.	10 µg/kg/day	100 µg/kg/day	Altered bone histopathology	[5]
Travoprost	28-Day toxicity studies in mice and rats	i.v.	1,000 µg/kg/day	N/A	No treatment-related effects on reproductive tissues.	[5]
Cloprostenol	1-Month rat study	s.c.	N/A	12.5 µg/kg/day	Ovarian vacuolization	[12]
Cloprostenol	3-Generation rat study	oral	15 µg/kg/day	20 µg/kg/day	Reduced neonatal viability	[12]
Cloprostenol	3-month rat study	oral	50 µg/kg/day	150 µg/kg/day	Ovarian vacuolization	[12]
Cloprostenol	3-Month study in marmosets	oral	50 µg/kg/day	150 µg/kg/day	Myocardial changes; increased testes weights	[12]
Cloprostenol or IC	28-Day study in rats and mice	i.p.	N/A	100 µg/kg/day	Degeneration of seminiferous tubules.	[58]
Cloprostenol	Developmental toxicity study in rats	oral	100 µg/kg/day	N/A	No evidence of adverse developmental effects.	[12]
Cloprostenol	Developmental toxicity study in rabbits	s.c.	0.25 µg/kg/day	N/A	No evidence of adverse developmental effects.	[12]

For conservatism, we identified the subcutaneous LOAEL of 0.12 µg/kg/day (0.00012 mg/kg/day) from the 3-generation rat study with travoprost as the point of departure for margin of safety calculations for IC [5]. This value is >100-fold lower than LOAELs for adverse effects on reproductive tissues following parenteral cloprostenol exposure, and is therefore consistent with OECD's recommendation to select the most conservative value from among the closest analogues [8]. Even though travoprost is not used as a cosmetic ingredient, use of cloprostenol as the sole surrogate for IC would result in a less conservative risk assessment because the identified N(L)OAELs for cloprostenol are higher than those for travoprost for the critical reproductive endpoints. Furthermore, expected differences in absorption and, potentially, distribution between IC and cloprostenol reduce confidence in the use of cloprostenol as the sole surrogate.

Based on high structural similarity, similar *in vivo* potency, available mechanism of action and receptor binding information for prostaglandin analogues, and similarity of *in silico* predictions for the evaluated endpoints, this report identifies travoprost as the most suitable surrogate for evaluation of IC's potential health effects, including carcinogenicity, reproductive toxicity and developmental toxicity.

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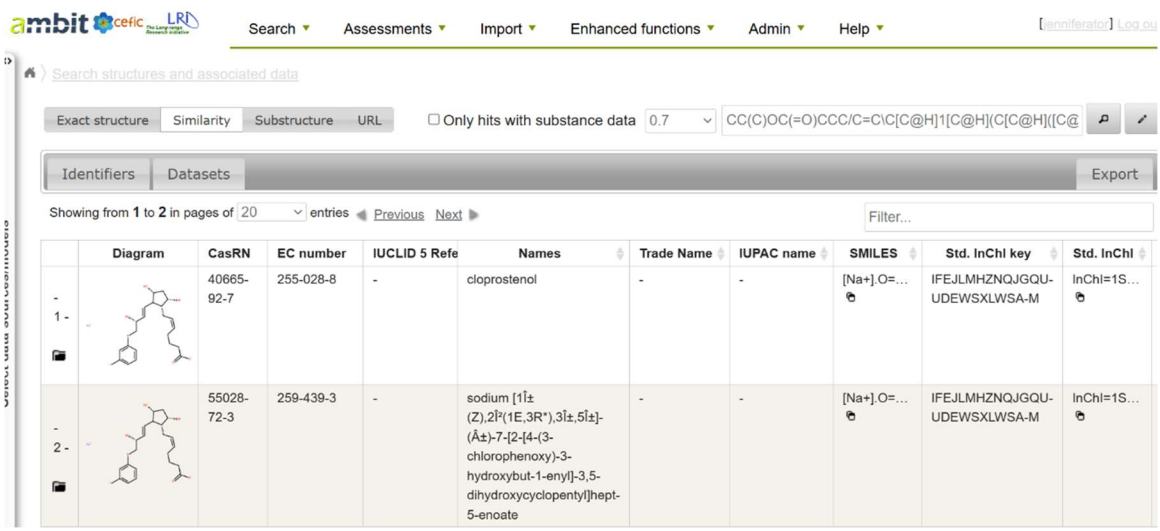
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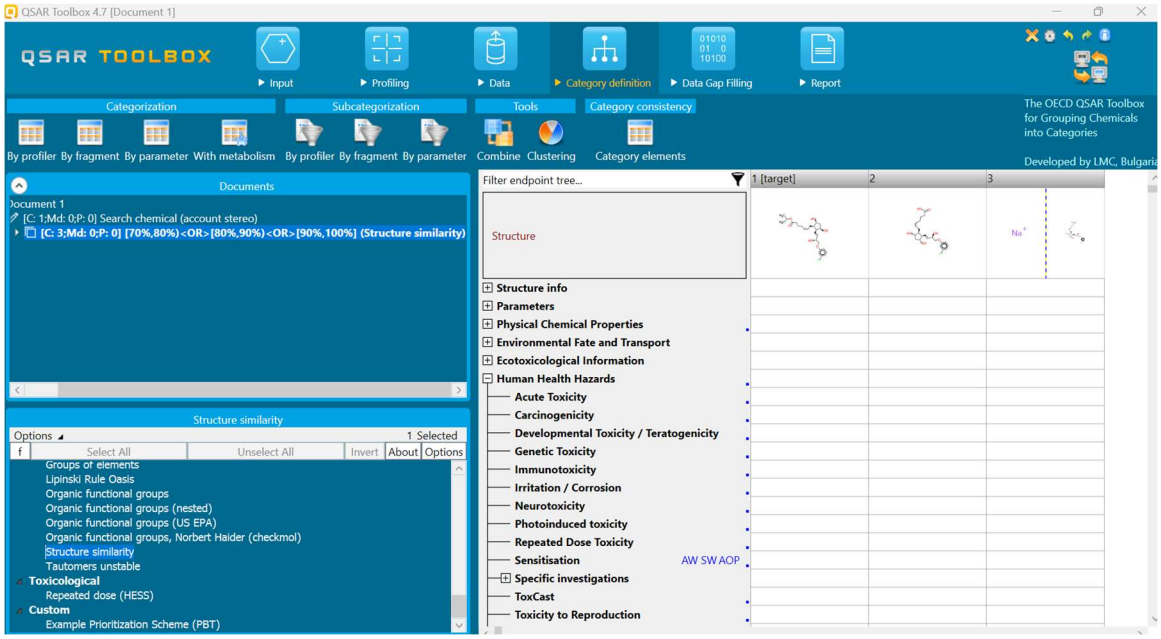
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APPENDIX A: STRUCTURAL SIMILARITY SEARCH RESULTS

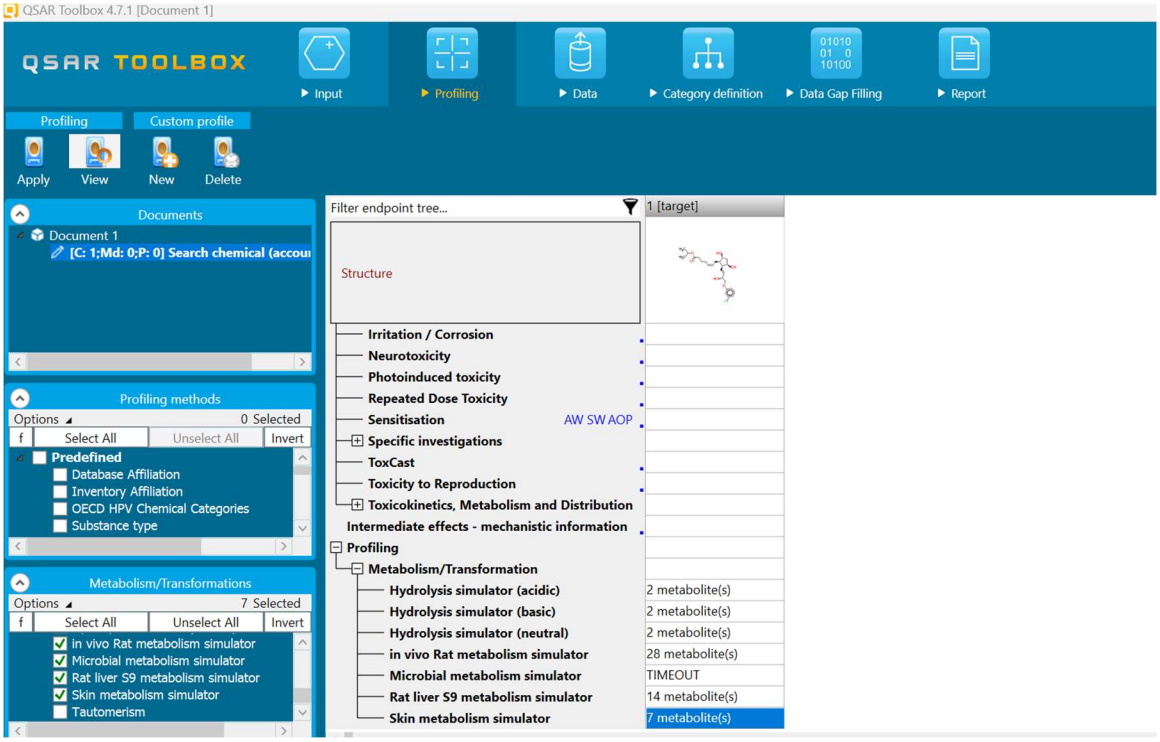




APPENDIX B: OECD QSAR TOOLBOX METABOLISM PROFILING

The assessment leveraged the OECD QSAR Toolbox metabolism profiling module to inform the pharmacokinetic profiles of IC and cloprostenol [56]. This module simulates non-enzymatic reactions as well as both Phase I and Phase II enzymatic reactions.[4] For each substance, the overall simulation results are provided first, and are followed by the individual chemical structures for the predicted skin metabolites (skin metabolism simulator), liver metabolites (rat liver s9 metabolism simulator), and other metabolites (i.e., only those *in vivo* rat metabolism simulator structures that are not among the liver or skin results).

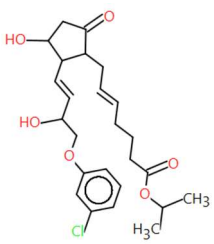
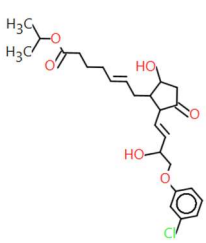
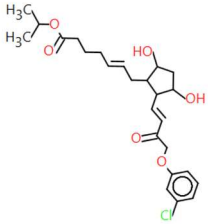
Overall results for IC



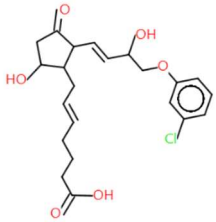
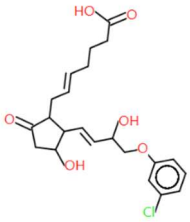
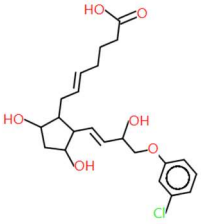
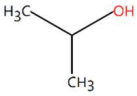
[4] https://qsartoolbox.org/resources/metabolisms/

IC: Skin metabolites

Oxidation Products

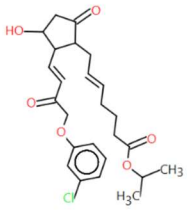
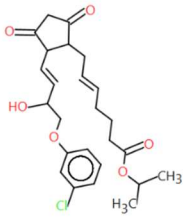
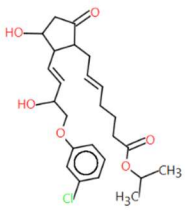
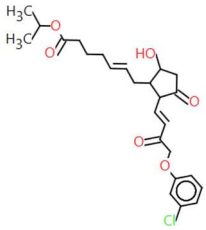
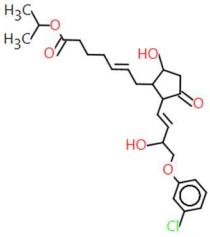
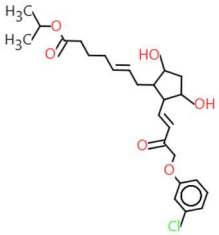




Hydrolysis Products (with or without additional oxidation)

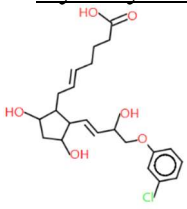
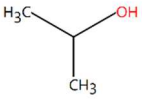
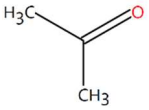





IC: Rat liver S9 metabolites

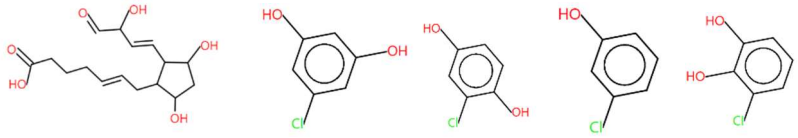
Oxidation Products

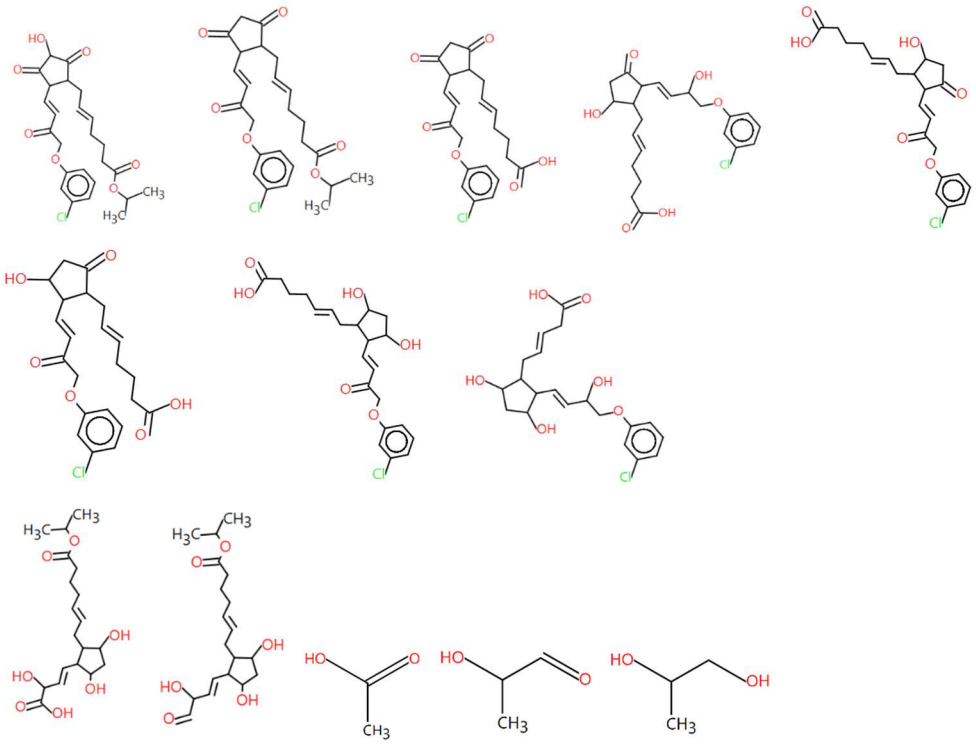
Hydrolysis Products (with or without additional oxidation)

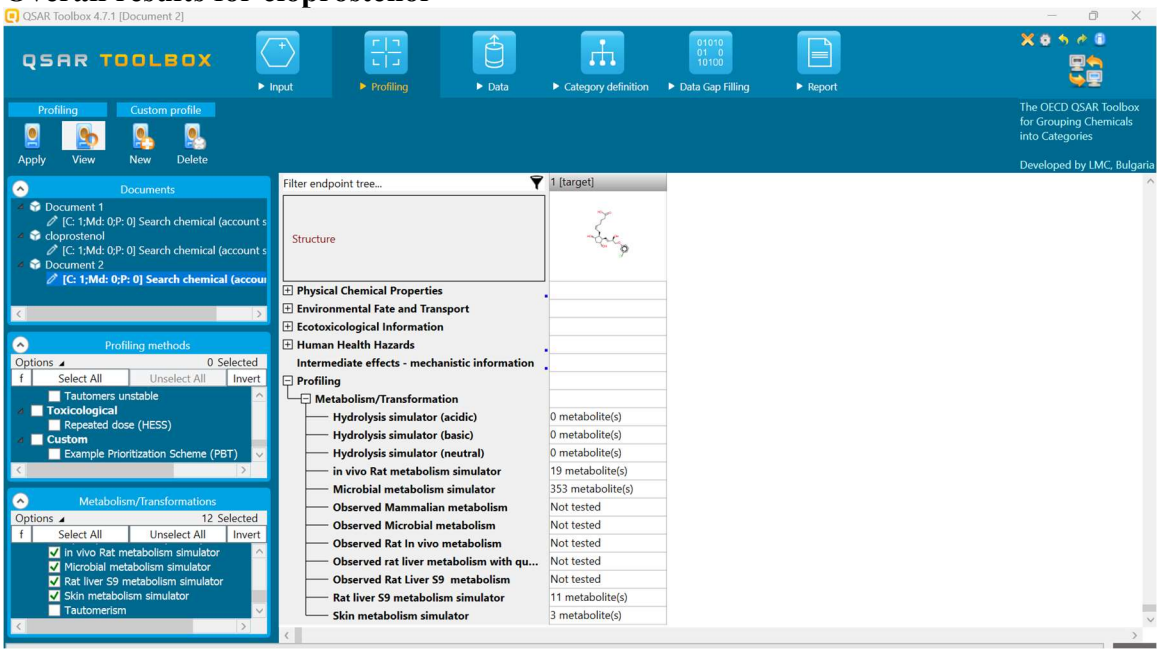
Ether Cleavage Products



IC: Additional *in vivo* rat metabolites

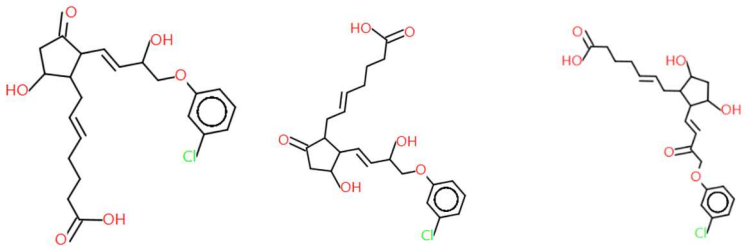


Overall results for cloprostenol



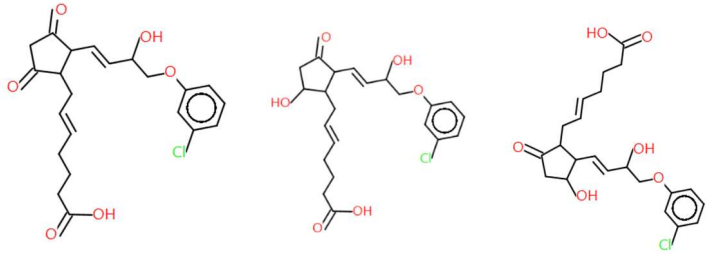
Cloprostenol: Skin metabolites

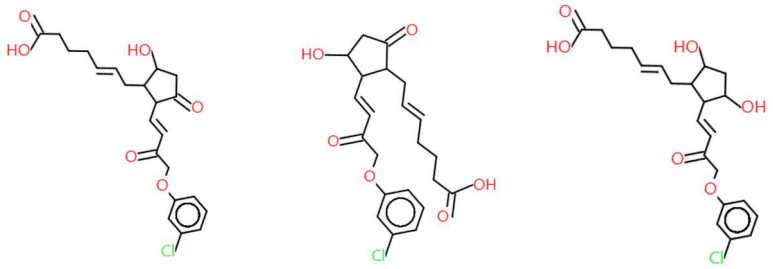
Oxidation Products



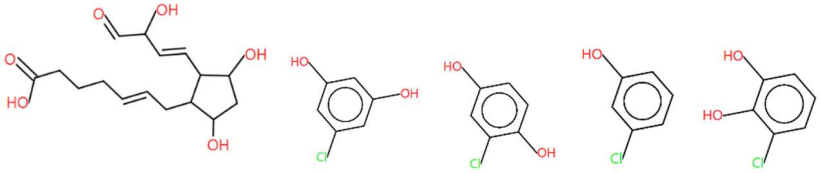
Cloprostenol: Rat liver S9 metabolites

Oxidation Products

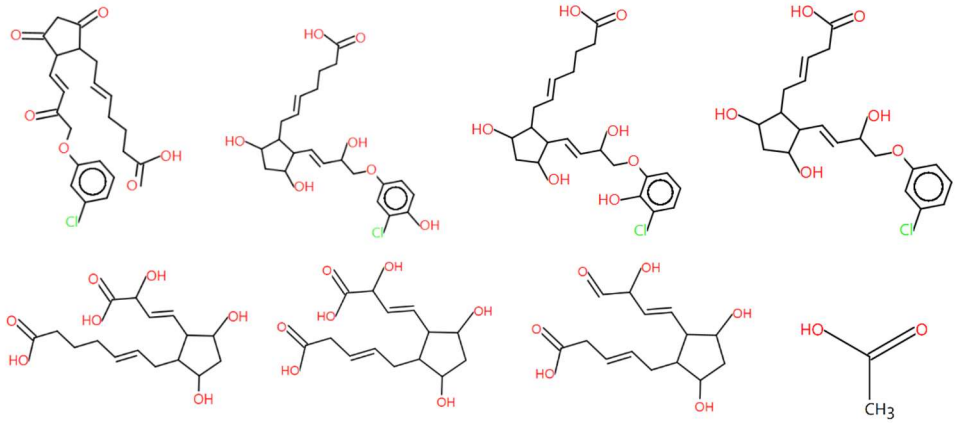




Ether Cleavage Products



Cloprostenol: Additional *in vivo* rat metabolites



Summary

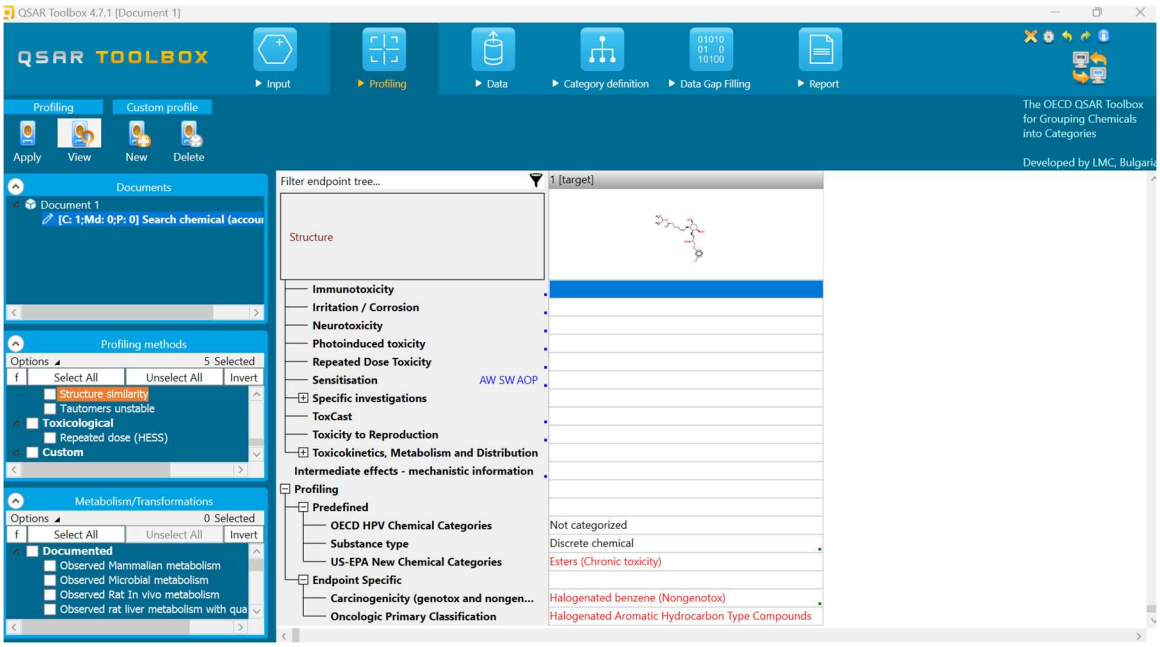
Both IC and cloprostenol are predicted to undergo oxidation of one or both ring hydroxy groups in the skin; the 15-hydroxy group on the omega chain is also predicted to undergo oxidation. IC is predicted to hydrolyze in the skin, with the acid hydrolysis product's hydroxy groups also oxidized.

In the liver, 14 metabolites are predicted for IC, 5 of which are also predicted for cloprostenol. Both substances are predicted to undergo direct oxidation and to undergo cleavage of the ether moiety, with the chlorinated cleavage products subsequently hydroxylated.

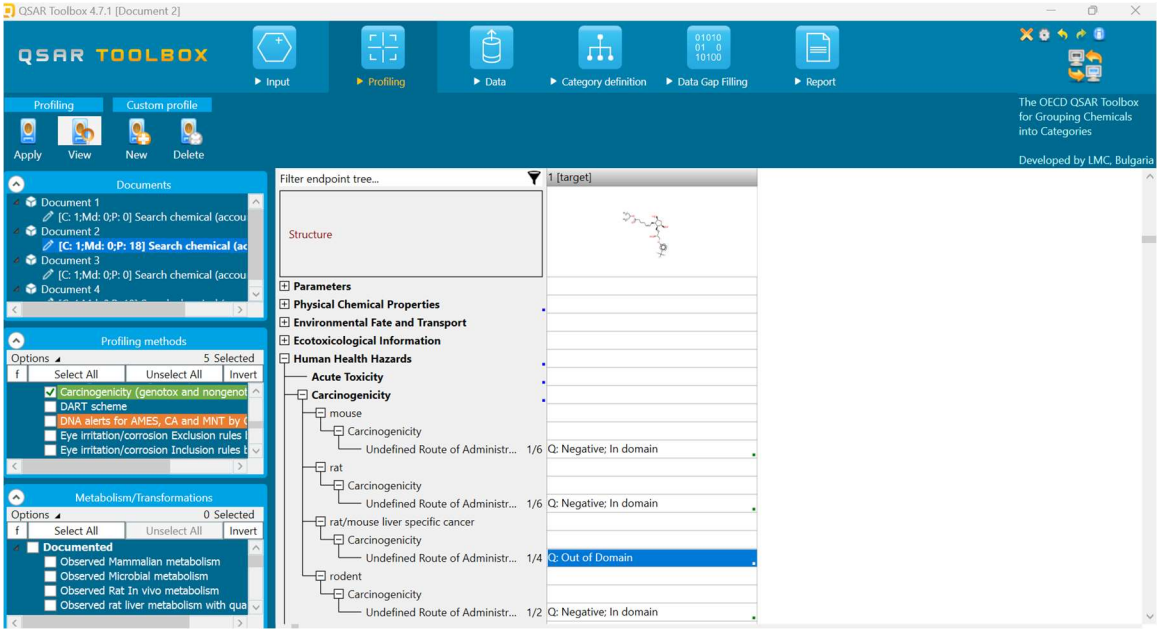
It is unclear whether the QSAR Toolbox metabolism simulation module accounts for the sequential metabolism that is expected to occur for topically-administered substances – i.e., initial metabolism in the skin, followed by metabolism in the liver or other tissues. The metabolism simulation module does not predict the relative abundance of each metabolite, nor the likelihood of their formation. It is also important to note that metabolite formation in humans may differ from metabolite formation in the rat.

APPENDIX C: OECD QSAR TOOLBOX IN SILICO PROFILING

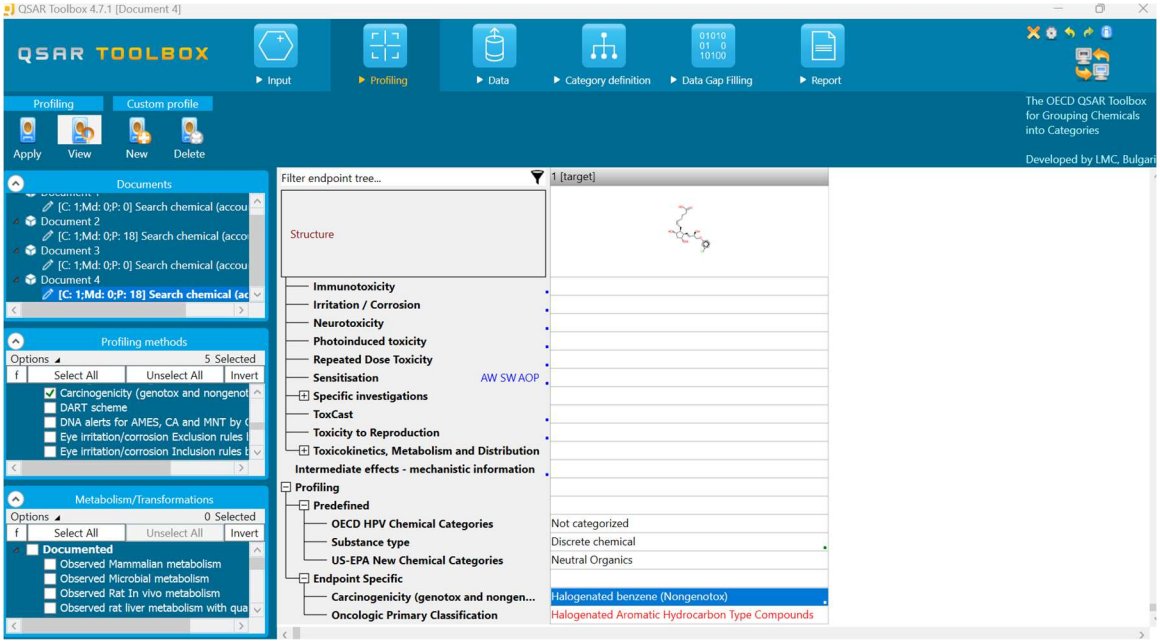
IC: Structural alerts



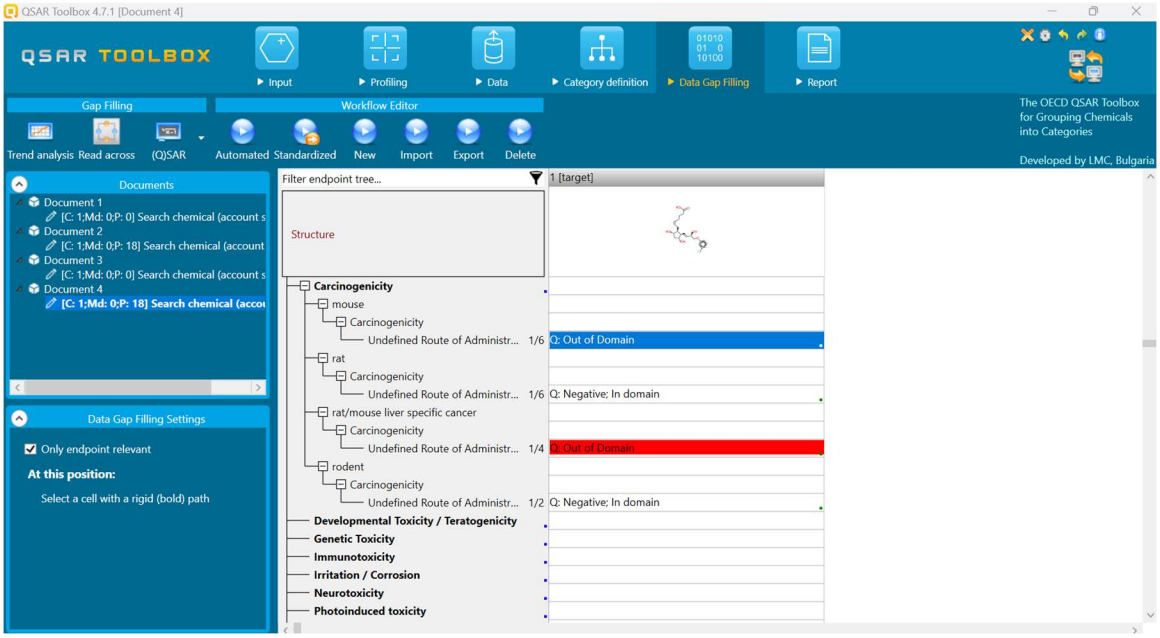
IC: Modeled carcinogenicity predictions



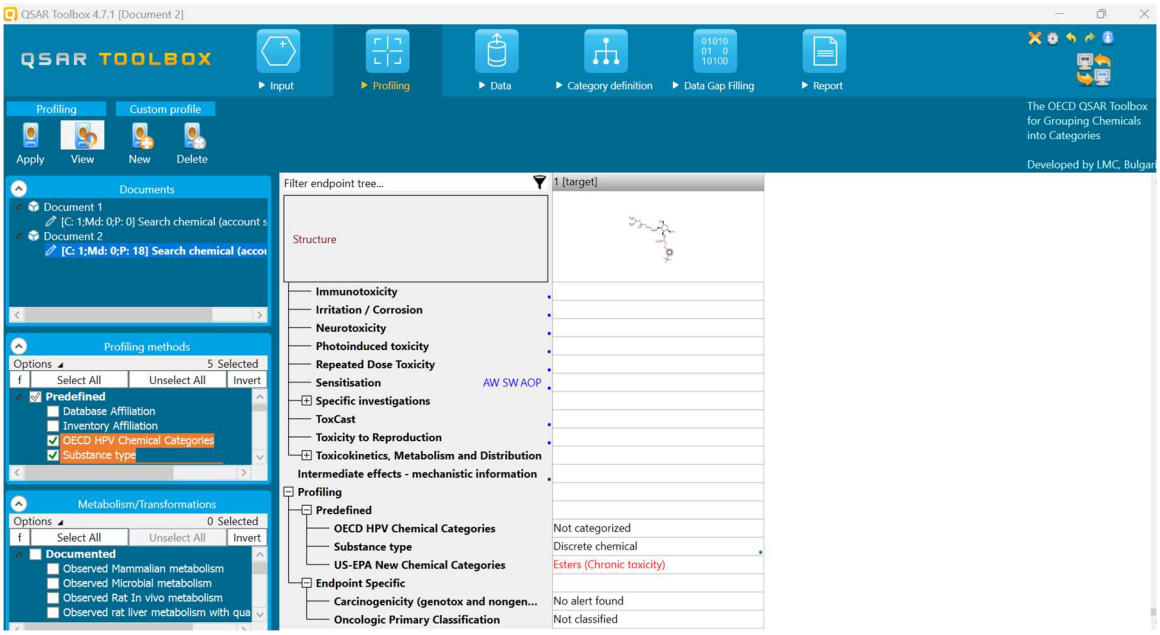
Cloprostenol: Structural alerts



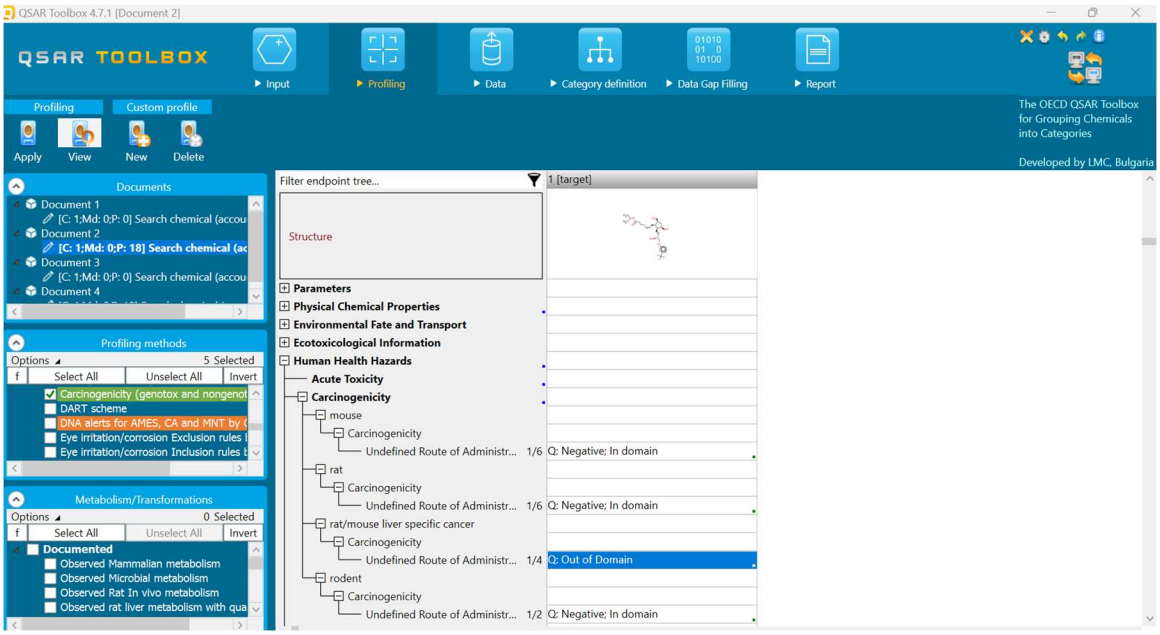
Cloprostenol: Modeled carcinogenicity predictions



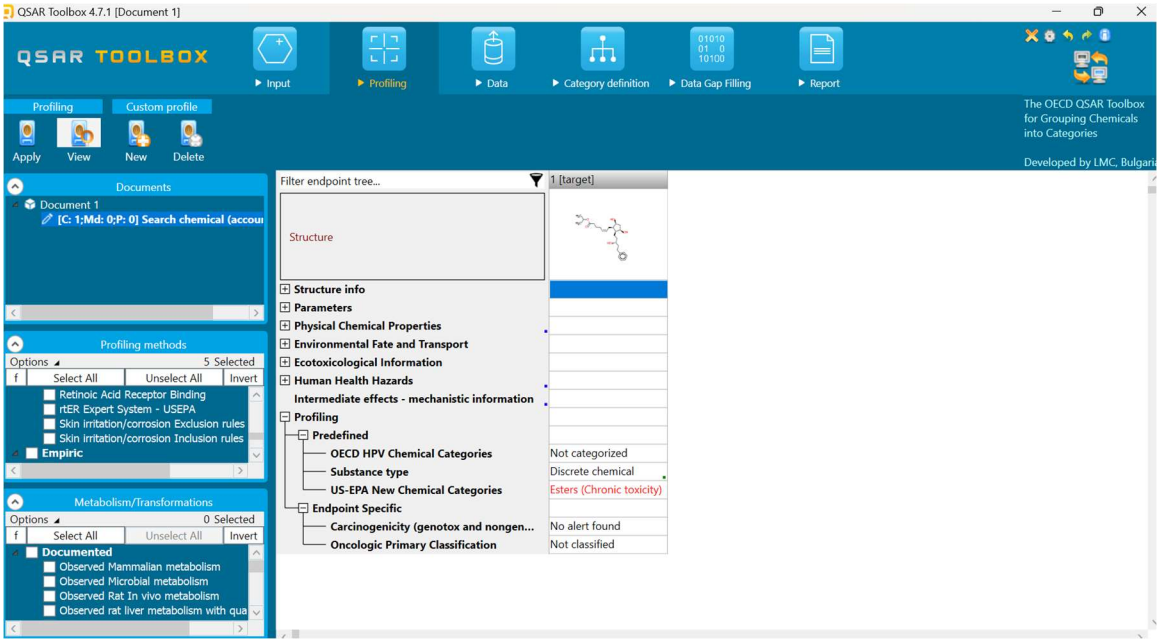
Travoprost: Structural alerts



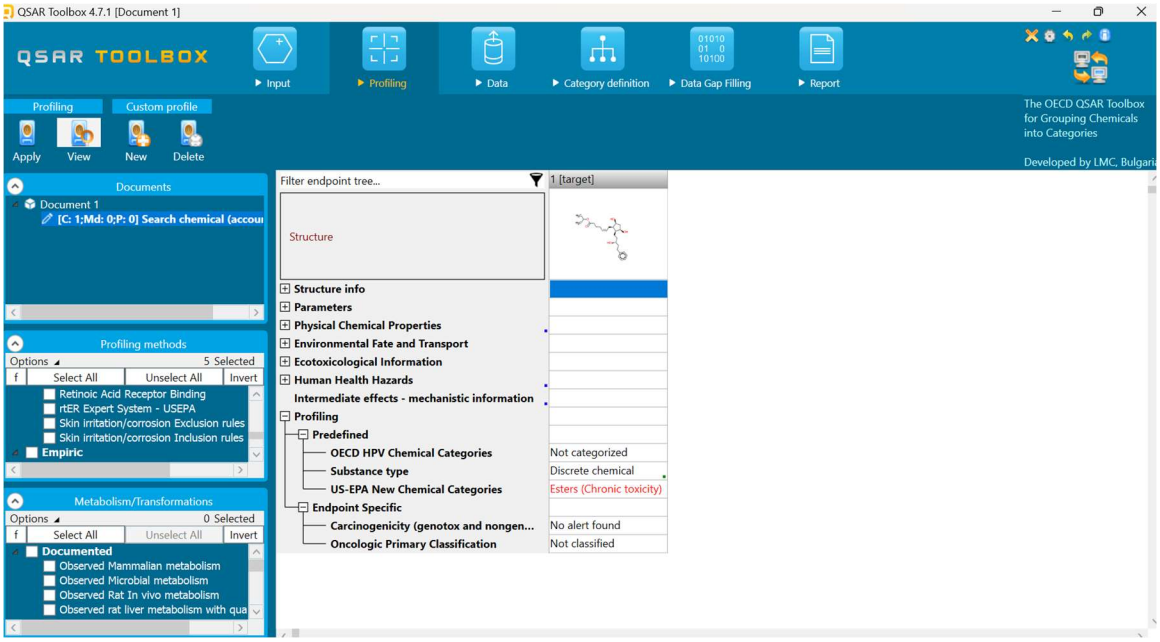
Travoprost: Modeled carcinogenicity predictions



Latanoprost: Structural alerts



Latanoprost: Modeled carcinogenicity predictions



APPENDIX D: REPRODUCTIVE AND DEVELOPMENTAL TOXICITY STUDY SUMMARIES

Studies considered as candidates for the point of departure are summarized individually, and are presented in the order in which they appear in Table 22.

Travoprost

The results of multiple studies in rats and mice identify reproduction and embryonic development as sensitive endpoints for parenterally-administered travoprost, although parental reproductive tissues do not appear to be directly affected [5].

One developmental toxicity study was available in which one generation of female Sprague-Dawley rats received doses of 0, 0.01, 0.03, and 0.1 µg/kg travoprost on GD 7 to PND 21 by subcutaneous injection. There were no effects on pup development. The NOAEL for this study was 0.1 µg/kg/day and there was no LOAEL [5].

In a 3-generation study, pregnant Sprague-Dawley rats were dosed by subcutaneous injection at 0, 0.12, 0.36, and 0.72 µg/kg/day on GD 7 to PND 21 [5]. Observed effects included a decrease in gestation length and an increase in litters with stillborn pups at all dose levels in the F0 generation and an increase in litter loss and a decrease in litters with viable pups and numbers of pups per litter were observed at 0.72 µg/kg/day. Effects observed in the F1 generation included decreases in pup survival on PND 1 to 4, physical development, and motor activity at all dose levels in the F1 generation. No effects were observed in the F2 generation [5]. Since all dose levels were affected, the LOAEL for this study is 0.12 µg/kg/day, and there is no NOAEL.

One developmental study in mice was identified [5]. Female CD-1(ICR)BR mice were given subcutaneous injections of 0, 0.1, 0.3, and 1 µg/kg/day 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; the LOAEL is therefore 1 µg/kg/day [5].

In another study in male and female Sprague-Dawley rats, animals were dosed by subcutaneous 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. Authors observed an increase in early fetal resorptions at 10 µg/kg/day. The NOAEL for this study was 3 µg/kg/day and the LOAEL was 10 µg/kg/day [5].

Pregnant Sprague-Dawley rats were dosed at 0, 1, 3, and 10 µg/kg/day travoprost by intravenous injection on GD 6-17 and were sacrificed on GD 20 [5]. Maternal body weight gain and gravid uterine weight were reduced. The incidence of total litter resorption was increased, as were the numbers of early and late resorptions. Premature delivery was also

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. Authors identified a NOAEL of 3 µg/kg/day for this study; the LOAEL is therefore 10 µg/kg/day [5].

There were no microscopic findings on male and female reproductive tissues in CD rats following 6 months' subcutaneous exposure to travoprost at up to 0.1 mg/kg/day. The critical effect in the 6-month study was altered bone histopathology (NOAEL = 0.01 mg/kg/day, LOAEL = 0.1 mg/kg/day) [5].

Twenty-eight day toxicity studies in CD-1 mice and CD rats revealed no effects on reproductive tissue histopathology at intravenous doses up to 1 mg/kg/day. Similarly, there were no microscopic findings on male and female reproductive tissues in CD rats following 13 weeks of intravenous exposure at doses up to 1 mg/kg/day. CD-1 mice exposed to travoprost at up to 1 mg/kg/day via intravenous and, subsequently, intraperitoneal injection had no evidence of treatment-related microscopic lesions in their reproductive tissues [5].

Cloprostenol

Daily subcutaneous injection of cloprostenol for one month resulted in vacuolization of ovarian luteal cells in female rats, which resolved upon cessation of treatment [12]. The LOAEL for this effect was 12.5 µg/kg/day, which was the lowest dose tested. There was no NOAEL.

A 3-generation oral-route study in rats did not identify adverse effects for cloprostenol on reproductive performance, although premature parturition, resulting in slightly reduced neonatal viability, was observed. Premature parturition has been attributed to cloprostenol's luteolytic effect late in gestation [12]. The NOAEL for the 3-generation study was 15 µg/kg/day and the LOAEL was 20 µg/kg/day.

Oral administration of cloprostenol for 3 months produced ovarian vacuolization in female rats; the NOAEL was 50 µg/kg/day, while the LOAEL was 150 µg/kg/day [12].

Effects observed in marmosets included unspecified myocardial changes and significantly increased testicular weights following oral exposure for 3 months. This study also yielded a NOAEL of 50 µg/kg/day and a LOAEL of 150 µg/kg/day [12].

Intraperitoneal injection of cloprostenol or IC (designated as CIPG and CIPG IE, respectively, by the authors) daily for 28 days resulted in microscopic effects on the testes in adult male rats at a dose of 100 µg/kg/day. Specific effects included dilation of testicular blood vessels, interstitial macrophage accumulation, and tubular degeneration. Such effects were also observed in adult rats and mice at a dose of 25 µg/kg/day, but the incidence and severity were unclear. Both test substances produced similar effects [58].

Cloprostenol does not adversely affect fetal development, based on results of an oral-route study in rats and a subcutaneous-route study in rabbits that yielded NOAELs of 100 µg/kg/day and 0.25 µg/kg/day, respectively, which were the highest doses tested in each species [12].

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March 25, 2025

Independent Ophthalmological Review of Clinical Photographs

Purpose: Three independent ophthalmologists (the "Panel Members") evaluated whether baseline and 8-month photographs ("Image Pairs") captured during an 8-month Ophthalmological In-Use Safety Evaluation of an Eyelash Serum containing 0.0044% isopropyl cloprostenate on 120[1] female participants (Study #C22-4792.01) (the "8-Month Clinical Study") show a pattern of iris stromal pigmentation darkening that is characteristic of prostaglandin-induced iris color change.

Methodology: Each Panel Member independently reviewed Image Pairs and performed the ophthalmological assessment via the Canfield Scientific Inc. ("Canfield") Clinical Services Website using identical Canfield-provided computers. The Image Pairs were cropped by a Canfield Graphics Technician to ensure the area of interest, namely the globe of each eye, was the primary focus and fully visible with only minimal lid and periorbital area visualized. Comparisons were made per side (e.g., right eye baseline and right eye 8-month photographs were reviewed independently from left eye baseline and left eye 8-month photographs). The Image Pairs were randomized in a specialized, Canfield proprietary, software image review program. For each Image Pair, Panel Members answered the following questions: (i) are the two images of good enough quality to make an assessment, (ii) are the two images of equal stromal iris pigmentation, and, if applicable (iii) which image shows a pattern of iris stromal pigmentation darkening that is characteristic of prostaglandin-induced iris color change? If unanimous consensus was reached on a particular Image Pair in the initial photographic review, that Image Pair finding was considered final. An automatic rescore was triggered if unanimous consent was not reached on a particular Image Pair in the initial photographic review. The Panel Members met virtually via Teams after the conclusion of the initial photographic review to evaluate the Image Pairs subject to the automatic rescore. If unanimous consensus was not reached on a particular Image Pair in the automatic rescore and adjudication, that Image Pair was considered "insufficient quality" to make a determination. If unanimous consensus was reached on a particular Image Pair in the automatic rescore and adjudication, that Image Pair finding was considered final.

Results: Panel Members unanimously found that 228 of the 228 Image Pairs (100%) were of sufficient quality to make an assessment and were of equal stromal iris pigmentation.

Conclusion: Image Pairs do not show any prostaglandin-induced iris color change.

I. Introduction

The Sponsor initially engaged Consumer Product Testing Company ("CPTC") to perform the 8-Month Clinical Study to evaluate the safety and ocular irritation potential of an eyelash serum containing 0.0044% isopropyl cloprostenate. In part, the 8-Month Clinical Study evaluated the

[1] 114 participants completed the 8-Month Clinical Study. Two participants were removed due to adverse events (meibomian gland dysfunction "probably test material related" that resolved without medical treatment and conjunctivitis "possibly test material related" that resolved without medical treatment). One participant was removed for protocol violations and three participants did not complete the 8-Month Clinical Study due to personal reasons unrelated to test material use.

potential for product-related change in iris pigmentation in a group of 120 healthy female volunteers. A CPTC bioinstrumentation technician captured VISIA-CR® Digital Imaging (Canfield) at baseline, 1-month, 2-month, 4-month and 8-month intervals to measure the potential for change in iris pigmentation. Images were catalogued using Mirror PhotoTOOLS software (Canfield) and color analysis was performed digitally using VAESTRO Image Analysis toolkit (Canfield). The 8-Month Clinical Study concluded that participants exhibited no statistically significant differences in visible color of the iris on the red/green/blue (RGB) scale (e.g., R/RGB (red color), G/RGB (green color), or B/RGB (blue color)) or in luminosity/brightness (L*) from baseline after 8 months of product use. In-depth digital analysis of the photographs did indicate a statistically significant increase in overall color change of the iris over the length of the study (delta E) that can be attributed to changes in two parameters, a* (redness /irritation) and b* (yellowness), which are of unclear clinical relevance with respect to the iris stromal pigmentation darkening that is characteristic of prostaglandin-induced iris color change.

In 2024, Sponsor engaged the Panel Members to independently evaluate the baseline and 8-month Image Pairs captured during the 8-Month Clinical Study to determine whether any of Images Pairs showed iris stromal pigmentation darkening that is characteristic of prostaglandin-induced iris color change. The Sponsor engaged Canfield to complete the following services: (i) study initiation, (ii) image archival and quality check, (iii) develop the secure web-based platform for the Panel Members to perform the ophthalmological assessment, (iv) data collection and (v) data transfer.

II. Panel Members

The Panel Members for the ophthalmological assessment were:

> **Louis Cantor, MD**: Professor Emeritus of Glaucoma Research and Education at Indiana University School of Medicine. Professor Emeritus of Ophthalmology at Indiana University School of Medicine. Former Chairman of Department of Ophthalmology at Indiana University School of Medicine.
>
> **Sheila Sanders, MD**: Director, Glaucoma Service and Glaucoma Fellowship Program at the University of Kentucky College of Medicine. Vice Chairman, Department of Ophthalmology at the University of Kentucky College of Medicine.
>
> **Ronald Gross, MD**: Professor of Ophthalmology at Baylor College of Medicine. Former Chairman of Ophthalmology at West Virginia University School of Medicine.

III. Methodology

A. Initial Photographic Review

Each Panel Member independently reviewed Image Pairs and performed the initial photographic evaluation via the Canfield Scientific Inc. ("Canfield") Clinical Services Website using identical Canfield-provided computers and monitors. The Image Pairs evaluated comprised cross-polarized baseline and cross-polarized 8-month photographs captured during the 8-Month Clinical Study.

The Image Pairs were cropped by a Canfield Graphics Technician to ensure the area of interest, namely the globe of each eye, was the primary focus and fully visible with only minimal lid and periorbital area visualized. Comparisons were made per side (e.g., right eye baseline vs. month 8 Image Pairs were reviewed independently from left eye baseline vs. month 8 Image Pairs). The Image Pairs were randomized in a specialized, Canfield proprietary, software image review program. Randomization occurred to the level of subject and visit (i.e., baseline and 8-month). Image placement to the top or bottom of the screen was also randomized. Select photographs, chosen by Sponsor and sent to Canfield, were included within the review as controls for reproducibility, including combinations of 15 baseline vs baseline, 15 month 8 vs month 8, and 30 baseline vs month 8 Image Pairs.

Each Panel Member answered the following questions on the Canfield Clinical Services Website during the initial photographic review:

Question	Answer Options
Q1*: Is the image of good enough quality to make an assessment?	Yes No
Q2**: Are the two (2) images of equal stromal iris pigmentation?	Yes No
Q3: Which image shows a pattern of iris stromal pigmentation darkening that is characteristic of prostaglandin induced iris color change?	Photo A Photo B

*If the Panel Member answered "Yes" to Q1, the Panel Member was prompted to answer Q2. If the Panel Member answered "No" to Q1, the session ended and the Panel Member was directed to the next Image Pair.

**If the Panel Member answered "No" to Q2, the Panel Member was prompted to answer Q3. If the Panel Member answered "Yes" to Q2, the session ended and the Panel Member was directed to the next Image Pair.

If unanimous consensus was reached on a particular Image Pair in the initial photographic review, that Image Pair finding was considered final. Canfield transferred the data for the initial photographic review to the Sponsor pursuant to the Canfield Data Transfer Specifications.

B. Automatic Rescore & Adjudication

An automatic rescore was triggered if unanimous consent was not reached on a particular Image Pair in the initial photographic review. The Panel Members met virtually via Teams after the conclusion of the initial photographic review to evaluate the Image Pairs subject to the automatic rescore. A Canfield representative projected each Image Pair subject to the automatic rescore. The Panel Members were provided with the following information for each Image Pair subject to the automatic rescore: (i) how each Panel Member graded the Image Pair in the initial photographic review and any comments a Panel Member included in the comments section for that particular Image Pair, (ii) if a Panel Member found iris color darkening in the initial photographic review,

whether they selected the baseline or 8-month photo as the image exhibiting iris color darkening, and (iii) any applicable reproducibility findings or comments for the Image Pair subject to the rescore. If unanimous consensus was not reached on a particular Image Pair after the automatic rescore & adjudication, that Image Pair was considered "insufficient quality" to make a determination. If unanimous consensus was reached on a particular Image Pair during the automatic rescore & adjudication, that Image Pair finding was considered final. Canfield transferred the data for the automatic rescore and adjudication to the Sponsor pursuant to the Canfield Data Transfer Specifications. All scores and rescores were maintained in an audit trail.

IV. Findings

A. Initial Photographic Review Findings

The Panel Members independently conducted the initial photographic evaluation remotely between January 8, 2025 and January 12, 2025. Panel Members unanimously found that 221 of the 228 Image Pairs were of sufficient quality to make an assessment and were of equal stromal iris pigmentation (i.e., no evidence of iris stromal pigmentation darkening that is characteristic of prostaglandin-induced iris color change). These unanimous findings were considered final.

The Panel Members did not reach a unanimous finding on seven Image Pairs during the initial photographic review.

In three of the seven instances, one Panel Member identified an Image Pair to be of insufficient quality to make an assessment, while the other two Panel Members found the same Image Pair of sufficient quality to make an assessment and of equal stromal iris pigmentation.

In four of the seven instances, one Panel Member identified a pattern of iris stromal pigmentation darkening that is characteristic of prostaglandin-induced iris color change, while the other two Panel Members found the same Image Pair of equal stromal iris pigmentation. In three of the four instances where one Panel Member identified iris stromal darkening during the initial photographic review, the Panel Member selected the baseline image as exhibiting iris color darkening, which could not possibly be the result of prostaglandin-induced iris color change.

In addition, although unanimous consensus was reached on a particular Image Pair during the initial photographic review (finding sufficient quality and equal iris color pigmentation), one Panel Member inconsistently identified iris color darkening in that same Image Pair when it was displayed as a reproducibility control (and selected the baseline image as exhibiting iris color darkening).

B. Automatic Rescore & Adjudication Findings

The Panel Members met virtually via Teams on February 8, 2025 to adjudicate the eight Image Pairs subject to the automatic rescore. A Canfield representative shared each Image Pair subject to the automatic rescore. The Panel Members were provided with the following information for each Image Pair subject to the automatic rescore: (i) how each Panel Member graded the Image Pair in the initial photographic review and any comments a Panel Member included in the comments

section for that particular Image Pair, (ii) if a Panel Member found iris color darkening in the initial photographic review, whether they selected the baseline or 8-month photo as the image exhibiting iris color darkening, and (iii) any applicable reproducibility findings or comments for the Image Pair subject to the rescore.

After reviewing the relevant Image Pairs and background information listed above (including that in four of the five instances where a Panel Member found iris color darkening in the initial photographic review, they selected the baseline photo as the image exhibiting iris color darkening which could not possibly be the result of prostaglandin-induced iris color change), the Panel Members unanimously found that the eight Image Pairs subject to the automatic rescore were of sufficient quality to make an assessment and did not display iris stromal pigmentation darkening that is characteristic of prostaglandin-induced iris color change.

V. Conclusion:

The Panel Members found that 228 of the 228 Image Pairs were of sufficient quality to make an assessment and were of equal stromal iris pigmentation. The Image Pairs do not show any evidence of iris stromal pigmentation darkening that is characteristic of prostaglandin-induced iris color change. This indicates that the statistically significant increase in overall color change of the iris over the length of the 8-Month Clinical Study (delta E) is not clinically relevant.

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CONSUMER PRODUCT TESTING COMPANY
cptclabs.com

FINAL REPORT

CLIENT: [redacted]

ATTENTION: [redacted]

TEST: Ophthalmological In-Use Safety Evaluation of an Eyelash Serum
Protocol No.: LPQU01-002
Protocol Date: 10/31/22

TEST MATERIAL: lash serum containing 0.0044% Isopropyl Cloprostenate [redacted]

STUDY NUMBER: [redacted]

Reviewed by: [redacted] M.D.
Board Certified Ophthalmologist

Approved by: [redacted] R.N.
Executive Vice President, Clinical Evaluations

Revision Date: 11/3/2023

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CONSUMER PRODUCT TESTING COMPANY
cptclabs.com

FDA Registration# 1000151293
DEA Registration# RC0199744 Schedule I-V
US EPA/RI DEP Registration# NJ0982726648
ISO/IEC 17025:2017 Accreditation # 80971

QUALITY ASSURANCE UNIT STATEMENT

Study Number: [redacted]

The Consumer Product Testing Company, Incorporated (CPTC) Quality Assurance Unit (QAU) is responsible for auditing the conduct, content and reporting of all clinical trials that are conducted at CPTC.

This trial has been conducted in accordance with the Declaration of Helsinki, the ICH Guideline E6 for *Good Clinical Practice*, the requirements of 21 CFR Parts 50 and 56, other applicable laws and regulations, CPTC Standard Operating Procedures, and the approved protocol.

The CPTC QAU has reviewed all data, records, and documents relating to this trial and also this Final Report. The following QAU representative signature certifies that all data, records, and documents relating to this trial and also this Final Report have been reviewed and are deemed to be acceptable, and that the trial conforms to all of the requirements as indicated above.

All records and documents pertaining to the conduct of this trial shall be retained in the CPTC archives for a minimum of five (5) years. At any time prior to the completion of the fifth archival year, a Sponsor may submit a written request to the CPTC QAU to obtain custody of trial 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, trial-related records shall be destroyed at the end of the CPTC archive period with no further notice in a manner that renders them useless.

[redacted]

Quality Assurance Representative

11-3-2023

Date

[redacted]

Objective:

The primary objective of the trial was to evaluate the safety and ocular irritation potential of an eyelash serum, when subjected to in-use conditions over a 1-month, 2-month, 4-month and 8-month period.

The secondary objectives of the trial were: (i) to evaluate the potential for change in ocular pigmentation over a 1-month, 2-month, 4-month and 8-month period; and (ii) to evaluate the potential for periorbital fat loss over the same period.

Participants:

One-hundred twenty female subjects, ranging in age from 18 to 55 years, were recruited and qualified for this trial. One-hundred fourteen subjects completed the trial.

- Subject #7 was a protocol violator and was removed from the trial.
- Subject #s 58, 81 and 85 did not complete the trial due to personal reasons unrelated to test material use.
- Subject #s 83 and 113 were removed from the trial by the Principal Investigator due to adverse events.

Inclusion Criteria:

1. Subjects who read, signed, and dated an Informed Consent Form that included a HIPAA statement;
2. Female subjects aged 18 to 55 years, inclusive;
3. Subjects who were willing to arrive at each Testing Facility visit with a clean face, wearing no eye make-up;
4. Subjects who were considered dependable and able to follow directions; and
5. Subjects must have signed a Non-Disclosure/Confidentiality Agreement ("N-DA") agreeing to keep their participation in the study confidential.

Exclusion Criteria:

1. Subjects who were in ill health, as determined by the Principal Investigator;
2. Subjects who were taking medication, other than birth control, such as any systemic or topical corticosteroids, immunosuppressants, anti-inflammatories, antihistamines, antibiotics, or other medication that, in the opinion of Investigator, may have influenced the purpose, integrity, or outcome of the trial;
3. Subjects who were pregnant, planning to become pregnant, or lactating during the course of the trial;
4. Subjects who experienced any type of ophthalmological issues within the past 6 months;
5. Subjects who had facial tattoos, piercings, or scars that would have interfered with photographs;
6. Subjects who had used eyelash serums within 6 months prior to trial initiation;

Exclusion Criteria (continued):

7. Subjects who had permanent injectable fillers in and around the eye area within 12 months;
8. Subjects who had undergone any facial surgeries (e.g., eyebrow lift) within 3 months of trial initiation;
9. Subjects who had eyelash lifts, eyelash extensions, or eyelash tints;
10. Subjects who had any visible disease which might have been confused with a reaction to the test material;
11. Subjects who had a history of adverse reactions to cosmetics, OTC drugs, or other personal care products;
12. Subjects who introduced the use of any new cosmetic, toiletry, personal care products during the trial; or
13. Subjects who were prone to dry eyes, styes, or undergoing chemotherapy.

Test Schedules:

Schedule	Initiation Date	Completion Date	Enrolled Subject #s
1	January 5, 2023	August 17, 2023	1 - 46
2	January 26, 2023	September 7, 2023	47 - 94
3	February 9, 2023	September 15, 2023	95 - 121

Test Material: [redacted] Lash Enhancing Serum

Trial Design: This was a single-center, baseline controlled, monadic clinical trial design of 8 months duration.

Descriptions of the trial timepoints are shown below:

Description	Baseline (Day 0) Supervised Application	Test Phase (1-Month)	Test Phase (2-Months)	Test Phase (4-Months)	Test Phase (8-Months)
Board Certified Ophthalmologist Evaluations	X	X	X	X	X
VISIA-CR®, Digital Imaging	X	X	X	X	X
Aeva 3D HE Imaging - Periorbital Volume Measurement	X	X	X	X	X
Distribution of Test Material, Daily Diaries, and Instruction for Use	X				
Return of unused Test Material and completed Daily Diaries					X

Instrumentation:

Environmentally-Controlled Rooms

The environmentally controlled rooms used were maintained at a temperature of 70 ± 5 °F (21.1 ± 2.8°C) and at a relative humidity of 40 ± 5%. Readings of temperature and humidity were electronically recorded with at least a 15-minute frequency.

Each subject equilibrated in the environmentally controlled room for at least 15 minutes prior to all measurements and photographs. All measurements were taken under these conditions.

VISIA-CR®, Digital Imaging

The VISIA-CR®, took a series of standardized, reproducible digital facial images, inside of a controlled lighting environment. Subject positioning was critical and was repeated at each time point. Items such as stool height and careful placement of the subject's chin and forehead into the imaging device was maintained. Also, the subject's hair was off their face, jewelry removed, and a black drape was used to standardize clothing.

Only the subjects' front view was captured with their eyes open using the following lighting parameters. Subjects removed contact lenses prior to image capture.

- Standard 1 - General purpose white light
- Standard 2 - Flat lighting
- Cross-polarized – Filters out surface reflections for superior visualization of sub-surface detail

Using the VISIA-CR®, a trained Bioinstrumentation technician captured standardized digital images of the eyes at baseline, after 1 month, 2 months, 4 months, and 8 months of test material usage. At the conclusion of the trial, all digital images were provided to the Sponsor.

The images captured with the VISIA-CR® were catalogued using MIRROR PhotoTools software (Canfield Scientific., Inc., Parsippany, NJ). The images for each subject were exported as a .dx2 file. These files were used for analysis using the VAESTRO™ Image analysis Toolkit. Color Analysis was performed, overall color was evaluated using ΔE, where $\Delta E = \sqrt{[(\Delta L^*)^2+(\Delta a^*)^2+(\Delta b^*)^2]}$. Therefore, an increase in ΔE represented an increase in color from baseline.

Aeva^{3D} -HE

Aeva^{3D}-HE is a high resolution, 3D Solution, measurement system for face topography, skin topography, and body morphological changes.

Instrumentation (continued):

Subjects were in a seated position in the Visio-4D bench for stable and repeatable alignment of the outer canthus area. The subjects' position was noted for re-positioning between the measurement time points, to ensure reliable and repeatable results.

The seating platform was remotely operated so that subjects could place the earplugs (part of Aeva^{3D}-HE, aiding with positioning) into their ears comfortably. Subjects were asked to make fine adjustments so that a green laser light emits through the lip corners. A plate was placed behind the subjects' head. As subjects were not able to move while taking the images, images were not captured until subjects' positioning and comfort was achieved.

Front view Aeva^{3D}-HE images were captured, using the 250 FOV Lens, for each subject. The view captured the subject's left and right orbital region. These images were used to assess measurements in periorbital volume.

Using Aeva^{3D}-HE, a trained Bioinstrumentation technician captured images at Baseline (pre-application) and after 1, 2, 4, and 8 months of test material usage.

Methodology:

Qualification (Day 0)

Potential subjects reported to the Testing Facility at their scheduled appointment time, with a clean face, wearing no eye or face make-up and executed an Informed Consent Form to become subjects. Subjects executed a Photographic Release Form and a Non-Disclosure Agreement.

The informed consent process fully appraised each potential subject of the risks and benefits associated with the research clinical trial and of the confidentiality requirements relating to the subject's clinical trial records. If the potential subject agreed to participate in the research clinical trial, then the potential subjects executed the Informed Consent Form (ICF). Staff who conducted the informed consent process also executed the form, after which the potential subject entered the clinical trial as a subject. Each subject received a signed copy of the fully executed ICF.

If at any time during the clinical trial the subject had questions, the ICF directed the subject to a Subject Rights Advocate, whose contact information was in the Informed Consent Form.

Subjects completed a Medical History Form to determine initial qualification.

Methodology (continued):

Subjects were screened by a Board-Certified Ophthalmologist to determine qualification. The Ophthalmologist studied and evaluated by gross and/or slit lamp examination each subject's eyelids, conjunctivae, corneas, anterior chambers, and pupillary reactions, in addition to measuring visual acuity. Observations also included evaluations of dryness, erythema, and edema which were recorded on CRFs. If applicable, the correct fit and condition of their contact lenses were evaluated. Findings were noted on individual subject CRFs.

Evaluation Key:

0	None
0.5	Barely perceptible
1	Mild
2	Moderate
3	Marked
4	Severe

Subjects presenting a score of moderate (2) or greater for erythema, edema, or dryness were disqualified. All evaluation scores were recorded on CRFs.

Test Phase (Day 0)

Qualified subjects acclimated, in an environmentally controlled room, for at least 15 minutes. A Bioinstrumentation technician captured VISIA-CR and Aeva^{3D}-HE images, as previously described.

Subjects were provided with an 8-month supply of the test material and a daily diary to document compliance and use. Written and verbal instructions for use and restrictions (listed below), supplied by the Sponsor, were provided to each subject. Subjects conducted the first application under Clinical Supervision.

Instructions:

- Arrive at each Testing Facility visit with a clean face, wearing no eye make-up.
- DO NOT use any eyelash serum other than the test material provided for the duration of this trial.
- You may wear your usual facial makeup products during the trial.
- Eyelash treatments such as eyelash lifts, eyelash extensions, and eyelash tints are prohibited during the trial.
- DO NOT introduce any new cosmetics, toiletry, or personal care products during the trial.
- CONTACT WEARERS: bring a contact lens case with solution to each visit. Contact lenses must be worn for the eye examination and then removed for image capture.

Methodology (continued):

Instructions for Use: *(Once Daily)*

- Apply once daily on clean, dry upper lash line only using a single stroke on your eyelid just above your upper lash line (like a liquid eyeliner).
- Do not apply to the lower lash line.
- Apply only with the product applicator.
- Use one dip into the bottle for both eyes and allow 1-2 minutes for the serum to fully dry before applying other products to your eye area.
- Use sparingly as dripping into the eye may cause irritation or other unwanted effects. If serum drips into the eye, immediately rinse thoroughly with cool water to completely remove serum from the eye.
- Apply every day for at least 3 months. After desired improvement is achieved, apply every other day for maintenance.
- The serum requires continued use to maintain benefits.

Keep out of reach of children. Do not let anyone else use the test material.

After each application of the test material, record the time under the APPLICATION column of the daily diary.

Report any adverse reactions or problems immediately to the Testing Facility staff.

Subjects were encouraged to document comments pertaining to the test material in their daily diaries.

Subjects agreed to use only the test material for all eyelash enhancement purposes for the duration of the trial. Eyelash treatments such as eyelash lifts, eyelash extensions, and eyelash tints were prohibited during the trial. Subjects were allowed to wear their usual facial makeup products during the trial. Subjects may not have introduced any new cosmetic, toiletry, or personal care products during the course of the trial.

Test Phase (1 Month)

After 1 month of test material use, subjects reported to the Testing Facility at their scheduled appointment time with a clean face, wearing no eye make-up.

The Board-Certified Ophthalmologist conducted an evaluation of each subject, as previously described.

Subjects acclimated in an environmentally controlled room, for at least 15 minutes. A Bioinstrumentation technician captured VISIA-CR and Aeva^{3D}-HE images, as previously described.

Methodology (continued):

Test Phase (2 Months)

After 2 months of test material use, subjects reported to the Testing Facility at their scheduled appointment time with a clean face, wearing no eye make-up.

The Board-Certified Ophthalmologist conducted an evaluation of each subject, as previously described.

Subjects acclimated in an environmentally controlled room, for at least 15 minutes. A Bioinstrumentation technician captured VISIA-CR and Aeva^{3D}-HE images, as previously described.

Test Phase (4 Months)

After 4 months of test material use, subjects reported to the Testing Facility at their scheduled appointment time with a clean face, wearing no eye make-up.

The Board-Certified Ophthalmologist conducted an evaluation of each subject, as previously described.

Subjects acclimated in an environmentally controlled room, for at least 15 minutes. A Bioinstrumentation technician captured VISIA-CR and Aeva^{3D}-HE images, as previously described.

Test Phase (8 Months)

After 8 months of test material use, subjects reported to the Testing Facility at their scheduled appointment time with a clean face, wearing no eye make-up.

Any unused test material and completed daily diaries were returned. Daily diaries were reviewed for completeness and retained by the Testing Facility.

The Board-Certified Ophthalmologist conducted a final evaluation of each subject, as previously described.

Subjects acclimated in an environmentally controlled room, for at least 15 minutes. A Bioinstrumentation technician captured VISIA-CR and Aeva^{3D}-HE images, as previously described.

Amendments:

Amendment 1

OLD VERSION:

5.2 Inclusion Criteria

3. Subjects must be willing to arrive at each Testing Facility with a clean face, wearing no eye make-up;

Amendments (continued):

5. Subjects shall signed a Non-Disclosure/Confidentiality Agreement ("NDA) agreeing to keep their participation in the study confidential.

NEW VERSION:

5.2 Inclusion Criteria

3. Subjects must be willing to arrive at each Testing Facility visit with a clean face, wearing no eye make-up;

5. Subjects must sign a Non-Disclosure/Confidentiality Agreement ("N-DA") agreeing to keep their participation in the study confidential.

ADDITION:

6.2.2 VISIA-CR®, Digital Imaging

Subjects will remove contact lenses prior image capture.

OLD VERSION:

6.2.3 Aeva3D -HE

Aeva3D-HE images will be captured, using the 160 FOV Lens, of the left and right sides of each subject's left and right orbital region. The images will be used to assess measurements in periorbital volume.

NEW VERSION:

6.2.3 Aeva3D -HE

Front view Aeva3D-HE images will be captured, using the 250 FOV Lens, for each subject. This view will capture the subject's left and right orbital region. These images will be used to assess measurements in periorbital volume.

REASON FOR CHANGE: To correct typographical errors and update image capture parameters.

Amendment 2

OLD VERSION:

6.4 Test Phase (Day 0)

Amendments (continued):

Instructions for Use: (Once Daily)

- Dip applicator into tube once for both eye applications.
- Apply once daily on a clean, dry upper eyelash line using a single stroke. (ex: apply like a liquid eyeliner)
- Allow 1-2 minutes for serum to dry

NEW VERSION:

6.4 Test Phase (Day 0)

Instructions for Use: (Once Daily)

- Apply once daily on a clean, dry upper eyelash line using a single stroke. (ex: apply like a liquid eyeliner)
- Allow 1-2 minutes for serum to dry before applying other products to your eye area.
- Use sparingly as dripping into the eye may cause irritation or other unwanted effects.
- If serum drips into the eye, immediately rinse thoroughly with cool water to completely remove serum from the eye.
- Apply every day for at least 3 months. After desired improvement is achieved, apply every other day for maintenance.
- The serum requires continued use to maintain benefits.

REASON FOR CHANGE: Per client request.

Deviations:

Subject #7 was enrolled in Schedule 1 of the trial. The subject deceptively presented as a new panelist at a later date and was enrolled a second time on the trial as subject # 51. On the medical history form as Subject # 51 the subject falsely stated she was not using an eyelash serum within the past 6 months. Due to the subject's deceptive behavior, Subject # 7/51 was removed from the trial by the Principal Investigator.

Per protocol, the environmentally controlled rooms were to be maintained at a relative humidity of 40 ± 5%. However, the following excursions in relative humidity occurred:

- On 4/6/2023 - 10 minutes excursion with maximum RH of 48.3% for the AP room
- On 8/17/2023 - 30 minutes excursion with maximum RH of 49.5% for the AP room
- On 9/7/2023 - 122 minutes excursion with maximum RH of 50.8% for the AP room and 46% for the AEVA room.

The Principal Investigator deemed these excursions to have no impact on trial results.

Deviations (continued):

Subject #s 103, 116, and 117 ended the trial 8 days earlier than initially scheduled and Subject #s 95-102, 104-112, 114, 115, 118-121 ended the trial 6 days earlier than initially scheduled due to the Board-Certified Ophthalmologist's availability. It was the opinion of the Principal Investigator that a few days difference for a 8 month trial did not impact trial results.

Adverse Events:

Subject #83 complained of left eye swelling that started on 1/27/23. Itching occurred on both lids on 1/27/23 and swelling of the right lid started on 1/31/23. Symptoms were reported on 2/2/23 and the Board-Certified Ophthalmologist examined the Subject on this date. The Ophthalmologist reported the Subject had meibomian gland dysfunction in both eyes. No erythema was noted on lids or eyes. The Subject was removed from the trial and instructed to seek follow up with her ophthalmologist if symptoms persisted. A follow-up phone call with the Subject on 2/13/23 revealed that all symptoms had resolved, and she did not deem it necessary to follow-up with her doctor. It was the opinion of the Principal Investigator that this event was probably test material related.

Subject #36 complained of a "ball" on the upper left eyelid on 3/2/23. Upon observation by the Board-Certified Ophthalmologist, an early hordeolum/chalazion on the left upper lid was found. The Ophthalmologist recommended warm compresses to the area. The Subject followed up by telephone and sent images to the testing facility on 3/9/23. All symptoms were resolved per subject and ophthalmologist. No further complaints were noted, and the subject continued on the trial. This event is considered resolved. It was the opinion of the Principal Investigator that this event was possibly test material related.

Subject #38 stated on 8/17/23, Month 8 visit, that 2 weeks prior she had sand blown into her eyes which caused irritation. Upon observation, the Board-Certified Ophthalmologist reported probable conjunctivitis, right eye greater than left with no corneal abrasion or adenopathy noted. The subject was recommended to see her eye doctor for follow up. The subject visited her eye doctor on 8/18/23 and was prescribed Tobramycin eye drops twice a day. All symptoms resolved as of 8/21/23 per subject, per telephone conversation on 8/22/23. This event is considered resolved. It was the opinion of the Principal Investigator that this event was unlikely related to the test material.

Subject #113 was evaluated by the Ophthalmologist at her 8-week visit and was diagnosed with conjunctivitis of her right eye and removed from the trial. A follow-up phone conversation on 4/11/23 revealed that all symptoms resolved as of 4/10/23. The subject did not follow-up with her primary doctor and had no further complaints. This event is considered resolved. It was the opinion of the Principal Investigator that this event was possibly test material related.

Statistical Analysis:

Within-groups statistical analysis was performed on the mean calculated ΔE values from the VISIA-CR® image analysis color data, and the periorbital volume measurements (total volume change) obtained from the Aeva3D-HE instrument. Prior to performing the analyses, diagnostic tests on the data was performed to determine whether normality and/or homogeneity of variances of the data are maintained. If the above conditions were maintained, a parametric Student's t-Test or Analysis of Variance was performed. If any of the above conditions were not maintained, a non-parametric equivalent to the above statistical tests was utilized. If statistical significance was observed and further statistical comparisons were required, multiple comparison testing was performed and appropriate adjustments to the *p*-values were made accordingly. For all of the above analyses, statistical significance was achieved at the 95% Confidence Level ($p < 0.050$).

Revisions:

At the client's request, the comments recorded by the subject in the daily diary were removed from the body of the report. The diaries are provided under separate cover. In addition, the summary was updated to include the RGB color space results to the summary section of the report.

Results:

Subject demographics are presented in Table 1.

Dermatological evaluations at baseline and after 1, 2, 4, and 8 months of test material usage are presented in Table 2.

- Dermatological examinations remained within normal limits throughout the test interval.

Statistical Analysis of Aeva3D-HE Positive volume (mm^3) values are presented in Table 3.

Left Orbital Region:

- When compared to baseline values for the left orbital side, there was a statistically significant decrease in volume after 1 month of test material use. There were no statistically significant differences in the left orbital volume after 2, 4, and 8 months of test material use.

Right Orbital Region:

- When compared to baseline values for the right orbital side, there was a statistically significant decrease in volume after 1 and 4 months of test material use. There were no statistically significant differences in the right orbital volume after 2 and 8 months of test material use.

VISIA-CR Image Analysis Color Data is presented in Table 4.

Results (continued):

For the Left Iris:

- When compared to baseline values, there were no statistically significant differences in R/RGB (red color), G/RGB (green color), or B/RGB (blue color) values after 1, 2, 4, and 8 months of test material use.
- When compared to baseline values, there was a statistically significant increase in the overall color change (ΔE) of the iris after 1, 2, 4, and 8, months of test material use. There were no statistically significant differences in L* (luminosity) values after 1, 2, 4, and 8, months of test material use. There were no statistically significant differences in a* (redness) values after 1, 2, and 4 months of test material use.

  However, there was a statistically significant increase in a* (redness) values after 8 months of test material use. There were no statistically significant differences in b* (yellowness) values after 1 and 2 months of test material use. However, there was a statistically significant increase in b* (yellowness) values after 4 and 8 months of test material use.

For the Right Iris:

- When compared to baseline values, there were no statistically significant differences in R/RGB (red color) after 1, 2, 4, and 8 months of test material use. When compared to baseline values, there were no statistically significant differences in G/RGB (green color) or B/RGB (blue color) after 1, 2, and 8 months of test material use. However, there was a statistically significant decrease in G/RGB (green color) and B/RGB (blue color) after 4 months of test material use.
- When compared to baseline values, there was a statistically significant increase in the overall color change (ΔE) of the iris after 1, 2, 4, and 8, months of test material use. There were no statistically significant differences in L* (luminosity) values after 1, 2, 4, and 8, months of test material use. There were no statistically significant differences in a* (redness) values after 1 and 2 months of test material use. However, there was a statistically significant increase in a* (redness) values after 4 and 8 months of test material use. There were no statistically significant differences in b* (yellowness) values after 1, 2, and 4 months of test material use. However, there was a statistically significant increase in b* (yellowness) values after 8 months of test material use.

Subject daily diaries are provided under separate cover.

Results (continued):

Transcribed ophthalmologist examination records and comments are provided under separate cover.

The following are *observations made by the ophthalmologist* between different trial timepoints. Note: for details pertaining to Adverse Events, see the Adverse Events section of this report:

Subject #	Comment / Observation
Baseline – 1 Month	
83	Meibomian gland dysfunction in both eyes *AE*
113	Erythema right and left conjunctiva
1 Month – 2 Months	
6	Cornea: Punctate epithelial erosion right
8	Cornea: Punctate epithelial erosion right and left
9	Conjunctiva: Mild conjunctival erythema both eyes
10	Conjunctiva: Mild conjunctival erythema both eyes
19	Cornea: Punctate epithelial erosion left inferior
26	Started a new prescription for glasses which improved her visual acuity score from 50-1/50-1 to 25/20
30	Plus 1 right eye tarsal erythema
34	Plus 1 bilateral tarsal erythema
36	Left upper lid chalazion *AE*
50	Lids: Increased meibomian gland dysfunction right and left
52	Cornea: Right and left punctate epithelial erosion
93	Conjunctiva: Mild erythema right and left
103	Lids: Meibomian gland dysfunction right and left
113	Conjunctivitis: right eye *AE*
2 Months – 4 Months	
40	Conjunctiva: Mild conjunctival erythema
45	Tarsal follicles bilateral
53	Conjunctiva: Mild erythema right and left
64	Conjunctiva: Mild erythema right and left
101	Conjunctiva: Conjunctival erythema and papillae
111	Conjunctiva: Conjunctival erythema
115	Conjunctiva: erythema right greater than left
120	Conjunctiva: Mild erythema and papillae
4 Months – 8 Months	
38	Conjunctiva: erythema right greater than left Cornea: Punctate epitheliopathy right eye *AE*
93	Lids: Meibomian gland dysfunction right and left
101	Conjunctiva: Erythema conjunctiva bilateral

Summary:

Under the conditions of this trial, 8 months of once daily usage of test material, [redacted] Lash Enhancing Serum, the following conclusions can be made:

- Safe for contact lens and non-contact lens wearers.
- Slight potential for transient ophthalmologic irritation.
- The total color change (ΔE) from baseline indicated a change in the color of the iris. When evaluating the individual color components, there were increases in the a* and b* values, red and yellow, respectively, after 8 months of usage. Although these increases were statistically significant, they were not clinically relevant.
- After 8 months of usage there were no statistically significant changes in the RGB color space values of the iris.
- After 8 months of usage there was no change in periorbital volume from baseline.

Table 1

Subject Demographics

Subject #	Subject ID	Age	Contact Lens
1	56135	53	No
2	87855	50	No
3	88888	18	No
4	86779	54	No
5	89104	52	No
6	88905	30	No
7	92956	54	No
8	92429	44	No
9	58976	31	No
10	91854	41	No
11	91927	54	No
12	75855	28	No
13	69699	37	No
14	66875	43	No
15	92592	41	No
16	87173	45	No
17	64690	18	No
18	92994	47	No
19	93102	35	No
20	25118	45	Yes (Soft)
21	72896	55	No
22	92978	22	No
23	85372	53	No
24	77457	39	No
25	89254	32	No
26	90669	53	No
27	68629	44	No
28	93146	41	No
29	47917	51	No
30	93150	40	No
31	93151	48	No
32	49868	55	Yes (Soft)
33	92615	40	No
34	76837	32	Yes (Soft)
35	89198	40	No

Did Not Complete: Subject #7, 58, 81, 83, 85 and 113

Table 1
(continued)

Subject Demographics

Subject #	Subject ID	Age	Contact Lens
36	80522	39	No
37	92575	43	No
38	92096	53	No
39	63350	20	No
40	93162	30	No
41	91523	40	No
42	66549	42	No
43	84377	53	No
44	92895	44	No
45	93104	51	No
46	60209	47	Yes (Soft)
47	21096	55	No
48	93197	47	No
49	47949	41	No
50	92889	37	No
52	92706	25	No
53	39164	43	No
54	93153	35	No
55	71893	51	No
56	91393	37	No
57	90080	36	No
58	93083	26	No
59	91137	33	No
60	92158	52	No
61	93210	32	Yes (Soft)
62	88490	40	Yes (Soft)
63	87998	33	No
64	93160	37	No
65	46058	40	No
66	70499	29	No
67	93152	42	No
68	91812	41	No

Did Not Complete: Subject #7, 58, 81, 83, 85 and 113

*Deviation: #51 – Subject # and data removed from the trial.

Table 1
(continued)

Subject Demographics

Subject #	Subject ID	Age	Contact Lens
69	56034	41	No
70	80386	32	No
71	63555	29	No
72	93198	31	No
73	92065	40	No
74	91636	34	Yes (Soft)
75	93018	42	No
76	77962	39	Yes (Soft)
77	90494	54	No
78	60639	46	No
79	92756	50	No
80	90099	41	No
81	78294	47	No
82	93044	37	No
83	93196	27	No
84	91200	43	No
85	92803	29	No
86	71209	52	No
87	91745	44	No
88	58577	52	No
89	92282	23	No
90	4284	52	No
91	92854	40	No
92	48073	49	No
93	92836	45	No
94	45824	52	No
95	61969	54	No
96	84567	54	No
97	92840	33	No
98	76099	54	No
99	92770	22	No
100	93235	45	No
101	93229	46	No
102	66648	32	Yes (Soft)

Did Not Complete: Subject #7, 58, 81, 83, 85 and 113

Table 1
(continued)

Subject Demographics

Subject #	Subject ID	Age	Contact Lens
103	75527	55	No
104	81232	51	No
105	92715	46	Yes (Soft)
106	47011	44	No
107	67311	54	No
108	62190	53	No
109	93213	53	No
110	93234	44	No
111	65407	55	No
112	42734	47	No
113	92456	38	No
114	91044	48	No
115	84560	55	No
116	87722	44	No
117	91445	44	No
118	72693	46	No
119	67863	42	No
120	92316	53	No
121	84404	51	Yes (Soft)

Did Not Complete: Subject #7, 58, 81, 83, 85 and 113

Table 2

DERMATOLOGICAL EVALUATIONS – QUALIFICATION (DAY 0)

TEST SITE: Eyelid (along upper lash line)
TEST MATERIAL: [redacted] Lash Enhancing Serum

EVALUATION SCALE

0 = NONE
0.5 = BARELY PERCEPTIBLE
1 = MILD
2 = MODERATE
3 = MARKED
4 = SEVERE

Any subject exhibiting a score of ≥2 for any one parameter will be disqualified.

Subject # & ID		ERYTHEMA		EDEMA		DRYNESS		COMMENTS
		Right	Left	Right	Left	Right	Left	
1.	56135	0	0	0	0	0	0	
2.	87855	0	0	0	0	0	0	
3.	88888	0	0	0	0	0	0	
4.	86779	0	0	0	0	0	0	
5.	89104	0	0	0	0	0	0	
6.	88905	0	0	0	0	0	0	
7.	92956	0	0	0	0	0	0	
8.	92429	0	0	0	0	0	0	
9.	58976	0	0	0	0	0	0	
10.	91854	0	0	0	0	0	0	
11.	91927	0	0	0	0	0	0	
12.	75855	0	0	0	0	0	0	
13.	69699	0	0	0	0	0	0	
14.	66875	0	0	0	0	0	0	

Table 2
(continued)

DERMATOLOGICAL EVALUATIONS – QUALIFICATION (DAY 0)

TEST SITE: Eyelid (along upper lash line)
TEST MATERIAL: [redacted] Lash Enhancing Serum

EVALUATION SCALE

0 = NONE
0.5 = BARELY PERCEPTIBLE
1 = MILD
2 = MODERATE
3 = MARKED
4 = SEVERE

Any subject exhibiting a score of ≥2 for any one parameter will be disqualified.

Subject # & ID		ERYTHEMA		EDEMA		DRYNESS		COMMENTS
		Right	Left	Right	Left	Right	Left	
15.	92592	0	0	0	0	0	0	
16.	87173	0	0	0	0	0	0	
17.	64690	0	0	0	0	0	0	
18.	92994	0	0	0	0	0	0	
19.	93102	0	0	0	0	0	0	
20.	25118	0	0	0	0	0	0	
21.	72896	1	1	0	0	0	0	
22.	92978	0	0	0	0	0	0	
23.	85372	0	0	0	0	0	0	
24.	77457	0	0	0	0	0	0	
25.	89254	0	0	0	0	0	0	
26.	90669	1	1	0	0	1	1	
27.	68629	0	0	0	0	0	0	
28.	93146	0	0	0	0	0	0	

Table 2
(continued)

DERMATOLOGICAL EVALUATIONS – QUALIFICATION (DAY 0)

TEST SITE: Eyelid (along upper lash line)
TEST MATERIAL: [redacted] Lash Enhancing Serum

EVALUATION SCALE

0 = NONE
0.5 = BARELY PERCEPTIBLE
1 = MILD
2 = MODERATE
3 = MARKED
4 = SEVERE

Any subject exhibiting a score of ≥2 for any one parameter will be disqualified.

Subject # & ID		ERYTHEMA		EDEMA		DRYNESS		COMMENTS
		Right	Left	Right	Left	Right	Left	
29.	47917	0	0	0	0	0	0	
30.	93150	0	0	0	0	0	0	
31.	93151	0	0	0	0	0	0	
32.	49868	1	1	0	0	0	0	
33.	92615	0	0	0	0	0	0	
34.	76837	0	0	0	0	0	0	
35.	89198	0	0	0	0	1	1	
36.	80522	0	0	0	0	0	0	
37.	92575	0	0	0	0	0	0	
38.	92096	0	0	0	0	1	1	
39.	63350	0	0	0	0	0	0	
40.	93162	0	0	0	0	0	0	
41.	91523	0	0	0	0	0	0	
42.	66549	0	0	0	0	0	0	

Table 2
(continued)

DERMATOLOGICAL EVALUATIONS – QUALIFICATION (DAY 0)

TEST SITE: Eyelid (along upper lash line)
TEST MATERIAL: [redacted] Lash Enhancing Serum

EVALUATION SCALE

0 = NONE
0.5 = BARELY PERCEPTIBLE
1 = MILD
2 = MODERATE
3 = MARKED
4 = SEVERE

Any subject exhibiting a score of ≥2 for any one parameter will be disqualified.

Subject # & ID		ERYTHEMA		EDEMA		DRYNESS		COMMENTS
		Right	Left	Right	Left	Right	Left	
43.	84377	0	0	0	0	0	0	
44.	92895	0	0	0	0	0	0	
45.	93104	0	0	0	0	0	0	
46.	60209	0	0	0	0	1	1	
47.	21096	0	0	0	0	0	0	
48.	93197	0	0	0	0	0	0	
49.	47949	0	0	0	0	0	0	
50.	92889	0	0	0	0	0	0	
51.	*	*	*	*	*	*	*	
52.	92706	0	0	0	0	0	0	
53.	39164	1	1	0	0	0	0	
54.	93153	0	0	0	0	0	0	
55.	71893	0	0	0	0	0	0	
56.	91393	0	0	0	0	0	0	

#51 will not be used – deviation

Table 2
(continued)

DERMATOLOGICAL EVALUATIONS – QUALIFICATION (DAY 0)

TEST SITE: Eyelid (along upper lash line)
TEST MATERIAL: [redacted] Lash Enhancing Serum

EVALUATION SCALE

0 = NONE
0.5 = BARELY PERCEPTIBLE
1 = MILD
2 = MODERATE
3 = MARKED
4 = SEVERE

Any subject exhibiting a score of ≥2 for any one parameter will be disqualified.

Subject # & ID		ERYTHEMA		EDEMA		DRYNESS		COMMENTS
		Right	Left	Right	Left	Right	Left	
57.	90080	1	1	0	0	0	0	
58.	93083	0	0	0	0	0	0	
59.	91137	0	0	0	0	0	0	
60.	92158	1	1	0	0	0	0	
61.	93210	0	0	0	0	0	0	
62.	88490	1	1	0	0	0	0	
63.	87998	0	0	0	0	0	0	
64.	93160	0	0	0	0	0	0	
65.	46058	0	0	0	0	0	0	
66.	70499	0	0	0	0	0	0	
67.	93152	0	0	0	0	0	0	
68.	91812	0	0	0	0	0	0	
69.	56034	0	0	0	0	0	0	
70.	80386	0	0	0	0	0	0	

Table 2
(continued)

DERMATOLOGICAL EVALUATIONS – QUALIFICATION (DAY 0)

TEST SITE: Eyelid (along upper lash line)
TEST MATERIAL [redacted] Lash Enhancing Serum

EVALUATION SCALE

0 = NONE
0.5 = BARELY PERCEPTIBLE
1 = MILD
2 = MODERATE
3 = MARKED
4 = SEVERE

Any subject exhibiting a score of ≥2 for any one parameter will be disqualified.

Subject # & ID		ERYTHEMA		EDEMA		DRYNESS		COMMENTS
		Right	Left	Right	Left	Right	Left	
71.	63555	0	0	0	0	0	0	
72.	93198	0	0	0	0	0	0	
73.	92065	0	0	0	0	0	0	
74.	91636	0	0	0	0	0	0	
75.	93018	1	1	0	0	0	0	
76.	77962	0	0	0	0	0	0	
77.	90494	0	0	0	0	0	0	
78.	60639	1	1	0	0	0	0	
79.	92756	0	0	0	0	0	0	
80.	90099	0	0	0	0	0	0	
81.	78294	1	1	0	0	0	0	
82.	93044	0	0	0	0	0	0	
83.	93196	0	0	0	0	0	0	
84.	91200	0	0	0	0	0	0	

Table 2
(continued)

DERMATOLOGICAL EVALUATIONS – QUALIFICATION (DAY 0)

TEST SITE: Eyelid (along upper lash line)
TEST MATERIAL: [redacted] Lash Enhancing Serum

EVALUATION SCALE

0 = NONE
0.5 = BARELY PERCEPTIBLE
1 = MILD
2 = MODERATE
3 = MARKED
4 = SEVERE

Any subject exhibiting a score of ≥2 for any one parameter will be disqualified.

Subject # & ID		ERYTHEMA		EDEMA		DRYNESS		COMMENTS
		Right	Left	Right	Left	Right	Left	
85.	92803	0	0	0	0	0	0	
86.	71209	0	0	0	0	0	0	
87.	91745	0	0	0	0	0	0	
88.	58577	1	1	0	0	0	0	
89.	92282	0	0	0	0	0	0	
90.	4284	0	0	0	0	0	0	
91.	92854	0	0	0	0	0	0	
92.	48073	0	0	0	0	0	0	
93.	92836	1	1	0	0	0	0	
94.	45824	0	0	0	0	0	0	
95.	61969	0	0	0	0	0	0	
96.	84567	0	0	0	0	0	0	
97.	92840	0	0	0	0	0	0	
98.	76099	0	0	0	0	0	0	

Table 2
(continued)

DERMATOLOGICAL EVALUATIONS – QUALIFICATION (DAY 0)

TEST SITE: Eyelid (along upper lash line)
TEST MATERIAL: [redacted] Lash Enhancing Serum

EVALUATION SCALE

0 = NONE
0.5 = BARELY PERCEPTIBLE
1 = MILD
2 = MODERATE
3 = MARKED
4 = SEVERE

Any subject exhibiting a score of ≥2 for any one parameter will be disqualified.

Subject # & ID		ERYTHEMA		EDEMA		DRYNESS		COMMENTS
		Right	Left	Right	Left	Right	Left	
99.	92770	0	0	0	0	0	0	
100.	93235	0	0	0	0	0	0	
101.	93229	0	0	0	0	0	0	
102.	66648	0	0	0	0	0	0	
103.	75527	0	0	0	0	0	0	
104.	81232	0	0	0	0	0	0	
105.	92715	0	0	0	0	0	0	
106.	47011	0	0	0	0	0	0	
107.	67311	0	0	0	0	0	0	
108.	62190	0	0	0	0	0	0	
109.	93213	0	0	0	0	0	0	
110.	93234	0	0	0	0	0	0	
111.	65407	0	0	0	0	0	0	
112.	42734	0	0	0	0	0	0	

Table 2
(continued)

DERMATOLOGICAL EVALUATIONS – QUALIFICATION (DAY 0)

TEST SITE: Eyelid (along upper lash line)
TEST MATERIAL: [redacted] Lash Enhancing Serum

EVALUATION SCALE

0 = NONE
0.5 = BARELY PERCEPTIBLE
1 = MILD
2 = MODERATE
3 = MARKED
4 = SEVERE

Any subject exhibiting a score of ≥2 for any one parameter will be disqualified.

Subject # & ID		ERYTHEMA		EDEMA		DRYNESS		COMMENTS
		Right	Left	Right	Left	Right	Left	
113.	92456	0	0	0	0	0	0	
114.	91044	0	0	0	0	0	0	
115.	84560	0	0	0	0	0	0	
116.	87722	0	0	0	0	0	0	
117.	91445	0	0	0	0	0	0	
118.	72693	0	0	0	0	0	0	
119.	67863	0	0	0	0	0	0	
120.	92316	0	0	0	0	0	0	
121.	84404	0	0	0	0	0	0	

Table 2
(continued)

DERMATOLOGICAL EVALUATIONS – WEEK 4

TEST SITE: Eyelid (along upper lash line)
TEST MATERIAL: [redacted] Lash Enhancing Serum

EVALUATION SCALE

0 = NONE
0.5 = BARELY PERCEPTIBLE
1 = MILD
2 = MODERATE
3 = MARKED
4 = SEVERE

Any subject exhibiting a score of ≥2 for any one parameter will be disqualified.

Subject # & ID		ERYTHEMA		EDEMA		DRYNESS		COMMENTS
		Right	Left	Right	Left	Right	Left	
1.	56135	0	0	0	0	0	0	
2.	87855	0	0	0	0	0	0	
3.	88888	0	0	0	0	0	0	
4.	86779	0	0	0	0	0	0	
5.	89104	0	0	0	0	0	0	
6.	88905	0	0	0	0	0	0	
7.	92956	-	-	-	-	-	-	Did Not Complete
8.	92429	0	0	0	0	0	0	
9.	58976	0	0	0	0	0	0	
10.	91854	0	0	0	0	0	0	
11.	91927	0	0	0	0	0	0	
12.	75855	0	0	0	0	0	0	
13.	69699	0	0	0	0	0	0	
14.	66875	0	0	0	0	0	0	

Table 2
(continued)

DERMATOLOGICAL EVALUATIONS – WEEK 4

TEST SITE: Eyelid (along upper lash line)
TEST MATERIAL: [redacted] Lash Enhancing Serum

EVALUATION SCALE

0 = NONE
0.5 = BARELY PERCEPTIBLE
1 = MILD
2 = MODERATE
3 = MARKED
4 = SEVERE

Any subject exhibiting a score of ≥2 for any one parameter will be disqualified.

Subject # & ID		ERYTHEMA		EDEMA		DRYNESS		COMMENTS
		Right	Left	Right	Left	Right	Left	
15.	92592	0	0	0	0	0	0	
16.	87173	0	0	0	0	0	0	
17.	64690	1	1	0	0	0	0	
18.	92994	0	0	0	0	0	0	
19.	93102	0	0	0	0	0	0	
20.	25118	0	0	0	0	0	0	
21.	72896	0	0	0	0	0	0	
22.	92978	0	0	0	0	0	0	
23.	85372	0	0	0	0	0	0	
24.	77457	0	0	0	0	0	0	
25.	89254	0	0	0	0	0	0	
26.	90669	0	0	0	0	0	0	
27.	68629	0	0	0	0	0	0	
28.	93146	0	0	0	0	0	0	

Table 2
(continued)

DERMATOLOGICAL EVALUATIONS – WEEK 4

TEST SITE: Eyelid (along upper lash line)
TEST MATERIAL: [redacted] Lash Enhancing Serum

EVALUATION SCALE

0 = NONE
0.5 = BARELY PERCEPTIBLE
1 = MILD
2 = MODERATE
3 = MARKED
4 = SEVERE

Any subject exhibiting a score of ≥2 for any one parameter will be disqualified.

Subject # & ID		ERYTHEMA		EDEMA		DRYNESS		COMMENTS
		Right	Left	Right	Left	Right	Left	
29.	47917	0	0	0	0	0	0	
30.	93150	0	0	0	0	0	0	
31.	93151	0	0	0	0	0	0	
32.	49868	0	0	0	0	0	0	
33.	92615	0	0	0	0	0	0	
34.	76837	0	0	0	0	0	0	
35.	89198	0	0	0	0	0	0	
36.	80522	0	0	0	0	0	0	
37.	92575	0	0	0	0	0	0	
38.	92096	0	0	0	0	0	0	
39.	63350	0	0	0	0	0	0	
40.	93162	0	0	0	0	0	0	
41.	91523	0	0	0	0	0	0	
42.	66549	0	0	0	0	0	0	

Table 2
(continued)

DERMATOLOGICAL EVALUATIONS – WEEK 4

TEST SITE: Eyelid (along upper lash line)
TEST MATERIAL: [redacted] Lash Enhancing Serum

EVALUATION SCALE

0 = NONE
0.5 = BARELY PERCEPTIBLE
1 = MILD
2 = MODERATE
3 = MARKED
4 = SEVERE

Any subject exhibiting a score of ≥2 for any one parameter will be disqualified.

Subject # & ID		ERYTHEMA		EDEMA		DRYNESS		COMMENTS
		Right	Left	Right	Left	Right	Left	
43.	84377	0	0	0	0	0	0	
44.	92895	0	0	0	0	0	0	
45.	93104	0	0	0	0	0	0	
46.	60209	0	0	0	0	0	0	
47.	21096	0	0	0	0	0	0	
48.	93197	0	0	0	0	0	0	
49.	47949	0	0	0	0	0	0	
50.	92889	0	0	0	0	0	0	
51.	*	*	*	*	*	*	*	
52.	92706	0	0	0	0	0	0	
53.	39164	0	0	0	0	0	0	
54.	93153	0	0	0	0	0	0	
55.	71893	0	0	0	0	0	0	
56.	91393	0	0	0	0	0	0	

#51 will not be used – deviation

Table 2
(continued)

DERMATOLOGICAL EVALUATIONS – WEEK 4

TEST SITE: Eyelid (along upper lash line)
TEST MATERIAL: [redacted] Lash Enhancing Serum

EVALUATION SCALE

0 = NONE
0.5 = BARELY PERCEPTIBLE
1 = MILD
2 = MODERATE
3 = MARKED
4 = SEVERE

Any subject exhibiting a score of ≥2 for any one parameter will be disqualified.

Subject # & ID		ERYTHEMA		EDEMA		DRYNESS		COMMENTS
		Right	Left	Right	Left	Right	Left	
57.	90080	0	0	0	0	0	0	
58.	93083	-	-	-	-	-	-	Did Not Complete
59.	91137	0	0	0	0	0	0	
60.	92158	0	0	0	0	0	0	
61.	93210	0	0	0	0	0	0	
62.	88490	0	0	0	0	0	0	
63.	87998	0	0	0	0	0	0	
64.	93160	0	0	0	0	0	0	
65.	46058	0	0	0	0	0	0	
66.	70499	0	0	0	0	0	0	
67.	93152	0	0	0	0	0	0	
68.	91812	0	0	0	0	0	0	
69.	56034	0	0	0	0	0	0	
70.	80386	0	0	0	0	0	0	

Table 2
(continued)

DERMATOLOGICAL EVALUATIONS – WEEK 4

TEST SITE: Eyelid (along upper lash line)
TEST MATERIAL [redacted] Lash Enhancing Serum

EVALUATION SCALE

0 = NONE
0.5 = BARELY PERCEPTIBLE
1 = MILD
2 = MODERATE
3 = MARKED
4 = SEVERE

Any subject exhibiting a score of ≥2 for any one parameter will be disqualified.

Subject # & ID		ERYTHEMA		EDEMA		DRYNESS		COMMENTS
		Right	Left	Right	Left	Right	Left	
71.	63555	0	0	0	0	0	0	
72.	93198	0	0	0	0	0	0	
73.	92065	0	0	0	0	0	0	
74.	91636	0	0	0	0	0	0	
75.	93018	0	0	0	0	0	0	
76.	77962	0	0	0	0	0	0	
77.	90494	0	0	0	0	0	0	
78.	60639	0	0	0	0	0	0	
79.	92756	0	0	0	0	0	0	
80.	90099	0	0	0	0	0	0	
81.	78294	0	0	0	0	0	0	
82.	93044	0	0	0	0	0	0	
83.	93196	-	-	-	-	-	-	Did Not Complete
84.	91200	0	0	0	0	0	0	

Table 2
(continued)

DERMATOLOGICAL EVALUATIONS – WEEK 4

TEST SITE: Eyelid (along upper lash line)
TEST MATERIAL: [redacted] Lash Enhancing Serum

EVALUATION SCALE

0 = NONE
0.5 = BARELY PERCEPTIBLE
1 = MILD
2 = MODERATE
3 = MARKED
4 = SEVERE

Any subject exhibiting a score of ≥2 for any one parameter will be disqualified.

Subject # & ID		ERYTHEMA		EDEMA		DRYNESS		COMMENTS
		Right	Left	Right	Left	Right	Left	
85.	92803	-	-	-	-	-	-	Did Not Complete
86.	71209	0	0	0	0	0	0	
87.	91745	0	0	0	0	0	0	
88.	58577	0	0	0	0	0	0	
89.	92282	0	0	0	0	0	0	
90.	4284	0	0	0	0	0	0	
91.	92854	0	0	0	0	0	0	
92.	48073	0	0	0	0	0	0	
93.	92836	0	0	0	0	0	0	
94.	45824	0	0	0	0	0	0	
95.	61969	0	0	0	0	0	0	
96.	84567	0	0	0	0	0	0	
97.	92840	0	0	0	0	0	0	
98.	76099	0	0	0	0	0	0	

Table 2
(continued)

DERMATOLOGICAL EVALUATIONS – WEEK 4

TEST SITE: Eyelid (along upper lash line)
TEST MATERIAL: [redacted] Lash Enhancing Serum

EVALUATION SCALE

0 = NONE
0.5 = BARELY PERCEPTIBLE
1 = MILD
2 = MODERATE
3 = MARKED
4 = SEVERE

Any subject exhibiting a score of ≥2 for any one parameter will be disqualified.

Subject # & ID		ERYTHEMA		EDEMA		DRYNESS		COMMENTS
		Right	Left	Right	Left	Right	Left	
99.	92770	0	0	0	0	0	0	
100.	93235	0	0	0	0	0	0	
101.	93229	0	0	0	0	0	0	
102.	66648	0	0	0	0	0	0	
103.	75527	0	0	0	0	0	0	
104.	81232	0	0	0	0	0	0	
105.	92715	0	0	0	0	0	0	
106.	47011	0	0	0	0	0	0	
107.	67311	0	0	0	0	0	0	
108.	62190	0	0	0	0	0	0	
109.	93213	0	0	0	0	0	0	
110.	93234	0	0	0	0	0	0	
111.	65407	0	0	0	0	0	0	
112.	42734	0	0	0	0	0	0	

Table 2
(continued)

DERMATOLOGICAL EVALUATIONS – WEEK 4

TEST SITE: Eyelid (along upper lash line)
TEST MATERIAL: [redacted] Lash Enhancing Serum

EVALUATION SCALE

0 = NONE
0.5 = BARELY PERCEPTIBLE
1 = MILD
2 = MODERATE
3 = MARKED
4 = SEVERE

Any subject exhibiting a score of ≥2 for any one parameter will be disqualified.

Subject # & ID		ERYTHEMA		EDEMA		DRYNESS		COMMENTS
		Right	Left	Right	Left	Right	Left	
113.	92456	0	0	0	0	0	0	
114.	91044	0	0	0	0	0	0	
115.	84560	0	0	0	0	0	0	
116.	87722	0	0	0	0	0	0	
117.	91445	0	0	0	0	0	0	
118.	72693	0	0	0	0	0	0	
119.	67863	0	0	0	0	0	0	
120.	92316	0	0	0	0	0	0	
121.	84404	0	0	0	0	0	0	

Table 2
(continued)

DERMATOLOGICAL EVALUATIONS – WEEK 8

TEST SITE: Eyelid (along upper lash line)
TEST MATERIAL: [redacted] Lash Enhancing Serum

EVALUATION SCALE

0 = NONE
0.5 = BARELY PERCEPTIBLE
1 = MILD
2 = MODERATE
3 = MARKED
4 = SEVERE

Any subject exhibiting a score of ≥2 for any one parameter will be disqualified.

Subject # & ID		ERYTHEMA		EDEMA		DRYNESS		COMMENTS
		Right	Left	Right	Left	Right	Left	
1.	56135	0	0	0	0	0	0	
2.	87855	0	0	0	0	0	0	
3.	88888	0	0	0	0	0	0	
4.	86779	0	0	0	0	0	0	
5.	89104	0	0	0	0	0	0	
6.	88905	0	0	0	0	0	0	
7.	92956	-	-	-	-	-	-	Did Not Complete
8.	92429	0	0	0	0	0	0	
9.	58976	0	0	0	0	0	0	
10.	91854	0	0	0	0	0	0	
11.	91927	0	0	0	0	0	0	
12.	75855	0	0	0	0	0	0	
13.	69699	0	0	0	0	0	0	
14.	66875	0	0	0	0	0	0	

Table 2
(continued)

DERMATOLOGICAL EVALUATIONS – WEEK 8

TEST SITE: Eyelid (along upper lash line)
TEST MATERIAL: [redacted] Lash Enhancing Serum

EVALUATION SCALE

0 = NONE
0.5 = BARELY PERCEPTIBLE
1 = MILD
2 = MODERATE
3 = MARKED
4 = SEVERE

Any subject exhibiting a score of ≥2 for any one parameter will be disqualified.

Subject # & ID		ERYTHEMA		EDEMA		DRYNESS		COMMENTS
		Right	Left	Right	Left	Right	Left	
15.	92592	0	0	0	0	0	0	
16.	87173	0	0	0	0	0	0	
17.	64690	1	1	0	0	0	0	
18.	92994	0	0	0	0	0	0	
19.	93102	0	0	0	0	0	0	
20.	25118	0	0	0	0	0	0	
21.	72896	0	0	0	0	0	0	
22.	92978	0	0	0	0	0	0	
23.	85372	0	0	0	0	0	0	
24.	77457	0	0	0	0	0	0	
25.	89254	0	0	0	0	0	0	
26.	90669	0	0	0	0	0	0	
27.	68629	0	0	0	0	0	0	
28.	93146	0	0	0	0	0	0	

Table 2
(continued)

DERMATOLOGICAL EVALUATIONS – WEEK 8

TEST SITE: Eyelid (along upper lash line)
TEST MATERIAL: [redacted] Lash Enhancing Serum

EVALUATION SCALE

0 = NONE
0.5 = BARELY PERCEPTIBLE
1 = MILD
2 = MODERATE
3 = MARKED
4 = SEVERE

Any subject exhibiting a score of ≥2 for any one parameter will be disqualified.

Subject # & ID		ERYTHEMA		EDEMA		DRYNESS		COMMENTS
		Right	Left	Right	Left	Right	Left	
29.	47917	0	0	0	0	0	0	
30.	93150	0	0	0	0	0	0	
31.	93151	0	0	0	0	0	0	
32.	49868	0	0	0	0	0	0	
33.	92615	0	0	0	0	0	0	
34.	76837	0	0	0	0	0	0	
35.	89198	0	0	0	0	0	0	
36.	80522	0	0	0	0	0	0	
37.	92575	0	0	0	0	0	0	
38.	92096	0	0	0	0	0	0	
39.	63350	0	0	0	0	0	0	
40.	93162	0	0	0	0	0	0	
41.	91523	0	0	0	0	0	0	
42.	66549	0	0	0	0	0	0	

Table 2
(continued)

DERMATOLOGICAL EVALUATIONS – WEEK 8

TEST SITE: Eyelid (along upper lash line)
TEST MATERIAL: [redacted] Lash Enhancing Serum

EVALUATION SCALE

0 = NONE
0.5 = BARELY PERCEPTIBLE
1 = MILD
2 = MODERATE
3 = MARKED
4 = SEVERE

Any subject exhibiting a score of ≥2 for any one parameter will be disqualified.

Subject # & ID		ERYTHEMA		EDEMA		DRYNESS		COMMENTS
		Right	Left	Right	Left	Right	Left	
43.	84377	0	0	0	0	0	0	
44.	92895	0	0	0	0	0	0	
45.	93104	0	0	0	0	0	0	
46.	60209	0	0	0	0	0	0	
47.	21096	0	0	0	0	0	0	
48.	93197	0	0	0	0	0	0	
49.	47949	0	0	0	0	0	0	
50.	92889	0	0	0	0	0	0	
51.	*	*	*	*	*	*	*	
52.	92706	0	0	0	0	0	0	
53.	39164	0	0	0	0	0	0	
54.	93153	0	0	0	0	0	0	
55.	71893	0	0	0	0	0	0	
56.	91393	0	0	0	0	0	0	

#51 will not be used – deviation

Table 2
(continued)

DERMATOLOGICAL EVALUATIONS – WEEK 8

TEST SITE: Eyelid (along upper lash line)
TEST MATERIAL: [redacted] Lash Enhancing Serum

EVALUATION SCALE

0 = NONE
0.5 = BARELY PERCEPTIBLE
1 = MILD
2 = MODERATE
3 = MARKED
4 = SEVERE

Any subject exhibiting a score of ≥2 for any one parameter will be disqualified.

Subject # & ID		ERYTHEMA		EDEMA		DRYNESS		COMMENTS
		Right	Left	Right	Left	Right	Left	
57.	90080	0	0	0	0	0	0	
58.	93083	-	-	-	-	-	-	Did Not Complete
59.	91137	0	0	0	0	0	0	
60.	92158	0	0	0	0	0	0	
61.	93210	0	0	0	0	0	0	
62.	88490	0	0	0	0	0	0	
63.	87998	0	0	0	0	0	0	
64.	93160	0	0	0	0	0	0	
65.	46058	0	0	0	0	0	0	
66.	70499	0	0	0	0	0	0	
67.	93152	0	0	0	0	0	0	
68.	91812	0	0	0	0	0	0	
69.	56034	0	0	0	0	0	0	
70.	80386	0	0	0	0	0	0	

Table 2
(continued)

DERMATOLOGICAL EVALUATIONS – WEEK 8

TEST SITE: Eyelid (along upper lash line)
TEST MATERIAL [redacted] Lash Enhancing Serum

EVALUATION SCALE

0 = NONE
0.5 = BARELY PERCEPTIBLE
1 = MILD
2 = MODERATE
3 = MARKED
4 = SEVERE

Any subject exhibiting a score of ≥2 for any one parameter will be disqualified.

Subject # & ID		ERYTHEMA		EDEMA		DRYNESS		COMMENTS
		Right	Left	Right	Left	Right	Left	
71.	63555	0	0	0	0	0	0	
72.	93198	0	0	0	0	0	0	
73.	92065	0	0	0	0	0	0	
74.	91636	0	0	0	0	0	0	
75.	93018	0	0	0	0	0	0	
76.	77962	0	0	0	0	0	0	
77.	90494	0	0	0	0	0	0	
78.	60639	0	0	0	0	0	0	
79.	92756	0	0	0	0	0	0	
80.	90099	0	0	0	0	0	0	
81.	78294	0	0	0	0	0	0	
82.	93044	0	0	0	0	0	0	
83.	93196	-	-	-	-	-	-	Did Not Complete
84.	91200	0	0	0	0	0	0	

Table 2
(continued)

DERMATOLOGICAL EVALUATIONS – WEEK 8

TEST SITE: Eyelid (along upper lash line)
TEST MATERIAL: [redacted] Lash Enhancing Serum

EVALUATION SCALE

0 = NONE
0.5 = BARELY PERCEPTIBLE
1 = MILD
2 = MODERATE
3 = MARKED
4 = SEVERE

Any subject exhibiting a score of ≥2 for any one parameter will be disqualified.

Subject # & ID		ERYTHEMA		EDEMA		DRYNESS		COMMENTS
		Right	Left	Right	Left	Right	Left	
85.	92803	-	-	-	-	-	-	Did Not Complete
86.	71209	0	0	0	0	0	0	
87.	91745	0	0	0	0	0	0	
88.	58577	0	0	0	0	0	0	
89.	92282	0	0	0	0	0	0	
90.	4284	0	0	0	0	0	0	
91.	92854	0	0	0	0	0	0	
92.	48073	0	0	0	0	0	0	
93.	92836	0	0	0	0	0	0	
94.	45824	0	0	0	0	0	0	
95.	61969	0	0	0	0	0	0	
96.	84567	0	0	0	0	0	0	
97.	92840	0	0	0	0	0	0	
98.	76099	0	0	0	0	0	0	

Table 2
(continued)

DERMATOLOGICAL EVALUATIONS – WEEK 8

TEST SITE: Eyelid (along upper lash line)
TEST MATERIAL: [redacted] Lash Enhancing Serum

EVALUATION SCALE

0 = NONE
0.5 = BARELY PERCEPTIBLE
1 = MILD
2 = MODERATE
3 = MARKED
4 = SEVERE

Any subject exhibiting a score of ≥2 for any one parameter will be disqualified.

Any subject Subject # & ID		ERYTHEMA		EDEMA		DRYNESS		COMMENTS
		Right	Left	Right	Left	Right	Left	
99.	92770	0	0	0	0	0	0	
100.	93235	0	0	0	0	0	0	
101.	93229	0	0	0	0	0	0	
102.	66648	0	0	0	0	0	0	
103.	75527	0	0	0	0	0	0	
104.	81232	0	0	0	0	0	0	
105.	92715	0	0	0	0	0	0	
106.	47011	0	0	0	0	0	0	
107.	67311	0	0	0	0	0	0	
108.	62190	0	0	0	0	0	0	
109.	93213	0	0	0	0	0	0	
110.	93234	0	0	0	0	0	0	
111.	65407	0	0	0	0	0	0	
112.	42734	0	0	0	0	0	0	

Table 2
(continued)

DERMATOLOGICAL EVALUATIONS – WEEK 8

TEST SITE: Eyelid (along upper lash line)
TEST MATERIAL: [redacted] Lash Enhancing Serum

EVALUATION SCALE

0 = NONE
0.5 = BARELY PERCEPTIBLE
1 = MILD
2 = MODERATE
3 = MARKED
4 = SEVERE

Any subject exhibiting a score of ≥2 for any one parameter will be disqualified.

Subject # & ID		ERYTHEMA		EDEMA		DRYNESS		COMMENTS
		Right	Left	Right	Left	Right	Left	
113.	92456*	0	0	0	0	0	0	
114.	91044	0	0	0	0	0	0	
115.	84560	0	0	0	0	0	0	
116.	87722	0	0	0	0	0	0	
117.	91445	0	0	0	0	0	0	
118.	72693	0	0	0	0	0	0	
119.	67863	0	0	0	0	0	0	
120.	92316	0	0	0	0	0	0	
121.	84404	0	0	0	0	0	0	

*Subject #113 removed from the trial after derm exam due to an adverse event.

Table 2
(continued)

DERMATOLOGICAL EVALUATIONS – WEEK 16

TEST SITE: Eyelid (along upper lash line)
TEST MATERIAL: [redacted] Lash Enhancing Serum

EVALUATION SCALE

0 = NONE
0.5 = BARELY PERCEPTIBLE
1 = MILD
2 = MODERATE
3 = MARKED
4 = SEVERE

Any subject exhibiting a score of ≥2 for any one parameter will be disqualified.

Subject # & ID		ERYTHEMA		EDEMA		DRYNESS		COMMENTS
		Right	Left	Right	Left	Right	Left	
1.	56135	0	0	0	0	0	0	
2.	87855	0	0	0	0	0	0	
3.	88888	0	0	0	0	0	0	
4.	86779	0	0	0	0	0	0	
5.	89104	0	0	0	0	0	0	
6.	88905	0	0	0	0	0	0	
7.	92956	-	-	-	-	-	-	Did Not Complete
8.	92429	0	0	0	0	0	0	
9.	58976	0	0	0	0	0	0	
10.	91854	0	0	0	0	0	0	
11.	91927	0	0	0	0	0	0	
12.	75855	0	0	0	0	0	0	
13.	69699	0	0	0	0	0	0	
14.	66875	0	0	0	0	0	0	

Table 2
(continued)

DERMATOLOGICAL EVALUATIONS – WEEK 16

TEST SITE: Eyelid (along upper lash line)
TEST MATERIAL: [redacted] Lash Enhancing Serum

EVALUATION SCALE

0 = NONE
0.5 = BARELY PERCEPTIBLE
1 = MILD
2 = MODERATE
3 = MARKED
4 = SEVERE

Any subject exhibiting a score of ≥2 for any one parameter will be disqualified.

Subject # & ID		ERYTHEMA		EDEMA		DRYNESS		COMMENTS
		Right	Left	Right	Left	Right	Left	
15.	92592	0	0	0	0	0	0	
16.	87173	0	0	0	0	0	0	
17.	64690	0	0	0	0	0	0	
18.	92994	0	0	0	0	0	0	
19.	93102	0	0	0	0	0	0	
20.	25118	0	0	0	0	0	0	
21.	72896	0	0	0	0	0	0	
22.	92978	0	0	0	0	0	0	
23.	85372	0	0	0	0	0	0	
24.	77457	0	0	0	0	0	0	
25.	89254	0	0	0	0	0	0	
26.	90669	0	0	0	0	0	0	
27.	68629	0	0	0	0	0	0	
28.	93146	0	0	0	0	0	0	

Table 2
(continued)

DERMATOLOGICAL EVALUATIONS – WEEK 16

TEST SITE: Eyelid (along upper lash line)
TEST MATERIAL: [redacted] Lash Enhancing Serum

EVALUATION SCALE

0 = NONE
0.5 = BARELY PERCEPTIBLE
1 = MILD
2 = MODERATE
3 = MARKED
4 = SEVERE

Any subject exhibiting a score of ≥2 for any one parameter will be disqualified.

Subject # & ID		ERYTHEMA		EDEMA		DRYNESS		COMMENTS
		Right	Left	Right	Left	Right	Left	
29.	47917	0	0	0	0	0	0	
30.	93150	0	0	0	0	0	0	
31.	93151	0	0	0	0	0	0	
32.	49868	0	0	0	0	0	0	
33.	92615	0	0	0	0	0	0	
34.	76837	0	0	0	0	0	0	
35.	89198	0	0	0	0	0	0	
36.	80522	0	0	0	0	0	0	
37.	92575	0	0	0	0	0	0	
38.	92096	0	0	0	0	0	0	
39.	63350	0	0	0	0	0	0	
40.	93162	0	0	0	0	0	0	
41.	91523	0	0	0	0	0	0	
42.	66549	0	0	0	0	0	0	

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Table 2
(continued)

DERMATOLOGICAL EVALUATIONS – WEEK 16

TEST SITE: Eyelid (along upper lash line)
TEST MATERIAL: [redacted] Lash Enhancing Serum

EVALUATION SCALE

0 = NONE
0.5 = BARELY PERCEPTIBLE
1 = MILD
2 = MODERATE
3 = MARKED
4 = SEVERE

Any subject exhibiting a score of ≥2 for any one parameter will be disqualified.

Subject # & ID		ERYTHEMA		EDEMA		DRYNESS		COMMENTS
		Right	Left	Right	Left	Right	Left	
43.	84377	0	0	0	0	0	0	
44.	92895	0	0	0	0	0	0	
45.	93104	0	0	0	0	0	0	
46.	60209	0	0	0	0	0	0	
47.	21096	0	0	0	0	0	0	
48.	93197	0	0	0	0	0	0	
49.	47949	0	0	0	0	0	0	
50.	92889	0	0	0	0	0	0	
51.	*	*	*	*	*	*	*	
52.	92706	0	0	0	0	0	0	
53.	39164	0	0	0	0	0	0	
54.	93153	0	0	0	0	0	0	
55.	71893	0	0	0	0	0	0	
56.	91393	0	0	0	0	0	0	

#51 will not be used – deviation

Table 2
(continued)

DERMATOLOGICAL EVALUATIONS – WEEK 16

TEST SITE: Eyelid (along upper lash line)
TEST MATERIAL: [redacted] Lash Enhancing Serum

EVALUATION SCALE

0 = NONE
0.5 = BARELY PERCEPTIBLE
1 = MILD
2 = MODERATE
3 = MARKED
4 = SEVERE

Any subject exhibiting a score of ≥2 for any one parameter will be disqualified.

Subject # & ID		ERYTHEMA		EDEMA		DRYNESS		COMMENTS
		Right	Left	Right	Left	Right	Left	
57.	90080	0	0	0	0	0	0	
58.	93083	-	-	-	-	-	-	Did Not Complete
59.	91137	0	0	0	0	0	0	
60.	92158	0	0	0	0	0	0	
61.	93210	0	0	0	0	0	0	
62.	88490	0	0	0	0	0	0	
63.	87998	0	0	0	0	0	0	
64.	93160	0	0	0	0	0	0	
65.	46058	0	0	0	0	0	0	
66.	70499	0	0	0	0	0	0	
67.	93152	0	0	0	0	0	0	
68.	91812	0	0	0	0	0	0	
69.	56034	0	0	0	0	0	0	
70.	80386	0	0	0	0	0	0	

Table 2
(continued)

DERMATOLOGICAL EVALUATIONS – WEEK 16

TEST SITE: Eyelid (along upper lash line)
TEST MATERIAL [redacted] Lash Enhancing Serum

EVALUATION SCALE

0 = NONE
0.5 = BARELY PERCEPTIBLE
1 = MILD
2 = MODERATE
3 = MARKED
4 = SEVERE

Any subject exhibiting a score of ≥2 for any one parameter will be disqualified.

Subject # & ID		ERYTHEMA		EDEMA		DRYNESS		COMMENTS
		Right	Left	Right	Left	Right	Left	
71.	63555	0	0	0	0	0	0	
72.	93198	0	0	0	0	0	0	
73.	92065	0	0	0	0	0	0	
74.	91636	0	0	0	0	0	0	
75.	93018	0	0	0	0	0	0	
76.	77962	0	0	0	0	0	0	
77.	90494	0	0	0	0	0	0	
78.	60639	0	0	0	0	0	0	
79.	92756	0	0	0	0	0	0	
80.	90099	0	0	0	0	0	0	
81.	78294	0	0	0	0	0	0	
82.	93044	0	0	0	0	0	0	
83.	93196	-	-	-	-	-	-	Did Not Complete
84.	91200	0	0	0	0	0	0	

Table 2
(continued)

DERMATOLOGICAL EVALUATIONS – WEEK 16

TEST SITE: Eyelid (along upper lash line)
TEST MATERIAL: [redacted] Lash Enhancing Serum

EVALUATION SCALE

0 = NONE
0.5 = BARELY PERCEPTIBLE
1 = MILD
2 = MODERATE
3 = MARKED
4 = SEVERE

Any subject exhibiting a score of ≥2 for any one parameter will be disqualified.

Subject # & ID		ERYTHEMA		EDEMA		DRYNESS		COMMENTS
		Right	Left	Right	Left	Right	Left	
85.	92803	-	-	-	-	-	-	Did Not Complete
86.	71209	0	0	0	0	0	0	
87.	91745	0	0	0	0	0	0	
88.	58577	0	0	0	0	0	0	
89.	92282	0	0	0	0	0	0	
90.	4284	0	0	0	0	0	0	
91.	92854	0	0	0	0	0	0	
92.	48073	0	0	0	0	0	0	
93.	92836	0	0	0	0	0	0	
94.	45824	0	0	0	0	0	0	
95.	61969	0	0	0	0	0	0	
96.	84567	0	0	0	0	0	0	
97.	92840	0	0	0	0	0	0	
98.	76099	0	0	0	0	0	0	

Table 2
(continued)

DERMATOLOGICAL EVALUATIONS – WEEK 16

TEST SITE: Eyelid (along upper lash line)
TEST MATERIAL: [redacted] Lash Enhancing Serum

EVALUATION SCALE

0 = NONE
0.5 = BARELY PERCEPTIBLE
1 = MILD
2 = MODERATE
3 = MARKED
4 = SEVERE

Any subject exhibiting a score of ≥2 for any one parameter will be disqualified.

Subject # & ID		ERYTHEMA		EDEMA		DRYNESS		COMMENTS
		Right	Left	Right	Left	Right	Left	
99.	92770	0	0	0	0	0	0	
100.	93235	0	0	0	0	0	0	
101.	93229	0	0	0	0	0	0	
102.	66648	0	0	0	0	0	0	
103.	75527	0	0	0	0	0	0	
104.	81232	0	0	0	0	0	0	
105.	92715	0	0	0	0	0	0	
106.	47011	0	0	0	0	0	0	
107.	67311	0	0	0	0	0	0	
108.	62190	0	0	0	0	0	0	
109.	93213	0	0	0	0	0	0	
110.	93234	0	0	0	0	0	0	
111.	65407	0	0	0	0	0	0	
112.	42734	0	0	0	0	0	0	

Table 2
(continued)

DERMATOLOGICAL EVALUATIONS – WEEK 16

TEST SITE: Eyelid (along upper lash line)
TEST MATERIAL: [redacted] Lash Enhancing Serum

EVALUATION SCALE

0 = NONE
0.5 = BARELY PERCEPTIBLE
1 = MILD
2 = MODERATE
3 = MARKED
4 = SEVERE

Any subject exhibiting a score of ≥2 for any one parameter will be disqualified.

Subject # & ID		ERYTHEMA		EDEMA		DRYNESS		COMMENTS
		Right	Left	Right	Left	Right	Left	
113.	92456	-	-	-	-	-	-	Did Not Complete
114.	91044	0	0	0	0	0	0	
115.	84560	0	0	0	0	0	0	
116.	87722	0	0	0	0	0	0	
117.	91445	0	0	0	0	0	0	
118.	72693	0	0	0	0	0	0	
119.	67863	0	0	0	0	0	0	
120.	92316	0	0	0	0	0	0	
121.	84404	0	0	0	0	0	0	

Table 2
(continued)

DERMATOLOGICAL EVALUATIONS – FINAL WEEK 32

TEST SITE: Eyelid (along upper lash line)
TEST MATERIAL: [redacted] Lash Enhancing Serum

EVALUATION SCALE

0 = NONE
0.5 = BARELY PERCEPTIBLE
1 = MILD
2 = MODERATE
3 = MARKED
4 = SEVERE

Any subject exhibiting a score of ≥2 for any one parameter will be disqualified.

Subject # & ID		ERYTHEMA		EDEMA		DRYNESS		COMMENTS
		Right	Left	Right	Left	Right	Left	
1.	56135	0	0	0	0	0	0	
2.	87855	0	0	0	0	0	0	
3.	88888	0	0	0	0	0	0	
4.	86779	0	0	0	0	0	0	
5.	89104	0	0	0	0	0	0	
6.	88905	0	0	0	0	0	0	
7.	92956	-	-	-	-	-	-	Did Not Complete
8.	92429	0	0	0	0	0	0	
9.	58976	0	0	0	0	0	0	
10.	91854	0	0	0	0	0	0	
11.	91927	0	0	0	0	0	0	
12.	75855	0	0	0	0	0	0	
13.	69699	0	0	0	0	0	0	
14.	66875	0	0	0	0	0	0	

Table 2
(continued)

DERMATOLOGICAL EVALUATIONS – FINAL WEEK 32

TEST SITE: Eyelid (along upper lash line)
TEST MATERIAL: [redacted] Lash Enhancing Serum

EVALUATION SCALE

0 = NONE
0.5 = BARELY PERCEPTIBLE
1 = MILD
2 = MODERATE
3 = MARKED
4 = SEVERE

Any subject exhibiting a score of ≥2 for any one parameter will be disqualified.

Subject # & ID		ERYTHEMA		EDEMA		DRYNESS		COMMENTS
		Right	Left	Right	Left	Right	Left	
15.	92592	0	0	0	0	0	0	
16.	87173	0	0	0	0	0	0	
17.	64690	0	0	0	0	0	0	
18.	92994	0	0	0	0	0	0	
19.	93102	0	0	0	0	0	0	
20.	25118	0	0	0	0	0	0	
21.	72896	0	0	0	0	0	0	
22.	92978	0	0	0	0	0	0	
23.	85372	0	0	0	0	0	0	
24.	77457	0	0	0	0	0	0	
25.	89254	0	0	0	0	0	0	
26.	90669	0	0	0	0	0	0	
27.	68629	0	0	0	0	0	0	
28.	93146	0	0	0	0	0	0	

Table 2
(continued)

DERMATOLOGICAL EVALUATIONS – FINAL WEEK 32

TEST SITE: Eyelid (along upper lash line)
TEST MATERIAL: [redacted] Lash Enhancing Serum

EVALUATION SCALE

0 = NONE
0.5 = BARELY PERCEPTIBLE
1 = MILD
2 = MODERATE
3 = MARKED
4 = SEVERE

Any subject exhibiting a score of ≥2 for any one parameter will be disqualified.

Subject # & ID		ERYTHEMA		EDEMA		DRYNESS		COMMENTS
		Right	Left	Right	Left	Right	Left	
29.	47917	0	0	0	0	0	0	
30.	93150	0	0	0	0	0	0	
31.	93151	0	0	0	0	0	0	
32.	49868	0	0	0	0	0	0	
33.	92615	0	0	0	0	0	0	
34.	76837	0	0	0	0	0	0	
35.	89198	0	0	0	0	0	0	
36.	80522	0	0	0	0	0	0	
37.	92575	0	0	0	0	0	0	
38.	92096	0	0	0	0	0	0	
39.	63350	0	0	0	0	0	0	
40.	93162	0	0	0	0	0	0	
41.	91523	0	0	0	0	0	0	
42.	66549	0	0	0	0	0	0	

Table 2
(continued)

DERMATOLOGICAL EVALUATIONS – FINAL WEEK 32

TEST SITE: Eyelid (along upper lash line)
TEST MATERIAL: [redacted] Lash Enhancing Serum

EVALUATION SCALE

0 = NONE
0.5 = BARELY PERCEPTIBLE
1 = MILD
2 = MODERATE
3 = MARKED
4 = SEVERE

Any subject exhibiting a score of ≥2 for any one parameter will be disqualified.

Subject # & ID		ERYTHEMA		EDEMA		DRYNESS		COMMENTS
		Right	Left	Right	Left	Right	Left	
43.	84377	0	0	0	0	0	0	
44.	92895	0	0	0	0	0	0	
45.	93104	0	0	0	0	0	0	
46.	60209	0	0	0	0	0	0	
47.	21096	0	0	0	0	0	0	
48.	93197	0	0	0	0	0	0	
49.	47949	0	0	0	0	0	0	
50.	92889	0	0	0	0	0	0	
51.	*	*	*	*	*	*	*	
52.	92706	0	0	0	0	0	0	
53.	39164	0	0	0	0	0	0	
54.	93153	0	0	0	0	0	0	
55.	71893	0	0	0	0	0	0	
56.	91393	0	0	0	0	0	0	

#51 will not be used – deviation

Table 2
(continued)

DERMATOLOGICAL EVALUATIONS – FINAL WEEK 32

TEST SITE: Eyelid (along upper lash line)
TEST MATERIAL: [redacted] Lash Enhancing Serum

EVALUATION SCALE

0 = NONE
0.5 = BARELY PERCEPTIBLE
1 = MILD
2 = MODERATE
3 = MARKED
4 = SEVERE

Any subject exhibiting a score of ≥2 for any one parameter will be disqualified.

Subject # & ID		ERYTHEMA		EDEMA		DRYNESS		COMMENTS
		Right	Left	Right	Left	Right	Left	
57.	90080	0	0	0	0	0	0	
58.	93083	-	-	-	-	-	-	Did Not Complete
59.	91137	0	0	0	0	0	0	
60.	92158	0	0	0	0	0	0	
61.	93210	0	0	0	0	0	0	
62.	88490	0	0	0	0	0	0	
63.	87998	0	0	0	0	0	0	
64.	93160	0	0	0	0	0	0	
65.	46058	0	0	0	0	0	0	
66.	70499	0	0	0	0	0	0	
67.	93152	0	0	0	0	0	0	
68.	91812	0	0	0	0	0	0	
69.	56034	0	0	0	0	0	0	
70.	80386	0	0	0	0	0	0	

Table 2
(continued)

DERMATOLOGICAL EVALUATIONS – FINAL WEEK 32

TEST SITE: Eyelid (along upper lash line)
TEST MATERIAL [redacted] Lash Enhancing Serum

EVALUATION SCALE

0 = NONE
0.5 = BARELY PERCEPTIBLE
1 = MILD
2 = MODERATE
3 = MARKED
4 = SEVERE

Any subject exhibiting a score of ≥2 for any one parameter will be disqualified.

Subject # & ID		ERYTHEMA		EDEMA		DRYNESS		COMMENTS
		Right	Left	Right	Left	Right	Left	
71.	63555	0	0	0	0	0	0	
72.	93198	0	0	0	0	0	0	
73.	92065	0	0	0	0	0	0	
74.	91636	0	0	0	0	0	0	
75.	93018	0	0	0	0	0	0	
76.	77962	0	0	0	0	0	0	
77.	90494	0	0	0	0	0	0	
78.	60639	0	0	0	0	0	0	
79.	92756	0	0	0	0	0	0	
80.	90099	0	0	0	0	0	0	
81.	78294	-	-	-	-	-	-	Did Not Complete
82.	93044	0	0	0	0	0	0	
83.	93196	-	-	-	-	-	-	Did Not Complete
84.	91200	0	0	0	0	0	0	

Table 2
(continued)

DERMATOLOGICAL EVALUATIONS – FINAL WEEK 32

TEST SITE: Eyelid (along upper lash line)
TEST MATERIAL: [redacted] Lash Enhancing Serum

EVALUATION SCALE
0 = NONE
0.5 = BARELY PERCEPTIBLE
1 = MILD
2 = MODERATE
3 = MARKED
4 = SEVERE

Any subject exhibiting a score of ≥2 for any one parameter will be disqualified.

Subject # & ID		ERYTHEMA		EDEMA		DRYNESS		COMMENTS
		Right	Left	Right	Left	Right	Left	
85.	92803	-	-	-	-	-	-	Did Not Complete
86.	71209	0	0	0	0	0	0	
87.	91745	0	0	0	0	0	0	
88.	58577	0	0	0	0	0	0	
89.	92282	0	0	0	0	0	0	
90.	4284	0	0	0	0	0	0	
91.	92854	0	0	0	0	0	0	
92.	48073	0	0	0	0	0	0	
93.	92836	0	0	0	0	0	0	
94.	45824	0	0	0	0	0	0	
95.	61969	0	0	0	0	0	0	
96.	84567	0	0	0	0	0	0	
97.	92840	0	0	0	0	0	0	
98.	76099	0	0	0	0	0	0	

Table 2
(continued)

DERMATOLOGICAL EVALUATIONS – FINAL WEEK 32

TEST SITE: Eyelid (along upper lash line)
TEST MATERIAL: [redacted]Lash Enhancing Serum

EVALUATION SCALE

0 = NONE
0.5 = BARELY PERCEPTIBLE
1 = MILD
2 = MODERATE
3 = MARKED
4 = SEVERE

Any subject exhibiting a score of ≥2 for any one parameter will be disqualified.

Subject # & ID		ERYTHEMA		EDEMA		DRYNESS		COMMENTS
		Right	Left	Right	Left	Right	Left	
99.	92770	0	0	0	0	0	0	
100.	93235	0	0	0	0	0	0	
101.	93229	0	0	0	0	0	0	
102.	66648	0	0	0	0	0	0	
103.	75527	0	0	0	0	0	0	
104.	81232	0	0	0	0	0	0	
105.	92715	0	0	0	0	0	0	
106.	47011	0	0	0	0	0	0	
107.	67311	0	0	0	0	0	0	
108.	62190	0	0	0	0	0	0	
109.	93213	0	0	0	0	0	0	
110.	93234	0	0	0	0	0	0	
111.	65407	0	0	0	0	0	0	
112.	42734	0	0	0	0	0	0	

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Table 2
(continued)

DERMATOLOGICAL EVALUATIONS – FINAL WEEK 32

TEST SITE: Eyelid (along upper lash line)
TEST MATERIAL: [redacted] Lash Enhancing Serum

EVALUATION SCALE

0 = NONE
0.5 = BARELY PERCEPTIBLE
1 = MILD
2 = MODERATE
3 = MARKED
4 = SEVERE

Any subject exhibiting a score of ≥2 for any one parameter will be disqualified.

Subject # & ID		ERYTHEMA		EDEMA		DRYNESS		COMMENTS
		Right	Left	Right	Left	Right	Left	
113.	92456	-	-	-	-	-	-	Did Not Complete
114.	91044	0	0	0	0	0	0	
115.	84560	0	0	0	0	0	0	
116.	87722	0	0	0	0	0	0	
117.	91445	0	0	0	0	0	0	
118.	72693	0	0	0	0	0	0	
119.	67863	0	0	0	0	0	0	
120.	92316	0	0	0	0	0	0	
121.	84404	0	0	0	0	0	0	

Table 3

Aeva3D-HE Positive volume (mm3) Left

[redacted] Lash Enhancing Serum

Subject #	Baseline	Month 1	Month 2	Month 4	Month 8	*Difference From Baseline At :* Month 1	Month 2	Month 4	Month 8
1	192.7	378 9	270 0	227 5	269 0	186 2	77 3	34 8	76 4
2	275 0	248 1	222 6	231 1	236 6	-26 9	-52 4	-43 9	-38.4
3	228 7	216 5	269 9	256 4	260 1	-12,2	41 2	27 7	31.4
4	228 5	259 9	273 3	255 8	363 5	31 4	44 8	27 3	134 9
5	417.1	542 4	452 0	452 6	380 3	125 4	35 0	35 5	-36 7
6	225.4	123 6	189 6	249 8	189.9	-101 8	-35 8	24.5	-35.5
8	399 6	488.4	516 8	477 4	447 2	88 9	117 2	77 9	47 6
9	292 2	271 2	247.9	297 6	304 4	-21 0	-44 2	5 4	12 3
10	482 2	523 1	527 6	555 4	446 1	40 8	45 4	73 2	-36 2
11	215 5	145 3	84 9	155 7	23 2	-70 2	-130 6	-59 7	-192 2
12	212 3	252.6	273 6	402 8	270 1	40 1	61.2	190 4	57 7
13	337 8	350 7	341 7	381 5	283 7	12 9	3 9	43 7	-54 1
14	205.7	215 7	346 9	339 3	297 2	10 0	141 2	133 6	91 5
15	312 6	226 3	236 3	272 0	278 7	-86 2	-76 3	-40 6	-33.8
16	263 6	316 3	340 7	309 9	348 3	52 7	77 1	46 4	84 7
17	331 1	240 6	227 1	222 0	252 1	-90 4	-103 9	-109 0	-79 0
18	554 2	384 9	388 1	439.2	463.5	-169 3	-166 1	-115 0	-90 7
19	377 1	448 1	528 2	445 5	439 5	71 0	151 1	68 5	62.4
20	311 0	281 3	317 0	222 1	216 9	-29 8	6 0	-88 9	-94 1
21	381 2	298 3	302 7	376 3	442 8	-83.0	-78 6	-4 9	61 6
22	199 0	136.7	163 4	164 4	161 1	-62 3	-35 6	-34 6	-37 9
23	521 2	561 0	537 2	552.5	652 1	39 8	16 0	31 3	130 9
24	415 8	408 6	576 1	458 4	491 6	-7 3	160 2	42 3	75 8
25	195 4	121 6	108 3	133 9	318 7	-73 9	-87 1	-61 5	123 3
26	409 7	415 3	396 8	462 0	465 0	5 6	-12 9	52 3	55 3
27	410 3	467 7	388 5	372 0	408 3	57 4	-21 8	-38 3	-2 0
28	559 0	544 9	507 8	514 8	559 8	-14 1	-51 1	-44 1	0 8
30	331 8	405 8	493 8	448 0	406 3	74 1	162 0	116 2	74 6
31	269 4	309 4	327 4	269 5	364 4	39 9	57 9	0 1	95 0
32	146 6	174 1	196 5	193 7	189 1	27 5	49 9	47 1	42 5
33	200 8	165 8	134 2	148 3	127 4	-35 0	-66 6	-52 5	-73 4
34	353 4	156 2	239 0	198 9	490 7	-197 3	-114 4	-154 6	137 3
35	417 1	479 2	417 4	429 0	337 9	62 0	0 3	11 9	-79 2
36	348 4	316 7	296 8	355 1	315 9	-31 7	-51 6	6 7	-32 5
37	221 0	256 8	173 8	231 3	266 3	35.7	-47 3	10 2	45 2
38	460 3	381 8	349.0	420 3	386 4	-78 5	-111 4	-40 1	-74 0
39	326 9	324 3	306 1	334 6	316 1	-2 6	-20 8	7.7	-10 8
40	141 1	129 7	105.4	76 7	145 9	-11 4	-35 7	-64 4	4 8
41	102 2	106 1	137 4	242 8	196 9	3 9	35 2	140 6	94 7
42	366 2	335 6	334 6	375 4	284 4	-30 5	-31 6	9 3	-81 8
43	298 7	326 9	241 1	274 6	348 6	28.2	-57 6	-24 1	49 9
44	480 2	304 9	384 8	345 1	436 7	-175 3	-95 4	-135 2	-43 5
45	208 5	339 6	333 0	213.8	254 0	131 2	124 5	5 3	45 5
46	335 2	441.7	371 7	452 0	450 6	106 5	36 5	116 8	115 4
47	610 3	593 2	595 7	610 1	588 9	-17 1	-14 6	-0 2	-21 4
48	331 4	300 2	204 5	265 1	235 8	-31 2	-126 9	-66 3	-95 6
49	425 2	339 8	395 1	432 4	848 3	-85 4	-30 1	-12 7	423 1
50	514 0	440 5	448 6	442 8	501 5	-73 4	-65 3	-71 2	-12 4
52	375 5	303 1	385 3	377 1	364 0	-72 4	9 8	1 6	-11 5
53	351 0	293 7	307 6	373.9	398 8	-57 3	-43 4	23 0	47 8
54	362 4	297 9	374 7	356 2	434 8	-64 5	12 3	-6 2	72 4
55	274 2	91 4	124 6	192 6	220 3	-182 8	-149 6	-81 6	-53 9
56	171 7	121 4	219 0	164 0	127 1	-50 3	47 3	-7 7	-44 6
57	160 6	301 7	196 4	103.7	174 8	141.1	35 8	-56 9	14 2
59	379 7	373 7	392 8	390 7	351 0	-5 9	13 1	11 0	-28 7
60	270 6	245 2	243 7	248.6	372.8	-25.4	-26 8	-21 9	102 3
61	378 7	323 8	282 0	298 8	381 3	-55 0	-96 8	-80 0	2 6

Table 3
(continued)
Aeva3D-HE Positive volume (mm3) Left

[redacted] **Lash Enhancing Serum**

62	304 7	294 7	367 8	340 5	255 3	-9 9	63 2	35 9	-49 3
63	513 9	510 9	542 6	534 0	541 5	-3 0	28 7	20.1	27 6
64	196 6	101 0	88 7	170 1	113 0	-95 6	-107 9	-26 5	-83 6
65	285.7	242 2	276 3	319 1	288 7	-43 4	-9 3	33 5	3 0
66	234 2	210 2	225 4	196 7	217 0	-24 0	-8 8	-37.5	-17 2
67	387 8	326 8	362 6	307 5	359 7	-61 1	-25 3	-80 4	-28.2
68	273 7	269 6	227 7	236 8	276 1	-4 1	-46 1	-36 9	2 4
69	255 2	270.6	262 3	357 3	318 1	15 4	7 1	102 1	62 9
70	439 3	366 3	433 7	427 8	384 9	-72 9	-5 5	-11 5	-54 4
71	256 3	241 0	218 3	249 1	203 7	-15 3	-38 0	-7 2	-52 6
72	167 1	179 1	88 2	157 7	136 5	12 0	-78 9	-9 3	-30 6
73	303 5	261 9	304 1	282 3	270 8	-41 6	0 5	-21 3	-32 7
74	274 4	270 9	265 2	394 1	272 9	-3 5	-9 2	119 7	-1 6
75	420 8	360 3	423.0	400 3	402 3	-60 5	2 2	-20 5	-18.5
76	389 0	411 1	689 5	390,1	365 6	22 1	300 5	1 1	-23.3
77	557 5	653 7	1110 6	594 9	772 7	96 1	553 1	37 3	215 1
78	385 6	364 5	346 9	385 8	407 6	-21 1	-38 7	0 1	22 0
79	274 6	366 6	252.0	291 8	292 9	92 0	-22 5	17 3	18 3
80	397 5	451 7	381 6	410,0	427 2	54 1	-15 9	12 5	29 6
82	336 1	299 7	321 8	327 3	342.1	-36 5	-14,3	-8 9	5 9
84	339,4	292 5	354 6	356 7	280 3	-46 9	15 3	17 4	-59 0
86	398 1	391 2	323 6	343 5	340 2	-6 9	-74 5	-54 6	-57 9
87	344 9	441 0	579 8	380 2	291 7	96 1	234 9	35 3	-53 2
88	277 0	305 4	451 7	414 3	318 4	28 4	174 7	137 3	41 4
89	393 0	378.0	398 1	374 4	391 0	-15 0	5 1	-18 6	-1 9
90	237 8	225 9	228.1	256 0	246 4	-11.9	-9 7	18 1	8 6
91	129 1	98 8	154 3	119 4	81 0	-30 3	25 2	-9 7	-48 2
92	188 2	138 2	22 7	319 8	211 2	-50 0	-165 5	131 5	23 0
93	274 1	269 8	281 2	290 1	231 9	-4 3	7 1	16 0	-22 2
94	386 1	195 9	355 7	363 6	259 4	-190 2	-30 4	-22 5	-126 7
95	280 3	317 5	279 4	272 2	229 9	37 4	-0 7	-7 8	-50 2
96	444 8	434 3	475 9	486 9	451 8	-10 5	31 1	42 1	7 0
97	215 5	249 9	95 9	84 3	0 0	34 5	-119 6	-131 2	-215 4
98	338 8	320 2	431 4	325.6	385 9	-18 6	92 6	-13 2	47,1
99	338 9	342 8	316 6	286 8	333 8	3 9	-22 3	-52 1	-5 1
100	162 2	139 3	168 8	156.6	185 0	-22 9	6 6	-5 6	22 8
101	382 4	361 3	357 6	388 0	727 7	-21 1	-24 8	5 7	345 3
102	258 9	240 2	267 3	274 0	256 7	-18 6	8 5	15 1	-2 2
103	274 5	12 4	8 5	83 2	6 5	-262 2	-266 0	-191 3	-268 0
104	299 3	298 1	275 3	329 6	308 9	-1 3	-24 1	30 3	9 6
105	562 9	565 2	622 8	604 7	533 7	2 3	59 9	41 8	-29 2
106	432 2	321 4	111 2	518 5	399 0	-110.8	-320.9	86 3	-33 2
107	445 9	429 1	485 8	366 9	360 9	-16 8	39 8	-79 0	-85 0
108	662 0	631 9	684.1	703 3	682 9	-30 1	22 1	41 3	20 9
109	355 8	411 7	340 3	426 6	340 6	55 9	-15 5	70 8	-15 8
110	317.0	367 7	347 5	50 0	89 3	50 6	30 5	-267 0	-227 8
111	263 5	220.0	248 4	204 9	266 7	-43 5	-15 1	-58 6	3 2
112	382 8	366 6	349 8	300 0	333 4	-16 2	-33 0	-82 8	-49 4
114	359 8	289 1	292 8	294 2	336 1	-70 7	-67 0	-65 6	-23 6
115	460 8	364 6	434 5	381 7	358 0	-96 3	-26 3	-79 2	-102 8
116	273.5	180 3	304 0	193 0	153 3	-93 3	30 5	-80 5	-120 2
117	372 8	363 1	368 6	358 3	377 1	-9 7	-4 2	-14 5	4 3
118	274 1	192 3	143 1	204 8	169 0	-81 8	-131 0	-69 3	-105 2
119	249 9	273 7	286 8	290 9	287 0	23 9	36 9	41 0	37 1
120	285 9	212 3	205 1	186 7	169 5	-73.6	-80 8	-99 1	-116 4
121	349 5	440 4	388 3	350,3	423.6	90 9	38 9	0,8	74 1
Mean =	**329.5**	**313.1**	**324.6**	**323.8**	**328.1**	**-16.4**	**-4.9**	**-5.7**	**-1.4**
Median =	**331.4**	**304.9**	**316.6**	**327.3**	**318.4**	**-15.3**	**-9.7**	**-0.2**	**-2.2**
Standard Deviation =	**109.0**	**123.9**	**154.9**	**124.8**	**146.0**	**71.4**	**100.6**	**69.3**	**91.5**
Maximum =	**662.0**	**653.7**	**1110.6**	**703.3**	**848.3**	**186.2**	**553.1**	**190.4**	**423.1**
Minimum =	**102.2**	**12.4**	**8.5**	**50.0**	**0.0**	**-262.2**	**-320.9**	**-267.0**	**-268.0**

Mean % Change From Baseline

Month 1	Month 2	Month 4	Month 8
-5 0%	-1 5%	-1.7%	-0 4%
Decrease	Decrease	Decrease	Decrease

Tallies & Percentages

	Month 1	Month 2	Month 4	Month 8
Total	113	113	113	113
Increased Values	39	50	56	53
Decreased Values	74	63	57	60
No Change	0	0	0	0
Increased Values	34 51%	44 25%	49 56%	46 90%
Decreased Values	65.49%	55 75%	50 44%	53.10%
No Change	0 00%	0 00%	0 00%	0.00%

Did Not Complete Subjects: 7, 51, 58, 81, 83, 85, 113
Note: Data for Subject #29 not included due to image noise

Table 3
(continued)
Aeva3D-HE Positive volume (mm3) Right

[redacted] **Lash Enhancing Serum**

						Difference From Baseline At :			
Subject #	**Baseline**	**Month 1**	**Month 2**	**Month 4**	**Month 8**	**Month 1**	**Month 2**	**Month 4**	**Month 8**
1	312 2	411 1	328 4	320 6	296 5	98 9	16 2	8 5	-15 6
2	222 1	172 9	126 6	158 2	159.1	-49 2	-95 4	-63 9	-63 0
3	263 5	245 8	283 0	265 8	225 0	-17 7	19.5	2 3	-38 4
4	264 2	281 4	315 2	288 3	288 7	17 2	51 0	24 2	24 5
5	266 5	410 9	306 1	297 7	268.9	144 5	39 7	31 2	2 4
6	181 0	118.1	167 6	175 1	155 0	-62 9	-13 5	-5 9	-26 1
8	324 0	380 9	429 1	388 5	332 3	56 9	105 1	64 5	8 3
9	259 9	247 6	240 9	234.1	278 4	-12 3	-19 0	-25 8	18 5
10	307 2	349 3	458 3	475 7	337 0	42 1	151 1	168 5	29 8
11	193 3	134 8	87 3	207 6	30 6	-58 4	-106 0	14 3	-162 7
12	205 9	238 4	260 2	361 1	290 8	32 5	54 3	155 2	85 0
13	371 4	363 6	357 8	330.2	311 2	-7 8	-13 7	-41 2	-60 2
14	214 5	214.6	286 3	295 7	330 0	0 0	71 8	81 1	115 4
15	355 1	209 5	273 7	240 1	281.0	-145 6	-81 4	-115 0	-74 1
16	244 1	228.5	218 7	212 7	282 1	-15 6	-25 3	-31.4	38.1
17	310 5	251 3	254 3	223 5	258 5	-59 2	-56 2	-86 9	-52 0
18	308 2	141.9	183 7	148 4	212.8	-166 3	-124 5	-159 8	-95 4
19	329 3	415.1	490 4	432 4	433 4	85 8	170 1	103 1	104 1
20	366 1	284 9	282 0	248 6	205 7	-81 2	-84 1	-117 6	-160 5
21	462 3	231 3	334 5	294 9	644 8	-231 0	-127 8	-167 4	182 5
22	134 0	39 6	65 2	28 9	38 7	-94.4	-68 8	-105 1	-95 3
23	611 1	609 1	615 3	629 3	646 8	-2 0	4 3	18 2	35 8
24	346 1	255 9	423 7	329 0	406 2	-90.2	77 6	-17 1	60 1
25	123 7	61 2	55 8	59 9	229 3	-62 4	-67 9	-63.8	105 6
26	436.5	484 6	427 2	527 4	487 1	48 1	-9 3	90 9	50.6
27	407 0	438 5	443 7	366 5	316 0	31 5	36 7	-40 5	-91 0
28	316 6	332 2	385 4	308 9	341 1	15 6	-11 2	-7 7	24 5
30	197 0	285 5	348 7	318 8	261 9	88.5	151 7	121 7	64 9
31	140 5	156 3	174 5	147 2	181 9	15 8	34 0	6 7	41 4
32	300 3	310 2	335 1	269 0	300 4	9 8	34 7	-31 3	0 1
33	126 6	87 5	64 5	98 9	74 3	-39 0	-62 1	-27 7	-52 3
34	279 2	67 4	73 3	59 9	402 0	-211 7	-205 9	-219 3	122 8
35	329 3	339 9	325.6	325 2	278 1	10 6	-3 7	-4 1	-51 2
36	223 9	189 9	182 5	212 8	175 2	-34 0	-41 4	-11 1	-48 7
37	185 4	208 2	81 2	190 4	83 0	22.8	-104 1	5 0	-102.4
38	428 6	305 0	324.7	372 4	417 0	-123 6	-103 9	-56 2	-11 6
39	241 8	225 7	238 1	223 0	247 7	-16 0	-3 7	-18 8	5 9
40	151 6	114 8	116 1	84 7	164 7	-36 8	-35 5	-66 9	13 1
41	131 4	120 5	151 3	311 0	208 6	-10 9	19.9	179 6	77 2
42	352 2	354 4	348 8	367 0	312 7	2 2	-3 4	14 8	-39 5
43	183 1	239 6	135 4	207 1	314 5	56 5	-47 7	24 0	131 4
44	446 0	326 5	353 7	417 8	424 7	-119 4	-92 2	-28 2	-21 3
45	141 3	274 1	275 5	163 1	179 6	132 8	134 2	21 8	38 3
46	440 3	597 9	507 1	573 8	576 7	157 6	66 7	133 5	136 4
47	450 2	450 1	441 7	498.0	449 1	0 0	-8 5	47 8	-1 1
48	424 9	308 3	314 1	375 8	284 7	-116 6	-310 8	-49 2	-140 3
49	307 5	184 7	266 9	274 0	649 7	-122 8	-40 6	-33 5	342 2
50	388 4	366 5	346 8	381 0	395 3	-21 9	-41 6	-7.4	6 9
52	358 5	299 8	385 3	335 3	336 4	-58 7	26 7	-23 2	-22 2
53	366 9	200 4	215 2	220 1	260 7	-166 5	-151 6	-146 8	-106 2
54	306 0	244 1	278 7	273 8	389 1	-61 9	-27 3	-32 3	83 1
55	278 5	164 6	190 7	188 7	274 0	-114 0	-87 8	-89 8	-4 5
56	176 3	179 5	257 1	155 1	168 3	3 2	80 8	-21 2	-8 0
57	181 1	247 9	249 0	138 7	188 1	66 8	67 9	-42.4	7 0
59	249 3	240 6	246 0	219 1	142 7	-8 7	-3 3	-30 2	-106.6
60	336 0	294.6	289 6	288 3	437.0	-41 4	-46 4	-47.8	101 0

Table 3
(continued)
Aeva3D-HE Positive volume (mm3) Right

[redacted] Lash Enhancing Serum

61	359 9	305 3	270 5	295.3	328.1	-54 5	-89 4	-64 6	-31 8
62	306 2	316 2	375 0	353 9	264 0	10 0	68 7	47 7	-42 2
63	413 3	413 5	418 5	396 8	421 9	0 1	5 2	-16 5	8 5
64	353 5	269 1	266 6	271 7	299.9	-84 3	-86 9	-81 7	-53 5
65	374 0	337 3	366 5	357 6	396 8	-36 8	-7.5	-16 4	22 7
66	210 5	191 6	194 8	180 5	197 9	-18 9	-15 6	-30 0	-12 6
67	250 8	219 1	266.5	199 7	221 6	-31 7	15 7	-51 1	-29.2
68	217 4	171 1	165 8	217 9	198 2	-46 3	-51.6	0 5	-19 3
69	235 0	19 1	7 6	17 4	24.7	-215 9	-227 4	-217 5	-210 2
70	302.1	209 6	408 3	280.9	274 2	-92 5	106 2	-21 2	-27 9
71	207 5	225 9	229 2	213 8	183 1	18 4	21 7	6 3	-24 4
72	192 4	180 6	177 5	146 9	173 6	-11 8	-15.0	-45 5	-18 8
73	357 2	348 4	363 4	321 7	320 3	-8.7	6 2	-35.5	-36 9
74	202 6	236 4	188 0	186 3	197 0	33 8	-14 6	-16 3	-5 6
75	261 6	169 5	206.7	276 4	175 4	-92.1	-54 9	14 8	-86 2
76	350 9	366 0	696 5	394 6	352 5	15 1	345 7	43,7	1 6
77	444 6	527 9	995.1	381 0	506 0	83 3	550 5	-63 6	61 4
78	258 5	255 8	221 0	237 3	235 6	-2 7	-37 5	-21 2	-22 9
79	173 9	662 8	176 0	197 9	213 0	488 9	2 1	24 0	39 1
80	335 7	518.3	334 3	391 6	380 2	182 6	-1 4	55 9	44 5
82	335 5	303 2	326 0	342 1	364 0	-32 3	-9.5	6 7	28 5
84	300 0	266 3	293 7	347 4	247 4	-33 7	-6 3	47 4	-52 6
86	331 4	315 6	245 3	275 1	242 1	-15 8	-86 1	-56 3	-89 3
87	331 5	340 7	433 2	310 1	310 9	9 2	101 7	-21 4	-20 6
88	253 9	497 9	511 1	480 8	314 5	244 1	257 3	226 9	60 6
89	351 7	394 4	378 7	340 8	363 5	42.7	27 0	-10 8	11 8
90	251 5	222 7	255 0	249 4	235.8	-28 8	3 5	-2 1	-15 7
91	171 7	24 2	320 6	75 3	40 6	-147 5	148 9	-96 4	-131 [
92	165 0	106 9	25 8	269 4	220 1	-58 2	-139 2	104 4	55 1
93	218 5	210.7	175 8	217 1	156 9	-7 8	-42 6	-1 4	-61 6
94	247 4	0 3	184 2	60 3	0 0	-247.2	-63 2	-187 1	-247 4
95	315 0	346 0	313 7	300 6	249 0	31 0	-1,3	-14 4	-66 0
96	207 1	234 1	267 9	260 1	231 6	27 0	60 8	53 0	24 5
97	302 2	306 6	147 2	66 5	2 3	4 5	-155 0	-235 6	-299 9
98	357 8	349 8	349 5	268 1	321 2	-8 0	-8 3	-89 6	-36,6
99	496 1	443 3	421 1	440 2	442 3	-52 8	-75 0	-55 9	-53 8
100	278 9	238 3	260 1	235 6	334 2	-40.6	-18 8	-43 3	55 3
101	221 3	180 8	149 3	247 5	455 5	-40 5	-72 0	26 2	234 2
102	187 3	159 3	201 0	208 6	208.1	-27 9	13 7	21 3	20 8
103	111 9	35 2	11 8	15 9	0 0	-76 7	-100 1	-96 0	-111 9
104	287 0	286.4	281 2	360 2	278 8	-0 6	-5 8	73 2	-8 2
105	443 9	448 1	483 1	487.6	445 4	4 2	39 2	43 7	1 4
106	389 5	337 0	170 3	497 1	359 5	-52 4	-219 2	107 7	-30 0
107	373 0	370 5	452 4	354 6	317 3	-2.5	79 4	-18 4	-55 7
108	356 1	360 4	497 4	337 6	390 5	4 3	141 3	-18 5	34 4
109	316 5	358 3	310 4	368 0	335 9	41 7	-6 1	51 5	19 4
110	479 7	500 1	516 1	578 7	422 2	20 5	36 5	99 0	-57 5
111	157 4	164 0	146 3	152.9	179 5	6 6	-11 1	-4 4	22 1
112	257 6	216 7	262 0	227 9	231 8	-40.8	4.4	-29 7	-25.8
114	322 9	262 3	269 6	271 9	299 6	-60 6	-53 3	-51 0	-23 4
115	419 6	351 6	458 3	356 3	351 2	-68 0	38 7	-63 4	-68 5
116	277 7	230 9	315 9	131 0	225 5	-46 9	38 2	-146 8	-52 3
117	318 6	288 1	298 9	273 5	304 7	-30 5	-19 6	-45 1	-13 8
118	250 0	168 8	185 4	218.6	225 1	-81 1	-84 6	-31 4	-24 8
119	232 0	270 8	246 6	294 4	204 1	38 7	14 6	62 4	-27.9
120	361 1	265 3	248 1	160 0	136 6	-95 8	-113 0	-201.1	-224 5
121	358 7	450 2	420 0	377 3	408 6	91 6	61 3	18 7	49.9
Mean =	**292.9**	**276.0**	**287.7**	**277.1**	**282.2**	**-16.9**	**-5.3**	**-15.8**	**-10.8**
Median =	**302.1**	**265.3**	**273.7**	**273.8**	**278.8**	**-15.6**	**-8.3**	**-18.4**	**-13.8**
Standard Deviation =	**95.0**	**126.1**	**143.9**	**120.9**	**128.1**	**91.9**	**105.0**	**80.1**	**87.0**
Maximum =	**611.1**	**662.8**	**995.1**	**629.3**	**649.7**	**488.9**	**550.5**	**226.9**	**342.2**
Minimum =	**111.9**	**0.3**	**7.6**	**15.9**	**0.0**	**-247.2**	**-310.8**	**-235.6**	**-299.9**

Mean % Change From Baseline

Month 1	Month 2	Month 4	Month 8
-5 8% Decrease	-1 8% Decrease	-5 4% Decrease	-3 7% Decrease

Tallies & Percentages

	Month 1	Month 2	Month 4	Month 8
Total	113	113	113	113
Increased Values	43	46	42	49
Decreased Values	70	67	71	64
No Change	0	0	0	0
Increased Values	38 05%	40 71%	37 17%	43 36%
Decreased Values	61 95%	59 29%	62,83%	56 64%
No Change	0 00%	0.00%	0 00%	0.00%

Did Not Complete Subject#s 7, 51, 58, 81, 83, 85, 113
Note: Data for Subject #29 not included due to image noise

[redacted]

Table 3
(continued)

Statistical Analysis of Aeva3D-HE Positive volume (mm3)

Within-Groups Analysis For :
[redacted] ***Lash Enhancing Serum (Left)***

Pre-Test	Normality	Brown-Forsythe	Friedman ANOVA
P-Value	< 0.050	Not Required	0.01

Pairwise Comparisons

Difference from Baseline	Test Type	P-value	Significance	Direction (Mean)
Month 1	Dunn's Method	**0.006**	**Yes**	**Decrease**
Month 2	Dunn's Method	0.610	No	Decrease
Month 4	Dunn's Method	1.000	No	Decrease
Month 8	Dunn's Method	1.000	No	Decrease

Within-Groups Analysis For :
[redacted] ***Lash Enhancing Serum (Right)***

Pre-Test	Normality	Brown-Forsythe	Friedman ANOVA
P-Value	< 0.050	Not Required	0.02

Pairwise Comparisons

Difference from Baseline	Test Type	P-value	Significance	Direction (Mean)
Month 1	Dunn's Method	**0.019**	**Yes**	**Decrease**
Month 2	Dunn's Method	0.370	No	Decrease
Month 4	Dunn's Method	**0.013**	**Yes**	**Decrease**
Month 8	Dunn's Method	0.103	No	Decrease

* = ANOVA (RM) compares the means across groups (the non-parametric version is Friedman). The null hypothesis is all means are equivalent and the alternative is that the mean of at least one group differs. If ANOVA is significant further pairwise comparisons can be made, if not then it is assumed there is no statistically significant difference among the mean (or ranks) and no further testing is done due to risk of type one error. It is possible that Friedman or RM ANOVA may be significant, but when running post-hoc test may not show any statistically significant difference due to only examining the comparisons to baseline, post-hoc test, small sample size, lack of power, or if there is borderline significance.

* = Dunn's method is a post-hoc test commonly run after Friedman RM ANOVA. It allows for comparisons between a control group, and it is a robust, non-parametric test that adjusts for multiple comparisons.

The images obtained from the Aeva^{3d}-HE instrument were used to measure the periorbital volume, the higher the value, the greater the volume.

Summary:

For subjects using the [redacted] Lash Enhancing Serum, when compared to baseline, there was a statistically significant decrease in the periorbital volume measurements after 1 month of test material usage on the left side. There was also a statistically sigificant decrease from baseline after month 1 and month 4 of test material usage on the right side.

Table 4

VISIA-CR Image Analysis Color Data- R/RGB * Left Iris

.01 – [redacted] Lash Enhancing Serum

						Difference From Baseline At :			
Subject #	**Baseline**	**Month 1**	**Month 2**	**Month 4**	**Month 8**	**Month 1**	**Month 2**	**Month 4**	**Month 8**
1	67 47	74 50	66 40	65 49	71 91	7 0	-1 1	-2 0	4 4
2	87 30	80 34	83 49	80 80	82 48	-7 0	-3 8	-6 5	-4 8
3	82 14	82 96	80 54	83 33	92 05	0 8	-1 6	1 2	9 9
4	86 45	85 06	91.52	83 16	82 08	-1 4	5 1	-3 3	-4 4
5	32.87	33 00	30 75	27 54	31 98	0 1	-2 1	-5 3	-0 9
6	32 62	31 49	26 52	26 78	31 54	-1 1	-6 1	-5 8	-1 1
8	30 98	29 39	30 49	31 25	28 08	-1.6	-0 5	0 3	-2 9
9	99 91	102 36	97 06	95 15	90 15	2 5	-2 8	-4 8	-9 8
10	83 35	91 31	81 81	86 71	82 72	8 0	-1 5	3 4	-0 6
11	24 15	23 34	25 65	32 66	25 00	-0 8	1 5	8 5	0 9
12	89 31	88 24	86 06	89 73	93 21	-1 1	-3 2	0 4	3 9
13	33 48	33 20	28 79	29 12	29 38	-0 3	-4 7	-4.4	-4 1
14	31 34	35 65	31 74	31 61	32 62	4 3	0 4	0 3	1 3
15	22 39	27 44	30 79	27 43	29 04	5 1	8 4	5 0	6 7
16	42.44	37 53	39 92	42 18	42 87	-4 9	-2 5	-0 3	0 4
17	67 49	65 45	72 73	69 41	68 01	-2.0	5 2	1 9	0 5
18	54 64	57 17	46 53	55.55	52 08	2 5	-8 1	0 9	-2 6
19	55 53	47 29	58 98	50.99	50 01	-8 3	3 4	-4 6	-5 5
20	78 09	89 03	79 31	76 23	73 72	10 9	1 2	-1 9	-4 4
21	77.35	79 31	86 82	86 91	89 20	2 0	9 5	9 6	11 9
22	52 34	54 28	53 25	53 02	54.50	1 9	0 9	0 7	2 2
23	81 68	84 73	82 21	78 26	77 36	3 0	0 5	-3 4	-4 3
24	28 84	26 10	27 03	29 55	23 94	-2 7	-1 8	0 7	-4 9
25	26 84	22 98	33 89	25 80	26 45	-3 9	7 0	-1 0	-0 4
26	83 50	88 69	84 77	84 91	83 73	5 2	1 3	1 4	0 2
27	51 46	48 60	48 38	53 60	53 94	-2 9	-3 1	2 1	2 5
28	45 83	46 19	48 33	50 29	46 75	0 4	2 5	4 5	0 9
29	61 72	67.98	71 45	75 03	72 82	6 3	9 7	13 3	11 1
30	19 45	20 89	22 31	26 36	20 64	1 4	2 9	6 9	1 2
31	26 28	24 78	21 86	24 72	25 66	-1 5	-4 4	-1 6	-0 6
32	59 27	57 60	55 75	59 61	59 51	-1 7	-3 5	0 3	0 2
33	47 32	44 71	48 45	43 14	46 71	-2 6	1 1	-4 2	-0 6
34	54 33	58 59	59 64	61 62	60 46	4 3	5 3	7 3	6 1
35	64 59	62 65	72 42	52 27	67 64	-1 9	7 8	-12 3	3 0
36	47 17	48 07	50 57	49 03	53 03	0 9	3 4	1 9	5 8
37	32 39	32 61	29 56	31 88	30 31	0 2	-2 8	-0 5	-2 1
38	92 66	88 70	93 49	99 24	86,80	-4 0	0 8	6 6	-5 9
39	89 05	87 15	91 51	91 50	93 72	-1 9	2 5	2 5	4 7
40	26 27	27 70	30 98	30 43	23 89	1 4	4.7	4 2	-2.4
41	30 16	29 28	27 53	30 91	28 76	-0 9	-2 6	0 7	-1 4
42	54 50	49 80	58 47	60 51	61 55	-4 7	4 0	6 0	7 0
43	36 72	33 60	33,93	34 43	34 23	-3 1	-2 8	-2 3	-2 5
44	67 58	70 78	65 39	65 73	71 57	3 2	-2 2	-1 9	4 0
45	27 49	31 40	33 43	32 98	30 40	3 9	5 9	5 5	2 9
46	55 43	66 64	59 80	64 53	75,03	11 2	4 4	9 1	19 6
47	61 11	43 72	52.38	50 89	47 01	-17 4	-8 7	-10 2	-14 1
48	31.08	32 31	26 63	30 17	29 50	1 2	-4 4	-0 9	-1 6
49	46.97	43 60	51 51	49 84	50 06	-3 4	4 5	2 9	3 1
50	78 50	76 81	73 86	74 91	79 60	-1 7	-4 6	-3 6	1 1
52	41 35	53 37	53 25	49 32	52 69	12 0	11 9	8 0	11 3
53	87 23	89 95	100 19	96 34	90 70	2 7	13 0	9 1	3 5
54	29 88	28,01	29 40	26 24	28 50	-1 9	-0 5	-3 6	-1 4
55	33,88	30 42	28 07	35 03	33 88	-3 5	-5 8	1 1	0 0
56	76 00	71.65	79 58	75 21	76 42	-4 4	3 6	-0 8	0 4
57	42,81	40,31	43 68	39 97	37 57	-2 5	0 9	-2 8	-5 2
59	30 39	30 25	33 01	35 75	44 31	-0 1	2.6	5 4	13 9
60	86.58	83 15	88 11	90.08	90 86	-3 4	1 5	3 5	4 3
61	46.74	48.81	53 12	45 11	46,91	2 1	6 4	-1 6	0 2
62	36 74	39.11	40 29	36.63	43 58	2 4	3.6	-0 1	6 8
63	71 46	71.79	67 54	68 42	78 21	0 3	-3 9	-3.0	6.7
64	47 06	47.99	46 70	46 66	44 88	0 9	-0.4	-0 4	-2 2
65	45 40	42.79	42.85	44 03	42 94	-2 6	-2 5	-1 4	-2 5
66	63,62	64 89	63 56	65 27	60 44	1 3	-0.1	1 6	-3 2
67	40 98	38.41	40.31	41 27	37.38	-2 6	-0 7	0 3	-3 6

Table 4
(continued)

VISIA-CR Image Analysis Color Data- R/RGB* Left Iris

.01 = [redacted] Lash Enhancing Serum

68	63 29	63 84	63 14	59 82	61 85	0 6	-0 1	-3 5	-1 4
69	70 99	72 41	73 30	72 13	70 24	1 4	2 3	1 1	-0 8
70	32 72	39 54	36 48	38 10	35 84	6 8	3 8	5 4	3 1
71	52.52	49 88	43 17	51 07	54 77	-2 6	-9 3	-1 4	2 3
72	47 14	53 46	49 96	51 82	56 83	6 3	2 8	4 7	9 7
73	37 16	37 86	33 07	35 33	34 76	0 7	-4 1	-1 8	-2 4
74	30 46	26 49	22 52	24 70	33 61	-4 0	-7 9	-5 8	3 2
75	43 15	38 01	38 85	40 22	41 29	-5 1	-4 3	-2 9	-1 9
76	58 60	58 17	60 26	59 78	59 84	-0 4	1 7	1 2	1 2
77	50 12	57 06	54.01	50.26	57.04	6 9	3 9	0 1	6 9
78	69 72	72 18	72 46	71 77	71 71	2 5	2 7	2 1	2 0
79	54 32	52 01	56 81	54 74	52 10	-2 3	2 5	0 4	-2 2
80	42.92	38 99	39 57	38 73	38 11	-3 9	-3 4	-4 2	-4 8
82	42 14	44 57	46 08	47 85	47 54	2 4	3 9	5 7	5 4
84	28 27	34 73	26 50	28 09	30 86	6 5	-1 8	-0 2	2 6
86	45 64	49 71	52 15	47 46	52 97	4 1	6 5	1 8	7 3
87	36 77	36 23	41 09	40 42	42 53	-0 5	4 3	3 6	5 8
88	102 87	111 34	100 48	107 96	110 92	8 5	-2.4	5 1	8 1
89	73 50	76 11	76 84	79 61	77 54	2 6	3 3	6 1	4 0
90	91 16	88 05	92.17	89 90	92 71	-3 1	1 0	-1 3	1 6
91	45.04	43 54	46 29	42 53	49 33	-1 5	1 3	-2 5	4 3
92	73 06	64 68	62 15	62 25	60 95	-8 4	-10 9	-10 8	-12 1
93	71 30	63 78	66.91	63 75	65 59	-7 5	-4 4	-7 5	-5 7
94	95 65	95 39	80 10	91 66	99 82	-0 3	-15 6	-4 0	4 2
95	77 24	73 10	74 82	77 22	75 99	-4.1	-2 4	0 0	-1 3
96	77 50	77 39	74 24	79 98	74.18	-0 1	-3 3	2 5	-3 3
97	20 06	25 23	19 63	20 63	24 44	5 2	-0 4	0 6	4 4
98	72 65	64 03	65 82	77 04	69 05	-8 6	-6 8	4 4	-3 6
99	49 68	47 76	52 14	51 59	52 14	-1 9	2 5	1 9	2 5
100	64 13	64 22	64 29	66 67	64 29	0 1	0 2	2 5	0 2
102	54 83	55 65	57 28	59 86	53 26	0 8	2 4	5 0	-1 6
102	72 55	64 95	66 04	57 02	69 24	-7 6	-6 5	-15 5	-3 3
103	56 10	53 49	61 66	57 06	56.07	-2 6	5 6	1 0	0 0
104	59 66	57 31	61 04	60 66	64 70	-2 4	1 4	1 0	5 0
105	98 67	92 65	91 95	102 30	98 59	-6 0	-6 7	3 6	-0 1
106	47 06	50 02	52 98	54 76	55 54	3 0	5 9	7 7	8 5
107	44 51	46 82	42.55	45 71	39 31	2 3	-2 0	1 2	-5 2
108	91 56	88 48	88 45	90 07	84 49	-3 1	-3 1	-1 5	-7 1
109	91 42	93 24	96 72	93 85	84 67	1 8	5 3	2 4	-6 7
110	90 20	89 53	92 55	92 16	94 25	-0 7	2 4	2 0	4 1
111	52 14	51 47	58.00	48 79	51 89	-0.7	5 9	-3 3	-0 2
112	35 77	34.57	38 19	35 24	36 05	-1 2	2 4	-0 5	0 3
114	51 19	52 87	57 15	53 66	54 03	1 7	6 0	2 5	2 8
115	47 38	45 53	46 75	48 35	46 72	-1 8	-0 6	1 0	-0 7
116	66 42	62 56	67 42	64 99	68 95	-3 9	1 0	-1 4	2 5
117	58 75	55.81	53 45	60 23	52 91	-2 9	-5 3	1.5	-5 8
118	78 21	82 76	78.86	81 72	86 36	4 6	0 6	3 5	8 2
119	50 12	49 89	50 80	48 38	49 97	-0 2	0 7	-1 7	-0 1
120	59 10	53 69	58.48	51.89	61 73	-5 4	-0 6	-7 2	2 6
121	34.90	39 65	32 81	40.88	46 04	4 8	-2 1	6 0	1 1 1
Mean =	**56.14**	**56.02**	**56.43**	**56.61**	**57.16**	**-0.12**	**0.29**	**0.46**	**1.02**
Median =	**53.42**	**53.12**	**53.73**	**52.64**	**53.98**	**-0.36**	**0.67**	**0.62**	**0.35**
Standard Deviation =	**21.30**	**21.39**	**21.35**	**21.51**	**21.45**	**4.41**	**4.74**	**4.61**	**5.25**
Maximum =	**102.87**	**111.34**	**100.48**	**107.96**	**110.92**	**12.01**	**12.96**	**13.31**	**19.61**
Minimum =	**19.45**	**20.89**	**19.63**	**20.63**	**20.64**	**-17.38**	**-15.55**	**-15.53**	**-14.10**

Mean % Change From Baseline

Month 1	Month 2	Month 4	Month 8
-0 2% Decrease	0.5% Increase	0 8% Increase	1.8% Increase

Tallies & Percentages

	Month 1	Month 2	Month 4	Month 8
Total	114	114	114	114
Increased Values	52	61	65	62
Decreased Values	62	53	49	52
No Change	0	0	0	0
Increased Values	45 61%	53 51%	57 02%	54 39%
Decreased Values	54 39%	46 49%	42 98%	45 61%
No Change	0.00%	0 00%	0 00%	0 00%

Did Not Complete. Subject#s 7, 51, 58, 81, 83, 85, 113

Mean R/RGB = Mean red value from the RGB color space, for the detected features within the masked region of interest (ROI)

Table 4
(continued)

VISIA-CR Image Analysis Color Data- R/RGB Right Iris

.01 = [redacted] Lash Enhancing Serum

						Difference From Baseline At :			
Subject #	**Baseline**	**Month 1**	**Month 2**	**Month 4**	**Month 8**	**Month 1**	**Month 2**	**Month 4**	**Month 8**
1	66 42	68 49	67 52	66 47	67 39	2 1	1.1	0 1	1 0
2	90.23	83 50	90 87	85 20	91 79	-6 7	0 6	-5 0	1 6
3	76 63	83 66	73 37	86 50	81.11	7 0	-3 3	9 9	4 5
4	82 80	82 23	84 83	84 36	84 85	-0 6	2 0	1.6	2 1
5	36.65	34 97	30.08	31 51	33 49	-1 7	-6 6	-5 1	-3 2
6	26 79	26 24	27 86	23 59	25.59	-0 6	1 1	-3 2	-1 2
8	32 75	27 59	31 24	30 27	25 63	-5 2	-1 5	-2 5	-7 1
9	87 67	93 58	88 77	86 73	89 53	5 9	1 1	-0 9	1 9
10	77 76	82 67	79 62	76 72	83 65	4 9	1 9	-1.0	5 9
11	26 96	26 22	23 98	28 53	27 37	-0 7	-3 0	1 6	0 4
12	90.96	88 81	86.36	99 65	96 40	-2 1	-4 6	8.7	5 4
13	28 89	29 32	27 31	27.02	27 91	0 4	-1 6	-1 9	-1 0
14	37 30	38 40	34 45	29 82	27 44	1 1	-2 9	-7 5	-9 9
15	21.96	31 52	31 46	22 87	27 30	9 6	9 5	0 9	5 3
16	39 83	36 57	37 65	39 07	39 16	-3 3	-2 2	-0 8	-0 7
17	69 88	66 44	74.82	75 98	73 07	-3 4	4 9	6 1	3 2
18	56 58	55 52	43 08	52 76	52 92	-1 1	-13 5	-3 8	-3 7
19	62 45	49 69	55 06	53 45	54 70	-12 8	-7 4	-9 0	-7 8
20	72 71	80 71	87 86	76 45	67 97	8.0	15 2	3 7	-4 7
21	82.42	88 30	96.43	101 73	99 14	5 9	14 0	19 3	16 7
22	54 18	54 06	54 68	54 73	54 96	-0 1	0 5	0 5	0 8
23	68 57	80 16	75 60	69 26	71 67	11 6	7 0	0 7	3 1
24	25 58	26 92	24 24	25 33	26 70	1 3	-1 3	-0 2	1 1
25	57 58	20 12	31 08	23 60	40 57	-37 5	-26 5	-34 0	-17 0
26	82.69	83 86	85 21	88 57	89 46	1 2	2 5	5 9	6 8
27	58 21	54 80	59 11	56 13	61 15	-3.4	0 9	-2 1	2 9
28	45.91	53 63	57 17	52 51	50 15	7 7	11 3	6 6	4 2
29	52 46	72 50	44 50	52 41	50 83	20 0	-8 0	-0 1	-1 6
30	22 06	21 17	23 43	23 17	22 17	-0.9	1 4	1 1	0,1
31	23 46	22 84	25 61	23 95	22 68	-0.6	2 1	0 5	-0 8
32	69 17	61 60	59 54	61 32	63 36	-7 6	-9 6	-7 8	-5 8
33	53 55	55 77	53.06	49 16	54 41	2 2	-0 5	-4 4	0 9
34	57 55	55 36	60 82	62 79	58 23	-2 2	3 3	5 2	0 7
35	72 71	75 21	74 06	59 09	77 59	2 5	1 4	-13 6	4 9
36	46 89	48 75	47 33	50 34	43 79	1 9	0 4	3 4	-3 1
37	29 31	30 87	27 92	29 55	30 38	1 6	-1 4	0 2	1 1
38	86 40	82 17	88 30	87 69	85 21	-4 2	1 9	1.3	-1 2
39	86 92	85 56	86 23	86 52	89 90	-1 4	-0 7	-0 4	3 0
40	27 56	27 13	29 09	27 74	25 05	-0 4	1 5	0 2	-2 5
41	27.90	35 52	25 13	27 17	25 47	7 6	-2 8	-0 7	-2 4
42	49 90	47 68	56 92	56 61	65 27	-2 2	7 0	6 7	15 4
43	33 24	30 19	32 14	32 02	31 65	-3 1	-1.1	-1 2	-1 6
44	75 63	75.92	69 05	73 00	74 07	0 3	-6 6	-2 6	-1 6
45	28 25	36 21	28.55	30 21	26 96	8 0	0 3	2 0	-1 3
46	72.21	87.16	63 41	61 92	93 00	15 0	-8 8	-10 3	20 8
47	64 27	59 15	62 66	59 38	53 76	-5 1	-1 6	-4 9	-10 5
48	30 74	27 52	28 88	28 15	31 90	-3 2	-1 9	-2 6	1 2
49	46.71	42 40	47 35	43 26	45 38	-4 3	0 6	-3 5	-1 3
50	71 61	70 97	70 34	68 54	72.80	-0.6	-1 3	-3 1	1 2
52	42.72	40 64	41.92	37 49	44 39	-2.1	-0,8	-5 2	1 7
53	72 21	79 35	75 26	76 67	78 32	7 1	3 0	4 5	6 1
54	27 39	26 76	27 82	25 24	27 16	-0 6	0 4	-2 1	-0 2
55	34 06	30 85	25 32	32 95	27 96	-3.2	-8 7	-1 1	-6 1
56	68 13	66 24	80 07	72 17	72,83	-1 9	11 9	4 0	4 7
57	30 79	31 43	34 08	33 04	29 73	0.6	3 3	2 2	-1 1
59	27,78	29,75	26 98	28 27	30.46	2 0	-0.8	0 5	2.7
60	78.87	82 63	74 94	80,68	77 92	3.8	-3 9	1,8	-0.9
61	42 96	44 76	47.79	42.32	42 49	1 8	4,8	-0 6	-0 5
62	33.13	31 46	31 15	32 30	34 95	-1 7	-2 0	-0,8	1 8
63	72 70	70 82	67 25	67 51	71 25	-1 9	-5 5	-5 2	-1 4
64	43 04	43 46	43 34	43 88	41.22	0,4	0,3	0 8	-1 8
65	41.15	37 29	37.47	38 82	39.33	-3 9	-3 7	-2 3	-1 8
66	57 25	61 76	60 01	60 15	60 44	4 5	2 8	2 9	3 2
67	36 66	37.15	34 58	36 69	36 16	0 5	-2 1	0 0	-0 5

Table 4
(continued)

VISIA-CR Image Analysis Color Data- R/RGB Right Iris

.01 = [redacted] Lash Enhancing Serum

68	50 98	56 33	49 27	50 86	53 08	5 3	-1 7	-0 1	2 1
69	73 94	73 79	73 38	74 95	72 30	-0 1	-0 6	1 0	-1 6
70	30 12	31 99	33 25	30.63	31 21	1 9	3 1	0 5	1 1
71	46 94	48 87	48 60	51 72	50 15	1 9	1.7	4 8	3 2
72	47 84	51 03	49 09	45 75	51 32	3 2	1 2	-2 1	3 5
73	36 57	38.28	36 67	36 09	37 24	1 7	0 1	-0 5	0 7
74	25 83	21 67	22 18	24 63	25 31	-4.2	-3 6	-1 2	-0 5
75	35 82	38 40	35 78	35 11	36 26	2 6	0.0	-0 7	0 4
76	50 72	55.92	53 30	53 80	51 29	5 2	2 6	3 1	0 6
77	52 66	47 45	54 28	51 37	48 66	-5 2	1 6	-1 3	-4 0
78	61 28	67 45	65.13	67 22	70.81	6 2	3 9	5 9	9 5
79	68 43	57 78	66 66	67 29	61 78	-10 7	-1 8	-1 1	-6 6
80	40 77	38 91	39 93	35 83	38 78	-1 9	-0 8	-4 9	-2 0
82	41 59	37 99	36 84	40 41	41 06	-3 6	-4 7	-1 2	-0 5
84	22 54	32 84	23 04	24 43	28 63	10 3	0 5	1 9	6 1
86	43 52	40 64	38 15	48 47	43 53	-2 9	-5 4	4 9	0 0
87	31 69	35 10	34 85	36 39	36.51	3 4	3 2	4 7	4 8
88	108 80	100 37	103 05	100 93	104 98	-8 4	-5 7	-7 9	-3 8
89	72.49	73 89	74 16	73 77	75 56	1 4	1 7	1 3	3 1
90	88 33	90 68	94 12	92 21	90 06	2 3	5 8	3 9	1 7
91	34 83	39 01	33 65	35.31	38 34	4 2	-1 2	0 5	3 5
92	68 25	64 29	57 75	72 12	54 33	-4 0	-10 5	3 9	-13 9
93	85 53	80 30	69.05	77 73	81 03	-5 2	-16 5	-7 8	-4 5
94	106 79	107 77	91 91	107 72	110 43	1 0	-14 9	0 9	3 6
95	80 77	77 16	87 32	82.11	83 32	-3.6	6 6	1 3	2 6
96	69.12	70 17	70 45	74 01	70 93	1 0	1 3	4 9	1 8
97	20 92	20 38	16.70	18 97	21.58	-0 5	-4 2	-1 9	0 7
98	61 33	61 05	69 89	55 26	64 72	-0 3	8 6	-6 1	3 4
99	47 38	49 72	51 95	49 76	52 56	2 3	4 6	2 4	5 2
100	55 73	48 54	54 53	51 13	50 40	-7 2	-1 2	-4 6	-5 3
101	55 42	52 67	58 00	55 77	53 41	-2 8	2 6	0 1	-2 0
102	70 45	67 35	72 67	50 87	66 85	-3 1	2 2	-19 6	-3 6
103	60 16	65 09	71 29	64 01	68 20	4 9	11 1	3 8	8 0
104	53 05	53 08	51 48	51 26	55 60	0 0	-1 6	-1 8	2 6
105	101 73	94 32	92 30	101 14	95 25	-7 4	-9 4	-0 6	-6 5
106	52 12	50 23	52 48	57 97	57 10	-1 9	0 4	5 9	5 0
107	41 78	41 78	45 42	39 89	37 03	0 0	3 6	-1 9	-4 7
108	84 39	85 78	91 30	91 22	90.34	1 4	6 9	6 8	5 9
109	94 44	85 84	85 06	79 23	88 85	-8 6	-9 4	-15 2	-5 6
110	88 26	89 07	88 50	89 77	90 45	0 8	0 2	1 5	2 2
111	48 58	58 09	54 08	48 00	53 33	9 5	5 5	-0 6	4.7
112	36 78	35 27	36 76	33 84	32 62	-1 5	0 0	-2 9	-4 2
114	55 06	57 73	55 45	59 13	60 02	2 7	0 4	4 1	5 0
115	52 66	53 21	54 80	44 85	44 17	0 5	2 1	-7 8	-8 5
116	64 41	63 68	64 76	66 12	67 79	-0 7	0 3	1.7	3 4
117	58 23	61 79	60 76	59 01	63 22	3 6	2 5	0 8	5 0
118	70 31	75 29	71 84	71 52	78 55	5 0	1 5	1 2	8 2
119	48 38	48 19	48 04	48 26	49 17	-0 2	-0 3	-0 1	0 8
120	57 31	55 42	54 25	51 25	58 14	-1 9	-3 1	-6 1	0 8
121	36 28	41 46	31 76	47 70	52 94	5 2	-4.5	11 4	16 7
Mean =	**55.17**	**55.33**	**54.81**	**54.63**	**55.87**	**0.16**	**-0.36**	**-0.54**	**0.70**
Median =	**53.30**	**53.85**	**54.27**	**52.06**	**53.37**	**-0.06**	**0.30**	**-0.09**	**0.78**
Standard Deviation =	**21.35**	**21.50**	**21.70**	**22.07**	**22.44**	**6.10**	**5.86**	**5.99**	**5.46**
Maximum =	**108.80**	**107.77**	**103.05**	**107.72**	**110.43**	**20.04**	**15.15**	**19.31**	**20.79**
Minimum =	**28.92**	**20.12**	**16.70**	**18.97**	**21.58**	**-37.45**	**-26.50**	**-33.98**	**-17.01**

Mean % Change From Baseline

Month 1	Month 2	Month 4	Month 8
0 3%	-0.7%	-1 0%	1 3%
Increase	Decrease	Decrease	Increase

Tallies & Percentages

	Month 1	Month 2	Month 4	Month 8
Total	114	114	114	114
Increased Values	57	60	56	66
Decreased Values	57	54	58	48
No Change	0	0	0	0
Increased Values	50.00%	52.63%	49.12%	57 89%
Decreased Values	50 00%	47 37%	50 88%	42 11%
No Change	0 00%	0 00%	0 00%	0 00%

Did Not Complete Subject#s 7, 51, 58, 81, 83, 85, 113

Mean R/RGB = Mean red value from the RGB color space, for the detected features within the masked region of interest (ROI)

[redacted]

Table 4
(continued)

.01[redacted] Lash Enhancing Serum

Within-Groups Analysis For :
VISIA-CR Image Analysis Color Data- R/RGB * Left Iris

Pre-Test	Normality	Brown-Forsythe	Friedman ANOVA
P-Value	P < 0.050	Not Required	0.092

Within-Groups Analysis For :
VISIA-CR Image Analysis Color Data- R/RGB * Right Iris

Pre-Test	Normality	Brown-Forsythe	Friedman ANOVA
P-Value	P < 0 050	Not Required	0 045

Pairwise Comparisons

Difference from Baseline	Test Type	P-value	Significance	Direction (Mean)
Month 1	Dunn's Method	1.000	No	Increase
Month 2	Dunn's Method	1.000	No	Decrease
Month 4	Dunn's Method	1.000	No	Decrease
Month 8	Dunn's Method	0.068	No	Increase

* = ANOVA (RM) compares the means across groups (the non-parametric version is Friedman). The null hypothesis is all means are equivalent and the alternative is that the mean of at least one group differs. If ANOVA is significant further pairwise comparisons can be made, if not then it is assumed there is no statistically significant difference among the mean (or ranks) and no further testing is done due to risk of type one error. It is possible that Friedman or RM ANOVA may be significant, but when running post-hoc test may not show any statistically significant difference due to only examining the comparisons to baseline, post-hoc test, small sample size, lack of power, or if there is borderline significance.

Mean R/RGB = Mean red value from the RGB color space, for the detected features within the masked region of interest (ROI)

Summary:

For subjects using [redacted] Lash Enhancing Serum, there were no statistically significant changes in the R/RGB values in either the left or the right eye when compared to baseline R/RBG values.

Table 4
(continued)

VISIA-CR Image Analysis Color Data - G/RGB * Value Left Iris

.01 = [redacted] Lash Enhancing Serum

						Difference From Baseline At :			
Subject #	Baseline	Month 1	Month 2	Month 4	Month 8	Month 1	Month 2	Month 4	Month 8
1	43 07	45 54	44 42	40 40	46 46	2 5	1 4	-2 7	3 4
2	100 75	90 78	94 44	90 07	89 86	-10 0	-6 3	-10 7	-10 9
3	86 85	89 44	84 57	88 31	96 19	2 6	-2 3	1 5	9 3
4	75 69	76 50	82 46	72 01	70 88	0.8	6 8	-3 7	-4 8
5	28.48	27 27	25.02	20.63	25 62	-1 2	-3 5	-7 9	-2 9
6	25 62	25 45	19 20	20 13	24 92	-0 2	-6 4	-5 5	-0 7
8	23 83	25 41	28 99	28 69	24 17	1 6	5 2	4 9	0 3
9	101 06	102 23	95 68	92 21	86 95	1 2	-5 4	-8 9	-14 1
10	88 03	101.63	86 23	93 32	90 16	13 6	-1 8	5 3	2 1
11	19 79	18 55	21 46	27 27	19 85	-1 2	1 7	7 5	0 1
12	97 19	91 53	93 12	96 12	99 82	-5 7	-4 1	-1 1	2 6
13	31 00	31 67	24.06	24 39	25.61	0 7	-6 9	-6 6	-5 4
14	21 71	29 93	23 27	22 82	24 54	8 2	1 6	1 1	2 8
15	15 99	24 78	26 84	22 16	25.56	8 8	10 8	6 2	9.6
16	30 49	25 26	27 01	29 13	29 95	-5 2	-3 5	-1 4	-0 5
17	42 39	42 90	50 05	44 60	41.15	0 5	7 7	2 2	-1 2
18	33 85	37 05	24 58	37 61	32 60	3 2	-9 3	3 8	-1 2
19	38 56	28 81	41.67	33 83	33 06	-9 8	3 1	-4 7	-5 5
20	87 65	103 24	93 13	87 62	84 36	15 6	5 5	0 0	-3 3
21	60 45	72 10	82 64	81 62	82 41	11 7	22 2	21 2	22 0
22	29 35	31 89	30 14	29 77	30 78	2 5	0 8	0 4	1 4
23	92 00	95 21	92 23	87 71	82 51	3 2	0 2	-4 3	-9 5
24	24 73	21 49	21 94	25 16	18 60	-3 2	-2 8	0 4	-6 1
25	22 61	18 79	31 16	22 10	21 51	-3 8	8 5	-0 5	-1 1
26	86 27	90 07	82 87	81.49	83 61	3 8	-3 4	-4 8	-2 7
27	31 11	29 31	29 17	35 36	33 55	-1 8	-1 9	4 2	2 4
28	31 43	34 58	35 56	35 56	27 34	3 1	4 1	4 1	-4 1
29	62 85	68 16	71 69	51 41	71 32	5 3	8 8	-11 4	8 5
30	17 70	18 17	20 27	25 26	18 25	0 5	2 6	7 6	0 5
31	24 77	23 50	19 77	22 68	23 01	-1 3	-5 0	-2 1	-1 8
32	35 90	33 10	31.10	34 94	34 06	-2 8	-4 8	-1 0	-1 8
33	28 63	27 37	29 86	25 35	26 82	-1 3	1 2	-3 3	-1 8
34	34 40	38 73	38 36	42 43	38 32	4 3	4 0	8 0	3 9
35	72 86	71 54	84 31	54 60	77 76	-1 3	11 4	-18 3	4 9
36	28 41	30 34	31 79	30 19	32 39	1 9	3 4	1 8	4 0
37	23 06	24 51	19 95	22 10	21 40	1 5	-3 1	-1 0	-1 7
38	103 44	98 82	103 39	109 38	95 80	-4 6	0 0	5 9	-7 6
39	71 14	72 27	77 26	76 18	75 09	1 1	6 1	5 0	3 9
40	20 78	23 77	27.18	27 46	17 33	3 0	6 4	6 7	-3 4
41	22 85	23 12	18 36	21 80	19 59	0 3	-4 5	-1 1	-3 3
42	34 69	29.05	36 39	35 79	39.18	-5 6	1 7	1 1	4,5
43	27 84	22 09	22 56	23 88	23 42	-5 7	-5 3	-4 0	-4 4
44	41 71	44.78	44.61	40 15	46 82	3 1	2 9	-1 6	5 1
45	23 41	30 40	29 45	29.87	27 88	7 0	6 0	6 5	4 5
46	34 84	47 03	38 78	40 56	48 11	12 2	3 9	5 7	13 3
47	63 00	43 30	56 06	53 67	49 34	-19 7	-6 9	-9 3	-13 7
48	27 74	27 72	22 67	27 21	26 38	0 0	-5 1	-0 5	-1 4
49	28 56	24 96	30 98	29 94	30 10	-3 6	2 4	1 4	1 5
50	70 75	70.68	68 18	67 09	70 41	-0 1	-2 6	-3 7	-0.3
52	29 11	34 74	31 92	31 87	31 58	5 6	2 8	2 8	2 5
53	85 43	82 39	95 28	90 14	80 58	-3 0	9 8	4 7	-4 8
54	25 96	21 79	24 20	20 15	22 23	-4 2	-1 8	-5 8	-3,7
55	29 27	25 18	19 88	31 81	28 28	-4 1	-9 4	2 5	-1 0
56	82 05	75 05	86.04	81 18	81 82	-7 0	4 0	-0 9	-0.2
57	35 80	32 92	38 02	30 30	30 13	-2 9	2 2	-5 5	-5 7
59	24 07	23 06	26 34	30 08	38 29	-1 0	2 3	6 0	14 2
60	84 32	82 42	85 77	88 83	87 09	-1 9	1,4	4 5	2 8
61	28 26	29 06	36.77	27.12	27 41	0 8	8 5	-1 1	-0 9
62	27.08	29 68	31 02	27 83	35 05	2 6	3,9	0 8	8 0
63	46 38	47 71	44 79	45 92	51 27	1 3	-1 6	-0 5	4.9
64	29 00	28 89	29 28	27 07	27 00	-0.1	0.3	-1 9	-2 0
65	28 94	26 92	26 77	26 12	26 26	-2.0	-2 2	-2 8	-2.7
66	52 23	49 16	48 67	46 26	48 55	-3 1	-3 6	-6 0	-3.7
67	24 55	25 12	30.15	30 22	21 84	0 6	5 6	5 7	-2 7

Table 4
(continued)
VISIA-CR Image Analysis Color Data - G/RGB * Value Left Iris

.01 = [redacted] Lash Enhancing Serum

68	41 01	45 83	40 81	37 20	39 84	4 8	-0 2	-3 8	-1 2
69	42 12	43 72	43 61	43 63	41 30	1 6	1 5	1 5	-0 8
70	22.46	30 70	27 33	27 86	24 70	8 2	4 9	5 4	2 2
71	37 67	33 74	28 15	35 24	35 82	-3 9	-9 5	-2 4	-1 9
72	29 30	34 29	31 06	32 44	38 16	5 0	1 8	3 1	8.9
73	27 28	25 48	22 11	23 71	23 68	-1.8	-5 2	-3 6	-3 6
74	27 84	21 04	17 28	19 72	29 45	-6 8	-10.6	-8 1	1 6
75	31 42	24 57	26 70	26.50	29 03	-6 9	-4 7	-4 9	-2 4
76	41 27	42.36	38 88	38 64	41 66	1 1	-2 4	-2 6	0 4
77	29 89	35 35	35 06	32.06	36 67	5 5	5 2	2 2	6.8
78	75 52	80 53	81 37	78 89	78 89	5 0	5 8	3.4	3 4
79	39 30	35.66	41.04	38 82	36 11	-3 6	1 7	-0 5	-3 2
80	32 38	26 57	26 38	25 35	24 67	-5.8	-6 0	-7 0	-7.7
82	26 54	30 57	31 13	32 35	31 66	4 0	4 6	5.8	5 1
84	22 66	33 98	20 47	23 22	26.50	11.3	-2 2	0,6	3 8
86	29 07	33 63	36 83	33 41	35 42	4 6	7 8	4 3	6 4
87	27 56	24.14	29 21	30 00	32 25	-3 4	1 6	2 4	4 7
88	101.81	113 64	100 20	105 39	112 34	11 8	-1 6	3 6	10 5
89	42 26	43 33	47 46	47 04	43 65	1 1	5 2	4 8	1 4
90	106 08	101.13	104.89	103 01	106 11	-4 9	-1 2	-3 1	0 0
91	29 43	29 53	28 18	27 06	31 28	0 1	-1 3	-2.4	1 8
92	51 00	46 13	41 75	39 34	37.90	-4 9	-9 3	-11 7	-13 1
93	75 01	62 07	67 45	64 31	65 89	-12 9	-7 6	-10 7	-9 1
94	68 23	67 14	57 57	62 61	69 86	-1 1	-10 7	-5 6	1 6
95	68 70	68 78	65 91	67 18	69 36	0.1	-2 8	-1 5	0 7
96	87.36	89 59	86 99	93 12	85 91	2 2	-0 4	5 8	-1 5
97	18.77	24 19	17 58	18 24	21 77	5 4	-1 2	-0 5	3 0
98	54 69	42 08	43 26	59 71	48 54	-12 6	-11 4	5 0	-6 1
99	41 04	39 83	43 70	43 20	42 33	-1 2	2 7	2 2	1 3
100	46 27	42 43	41 86	45 71	44 75	-3 8	-4 4	-0 6	-1 5
101	34 90	34 43	36 69	37 01	31 92	-0 5	1 8	2 1	-3 0
102	39 39	38.10	39 76	28 18	40 98	-1 3	0 4	-11 2	1 6
103	37 55	33 78	39 95	36 57	35 13	-3 8	2 4	-1 0	-2 4
104	40 37	38 20	43 17	40 36	46 35	-2 2	2 8	0 0	6 0
105	86 31	89 06	88 92	82 64	92 55	2 7	2 6	-3 7	6 2
106	26 71	32 40	31 39	30 81	32 06	5 7	4 7	4 1	5 4
107	25 49	29 66	26 82	28 03	27 27	4 2	1 3	2 5	1 8
108	99 96	97 78	98 62	99 25	93 17	-2 2	-1 3	-0 7	-6 8
109	91 22	94 93	97 34	93 86	84 91	3 7	6 1	2 6	-6 3
110	97 16	95 18	100.97	99 04	99 60	-2 0	3.8	1 9	2 4
111	30 51	32 46	38 52	28 51	34 85	1 9	8 0	-2 0	4 3
112	27 04	25 72	30 76	27 33	27 15	-1 3	3,7	0 3	0 1
114	31 55	36 16	37 45	35 67	34 57	4 6	5 9	4 1	3 0
115	34 18	30 47	31 82	34 31	32 98	-3 7	-2 4	0 1	-1 2
116	40 53	39.48	44 24	40 97	45 68	-1 1	3 7	0 4	5 1
117	62.57	60 89	56 91	62 21	55 97	-1 7	-5 7	-0.4	-6 6
118	55 88	59 72	55.49	60 04	65 78	3 8	-0 4	4 2	9 9
119	30 67	31 19	32 18	29 22	30 42	0 5	1 5	-1 4	-0 2
120	37 83	33 55	38.66	31 72	42 02	-4 3	0 8	-6 1	4 2
121	22.49	24 44	22 20	26 88	31 70	1 9	-0 3	4 4	9 2
Mean =	**45.86**	**46.04**	**46.37**	**45.65**	**46.25**	**0.18**	**0.51**	**-0.21**	**0.39**
Median =	**34.77**	**34.36**	**37.14**	**35.30**	**35.62**	**0.09**	**1.28**	**-0.20**	**0.05**
Standard Deviation =	**25.26**	**25.53**	**25.61**	**25.01**	**24.82**	**5.36**	**5.41**	**5.31**	**5.67**
Maximum =	**106.68**	**113.64**	**104.89**	**109.38**	**112.34**	**15.59**	**22.19**	**21.17**	**21.97**
Minimum =	**15.99**	**18.17**	**17.28**	**18.24**	**17.33**	**-19.69**	**-11.42**	**-18.26**	**-14.11**

Mean % Change From Baseline

Month 1	Month 2	Month 4	Month 8
0 4% Increase	1 1% Increase	-0 4% Decrease	0 9% Increase

Tallies & Percentages

	Month 1	Month 2	Month 4	Month 8
Total	114	114	114	114
Increased Values	58	63	55	58
Decreased Values	56	51	59	56
No Change	0	0	0	0
Increased Values	50 88%	55 26%	48 25%	50 88%
Decreased Values	49 12%	44.74%	51 75%	49 12%
No Change	0 00%	0 00%	0 00%	0.00%

Did Not Complete Subject#s 7, 51, 58, 81, 83, 85, 113

Mean G/RGB = Mean Green value from the RGB color space, for the detected features within the masked region of interest (ROI)

Table 4
(continued)

VISIA-CR Image Analysis Color Data- G/RGB Values Rigist Iris

.01 = [redacted] Lash Enhancing Serum

Subject #	Baseline	Month 1	Month 2	Month 4	Month 8	*Difference From Baseline At :* Month 1	Month 2	Month 4	Month 8
1	38 06	37 42	40 37	37 54	38 77	-0.6	2 3	-0 5	0 7
2	102 60	92 81	103 61	94 69	102.10	-9 8	1 0	-7 9	-0 5
3	82 82	92 48	78.63	94 57	87 30	9 7	-4 2	11 7	4 5
4	73.44	71 55	74 21	73 21	73 37	-1 9	0 8	-0 2	-0 1
5	30 87	26 63	23 55	22.97	25 93	-4 2	-7 3	-7 9	-5 0
6	19 38	19 03	18 98	15 23	17.70	-0 3	-0 4	-4.1	-1 7
8	30 05	21 71	27 79	25 66	21 26	-8 3	-2 3	-4 4	-8 8
9	89 58	95 38	88 07	84 04	88 89	5 8	-1 5	-5 5	-0 7
10	82 92	91 68	85 92	83 35	90 73	8 8	3 0	0 4	7 8
11	21 92	21 03	18 60	21 24	21 22	-0.9	-3 3	-0 7	-0 7
12	96 31	91 64	90 99	105 14	100.93	-4 7	-5 3	8 8	4 6
13	26 86	27 49	23 30	23 05	24.61	0 6	-3 6	-3 8	-2 2
14	28.84	32 43	27.27	21.91	19 95	3 6	-1 6	-6 9	-8 9
15	16 46	28 41	25 46	16 06	21 12	11.9	9 0	-0 4	4 7
16	27 63	24 49	24 35	26 41	26.41	-3 1	-3 3	-1 2	-1 2
17	47 57	45 75	56 60	53,53	49 87	-1 8	9 0	6.0	2 3
18	31 62	31 19	22.89	29 06	31 39	-0 4	-8 7	-2 6	-0 2
19	43 39	28 14	33 18	35 18	34 32	-15 3	-10 2	-8 2	-9 1
20	79 47	93 86	102 91	87 90	76 92	14 4	23 4	8.4	-2.6
21	60 01	75 83	90 23	97 66	88 59	15 8	30 2	37 6	28 6
22	27 91	27 65	29 99	28 12	27 71	-0 3	2 1	0 2	-0 2
23	74 35	88 86	84.81	76 98	75 70	14 5	10 5	2 6	1 4
24	21 31	22 81	19 68	21 24	22 19	1 5	-1 6	-0 1	0 9
25	31 35	16 34	26 43	19 83	24 09	-15 0	-4 9	-11 5	-7.3
26	84 12	84 81	85 62	88 58	89 16	0 7	1 5	4 5	5 0
27	37 09	33 22	38 44	35 08	39.01	-3 9	1 4	-2 0	1 9
28	27 30	39 50	39 16	30 67	30 27	12 2	11 9	3 4	3 0
29	51 55	71 55	45 09	47 41	50 13	20 0	-6 5	-4 1	-1 4
30	17 08	14 91	18 88	18 44	16 40	-2 2	1 8	1 4	-0 7
31	19 81	19 86	22.66	20 39	19 03	0 0	2 9	0 6	-0 8
32	49 65	35 60	33 45	35 18	36 92	-14 1	-16 2	-14 5	-12 7
33	30.04	30 35	29 77	27 29	29 26	0 3	-0 3	-2 8	-0 8
34	39 76	33 61	39 27	41 07	35 20	-6 2	-0 5	1 3	-4.6
35	77 96	85 88	85 51	63 15	89 06	7 9	7 6	-14 8	11.1
36	28 76	28 62	25.86	31 91	24 04	-0 1	-2 9	3 1	-4 7
37	19 34	23 27	18 43	19 30	21 16	3 9	-0 9	0 0	1 8
38	95 84	91 54	99 02	96 36	94 55	-4 3	3 2	0 5	-1 3
39	69 68	67 78	69 14	68 17	72 11	-1 9	-0 5	-1 5	2 4
40	20 66	21 05	21 78	19 60	17.11	0 4	1 1	-1 1	-3 6
41	20 49	30 19	16 44	20 20	17 15	9 7	-4 0	-0 3	-3 3
42	30.02	27 70	33.98	32 39	41 55	-2 3	4 0	2 4	11 5
43	23 44	18,46	20 61	21 17	20 53	-5 0	-2 8	-2 3	-2 9
44	52 50	51 60	46 68	47 72	50 15	-0 9	-5 8	-4 8	-2 3
45	25 56	35 82	24 84	27 72	22 97	10 3	-0 7	2 2	-2 6
46	43 35	56 69	37 73	36 96	57 72	13 3	-5 6	-6 4	14 4
47	68 29	61 51	66 79	61 54	54 09	-6 8	-1 5	-6 7	-14 2
48	28 16	22 64	25 56	23 84	29 33	-5 5	-2 6	-4 3	1 2
49	27 62	24.66	27 43	25 87	26 00	-3 0	-0 2	-1 8	-1 6
50	69.94	68 78	68,66	63 95	69,93	-1 2	-1 3	-6 0	0 0
52	28 77	26 82	24.95	24.39	26 21	-2 0	-3 8	-4 4	-2 6
53	67 61	68 32	63 13	66 53	63.10	0 7	-4 5	-1 1	-4 5
54	22 71	21 75	22 75	18,91	21 22	-1.0	0 0	-3 8	-1 5
55	29 31	26 70	17 94	28 79	20 91	-2 6	-11 4	-0 5	-8 4
56	74 44	69 12	87 36	78 69	78 31	-5 3	12 9	4 2	3 9
57	24 57	25.11	30 27	25 85	22 03	0.5	5 7	1 3	-2 5
59	21 04	23 65	19,46	20 49	23 64	2 6	-1 6	-0 5	2 6
60	67.07	70 34	61 70	69 00	64 74	3 3	-5.4	1 9	-2 3
61	26 22	28 55	32 03	26.30	24 73	2 3	5.8	0 1	-1 5
62	23 68	21 68	21 39	23 20	26 52	-2.0	-2 3	-0 5	2 8
63	42 69	42 73	39 88	39 46	42.17	0 0	-2 8	-3 2	-0 5
64	27 52	27 30	28 20	27 31	25 35	-0 2	0.7	-0 2	-2 2
65	25.18	21 84	22 38	23 20	23 01	-3.3	-2 8	-2.0	-2 2
66	43 89	48 98	44 32	43 76	47.75	5.1	0.4	-0.1	3 9

Table 4
(continued)

VISIA-CR Image Analysis Color Data - G/RGB Values Right Iris

.01 = [redacted] Lash Enhancing Serum

67	24 50	26 14	21 74	22 33	22 65	1 6	-2 8	-2 2	-1 9
68	30 32	37 33	28 73	28 83	32 03	7 0	-1.6	-1 5	1 7
69	43 80	43 76	42 14	46 84	42 40	0 0	-1 7	3 0	-1 4
70	20.08	23 86	24 49	21 13	21.96	3 8	4 4	1 0	1 9
71	32 53	33 97	31 92	34 75	30 46	1 4	-0.6	2 2	-2 1
72	27 37	31 44	29 08	26 15	30 84	4 1	1 7	-1 2	3 5
73	23 74	24 77	22 93	22 11	23.07	1 0	-0 8	-1 6	-0 7
74	20.24	15 21	16 42	17 91	18 52	-5 0	-3 8	-2 3	-1.7
75	24 58	24 09	23 47	22 25	24 77	-0 5	-1 1	-2 3	0 2
76	36 31	42 36	35 24	34 07	35.42	6 0	-1 1	-2 2	-0 9
77	33 05	27 92	34 59	34 22	32 06	-5 1	1 5	1 2	-1 0
78	56 87	73 70	69 74	71 35	76 00	16 8	12.9	14 5	19 1
79	48 32	36 95	49 31	44 93	41 75	-11 4	1 0	-3 4	-6 6
80	25 10	23 22	23 86	20 61	22 14	-1 9	-1 2	-4 5	-3 0
82	24 98	24 46	21 97	26 00	24 58	-0 5	-3 0	1 0	-0 4
84	17 29	32 17	17 94	18 13	25 18	14 9	0 6	0 8	7 9
86	24 52	27 41	25 27	33.33	27 37	2 9	0 7	8 8	2 9
87	24 01	22 51	22 41	23 35	24 19	-1 5	-1 6	-0 7	0 2
88	110 82	102 59	104 77	97 80	105 37	-8 2	-6.0	-13 0	-5 4
89	39 07	38 91	43 60	38 43	38 93	-0 2	4 5	-0 6	-0 1
90	100 72	104 15	105 74	104 77	102 74	3 4	5 0	4 1	2 0
91	21 09	27 84	20 30	21 70	24 71	6 8	-0 8	0 6	3 6
92	40 28	41 47	33 82	45 46	32 69	1 2	-6 5	5 2	-7 6
93	93 14	81 26	70 94	80 88	83 61	-11.9	-22 2	-12 3	-9 5
94	81 51	84 10	64 32	82 50	86 01	2 6	-17 2	1 0	4 5
95	71 00	69 19	76 90	68 14	73 06	-1 8	5 9	-2 9	2.1
96	79 90	81 44	80 44	86 52	81 02	1 5	0 5	6 6	1 1
97	20 28	19 49	16 24	17 36	19 79	-0 8	-4 0	-2 9	-0 5
98	57 64	56 35	63 69	47 73	57 63	-1.3	6 1	-9 9	0 0
99	38 04	39 46	42 62	40 59	42 35	1 4	4 6	2 5	4 3
100	44 53	31 11	38 30	36 73	36 99	-13 4	-6 2	-7 8	-7 5
101	34 08	30 80	35 19	33 59	31 38	-3 3	1 1	-0 5	-2 7
102	35 91	34 33	36 32	24 31	37 55	-1 6	0 4	-11 6	1 6
103	37 74	43 71	49 77	41 35	46 07	6 0	12 0	3 6	8 3
104	33 16	32 74	31 92	30 79	36 21	-0 4	-1 2	-2 4	3 0
105	88 90	85 84	87 45	84 78	85 20	-3 1	-1.4	-4 1	-3 7
106	27 69	28 09	27 94	31 07	31 79	0 4	0.3	3 4	4 1
107	26 29	24 07	34 48	24 36	26 12	-2.2	8 2	-1 9	-0 2
108	85 00	93 30	95 98	97 36	98 08	8 3	11.0	12.4	13 1
109	95 01	85 25	84 27	72.90	90 34	-9 8	-10 7	-22 1	-4 7
110	91 42	92 48	93 04	93 58	94 29	1 1	1 6	2 2	2.9
111	25 75	40 71	33 92	26 27	35 88	15.0	8 2	0 5	10 1
112	29.48	26 30	28.33	25 40	23 30	-3 2	-1 1	-4 1	-6 2
114	41 63	45 58	39 88	43 76	42 59	3 9	-1 7	2 1	1 0
115	35 26	39 49	42 83	32 50	29 62	4 2	7.6	-2 8	-5 6
116	38 49	42 26	41 81	42 90	45 88	3 8	3 3	4 4	7 4
117	58 71	62 41	60 28	58 87	63 16	3 7	1 6	0 2	4 4
118	48 15	48 27	46 74	47 78	52 34	0 1	-1 4	-0 4	4 2
119	27 85	28 27	26 59	27 61	27 98	0 4	-1.3	-0 2	0 1
120	33 82	35 27	31 21	28 71	35.59	1 4	-2 6	-5 1	1 8
121	23 50	27 83	20 01	32 51	38 05	4 3	-3 5	9 0	14 5
Mean =	**44.04**	**44.76**	**44.08**	**43.26**	**44.43**	**0.72**	**0.04**	**-0.78**	**0.39**
Median =	**33.49**	**33.79**	**33.95**	**32.92**	**34.76**	**0.00**	**-0.30**	**-0.53**	**-0.31**
Standard Deviation =	**24.56**	**25.07**	**25.62**	**25.34**	**25.69**	**6.65**	**6.74**	**6.55**	**5.97**
Maximum =	**110.82**	**104.15**	**105.74**	**105.14**	**105.37**	**20.00**	**30.22**	**37.65**	**28.58**
Minimum =	**16.46**	**14.91**	**16.24**	**15.23**	**16.40**	**-15.26**	**-22.20**	**-22.11**	**-14.20**

Mean % Change From Baseline

Month 1	Month 2	Month 4	Month 8
1 6%	0 1%	-1.8%	0 9%
Increase	Increase	Decrease	Increase

Tallies & Percentages

	Month 1	Month 2	Month 4	Month 8
Total	114	114	114	114
Increased Values	57	49	44	50
Decreased Values	57	65	70	64
No Change	0	0	0	0
Increased Values	50 00%	42 98%	38 60%	43 86%
Decreased Values	50 00%	57 02%	61 40%	56 14%
No Change	0.00%	0 00%	0 00%	0.00%

Did Not Complete Subjects 7, 51, 58, 81, 83, 85, 113

Mean G/RGB = Mean Green value from the RGB color space, for the detected features within the masked region of interest (ROI)

[redacted]

Table 4
(continued)

.01[redacted]Lash Enhancing Serum

Within-Groups Analysis For :
VISIA-CR Image Analysis Color Data - G/RGB * Value Left Iris

Pre-Test	Normality	Brown-Forsythe	Friedman ANOVA
P-Value	P < 0.050	Not Required	0.710

Within-Groups Analysis For :
VISIA-CR Image Analysis Color Data - G/RGB * Value Right Iris

Pre-Test	Normality	Brown-Forsythe	Friedman ANOVA
P-Value	P < 0.050	Not Required	0.036

Pairwise Comparisons

Difference from Baseline	Test Type	P-value	Significance	Direction (Mean)
Month 1	Dunn's Method	1.000	No	Increase
Month 2	Dunn's Method	0.314	No	Increase
Month 4	Dunn's Method	**0.018**	**Yes**	**Decrease**
Month 8	Dunn's Method	1.000	No	Increase

* = ANOVA (RM) compares the means across groups (the non-parametric version is Friedman). The null hypothesis is all means are equivalent and the alternative is that the mean of at least one group differs. If ANOVA is significant further pairwise comparisons can be made, if not then it is assumed there is no statistically significant difference among the mean (or ranks) and no further testing is done due to risk of type one error. It is possible that Friedman or RM ANOVA may be significant, but when running post-hoc test may not show any statistically significant difference due to only examining the comparisons to baseline, post-hoc test, small sample size, lack of power, or if there is borderline significance.

Mean G/RGB = Mean Green value from the RGB color space, for the detected features within the masked region of interest (ROI)

Summary:

For subjects using [redacted]Lash Enhancing Serum, there was no statistically significant change in the G/RGB values in the left eye when compared to baseline. There was a statistically significant increase in the right eye at month 4 indicating an increase in the mean green value in the RGB color space; however, there was then no difference when comparing baseline to month 8.

Table 4
(continued)

VISIA-CR Image Analysis Color Data- B/RGB * Value Left Iris

.01 - [redacted] Lash Enhancing Serum

						Difference From Baseline At :			
Subject #	**Baseline**	**Month 1**	**Month 2**	**Month 4**	**Month 8**	**Month 1**	**Month 2**	**Month 4**	**Month 8**
1	31 73	30 58	33 86	29 01	33 77	-1 1	2 1	-2 7	2 0
2	109 60	97.04	101 64	96 70	94 99	-12 6	-8 0	-12 9	-14 6
3	83 34	87 07	83.22	85 27	89 92	3 7	-0 1	1 9	6 6
4	55 11	57 32	61.19	52 56	53 13	2 2	6 1	-2 6	-2 0
5	25 23	23 86	21 67	17 20	22 06	-1 4	-3.6	-8 0	-3 2
6	21 23	21 29	15 28	16 60	20 81	0 1	-5 9	-4 6	-0 4
8	18 97	22.01	25 79	26 72	20 50	3 0	6 8	7 8	1 5
9	85.18	84 59	79 42	76 95	73 55	-0 6	-5 8	-8 2	-11 6
10	88 56	102 69	86 85	93 85	91 92	14 1	-1.7	5 3	3 4
11	16 73	15 43	18 50	22 24	16.83	-1 3	1 8	5 5	0 1
12	93 44	86 14	89 33	91 80	95 27	-7 3	-4 1	-1 6	1 8
13	27 97	28 68	20.86	21 10	22.28	0 7	-7 1	-6 9	-5 7
14	18 05	27 76	20 08	19 43	21 44	9 7	2 0	1 4	3 4
15	12 12	22 24	23 30	18 66	22 20	10 1	11 2	6 5	10 1
16	26 59	21 88	22 70	24 73	25 08	-4 7	-3 9	-1 9	-1 5
17	28 64	30 48	38 65	31 31	28 82	1 8	10 0	2 7	0 2
18	25 68	29 41	17 06	30 38	24 55	3 7	-8 6	4 7	-1 1
19	29 43	19 98	32 41	24 58	24 28	-9 5	3 0	-4 9	-5 1
20	93 89	109 64	101 54	94 80	91 35	15 8	7 7	0 9	-2 5
21	41 96	55 15	66 69	65 41	65 12	13 2	24 7	23 4	23 2
22	20 73	23 82	21 52	20 83	22 03	3 1	0 8	0 1	1 3
23	96 73	99 99	96.99	93 69	85 47	3 3	0 3	-3 0	-11 3
24	21 34	18.09	18 39	21 98	15 29	-3 3	-3 0	0 6	-6 0
25	19 19	15 48	27 65	18 75	17 84	-3 7	8 5	-0 4	-1 3
26	80 23	80 61	70 59	68 67	73 40	0 4	-9 6	-11 6	-6 8
27	22 52	22 51	22 50	28 56	24 06	0 0	0 0	6 0	1 5
28	26 68	30 08	29 74	30.29	19 91	3 4	3 1	3 6	-6 8
29	54 71	54 95	59.64	39.89	58 27	0 2	4 9	-14 8	3 6
30	14.54	14 93	17 12	22 03	14 68	0 4	2 6	7 5	0 1
31	21 22	21 01	16 89	19 11	19 59	-0 2	-4 3	-2 1	-1 6
32	24 99	22 45	19 85	24 16	22 57	-2 5	-5 1	-0 8	-2 4
33	21 09	20 54	22 54	17 88	19 01	-0 6	1 5	-3 2	-2 1
34	23.88	28 49	28 16	33 51	28 25	4 6	4 3	9 6	4 4
35	80 18	79 49	90 80	57 78	85 89	-0 7	10 6	-22 4	5 7
36	20 65	24 12	24 97	22 92	24 27	3 5	4 3	2 3	3 6
37	18 38	20 79	15 53	17 54	17 07	2 4	-2 9	-0 8	-1 3
38	103 71	97 69	101 58	106 73	94 34	-6 0	-2 1	3 0	-9 4
39	47 98	50 67	55 42	53 22	51 31	2 7	7 4	5 2	3 3
40	17 63	20 57	24 14	23 97	13 44	2 9	6 5	6 3	-4 2
41	19 61	19 72	14 60	17 74	15 46	0 1	-5 0	-1 9	-4 1
42	24 35	18 51	25 18	23 01	25 68	-5.8	0 8	-1 3	1 3
43	23 83	17 10	17 83	19 54	18 40	-6 7	-6 0	-4 3	-5 4
44	26 50	29.10	31.37	25 74	29 21	2 6	4 9	-0 8	2 7
45	20 20	27 48	24 51	24 98	24 58	7 3	4 3	4 8	4 4
46	30.35	41 87	32.35	31 19	41 38	11 5	2.0	0.8	11 0
47	60 71	43 17	58 04	54 77	50 49	-17 5	-2 7	-5 9	-10 2
48	24 66	23 99	19 31	23 82	22 89	-0 7	-5 4	-0 8	-1 8
49	21 17	18 03	23 16	21 70	22 08	-3 1	2 0	0 5	0 9
50	55 70	56 42	55.60	53 14	54 10	0 7	-0 1	-2 6	-1 6
52	23 72	26 72	23 14	23 97	22 11	3 0	-0 6	0.3	-1 6
53	69 15	64 76	77 42	71 71	63.54	-4 4	8 3	2 6	-5 6
54	22 91	18 74	20 89	16 76	19 07	-4 2	-2 0	-6 1	-3 8
55	26 44	21 88	16.08	28 38	25 10	-4 6	-10 4	1 9	-1 3
56	83 82	76 44	87 57	82 66	83 08	-7 4	3 8	-1 2	-0 7
57	30 94	28 15	32 82	25 73	26 92	-2.8	1 9	-5 2	-4 0
59	20 58	19 45	22 98	26 65	30 82	-1 1	2 4	6 1	10 2
60	72 26	72 60	71.11	75 18	70 37	0.3	-1 1	2 9	-1.9
61	20 62	20 98	29 69	19 84	19 79	0 4	9 1	-0 8	-0 8
62	23 38	26 46	27.75	24 26	31 25	3 1	4 4	0 9	7 9
63	29.44	30.47	29.45	28 72	33 48	1 0	0 0	-0 7	4 0
64	21 84	20 89	22 16	18 88	20 21	-0 9	0 3	-3 0	-1 6
65	19 97	18 66	18 42	16 92	17 31	-1 3	-1 6	-3 0	-2 7
66	39 15	34 99	34 37	30 18	36 82	-4 2	-4 8	-9 0	-2 3

Table 4
(continued)
VISIA-CR Image Analysis Color Data- B/RGB * Value Left Iris

.01 – [redacted] Lash Enhancing Serum

67	17 62	19.12	25 09	25 04	14 93	1 5	7 5	7 4	-2 7
68	28 67	35 73	28 14	24 64	27 37	7 1	-0 5	-4 0	-1 3
69	26 52	26 31	25 70	27 15	24 73	-0 2	-0 8	0 6	-1 8
70	19 19	27 29	23 53	23 76	20 43	8 1	4 3	4 6	1 2
71	31 88	27 11	21 75	28 67	25 38	-4 8	-10 1	-3 2	-6 5
72	22 23	27 48	24 52	25 51	31 02	5 2	2 3	3 3	8 8
73	22 73	20 23	18 20	18 47	18.70	-2.5	-4 5	-4 3	-4 0
74	24 48	17 31	14 16	15 87	25 76	-7 2	-10 3	-8 6	1 3
75	26 76	19 10	21 95	20 65	24 01	-7.7	-4 8	-6 1	-2 7
76	29 19	31 32	26 42	26 72	29 09	2 1	-2 8	-2 5	-0 1
77	20 54	25 36	26 49	23 90	27 38	4 8	6 0	3 4	6 8
78	83 32	88 64	89 82	85 82	86 30	5 3	6 5	2 5	3 0
79	27 80	24 99	29 95	27 69	25 07	-2 8	2 1	-0 1	-2 7
80	27 79	22 77	22 25	21 09	19 87	-5.0	-5 5	-6 7	-7 9
82	21 49	25 52	25 64	26 35	25 40	4 0	4 2	4 9	3 9
84	17 91	30 73	16 13	19 31	22 71	12 8	-1 8	1 4	4 8
86	23.04	27 31	29 50	27 94	28.48	4 3	6 5	4 9	5 4
87	24 36	20 55	24 19	26 38	28 41	-3 8	-0 2	2 0	4 1
88	89 92	101 34	89 77	92 58	100 15	11 4	-0 2	2 7	10 2
89	25 95	25 75	31 15	27 59	24 93	-0 2	5 2	1 6	-1 0
90	112 51	105 46	109 14	108 25	110.98	-7 1	-3 4	-4 3	-1 5
91	22 39	23 69	20 27	20.03	23 45	1 3	-2.1	-2 4	1.1
92	34.25	31 14	27 06	25 14	24 04	-3 1	-7 2	-9 1	-10 2
93	73 99	59 99	65 71	61 81	63 55	-14 0	-8 3	-12 2	-10 4
94	38 90	39 20	41 10	37 14	39 08	0 3	2 2	-1 8	0 2
95	54 27	55 58	50 37	51 45	54 94	1 3	-3 9	-2 8	0 7
96	96 85	100 06	98 58	103 50	96 91	3 2	1 7	6 6	0 1
97	16 14	21 61	14 71	14 90	18 38	5 5	-1 4	-1 2	2 2
98	42 57	29 87	32 94	45 08	35 71	-12 7	-9 6	2 5	-6 9
99	32.57	30 86	33 76	33.89	34 04	-1 7	1 2	1 3	1 5
100	32 83	27 05	26 58	29 98	30 96	-5 8	-6 2	-2 8	-1 9
101	25 93	24 37	26 04	25 25	21 70	-1 6	0 1	-0 7	-4 2
102	24 25	27.75	29 93	18 55	29 48	3 5	5 7	-5 7	5 2
103	28.53	23 20	28 30	25 42	23 56	-5 3	-0 2	-3 1	-5 0
104	30 71	28 33	34.46	28 75	35 80	-2 4	3 8	-2 0	5 1
105	62 79	70 70	71 60	55 50	72 15	7 9	8 8	-7 3	9 4
106	19 31	26 47	23 73	22 16	23 89	7 2	4 4	2 8	4 6
107	18 18	22 46	19 78	20 99	21 86	4 3	1 6	2 8	3 7
108	98 86	97 67	98 85	99 45	93 87	-1 2	0 0	0 6	-5 0
109	81 47	86 88	86 65	83 01	78 45	5 4	5 2	1 5	-3 0
110	94 60	91 95	97 78	95 60	96 27	-2 6	3 2	1 0	1 7
111	21 76	22.38	27 62	19 25	27 44	0 6	5 9	-2 5	5 7
112	23 27	21 94	27 23	23 50	23 16	-1 3	4 0	0 2	-0 1
114	22 61	27 37	27 83	27 10	24 71	4 8	5 2	4 5	2 1
115	28 46	23 88	24 70	27 23	26 91	-4 6	-3 8	-1 2	-1 6
116	25 30	23 98	27 86	23 99	28 27	-1 3	2 6	-3 3	3 0
117	67 82	67 11	62 60	65 55	62 01	-0 7	-5 2	-2 3	-5 8
118	37 01	40 25	35 00	39 41	45 82	3 2	-2 0	2 4	8 8
119	23 78	25 24	25 64	21 71	23 28	1 5	1 9	-2 1	-0 5
120	27 48	24 81	28 97	22 73	31 88	-2 7	1 5	-4 7	4.4
121	18.82	19 89	19.12	22 24	25 26	1 1	0 3	3 4	6 4
Mean =	**38.87**	**39.13**	**39.46**	**38.25**	**38.83**	**0.27**	**0.59**	**-0.62**	**-0.04**
Median =	**26.51**	**27.20**	**27.63**	**26.36**	**26,33**	**0.17**	**0.55**	**-0.75**	**-0.46**
Standard Deviation =	**26.39**	**26.45**	**26.58**	**25.82**	**25.65**	**5.64**	**5.58**	**5.56**	**5.50**
Maximum =	**112.51**	**109.64**	**109.14**	**108.25**	**110.98**	**15.75**	**24.73**	**23.45**	**23.16**
Minimum =	**12.12**	**14.93**	**14.16**	**14.90**	**13.44**	**-17.54**	**-10.35**	**-22.40**	**-14.61**

Mean % Change From Baseline

Month 1	Month 2	Month 4	Month 8
0 7% Increase	1.5% Increase	-1 6% Decrease	-0 1% Decrease

Tallies & Percentages

	Month 1	Month 2	Month 4	Month 8
Total	114	114	114	114
Increased Values	59	62	53	54
Decreased Values	55	52	61	60
No Change	0	0	0	0
Increased Values	51 75%	54 39%	46 49%	47 37%
Decreased Values	48 25%	45 61%	53.51%	52 63%
No Change	0 00%	0 00%	0 00%	0 00%

Did Not Complete: Subject#s 7, 51, 58, 81, 85 and 113

Mean B/RGB = Mean Blue value from the RGB color space, for the detected features within the masked region of interest (ROI)

Table 4
(continued)
VISIA-CR Image Analysis Color Data - B/RGB Values Right Iris

.01 = [redacted] Lash Enhancing Serum

						Difference From Baseline At :			
Subject #	**Baseline**	**Month 1**	**Month 2**	**Month 4**	**Month 8**	**Month 1**	**Month 2**	**Month 4**	**Month 8**
1	24 91	22 83	27 94	24 00	25 29	-2 1	3 0	-0 9	0 4
2	108 87	97 84	109 74	100 46	107 27	-11 0	0 9	-8 4	-1 6
3	82 22	92 96	80.08	93 65	86 10	10 7	-2 1	11 4	3 9
4	53 15	52 00	54 00	53 42	54 52	-1 1	0 8	0 3	1 4
5	27 91	22 91	20 33	19 16	22 18	-5 0	-7 6	-8 8	-5.7
6	15 73	15 68	15 34	11.79	14 12	-0 1	-0 4	-3 9	-1 6
8	26 66	17 33	23.74	20 91	17 36	-9.3	-2 9	-5 8	-9 3
9	77 61	82 55	74 55	69 93	75 59	4 9	-3 1	-7 7	-2 0
10	86.94	94 52	89 19	87 48	94 21	7 6	2 2	0 5	7 3
11	19 28	18 04	15 61	16 67	18 29	-1 2	-3 7	-2 6	-1 0
12	89 21	84 19	84 07	98 06	93 57	-5 0	-5 1	8 9	4 4
13	23 89	24 94	20.17	20 15	21 48	1 1	-3 7	-3 7	-2 4
14	23 14	29 34	24 04	19 19	17 22	6 2	0 9	-4 0	-5 9
15	13 57	25 77	21 47	12.64	17 85	12 2	7 9	-0 9	4 3
16	24 08	20 57	20,27	22 67	21 91	-3 5	-3 8	-1.4	-2 2
17	32 77	31 78	44 31	38 98	34 99	-1 0	11 5	6 2	2 2
18	21 90	21 47	15 79	20 14	22 88	-0 4	-6 1	-1 8	1 0
19	33.13	18 36	23 06	26 33	24 02	-14 8	-10 1	-6 8	-9 1
20	85 19	100 74	110 30	95 34	83 79	15 5	25 1	10 1	-1 4
21	38 73	55 26	71 68	79 97	66 16	16 5	33 0	41 2	27 4
22	17 92	18 13	21 29	17 98	17 30	0 2	3 4	0 1	-0 6
23	77.63	94 94	90 94	83 12	77 52	17 3	13 3	5 5	-0 1
24	18 33	19 81	16 55	18 10	18 83	1 5	-1 8	-0 2	0 5
25	19 27	13 00	22 48	16 77	16 31	-6 3	3 2	-2 5	-3 0
26	75 20	75 64	73 94	76 23	76 58	0 4	-1 3	1 0	1 4
27	27 67	24 30	28 53	26 11	27 57	-3 4	0 9	-1 6	-0 1
28	20 38	34 13	33 23	21 99	23 28	13 7	12 8	1 6	2 9
29	45 08	65 68	41.14	39 68	44 56	20 6	-3 9	-5 4	-0 5
30	13 68	11 33	15 43	15 01	12 57	-2 3	1 8	1 3	-1 1
31	16 69	17 49	19 52	17 09	15 89	0 8	2 8	0 4	-0 8
32	39 77	23 02	20 91	23 10	23 82	-16 8	-18 9	-16 7	-15 9
33	21 31	21 13	21.48	18 78	19 78	-0 2	0 2	-2 5	-1 5
34	30.43	22 87	28 37	29 99	24 42	-7 6	-2 1	-0 4	-6 0
35	77 03	89 40	89 09	66 40	90 78	12 4	12 1	-10 6	13 7
36	21 31	20.84	17 86	24 85	16 44	-0 5	-3 5	3 5	-4 9
37	15 33	19 91	14 78	15 33	17 52	4 6	-0 5	0 0	2 2
38	97 09	93 72	99 84	96 58	95 90	-3 4	2 8	-0 5	-1 2
39	47 57	46 71	48 91	45 98	49 71	-0 9	1 3	-1 6	2 1
40	17 34	17.96	18 39	15 49	13 14	0.6	1 0	-1 9	-4 2
41	17 17	26 28	12 94	16 41	13,05	9 1	-4 2	-0 8	-4 1
42	20 38	17 40	22 07	19 81	28 24	-3.0	1 7	-0 6	7 9
43	19.64	13 87	16 35	16 97	16 08	-5 8	-3 3	-2 7	-3 6
44	33 89	32 21	28 56	28 70	30 55	-1 7	-5 3	-5 2	-3 3
45	22 61	34 36	21 34	23 65	19 98	11 8	-1 3	1 0	-2 6
46	35 79	46 12	30 13	28 50	44 91	10 3	-5 7	-7 3	9 1
47	67.30	60 08	67 04	60 35	52.00	-7 2	-0 3	-7 0	-15 3
48	25 60	18 99	22 25	20 32	26 11	-6 6	-3 4	-5 3	0 5
49	20 75	17 82	20 62	19 27	18 61	-2 9	-0 1	-1 5	-2 1
50	60 36	59 49	59 42	54 15	59 21	-0 9	-0 9	-6 2	-1 2
52	22.82	21 09	17.69	18 83	18 39	-1 7	-5.1	-4 0	-4 4
53	54 12	51 49	46 63	49 29	45 98	-2 6	-7 5	-4 8	-8 1
54	19 93	18 65	19 54	16 01	18 19	-1 3	-0 4	-3 9	-1 7
55	26 36	23 81	14 23	25.70	17 22	-2 5	-12 1	-0 7	-9 1
56	76,73	72,07	88 31	79 99	80 39	-4.7	11 6	3 3	3 7
57	21 26	21 26	26 13	21 70	18 97	0.0	4 9	0 4	-2.3
59	17 84	20 56	16 07	16 67	20.03	2 7	-1 8	-1 2	2 2
60	50 25	51 00	42 90	50 11	44.82	0.7	-7 3	-0 1	-5 4
61	19,47	21 87	24 92	19 39	17 66	2.4	5.4	-0 1	-1 8
62	21 13	19 25	18.40	19 44	23 30	-1.9	-2 7	-1 7	2 2
63	27 47	28.32	25.87	24 75	26 61	0 9	-1 6	-2 7	-0 9
64	21 55	21 00	22.62	20.61	19 08	-0 6	1 1	-0 9	-2 5
65	18.61	15 10	16 31	16 32	16 13	-3 5	-2 3	-2 3	-2 5
66	31 33	35 23	30 26	29.18	35 07	3 9	-1 1	-2 2	3.7
67	19 43	21.58	16 02	16 15	16 89	2 1	-3 4	-3 3	-2 5

Table 4
(continued)
VISIA-CR Image Analysis Color Data - B/RGB Values Right Iris

.01 = [redacted] Lash Enhancing Serum

68	21 55	28 65	19 19	18 16	22 46	7 1	-2 4	-3 4	0 9
69	24 78	23 88	22 17	29 95	24 13	-0.9	-2 6	5 2	-0 7
70	16 82	20 62	20 35	17 27	18 42	3.8	3 5	0 4	1.6
71	27 36	27 41	24 29	25 54	21 05	0 0	-3 1	-1 8	-6 3
72	19 93	24 56	22 04	19 60	22 88	4 6	2 1	-0 3	3 0
73	17 96	19.16	17 38	16 44	16 91	1 2	-0 6	-1 5	-1 0
74	17 06	12 24	13 38	14 21	15 34	-4 8	-3 7	-2 8	-1 7
75	20 82	18 35	19 37	17 50	20 02	-2 5	-1 4	-3 3	-0 8
76	26.93	32 01	24 81	23 69	25 50	5 1	-2 1	-3 2	-1 4
77	24.77	19 39	25.94	26 72	24 39	-5 4	1 2	2 0	-0 4
78	58 65	80 17	75 10	76 75	80 99	21 5	16 5	18 1	22 3
79	31 73	24 00	36 86	27 94	25 52	-7 7	5 1	-3 8	-6 2
80	19 69	18 29	17 51	15 33	16 63	-1 4	-2 2	-4 4	-3 1
82	18.83	19 83	16.39	21 13	18 36	1 0	-2 4	2 3	-0 5
84	13 46	28 97	14 02	14 29	21 52	15.5	0 6	0 8	8 1
86	19 00	23 62	21 26	28 44	22 06	4 6	2 3	9 4	3 1
87	21 07	18 77	18 74	19 95	20 70	-2 3	-2 3	-1 1	-0 4
88	98 83	90 33	93 39	82 98	90 06	-8 5	-5 4	-15 8	-8 8
89	23 39	22 45	28 95	21 27	21 28	-0 9	5 6	-2 1	-2 1
90	103 14	107 14	106 55	107.32	105 88	4 0	3 4	4 2	2 7
91	15 38	23 68	14 70	16 25	18 20	8 3	-0 7	0 9	2 8
92	24.64	26 85	20 73	28 77	22 87	2 2	-3 9	4 1	-1 8
93	93 56	78 62	70 43	80.19	81 40	-14 9	-23 1	-13 4	-12 2
94	48 69	52 77	40 85	50 35	52 82	4 1	-7 8	1 7	4 1
95	52 85	52 40	55 18	47 27	53.36	-0 4	2 3	-5.6	0 5
96	89.68	92.05	90 16	96 82	90 76	2.4	0 5	7 1	1 1
97	18 13	17 08	13 91	14 20	16 58	-1 0	-4 2	-3 9	-1 6
98	49.89	47 77	53 76	37 15	48 07	-2 1	3 9	-12 7	-1 8
99	28 98	28 80	31 59	30 56	32 36	-0 2	2 6	1 6	3 4
100	35 47	21 10	27 23	25 32	27 34	-14 4	-8 2	-10 2	-8 1
101	24 29	21 32	23 98	23 08	20 63	-3 0	-0 3	-1 2	-3 7
102	21 57	21 47	20.99	16 36	25 89	-0 1	-0 6	-5 2	4 3
103	27 08	31 47	37.84	29 38	34 84	4 4	10 8	2 3	7 8
104	24 74	23 67	24 08	21 74	25 66	-1 1	-0 7	-3 0	0 9
105	64 67	66.03	68 33	58 77	64 56	1.4	3 7	-5 9	-0 1
106	18 63	20 67	19 25	20 84	22.79	2 0	0 6	2 2	4 2
107	20 15	17 05	28 41	17 98	21 54	-3 1	8 3	-2 2	1.4
108	76 14	90 39	89 20	94 16	94 63	14 3	13 1	18 0	18 5
109	82 81	74 45	73 44	59.48	79 13	-8 4	-9 4	-23 3	-3 7
110	83 36	83 54	84 88	85 13	85 85	0 2	1 5	1 8	2 5
111	17 66	31 09	23 28	17 21	28 30	13 4	5 6	-0 5	10 6
112	26 26	22 26	24 02	21 69	18 99	-4 0	-2 2	-4 6	-7 3
114	36 44	39 34	32.81	37 15	32 39	2 9	-3.6	0 7	-4 1
115	27 87	31.60	36 34	27 67	23 86	3 7	8 5	-0 2	-4 0
116	22 14	25 38	25 55	26 98	28 35	3 2	3 4	4 8	6 2
117	54 16	59 26	57 21	54 28	60.07	5 1	3 0	0 1	5 9
118	30 93	30 24	28 57	28 48	32 08	-0 7	-2 4	-2 4	1 2
119	20 14	22 21	19 03	19 70	19 72	2 1	-1 1	-0 4	-0 4
120	23 95	27 18	22 40	20.19	25 59	3 2	-1 6	-3 8	1 6
121	19 12	22 12	15 97	26 04	30 65	3 0	-3 2	6 9	11 5
Mean =	**36.71**	**37.63**	**36.90**	**35.70**	**36.68**	**0.92**	**0.19**	**-1.01**	**-0.03**
Median =	**24.78**	**24.43**	**24.06**	**23.85**	**24.07**	**-0.08**	**-0.62**	**-1.31**	**-0.73**
Standard Deviation =	**24.82**	**25.77**	**26.32**	**25.69**	**25.90**	**7.91**	**7.00**	**6.99**	**6.17**
Maximum =	**108.87**	**107.14**	**110.30**	**107.32**	**107.27**	**21.52**	**32.95**	**41.24**	**27.43**
Minimum =	**13.46**	**11.33**	**12.94**	**11.79**	**12.57**	**-16.75**	**-23.14**	**-23.33**	**-15.95**

Mean % Change From Baseline

Month 1	Month 2	Month 4	Month 8
2 5% Increase	0 5% Increase	-2 8% Decrease	-0 1% Decrease

Tallies & Percentages

	Month 1	Month 2	Month 4	Month 8
Total	114	114	114	114
Increased Values	56	49	39	47
Decreased Values	58	65	75	67
No Change	0	0	0	0
Increased Values	49 12%	42 98%	34 21%	41 23%
Decreased Values	50.88%	57.02%	65.79%	58 77%
No Change	0 00%	0.00%	0 00%	0 00%

Did Not Complete Subjects 7, 51, 58, 81, 85 and 113

Mean B/RGB = Mean Blue value from the RGB color space, for the detected features within the masked region of interest (ROI)

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Table 4
(continued)

.01[redacted] Lash Enhancing Serum

Within-Groups Analysis For :
VISIA-CR Image Analysis Color Data - B/RGB * Value Left Iris

Pre-Test	Normality	Brown-Forsythe	Friedman ANOVA
P-Value	P < 0.050	Not Required	0.368

Within-Groups Analysis For :
VISIA-CR Image Analysis Color Data - B/RGB * Value Right Iris

Pre-Test	Normality	Brown-Forsythe	Friedman ANOVA
P-Value	<0.050	Not Required	0.003

Pairwise Comparisons

Difference from Baseline	Test Type	P-value	Significance	Direction (Mean)
Month 1	Dunn's Method	1.000	No	Increase
Month 2	Dunn's Method	0.131	No	Increase
Month 4	Dunn's Method	**0.001**	Yes	**Decrease**
Month 8	Dunn's Method	0.668	No	Decrease

* = ANOVA (RM) compares the means across groups (the non-parametric version is Friedman). The null hypothesis is all means are equivalent and the alternative is that the mean of at least one group differs. If ANOVA is significant further pairwise comparisons can be made, if not then it is assumed there is no statistically significant difference among the mean (or ranks) and no further testing is done due to risk of type one error. It is possible that Friedman or RM ANOVA may be significant, but when running post-hoc test may not show any statistically significant difference due to only examining the comparisons to baseline, post-hoc test, small sample size, lack of power, or if there is borderline significance.

Mean B/RGB = Mean Blue value from the RGB color space, for the detected features within the masked region of interest (ROI)

Summary:

For subjects using [redacted] Lash Enhancing Serum, there was no statistically significant change in the B/RGB values in the left eye when compared to baseline. In the right eye, there was a statistically significant decrease at month 4 indicating a decrease in the mean blue value within the ROI.

Table 4
(continued)
VISIA-CR Image Analysis Color Data - Delta E Value Left Iris

.01 = [redacted] Lash Enhancing Serum

Subject #	Month 1	Month 2	Month 4	Month 8
1	3 98	1 58	1 19	1 98
2	4 15	2 63	4 40	4 84
3	1 28	1 61	0 65	4 40
4	1 52	2.82	1 70	2.63
5	0 82	1 67	3 81	1 52
6	0 65	3 58	3 25	0 60
8	1 78	3 41	3 09	1 95
9	1 32	2 26	3 76	5 98
10	5 54	0 77	2 17	1 50
11	0 56	0 84	4 70	0 51
12	2 79	1 64	0 73	1.45
13	0 54	3 36	3 21	2 59
14	3 88	0 73	0 53	1 37
15	3 90	4 94	2 82	4 34
16	2 68	1 64	0 83	0.81
17	1 74	3.70	1 00	1 19
18	1 49	4 49	2 19	1 16
19	4 57	1 48	2 23	2 65
20	6 33	2 64	0 95	1 69
21	6 36	10 23	9 75	9 59
22	1 17	0 42	0 40	0 98
23	1 33	0 19	1 95	4.09
24	1 59	1 31	0 31	3 01
25	2 01	3 91	0 55	0 65
26	2 76	4 16	4 91	2 73
27	1 77	1 90	2 32	1 34
28	1 89	2 08	2 12	3 48
29	4 00	4 71	13 38	5 30
30	0 58	1 32	3 59	0 54
31	1 30	2 62	1 00	0 94
32	1 30	2 18	0 90	1 54
33	1 38	0 59	1 99	0 96
34	2 01	2 20	3 68	2 44
35	0 83	4 83	8 06	2 04
36	1 28	1 66	0 89	2 58
37	0 83	1 68	0 50	1 15
38	1 93	1 19	3 10	3 09
39	1 94	3.03	2 30	1 83
40	1 54	3 09	3 28	1 52
41	0 70	2 11	1 03	1 55
42	2 52	2 01	4 01	4 03
43	2 82	2 59	1 93	2 17
44	1 44	3 35	0 93	2 67
45	3 37	3 49	3 50	2 21
46	5 29	2 33	5 12	7 69
47	9.03	4 50	5 01	6 81
48	1.00	2 46	0 41	0 73
49	1 92	2 10	1 44	1 57
50	1 00	2 37	1.76	1 07
52	5 79	6 71	4 27	6 66
53	2 95	4 93	3 65	4 19
54	2,08	0 95	2 80	1 94
55	1 94	4 51	1 45	0 66
56	2 95	1 67	0 38	0 40
57	1 39	1 17	2 73	3 06
59	0 58	1 24	2 98	7.85
60	1 83	1 57	2 06	3 06
61	1 07	3 93	0 88	0 64
62	1 27	1 91	0.65	3 80
63	0 73	2 04	1,49	2 71
64	0 97	0 35	1 27	1 14
65	1.36	1 33	1 41	1 36
66	2 67	2 43	5 08	1 80

Table 4
(continued)
VISIA-CR Image Analysis Color Data - Delta E Value Left Iris

.01 = [redacted] Lash Enhancing Serum

67	2 03	4 05	3 67	1 87
68	3 32	0 23	1 71	0 57
69	1 24	1 69	0 82	0 65
70	4 05	2 48	2 97	1 71
71	1 87	4 58	1 22	3 83
72	2 71	1 18	1 96	4 41
73	1 48	2 65	1 77	1 72
74	3 23	5 08	3.84	1 49
75	3 25	2 32	2 39	1 13
76	1 16	2 40	2 23	0 67
77	3 11	2 38	1 53	3 27
78	2 17	2 54	1 57	1 50
79	1 87	1 01	0 55	1 54
80	2 84	2 96	3 45	3 72
82	1 96	2 24	2 90	2 78
84	5 40	1 16	0 57	1 76
86	2 26	3 79	2 20	3 55
87	2 08	2 60	1 74	2 77
88	4 67	1 22	1 86	4 15
89	1 46	2 35	3 13	2 50
90	2 18	1 45	1 37	1 04
91	1 36	1 62	1 27	2 15
92	3 57	4 73	5 47	6 20
93	5 57	3 23	4 53	3 92
94	0 88	9 78	3 10	1 84
95	2 30	1 30	1 09	1 07
96	1 23	1 71	2 44	1 43
97	2 65	0 55	0 53	2 02
98	5 59	5 23	2 66	2 84
99	0 79	1 54	1 13	0 99
100	2 99	3 28	2 54	1 13
101	0 99	1 50	3 02	1 58
102	5 21	5 48	7 71	3 87
103	1 96	3 15	1 91	2 39
104	1 03	1 50	1 42	2 73
105	5 55	6 09	4 55	4 11
106	2 82	2 69	3 72	3 85
107	2 03	1 96	1 28	4 38
108	1 25	1 34	0 92	3 13
109	1 79	2 60	1 30	3 41
110	0 93	1 59	0 96	1 47
111	1 63	3 91	1 42	3 14
112	0 64	1 78	0 49	0 17
114	2 41	2 90	1 94	1 65
115	1 88	1.52	0 98	0 64
116	1 75	2 07	1 32	2 72
117	1 27	2 58	1 36	3 00
118	1 86	1 18	2 03	4 26
119	0 72	0 81	0 75	0 14
120	2 62	0 99	3 36	1 96
121	2 48	1 19	3.11	6 00
Mean =	**2.33**	**2.56**	**2.42**	**2.52**
Median =	**1.90**	**2.22**	**1.95**	**2.00**
Standard Deviation =	**1.55**	**1.67**	**1.94**	**1.75**
Maximum =	**9.03**	**10.23**	**13.38**	**9.59**
Minimum =	**0.54**	**0.19**	**0.31**	**0.14**

Did Not Complete Subject#s 7, 51, 58, 81, 83, 85, 113

L* values are a measure of the luminosity of the test site The higher the value, the greater the lightness of the test site
a* values are a measure of the redness of the test site The higher the value, the greater the redness of the test site.
b* values are a measure of the yellowness of the test site The higher the value, the greater the yellowness of the test site

Table 4
(continued)
VISIA-CR Image Analysis Color Data - L* Value Left Iris

.01 = [redacted] Lash Enhancing Serum

Subject #	Baseline	Month 1	Month 2	Month 4	Month 8	*Difference From Baseline At :* Month 1	Month 2	Month 4	Month 8
1	20.38	22 07	20 63	19 27	22 01	1 7	0 2	-1 1	1 6
2	41 73	37 76	39 26	37 57	37 61	-4 0	-2 5	-4 2	-4 1
3	36 35	37 31	35 49	36 96	40 23	1 0	-0 9	0 6	3 9
4	32 83	32 94	35.47	31 32	30 86	0 1	2 6	-1 5	-2 0
5	10 91	10 48	9 38	7 38	9 75	-0 4	-1 5	-3 5	-1 2
6	9 82	9 62	6 79	7 22	9 42	-0 2	-3.0	-2 6	-0 4
8	8 96	9 34	10 84	10 87	8 73	0 4	1 9	1 9	-0 2
9	42 30	42 85	40 31	39 01	36 88	0 6	-2 0	-3.3	-5 4
10	36 93	42 08	36 19	38 96	37 62	5 1	-0 7	2 0	0 7
11	6 73	6 21	7 47	10 38	6 83	-0 5	0 7	3 7	0 1
12	40 38	38 44	38.77	40 07	41 56	-1 9	-1 6	-0 3	1 2
13	11.94	12 14	8 78	8.93	9 42	0 2	-3 2	-3.0	-2 5
14	8 30	11.81	8 88	8 71	9.48	3 5	0 6	0 4	1 2
15	5 41	8 99	10 07	8 02	9 45	3 6	4 7	2.6	4 0
16	12 89	10 40	11 33	12 37	12 74	-2 5	-1 6	-0 5	-0 1
17	20 13	20 03	23 21	21.09	19 85	-0 1	3 1	1.0	-0 3
18	15 67	17 09	11 52	17 03	14 89	1 4	-4 1	1 4	-0 8
19	17 29	12 92	18 74	15 09	14 71	-4 4	1 5	-2 2	-2 6
20	36 50	42 61	38 47	36 35	35 04	6 1	2 0	-0 2	-1 5
21	27 00	30 97	35 13	34 84	35 28	4 0	8 1	7 8	8 3
22	13 92	15 03	14 31	14 16	14 72	1 1	0 4	0 2	0 8
23	38 23	39 55	38 36	36 54	34 64	1 3	0 1	-1 7	-3 6
24	9 03	7 55	7 83	9 31	6 25	-1 5	-1 2	0 3	-2 8
25	8 29	6 48	12 02	7 95	7 70	-1 8	3 7	-0 3	-0 6
26	36 23	37 89	35 14	34 70	35 29	1 7	-1 1	-1 5	-0 9
27	14 34	13 36	13 28	16 06	15 48	-1 0	-1 1	1 7	1 1
28	13 65	14 77	15 34	15 63	12 40	1 1	1 7	2 0	-1 3
29	26 17	28 53	30 03	23 92	30 05	2 4	3 9	-2 3	3 9
30	5 66	5 93	6 84	9 06	5 93	0 3	1 2	3 4	0 3
31	8 77	8 19	6 57	7 84	8 05	-0 6	-2 2	-0 9	-0 7
32	16 97	15 89	14 98	16 77	16 47	-1 1	-2 0	-0 2	-0 5
33	12 92	12 12	13 50	11 20	12 24	-0 8	0 6	-1 7	-0 7
34	15 74	17 74	17 80	19 35	17 88	2 0	2 1	3 6	2 1
35	30 38	29 75	34 92	22 65	32 33	-0 6	4 5	-7 7	2 0
36	12 82	13 63	14 47	13 70	15 00	0 8	1 7	0 9	2 2
37	8 87	9 43	7 42	8 47	8 05	0 6	-1 5	-0 4	-0 8
38	42 80	40 94	42 80	45 19	39 79	-1 9	0 0	2 4	-3 0
39	31 66	31 80	33 83	33 48	33 39	0 1	2 2	1 8	1 7
40	7 29	8 57	10 20	10 24	5 83	1 3	2 9	2 9	-1 5
41	8.53	8 53	6 62	8 22	7 17	0 0	-1 9	-0 3	-1 4
42	15 78	13 34	16 92	17 07	18 25	-2 4	1 1	1 3	2 5
43	11 17	8 69	8 91	9 45	9 24	-2 5	-2 3	-1 7	-1 9
44	19 93	21 32	20 53	19 20	22 04	1 4	0 6	-0 7	2 1
45	8 42	11.48	11 32	11 43	10 44	3 1	2 9	3 0	2 0
46	16 18	21 34	18 04	19 26	22 86	5 2	1 9	3 1	6 7
47	26 38	17 69	23 21	22 16	20 26	-8 7	-3 2	-4 2	-6 1
48	10 42	10 55	8 01	10 11	9 74	0 1	-2 4	-0 3	-0 7
49	12 83	11 16	14 31	13 69	13 79	-1 7	1 5	0 9	1 0
50	30 47	30 28	29 16	28 90	30 46	-0 2	-1 3	-1 6	0 0
52	12 25	15 79	14.85	14 27	14 64	3 5	2 6	2.0	2.4
53	35 98	35 35	40 43	38 43	34 91	-0 6	4.5	2 5	-1 1
54	9 69	7 92	8 97	7 09	8 13	-1 8	-0 7	-2 6	-1 6
55	11 33	9 41	7 15	12 41	10 97	-1 9	-4 2	1 1	-0.4
56	34 33	31.54	35 96	33 97	34 28	-2 8	1 6	-0.4	-0 1
57	14 73	13 39	15 63	12 43	12 09	-1 3	0 9	-2 3	-2 6
59	9 03	8 65	10 19	11 87	15.75	-0 4	1 2	2 8	6 7
60	35.73	34 83	36 29	37 48	36 94	-0 9	0 6	1 7	1.2
61	12 71	13 28	16 43	12 09	12 46	0 6	3 7	-0 6	-0 2
62	10.89	12 15	12 78	11 15	14 58	1 3	1 9	0 3	3.7
63	21 86	22 31	20.83	21 28	24.29	0 4	-1 0	-0 6	2 4
64	12 96	13 08	13 01	12 29	11 99	0.1	0 1	-0 7	-1.0
65	12 66	11 62	11 59	11 55	11.42	-1.0	-1 1	-1 1	-1 2
66	22.73	21 91	21 59	21.01	21 19	-0 8	-1.1	-1 7	-1.5

Table 4
(continued)
VISIA-CR Image Analysis Color Data - L* Value Left Iris

				.01 % [redacted] Lash Enhancing Serum					
67	10 61	10 44	12 46	12 64	9 16	-0 2	1 9	2 0	-1 4
68	19.08	20 73	18 99	17 39	18 51	1 7	-0 1	-1 7	-0 6
69	20 58	21 23	21 33	21 19	20 20	0 6	0 7	0 6	-0 4
70	8 72	12 56	10 96	11 35	9 94	3 8	2 2	2 6	1 2
71	16 66	14.97	12 15	15 62	16 26	-1 7	-4 5	-1 0	-0 4
72	13.11	15 66	14 12	14 83	17 39	2 6	1 0	1 7	4 3
73	11 00	10 46	8 64	9 49	9 40	-0 5	-2.4	-1 5	-1 6
74	10 40	7 44	5 67	6 75	11 33	-3.0	-4 7	-3 7	0 9
75	13 28	10 18	11 05	11 15	12 19	-3 1	-2 2	-2 1	-1 1
76	18 54	18 88	18 03	17 84	18 82	0 3	-0 5	-0 7	0 3
77	13 72	16 49	15 96	14 45	16 91	2 8	2 2	0 7	3 2
78	31 64	33 59	33 91	32 96	32 98	1 9	2 3	1 3	1 3
79	17 32	15 83	18 23	17 21	15 96	-1 5	0 9	-0 1	-1 4
80	13 58	11 05	11 06	10 58	10 24	-2 5	-2 5	-3 0	-3 3
82	11.49	13 20	13 59	14 22	13 97	1 7	2.1	2 7	2 5
84	8 21	13 19	7 22	8 41	9 92	5 0	-1 0	0 2	1 7
86	12 80	14 88	16 24	14 53	15 93	2 1	3 4	1 7	3 1
87	11 08	9 80	12 21	12 41	13 47	-1 3	1 1	1 3	2 4
88	42 91	47 37	42 15	44 46	46 93	4 5	-0 8	1 6	4 0
89	20 98	21 68	22 99	23 25	21 99	0 7	2 0	2 3	1 0
90	43 74	41 81	43 39	42 60	43 84	-1 9	-0 4	-1 1	0 1
91	12.84	12 65	12 61	11 68	14 08	-0 2	-0 2	-1 2	1 2
92	23 52	20 88	19 16	18 45	17 79	-2 6	-4 4	-5 1	-5 7
93	31.44	26 30	28 45	27.06	27 75	-5 1	-3 0	-4 4	-3 7
94	31 53	31 18	26 47	29 39	32 53	-0 4	-5 1	-2 1	1 0
95	29 69	29 23	28 50	29 16	29 76	-0 5	-1 2	-0 5	0 1
96	36 50	37 27	36 18	38 67	35 79	0 8	-0 3	2 2	-0 7
97	6 06	8 49	5 65	5 90	7 50	2 4	-0 4	-0 2	1 4
98	24 66	19 58	20 24	26 75	22 24	-5 1	-4 4	2 1	-2 4
99	17 37	16 71	18 55	18 33	18 13	-0 7	1 2	1 0	0 8
100	20 78	19.64	19.48	20 99	20 40	-1 1	-1 3	0 2	-0 4
101	16 03	15 98	16 93	17 39	14 84	0 0	0 9	1 4	-1 2
102	20.04	18 57	19 26	14 34	20 05	-1 5	-0 8	-5 7	0 0
103	17 03	15 44	18 56	16 84	16 24	-1 6	1 5	-0 2	-0 8
104	18 46	17 43	19 57	18 57	21 04	-1 0	1 1	0 1	2 6
105	37 52	37 76	37 67	36 76	39 46	0 2	0 1	-0 8	1 9
106	12 29	14 59	14 69	14 78	15 29	2 3	2 4	2 5	3 0
107	11 47	13 18	11 59	12 48	11 29	1 7	0 1	1 0	-0 2
108	41 56	40 62	40 91	41 24	38 79	-0 9	-0 7	-0 3	-2 8
109	38 46	39,87	40 92	39 52	35 82	1 4	2 5	1 1	-2 6
110	40 40	39 67	41 82	41 16	41 51	-0 7	1 4	0 8	1 1
111	14 23	14.70	17 57	13 06	15 58	0 5	3 3	-1 2	1 3
112	10 75	10 13	12 39	10 78	10.82	-0 6	1 6	0 0	0 1
114	14 38	16 11	17.11	16 07	15.74	1 7	2 7	1 7	1 4
115	14 78	13 25	13 86	14 91	14 29	-1 5	-0 9	0 1	-0 5
116	19 40	18 50	20 64	19 29	21 28	-0 9	1 2	-0 1	1 9
117	26 21	25 36	23 71	26 17	23 36	-0 9	-2 5	0 0	-2 9
118	25 65	27.41	25.59	27 34	29 70	1 8	-0 1	1 7	4 1
119	14 02	14 17	14 61	13 28	13 92	0 2	0 6	-0 7	-0 1
120	17 57	15 44	17 75	14 58	19 27	-2 1	0 2	-3 0	1 7
121	9 08	10 43	8 71	11 39	13 73	1 3	-0 4	2 3	4 7
Mean =	**19.62**	**19.66**	**19.81**	**19.60**	**19.87**	**0.04**	**0.20**	**-0.02**	**0.25**
Median =	**15.76**	**15.73**	**16.92**	**15.84**	**16.10**	**-0.08**	**0.21**	**-0.07**	**-0.03**
Standard Deviation =	**10.76**	**10.84**	**10.89**	**10.67**	**10.61**	**2.26**	**2.30**	**2.24**	**2.44**
Maximum =	**43.74**	**47.37**	**43.39**	**45.19**	**46.93**	**6.11**	**8.13**	**7.84**	**8.28**
Minimum =	**5.41**	**5.93**	**5.65**	**5.90**	**5.83**	**-8.69**	**-5.06**	**-7.72**	**-6.12**

Mean % Change From Baseline

Month 1	Month 2	Month 4	Month 8
0 2% Increase	1 0% Increase	-0 1% Decrease	1 3% Increase

Did Not Complete Subjects 7, 51, 58, 81, 83, 85, 113

Tallies & Percentages

	Month 1	Month 2	Month 4	Month 8
Total	114	114	114	114
Increased Values	56	63	56	56
Decreased Values	58	51	58	58
No Change	0	0	0	0
Increased Values	49 12%	55 26%	49 12%	49.12%
Decreased Values	50 88%	44 74%	50 88%	50 88%
No Change	0 00%	0.00%	0 00%	0 00%

L* values are a measure of the luminosity of the test site. The higher the value, the greater the lightness of the test site
a* values are a measure of the redness of the test site. The higher the value, the greater the redness of the test site
b* values are a measure of the yellowness of the test site. The higher the value, the greater the yellowness of the test site.

Table 4
(continued)
VISIA-CR Image Analysis Color Data - a* Values Left Iris

.01 = [redacted] Lash Enhancing Serum

Subject #	Baseline	Month 1	Month 2	Month 4	Month 8	*Difference From Baseline At :* Month 1	Month 2	Month 4	Month 8
1	11 38	13 19	10 17	11 80	11.64	1 8	-1 2	0 4	0 3
2	-2 51	-1 92	-1.93	-1 43	-0 91	0 6	0 6	1 1	1 6
3	-1 38	-1 89	-0.78	-1 42	-1 45	-0 5	0 6	0 0	-0 1
4	3 28	2 30	2 32	3 54	3 72	-1 0	-1 0	0 3	0 4
5	2 24	2.92	2 90	3 41	3 22	0 7	0 7	1 2	1 0
6	3 49	3 01	3 45	3 13	3 31	-0 5	0 0	-0 4	-0 2
8	3 32	1 94	0 74	1 47	1 80	-1 4	-2 6	-1 9	-1 5
9	-1 51	-1 15	-0 50	0 30	0 66	0 4	1 0	1 8	2.2
10	-0 61	-2 66	-0 55	-1 38	-1 57	-2 1	0 1	-0 8	-1.0
11	1 96	2 09	2.03	2 50	2 42	0 1	0 1	0 5	0 5
12	-2 57	-0 96	-2 31	-2 07	-2 15	1 6	0 3	0 5	0 4
13	1 30	0.83	2.38	2 38	1.87	-0 5	1 1	1 1	0 6
14	4 75	3 10	4 33	4 42	4 05	-1 6	-0 4	-0 3	-0 7
15	2 76	1 30	1 87	2 42	1 57	-1 5	-0 9	-0.3	-1 2
16	6.13	6 47	6 64	6 67	6 52	0 3	0 5	0 5	0 4
17	11 49	10 30	10 25	11 30	12 55	-1 2	-1 2	-0 2	1 1
18	10 13	9 77	11 20	8 72	9 53	-0 4	1 1	-1 4	-0 6
19	7 97	9 04	7.96	8 16	8 11	1 1	0 0	0 2	0 1
20	-1 63	-3 23	-2 82	-2 17	-1 96	-1 6	-1 2	-0 5	-0 3
21	6 56	2 04	0 91	1 27	1 94	-4 5	-5 6	-5 3	-4 6
22	11 42	11 06	11 45	11 51	11 74	-0 4	0 0	0 1	0 3
23	-2 18	-2 19	-2 05	-1 65	-0 49	0.0	0 3	0 5	1 7
24	1 99	2 15	2 38	2 13	2 41	0 2	0 4	0 1	0 4
25	1 96	1 76	1 33	1 67	2 24	-0 2	-0 6	-0 3	0 3
26	-0 99	-0 82	0 21	0 82	-0 34	0 2	1 2	1 8	0 6
27	9 99	9 68	9 65	8 97	9 84	-0 3	-0 3	-1 0	-0 1
28	7 32	5 88	6 26	7 33	9 75	-1 4	-1.1	0 0	2 4
29	-0 86	-1 09	-0 91	10 79	-0 20	-0 2	0 0	11 7	0 7
30	0 62	1 04	0 78	0 42	0 83	0 4	0 2	-0 2	0 2
31	0 61	0.64	0 81	0 79	1 18	0 0	0 2	0 2	0 6
32	11 16	11.87	11 99	11 87	12 25	0 7	0 8	0 7	1.1
33	9 31	8 73	9 24	8 96	9 99	-0 6	-0 1	-0 3	0 7
34	9 45	9 35	10 10	9 01	10 47	-0 1	0 7	-0 4	1 0
35	-1 10	-1 24	-2 54	0 41	-1 62	-0 1	-1 4	1 5	-0 5
36	9 32	8 92	9 36	9 38	10 15	-0 4	0 0	0.1	0 8
37	4 64	4 11	4 71	4 88	4 32	-0 5	0 1	0 2	-0 3
38	-2 99	-3 04	-2 99	-3 14	-2 69	-0 1	0.0	-0 1	0 3
39	6 40	5 06	4 67	5 08	6 61	-1 3	-1 7	-1 3	0 2
40	2 63	1 88	1 97	1 47	2 86	-0 7	-0 7	-1 2	0 2
41	3 76	3 11	4 44	4 60	4.51	-0 7	0 7	0 8	0 7
42	9 36	10 00	10 43	11 66	10 28	0 6	1 1	2 3	0 9
43	4 55	5 88	5 83	5 42	5 50	1 3	1 3	0 9	1 0
44	11 81	11 72	9 28	11 79	10 85	-0 1	-2 5	0 0	-1 0
45	1 93	0 54	1 68	1 26	1 16	-1 4	-0 2	-0 7	-0 8
46	10 38	9 36	10 26	11 41	12 63	-1 0	-0 1	1 0	2 3
47	-0 17	0 96	-0 43	-0 22	-0 08	1 1	-0 3	0 0	0 1
48	1 70	2 27	1 88	1 46	1 48	0 6	0 2	-0 2	-0 2
49	9 15	9 47	10 15	9 81	9 85	0 3	1 0	0 7	0 7
50	2 41	1.74	1 72	2 55	2 95	-0 7	-0 7	0 1	0 5
52	6 09	9 00	10 42	8 50	10 26	2 9	4 3	2.4	4 2
53	-0 35	2.10	0 88	1 40	3 37	2 4	1 2	1 8	3 7
54	1.89	2 98	2 48	2 84	3 04	1 1	0 6	0 9	1 1
55	2 45	2 61	4 06	1 65	2 89	0 2	1 6	-0 8	0 4
56	-1 13	-0 16	-1 29	-1 16	-0 94	1 0	-0 2	0.0	0 2
57	3 37	3 59	2 63	4 83	3 87	0 2	-0 7	1 5	0 5
59	3 10	3 53	3 32	2 86	2 47	0 4	0 2	-0 2	-0 6
60	0 37	-0 02	0 05	-0 26	0 44	-0 4	-0 3	-0 6	0 1
61	9 21	9 80	7 96	9.05	9 78	0 6	-1 3	-0 2	0 6
62	4 97	4.90	4 79	4 48	4 31	-0.1	-0.2	-0 5	-0 7
63	11 09	10 51	10 11	9 79	11 78	-0 6	-1 0	-1.3	0 7
64	9 01	9 47	8 67	9 79	8 98	0 5	-0 3	0 8	0 0
65	7 96	7 80	7 90	8 83	8 19	-0 2	-0 1	0 9	0 2
66	4.45	6 57	6 15	8 11	4 88	2 1	1 7	3 7	0 4

Table 4
(continued)
VISIA-CR Image Analysis Color Data - a* Values Left Iris

.01 = [redacted] Lash Enhancing Serum

67	8 34	6 74	5 03	5 57	7 94	-1 6	-3 3	-2 8	-0 4
68	10 24	8 16	10 25	10 54	10 14	-2 1	0.0	0 3	-0 1
69	13 28	12 97	13 44	12 96	13 27	-0 3	0 2	-0 3	0 0
70	5 38	4 55	4 68	5 24	5 76	-0 8	-0 7	-0 1	0 4
71	7 26	7 92	7 54	7 76	8 93	0 7	0.3	0 5	1 7
72	8 90	9 45	9 44	9 61	9 05	0 6	0 5	0 7	0 2
73	5 05	6 24	5 67	5 93	5 67	1 2	0 6	0 9	0 6
74	1 25	2 52	2 28	2,21	2 05	1 3	1 0	1 0	0 8
75	5 92	6 85	6 21	6 90	6 20	0 9	0 3	1 0	0 3
76	7 73	7 03	9 86	9 79	8.11	-0 7	2 1	2 1	0 4
77	9 90	10 39	9 12	8 87	9 72	0 5	-0 8	-1 0	-0 2
78	0 05	-0.88	-1 03	-0 64	-0 57	-0 9	-1 1	-0 7	-0 6
79	6 67	7 59	7 11	7 19	7 31	0 9	0 4	0 5	0.6
80	5 31	6 45	6,83	6 95	6 94	1 1	1 5	1 6	1 6
82	8 01	7 07	7 50	7 74	7 90	-0.9	-0 5	-0 3	-0.1
84	2 50	0 42	2 76	2 23	2 08	-2 1	0 3	-0 3	-0 4
86	8 34	7 90	7 34	7 04	8 54	-0 4	-1.0	-1 3	0 2
87	4 76	6 32	5,96	5 35	5 25	1 6	1 2	0 6	0 5
88	-0 03	-1 37	-0 15	0 52	-1 00	-1 3	-0 1	0 5	-1 0
89	14 40	15.01	13 24	14 60	15 48	0 6	-1 2	0 2	1 1
90	-3 45	-3 21	-3 00	-3 03	-3 17	0 2	0 5	0 4	0 3
91	7 72	6 99	9 00	7 73	8 88	-0 7	1 3	0 0	1 2
92	9 42	7 94	9 06	10 52	10 66	-1 5	-0 4	1 1	1 2
93	-0 73	1 34	0 44	0 26	0 44	2 1	1 2	1 0	1 2
94	10 63	11 09	9 53	11 82	11 62	0,5	-1 1	1 2	1 0
95	2 93	1 14	2 92	3 40	1,93	-1 8	0,0	0 5	-1.0
96	-1 16	-1 86	-1 88	-2 19	-1 61	-0 7	-0 7	-1 0	-0 4
97	0 51	0 51	0 88	0 86	1 13	0 0	0 4	0 4	0 6
98	7 79	10 08	10 54	7 21	9 10	2 3	2 8	-0 6	1 3
99	3 69	3 28	3 40	3,45	4 31	-0 4	-0 3	-0 2	0 6
100	7 83	9 71	10 07	9 12	8 63	1 9	2 2	1 3	0 8
101	9 62	10 20	9 73	10 76	10 33	0 6	0 1	1 1	0 7
102	15 56	12 87	12 59	14 39	13 35	-2 7	-3 0	-1 2	-2 2
103	8 78	9 36	10 07	9 62	9 85	0.6	1 3	0 8	1 1
104	9,01	8 97	8 32	9 35	8 30	0 0	-0 7	0 3	-0 7
105	3 82	0.16	0 07	7 01	1.17	-3,7	-3 7	3 2	-2 6
106	10 28	8 84	10 72	11 86	11 61	-1 4	0.4	1 6	1 3
107	9 63	8 54	7 88	8 86	6 05	-1 1	-1 8	-0 8	-3 6
108	-2 29	-2,50	-2 81	-2 42	-2 22	-0 2	-0 5	-0 1	0 1
109	-0 20	-0 72	-0 62	-0 38	0 05	-0 5	-0 4	-0 2	0 2
110	-2 01	-1 60	-2 64	-2 10	-1 43	0 4	-0 6	-0 1	0 6
111	10 66	9 11	9 07	10 00	8 27	-1 6	-1 6	-0 7	-2 4
112	4 47	4,54	3 79	4 01	4 53	0 1	-0.7	-0 5	0 1
114	9 59	7 95	9 34	8 65	9 30	-1 6	-0 3	-0 9	-0 3
115	6 50	7 42	7 29	6 78	6 76	0 9	0 8	0 3	0 3
116	11 89	10 46	10 25	10 75	10 16	-1 4	-1 6	-1 1	-1 7
117	0 21	-0.15	0 39	0 64	0 63	-0 4	0 2	0 4	0 4
118	9 23	9 41	9 62	8.64	8 03	0 2	0 4	-0 6	-1 2
119	9 72	9 41	9 30	9 56	9 76	-0 3	-0 4	-0 2	0.0
120	10 03	9 76	9 34	9 82	9 11	-0 3	-0 7	-0 2	-0 9
121	6 17	7 52	5 27	7 11	7 07	1.4	-0 9	0 9	0 9
Mean =	**5.10**	**4.96**	**4.99**	**5.37**	**5.33**	**-0.14**	**-0.11**	**0.27**	**0.23**
Median =	**5.01**	**5.47**	**4.91**	**5.49**	**5.37**	**-0.12**	**-0.01**	**0.14**	**0.31**
Standard Deviation =	**4.58**	**4.60**	**4.54**	**4.57**	**4.60**	**1.19**	**1.26**	**1.55**	**1.16**
Maximum =	**15.56**	**15.01**	**13.44**	**14.60**	**15.48**	**2.91**	**4.33**	**13.66**	**4.17**
Minimum =	**-3.45**	**-3.23**	**-3.00**	**-3.14**	**-3.17**	**-4.52**	**-5.64**	**-5.29**	**-4.62**

Mean % Change From Baseline

Month 1	Month 2	Month 4	Month 8
-2.7%	-2 2%	5 3%	4 5%
Decrease	Decrease	Increase	Increase

Tallies & Percentages

	Month 1	Month 2	Month 4	Month 8
Total	114	114	114	114
Increased Values	51	55	62	77
Decreased Values	63	59	52	37
No Change	0	0	0	0
Increased Values	44 74%	48 25%	54.39%	67 54%
Decreased Values	55.26%	51.75%	45.61%	32 46%
No Change	0 00%	0 00%	0 00%	0.00%

Did Not Complete Subject#s:7, 51, 58, 81, 83, 85, 113

L* values are a measure of the luminosity of the test site The higher the value, the greater the lightness of the test site.
a* values are a measure of the redness of the test site The higher the value, the greater the redness of the test site
b* values are a measure of the yellowness of the test site. The higher the value, the greater the yellowness of the test site.

Table 4
(continued)
VISIA-CR Image Analysis Color Data - b* Values Left Iris

.01 = [redacted] Lash Enhancing Serum

Subject #	Baseline	Month 1	Month 2	Month 4	Month 8	*Difference From Baseline At :* Month 1	Month 2	Month 4	Month 8
1	16 65	19 77	15 66	16 73	17 75	3 1	-1 0	0 1	1 1
2	2 56	3 58	3 27	3 48	4 52	1 0	0.7	0 9	2 0
3	9 22	8 55	8.81	9.02	11 30	-0 7	-1 2	-0 2	2 1
4	20 31	19 14	20 60	19 59	18 61	-1 2	0 3	-0 7	-1 7
5	6 45	6.62	6 29	5 59	6 58	0 2	-0 2	-0.9	0 1
6	7 37	6 99	5 46	5 47	6 96	-0 4	-1 9	-1.9	-0 4
8	6.93	5 88	5 74	5.37	5 73	-1 0	-1.2	-1 6	-1 2
9	16 94	18 09	17 28	16 84	15 67	1 1	0 3	-0 1	-1 3
10	7 17	6 98	6.99	7 20	6 25	-0 2	-0 2	0 0	-0 9
11	4 61	4 45	5 00	7 51	4 81	-0 2	0 4	2 9	0 2
12	9 53	10 73	9 42	9 97	10 28	1 2	-0 1	0 4	0 7
13	6 26	6 07	5.89	6 00	6 05	-0 2	-0.4	-0 3	-0 2
14	6 29	6 22	6 15	6 30	6 25	-0 1	-0 1	0 0	0 0
15	4 55	5 02	5 90	5 56	5 59	0 5	1 4	1 0	1 0
16	8 84	7 90	8 90	9 22	9 53	-0 9	0 1	0 4	0 7
17	18 14	16 88	16 50	17 91	17 68	-1 3	-1 6	-0 2	-0 5
18	13 48	13 23	12 13	12 50	12 87	-0 2	-1 3	-1 0	-0 6
19	13 45	12 64	13 77	13 15	12 85	-0 8	0 3	-0 3	-0 6
20	3 48	3 89	2 17	2 72	2 69	0 4	-1 3	-0 8	-0 8
21	19 71	17 64	17 12	17 33	18 26	-2 1	-2 6	-2 4	-1 5
22	13 85	13 74	14 00	14 16	14 32	-0 1	0 1	0 3	0 5
23	4 45	4 61	4.49	3 63	5 47	0 2	0 0	-0 8	1 0
24	5 70	5 15	5 37	5 68	4 63	-0 5	-0 3	0 0	-1 1
25	5 31	4.47	6 30	4 99	5 35	-0.8	1 0	-0 3	0 0
26	10 79	13 00	14 62	15.09	13 27	2 2	3 8	4 3	2 5
27	13 49	12 05	11 95	12 31	14 18	-1 4	-1 5	-1 2	0.7
28	9 67	9 18	10 27	10 43	11 82	-0 5	0 6	0 8	2 1
29	10 87	14 09	13 57	17 06	14 41	3 2	2 7	6 2	3 5
30	3 71	4 00	4 28	4 87	4 14	0 3	0 6	1 2	0 4
31	5 54	4 38	4 12	5 20	5 30	-1 2	-1 4	-0 3	-0 2
32	15 56	15 76	15 89	16 09	16 53	0 2	0 3	0 5	1 0
33	12 15	11 19	12 20	11 22	12 29	-1 0	0 1	-0 9	0 1
34	14 52	14 64	14 97	13 95	15 12	0 1	0 4	-0 6	0 6
35	1 97	1 44	2 75	3 72	1.64	-0 5	0 8	1 8	-0 3
36	12 27	11 37	12 15	12 17	13 36	-0 9	-0 1	-0 1	1 1
37	7 16	6 85	6 33	6 99	6 42	-0 3	-0 8	-0 2	-0 7
38	7 45	7 96	8 64	9 40	8 09	0 5	1 2	2 0	0 6
39	22 75	21 35	21 53	22 28	23 31	-1 4	-1 2	-0 5	0 6
40	5 09	5 51	5 93	5 95	4 75	0 4	0 8	0 9	-0 3
41	6 14	5 89	5 54	6 65	6 04	-0 3	-0 6	0 5	-0 1
42	14 06	13 90	15 34	17 08	17 11	-0 2	1 3	3 0	3 0
43	7 58	7 66	7 58	7 48	7.86	0 1	0 0	-0 1	0 3
44	19 14	19 51	17 04	18 58	20 46	0 4	-2 1	-0 6	1 3
45	5 30	5 59	7 22	6 95	5 76	0 3	1 9	1 7	0 5
46	11 44	12 02	12 85	15 40	14 52	0 6	1 4	4 0	3 1
47	7 50	5 32	4 32	4 80	4 52	-2 2	-3 2	-2 7	-3 0
48	5 90	6 72	5 39	6 05	6 04	0 8	-0 5	0 1	0 1
49	11 95	11 06	13 05	12 90	12 97	-0 9	1 1	0 9	1 0
50	16 52	15.81	14 68	15 76	17 45	-0 7	-1 8	-0 8	0 9
52	9 52	13 07	13 93	12.41	14 13	3 5	4 4	2 9	4 6
53	16 90	18 42	18 64	18 95	18 50	1 5	1 7	2 0	1 6
54	5 60	5 42	5 81	5.18	5 61	-0 2	0 2	-0 4	0 0
55	6 32	6,11	5 74	6 85	6 65	-0 2	-0 6	0 5	0 3
56	5 91	6 01	6 23	6 03	6 25	0 1	0 3	0 1	0 3
57	8.73	8.44	8 78	8 48	7 26	-0 3	0 0	-0 2	-1 5
59	6 18	6 18	6 56	7.07	10 18	0 0	0 4	0 9	4 0
60	14 71	13 17	16 14	15 60	17 52	-1 5	1 4	0 9	2 8
61	12 06	12.74	12 11	11.45	12 25	0 7	0 1	-0 6	0 2
62	7 64	7 73	7 89	7 30	8 29	0 1	0 2	-0.3	0 6
63	20 09	20 08	18 63	19 68	21 08	0 0	-1 5	-0 4	1 0
64	11 49	12.34	11 45	12 22	10 89	0 8	0 0	0 7	-0.6
65	12 24	11 38	11.47	12 19	11 74	-0 9	-0 8	-0 1	-0 5
66	15 29	16 70	16 59	18 36	14 48	1 4	1 3	3 1	-0 8
67	10 38	9 15	8 97	9 08	9 26	-1 2	-1.4	-1 3	-1 1

Table 4
(continued)
VISIA-CR Image Analysis Color Data - b* Values Left Iris

.01 = [redacted] Lash Enhancing Serum

68	16 58	14 59	16 80	16.57	16 57	-2 0	0 2	0 0	0 0
69	20 13	21 14	21 64	20 58	20.66	1 0	1 5	0.5	0.5
70	6 74	7 75	7 56	8 12	7 89	1 0	0 8	1 4	1 1
71	11 07	11 53	10 35	11 47	14 49	0 5	-0 7	0 4	3 4
72	11 67	12 37	11 94	12 27	12 74	0 7	0 3	0 6	1 1
73	7 95	8 64	6 92	8 15	7 86	0 7	-1.0	0 2	-0 1
74	5 76	5.50	4 23	5 10	6 61	-0 3	-1 5	-0 7	0 9
75	9 24	9 01	8 67	9 69	9 19	-0 2	-0.6	0 5	0 0
76	15 49	14 63	16 50	16.00	15 97	-0 9	1 0	0 5	0 5
77	13 63	14 96	13 43	12 74	14 34	1 3	-0 2	-0 9	0 7
78	2 25	2 11	1.91	2 76	2 51	-0 1	-0 3	0 5	0 3
79	14 49	13 84	14 43	14 37	14 15	-0 6	-0 1	-0 1	-0 3
80	8 98	8 40	8 69	8 54	8 75	-0 6	-0 3	-0 4	-0 2
82	9.85	9 87	10 43	10 77	11 11	0 0	0 6	0 9	1 3
84	6 33	6 49	5 77	5 87	6 37	0 2	-0 6	-0.5	0 0
86	10 44	11 20	11 67	10 08	12 10	0.8	1 2	-0 4	1 7
87	7 10	7 60	9 11	8 05	8 40	0 5	2 0	0 9	1 3
88	15 27	15 60	14 31	16 13	15 59	0 3	-1 0	0 9	0 3
89	21 05	22 16	20 72	23 18	23.06	1 1	-0 3	2 1	2 0
90	3 98	4 95	5 32	4 61	4 98	1 0	1 3	0 6	1 0
91	11 11	9 97	12 07	10 61	12 43	-1 1	1 0	-0 5	1 3
92	19 48	17 59	17 68	17 76	17 45	-1 9	-1 8	-1 7	-2 0
93	7.29	7 87	7 68	7 88	7 93	0 6	0 4	0 6	0 6
94	27 82	27 17	19 53	25 92	29 02	-0 7	-8 3	-1 9	1 2
95	16 31	14 94	16 82	17 13	15 92	-1 4	0 5	0 8	-0 4
96	1 74	1 08	0 22	1 27	0 59	-0 7	-1 5	-0 5	-1 2
97	3 71	4 78	3 73	4 08	4 99	1.1	0 0	0 4	1 3
98	16 15	16 57	15 70	17 70	16 84	0 4	-0 5	1 5	0 7
99	11 59	11 68	12 54	12 15	11 78	0 1	1 0	0 6	0 2
100	16 33	18 35	18 34	18 51	17 04	2 0	2 0	2 2	0 7
101	13 86	14 65	15 06	16 30	14 61	0 8	1 2	2 4	0 8
102	20 74	16 53	16 20	15.68	17 56	-4 2	-4 5	-5 1	-3 2
103	13 66	14 64	16 09	15 36	15 64	1 0	2 4	1 7	2 0
104	14 46	14 47	13 72	15 83	15 02	0 0	-0 7	1 4	0 6
105	22 59	18 42	17 79	25 75	20 11	-4 2	-4 8	3 2	-2 5
106	12 09	11 30	13 23	14 36	14 11	-0 8	1 1	2 3	2 0
107	11 49	11 56	10 63	11 55	8 98	0 1	-0 9	0 1	-2 5
108	8 31	7 52	7 27	7 46	6 87	-0 8	-1 0	-0 9	-1 4
109	13 48	12 52	14 18	14 20	11 33	-1 0	0 7	0 7	-2 1
110	9 02	9 43	9 38	9 60	9 80	0.4	0 4	0 6	0 8
111	13 66	13 80	14 94	13 22	12 13	0 1	1 3	-0 4	-1 5
112	7 37	7 23	7 46	7 20	7 51	-0 1	0 1	-0 2	0.1
114	13 12	12 82	14 06	13 05	14 01	-0 3	0 9	-0 1	0 9
115	10 41	10 99	11 33	11 33	10 72	0 6	0 9	0 9	0 3
116	19 02	18 58	19 27	19 67	19 94	-0 4	0 3	0 7	0 9
117	2 99	2 12	2 35	4 28	2 14	-0 9	-0 6	1 3	-0.8
118	20 80	21 38	21 91	21 76	21 33	0 6	1 1	1 0	0 5
119	12 14	11 50	11 79	12 15	12 24	-0 6	-0 4	0 0	0 1
120	15 25	13 76	14 55	13 72	14.92	-1 5	-0 7	-1 5	-0 3
121	7 05	8 63	6 37	8 90	10 73	1 6	-0 7	1.9	3 7
Mean =	**11.09**	**11.01**	**11.02**	**11.44**	**11.48**	**-0.1**	**-0.1**	**0.4**	**0.4**
Median =	**10.83**	**11.19**	**11.46**	**11.39**	**11.76**	**-0.1**	**0.0**	**0.2**	**0.4**
Standard Deviation =	**5.39**	**5.28**	**5.18**	**5.46**	**5.49**	**1.2**	**1.6**	**1.4**	**1.4**
Maximum =	**27.82**	**27.17**	**21.91**	**25.92**	**29.02**	**3.5**	**4.4**	**6.2**	**4.6**
Minimum =	**1.74**	**1.08**	**0.22**	**1.27**	**0.59**	**-4.2**	**-8.3**	**-5.1**	**-3.2**

Mean % Change From Baseline

Month 1	Month 2	Month 4	Month 8
-0 4%	-0 4%	1 8%	2 0%
Decrease	Decrease	Increase	Increase

Tallies & Percentages

	Month 1	Month 2	Month 4	Month 8
Total	114	114	114	114
Increased Values	55	60	64	74
Decreased Values	59	54	50	40
No Change	0	0	0	0
Increased Values	48 25%	52 63%	56.14%	64 91%
Decreased Values	51.75%	47 37%	43 86%	35.09%
No Change	0 00%	0 00%	0 00%	0 00%

Did Not Complete Subject#s 7, 51, 58, 81, 83, 85, 113

L* values are a measure of the luminosity of the test site The higher the value, the greater the lightness of the test site
a* values are a measure of the redness of the test site The higher the value, the greater the redness of the test site
b* values are a measure of the yellowness of the test site. The higher the value, the greater the yellowness of the test site

Table 4
(continued)

.01[redacted]Lash Enhancing Serum (Left)

Within-Groups Analysis For :
VISIA-CR Image Analysis Color Data - Delta E Value

Pre-Test	Normality	Brown-Forsythe	Friedman ANOVA
P-Value	P < 0 050	Not Required	0 001

Pairwise Comparisons

Difference from Baseline	Test Type	P-value	Significance	Direction (Mean)
Month 1	Dunn's Method	**0.001**	Yes	Increase
Month 2	Dunn's Method	**0.001**	Yes	Increase
Month 4	Dunn's Method	**0.001**	Yes	Increase
Month 8	Dunn's Method	**0.001**	Yes	Increase

Within-Groups Analysis For :
VISIA-CR Image Analysis Color Data - Delta L Value

Pre-Test	Normality	Brown-Forsythe	Friedman ANOVA
P-Value	P < 0 050	Not Required	0 896

Within-Groups Analysis For :
VISIA-CR Image Analysis Color Data - Delta A Value

Pre-Test	Normality	Brown-Forsythe	Friedman ANOVA
P-Value	P < 0 050	Not Required	0 001

Pairwise Comparisons

Difference from Baseline	Test Type	P-value	Significance	Direction (Mean)
Month 1	Dunn's Method	1 000	No	Decrease
Month 2	Dunn's Method	1 000	No	Decrease
Month 4	Dunn's Method	0 238	No	Increase
Month 8	Dunn's Method	**0.008**	Yes	Increase

Within-Groups Analysis For :
VISIA-CR Image Analysis Color Data - Delta B Value

Pre-Test	Normality	Brown-Forsythe	Friedman ANOVA
P-Value	P < 0 050	Not Required	0 001

Pairwise Comparisons

Difference from Baseline	Test Type	P-value	Significance	Direction (Mean)
Month 1	Dunn's Method	1 000	No	Decrease
Month 2	Dunn's Method	1 000	No	Decrease
Month 4	Dunn's Method	**0.042**	Yes	Increase
Month 8	Dunn's Method	**0.001**	Yes	Increase

* = ANOVA (RM) compares the means across groups (the non-parametric version is Friedman). The null hypothesis is all means are equivalent and the alternative is that the mean of at least one group differs If ANOVA is significant further pairwise comparisons can be made, if not then it is assumed there is no statistically significant difference among the mean (or ranks) and no further testing is done due to risk of type one error. It is possible that Friedman or RM ANOVA may be significant, but when running post-hoc test may not show any statistically significant difference due to only examining the comparisons to baseline, post-hoc test, small sample size, lack of power, or if there is borderline significance

* = Dunn's method is a post-hoc test commonly run after Friedman RM ANOVA. It allows for comparisons between a control group, and it is a robust, non-parametric test that adjusts for multiple comparisons.

ΔE is a measure of total color change of the test site from baseline The larger the value, the greater the change in color ΔE does not provide the direction, or individual component of the change. Subsequently, the individual changes in the L * a* b* values from baseline are evaluated
L* values are a measure of the luminosity of the test site. The higher the value, the greater the lightness of the test site
a* values are a measure of the redness of the test site The higher the value, the greater the redness of the test site
b* values are a measure of the yellowness of the test site. The higher the value, the greater the yellowness of the test site.

Summary:
For subjects using[redacted]Lash Enhancing Serum, there was an overall increase in ΔE values of the left eye indicating an increase in color change from baseline at all timepoints When evaluating the individual L* a* b* color values, there were no statistically significant differences in ocular pigmentation from baseline when looking at the change in L* When evaluating the a* of the left eye compared to baseline, there was a significant difference at month 8, which represents an increase in redness at month 8 There is also a statistically significant increase at months 4 and 8 when compared to baseline for the delta b color value, indicating an increase in yellowness

Table 4
(continued)
VISIA-CR Image Analysis Color Data - Delta E Value Right Iris

.01 = [redacted] Lash Enhancing Serum

Subject #	Month 1	Month 2	Month 4	Month 8
1	1 96	1 23	0 47	0 49
2	3 95	0 42	3 23	0 99
3	3 91	2 16	4 79	1 97
4	1 00	0 77	0 89	1 23
5	2 19	3 60	3 77	2 35
6	0 31	0 82	1 96	0 82
8	3.87	1 02	1 94	4 12
9	2 51	1 54	3 04	1 42
10	3 78	1.35	0 68	3 26
11	0 41	1 64	1 68	0 73
12	1 99	2 20	3 65	2 07
13	0.36	1 80	1 93	1 19
14	1 99	1 96	4 18	5 30
15	5 78	4 86	0 53	2 62
16	1 61	1 57	0 58	0.74
17	1 50	4 51	2 65	1 24
18	0 50	6 71	1 84	2 38
19	7 01	4 62	4 18	4.09
20	5 99	9 52	3 66	1 79
21	7 59	13 73	16 73	12 22
22	0 29	1 36	0 30	0 63
23	6 11	4 51	1 97	1 45
24	0 73	0 77	0 18	0 65
25	19 22	15 17	17 90	8 44
26	0 44	1 84	2 99	3 35
27	1 81	0 65	1 03	1 78
28	5 75	5 58	3 38	1 92
29	8 79	3 78	2 64	0.93
30	0 89	0 91	0 67	0 30
31	0 54	1 38	0 32	0 45
32	6 88	7 67	7 01	6 62
33	1 31	0 35	1 98	1 08
34	3 36	2 44	2 83	3.49
35	4 17	4 25	7 08	4 90
36	1 23	2 08	1 54	2 29
37	1 97	0 78	0 22	0 84
38	1 91	1 33	0 74	0 54
39	0 99	1 06	0 82	1.18
40	0.49	0 72	0 65	1 62
41	4 91	1 85	0 45	1 51
42	1 06	3 79	4 22	7 27
43	2 41	1 47	1 12	1 44
44	0 91	2 73	2 25	1 21
45	4 72	0 62	1 29	1 28
46	6 29	3 36	3 82	8 72
47	2 90	1 01	2 90	6 13
48	2.63	1 26	2 05	0.86
49	1.97	0.47	1 61	0 76
50	0 53	0 60	2 65	0.91
52	1 06	2 26	2 59	2 75
53	4 08	4 38	3 62	5 95
54	0 45	0 41	1 80	0 82
55	1.41	5 41	0 48	3 96
56	2 50	5.55	1.95	1.83
57	0 37	2 89	1 03	1 37
59	1 25	0 74	0 61	1 41
60	2 17	2 57	1 49	2.13
61	1 12	2 79	0 42	0 81
62	1 08	1 15	0 59	1 48
63	1 29	2 38	2 06	0 58
64	0 40	0 39	0 79	1 05
65	2.03	1 97	1 18	1 11
66	2 40	1 80	2 28	1 73

Table 4
(continued)
VISIA-CR Image Analysis Color Data - Delta E Value Right Iris

.01 = [redacted] Lash Enhancing Serum

67	0 90	1 28	1 41	1 01
68	3 21	0 80	1 33	1 04
69	0 47	1 09	2 17	0 80
70	1 96	2 42	0 70	1 05
71	1 26	2 19	3 27	4 10
72	1 89	0 79	1 26	1 78
73	0 78	0 60	0 94	0 81
74	2 40	1 93	1 10	0 91
75	2 30	0 74	1 28	0.58
76	2 85	2 25	3 15	0 95
77	2 52	0 78	1 59	2 13
78	8 13	6 43	6 87	8 62
79	5 84	3 07	1 82	3 02
80	1 13	0 63	2 70	1.53
82	2 48	2 46	1 46	0 23
84	6 97	0 33	0 90	3.76
86	3 37	3 96	4 19	1 73
87	2 94	2 89	3 30	2 99
88	3 37	2 48	5 39	2 71
89	1 09	2 50	1 51	2 39
90	1 36	2 42	1 65	0 87
91	3 23	0 64	0 29	2 16
92	3 13	4.87	2 26	7 52
93	5 26	9 17	5 04	4 19
94	1 53	8 70	0 51	1.84
95	1 69	3 35	2 80	1 32
96	0 75	0 47	2 73	0 64
97	0 40	1 92	1 29	0 77
98	0.79	3 64	4 72	2 07
99	1 43	2 52	1 34	2 31
100	6 48	3 85	3 95	3 45
101	1 58	1 45	0 69	1 28
102	1 69	1 35	10 38	3 66
103	2 86	5 46	1 94	3 82
104	0 55	0 79	1 13	1 90
105	3 98	5 52	2 45	3 23
106	1 78	0 22	2 80	2 20
107	1 55	4 18	0 95	3 14
108	5 10	4 57	5 87	6 21
109	4 14	4 51	9 20	2 22
110	0 67	0 79	0 91	1 20
111	7 15	4 27	0 74	5 05
112	1 61	0 97	1 95	2 97
114	1 92	1 79	1 93	4 12
115	2 52	4 17	4 27	3.86
116	2 76	2.04	2 30	3 67
117	1 79	1 24	0 35	2 22
118	2 89	1.87	1 59	3 89
119	1 14	0 71	0 14	0 55
120	2 38	1.65	2 98	0 87
121	2 88	2 19	5 66	8 29
Mean =	**2.70**	**2.68**	**2.55**	**2.49**
Median =	**1.97**	**1.94**	**1.94**	**1.78**
Standard Deviation =	**2.52**	**2.51**	**2.71**	**2.16**
Maximum =	**19.22**	**15.17**	**17.90**	**12.22**
Minimum =	**0.29**	**0.22**	**0.14**	**0.23**

Did Not Complete Subject#s.7, 51, 58, 81, 83, 85, 113

L* values are a measure of the luminosity of the test site The higher the value, the greater the lightness of the test site
a* values are a measure of the redness of the test site The higher the value, the greater the redness of the test site
b* values are a measure of the yellowness of the test site. The higher the value, the greater the yellowness of the test site

Table 4
(continued)

VISIA-CR Image Analysis Color Data - L* Value Right Iris

.01 = [redacted] Lash Enhancing Serum

Subject #	Baseline	Month 1	Month 2	Month 4	Month 8	*Difference From Baseline At :* Month 1	Month 2	Month 4	Month 8
1	18 70	18 83	19 58	18 55	19 07	0 1	0 9	-0 2	0 4
2	42 48	38 65	42 86	39 40	42 41	-3 8	0 4	-3 1	-0 1
3	34 60	38 43	32 96	39 30	36 47	3 8	-1 6	4 7	1 9
4	31 73	31 07	32 19	31 83	31 94	-0 7	0 5	0 1	0 2
5	12 25	10 50	8 77	8 72	10 04	-1 8	-3 5	-3 5	-2 2
6	6 91	6 74	6 93	5 19	6 18	-0 2	0 0	-1 7	-0 7
8	11 44	7 80	10.44	9 60	7 43	-3 6	-1 0	-1 8	-4 0
9	37 59	39 98	37 19	35.67	37 52	2 4	-0 4	-1 9	-0 1
10	34 85	38 16	36 00	34 93	37 96	3 3	1 2	0 1	3 1
11	7 85	7 45	6 28	7 76	7 59	-0 4	-1 6	-0 1	-0 3
12	40 18	38 45	38 01	43 79	42 15	-1 7	-2 2	3 6	2 0
13	9 87	10 16	8 33	8 20	8 87	0 3	-1 5	-1 7	-1 0
14	11 52	13 04	10 66	8 17	7 20	1 5	-0 9	-3 3	-4 3
15	5 38	10 75	9 66	5 38	7 61	5 4	4 3	0 0	2 2
16	11 54	9 97	10 09	11.01	11 00	-1 6	-1 5	-0 5	-0 5
17	22 05	21 02	25 56	24 70	23 19	-1 0	3 5	2 7	1 1
18	15 28	14 98	10 42	13 90	14 65	-0 3	-4 9	-1 4	-0 6
19	19 77	13 09	15 50	15 89	15 79	-6 7	-4 3	-3 9	-4 0
20	33 26	38 83	42 39	36 47	32 05	5 6	9 1	3 2	-1 2
21	27.40	33 07	38.43	41 36	38 11	5 7	11 0	14 0	10 7
22	13 78	13 69	14 52	13 93	13 84	-0 1	0 7	0 2	0 1
23	31 06	37 08	35 32	32 13	31 78	6 0	4 3	1 1	0 7
24	7 46	8 16	6 72	7 44	7 91	0 7	-0 7	0 0	0 5
25	15 30	5 31	9 95	6 92	10 59	-10 0	-5 3	-8.4	-4 7
26	35 38	35.70	36 05	37 30	37 57	0 3	0 7	1 9	2 2
27	17 23	15 51	17 76	16 29	18 22	-1 7	0 5	-0 9	1 0
28	12 27	17 43	17 81	14 33	13 93	5 2	5 5	2 1	1 6
29	21 42	30 16	18 31	19 93	20 75	8 7	-3 1	-1 5	-0 7
30	5 60	4 83	6 41	6 21	5 41	-0 8	0 8	0 6	-0 2
31	6 71	6 63	7 98	6 96	6 30	-0 1	1 3	0 2	-0 4
32	22 67	17 23	16 26	17 07	17 89	-5 4	-6 4	-5 6	-4.8
33	14 35	14.79	14 18	12 78	14 23	0 4	-0 2	-1 6	-0 1
34	17 98	15 66	18 21	19 08	16 61	-2 3	0 2	1 1	-1 4
35	32 63	35 59	35 40	26 35	36 86	3 0	2 8	-6 3	4 2
36	12 89	13 14	12 04	14 42	10 89	0 3	-0 8	1 5	-2 0
37	7 22	8 82	6 70	7 20	8 00	1 6	-0 5	0 0	0 8
38	39 79	38 04	40 98	40 05	39 26	-1 8	1 2	0 3	-0 5
39	30 94	30 20	30 72	30 43	32 04	-0 7	-0 2	-0 5	1 1
40	7 43	7 58	8 02	7 12	5 89	0 1	0 6	-0 3	-1 5
41	7 39	11 86	5 73	7 22	5 96	4 5	-1 7	-0 2	-1 4
42	13 67	12 62	16.00	15 46	19 54	-1 1	2 3	1 8	5 9
43	9 16	6 98	8 00	8 19	7 89	-2 2	-1 2	-1 0	-1 3
44	24 28	24 05	21 61	22 48	23 36	-0 2	-2 7	-1 8	-0 9
45	9 36	14 03	9 08	10 34	8 25	4 7	-0 3	1 0	-1 1
46	21 02	26 84	18 05	17 64	28 02	5 8	-3 0	-3 4	7 0
47	28 51	25 68	27 87	25 70	22 55	-2 8	-0 6	-2 8	-6 0
48	10 58	8 13	9 37	8 65	11 13	-2 5	-1 2	-1 9	0 5
49	12 50	10 88	12 54	11 40	11 77	-1 6	0 0	-1 1	-0 7
50	29 53	29 09	28 99	27 22	29 62	-0.4	-0 5	-2 3	0 1
52	12 30	11 36	10.89	10.05	11 68	-0 9	-1 4	-2 2	-0 6
53	28 80	29 78	27 64	28 89	27 99	1 0	-1 2	0 1	-0 8
54	8 18	7 76	8 24	6 56	7 61	-0 4	0 1	-1 6	-0 6
55	11 35	10 04	6 19	11 05	7.55	-1 3	-5 2	-0 3	-3 8
56	31 09	29 09	36 44	32 86	32 82	-2.0	5 3	1 8	1 7
57	9 22	9 49	11.70	9 95	8 17	0 3	2 5	0 7	-1 0
59	7.64	8 81	7 01	7 49	8 88	1 2	-0 6	-0.2	1 2
60	29 29	30 72	27 08	30 08	28 39	1 4	-2 2	0 8	-0 9
61	11 47	12 52	14 09	11 40	10 91	1 1	2 6	-0.1	-0 6
62	9 22	8 28	8 13	8 93	10 47	-0 9	-1 1	-0 3	1 2
63	20.98	20 73	19 35	19 26	20.62	-0 3	-1 6	-1 7	-0.4
64	11 93	11 92	12 20	11.97	10.93	0 0	0 3	0 0	-1 0
65	10.84	9 13	9 35	9 82	9 83	-1 7	-1 5	-1.0	-1 0
66	19 23	21 45	19 72	19 55	20 90	2 2	0 5	0 3	1 7

Table 4
(continued)
VISIA-CR Image Analysis Color Data - L* Value Right Iris

				.01 = [redacted] Lash Enhancing Serum					
67	9 97	10.64	8.74	9 24	9 26	0 7	-1 2	-0 7	-0 7
68	14 00	17 01	13 22	13 48	14 84	3 0	-0 8	-0 5	0 8
69	21.45	21 42	20 89	22 54	20 82	0 0	-0 6	1 1	-0 6
70	7 52	9 14	9 52	7 96	8 34	1 6	2 0	0 4	0 8
71	14 16	14 88	14 15	15 47	13 89	0 7	0 0	1 3	-0 3
72	12 60	14 40	13 34	11 90	14.23	1 8	0 7	-0 7	1 6
73	9 68	10 29	9.43	9 05	9.55	0 6	-0 3	-0 6	-0 1
74	7 12	4 98	5 40	6 12	6 44	-2 1	-1 7	-1 0	-0 7
75	9 90	10 07	9 50	8 97	10 01	0.2	-0 4	-0 9	0 1
76	15 90	18 59	15 91	15 61	15 68	2 7	0 0	-0 3	-0 2
77	15 13	12 70	15 86	15 34	14 22	-2 4	0 7	0 2	-0 9
78	24 30	30 82	29 20	29 95	31.89	6 5	4.9	5 7	7 6
79	22 06	17 02	22 19	20 85	19 09	-5 0	0 1	-1 2	-3.0
80	10 79	9.88	10 22	8 51	9 48	-0 9	-0 6	-2 3	-1 3
82	10 85	10 17	9 15	11 04	10.64	-0 7	-1 7	0 2	-0 2
84	5 69	12 25	5.97	6 20	9 19	6 6	0 3	0 5	3.5
86	11 03	11 58	10 48	14 68	11 96	0 5	-0 6	3 6	0 9
87	9 18	9.09	9 01	9 56	9 89	-0.1	-0 2	0 4	0 7
88	46 28	42 94	43 88	41 42	44,18	-3 3	-2 4	-4 9	-2 1
89	19 94	20 11	21 52	19 95	20 38	0 2	1 6	0 0	0 4
90	41 62	42.95	43 68	43 26	42 44	1 3	2 1	1 6	0 8
91	8 52	11 48	8 07	8 81	10 26	3 0	-0 5	0 3	1.7
92	19 62	19 37	16 07	21 65	15 24	-0 3	-3 5	2 0	-4 4
93	38 81	34 33	29.85	34 00	35 20	-4 5	-9 0	-4 8	-3 6
94	36 77	37 68	29 98	37 15	38 52	0 9	-6 8	0 4	1 7
95	30 75	29 75	33 28	29 96	31 66	-1 0	2 5	-0 8	0 9
96	33 27	33 90	33 56	35 95	33 80	0 6	0 3	2 7	0 5
97	6 72	6 33	4 94	5 46	6 50	-0 4	-1 8	-1 3	-0 2
98	24 40	23 92	27 32	20 31	24 74	-0 5	2 9	-4 1	0 3
99	16 03	16 78	18 12	17 18	18 13	0 7	2 1	1 1	2 1
100	19 29	13 85	17 05	16 06	16 07	-5 4	-2 2	-3 2	-3 2
101	15 85	14 40	16 56	15 73	14 68	-1 5	0 7	-0 1	-1 2
102	18 75	17 81	19 21	12 12	18 67	-0 9	0 5	-6 6	-0 1
103	17 69	20 23	22 97	19 35	21 43	2 5	5 3	1 7	3 7
104	15 23	15 08	14 61	14 20	16 52	-0 1	-0 6	-1 0	1 3
105	38 65	36 98	37 25	37 28	36 87	-1 7	-1 4	-1 4	-1 8
106	13 38	13 24	13 53	15 34	15 45	-0 1	0 1	2 0	2 1
107	11 33	10 58	14 58	10 39	10 59	-0 7	3 2	-0 9	-0 7
108	35 87	38 83	40 13	40 62	40 80	3 0	4 3	4 7	4 9
109	39 95	36 00	35.62	31 20	37 94	-4 0	-4 3	-8 7	-2 0
110	38 29	38 70	38 83	39 12	39 42	0 4	0 5	0.8	1 1
111	12 20	18 34	15 56	12 25	16 16	6.1	3 4	0 1	4 0
112	11 79	10 41	11 34	9.93	8 99	-1 4	-0 4	-1 9	-2 8
114	18 34	19 96	17 77	19 54	19 19	1 6	-0 6	1 2	0 9
115	15 84	17 28	18 66	13 85	12 80	1 4	2 8	-2 0	-3 0
116	18 51	19 53	19 57	20 09	21 22	1 0	1 1	1 6	2 7
117	24 47	26 15	25 34	24 64	26 59	1 7	0 9	0 2	2 1
118	22 26	22 99	22 05	22 28	24 63	0 7	-0 2	0 0	2 4
119	12 82	12 96	12 39	12 71	12 98	0 1	-0 4	-0.1	0 2
120	16 07	16 27	14 81	13 56	16 74	0 2	-1 3	-2 5	0 7
121	9 61	11 77	7 79	14 20	16 76	2 2	-1 8	4.6	7 1
Mean =	**18.96**	**19.15**	**18.85**	**18.54**	**19.08**	**0.25**	**-0.05**	**-0.36**	**0.18**
Median =	**15.57**	**15.30**	**15.89**	**15.34**	**15.74**	**0.06**	**-0.24**	**-0.13**	**-0.10**
Standard Deviation =	**10.53**	**10.73**	**10.96**	**10.90**	**11.04**	**2.86**	**2.80**	**2.75**	**2.55**
Maximum =	**46.28**	**42.95**	**43.88**	**43.79**	**44.18**	**8.74**	**11.03**	**13.96**	**10.71**
Minimum =	**5.38**	**4.83**	**4.94**	**5.19**	**5.41**	**-9.99**	**-8.96**	**-8.75**	**-5.97**

Mean % Change From Baseline

Month 1	Month 2	Month 4	Month 8
1.3%	-0 3%	-1 9%	0.9%
Increase	Decrease	Decrease	Increase

Did Not Complete Subject#s 7, 51, 58, 81, 83, 85, 113

Tallies & Percentages

	Month 1	Month 2	Month 4	Month 8
Total	114	114	114	114
Increased Values	57	52	50	54
Decreased Values	57	62	64	60
No Change	0	0	0	0
Increased Values	50 00%	45 61%	43 86%	47 37%
Decreased Values	50 00%	54 39%	56 14%	52 63%
No Change	0 00%	0 00%	0 00%	0.00%

L* values are a measure of the luminosity of the test site The higher the value, the greater the lightness of the test site
a* values are a measure of the redness of the test site The higher the value, the greater the redness of the test site
b* values are a measure of the yellowness of the test site The higher the value, the greater the yellowness of the test site

Table 4
(continued)

VISIA-CR Image Analysis Color Data - a* Values Right Iris

.01 = [redacted] Lash Enhancing Serum

Subject #	Baseline	Month 1	Month 2	Month 4	Month 8	*Difference From Baseline At :* Month 1	Month 2	Month 4	Month 8
1	13 39	14.66	12 74	13 67	13 46	1 3	-0 6	0 3	0 1
2	-2 55	-1 67	-2 70	-1 59	-1 95	0.9	-0 1	1 0	0 6
3	-1 57	-2 29	-0 90	-2 20	-1 61	-0 7	0 7	-0 6	0 0
4	2 59	3 30	3 18	3 47	3 72	0 7	0 6	0 9	1 1
5	3 08	4 28	3 32	4 37	3 87	1 2	0 2	1 3	0 8
6	3 44	3 35	4 23	3 61	3 65	-0 1	0 8	0 2	0 2
8	1 34	2 63	1 52	1 89	1 89	1.3	0 2	0 6	0 6
9	-1 45	-1 44	-0 52	0 28	-0.51	0 0	0 9	1 7	0 9
10	-0 30	-2 00	-0.88	-0 92	-1 12	-1 7	-0.6	-0 6	-0 8
11	2 48	2 44	2 50	3 46	3 07	0 0	0 0	1 0	0.6
12	-2 08	-1.12	-1 83	-2.01	-1 72	1.0	0 3	0 1	0 4
13	1 01	0 99	1 95	1 97	1.65	0 0	0 9	1.0	0 6
14	3 90	3 12	3 61	4 08	3 70	-0 8	-0 3	0 2	-0 2
15	2 41	1 66	2 80	2 85	2 88	-0 7	0 4	0 4	0 5
16	6 36	6 31	6.95	6 60	6 55	-0 1	0 6	0 2	0 2
17	9 82	9 14	7 82	9 73	10.18	-0 7	-2 0	-0 1	0 4
18	12 23	11 94	10 33	11 78	10 58	-0 3	-1 9	-0 4	-1 6
19	8 80	10 63	10 55	8 75	9 71	1 8	1 7	0 0	0 9
20	-0 69	-2 85	-3 35	-2 15	-1 41	-2 2	-2 7	-1 5	-0 7
21	8 97	4 19	1 45	0 52	3 11	-4 8	-7 5	-8 5	-5 9
22	13 09	13 21	12 30	13 24	13 57	0 1	-0 8	0 2	0 5
23	-0 76	-1 32	-1 54	-1 04	-0 26	-0 6	-0.8	-0 3	0 5
24	1 98	1 95	2 02	1 82	2 07	0 0	0 0	-0 2	0 1
25	12 47	1 44	2 20	1 64	7 91	-11 0	-10 3	-10 8	-4 6
26	-0 87	-0 69	-0 79	-0 67	-0 53	0 2	0 1	0 2	0 3
27	10 07	10 50	9 76	10 16	10 34	0.4	-0 3	0 1	0 3
28	9 38	6 97	8 85	10 75	9 95	-2 4	-0 5	1 4	0 6
29	0 21	0.58	-0 21	2 01	0 23	0 4	-0.4	1 8	0 0
30	2 07	2 45	1 86	1 96	2 29	0 4	-0 2	-0 1	0 2
31	1 55	1 37	1 31	1 52	1 55	-0 2	-0 2	0 0	0 0
32	8 92	12 36	12 52	12 49	12 51	3 4	3 6	3 6	3 6
33	11 66	12 57	11 60	10 95	12 47	0 9	-0 1	-0 7	0 8
34	8 37	10 41	10 09	10 14	11 00	2 0	1 7	1 8	2 6
35	-1 26	-2 61	-2.93	-0 25	-3 22	-1 4	-1 7	1 0	-2 0
36	9 02	10 06	10 82	9 10	10 05	1 0	1 8	0 1	1 0
37	4 93	3 78	4 67	5 14	4 65	-1 1	-0 3	0 2	-0 3
38	-2 40	-2 27	-2 93	-2 26	-2.35	0 1	-0 5	0 1	0 0
39	6 14	6.53	6 27	6 73	6 35	0 4	0 1	0 6	0 2
40	3 31	2 88	3 58	3 78	3 53	-0 4	0 3	0 5	0 2
41	3 69	2 62	3 94	3 29	3 76	-1 1	0 2	-0 4	0 1
42	9 59	9 70	10 91	11 59	10 88	0 1	1 3	2 0	1 3
43	4 97	5.87	5 88	5 51	5 65	0 9	0 9	0 5	0 7
44	9 73	10 29	9 59	10 95	10 13	0 6	-0 1	1 2	0 4
45	1 29	0 60	1 68	1 01	1 93	-0 7	0 4	-0 3	0 6
46	13 80	13 80	12 42	12 05	15 80	0 0	-1 4	-1 8	2 0
47	-0 92	-0.35	-0 74	-0 21	0 43	0 6	0 2	0.7	1 4
48	1 38	2 31	1 59	2 04	1 27	0.9	0 2	0 7	-0.1
49	9 60	9 03	10 04	8 81	9 79	-0 6	0 4	-0 8	0 2
50	0 31	0 59	0 35	1 59	0 71	0 3	0 0	1 3	0 4
52	6 96	6 99	8 56	6 68	9 10	0 0	1 6	-0 3	2 1
53	1 19	3.81	4 39	3 31	5 80	2 6	3 2	2 1	4 6
54	2 27	2 36	2 41	2 98	2 84	0 1	0 1	0 7	0 6
55	2 48	2 12	3 38	2 13	3 39	-0 4	0 9	-0 4	0 9
56	-1 23	0 25	-1 71	-1 45	-0 89	1 5	-0 5	-0 2	0 3
57	3 18	3 13	1.80	3 56	3 94	-0 1	-1 4	0 4	0.8
59	3 21	2 99	3 55	3 71	3 34	-0 2	0 3	0 5	0 1
60	4 09	4 06	4 65	3 81	4 45	0 0	0 6	-0 3	0.4
61	8.45	8 12	7 76	8 06	9 01	-0 3	-0 7	-0.4	0 6
62	5 07	5 25	5.12	4 60	4 38	0 2	0 1	-0 5	-0 7
63	13 82	12.94	12 71	13 02	13 36	-0.9	-1 1	-0.8	-0 5
64	7 86	8 19	7 68	8 33	8 08	0 3	-0 2	0 5	0.2
65	8.08	7 86	7 72	7 92	8 30	-0 2	-0.4	-0.2	0 2
66	5 62	5 13	6.66	6 98	5.20	-0 5	1 0	1 4	-0.4

Table 4
(continued)
VISIA-CR Image Analysis Color Data - a* Values Right Iris

.01 = [redacted] Lash Enhancing Serum

67	6.20	5 64	6 47	7 27	6 89	-0 6	0 3	1 1	0 7
68	10 16	9 03	10 09	10 78	10 21	-1 1	-0 1	0 6	0 1
69	13 59	13 48	14 16	12 60	13 59	-0 1	0 6	-1 0	0 0
70	5.25	4 24	4.47	4 89	4 79	-1 0	-0 8	-0 4	-0 5
71	7.28	7 34	8 16	8 10	9 62	0 1	0 9	0 8	2 3
72	10 30	9 75	10 03	9 99	10 12	-0 6	-0 3	-0 3	-0 2
73	6 53	6 89	7 06	7 20	7 24	0 4	0 5	0 7	0 7
74	2 59	2 69	2 47	3 05	3 19	0 1	-0 1	0 5	0 6
75	5 84	7 30	6 39	6 64	5 85	1 5	0.6	0 8	0 0
76	6 63	5 96	8 45	9 37	7 35	-0.7	1 8	2 7	0.7
77	9 56	9 70	9.52	8 36	8 09	0 1	0.0	-1 2	-1 5
78	3 29	-0 45	0 00	0 22	-0.26	-3 7	-3 3	-3 1	-3 5
79	8 54	9 67	7 64	9 76	8 73	1 1	-0 9	1 2	0 2
80	8 05	8 18	8 21	7 93	8 69	0 1	0,2	-0 1	0 6
82	8 47	6 91	7 58	7 40	8 41	-1 6	-0 9	-1 1	-0 1
84	2 16	0 37	2 13	2 79	1 62	-1 8	0 0	0 6	-0 5
86	9 70	6 87	6 58	7 64	8 26	-2 8	-3 1	-2 1	-1 4
87	3 98	6 57	6 55	6 89	6 42	2 6	2 6	2 9	2 4
88	-1 25	-1 46	-1,11	0 46	-1 05	-0 2	0 1	1 7	0 2
89	15 68	16 41	14 12	16.57	17 14	0 7	-1 6	0 9	1 5
90	-3 28	-3 54	-3 16	-3 26	-3 25	-0 3	0 1	0 0	0 0
91	7 04	5 74	6 84	6 98	6 84	-1 3	-0 2	-0 1	-0 2
92	12 91	10 32	11 36	11 97	10 52	-2 6	-1 5	-0 9	-2 4
93	-1 84	0 26	0 09	-0 37	-0 32	2 1	1 9	1 5	1 5
94	8 87	8 15	11 22	8 87	8 31	-0 7	2 4	0 0	-0 6
95	3 01	2 28	2 86	4 72	3 03	-0 7	-0 2	1 7	0 0
96	-1 55	-1 55	-1 23	-2.05	-1 26	0 0	0 3	-0 5	0 3
97	0 30	0 38	0 12	0 54	0 65	0 1	-0 2	0 2	0 4
98	1 39	1 71	2 34	2.89	2 75	0 3	0 9	1 5	1 4
99	3 99	4 26	3 70	3 76	4 28	0 3	-0 3	-0 2	0 3
100	4 91	8 32	7 35	6 40	6 07	3 4	2 4	1 5	1 2
101	10 28	10 71	10 87	10 66	10 65	0 4	0 6	0 4	0 4
102	16 46	15 89	17 27	13 52	13 99	-0 6	0 8	-2 9	-2 5
103	10 61	9 71	9 64	10 47	10 12	-0 9	-1 0	-0 1	-0 5
104	9 71	9 89	9 64	10 02	9 14	0 2	-0 1	0 3	-0 6
105	4 04	2 46	0 77	5 52	3 12	-1 6	-3 3	1 5	-0 9
106	12 21	11.15	12 28	13 22	12 49	-1 1	0 1	1 0	0 3
107	7 87	9 04	5 25	7 89	5 53	1 2	-2 6	0 0	-2 3
108	-0 31	-2 25	-1 58	-1 76	-2 41	-1 9	-1 3	-1 4	-2 1
109	-0.84	-0 24	-0 16	1 93	-1 14	0 6	0 7	2 8	-0 3
110	-1 40	-1 62	-1 97	-1 71	-1 70	-0 2	-0 6	-0 3	-0 3
111	11 54	8 02	9 59	10 86	8 43	-3 5	-2 0	-0 7	-3 1
112	3 77	4 59	4 22	4 30	4 71	0 8	0 5	0 5	0 9
114	6 54	5 66	7 40	7 28	7 94	-0 9	0 9	0 7	1 4
115	8 42	6 37	5 63	6 21	7 30	-2 0	-2 8	-2 2	-1 1
116	11 90	9 39	10 24	10 35	9 43	-2 5	-1 7	-1 5	-2 5
117	-0 09	0 13	0 60	0 18	0 44	0 2	0 7	0 3	0 5
118	9 52	11 92	10 97	10 16	11 18	2 4	1 5	0 6	1 7
119	10 29	10 14	10 84	10 33	10 58	-0 2	0 5	0 0	0 3
120	11 38	9 79	11 37	11 24	10 84	-1 6	0 0	-0 1	-0.5
121	6 37	6 83	5 78	7 49	7 14	0 5	-0 6	1 1	0.8
Mean =	**5.44**	**5.21**	**5.28**	**5.55**	**5.55**	**-0.23**	**-0.16**	**0.11**	**0.11**
Median =	**5.16**	**5.19**	**5.19**	**5.52**	**5.59**	**-0.03**	**0.04**	**0.21**	**0.25**
Standard Deviation =	**4.81**	**4.79**	**4.79**	**4.65**	**4.77**	**1.66**	**1.70**	**1.71**	**1.38**
Maximum =	**16.46**	**16.41**	**17.27**	**16.57**	**17.14**	**3.45**	**3.60**	**3.57**	**4.61**
Minimum =	**-3.28**	**-3.54**	**-3.35**	**-3.26**	**-3.25**	**-11.03**	**-10.27**	**-10.82**	**-5.86**

Mean % Change From Baseline

Month 1	Month 2	Month 4	Month 8
-4 2%	-3.0%	1 9%	2.0%
Decrease	Decrease	Increase	Increase

Did Not Complete Subject#s: 7, 51, 58, 81, 83, 85, 133

Tallies & Percentages

	Month 1	Month 2	Month 4	Month 8
Total	114	114	114	114
Increased Values	53	59	69	78
Decreased Values	61	55	45	36
No Change	0	0	0	0
Increased Values	46 49%	51.75%	60 53%	68.42%
Decreased Values	53.51%	48 25%	39.47%	31.58%
No Change	0 00%	0 00%	0 00%	0.00%

L* values are a measure of the luminosity of the test site. The higher the value, the greater the lightness of the test site
a* values are a measure of the redness of the test site. The higher the value, the greater the redness of the test site
b* values are a measure of the yellowness of the test site. The higher the value, the greater the yellowness of the test site

Table 4
(continued)

VISIA-CR Image Analysis Color Data - b* Values Right Iris

.01 - [redacted] Lash Enhancing Serum

						Difference From Baseline At :			
Subject #	**Baseline**	**Month 1**	**Month 2**	**Month 4**	**Month 8**	**Month 1**	**Month 2**	**Month 4**	**Month 8**
1	18 40	19 89	17 83	18 75	18 72	1 5	-0 6	0 3	0 3
2	4 15	4 51	4 24	4 26	4.93	0.4	0 1	0 1	0 8
3	7 20	6 91	5 97	7 87	7 82	-0 3	-1 2	0 7	0 6
4	19 81	19 55	20 00	19.82	19 37	-0 3	0 2	0 0	-0 4
5	6 85	7 36	6 00	6 71	6 99	0 5	-0 9	-0 1	0 1
6	5 31	5.07	5 52	4 38	5 00	-0 2	0 2	-0 9	-0 3
8	6 04	5 88	6 11	6 27	5 30	-0 2	0 1	0 2	-0 7
9	14 24	15 00	15 39	15 84	15 31	0 8	1 2	1 6	1 1
10	4 94	5 61	5 35	4 68	5 46	0 7	0.4	-0 3	0 5
11	4 99	4 94	4 55	6 35	5 34	-0 1	-0 4	1.4	0 3
12	11 58	11 86	11 28	12 06	12 09	0 3	-0 3	0 5	0 5
13	5 54	5 33	5 44	5 31	5 65	-0 2	-0 1	-0 2	0 1
14	8 20	7 19	6 46	5 70	5 13	-1 0	-1 7	-2 5	-3 1
15	4 01	6 02	6 29	4 30	5 30	2 0	2 3	0 3	1 3
16	8 33	7 98	8 34	8 38	8 81	-0 4	0 0	0 1	0 5
17	18 32	17 46	16 33	18 35	18 61	-0 9	-2 0	0 0	0 3
18	15 19	14 93	10 98	14 05	13 60	-0 3	-4 2	-1 1	-1 6
19	14 81	13 77	14 77	13.27	14.61	-1 0	0 0	-1 5	-0 2
20	3 55	3 10	3 23	2 60	2 45	-0 5	-0 3	-1 0	-1 1
21	22 17	20 56	18 97	18 49	21 75	-1 6	-3 2	-3.7	-0 4
22	15 19	14 94	14 36	15 40	15 59	-0 3	-0 8	0 2	0 4
23	4 64	3 76	3 37	3 00	5 79	-0 9	-1 3	-1 6	1 2
24	4 87	5 09	4 63	4 79	5 32	0 2	-0 2	-0 1	0 5
25	16 06	3 89	6 27	4 52	10 75	-12 2	-9 8	-11 5	-5 3
26	12 39	12.64	14 10	14 67	14 90	0 2	1 7	2 3	2 5
27	14,61	14 23	14 82	14 18	16 06	-0 4	0 2	-0 4	1 5
28	11 46	10 66	11 90	13 75	12 27	-0 8	0 4	2 3	0 8
29	9 58	10 49	7 48	10.82	8 94	0 9	-2 1	1 2	-0.6
30	4 23	3 95	4 56	4 47	4 32	-0 3	0 3	0 2	0 1
31	4 54	4 04	5 02	4 75	4 37	-0 5	0 5	0 2	-0 2
32	14 96	17 36	17 14	17 19	17 80	2 4	2 2	2 2	2 8
33	14 17	14 99	13 86	13 18	14 88	0 8	-0 3	-1 0	0 7
34	13 83	15 16	15 55	15 74	15 66	1 3	1 7	1 9	1 8
35	7 20	4 59	4 45	4 08	5 69	-2 6	-2 7	-3 1	-1 5
36	11 82	12 42	12 43	11 99	11 39	0 6	0 6	0 2	-0 4
37	6 10	6 23	5 58	6 14	6 25	0 1	-0 5	0 0	0 2
38	6 61	5 86	6 88	7 29	6 49	-0.7	0 3	0 7	-0 1
39	21 96	21 42	20 92	22 20	22 32	-0 5	-1 0	0 2	0 4
40	5 40	5 20	5 71	5 71	4 93	-0 2	0 3	0 3	-0 5
41	5 59	7 31	4 79	5 48	5 09	1 7	-0 8	-0 1	-0 5
42	13 43	13 55	16 11	16.68	17 52	0 1	2 7	3 2	4 1
43	6 92	6 42	6.92	6 78	6 87	-0 5	0 0	-0.1	0 0
44	20 75	21 42	20 18	21 34	21 43	0 7	-0 6	0 6	0 7
45	5,25	5 25	5 65	6,06	5 22	0 0	0 4	0 8	0 0
46	15 05	17 44	14 30	14 69	19 85	2 4	-0.8	-0 4	4 8
47	6 76	6 83	6 00	6 74	7 10	0 1	-0 8	0 0	0 3
48	5 52	5 72	5 77	5 68	6 18	0 2	0 2	0 2	0 7
49	11 66	10 69	11 82	10 79	11 79	-1 0	0 2	-0 9	0 1
50	12 52	12 39	12 26	12 80	13 33	-0 1	-0 3	0 3	0 8
52	10 10	9.63	10.85	8 84	11 73	-0 5	0 7	-1 3	1 6
53	15 09	18 05	17 85	18 02	18 76	3 0	2.8	2 9	3 7
54	5 11	5 26	5 49	4 77	5 26	0 1	0 4	-0 3	0 2
55	6.32	5 97	4 98	6 22	5 67	-0 4	-1.3	-0 1	-0 6
56	5 09	4 84	6 50	5 87	5 57	-0 2	1 4	0 8	0 5
57	6 33	6 56	6.84	6 95	5 87	0 2	0 5	0 6	-0.5
59	5 48	5.88	5 31	5 79	6.14	0.4	-0 2	0 3	0 7
60	18,02	19 65	19 20	19 25	19,92	1 6	1 2	1 2	1.9
61	10 75	10 93	11 39	10 59	10 91	0 2	0 6	-0 2	0 2
62	6 55	6 05	6 20	6 74	6 98	-0 5	-0 3	0 2	0 4
63	20 14	19 23	18 80	19 35	20 12	-0.9	-1 3	-0.8	0 0
64	10 18	10.40	9 98	10 81	9 97	0 2	-0 2	0 6	-0 2
65	10 36	9 30	9 12	9 81	9 95	-1.1	-1 2	-0 5	-0 4
66	15 07	15.85	16.46	16 87	15 16	0 8	1 4	1 8	0 1
67	8 31	8 07	8 12	8 85	8 50	-0 2	-0 2	0.5	0 2

Table 4
(continued)

VISIA-CR Image Analysis Color Data - b* Values Right Iris

.01 = [redacted] Lash Enhancing Serum

68	13 54	13 65	13 70	14 60	14 17	0 1	0 2	1 1	0 6
69	22 34	22 79	23.07	20 74	21 85	0 5	0 7	-1 6	-0 5
70	6 02	6 49	7 13	6 43	6 48	0.5	1 1	0 4	0 5
71	9 90	10 93	11 91	12 78	13 24	1 0	2 0	2 9	3 3
72	12 35	12 28	12 29	11 35	13 05	-0 1	-0 1	-1 0	0 7
73	8.70	9.00	8.56	8 51	9 07	0 3	-0 1	-0 2	0 4
74	4 94	3 87	4 08	4 89	4 89	-1 1	-0 9	0 0	-0 1
75	7 53	9 29	7 81	7 89	8 10	1 8	0 3	0 4	0 6
76	13 08	13 75	14 41	14 63	13 66	0 7	1 3	1 5	0 6
77	13 32	12 70	13 61	12 29	12 08	-0 6	0 3	-1 0	-1 2
78	5 89	2 77	3 33	3 48	3 89	-3 1	-2 6	-2 4	-2 0
79	18 91	16 20	15 99	19 50	18 48	-2,7	-2 9	0 6	-0 4
80	9 72	9 06	9 93	8 27	9 23	-0 7	0 2	-1 5	-0 5
82	10 17	8 37	8 64	9 19	10 14	-1.8	-1 5	-1 0	0 0
84	4 48	6 01	4 65	4 88	5 74	1 5	0 2	0 4	1 3
86	10 17	8 43	7 78	10 12	9 87	-1 7	-2.4	0 0	-0 3
87	6 11	7 51	7 43	7 63	7.70	1 4	1 3	1 5	1 6
88	15 33	15 04	14 77	16 93	17 03	-0 3	-0 6	1 6	1 7
89	21 14	21 94	20 01	22 36	22 99	0 8	-1 1	1 2	1 8
90	5 99	5 79	7 25	6 18	5 71	-0 2	1 3	0 2	-0 3
91	8 12	8 25	7 72	8 15	9 39	0 1	-0 4	0 0	1 3
92	19 88	18 14	16 92	20 17	14 24	-1 7	-3 0	0 3	-5 6
93	7 16	8 95	7 02	7 58	8,65	1 8	-0 1	0 4	1 5
94	29 43	28 43	24 51	29 08	29 55	-1 0	-4 9	-0 3	0 1
95	18 62	17 46	20 81	20 70	19 58	-1 2	2 2	2 1	1 0
96	0 87	0 48	1 05	0 88	1 08	-0 4	0 2	0 0	0 2
97	3 66	3 67	2 98	3 69	4 31	0 0	-0 7	0 0	0 6
98	11 17	11 70	13 13	12 99	12 69	0 5	2 0	1 8	1 5
99	11 91	13 10	13 29	12 57	12 83	1 2	1 4	0 7	0 9
100	12 57	13 48	14 54	14 29	13 03	0 9	2 0	1 7	0 5
101	14 61	14 15	15 72	15 16	14 97	-0 5	1 1	0 6	0 4
102	20 59	19 30	21 57	13 16	17 90	-1 3	1 0	-7 4	-2 7
103	15 57	16 55	16 58	16 57	16 21	1 0	1 0	1 0	0 6
104	13 34	13 84	12 86	13 67	14 62	0 5	-0 5	0 3	1 3
105	23 25	20 01	19 03	24 64	20 73	-3 2	-4 2	1 4	-2 5
106	14 12	12 71	13 97	15 86	14 83	-1 4	-0 2	1 7	0 7
107	9 96	10 66	9 83	9 84	8 00	0 7	-0 1	-0 1	-2 0
108	12 65	8 98	11 59	9 52	9 50	-3 7	-3 1	-3 1	-3 2
109	14.85	13 79	13 79	15 49	13 97	-1 1	-1 1	0 6	-0 9
110	12 09	12 57	12 03	12 32	12 35	0 5	-0 1	0 2	0 3
111	12 94	14.01	14 72	13 20	12 57	1 1	1 8	0 3	-0.4
112	7 39	7 55	8 11	7 17	7 09	0 2	0 7	-0 2	-0.3
114	10 65	11 17	12 12	11 97	14 44	0 5	1 5	1 3	3 8
115	12 43	12 14	11 15	9 36	10 34	-0 3	-1 3	-3 1	-2 1
116	19 69	19 15	19 14	19 06	19 78	-0 5	-0 6	-0 6	0 1
117	8 67	8 10	8.11	8 81	8 23	-0 6	-0 6	0 1	-0 4
118	19 67	21 11	20 83	21 13	22 28	1 4	1 2	1 5	2 6
119	12 44	11 32	12 34	12 36	12 88	-1 1	-0 1	-0 1	0 4
120	15 23	13 47	14 17	13 63	15 11	-1 8	-1 1	-1 6	-0 1
121	7 64	9 49	6 57	10 75	11.76	1 9	-1 1	3 1	4 1
Mean =	**11.23**	**11.07**	**11.01**	**11.26**	**11.50**	**-0.2**	**-0.2**	**0.0**	**0.3**
Median =	**10.70**	**10.66**	**11.06**	**10.77**	**11.15**	**-0.1**	**-0.1**	**0.2**	**0.3**
Standard Deviation =	**5.54**	**5.54**	**5.41**	**5.71**	**5.68**	**1.6**	**1.7**	**1.8**	**1.6**
Maximum =	**29.43**	**28.43**	**24.51**	**29.08**	**29.55**	**3.0**	**2.8**	**3.2**	**4.8**
Minimum =	**0.87**	**0.48**	**1.05**	**0.88**	**1.08**	**-12.2**	**-9.8**	**-11.5**	**-5.6**

Mean % Change From Baseline

Month 1	Month 2	Month 4	Month 8
-0 8%	-1 2%	0 1%	1.4%
Decrease	Decrease	Increase	Increase

Tallies & Percentages

	Month 1	Month 2	Month 4	Month 8
Total	114	114	114	114
Increased Values	54	54	70	71
Decreased Values	60	60	44	43
No Change	0	0	0	0
Increased Values	47 37%	47 37%	61 40%	62 28%
Decreased Values	52 63%	52 63%	38.60%	37 72%
No Change	0.00%	0 00%	0 00%	0 00%

Did Not Complete Subjects: 7, 51, 58, 81, 83, 85, 113

L* values are a measure of the luminosity of the test site The higher the value, the greater the lightness of the test site
a* values are a measure of the redness of the test site. The higher the value, the greater the redness of the test site
b* values are a measure of the yellowness of the test site The higher the value, the greater the yellowness of the test site

Table 4
(continued)

.01 [redacted] Lash Enhancing Serum (Right)

Within-Groups Analysis For :
VISIA-CR Image Analysis Color Data - Delta E Value

Pre-Test	Normality	Brown-Forsythe	Friedman ANOVA
P-Value	P < 0 050	Not Required	0 001

Pairwise Comparisons

Difference from Baseline	Test Type	P-value	Significance	Direction (Mean)
Month 1	Dunn's Method	**0.001**	**Yes**	**Increase**
Month 2	Dunn's Method	**0.001**	**Yes**	**Increase**
Month 4	Dunn's Method	**0.001**	**Yes**	**Increase**
Month 8	Dunn's Method	**0.001**	**Yes**	**Increase**

Within-Groups Analysis For :
VISIA-CR Image Analysis Color Data - Delta L Value

Pre-Test	Normality	Brown-Forsythe	Friedman ANOVA
P-Value	P < 0 050	Not Required	0 151

Within-Groups Analysis For :
VISIA-CR Image Analysis Color Data - Delta A Value

Pre-Test	Normality	Brown-Forsythe	Friedman ANOVA
P-Value	P < 0 050	Not Required	0 001

Pairwise Comparisons

Difference from Baseline	Test Type	P-value	Significance	Direction (Mean)
Month 1	Dunn's Method	1 000	No	Decrease
Month 2	Dunn's Method	1 000	No	Decrease
Month 4	Dunn's Method	**0.004**	**Yes**	**Increase**
Month 8	Dunn's Method	**0.001**	**Yes**	**Increase**

Within-Groups Analysis For :
VISIA-CR Image Analysis Color Data - Delta B Value

Pre-Test	Normality	Brown-Forsythe	Friedman ANOVA
P-Value	P < 0 050	Not Required	0 001

Pairwise Comparisons

Difference from Baseline	Test Type	P-value	Significance	Direction (Mean)
Month 1	Dunn's Method	1 000	No	Decrease
Month 2	Dunn's Method	1 000	No	Decrease
Month 4	Dunn's Method	0.375	No	Increase
Month 8	Dunn's Method	**0.003**	**Yes**	**Increase**

* = ANOVA (RM) compares the means across groups (the non-parametric version is Friedman). The null hypothesis is all means are equivalent and the alternative is that the mean of at least one group differs. If ANOVA is significant further pairwise comparisons can be made, if not then it is assumed there is no statistically significant difference among the mean (or ranks) and no further testing is done due to risk of type one error. It is possible that Friedman or RM ANOVA may be significant, but when running post-hoc test may not show any statistically significant difference due to only examining the comparisons to baseline, post-hoc test, small sample size, lack of power, or if there is borderline significance.

* = Dunn's method is a post-hoc test commonly run after Friedman RM ANOVA. It allows for comparisons between a control group, and it is a robust, non-parametric test that adjusts for multiple comparisons.

ΔE is a measure of total color change of the test site from baseline The larger the value, the greater the change in color. ΔE does not provide the direction, or individual component of the change Subsequently, the individual changes in the L * a* b* values from baseline are evaluated

L* values are a measure of the luminosity of the test site The higher the value, the greater the lightness of the test site
a* values are a measure of the redness of the test site The higher the value, the greater the redness of the test site
b* values are a measure of the yellowness of the test site. The higher the value, the greater the yellowness of the test site.

Summary:

For subjects using [redacted] Lash Enhancing Serum, there was an overall increase in ΔE values of the right eye indicating an increase in color change from baseline at all timepoints When evaluating the individual L* a* b* values, there were no statistically significant differences in ocular pigmentation from baseline when looking at the change in L* When evaluating the a* value of the test site on the right side compared to baseline, there was a significant difference at month 4 and month 8 These changes represent a increase in redness at month 4 and an increase in redness at month 8 There is also a statistically significant increase at month 8 when compared to baseline for the b* value, indicating more yellowing of the test site

CONSUMER PRODUCT TESTING COMPANY
cptclabs.com

FDA Registration# 1000151293
DEA Registration# RC0199744 Schedule I-V
US EPA/NJ DEP Registration# NJ0882276648
ISO/IEC 17025:2017 Accreditation # 80171

[redacted]

Lash serum containing IC #1

Lash serum containing IC #2

Dear [redacted]

Per your request, I reviewed the data from three studies we conducted to generate an average amount of Isopropyl Cloprostenate (IC) per usage of [redacted] and [redacted] cosmetic products. In our study number Q22-6783 the average concentration of IC was 0.0044% and, in our study, Q23-2055 the average weight of application was 0.19mg, making the average amount of IC 0.0000084mg per usage for the [redacted] cosmetic product. In our Q23-1189 the average concentration of IC was 0.0048% and, in our study, Q23-2055 the average weight of application was 0.26mg, making the average amount of IC 0.000013mg per usage for the [redacted] cosmetic product. I have attached the noted studies for your review.

Please let me know if you need additional information.

Lash serum containing IC #1

Best regards,

Lash serum containing IC #2

[redacted]
President

[redacted]

Distributed for Comment Only -- Do Not Cite or Quote

CONSUMER PRODUCT TESTING COMPANY
cptclabs.com

ANALYSIS REPORT

[redacted]

Analysis #: Q22-6783
T.A. #(s):
Data: [redacted]

Date Received: 12/29/2022
Date(s) Analyzed: 01/03/2023 – 01/04/2023

Collected By: Client
P.O. Auth.: N/A

Lash serum containing IC #1

Q22-6783.01 [redacted]

Analysis	Specification	Results	Method
[redacted] Assay	Report results	Prep 1 : 0.0043% Prep 2 : 0.0046%	HPLC

Comments:

Questions? Please contact the Analytical Services Division at [redacted]

This study was conducted according to Consumer Product Testing Company Standard Operating Procedures.

Approved By: [redacted]
1/04/2023
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Quality Assurance

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FDA Registration# 1000151293
DEA Registration# RC0199744 Schedule I-V
US EPA/NJ DEP Registration# NJD982726648
ISO/IEC 17025:2017 Accredited

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ANALYSIS REPORT

[redacted]

Analysis #: Q23-2055
T.A. #(s):
Data: [redacted]

Date Received: 04/19/2023
Date(s) Analyzed: 05/01/2023

Collected By: Client
P.O. Auth.: N/A

Lash serum containing IC #1

Q23-2055.01 [redacted]

Analysis	Weight #	Specification	Results	Method
Average Weight Usage Test	1	Report results	0.18 mg	[redacted] Method
	2	Report results	0.19 mg	
	3	Report results	0.29 mg	
	4	Report results	0.24 mg	
	5	Report results	0.18 mg	
	6	Report results	0.25 mg	
	7	Report results	0.16 mg	
	8	Report results	0.19 mg	
	9	Report results	0.15 mg	
	10	Report results	0.14 mg	
	11	Report results	0.29 mg	
	12	Report results	0.20 mg	
	13	Report results	0.12 mg	
	14	Report results	0.14 mg	
	15	Report results	0.20 mg	
	Average	Report results	0.19 mg	

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ANALYSIS REPORT

[redacted]

Analysis #: Q23-1189
T.A. #(s):
Data: [redacted]

Date Received: 03/07/2023
Date(s) Analyzed: 03/20/2023 – 03/21/2023

Collected By: Client
P.O. Auth.: N/A

Lash serum containing IC #2

Q23-1189.01 [redacted]

Analysis	Specification	Results	Method
[redacted] Assay	Report results	Prep 1: 0.0049% Prep 2: 0.0047%	HPLC

Comments:

Questions? Please contact the Analytical Services Division at [redacted]

This study was conducted according to Consumer Product Testing Company Standard Operating Procedures.

Approved By:
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3/21/2023
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Manager,
Analytical Services

Approved By:
[redacted]
03/21/23
Quality Assurance

[redacted]

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DEA Registration# RC0199744 Schedule I-V
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DEA Registration# RC0198744 Schedule I-V
US EPA/NJ DEP Registration# NJ0982726646
ISO/IEC 17025:2017 Accreditation # 80071

Study #: Q23-2055.01-.02
Study Title: Lash Products

ANALYSIS REPORT

Q23-2055.02 Lash serum containing IC #2 [redacted]

Analysis	Weight #	Specification	Results	Method
Average Weight Usage Test	1	Report results	0.50 mg	[redacted] Method
	2	Report results	0.42 mg	
	3	Report results	0.28 mg	
	4	Report results	0.40 mg	
	5	Report results	0.25 mg	
	6	Report results	0.10 mg	
	7	Report results	0.14 mg	
	8	Report results	0.21 mg	
	9	Report results	0.18 mg	
	10	Report results	0.10 mg	
	11	Report results	0.28 mg	
	12	Report results	0.49 mg	
	13	Report results	0.23 mg	
	14	Report results	0.18 mg	
	15	Report results	0.15 mg	
	Average	Report results	0.26 mg	

Comments:

Questions? Please contact the Analytical Services Division at [redacted]

This study was conducted according to Consumer Product Testing Company Standard Operating Procedures.

Approved By: [redacted]
5/02/2023
[redacted]
Manager,
Analytical Services

Approved By: [redacted]
05/02/23
Quality Assurance

[redacted]

[redacted]

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FDA Registration# 1000151293
DEA Registration# RC0199744 Schedule I-V
US EPA/HI DEP Registration# HID932726648
ISO/IEC 17025:2017 Accreditation # 80971

Average Sample Weight Usage Testing

1. Dip applicator into fresh bottle once, don't double dip.

2. Wipe off excessive serum by swiping applicator along the rim of the container to ensure the applicator is not dripping.

4. Take a clean pre-weighed Helioscreen PMMA HD 6 plate.

5. Swipe applicator containing product in petri dish or slide. The swipe should be 1 inch.

6. Do not double dip. Swipe applicator a second time in a separate part of the petri dish or slide. The swipe should be 1 inch. (Note the second swipe approximates a user applying the serum to both upper eyelashes)

7. Weight plate

8. Determine amount of product on slide.

9. Repeat for 15 total applications.

We will perform the described experiment and record results. The results of the analysis will be provided on a Certificate of Analysis (CoA). We will report all sample weights and the average.

[redacted]

Test Report No. 202402011
Report Date November 8, 2024

[redacted]

Approval Date	May 17, 2024	**Delivery Conditions**	Satisfactory, Tested as Received
Date of Receipt	May 23, 2024	**Testing Date Range**	05-23-2024 to 09-06-2024
Test Request Form #	202402011		

Eurofins ID	Sample Name	Lot/Batch Number	Supplier/Manufacturer	Country of Origin
202402011-1 / 24-0055B	[redacted]	N/A	N/A	N/A

lash serum containing 0.005% Isopropyl Cloprostenate

The following test item(s) was/were performed on submitted sample(s) and/or component(s) confirmed by applicant

TEST REQUESTED	RESULT
Dermal: Absorption Development - 4 donors	See Attachment
Panel - Dermal: Method evaluation non-GLP	See Attachment
Panel - Dermal: Supply and preparation of skin	See Attachment
Panel - Dermal: Bioanalysis - non-GLP - 5 analytical batches	See Attachment
Panel - Dermal: In-vitro absorption study, experimentation	See Attachment

Analysis completed by Eurofins Subcontract Laboratory

Signed for and on behalf of
Eurofins MTS Consumer Product Testing US, Inc.

[redacted]

[redacted] **/ Project Coordinator**

11-08-2024

This report relates to the above mentioned test item(s) and the extent to tests performed. This test report is not permitted to be reproduced except in full, without written permission of the test facility. This test report does not entitle any safety marks on this or similar products. The sample and the information regarding sample have been provided by the client. All information related to the sample are under liability of the client and have not been checked by Eurofins MTS Consumer Product Testing US, Inc.

Eurofins MTS Consumer Product Testing US, LLC
22310 20th Ave SE, Bothell WA 98021
Phone: (425) 686-3575 Web: www.EurofinsUS.com

ATTACHMENT

REPORT

Title	Qualification of an analytical method for Isopropyl cloprostenate and its metabolite: quantification in Washing, *Stratum corneum*, Skin and Receptor Fluid samples in the context of *in vitro* skin absorption study.
Eurofins|ADME BIOANALYSES reference	24-0055A
Name of product	Isopropyl cloprostenate and its metabolite
Test Facility	Eurofins|ADME BIOANALYSES 75A, Avenue de Pascalet 30310 Vergèze France
Sponsor	Eurofins Product Testing US Inc. 11822 N Creek Pkwy #110, Bothell, WA 98011, USA
Date of the experiment	Protocol signature: April 08th, 2024 Start of the experiments: April 26th, 2024 Completion of the experiments: July 22nd, 2024
Document status	Final
Date of version	November 07th, 2024

APPROVAL PAGE

This report has been approved by the Analytical Site. The following signatures document those approvals:

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Principal Scientist

[redacted]

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SUMMARY

OBJECTIVE:

The objective of this study was to qualify an analytical method for the quantification of Isopropyl cloprostenate and Cloprostenol in washing, receptor fluid, *Stratum Corneum* (tape stripping) and skin samples. This study was done in the context of *in vitro* skin penetration study.

ANALYTICAL METHOD:

The qualification of an analytical method referenced as PKH/MOA/1745 (APPENDIX C), using LC-MS/MS techniques, for Isopropyl cloprostenate and Cloprostenol detection was performed in washing, strips (*stratum corneum*), skin and receptor fluid.

The results obtained during this qualification demonstrated that the analytical method can be used to measure Isopropyl cloprostenate and Cloprostenol concentrations.

Calibration ranges were:

- **Washing**: For Isopropyl cloprostenate: 1 - 100 ng/mL
  For Cloprostenol (metabolite): 0.5 - 50 ng/mL
- **Strips**: For Isopropyl cloprostenate: 0.1 - 50 ng/mL
  For Cloprostenol (metabolite): 0.25 - 125 ng/mL
- **Skin**: For Isopropyl cloprostenate: 0.05 - 25 ng/mL
  For Cloprostenol (metabolite): 1 - 500 ng/mL
- **Receptor fluid:** For Isopropyl cloprostenate and Cloprostenol (metabolite): 1 to 500 ng/mL

The qualification has been conducted and documented so that the qualified method can be used in support of dermal absorption.

RESULTS:

Table 1. Summary of the Isopropyl cloprostenate assay analytical method qualification

Reference compound	Isopropyl cloprostenate		
Internal Standard	Irbesartan (for Washing matrix only)		
Detection method	LC-MS/MS		
Regression (weighting)	Linear ($1/x^2$)		
	Washing		
Matrix	3 half cotton buds wetted with water containing 10% of Tween 80, 2 dry half cotton buds		
Calibration range	1 to 100 ng/mL		
	Tested concentration (ng/mL)		
	3	**50**	**80**
Intra-run precision (%) *Repeatability*	9.38	8.54	7.48
Intra-run deviation (%) *Repeatability*	-18.26	-6.92	-6.56
Recovery ± SD (CV%) *QC (matrix 15min RT) vs QC (solvent)*	68.94% ± 9.30% (CV 13.49%)	-	92.79% ± 6.84% (CV 7.37%)
Overall recovery ± SD (CV%)	80.87 % ± 14.69 % (CV 18.16 %)		
Specificity	The method is specific as no interference was observed in the blank matrix.		

	For the specificity in presence of metabolite, the impact of concomitant drug should be evaluated according to the concentrations obtained during assays (see section 7.5 in current report).		
Carry over	No carry over was observed. No specific precaution necessary.		
Long term stability at target temperature -20°C	Long term stability was tested for 29 days at -20°C for 3 LLOQ and 0.8 ULOQ At 3 LLOQ, the mean intrarun %CV was +6.48% (criteria < 20%) and the mean intrarun %deviation was -36.68% (criteria < 20.00%). At 0.8 ULOQ, the mean intrarun %CV was +1.82% (criteria <20%) and the mean intrarun %deviation criteria was -34.98% (criteria < 20.00%). Considering these results, the washing samples will be preferably analysed on a period inferior to 29 days. The washing samples of the application study 24-0055B were analysed the day of cells dismantling.		
	Strips		
Matrix	5 pieces of Scotch Magic 3M striped on human skin		
Calibration range	0.1 to 50 ng/mL		
	Tested concentration (ng/mL)		
	0.3	**25**	**40**
Intra-run precision (%) *Repeatability*	3.86	2.23	3.11
Intra-run deviation (%) *Repeatability*	13.95	13.06	6.59
Recovery ± SD (CV%) *QC (matrix 15min RT) vs QC (solvent)*	97.24% ± 4.18% (CV 4.30%)	-	96.24% ± 4.33% (CV 4.50%)
Overall recovery ± SD (CV%)	96.74% ± 4.09% (CV 4.23%)		
Specificity	The method is specific as no interference was observed in the blank matrix. For the specificity in presence of metabolite, the impact of concomitant drug should be evaluated according to the concentrations obtained during assays (see section 7.5 in current report).		
Carry over	No carry over was observed. No specific precaution necessary.		
Long term stability at target temperature -20°C	Long term stability was tested for 6 days at -20°C for 3 LLOQ and 0.8 ULOQ. At 3 LLOQ, the mean intrarun %CV was +18.33% (criteria <20%) and the mean intrarun% deviation was +22.93% (criteria < 20%). At 0.8 ULOQ, the intrarun %CV was +6.15% (criteria < 20%) and the intrarun% mean deviation was +8.65% (criteria <20%) The mean intrarun% did not fulfilled the acceptance criteria at 3 LLOQ. However, the overall results and the positive mean intrarun% deviation show no degradation in strips on a period of 6 days.		
	Skin		
Matrix	Human dermatomed skin 1 cm²		
Calibration range	0.05 to 25 ng/mL		
	Tested concentration (ng/mL)		
	0.15	**12.5**	**20**
Intra-run precision (%) *Repeatability*	10.20	7.66	4.67
Intra-run deviation (%) *Repeatability*	-4.65	-3.70	-0.21
Recovery ± SD (CV%) *QC (matrix 15min RT) vs QC (solvent)*	94.93% ± 6.56% (CV 6.91%)	-	91.64% ± 7.35% (CV 8.02%)
Overall recovery ± SD (CV%)	93.29% ± 6.86% (CV 7.35%)		
Long term stability at target temperature -20°C	Long term stability was tested for 17 days at -20°C for 3 LLOQ and 0.8 ULOQ. At 3 LLOQ, the mean intrarun %CV was +11.54 (criteria <20%) and the intrarun% mean deviation was +46% (criteria <20%). At 0.8 ULOQ, the mean intrarun %CV was +3.63% (criteria <20%) and the intrarun% mean deviation was +26.13 % (criteria <20%). The mean intrarun% did not fulfilled the acceptance criteria at 3 LLOQ and 0.8 ULOQ. However, the overall results and the positive intrarun% mean deviation show no degradation in skin on a period of 17 days.		
Specificity	The method is specific as no interference was observed in the blank matrix.		

Carry over	No carry over was observed. No specific precaution necessary.		
	Receptor fluid		
Matrix	PBS 0.01M pH 7.4		
Calibration range	1 to 500 ng/mL		
	Tested concentration (ng/mL)		
	3	**250**	**400**
Intra-run precision (%) *Repeatability (run 6)*	10.72	18.29	6.73
Intra-run deviation (%) *Repeatability (run 6)*	-2.56	1.07	4.65
Long term stability at target temperature -20°C	Long term stability was tested for 17 days at -20°C for 3 LLOQ and 0.8 ULOQ At 3 LLOQ, the mean intrarun %CV was +18.95% (criteria <20%) and the mean intrarun% deviation was +6.78 (criteria <20%). At 0.8 ULOQ, the mean intrarun %CV was +6.43% (criteria <20%) and the mean intrarun% deviation was +25.08% (criteria <20%). The mean intrarun% did not fulfilled the acceptance criteria at 0.8 ULOQ. However, the overall results and the positive intrarun% mean deviation show no degradation in receptor fluid on a period of 17 days.		
Specificity	The method is specific as no interference was observed in the blank matrix. For the specificity in presence of metabolite, the impact of concomitant drug should be evaluated according to the concentrations obtained during assays (see section 7.5 in current report).		
Carry over	No carry over was observed. No specific precaution necessary.		

Table 2. Summary of the Cloprostenol assay analytical method qualification

Reference compound	Cloprostenol		
Internal Standard	Prostaglandin F2Alpha-d4		
Detection method	LC-MS/MS		
Regression (weighting)	Linear ($1/x^2$)		
	Washing		
Matrix	3 half cotton buds wetted with water containing 10% of Tween 80, 2 dry half cotton buds		
Calibration range	0.5 to 50 ng/mL		
	Tested concentration (ng/mL)		
	1.5	**25**	**40**
Intra-run precision (%) *Repeatability*	7.15	9.78	7.41
Intra-run deviation (%) *Repeatability*	-1.61	0.00	-5.07
Recovery ± SD (CV%) *QC (matrix 15 min RT) vs QC (solvent)*	80.12% ± 8.84% (CV 11.03%)	-	85.25% ± 8.07% (CV 9.46%)
Overall recovery ± SD (CV%)	82.69 % ± 8.50 % (CV 10.28 %)		
Carry over	No carry over was observed. No specific precaution necessary.		
Long term stability at target temperature -20°C	Long term stability was tested for 29 days at -20°C for 3 LLOQ and 0.8 ULOQ At 3 LLOQ, the stability criteria were fulfilled in regard with a mean intrarun% %CV of +6.28% (criteria <20%) and a mean intrarun% deviation of -18.85% (criteria <20%). At 0.8ULOQ, the mean intrarun %CV was +1.44% (criteria <20%) and the mean intrarun% deviation of -22.00% (criteria <20%). Considering these results, the washing samples should be preferably analysed on a period inferior to 29 days. The washing samples of the application study 24-0055B were analysed the day of cells dismantling.		
	Strips		
Matrix	5 pieces of Scotch Magic 3M striped on human skin		
Calibration range	0.25 to 125 ng/mL		

	Tested concentration (ng/mL)		
	0.75	**62.5**	**100**
Intra-run precision (%) *Repeatability*	6.92	0.82	3.40
Intra-run deviation (%) *Repeatability*	-13.57	3.70	0.22
Recovery ± SD (CV%) *QC (matrix 15 min RT) vs QC (solvent)*	105.82% ± 7.32% (CV 6.91%)	-	96.06% ± 4.08% (CV 4.25%)
Overall recovery ± SD (CV%)	100.94 % ± 7.61 % (CV 7.54 %)		
Specificity	The method is specific as no interference was observed in the blank matrix. For the specificity in presence of metabolite, the impact of concomitant drug should be evaluated according to the concentrations obtained during assays (see section 7.5 in current report).		
Carry over	No carry over was observed. No specific precaution necessary.		
Long term stability at target temperature -20°C	Long term stability was tested for 6 days at -20°C for 3 LLOQ and 0.8 ULOQ At 3 LLOQ, the mean intrarun %CV was +6.71% (criteria <20%) and the mean intrarun% deviation was -0.28 (criteria <20%). At 0.8 ULOQ, the mean intrarun %CV was +1.88% (criteria <20%) and the mean intrarun% deviation of -1.72% (criteria <20%). The results show stability of Cloprostenol in the strips samples on a 6 days period.		
	Skin		
Matrix	Human dermatomed skin 1 cm²		
Calibration range	1 to 500 ng/mL		
	Tested concentration (ng/mL)		
	3	**250**	**400**
Intra-run precision (%) *Repeatability*	1.43	1.95	2.01
Intra-run deviation (%) *Repeatability*	3.08	6.07	5.83
Recovery ± SD (CV%) *QC (matrix 15 min RT) vs QC (solvent)*	95.89% ± 3.93% (CV 4.10%)	-	95.65% ± 3.85% (CV 4.03%)
Overall recovery ± SD (CV%)	95.77 % ± 3.71 % (CV 3.88 %)		
Long term stability at target temperature -20°C	Long term stability was tested for 17 days at -20°C for 3 LLOQ and 0.8 ULOQ. At 3 LLOQ, the mean intrarun %CV was +4.71% (criteria <20%) and the mean intrarun% deviation was +6.87% (criteria <20%). At 0.8 ULOQ, the mean intrarun %CV was +4.09% (criteria <20%) and the mean intrarun% deviation was +0.01% (criteria <20%). The results show stability of Cloprostenol in the skin samples on a 17 days period.		
Specificity	The method is specific as no interference was observed in the blank matrix. For the specificity in presence of metabolite, the impact of concomitant drug should be evaluated according to the concentrations obtained during assays (see section 7.5 in current report).		
Carry over	No carry over was observed. No specific precaution necessary.		
	Receptor fluid		
Matrix	PBS 0.01M pH 7.4		
Calibration range	1 to 500 ng/mL		
	Tested concentration (ng/mL)		
	3	**250**	**400**
Intra-run precision (%) *Repeatability (run 6)*	5.07	1.30	4.15
Intra-run deviation (%) *Repeatability (run 6)*	-4.12	7.52	8.27
Long term stability at target temperature -20°C (and 16h 32°C)	Long term stability was tested for 17 days at -20°C for 3 LLOQ and 0.8 ULOQ. At 3 LLOQ, the mean intrarun %CV was 2.69% (criteria <20%) and the mean intrarun% deviation was -12.80% (criteria <20%).		

	At 0.8 ULOQ, the mean intrarun %CV was +3.12% (criteria <20%) and a mean intrarun% deviation was +1.92% (criteria <20%). The results show stability of Cloprostenol in the receptor fluid samples on a 17 days period.
Specificity	The method is specific as no interference was observed in the blank matrix. For the specificity in presence of metabolite, the impact of concomitant drug should be evaluated according to the concentrations obtained during assays (see section 7.5 in current report).
Carry over	No carry over was observed. No specific precaution necessary.

CONCLUSION:

The objective of this study was to qualify an analytical method for the analysis of Isopropyl cloprostenate and its metabolite Cloprostenol in washing, receptor fluid, *Stratum Corneum* (tape stripping) and skin samples. This study was done in the context of *in vitro* skin penetration study.

The theoretical Isopropyl cloprostenate concentration was used to perform calculations.

The analytical method using LC-MS/MS and referenced as PKH/MOA/1745 for the quantification of Isopropyl cloprostenate and its metabolite in the different generated samples was qualified in this study and can be used in support of dermal absorption.

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GLOSSARY

BLQ	:	Below the Limit of Quantification
Cmean	:	Mean concentration
Cmeas	:	Measured or back calculated concentration
Cn	:	Nominal concentration
IS	:	Internal standard
LLOQ	:	Lower Limit Of Quantification
MeOH	:	Methanol
MRM	:	Multiple Reaction Monitoring
n	:	Number of determinations or replicates
PBS	:	Phosphate Buffered Saline
QC	:	Quality Control samples
RT	:	Retention Time
S0	:	Blank matrix sample
S0SI	:	Blank matrix spiked with internal standard
SD	:	Standard Deviation
Series	:	Analytical batch or run
SOP	:	Standard Operating Procedure
ULOQ	:	Upper Limit Of Quantification
Deviation %	:	Deviation (%) = $\frac{Cmeas - Cn}{Cn} x100$
Precision%	:	Precision (%) = $\frac{SD}{Cmean} x100$

1. Objective

The objective of this study was to qualify an analytical method for the quantification of Isopropyl cloprostenate and Cloprostenol in washing, receptor fluid, *Stratum Corneum* (tape stripping) and skin samples. This study was done in the context of *in vitro* skin penetration study.

2. Location and regulatory compliance

This study was conducted at Eurofins|ADME BIOANALYSES – 75A, Avenue de Pascalet – 30310 Vergèze – France under the responsibility of [redacted]

The study was performed according to:

- Eurofins|ADME BIOANALYSES Standard Operating Procedures,
- this protocol and its amendment referenced 24-0055A.

Good Laboratory Practices was not required for this study.

3. Modification and deviation of the final protocol

No deviation to protocol 24-0055A were reported. The deviations to 24-0055B protocol were reported in the 24-0055B report.

4. Solubility test of Isopropyl Cloprostenate and Cloprostenol in receptor fluid

For *in vitro* skin absorption studies, the molecule should be adequately soluble in the selected receptor fluid so that it does not act as a barrier to absorption, and the receptor fluid should maintain the skin barrier integrity. Therefore, the solubility of Isopropyl cloprostenate and Cloprostenol in the receptor fluid was demonstrated as not being a rate limiting factor. According to the guideline, ENV/JM/MONO(2004)2, the solubility should be at least 10-fold higher than the maximal concentration expected in the receptor fluid at the end of the *in vitro* study.

For information, the SCCS/1635/21 (§ 3.4 Safety Evaluation – Isopropyl cloprotenate – Applicant #2 provides an estimation of Isopropyl cloprostenate dermal absorption of 10% following the application of a formulation at 0.007%:

Considering the maximal Isopropyl cloprostenate concentration in test item preparation (0.005% w/w) and a projected amount applied of 5 mg/cm², the maximal concentration of Isopropyl cloprostenate and Cloprostenol (100% of absorption in receptor fluid) in receptor fluid (24 mL of receptor fluid, dynamic cells with a flow at 1 mL/h) was estimated to be 10.4 ng/mL. Isopropyl cloprostenate and Cloprostenol solubility should be **at least** 104 ng/mL (10-fold the maximal estimated concentration corresponding to 1000% of dose applied) to confirm that the receptor fluid selected is suitable for the study.

The actual amount applied of formulation in 24-0055B study was 10 mg/cm² corresponding to a maximal concentration of Isopropyl cloprostenate and Cloprostenol (100% of absorption in receptor fluid) in receptor fluid (24 mL of receptor fluid, dynamic cells with a flow at 1 mL/h) estimated to be 20.8 ng/mL. Isopropyl cloprostenate and Cloprostenol solubility should be **at least** 208 ng/mL (10-fold the maximal estimated concentration corresponding to 1000% of dose applied) to confirm that the receptor fluid selected is suitable for the study.

The selection of the receptor fluid was determined by the estimation of the solubility of Isopropyl cloprostenate and Cloprostenol in the following receptor fluids, allowed by the guidelines for moderate solubility in water:

- RF1: PBS 0.01M pH7.4
- RF2: 5% BSA in PBS 0.01 M* pH* 7.4

The solubility test results of Isopropyl cloprostenate are presented below.

Table 3. Isopropyl cloprostenate solubility in receptor fluid

Receptor Fluid	RF1: PBS 0.01M pH7.4 Concentration of Isopropyl Cloprostenate (ng/mL)		Minimum required (µg/mL)
Dilution factor	*2500*	*25 000*	
A	40750.0 50427.5	41677.5 32672.5	**0.104**
B	33920.0 53572.5	45947.5 44732.5	
Mean concentration ng/mL	**44667.500**	**41257.500**	
SD	**9006**	**5999**	
CV (%)	**20.16**	**14.54**	
Mean Concentration (µg/mL)	**44.668**	**41.258**	

Receptor Fluid	RF2: 5% BSA in PBS 0.01M pH 7.4 Concentration of Isopropyl Cloprostenate (ng/mL)		Minimum required (µg/mL)
Dilution factor	*2500*	*25 000*	
A	670750 659475	764775 811850	**0.104**
B	726150 706800	783400 862850	
Mean concentration ng/mL	**690794**	**805719**	
SD	**31032**	**42724**	
CV (%)	**4.49**	**5.30**	
Mean Concentration (µg/mL)	**690.794**	**805.719**	

Table 4. Cloprostenol solubility in receptor fluid

Receptor Fluid	RF1: RF1: PBS 0.01M pH7.4 Concentration of Cloprostenol (ng/mL)		Minimum required (µg/mL)
Dilution factor	*2500*	*25 000*	
A	1015775 1043925	1016975 989400	**0.104**
B	1055850 1044350	1100000 1033800	
Mean concentration ng/mL	**1039975**	**1035044**	
SD	**17053**	**47013**	
CV (%)	**1.64**	**4.54**	
Mean Concentration (µg/mL)	**1039.975**	**1035.044**	

Receptor Fluid	RF2: 5% BSA in PBS 0.01M pH 7.4 Concentration of Cloprostenol (ng/mL)		Minimum required (µg/mL)
Dilution factor	*2500*	*25 000*	
A	835200 929725	821075 890350	**0.104**
B	964525 1053075	949650 1026725	
Mean concentration ng/mL	**945631**	**921950**	
SD	**90091**	**87406**	
CV (%)	**9.53**	**9.48**	
Mean Concentration (µg/mL)	**945.631**	**921.950**	

Table 5. Filtration results for Isopropyl cloprostenate for both receptor fluids

	Peak Area of Isopropyl Cloprostenate for QC at 500 ng/mL in RF1	
	Before filtration	After filtration
injection 1	19698.7	2587.4
injection 2	23356.0	3054.0
injection 3	13973.8	2241.8
Mean	**19009.5**	**2627.7**
Difference (%)	*-86.18*	

	Peak Area of Cloprostenol for QC at 500 ng/mL in RF1	
	Before filtration	After filtration
injection 1	7006701.9	6555862.9
injection 2	5832953.8	6927928.7
injection 3	6272042.5	6597990.5
Mean	**6370566.1**	**6693927.4**
Difference (%)	*5.08*	

Table 6. Filtration results for Cloprostenol for both receptor fluids

	Peak Area of Isopropyl Cloprostenate for QC at 500 ng/mL in RF2	
	Before filtration	After filtration
injection 1	351974.6	365026
injection 2	312158.3	384031.6
injection 3	339474.0	327079.0
Mean	**334535.6**	**358712.2**
Difference (%)	7.23	

	Peak Area of Cloprostenol for QC at 500 ng/mL in RF2	
	Before filtration	After filtration
injection 1	8205386.1	8714319.5
injection 2	7103815.3	9014578.2
injection 3	7037363.5	8218755.1
Mean	**7448855.0**	**8649217.6**
Difference (%)	16.11	

Comments: The results of the solubility test were in the acceptance criteria for Isopropyl Cloprostenate and Cloprostenol for both receptor fluids. The absorption to the filter was analysed concomitantly to the solubility test. For Isopropyl Cloprostenate, absorption at the filter was observed following the filtration experiment for receptor fluid RF1. However, the Isopropyl cloprostenate measured concentrations during the solubility test corresponded to the targeted concentration. The measured concentrations during the solubility test was not impacted by the absorption to the filter. Besides, no filtration is performed for the study samples. Consequently, the absorption to the filter observed in the experiment of filtration has no impact on the receptor fluid selection and has no impact on the analysis of the study samples.
For Cloprostenol, no absorption at the filter was observed following both filtration experiment that were performed concomitantly to the solubility test.

Isopropyl cloprostenate had solubility higher than required threshold (104 ng/mL or 208 ng/mL) for both receptor fluids. Therefore, the most physiological receptor fluid was preferred (RF1: PBS 0.01M pH 7.4).

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5. Tests and acceptance criteria

Tests were performed in accordance with the tests described in the protocol.

All the tests and their acceptance criteria are detailed in the protocol (APPENDIX B).

6. Summary of the analytical runs

The summary of the analytical runs is presented in Table 80.

7. Qualification results

7.1. Selectivity

The selectivity of the method was tested by the analysis for the skin matrix from 1 donor (n=1) and 1 replicate for receptor fluid, washing and *stratum corneum* matrices.

Table 7. Isopropyl cloprostenate selectivity for washing

Run identification	Area Interference	Area ISOPROPYL CLOPROSTENATE	% Interference
G260424-W-ISOCLO-Select	2135	17669	12.08

Table 8. Irbesartan selectivity for washing

Run identification	Area Interference	Area IRBESARTAN	% Interference
G260424-W-ISOCLO-Select	17476	8406610	0.21

Table 9. Isopropyl cloprostenate selectivity for skin

Run identification	Batches	Area Interference	Area ISOPROPYL CLOPROSTENATE	% Interference
G170524-ISOCLO-S	1	140	11131	1.26

Table 10. Isopropyl cloprostenate selectivity for strips

Run identification	Batches	Area Interference	Area ISOPROPYL CLOPROSTENATE	% Interference
G210524-Strip-Select	1	196	16682	1.17

Table 11. Isopropyl cloprostenate selectivity for receptor fluid

Run identification	Batches	Area Interference	Area ISOPROPYL CLOPROSTENATE	% Interference
G170524-RF-ISOCLO-Select	RP040-170524	110	5852	1.87

Table 12. Cloprostenol selectivity for washing

Run identification	Area Interference	Area CLOPROSTENOL	% Interference
G260424-W-CLO-Select	1735	49428	3.51

Table 13. Prostaglandin F2Alpha-d4 selectivity for washing

Run identification	Area Interference	Area PTGF2alpha-d4	% Interference
G260424-W-CLO-Select	2220	4269267	0.05

Table 14. Cloprostenol selectivity for skin

Run identification	Batches	Area Interference	Area CLOPROSTENOL	% Interference
G170524-CLO-S	1	12364	155513	7.95

Table 15. Prostaglandin F2Alpha-d4 selectivity for skin

Run identification	Batches	Area Interference	Area PTGF2alpha-d4	% Interference
G170524-CLO-S	1	27220	4534935	0.60

Table 16. Cloprostenol selectivity for strips

Run identification	Batches	Area Interference	Area CLOPROSTENOL	% Interference
G210524-Strip-Select	1	63	3760	1.67

Table 17. Prostaglandin F2Alpha-d4 selectivity for strips

Run identification	Batches	Area Interference	Area PTGF2ALPHA-D4	% Interference
G210524-Strip-Select	1	3954	5115086	0.08

Table 18. Cloprostenol selectivity for receptor fluid

Run identification	Batches	Area Interference	Area CLOPROSTENOL	% Interference
G170524-RF-CLO-Select	RP040-170524	4846	94661	5.12

Table 19. Prostaglandin F2Alpha-d4 selectivity for receptor fluid

Run identification	Batches	Area Interference	Area PTGF2alpha-d4	% Interference
G170524-RF-CLO-Select	RP040-170524	1234	4247040	0.03

The interference results were within the acceptance criteria.

Method selectivity regarding Isopropyl cloprostenate and its metabolite signal was demonstrated in all matrices as no interference greater than 20% for Isopropyl cloprostenate and its metabolite was observed in any of the tested blank matrix.

Method selectivity regarding Irbesartan signal was demonstrated in strips, receptor fluid and washing as no interference greater than 5% for internal standard was observed in any of the tested blank matrix.

7.2. Concentration-response relationship

The concentration-response relationship was determined from the calibration standards at concentrations ranging from the LLOQ up to the ULOQ on one run.

All the runs used for the study are presented.

Table 20. Isopropyl cloprostenate Curve parameters and Concentration-response relationship (ng/mL) for washing

Run Date	Curve Number	Slope	Intercept	R-Squared
26-Apr-2024	2	0.001542	0.0005702	0.9865

Run Date	Curve Number	S0.5/1 1.0000 ng/mL	%Deviation	S1/2 2.0000 ng/mL	%Deviation	S2.5/5 5.0000 ng/mL	%Deviation	S5/10 10.000 ng/mL	%Deviation	S10/20 20.000 ng/mL	%Deviation	S25/50 50.000 ng/mL	%Deviation	S45/90 90.000 ng/mL	%Deviation	S50/100 100.00 ng/mL	%Deviation
26-Apr-2024	2	1.0924	9.24	2.2847	14.24	4.5352	-9.30	10.203	2.03	20.650	3.25	51.090	2.18	86.807	-3.55	87.120	-12.88
		0.85637	-14.36	2.0785	3.93	4.0269	-19.46	10.930	9.30	21.913	9.57	54.451	8.90	89.038	-1.07	97.990	-2.01

Run Date	Curve Number	Slope	Intercept	R-Squared
24-May-2024	22	0.006318	-0.0001117	0.9738

Run Date	Curve Number	S0.5/1 1.0000 ng/mL	%Deviation	S1/2 2.0000 ng/mL	%Deviation	S2.5/5 5.0000 ng/mL	%Deviation	S5/10 10.000 ng/mL	%Deviation	S10/20 20.000 ng/mL	%Deviation	S25/50 50.000 ng/mL	%Deviation	S45/90 90.000 ng/mL	%Deviation	S50/100 100.00 ng/mL	%Deviation
24-May-2024	22	*0.083043		1.6900	-15.50	4.1949	-16.10	8.1157	-18.84	22.855	14.28	45.768	-8.46	81.997	-8.89	88.159	-11.84
		*0.076396		2.3322	16.61	5.7269	14.54	10.932	9.32	**28.410		56.356	12.71	94.879	5.42	106.76	6.76

Reasons Deactivated

* Chromatographic profile not acceptable

** Out of acceptance criteria

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Table 21. Isopropyl cloprostenate Curve parameters and Concentration-response relationship (ng/mL) for skin

Run Date	Curve Number	Slope	Intercept	R-Squared
17-May-2024	16	140500	4312	0.9953

Run Date	Curve Number	S1/0.05 0.050000 ng/mL	%Deviation	S2/0.1 0.10000 ng/mL	%Deviation	S10/0.5 0.50000 ng/mL	%Deviation	S50/2.5 2.5000 ng/mL	%Deviation	S100/5 5.0000 ng/mL	%Deviation	S250/12.5 12.500 ng/mL	%Deviation	S450/22.5 22.500 ng/mL	%Deviation	S500/25 25.000 ng/mL	%Deviation
17-May-2024	16	0.048521	-2.96	0.10836	8.36	0.49293	-1.41	2.4249	-3.00	4.9533	-0.93	12.239	-2.09	23.142	2.85	25.489	1.96
		0.053519	7.04	0.086116	-13.88	0.43892	-12.22	2.5806	3.22	5.1123	2.25	12.770	2.16	23.347	3.76	26.225	4.90

Run Date	Curve Number	Slope	Intercept	R-Squared
03-Jun-2024	28	51910	-867.3	0.9867

Run Date	Curve Number	S1/0.05 0.050000 ng/mL	%Deviation	S2/0.1 0.10000 ng/mL	%Deviation	S10/0.5 0.50000 ng/mL	%Deviation	S50/2.5 2.5000 ng/mL	%Deviation	S100/5 5.0000 ng/mL	%Deviation	S250/12.5 12.500 ng/mL	%Deviation	S450/22.5 22.500 ng/mL	%Deviation	S500/25 25.000 ng/mL	%Deviation
03-Jun-2024	28	*0.10562		0.080838	-19.16	0.52462	4.92	2.4374	-2.50	5.0199	0.40	14.316	14.53	24.257	7.81	27.848	11.39
		0.059334	18.67	0.082419	-17.58	0.46255	-7.49	2.4644	-1.42	4.7453	-5.09	12.326	-1.39	22.718	0.97	23.991	-4.04

Reason Deactivated

* Out of acceptance criteria

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Table 22. Isopropyl cloprostenate Curve parameters and Concentration-response relationship (ng/mL) for strips

Run Date	Curve Number	Slope	Intercept	R-Squared
21-May-2024	20	119000	1937	0.9976
11-Jul-2024	43	57360	1883	0.9948
17-Jul-2024	55	118000	2529	0.9754

Run Date	Curve Number	S0.25/0.1 0.10000 ng/mL	%Deviation	S0.5/0.2 0.20000 ng/mL	%Deviation	S5/2 2.0000 ng/mL	%Deviation	S25/10 10.000 ng/mL	%Deviation	S50/20 20.000 ng/mL	%Deviation	S62.5/25 25.000 ng/mL	%Deviation	S112.5/45 45.000 ng/mL	%Deviation	S125/50 50.000 ng/mL	%Deviation
21-May-2024	20	0.10523	5.23	0.19651	-1.75	2.0676	3.38	9.9719	-0.28	22.027	10.14	25.461	1.84	42.628	-5.27	49.006	-1.99
		0.096760	-3.24	0.19495	-2.53	1.9719	-1.41	9.9434	-0.57	21.182	5.91	24.890	-0.44	41.936	-6.81	48.881	-2.24
11-Jul-2024	43	0.10982	9.82	0.17989	-10.06	2.1335	6.68	10.534	5.34	20.303	1.52	24.935	-0.26	44.850	-0.33	48.197	-3.61
		0.10110	1.10	0.17286	-13.57	2.1947	9.74	10.345	3.45	20.111	0.56	24.836	-0.66	43.546	-3.23	46.755	-6.49
17-Jul-2024	55	0.092883	-7.12	*0.14659		1.7926	-10.37	9.7430	-2.57	16.492	-17.54	21.667	-13.33	37.923	-15.73	42.360	-15.28
		*0.14471		0.22752	13.76	2.3329	16.65	*13.121		23.298	16.49	*30.629		52.035	15.63	59.705	19.41

Reason Deactivated

* Out of acceptance criteria

Table 23. Isopropyl cloprostenate Curve parameters and Concentration-response relationship (ng/mL) for receptor fluid

Run Date	Curve Number	Slope	Intercept	R-Squared
26-Apr-2024	4	0.002904	0.001410	0.9924
17-May-2024	18	4361	-1437	0.9915

Run Date	Curve Number	S1 1.0000 ng/mL	%Deviation	S2 2.0000 ng/mL	%Deviation	S10 10.000 ng/mL	%Deviation	S50 50.000 ng/mL	%Deviation	S100 100.00 ng/mL	%Deviation	S250 250.00 ng/mL	%Deviation	S450 450.00 ng/mL	%Deviation	S500 500.00 ng/mL	%Deviation
26-Apr-2024	4	0.96632	-3.37	2.0679	3.40	10.451	4.51	52.031	4.06	106.04	6.04	236.80	-5.28	421.67	-6.30	427.59	-14.48
						11.143	11.43							*258.23			
17-May-2024	18	0.99244	-0.76	1.9529	-2.36	*7.2694		*37.351		92.528	-7.47	279.38	11.75	491.92	9.32	462.56	-7.49
		1.0515	5.15	1.9091	-4.55	9.0346	-9.65	*37.124		100.63	0.63	273.62	9.45	485.83	7.96	439.99	-12.00

Reason Deactivated

* Out of acceptance criteria

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Table 24. Cloprostenol Curve parameters and Concentration-response relationship (ng/mL) for washing

Run Date	Curve Number	Slope	Intercept	R-Squared
26-Apr-2024	1	0.02081	0.001251	0.9855

Run Date	Curve Number	S0.5/1 0.50000 ng/mL	%Deviation	S1/2 1.0000 ng/mL	%Deviation	S2.5/5 2.5000 ng/mL	%Deviation	S5/10 5.0000 ng/mL	%Deviation	S10/20 10.000 ng/mL	%Deviation	S25/50 25.000 ng/mL	%Deviation	S45/90 45.000 ng/mL	%Deviation	S50/100 50.000 ng/mL	%Deviation
26-Apr-2024	1	0.56681	13.36	1.0045	0.45	*1.9376		5.3620	7.24	11.125	11.25	25.113	0.45	48.799	8.44	43.131	-13.74
		0.46164	-7.67	0.85220	-14.78	*1.8597		4.9310	-1.38	10.868	8.68	26.232	4.93	46.163	2.58	40.093	-19.81

Reason Deactivated

* Out of acceptance criteria

Run Date	Curve Number	Slope	Intercept	R-Squared
24-May-2024	21	0.01882	0.0001333	0.9951

Run Date	Curve Number	S0.5/1 0.50000 ng/mL	%Deviation	S1/2 1.0000 ng/mL	%Deviation	S2.5/5 2.5000 ng/mL	%Deviation	S5/10 5.0000 ng/mL	%Deviation	S10/20 10.000 ng/mL	%Deviation	S25/50 25.000 ng/mL	%Deviation	S45/90 45.000 ng/mL	%Deviation	S50/100 50.000 ng/mL	%Deviation
24-May-2024	21	*-0.0060199		0.98008	-1.99	2.4673	-1.31	4.4864	-10.27	**12.539		26.555	6.22	43.922	-2.40	48.232	-3.54
		*-0.0038417		1.0380	3.80	2.5999	4.00	4.6462	-7.08	**12.989		27.563	10.25	45.585	1.30	50.506	1.01

Reasons Deactivated

* Chromatography not acceptable

** Out of acceptance criteria

Run 21: Validated in 24-0055B study

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Table 25. Cloprostenol Curve parameters and Concentration-response relationship (ng/mL) for skin

Run Date	Curve Number	Slope	Intercept	R-Squared
17-May-2024	15	0.03447	0.001068	0.9977

Run Date	Curve Number	S1/0.05 1.0000 ng/mL	%Deviation	S2/0.1 2.0000 ng/mL	%Deviation	S10/0.5 10.000 ng/mL	%Deviation	S50/2.5 50.000 ng/mL	%Deviation	S100/5 100.00 ng/mL	%Deviation	S250/12.5 250.00 ng/mL	%Deviation	S450/22.5 450.00 ng/mL	%Deviation	S500/25 500.00 ng/mL	%Deviation
17-May-2024	15	0.96388	-3.61	1.7854	-10.73	9.6660	-3.34	50.094	0.19	95.731	-4.27	253.74	1.50	454.21	0.94	488.27	-2.35
		1.0714	7.14	2.0905	4.53	9.8955	-1.05	50.342	0.68	100.92	0.92	258.09	3.24	458.69	1.93	521.44	4.29

Run Date	Curve Number	Slope	Intercept	R-Squared
03-Jun-2024	27	0.04389	0.0009458	0.9949

Run Date	Curve Number	S1/0.05 1.0000 ng/mL	%Deviation	S2/0.1 2.0000 ng/mL	%Deviation	S10/0.5 10.000 ng/mL	%Deviation	S50/2.5 50.000 ng/mL	%Deviation	S100/5 100.00 ng/mL	%Deviation	S250/12.5 250.00 ng/mL	%Deviation	S450/22.5 450.00 ng/mL	%Deviation	S500/25 500.00 ng/mL	%Deviation
03-Jun-2024	27	1.0556	5.56	2.0858	4.29	10.977	9.77	54.463	8.93	109.69	9.69	239.53	-4.19	455.53	1.23	519.97	3.99
		0.94764	-5.24	1.8696	-6.52	9.7533	-2.47	46.361	-7.28	99.703	-0.30	228.10	-8.76	424.01	-5.78	485.35	-2.93

Table 26. Cloprostenol Curve parameters and Concentration-response relationship (ng/mL) for strips

Run Date	Curve Number	Slope	Intercept	R-Squared
21-May-2024	19	0.003830	0.0007753	0.9912
11-Jul-2024	42	0.03296	-0.0003100	0.9978
17-Jul-2024	54	0.02270	-0.000007062	0.9971

Run Date	Curve Number	S0.25/0.1 0.25000 ng/mL	%Deviation	S0.5/0.2 0.50000 ng/mL	%Deviation	S5/2 5.0000 ng/mL	%Deviation	S25/10 25.000 ng/mL	%Deviation	S50/20 50.000 ng/mL	%Deviation	S62.5/25 62.500 ng/mL	%Deviation	S112.5/45 112.50 ng/mL	%Deviation	S125/50 125.00 ng/mL	%Deviation
21-May-2024	19	0.29314	17.26	*0.34876		4.9350	-1.30	26.081	4.32	55.147	10.29	67.631	8.21	116.64	3.68	132.47	5.98
		0.20776	-16.90	*0.37774		4.6462	-7.08	24.334	-2.66	48.654	-2.69	59.662	-4.54	100.93	-10.28	119.65	-4.28
11-Jul-2024	42	0.23881	-4.48	0.48110	-3.78	4.8971	-2.06	23.541	-5.84	48.271	-3.46	62.032	-0.75	116.02	3.13	117.60	-5.92
		0.25798	3.19	0.52999	6.00	5.3340	6.68	24.983	-0.07	50.248	0.50	65.768	5.23	116.34	3.41	122.77	-1.78
17-Jul-2024	54	0.25882	3.53	0.54795	9.59	4.7574	-4.85	24.706	-1.18	50.671	1.34	66.663	6.66	114.12	1.44	126.98	1.58
		0.23301	-6.80	0.48716	-2.57	5.0183	0.37	24.833	-0.67	45.141	-9.72	63.834	2.13	108.98	-3.13	127.82	2.26

Reason Deactivated

* Out of acceptance criteria

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Table 27. Cloprostenol Curve parameters and Concentration-response relationship (ng/mL) for receptor fluid

Run Date	Curve Number	Slope	Intercept	R-Squared
26-Apr-2024	3	0.01792	-0.00002388	0.9979
17-May-2024	17	0.02077	0.001146	0.9995

Run Date	Curve Number	S1 1.0000 ng/mL	%Deviation	S2 2.0000 ng/mL	%Deviation	S10 10.000 ng/mL	%Deviation	S50 50.000 ng/mL	%Deviation	S100 100.00 ng/mL	%Deviation	S250 250.00 ng/mL	%Deviation	S450 450.00 ng/mL	%Deviation	S500 500.00 ng/mL	%Deviation
26-Apr-2024	3	1.0233	2.33	1.9412	-2.94	9.5486	-4.51	49.833	-0.33	101.37	1.37	240.75	-3.70	465.24	3.39	544.80	8.96
						9.5766	-4.23							448.54	-0.32		
17-May-2024	17	0.99266	-0.73	2.0603	3.02	9.8719	-1.28	51.844	3.69	100.19	0.19	247.52	-0.99	443.44	-1.46	489.21	-2.16
		0.98592	-1.41	2.0272	1.36	9.9768	-0.23	50.799	1.60	101.03	1.03	256.56	2.62	435.21	-3.29	490.22	-1.96

Reason Deactivated

* Out of acceptance criteria

The best fit for the calibration curve could be achieved using the linear equation y = *a*x + *b* with a $1/x^2$ weighting factor over the range of:

Isopropyl cloprostenate:

- 1 to 100 ng/mL for Washing
- 0.05 to 25 ng/mL for Skin
- 0.1 to 50 ng/mL for Strip
- 1 to 500 ng/mL for Receptor fluid

Cloprostenol:

- 0.5 to 50 ng/mL for Washing
- 1 to 500 ng/mL for Skin
- 0.25 to 125 ng/mL for Strip
- 1 to 500 ng/mL for Receptor fluid

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7.3. Intra-run precision and deviation (repeatability)

The intra-run precision and deviation of the assay method were tested for each concentration levels.

QC samples were prepared in each matrix n=6 within the same run at 3LLOQ, 0.5ULOQ and 0.8ULOQ concentration levels.

Table 28. Isopropyl cloprostenate intra-run (repeatability) precision and deviation of the method for washing

Run Date	Curve Number	L 3.0000 ng/mL	%Deviation	M 50.000 ng/mL	%Deviation	H 80.000 ng/mL	%Deviation
26-Apr-2024	2	2.5538	-14.87	~39.531	-20.94	80.947	1.18
		2.4617	-17.94	46.951	-6.10	68.951	-13.81
		~2.2056	-26.48	50.374	0.75	73.058	-8.68
		~2.1745	-27.52	49.167	-1.67	77.258	-3.43
		2.5343	-15.52	44.585	-10.83	68.062	-14.92
		2.7827	-7.24	48.623	-2.75	80.253	0.32
Intrarun Mean		2.4521		46.539		74.755	
Intrarun SD		0.22992		3.9763		5.5892	
Intrarun %CV		9.38		8.54		7.48	
Intrarun %Deviation		-18.26		-6.92		-6.56	
n		6		6		6	

~ > 20%Deviation

Table 29. Isopropyl cloprostenate intra-run (repeatability) precision and deviation of the method for skin

Run Date	Curve Number	L 0.15000 ng/mL	%Deviation	M 12.500 ng/mL	%Deviation	H 20.000 ng/mL	%Deviation
17-May-2024	16	0.14069	-6.21	12.348	-1.22	20.789	3.95
		0.13611	-9.26	11.018	-11.86	20.096	0.48
		0.12965	-13.57	10.942	-12.46	20.528	2.64
		0.12977	-13.49	12.088	-3.30	18.407	-7.97
		0.16348	8.99	13.347	6.78	20.638	3.19
		0.15845	5.63	12.483	-0.14	19.289	-3.56
Intrarun Mean		0.14303		12.038		19.958	
Intrarun SD		0.014589		0.92214		0.93275	
Intrarun %CV		10.20		7.66		4.67	
Intrarun %Deviation		-4.65		-3.70		-0.21	
n		6		6		6	

Table 30. Isopropyl cloprostenate intra-run (repeatability) precision and deviation of the method for strips

Run Date	Curve Number	L 0.30000 ng/mL	%Deviation	M 25.000 ng/mL	%Deviation	H 40.000 ng/mL	%Deviation
21-May-2024	20	0.34739	15.80	29.087	16.35	43.036	7.59
		0.32503	8.34	28.374	13.50	43.182	7.96
		0.34165	13.88	28.000	12.00	44.840	12.10
		0.35875	19.58	27.438	9.75	41.609	4.02
		0.35044	16.81	27.823	11.29	41.843	4.61
		0.32776	9.25	28.859	15.44	41.298	3.25
Intrarun Mean		0.34184		28.264		42.635	
Intrarun SD		0.013203		0.63086		1.3259	
Intrarun %CV		3.86		2.23		3.11	
Intrarun %Deviation		13.95		13.06		6.59	
n		6		6		6	

Table 31. Isopropyl cloprostenate intra-run (repeatability) precision and deviation of the method for receptor fluid

Run Date	Curve Number	L 3.0000 ng/mL	%Deviation	M 250.00 ng/mL	%Deviation	H 400.00 ng/mL	%Deviation
17-May-2024	18	~2.3928	-20.24	225.95	-9.62	419.54	4.89
		3.2766	9.22	~335.75	34.30	463.26	15.82
		2.8620	-4.60	269.17	7.67	385.96	-3.51
		3.1777	5.92	222.24	-11.10	397.46	-0.64
		3.0042	0.14	209.50	-16.20	407.98	2.00
		2.8258	-5.81	253.41	1.36	437.40	9.35
Intrarun Mean		2.9232		252.67		418.60	
Intrarun SD		0.31330		46.214		28.192	
Intrarun %CV		10.72		18.29		6.73	
Intrarun %Deviation		-2.56		1.07		4.65	
n		6		6		6	

~ > 20%Deviation

Table 32. Cloprostenol intra-run (repeatability) precision and deviation of the method for washing

Run Date	Curve Number	L 1.5000 ng/mL	%Deviation	M 25.000 ng/mL	%Deviation	H 40.000 ng/mL	%Deviation
26-Apr-2024	1	1.5620	4.13	22.677	-9.29	39.152	-2.12
		1.4784	-1.44	22.076	-11.70	38.716	-3.21
		1.3185	-12.10	26.084	4.34	39.169	-2.08
		1.3866	-7.56	28.177	12.71	38.223	-4.44
		1.5936	6.24	24.043	-3.83	32.381	-19.05
		1.5160	1.07	26.947	7.79	40.182	0.46
Intrarun Mean		1.4759		25.001		37.971	
Intrarun SD		0.10548		2.4463		2.8137	
Intrarun %CV		7.15		9.78		7.41	
Intrarun %Deviation		-1.61		0.00		-5.07	
n		6		6		6	

Table 33. Cloprostenol intra-run (repeatability) precision and deviation of the method for skin

Run Date	Curve Number	L 3.0000 ng/mL	%Deviation	M 250.00 ng/mL	%Deviation	H 400.00 ng/mL	%Deviation
17-May-2024	15	3.0833	2.78	265.68	6.27	413.45	3.36
		3.0828	2.76	273.41	9.36	420.72	5.18
		3.0283	0.94	260.10	4.04	417.25	4.31
		3.1331	4.44	262.91	5.16	423.83	5.96
		3.0752	2.51	260.36	4.14	427.00	6.75
		3.1518	5.06	268.63	7.45	437.70	9.43
Intrarun Mean		3.0924		265.18		423.33	
Intrarun SD		0.044192		5.1784		8.5042	
Intrarun %CV		1.43		1.95		2.01	
Intrarun %Deviation		3.08		6.07		5.83	
n		6		6		6	

Table 34. Cloprostenol intra-run (repeatability) precision and deviation of the method for strips

Run Date	Curve Number	L 0.75000 ng/mL	%Deviation	M 62.500 ng/mL	%Deviation	H 100.00 ng/mL	%Deviation
21-May-2024	19	~0.57252	-23.66	65.119	4.19	98.928	-1.07
		0.64171	-14.44	64.148	2.64	96.252	-3.75
		0.67461	-10.05	64.873	3.80	97.074	-2.93
		0.70751	-5.67	64.371	2.99	102.13	2.13
		0.64041	-14.61	64.716	3.55	105.17	5.17
		0.65268	-12.98	65.630	5.01	101.79	1.79
Intrarun Mean		0.64824		64.810		100.22	
Intrarun SD		0.044868		0.53135		3.4037	
Intrarun %CV		6.92		0.82		3.40	
Intrarun %Deviation		-13.57		3.70		0.22	
n		6		6		6	

Table 35. Cloprostenol intra-run (repeatability) precision and deviation of the method for receptor fluid

Run Date	Curve Number	L 3.0000 ng/mL	%Deviation	M 250.00 ng/mL	%Deviation	H 400.00 ng/mL	%Deviation
07-May-2024	9	2.6715	-10.95	264.02	5.61	461.03	15.26
		2.7594	-8.02	270.36	8.14	417.72	4.43
		3.0657	2.19	267.04	6.82	418.75	4.69
		2.8482	-5.06	272.48	8.99	418.03	4.51
		2.9792	-0.69	266.41	6.56	437.69	9.42
		2.9337	-2.21	272.40	8.96	445.15	11.29
Intrarun Mean		2.8763		268.79		433.06	
Intrarun SD		0.14571		3.4812		17.977	
Intrarun %CV		5.07		1.30		4.15	
Intrarun %Deviation		-4.12		7.52		8.27	
n		6		6		6	

Intra-run accuracy and precision were within the acceptance criteria specified in the protocol. The repeatability of the analytical method was demonstrated.

7.4. Specificity: contribution of internal standard

The SI contribution was assessed with the study of the S0SI injected during repeatability tests.

Table 36. Contribution of IS on Isopropyl cloprostenate and vice-versa for washing

At retention time of Isopropyl Cloprostenate:

Run identification	Area of ISOPROPYL CLOPROSTENATE peak in S0SI samples	Area of ISOPROPYL CLOPROSTENATE peak in samples spiked with ISOPROPYL CLOPROSTENATE at LLOQ	% Interference
G260424-W-ISOCLO	331	3259	10.16

Table 37. Contribution of IS on Cloprostenol and vice-versa for washing

At retention time of Cloprostenol:

Run identification	Area of CLOPROSTENOL peak in S0SI samples	Area of CLOPROSTENOL peak in samples spiked with CLOPROSTENOL at LLOQ	% Interference
G260424-W-CLO	5871	66485	8.83

Table 38. Contribution of IS on Cloprostenol and vice-versa for skin

At retention time of Cloprostenol:

Run identification	Area of CLOPROSTENOL peak in S0SI samples	Area of CLOPROSTENOL peak in samples spiked with CLOPROSTENOL at LLOQ	% Interference
G170524-CLO-S	867	155513	0.56

Table 39. Contribution of IS on Cloprostenol and vice-versa for strip

At retention time of Cloprostenol:

Run identification	Area of CLOPROSTENOL peak in S0SI samples	Area of CLOPROSTENOL peak in samples spiked with CLOPROSTENOL at LLOQ	% Interference
G210524-CLO-ST	1522	11263	13.51

Table 40. Contribution of IS on Cloprostenol and vice-versa for receptor fluid

At retention time of Cloprostenol:

Run identification	Area of CLOPROSTENOL peak in S0SI samples	Area of CLOPROSTENOL peak in samples spiked with CLOPROSTENOL at LLOQ	% Interference
G170524-RF-CLO	2319	89550	2.59

In matrix samples spiked with IS Isopropyl Cloprostenate only, no interference above 20.00% was detected at Isopropyl Cloprostenate nor Cloprostenol retention times in the MRM trace specific for Isopropyl Cloprostenate nor Cloprostenol in each S0SI samples (≤20%). The method in these matrices is thus specific for Isopropyl Cloprostenate in presence of its internal standard.

7.5. Specificity in presence of a metabolite

The specificity of the method for the test substance Isopropyl Cloprostenate and Cloprostenol was assessed by the study of the peak area obtained at the retention time of analyte in the MRM traces specific for analyte in different conditions:

Table 41. Specificity of the analytical method for Isopropyl Cloprostenate in the presence of metabolite for washing

At retention time of Isopropyl Cloprostenate:

Run identification	Area of ISOPROPYL CLOPROSTENATE peak in samples spiked with CLOPROSTENOL at ULOQ without IS	Area of ISOPROPYL CLOPROSTENATE peak in samples spiked with ISOPROPYL CLOPROSTENATE at LLOQ without IS	% Interference
G260424-W-ISOCLO-Contri	4284	11822	36.24
G260424-W-ISOCLO-Contri	2207	13120	16.82
G260424-W-ISOCLO-Contri	4880	13669	35.70
Mean	3790	12871	29.45

Table 42. Specificity of the analytical method for Isopropyl Cloprostenate in the presence of metabolite for skin

At retention time of Isopropyl Cloprostenate:

Run identification	Area of ISOPROPYL CLOPROSTENATE peak in samples spiked with CLOPROSTENOL at ULOQ without IS	Area of ISOPROPYL CLOPROSTENATE peak in samples spiked with ISOPROPYL CLOPROSTENATE at LLOQ without IS	% Interference
G170524-S-ISOCLO	167	4593	3.64
G170524-S-ISOCLO	62	5929	1.04
G170524-S-ISOCLO	553	3234	17.09
Mean	261	4585	5.68

Table 43. Specificity of the analytical method for Isopropyl Cloprostenate in the presence of metabolite for strips

At retention time of Isopropyl Cloprostenate:

Run identification	Area of ISOPROPYL CLOPROSTENATE peak in samples spiked with ClOPROSTENOL at ULOQ without IS	Area of ISOPROPYL CLOPROSTENATE peak in samples spiked with ISOPROPYL CLOPROSTENATE at LLOQ without IS	% Interference
G210524-Strip-Contri-ISOCLO	332	7553	4.39
G210524-Strip-Contri-ISOCLO	383	8072	4.74
G210524-Strip-Contri-ISOCLO	333	7933	4.20
Mean	349	7853	4.45

Table 44. Specificity of the analytical method for Isopropyl Cloprostenate in the presence of metabolite for receptor fluid

At retention time of Isopropyl Cloprostenate:

Run identification	Area of ISOPROPYL CLOPROSTENATE peak in samples spiked with CLOPROSTENOL at ULOQ without IS	Area of ISOPROPYL CLOPROSTENATE peak in samples spiked with ISOPROPYL CLOPROSTENATE at LLOQ without IS	% Interference
G170524-RF-ISOCLO	165	3717	4.43
G170524-RF-ISOCLO	557	3988	13.96
G170524-RF-ISOCLO	97	3837	2.54
Mean	273	3847	7.10

Table 45. Specificity of the analytical method for Cloprostenol in the presence of metabolite for washing

At retention time of Cloprostenol:

Run identification	Area of CLOPROSTENOL peak in samples spiked with ISOPROPYL CLOPROSTENATE at ULOQ without IS	Area of CLOPROSTENOL peak in samples spiked with CLOPROSTENOL at LLOQ without IS	% Interference
G260424-W-CLO	40604	44256	91.75
G260424-W-CLO	34135	43746	78.03
G260424-W-CLO	37256	44074	84.53
Mean	37332	44025	84.80

Table 46. Specificity of the analytical method for Cloprostenol in the presence of metabolite for skin

At retention time of Cloprostenol:

Run identification	Area of CLOPROSTENOL peak in samples spiked with ISOPROPYL CLOPROSTENATE at ULOQ without IS	Area of CLOPROSTENOL peak in samples spiked with CLOPROSTENOL at LLOQ without IS	% Interference
G170524-S-CLO-Contri	3619	178195	2.03
G170524-S-CLO-Contri	27563	157186	17.54
G170524-S-CLO-Contri	23789	223829	10.63
Mean	18324	186403	9.83

Table 47. Specificity of the analytical method for Cloprostenol in the presence of metabolite for strips

At retention time of Cloprostenol:

Run identification	Area of CLOPROSTENOL peak in samples spiked with ISOPROPYL CLOPROSTENATE at ULOQ without IS	Area of CLOPROSTENOL peak in samples spiked with CLOPROSTENOL at LLOQ without IS	% Interference
G210524-Strip-Contri-CLO	5133	4953	103.63
G210524-Strip-Contri-CLO	3932	4778	82.28
G210524-Strip-Contri-CLO	4475	4695	95.32
Mean	4513	4809	93.85

Table 48. Specificity of the analytical method for Cloprostenol in the presence of metabolite for receptor fluid

At retention time of Cloprostenol:

Run identification	Area of CLOPROSTENOL peak in samples spiked with ISOPROPYL CLOPROSTENATE at ULOQ without IS	Area of CLOPROSTENOL peak in samples spiked with CLOPROSTENOL at LLOQ without IS	% Interference
G170524-RF-CLO-Contri	12953	50672	25.56
G170524-RF-CLO-Contri	13562	51682	26.24
G170524-RF-CLO-Contri	14153	44694	31.67
Mean	13556	49016	27.66

The contribution of Isopropyl Cloprostenate was within predefined acceptance criteria (<20.00%) for skin matrix, receptor fluid and strip matrix. The contribution of Isopropyl Cloprostenate was not within predefined acceptance criteria (>20.00%) for washing matrix.

The contribution of Cloprostenol was within predefined acceptance criteria (<20.00%) for skin matrix. The contribution of Cloprostenol was not within predefined acceptance criteria (>20.00%) for washing matrix, strip matrix and receptor fluid.

According to the concentrations obtained during assays the impact of concomitant drug should be evaluated. Taking into account the measured concentrations for Isopropyl Cloprostenate and Cloprostenol in washing samples, strips samples and receptor fluid samples in study 24-0055B (presented in 24-0055B report), the observed contribution of the parent and metabolite during the method qualification may be considered to have minor impact on the study results.

7.6. Carry over

One S0 sample was three times injected immediately following the last higher calibration standard (ULOQ) of at least one calibration curve.

Table 49. Carry-over of Isopropyl cloprostenate for washing

Run identification	Area of ISOPROPYL CLOPROSTENATE peak in S0	Area of ISOPROPYL CLOPROSTENATE peak in LLOQ calibration standard	% interference
G260424-W-ISOCLO	114	3259	3.49
G260424-W-ISOCLO	191	3259	5.85
G260424-W-ISOCLO	534	3259	16.38

Table 50. Carry-over of internal standard for washing

Run identification	Area of IRBESARTAN peak in S0	Area of IRBESARTAN peak in S0SI	% interference
G260424-W-ISOCLO	1953	1016875	0.19
G260424-W-ISOCLO	16180	1016875	1.59
G260424-W-ISOCLO	7009	1016875	0.69

Table 51. Carry-over of Isopropyl cloprostenate for skin

Run identification	Area of ISOPROPYL CLOPROSTENATE peak in S0	Area of ISOPROPYL CLOPROSTENATE peak in LLOQ calibration standard	% interference
G170524-S-ISOCLO	27	11131	0.24
G170524-S-ISOCLO	189	11131	1.70
G170524-S-ISOCLO	178	11131	1.60

Table 52. Carry-over of Isopropyl cloprostenate for strips

Run identification	Area of ISOPROPYL CLOPROSTENATE peak in S0	Area of ISOPROPYL CLOPROSTENATE peak in LLOQ calibration standard	% interference
G210524-ISOCLO-ST	321	14463	2.22
G210524-ISOCLO-ST	1122	14463	7.76
G210524-ISOCLO-ST	1813	14463	12.54

Table 53. Carry-over of Isopropyl cloprostenate for receptor fluid

Run identification	Area of ISOPROPYLE CLOPROSTENATE peak in S0	Area of ISOPROPYLE CLOPROSTENATE peak in LLOQ calibration standard	% interference
G170524-RF-CLO	233	2801	8.31
G170524-RF-CLO	90	2801	3.23
G170524-RF-CLO	88	2801	3.14

Table 54. Carry-over of Cloprostenol for washing

Run identification	Area of CLOPROSTENOL peak in S0	Area of CLOPROSTENOL peak in LLOQ calibration standard	% interference
G260424-W-CLO	2157	66485	3.24
G260424-W-CLO	1881	66485	2.83
G260424-W-CLO	3176	66485	4.78

Table 55. Carry-over of internal standard for washing

Run identification	Area of PTGF2alpha-d4 peak in S0	Area of PTGF2alpha-d4 peak in S0SI	% interference
G260424-W-CLO	189	4883378	0.00
G260424-W-CLO	515	4883378	0.01
G260424-W-CLO	1026	4883378	0.02

Table 56. Carry-over of Cloprostenol for skin

Run identification	Area of CLOPROSTENOL peak in S0	Area of CLOPROSTENOL peak in LLOQ calibration standard	% interference
G170524-CLO-S	12364	155513	7.95
G170524-CLO-S	41704	155513	26.82
G170524-CLO-S	63753	155513	41.00

Table 57. Carry-over of internal standard for skin

Run identification	Area of PTGF2alpha-d4 peak in S0	Area of PTGF2alpha-d4 peak in S0SI	% interference
G170524-CLO-S	1342	4534935	0.03
G170524-CLO-S	1334	4534935	0.03
G170524-CLO-S	10070	4534935	0.22

Table 58. Carry-over of Cloprostenol for strips

Run identification	Area of CLOPROSTENOL peak in S0	Area of CLOPROSTENOL peak in LLOQ calibration standard	% interference
G210524-CLO-ST	233	11263	2.07
G210524-CLO-ST	317	11263	2.81
G210524-CLO-ST	656	11263	5.82

Table 59. Carry-over of internal standard for strips

Run identification	Area of PTGF2ALPHA-D4 peak in S0	Area of PTGF2ALPHA-D4 peak in S0SI	% interference
G210524-CLO-ST	7753	5565895	0.14
G210524-CLO-ST	5502	5565895	0.10
G210524-CLO-ST	3727	5565895	0.07

Table 60. Carry-over of Cloprostenol for receptor fluid

Run identification	Area of CLOPROSTENOL peak in S0	Area of CLOPROSTENOL peak in LLOQ calibration standard	% interference
G170524-RF-CLO	273	89550	0.31
G170524-RF-CLO	214	89550	0.24
G170524-RF-CLO	No peak	89550	0.00

Table 61. Carry-over of internal standard for receptor fluid

Run identification	Area of PTGF2alpha-d4 peak in S0	Area of PTGF2alpha-d4 peak in S0SI	% interference
G170524-RF-CLO	461	4037888	0.01
G170524-RF-CLO	550	4037888	0.01
G170524-RF-CLO	55	4037888	0.00

No interference greater than 20% was observed at the retention time and in the MRM trace of Isopropyl cloprostenate and its metabolite in the three blanks injected after the highest calibration standard for each matrix.

An exception concerns the carry-over of Cloprostenol in skin matrix: the first S0 was within the acceptance criteria (<20%) whereas both following S0 were out of acceptance criteria (>20%) which may be explained by the hypothesis of a pollution in both of these S0 samples.

No special preventive action against carry-over of analyte appears to be necessary during other sample analysis.

7.7. Recovery

Recoveries of Isopropyl cloprostenate and its metabolite and IS were consistent, precise and reproducible for strips, skin and washing matrices, as precision on normalized recoveries were <20%.

Table 62. Isopropyl cloprostenate Recovery for washing

Run identification/ Run number	Replicate	Date of extraction	Area spiked after 3 LLOQ	Area spiked before 3 LLOQ	Recovery (%)* 3 LLOQ	Area spiked after 0.8ULOQ	Area spiked before 0.8ULOQ	Recovery (%)* 0.8ULOQ
G260424-ISOCLO	n=6	26/04/2024	33609	27248	81.1	1009857	998837	98.9
		26/04/2024	40834	26283	64.4	1050941	985080	93.7
		26/04/2024	40212	24130	60.0	1022955	927085	90.6
		26/04/2024	42343	24829	58.6	1030410	996851	96.7
		26/04/2024	40050	29048	72.5	1089763	873078	80.1
		26/04/2024	42280	32573	77.0	1112975	1075299	96.6
	Mean		**39888**	**27352**	**68.94**	**1052817**	**976038**	**92.79**
	SD		3230.89	3099.25	9.30	40563.03	69115.39	6.84
	CV%		8.10	11.33	**13.49**	3.85	7.08	**7.37**
Overall recovery* (%)								
	Mean				**80.87**			
	SD				14.69			
	CV%				**18.16**			

* Matrice vs mean solvent
L: low level 3LLOQ
H: high level 0.3ULOQ

Table 63. IS Isopropyl cloprostenate Recovery for washing

Run identification/ Run number	Replicate	Date of extraction	Area spiked after 3 LLOQ	Area spiked before 3 LLOQ	Recovery (%)* 3 LLOQ	Area spiked after 0.8ULOQ	Area spiked before 0.8ULOQ	Recovery (%)* 0.8ULOQ
G260424-ISOCLO	n=6	26/04/2024	6899528	6042744	87.6	7798592	7964011	102.1
		26/04/2024	7256397	6019025	82.9	8091655	9213530	113.9
		26/04/2024	7193601	6074437	84.4	8013401	8186086	102.2
		26/04/2024	7613792	6327338	83.1	8094516	8325921	102.9
		26/04/2024	7819116	6485044	82.9	8101340	8272008	102.1
		26/04/2024	7899202	6700040	84.8	8362198	8647469	103.4
	Mean		**7446939**	**6274771**	**84.31**	**8076950**	**8434838**	**104.42**
	SD		392693.96	278283.21	1.80	181112.19	441249.34	4.66
	CV%		5.27	4.43	**2.13**	2.24	5.23	**4.46**
Overall recovery* (%)								
	Mean				**94.36**			
	SD				11.03			
	CV%				**11.69**			

* Matrice vs mean solvent
L: low level 3LLOQ
H: high level 0.3ULOQ

Table 64. Isopropyl cloprostenate Recovery for skin

Run identification/ Run number	Replicate	Date of extraction	Area spiked after 3 LLOQ	Area spiked before 3 LLOQ	Recovery (%)* 3 LLOQ	Area spiked after 0.8ULOQ	Area spiked before 0.8ULOQ	Recovery (%)* 0.8ULOQ
G170524-S-ISOCLO	n=6	17/05/2024	24160	24085	99.7	2962679	2926026	98.8
		17/05/2024	25642	23441	91.4	3264341	2828647	86.7
		17/05/2024	22554	22534	99.9	2959574	2889416	97.6
		17/05/2024	27073	22550	83.3	2945433	2591318	88.0
		17/05/2024	28418	27288	96.0	2975134	2904830	97.6
		17/05/2024	26783	26580	99.2	3344443	2715188	81.2
	Mean		**25771**	**24413**	**94.93**	**3075267**	**2809237**	**91.64**
	SD		2130.06	2050.14	6.56	179526.71	131085.55	7.35
	CV%		8.27	8.40	**6.91**	5.84	4.67	**8.02**
Overall recovery* (%)								
	Mean				**93.29**			
	SD				6.86			
	CV%				**7.35**			

* Matrice vs mean solvent
L: low level 3LLOQ
H: high level 0.3ULOQ

Table 65. IS Isopropyl cloprostenate Recovery for skin

Run identification/ Run number	Replicate	Date of extraction	Area spiked after 3 LLOQ	Area spiked before 3 LLOQ	Recovery (%)* 3 LLOQ	Area spiked after 0.8ULOQ	Area spiked before 0.8ULOQ	Recovery (%)* 0.8ULOQ
G170524-S-ISOCLO	n=6	17/05/2024	4700376	4670154	99.4	4386779	4509097	102.8
		17/05/2024	4741343	4813381	101.5	4500462	4324187	96.1
		17/05/2024	4790467	4744224	99.0	4660466	4571669	98.1
		17/05/2024	4645974	4560998	98.2	4490421	4534472	101.0
		17/05/2024	4774215	4678309	98.0	4418191	4232754	95.8
		17/05/2024	4544764	4625887	101.8	4548707	4324554	95.1
	Mean		**4699523**	**4682159**	**99.64**	**4500838**	**4416122**	**98.14**
	SD		92105.50	88404.26	1.64	97694.69	139503.73	3.12
	CV%		1.96	1.89	**1.65**	2.17	3.16	**3.18**
Overall recovery* (%)								
	Mean				**98.89**			
	SD				2.51			
	CV%				**2.53**			

* Matrice vs mean solvent
L: low level 3LLOQ
H: high level 0.3ULOQ

Table 66. Isopropyl cloprostenate Recovery for strips

Run identification/ Run number	Replicate	Date of extraction	Area spiked after 3 LLOQ	Area spiked before 3 LLOQ	Recovery (%)* 3 LLOQ	Area spiked after 0.8ULOQ	Area spiked before 0.8ULOQ	Recovery (%)* 0.8ULOQ
G210524-ISOCLO-ST	n=6	21/05/2024	46490	43288	93.1	5390396	5142002	95.4
		21/05/2024	42723	40625	95.1	5309125	5124605	96.5
		21/05/2024	44833	42604	95.0	5093582	5329299	104.6
		21/05/2024	43276	44639	103.1	5233592	4954734	94.7
		21/05/2024	42846	43650	101.9	5321381	4982543	93.6
		21/05/2024	43027	40950	95.2	5311763	4917649	92.6
	Mean		**43866**	**42626**	**97.24**	**5276640**	**5075139**	**96.24**
	SD		1497.69	1571.69	4.18	102561.67	154524.71	4.33
	CV%		3.41	3.69	**4.30**	1.94	3.04	**4.50**
Overall recovery* (%)								
	Mean				**96.74**			
	SD				4.09			
	CV%				**4.23**			

* Matrice vs mean solvent
L: low level 3LLOQ
H: high level 0.3ULOQ

Table 67. Cloprostenol Recovery for washing

Run identification/ Run number	Replicate	Date of extraction	Area spiked after 3 LLOQ	Area spiked before 3 LLOQ	Recovery (%)* 3 LLOQ	Area spiked after 0.8ULOQ	Area spiked before 0.8ULOQ	Recovery (%)* 0.8ULOQ
G260424-CLO	n=6	26/04/2024	159838	129279	80.9	3683952	3254415	88.3
		26/04/2024	147611	134276	91.0	3472063	3393292	97.7
		26/04/2024	172954	114734	66.3	3752870	3210714	85.6
		26/04/2024	149937	110407	73.6	3687271	3063710	83.1
		26/04/2024	151408	129664	85.6	3662296	2668027	72.9
		26/04/2024	153896	128172	83.3	4021496	3376187	84.0
	Mean		**155941**	**124422**	**80.12**	**3713325**	**3161057**	**85.25**
	SD		9325.23	9513.25	8.84	178261.54	269796.40	8.07
	CV%		5.98	7.65	**11.03**	4.80	8.54	**9.46**
Overall recovery* (%)								
	Mean				**82.69**			
	SD				8.50			
	CV%				**10.28**			

* Matrice vs mean solvent
L: low level 3LLOQ
H: high level 0.3ULOQ

Table 68. IS Cloprostenol Recovery for washing

Run identification/ Run number	Replicate	Date of extraction	Area spiked after 3 LLOQ	Area spiked before 3 LLOQ	Recovery (%)* 3 LLOQ	Area spiked after 0.8ULOQ	Area spiked before 0.8ULOQ	Recovery (%)* 0.8ULOQ
G260424-CLO	n=6	26/04/2024	3610200	3829081	106.1	3706626	3987504	107.6
		26/04/2024	3980707	4193220	105.3	3354034	4204442	125.4
		26/04/2024	3702102	3998478	108.0	3541063	3932259	111.0
		26/04/2024	3874151	3666620	94.6	3687750	3845029	104.3
		26/04/2024	3629069	3767095	103.8	3545327	3951363	111.5
		26/04/2024	3610716	3907278	108.2	4005077	4030829	100.6
	Mean		**3734491**	**3893629**	**104.34**	**3639979**	**3991904**	**110.06**
	SD		156881.18	185819.57	5.03	219330.07	121227.14	8.55
	CV%		4.20	4.77	**4.82**	6.03	3.04	**7.77**
Overall recovery* (%)								
	Mean				**107.20**			
	SD				7.32			
	CV%				**6.83**			

* Matrice vs mean solvent
L: low level 3LLOQ
H: high level 0.3ULOQ

Table 69. Cloprostenol Recovery for skin

Run identification/ Run number	Replicate	Date of extraction	Area spiked after 3 LLOQ	Area spiked before 3 LLOQ	Recovery (%)* 3 LLOQ	Area spiked after 0.8ULOQ	Area spiked before 0.8ULOQ	Recovery (%)* 0.8ULOQ
G170524-S-CLO	n=6	17/05/2024	516863	501339	97.0	67466006	64265120	95.3
		17/05/2024	528443	516621	97.8	68142478	62713711	92.0
		17/05/2024	566346	500290	88.3	68867029	65756614	95.5
		17/05/2024	498527	497444	99.8	65536389	66249116	101.1
		17/05/2024	519614	500899	96.4	68383262	62304625	91.1
		17/05/2024	528135	507492	96.1	65956892	65250540	98.9
	Mean		**526321**	**504014**	**95.89**	**67392009**	**64423288**	**95.65**
	SD		22435.62	6998.94	3.93	1358663.43	1626785.78	3.85
	CV%		4.26	1.39	**4.10**	2.02	2.53	**4.03**
Overall recovery* (%)								
	Mean				**95.77**			
	SD				3.71			
	CV%				**3.88**			

* Matrice vs mean solvent
L: low level 3LLOQ
H: high level 0.3ULOQ

Table 70. Cloprostenol Recovery for strips

Run identification/ Run number	Replicate	Date of extraction	Area spiked after 3 LLOQ	Area spiked before 3 LLOQ	Recovery (%)* 3 LLOQ	Area spiked after 0.8ULOQ	Area spiked before 0.8ULOQ	Recovery (%)* 0.8ULOQ
G210524-CLO-ST	n=6	21/05/2024	17842	16887	94.6	1930061	1889621	97.9
		21/05/2024	17332	18099	104.4	1960858	1816926	92.7
		21/05/2024	17743	18441	103.9	1943088	1853261	95.4
		21/05/2024	16277	19096	117.3	1916862	1982721	103.4
		21/05/2024	16206	17492	107.9	2101043	1964472	93.5
		21/05/2024	16172	17248	106.7	2030345	1897537	93.5
	Mean		**16929**	**17877**	**105.82**	**1980376**	**1900756**	**96.06**
	SD		797.32	822.14	7.32	71266	63554	4.08
	CV%		4.71	4.60	**6.91**	3.60	3.34	**4.25**
Overall recovery* (%)								
	Mean				**100.94**			
	SD				7.61			
	CV%				**7.54**			

* Matrice vs mean solvent
L: low level 3LLOQ
H: high level 0.3ULOQ

Table 71. IS Cloprostenol Recovery for strips

Run identification/ Run number	Replicate	Date of extraction	Area spiked after 3 LLOQ	Area spiked before 3 LLOQ	Recovery (%)* 3 LLOQ	Area spiked after 0.8ULOQ	Area spiked before 0.8ULOQ	Recovery (%)* 0.8ULOQ
G210524-CLO-ST	n=6	21/05/2024	5184306	5689240	109.7	4832932	4977128	103.0
		21/05/2024	5130413	5598602	109.1	4922150	4918431	99.9
		21/05/2024	5107149	5490695	107.5	4831542	4974355	103.0
		21/05/2024	4884637	5479798	112.2	4733633	5058827	106.9
		21/05/2024	4936746	5419356	109.8	4799855	4867868	101.4
		21/05/2024	4908094	5267110	107.3	5068180	4857563	95.8
	Mean		**5025224**	**5490800**	**109.28**	**4864715**	**4942362**	**101.67**
	SD		129919.95	145782.14	1.78	116787.49	76301.19	3.67
	CV%		2.59	2.66	**1.63**	2.40	1.54	**3.61**
Overall recovery* (%)								
	Mean				**105.47**			
	SD				4.83			
	CV%				**4.58**			

* Matrice vs mean solvent
L: low level 3LLOQ
H: high level 0.3ULOQ

7.8. Long term stability at -20°C

Table 72. Isopropyl cloprostenate long term stability stored for at -20°C for washing

Run Date	Curve Number	L*-LT 3.0000 ng/mL	%Deviation	H*-LT 80.000 ng/mL	%Deviation
24-May-2024	22	~1.9757	-34.14	~51.933	-35.08
		~1.7913	-40.29	~53.071	-33.66
		~2.0089	-33.04	~50.898	-36.38
		~1.8707	-37.64	~53.070	-33.66
		~1.7262	-42.46	~52.091	-34.89
		~2.0241	-32.53	~51.022	-36.22
Intrarun Mean		1.8995		52.014	
Intrarun SD		0.12318		0.94598	
Intrarun %CV		6.48		1.82	
Intrarun %Deviation		-36.68		-34.98	
n		6		6	

~ > 20%Deviation

Run 22: long term stability 29 days - Validated in 24-0055B study

Table 73. Isopropyl cloprostenate long term stability stored for at -20°C for skin

Run Date	Curve Number	L*-LT 0.15000 ng/mL	%Deviation	H*-LT 20.000 ng/mL	%Deviation
03-Jun-2024	28	~0.23682	57.88	~26.238	31.19
		~0.21174	41.16	~25.282	26.41
		~0.20156	34.37	~25.998	29.99
		~0.23712	58.08	~25.544	27.72
		~0.24802	65.35	23.881	19.41
		0.18201	21.34	24.408	22.04
Intrarun Mean		0.21955		25.225	
Intrarun SD		0.025343		0.91679	
Intrarun %CV		11.54		3.63	
Intrarun %Deviation		46.37		26.13	
n		6		6	

Run 28: long term stability 17 days -- Validated in 24-0055B study

Table 74. Isopropyl cloprostenate long term stability stored for at -20°C for strips

17-Jul-2024	55	0.30065	0.22	39.796	-0.51
		~0.42103	40.34	40.930	2.33
		0.33033	10.11	42.983	7.46
		~0.42343	41.14	45.373	13.43
		0.29445	-1.85	45.243	13.11
		~0.44289	47.63	46.440	16.10
Intrarun Mean		0.36880		43.461	
Intrarun SD		0.067608		2.6748	
Intrarun %CV		18.33		6.15	
Intrarun %Deviation		22.93		8.65	
n		6		6	

~ > 20%Deviation

Run 55: long term stability 6 days -- Validated in 24-0055B study

Table 75. Isopropyl cloprostenate long term stability stored for at -20°C for receptor fluid

Run Date	Curve Number	L*-LT 3.0000 ng/mL	%Deviation	H*-LT 400.00 ng/mL	%Deviation
03-Jun-2024	26	3.5409	18.03	471.06	17.77
		3.3117	10.39	~497.93	24.48
		~3.9521	31.74	~563.32	40.83
		3.4780	15.93	~489.26	22.32
		2.4329	-18.90	~493.08	23.27
		2.5047	-16.51	~487.14	21.79
Intrarun Mean		3.2034		500.30	
Intrarun SD		0.60720		32.185	
Intrarun %CV		18.95		6.43	
Intrarun %Deviation		6.78		25.08	
n		6		6	

Run 26: long term stability 17 days -- Validated in 24-0055B study

Table 76. Cloprostenol long term stability stored for at -20°C for washing

Run Date	Curve Number	L*-LT 1.5000 ng/mL	%Deviation	H*-LT 40.000 ng/mL	%Deviation
24-May-2024	21	1.2803	-14.65	~31.257	-21.86
		1.2260	-18.27	~31.483	-21.29
		1.2290	-18.07	~30.445	-23.89
		1.2454	-16.97	~31.014	-22.47
		~1.0666	-28.89	~31.244	-21.89
		1.2561	-16.26	~31.769	-20.58
Intrarun Mean		1.2172		31.202	
Intrarun SD		0.076403		0.45025	
Intrarun %CV		6.28		1.44	
Intrarun %Deviation		-18.85		-22.00	
n		6		6	

~ > 20%Deviation

Run 21: long term stability 29 days - Validated in 24-0055B study

Table 77. Cloprostenol long term stability stored for at -20°C for skin

Run Date	Curve Number	L*-LT 3.0000 ng/mL	%Deviation	H*-LT 400.00 ng/mL	%Deviation
03-Jun-2024	27	3.1415	4.72	409.16	2.29
		3.3162	10.54	408.40	2.10
		3.1590	5.30	401.11	0.28
		3.2398	7.99	408.37	2.09
		2.9736	-0.88	405.87	1.47
		3.4069	13.56	367.21	-8.20
Intrarun Mean		3.2062		400.02	
Intrarun SD		0.15101		16.343	
Intrarun %CV		4.71		4.09	
Intrarun %Deviation		6.87		0.01	
n		6		6	

Run 40: long term stability 17 days

Table 78. Cloprostenol long term stability stored for at -20°C for strips

17-Jul-2024	54	0.73546	-1.94	94.783	-5.22
		0.72973	-2.70	98.327	-1.67
		0.71269	-4.97	98.229	-1.77
		0.76444	1.93	99.433	-0.57
		0.70418	-6.11	98.846	-1.15
		0.84104	12.14	100.08	0.08
Intrarun Mean		0.74792		98.283	
Intrarun SD		0.050169		1.8506	
Intrarun %CV		6.71		1.88	
Intrarun %Deviation		-0.28		-1.72	
n		6		6	

~ > 20%Deviation

Run 54: long term stability 6 days

Table 79. Cloprostenol long term stability stored for at -20°C for receptor fluid

Run Date	Curve Number	L*-LT 3.0000 ng/mL	%Deviation	H*-LT 400.00 ng/mL	%Deviation
03-Jun-2024	25	2.7167	-9.44	401.74	0.44
		2.5188	-16.04	397.33	-0.67
		2.5959	-13.47	424.17	6.04
		2.6529	-11.57	415.20	3.80
		2.6461	-11.80	391.43	-2.14
		2.5652	-14.49	416.17	4.04
Intrarun Mean		2.6159		407.67	
Intrarun SD		0.070485		12.704	
Intrarun %CV		2.69		3.12	
Intrarun %Deviation		-12.80		1.92	
n		6		6	

Run 25: long term stability 17 days

Therefore, it can be concluded that Isopropyl cloprostenate and its metabolite is stable in specimens stored at -20°C for:

- a period of 17 days in Methanol for skin
- a period of 17 days for receptor fluid
- a period of 6 days in Methanol for strips
- a period inferior to 29 days in Methanol for washing.

It is recommended to analyse the washing samples on a short period following cells dismantling, as performed in study 24-0055B.

7.9. Assay organization

Each series of assays was organized as follows:

- Blank matrix not spiked (S0).
- S0SI in case of available isotopic internal standard
- At least 8 calibration standards different from zero prepared on the same day in the matrix.
- Quality Controls.
- Blank matrix not spiked (S0).
- S0SI in case of available isotopic internal standard
- At least 8 calibration standards different from zero prepared on the same day in the matrix.

8. Conclusion

The objective of this study was to qualify an analytical method for the analysis of Isopropyl cloprostenate and its metabolite Cloprostenol in washing, receptor fluid, Stratum Corneum (tape stripping) and skin samples. This study was done in the context of in vitro skin penetration study.

The theoretical Isopropyl cloprostenate concentration was used to perform calculations.

The analytical method using LC-MS/MS and referenced as PKH/MOA/1745 for the quantification of Isopropyl cloprostenate and its metabolite in the different generated samples was qualified in this study to be used in support of dermal absorption.

9. Archiving procedure

All the documentation relative to this study will be archived in accordance with the Protocol (APPENDIX B).

APPENDIX A

RUN SUMMARY

A summary of accepted series included in the qualification study is presented below:

Table 80. Summary of analytical runs

Calibration curve	Watson run ID	Biological Matrix	Tests performed	Results
G260424-W-CLO	1	Washing	selectivity recovery carry over contribution of IS specificity in presence of metabolite linearity repeatability T0 long term stability	Accepted
G260424-W-ISOCLO	2	Washing	selectivity recovery carry over contribution of IS specificity in presence of metabolite linearity repeatability T0 long term stability	Accepted
G260424-Solubilite-neg	3	Receptor fluid	Solubility Filter	Accepted
G260424-Solubilité-pos	4	Receptor fluid	Solubility Filter	Accepted
G070524-LR	9	Receptor fluid	carry over contribution of IS specificity in presence of metabolite linearity repeatability T0 long term stability	Accepted
G170524-S-CLO	15	Skin	selectivity recovery carry over contribution of IS specificity in presence of metabolite linearity repeatability T0 long term stability	Accepted
G170524-S-ISOCLO	16	Skin	selectivity recovery carry over contribution of IS specificity in presence of metabolite linearity repeatability T0 long term stability	Accepted
G170524-RF-CLO	17	Receptor fluid	selectivity recovery carry over contribution of IS specificity in presence of metabolite linearity repeatability T0 long term stability	Accepted

Calibration curve	Watson run ID	Biological Matrix	Tests performed	Results
G170524-RF-ISOCLO	18	Receptor fluid	selectivity recovery carry over contribution of IS specificity in presence of metabolite linearity repeatability T0 long term stability	Accepted
G210524-Strip-CLO	19	Strips	selectivity recovery carry over contribution of IS specificity in presence of metabolite linearity repeatability	Accepted
G210524-Strip-ISOCLO	20	Strips	selectivity recovery carry over contribution of IS specificity in presence of metabolite linearity repeatability	Accepted
G240524-W-CLO*	21	Washing	Long term stability 29 days	Accepted
G240524-W-ISOCLO*	22	Washing	Long term stability 29 days	Accepted
G0306-24-RF-Clo	25	Receptor fluid	Long term stability 17 days	Accepted
G0306-24-RF-Isoclo	26	Receptor fluid	Long term stability 17 days	Accepted
G0306-24-S-Clo	27	Skin	Long term stability 17 days	Accepted
G0306-24-S-Isoclo	28	Skin	Long term stability 17 days	Accepted
G110724-ST-CLO	42	Strips	T0 long term stability	Accepted
G110724-ST-IC	43	Strips	T0 long term stability	Accepted
G170724-ST-CLO	54	Strips	Long term stability 6 days	Accepted
G170724-ST-IC	55	Strips	Long term stability 6 days	Accepted

*Run validated in 24-0055B

Rejected or failed runs, not included in the qualification results, are not presented in the table above.

APPENDIX B

FINAL PROTOCOL

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PROTOCOL

Title	Qualification of an analytical method for Isopropyl cloprostenate and its metabolite: quantification in Washing, *Stratum corneum*, Skin and Receptor Fluid samples in the context of *in vitro* skin absorption study.
Eurofins|ADME BIOANALYSES reference	24-0055A
Name of product	Isopropyl cloprostenate and its metabolite
Test Facility	Eurofins|ADME BIOANALYSES 75A, Avenue de Pascalet 30310 Vergèze France
Sponsor Monitor	Eurofins Product Testing US Inc. 11822 N Creek Pkwy #110, Bothell, WA 98011, USA
Document status	Final
Date of version	April 08th, 2024

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Protocol
Eurofins|ADME BIOANALYSES reference: 24-0055A
Confidentiality level: high

APPROVAL PAGE

This protocol has been approved by the Analytical Site. The following signatures document those approvals:

Analytical Site

Eurofins|ADME BIOANALYSES
75A, Avenue de Pascalet
30310 Vergèze
France

Analytical Site Management

Date and signature

[redacted]

Principal Scientist

Date and signature

[redacted]

Deputy Principal Scientist

Date and signature

[redacted]

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APPROVAL PAGE (continued)

This protocol has been approved by the Sponsor. The following signatures document those approvals:

Sponsor Monitor [redacted]

Eurofins Product Testing US Inc.
11822 N Creek Pkwy #110, Bothell, WA 98011, USA
e-mail: [redacted]

[redacted]

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GLOSSARY

BLQ	:	Below the Limit of Quantification
BSA	:	Bovine Serum Albumin
Cmean	:	Mean concentration
Cmeas	:	Measured or back calculated concentration
Cn	:	Nominal concentration
LLOQ	:	Lower Limit Of Quantification
MRM	:	Multiple Reaction Monitoring
n	:	Number of determinations or replicates
PBS	:	Phosphate Buffered Saline
QC	:	Quality Control samples
RT	:	Retention Time
S0	:	Blank matrix sample
SD	:	Standard Deviation
Series	:	Analytical batch or run
SOP	:	Standard Operating Procedure
ULOQ	:	Upper Limit Of Quantification
Deviation %	:	Deviation (%) = $\frac{Cmeas - Cn}{Cn} x100$
Precision%	:	Precision (%) = $\frac{SD}{Cmean} x100$

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1. OBJECTIVES

The objective of this study is to qualify an analytical method for the quantification of Isopropyl cloprostenate and Cloprostenol in washing, receptor fluid, *Stratum Corneum* (tape stripping) and skin samples. This study is done in the context of *in vitro* skin penetration study.

2. LOCATION AND REGULATORY COMPLIANCE

This study will be conducted at Eurofins|ADME BIOANALYSES – 75A, Avenue de Pascalet – 30310 Vergèze – France under the responsibility of [redacted].

The study will be performed according to:

- Eurofins|ADME BIOANALYSES Standard Operating Procedures, this protocol referenced 24-0055A and its eventual amendment.

Good Laboratory Practices are not required for this study.

3. MODIFICATION AND DEVIATION OF THE FINAL PROTOCOL

All unplanned deviation(s) from the study plan that may occur during the progression of the experiment will be noted in the paragraph "deviation(s) to the study plan" in the final report.

In case of deviation impacting the results, the Sponsor will be informed during the study.

4. EXPECTED SCHEDULE

Proposed start of the method qualification:	April, 2024 at the latest
Proposed start of the dermal application:	After completion of method qualification, 2024
Expected delivery of the results:	Two weeks after the end of experiments, 2024
Expected draft report:	Four weeks after the end of experiments
Expected final report:	1 month after receipt of finalization approval

Any modification to this schedule will not require an amendment to the final protocol.

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5. TEST SUBSTANCE

	Reference item		Internal Standard
Name	Isopropyl cloprostenate	Cloprostenol	Prostaglandin F2Alpha-d4
Batch number	0694748-3	0412491-8	0482283
Supplier	Bertin Technologies	Bertin Technologies	Bertin Technologies
Molecular weight (g/mol)	467	424.9	358.5
Corrective factor	1	1.008	1
Purity	100	99.2	100
Storage conditions	Target temperature -20°C	Target temperature -20°C	Target temperature -20°C
Retest or expiring date	01/11/25	28/08/25	07/05/25
Traceability	Details of the reference item used will be fully described in the raw data and final report. The certificates of analysis will be reproduced in the final report.		
Handling	The analytical reference item will be handled according to the SOP EER/GES/002P in use at Eurofins|ADME BIOANALYSES. All safety precautions will be taken to prevent any accidental injection, swallowing or inhalation of the tested compounds. The pure standard compounds will be handled while wearing a lab-coat, glasses, mask and gloves.		
Responsibility	All safety information relative to the manipulation of reference item will be documented by the suppliers. Reference item will be sent with the corresponding information (certificate of analysis, labeling, purity, water content, expiry date). Compliance with this information will be the responsibility of the suppliers.		

The certificate of analysis will be presented in the report.

According to the availability of reference item, the batch number, and/or purity and corrective factor could be modified. New reference or batch of reference item could be ordered during the study, if necessary.

6. BLANK MATRIX

The following test systems will be used:

Matrix: **Washing**
3 half cotton buds wetted with water containing 10% of Tween 80
2 dry half cotton buds
Storage: Ambient temperature

Matrix: **Strips on human skin**
Scotch: 5 Scotch Magic 3M stripped on human skin.
Storage: Ambient temperature

Matrix: Skin: 1 cm² (dermis and epidermis not separated for blank matrix) corresponding to the application area for dynamic Bronaugh cells
Skin: Frozen Dermatomed skin (300-400 µm)
Species: Human
Supplier: Tissue banks or private hospitals
Storage: Target temperature -20°C

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Matrix: **Receptor fluid**
One of the receptor fluid below:
RF1: PBS 0.01M pH7.4
RF2: 5BSA in PBS 0.01 M* pH* 7.4
Storage: Target temperature 4°C or ambient temperature

7. SOLUBILITY TEST OF Isopropyl cloprostenate and Cloprostenol IN RECEPTOR FLUID

For *in vitro* skin absorption studies, the molecule should be adequately soluble in the selected receptor fluid so that it does not act as a barrier to absorption, and the receptor fluid should maintain the skin barrier integrity. Therefore, the solubility of Isopropyl cloprostenate and Cloprostenol in the receptor fluid will be demonstrated as not being a rate limiting factor. According to the guideline, ENV/JM/MONO(2004)2, the solubility should be at least 10-fold higher than the maximal concentration expected in the receptor fluid at the end of the *in vitro* study.

For information, the SCCS/1635/21 (§ 3.4 Safety Evaluation – Isopropyl cloprotenate – Applicant #2 provides an estimation of Isopropyl cloprostenate dermal absorption of 10% following the application of a formulation at 0.007%:

Considering the maximal Isopropyl cloprostenate concentration in test item preparation (0.005% w/w) and the amount applied (5 mg/cm²), the maximal concentration of Isopropyl cloprostenate and Cloprostenol (100% of absorption in receptor fluid) in receptor fluid (24 mL of receptor fluid, dynamic cells with a flow at 1 mL/h) is estimated to be 10.4 ng/mL.

Isopropyl cloprostenate and Cloprostenol solubility should be **at least** 104 ng/mL (10-fold the maximal estimated concentration corresponding to 1000% of dose applied) to confirm that the receptor fluid selected is suitable for the study.

The selection of the receptor fluid will be determined by the estimation of the solubility of Isopropyl cloprostenate and Cloprostenol in the following receptor fluids, allowed by the guidelines for moderate solubility in water:

- RF1: PBS 0.01M pH7.4
- RF2: 5% BSA in PBS 0.01 M* pH* 7.4

The estimation of the solubility of Isopropyl cloprostenate and Cloprostenol in each receptor fluid tested will be tested as follow:

- 2 mg of Isopropyl cloprostenate or Cloprostenol will be precisely weighed in separate vials and receptor fluid will be added in order to obtain a concentration at 1 mg/mL. Prepared in duplicate.
- the solutions will be mixed for 1 hour at 32°C ± 1°C after which the vials will be left standing for 1 hour at 32°C ± 1°C,
- the solutions will be filtered by using for exemple Chromafil® Xtra H-PTFE 0.2µm or GHP ACRODISC 0.2µm (supplier: Pall) syringe filter.
- The absorption to the filter will be checked by comparison of Isopropyl cloprostenate and Cloprostenol samples (prepared in receptor fluid at a defined concentration, injected in n=3) peak area ratio before and after filtration, the deviation will be inferior to 20%. If acceptance criteria are not fulfilled then an investigation will be made to evaluate the impact.
- Two aliquots of filtrate each will be analysed.
- For each vials, two successive dilutions of the filtrate will be performed and analyzed with a calibration curve prepared in appropriate solvent, the solvent will be specified on the report.

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The organization of the run will be as follow:

- 1 blank solvent not spiked with the product to be analyzed (S0).
- 1 blank solvent not spiked with the product to be analyzed and with the internal standard (S0SI).
- At least 8 calibration standards different from zero prepared on the same day in the solvent.
- 3 blanks solvent not spiked with the product to be analyzed nor with the internal standard (S0).
- The specimens of the solubility test
- The specimens of the test of absorption to the filter
- At least 3 S0
- Reinjection of 2 calibration standards

The run organization (for example, number of S0 samples injected) may be adjusted after completion of the method development.

Criteria for run acceptance:

- *deviation for the calibration standards will range between ± 20.00% of the nominal value for all concentrations, and ± 25.00% for LLOQ*
- *LLOQ and ULOQ will be included in the calibration curve, but if the LLOQ or the ULOQ will be outside the acceptance criteria, the calibration standard after the LLOQ will became the new LLOQ and the calibration standard just before the ULOQ will became the new ULOQ.*
- *75% calibration standards should be included in the final calibration curve*
- *At least one of two reinjected calibration standards will range between ± 20.00% of the nominal value*

The results of the solubility test for each receptor fluid tested will be communicated to sponsor for the determination of the appropriate receptor fluid.

8. QUALIFICATION OF THE ANALYTICAL METHOD

8.1. Description

The qualification of an analytical method referenced as PKH/MOA/1745 using LC-MS/MS techniques, for Isopropyl cloprostenate and Cloprostenol detection will be performed in washing, strips (*stratum corneum*), skin and receptor fluid.

For information, the SCCS/1635/21 (§ 3.4 Safety Evaluation – Isopropyl cloprotenate – Applicant #2) provides an estimation of Isopropyl cloprostenate dermal absorption of 10% following the application of a formulation at 0.007%.

According to the tested dose (0.005% w/w of Isopropyl cloprostenate) and the amount applied (5 mg/cm² on an area of 1 cm²), the target calibration ranges will be:

- **Washing**: For Isopropyl cloprostenate: 1 - 100 ng/mL
  Corresponding to target 0.4 – 40 % of the applied dose of Isopropyl cloprostenate for the formulation at 0.005% w/w.

  For Cloprostenol (metabolite): 0.5 - 50 ng/mL
  Expected extraction volume = 10 mL

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- **Strips**: For Isopropyl cloprostenate: 0.1 - 50 ng/mL
  Corresponding to target 0.04% - 10% of the applied dose for the formulation at 0.005% w/w.
  Expected extraction volume = 5 mL

  For Cloprostenol (metabolite): 0.25 – 125 ng/mL

- **Skin**: For Isopropyl cloprostenate: 0.05 - 25 ng/mL
  Expected extraction volume = 5 mL
  Corresponding to target.0.02% - 10% of the applied dose for the formulation at 0.005% w/w.

  For Cloprostenol (metabolite): 1 - 500 ng/mL

- **Receptor fluid:** For Isopropyl cloprostenate and Cloprostenol (metabolite): 1 to 500 ng/mL
  Expected Bronaugh cells volume collected around 24 mL (flow rate of 1 mL/h)
  Corresponding to target 0.4 - 20% of the applied dose for the formulation at 0.005% w/w.

Since the calibration range and solvent are the same than for strips, the qualification of the method for RCD (donor compartment washing) will be covered by the qualification of the method for strips.

The analytical method qualified for skin will be used for the analysis of remaining skin.

Depending on the sensitivity of the analytical method, the range and the extraction volumes could be modified. The Sponsor will be informed. These modifications will not require an amendment to the final protocol.

To qualify the analytical method for Isopropyl cloprostenate and Cloprostenol, the following criteria will be checked in each matrix:

- Selectivity
- Linearity
- Carry over
- Intra-run precision (repeatability)
- Specificity (Internal standard contribution)
- Specificity in presence of a metabolite
- Recovery
- Long-term stability (at least 7 days)

Only the qualified method will be presented in the report.

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8.2. Selectivity

The selectivity of the method will be tested by the analysis for the skin matrix from 1 donor (n=1) and 1 replicate for receptor fluid, washing and *stratum corneum* matrices.

The matrix will be spiked with Isopropyl cloprostenate and Cloprostenol at LLOQ then extracted and compared to the corresponding blank (S0).

The MRM chromatograms will be examined for the presence of peaks at the retention times (RT) of Isopropyl cloprostenate and its metabolite. The area of any peak present at the respective RT will be measured.

Acceptance criteria:

- *Area of possible interferences at the Isopropyl cloprostenate retention time in the MRM traces specific for Isopropyl cloprostenate will have to be < 20.00% of the Isopropyl cloprostenate peak area in matrix spiked with Isopropyl cloprostenate at LLOQ.*
- *Area of possible interferences at the cloprostenol retention time in the MRM traces specific for cloprostenol will have to be < 20.00% of the cloprostenol peak area in matrix spiked with cloprostenol at LLOQ.*

If acceptance criteria are not met, the test could be conducted again or an investigation will be conducted.

8.3. Concentration-response relationship (linearity)

Only one stock solution will be prepared for calibration standards and QCs.

The concentration-response relationship will be determined from the calibration standards at concentrations ranging from the LLOQ up to the ULOQ on one run.

At least eight calibration points different from zero, prepared the same day of the analysis, will be prepared in each matrix.

The weighting factor of the calibration curve will be determined during the qualification according to the results of calibration curve regression fits. The simplest model that adequately describes the concentration-response relationship will be used.

Acceptance criteria:

- *Deviation for the calibration standards should be between ± 25.00% of the nominal value for the LLOQ concentration level and between ± 20.00% of the nominal values for the other concentration levels*
- *LLOQ and ULOQ should be included in the calibration curve,*
- *75% calibration standards should be included from the final calibration curve.*

If acceptance criteria are not met, the test could be conducted again or an investigation will be conducted.

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8.4. Intra-run precision and deviation (repeatability)

Only one stock solution will be prepared for calibration standards and QCs.

The intra-run precision and deviation of the assay method will be tested for each concentration levels on at least one run.

QC samples will be prepared in n=6 within the same run at 3LLOQ, 0.5ULOQ and 0.8ULOQ concentration for each matrix.

All QC results will be used for precision and mean deviation calculation except those rejected for defined analytical problem.

Acceptance criteria:

- *Mean precision (CV %) will be calculated and will have to be <20.00%,*
- *Mean deviation will be calculated and should be within ± 20.00%.*

If acceptance criteria are not met, the test could be conducted again or an investigation will be conducted.

8.5. Specificity: contribution of internal standard

The SI contribution will be assessed with the study of the S0SI injected during repeatability tests.

Acceptance criteria:

- *peak area obtained at the retention time of analyte and its metabolite in the MRM traces specific for analyte in each S0SI should be inferior to 20.00% of the peak area obtained for analyte and its metabolite in the first calibration standard (LLOQ).*

If acceptance criteria are not met, the test could be conducted again or an investigation will be conducted.

8.6. Specificity in presence of a metabolite

The specificity of the method for the test susbtance Isopropyl Cloprostenate and Cloprostenol will be assessed by the study of the peak area obtained at the retention time of analyte in the MRM traces specific for analyte in the following conditions:

- Test substance at ULOQ without SI (n=3)
- Test substance at LLOQ without SI (n=3)
- The metabolite at the LLOQ without SI (n=3)
- The metabolite at the ULOQ without SI (n=3)

Acceptance criteria:

- *the mean signal obtained in the samples spiked with the metabolite should be inferior to 20.00% of the mean signal obtained for the parent at the LLOQ at the retention time of analyte in the MRM traces specific for analyte.*

If acceptance criteria are not met, the test could be conducted again or an investigation will be conducted.

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8.7. Carry over

One S0 sample will be injected three times injected immediately following the last higher calibration standard (ULOQ) of at least one calibration curve, in at least one run.

The MRM chromatograms will be examined for the presence of peaks at the retention time of Isopropyl Cloprostenate, and cloprostenol. The area of any peak present will be measured.

Acceptance criteria:

- *peak area obtained at the retention time of analyte or metabolite in the MRM traces specific for analyte and metabolite in each S0 should be inferior to 20.00% of the peak area obtained for analyte and metabolite in the first calibration standard (LLOQ).*

If acceptance criteria are not fulfilled, then additional tests may be realized. If necessary, the method or the run organization will be modified.

8.8. Recovery

In order to evaluate the recovery (linked to extraction rate in strips, skin and washing matrices), QCs will be prepared in these matrices extracted then spiked at two concentrations (3 LLOQ and 0.8 ULOQ) n=6 and analyzed in the same run than QCs prepared in these matrices spiked then extracted.

The recovery (%) corresponds to:

$$\frac{\text{peak area or area ratio spiked before extraction}}{\text{peak area or area ratio spiked after extraction}} \times 100$$

Acceptance criteria:

– Mean recovery precision (CV%) calculated on area ratios should be < 20.00% at each concentration level.

If acceptance criteria are not met, the test could be conducted again or an investigation will be conducted.

8.9. Long-term Stability at -20°C

As all samples will not be analysed the day of cell dismantling, the Isopropyl Cloprostenate and Cloprostenol stability will be evaluated in matrices at -20°C.

Stability QCs will be prepared at 3 LLOQ (n=6) and 0.8 ULOQ (n=6) in each matrix.

Then, stability QCs will be stored at target temperature -20°C.

Freshly prepared calibration curve and QC samples at the same concentrations will be used for quantification.

Acceptance criteria:

The test substance will be stable in the condition tested if:
– Mean deviation is within ± 20.00% of the nominal concentrations
– Mean precision (%CV) is < 20.00%

If acceptance criteria are not fulfilled at the tested period, a shorter stability period at -20°C could be tested (if possible) or the sponsor will be informed and a corrective factor could be applied on the concentrations of the samples in the dermal study.

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8.10. Assay organization

Each series of assays will be organized as follows:

- Blank matrix not spiked (S0).
- S0SI in case of available isotopic internal standard
- At least 8 calibration standards different from zero prepared on the same day in the matrix.
- Blank matrix not spiked (S0).
- Quality Controls.

Depending whether an isotopic internal standard is used for LC-MS/MS analysis, S0 and calibration standards may be injected after the last QC.

9. DETERMINATION OF THE CONCENTRATIONS

Concentrations will be calculated using Watson® 7.5 SP1 (HF1) directly from peak area obtained after automatic integration of chromatograms by LabSolutions V6.60.

Concentrations of the QC samples will be calculated by interpolation with the weighed calibration curves prepared in the same conditions and assessed daily after automatic integration. Results of concentrations, once accepted, will be expressed in "ng/mL".

Statistic (mean, SD, precision and deviation) will be calculated with Watson® except for specificity, carry-over and recovery which will be calculated with Excel®.

The concentration results will be reported with at least 5 significant figures in Excel® tables.

10. SENDING OF RESULTS AND DOCUMENT

FileXchange secure transfer platform will be used for the transfer of results and all documents associated to this study. Access to documents to be downloaded through the FileXchange platform will be protected by a password. This password will be sent in a separate mail.

11. FINAL REPORT

The study will be presented in one final report written in English. A final report (in PDF format with Principal Scientist and Analytical Site Management approvals) will be sent to the Sponsor by FileXchange (secure server).

- Margins: right: 1.8 cm / left: 1.8 cm / top: 2.5 cm and bottom 2.5 cm.
- Pagination: page number/total number of pages
- Heading: On the top of the sheet: "Eurofins|ADME BIOANALYSES reference: 24-0055A - Type of document."

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12. ARCHIVING PROCEDURE

All study data and associated study specific documentation will be retained in secure storage by Eurofins ADME BIOANALYSES for a period of 1 year from completion of the study (issue of final results' document or report) after which time it will be securely destroyed. The Sponsor will not be systematically informed in advance of this destruction. Extended storage may be requested by the Sponsor in advance but will be subject to additional cost agreements.
Contents are as follows:

- Signed protocol and its possible amendments
- All relevant correspondence
- Internal memoranda
- Analytical records
- Signed final report and its possible amendments
- List of staff involved
- Laboratory notebook(s)
- All raw data relevant to the study

APPENDIX C

ANALYTICAL METHOD PKH/MOA/1745

Software

Data acquisition: capture and integration of chromatographic peak area	Analyst® 1.6.3 (Applied Biosystems, France) LabSolutions V6.60
Analysis software: concentrations calculation, data analysis, store concentration data, statistical calculation except for carry over, recovery and selectivity.	Watson® 7.5.SP1 (Thermo Electron Corporation, USA)
Storage samples tracking	Watson® 7.5.SP1 (Thermo Electron Corporation, USA)
Statistical calculation for carry over	Excel® (2010)
Weighting software	LABX® 6.0.0

Test substances

	Reference items		Internal Standard
Name	Isopropyl cloprostenate	Cloprostenol	Prostaglandin F2Alpha-d4
Batch number	0694748-3	0412491-8	0482283
Supplier	Bertin Technologies	Bertin Technologies	Bertin Technologies
Molecular weight (g/mol)	467	424.9	358.5
Corrective factor	1	1.008	1
Purity	100	99.2	100
Storage conditions	Target temperature -20°C	Target temperature -20°C	Target temperature -20°C
Retest or expiring date	01/11/25	28/08/25	07/05/25
Traceability	Details of the reference items used were fully described in the raw data and final report. The certificates of analysis are preseneted in APPENDIX D.		
Handling	The analytical reference items and internal standard were handled according to the SOP EER/GES/002P in use at Eurofins|ADME BIOANALYSES. All safety precautions were taken to prevent any accidental injection, swallowing or inhalation of the tested compounds. The pure standard compounds were handled while wearing a lab-coat, glasses, mask and gloves.		
Responsibility	All safety information relative to the manipulation of reference items and internal standard were documented by the suppliers. Reference items and internal standard were sent with the corresponding information (certificate of analysis, labeling, purity, water content, expiry date). Compliance with this information was the responsibility of the suppliers.		

Blanks matrix

The following matrix were used:

Matrix: **Washing**
3 half cotton buds wetted with water containing 10% of Tween 80
2 dry half cotton buds
Storage: ambient temperature

Matrix: **Strips on human skin**
Scotch: 5 Scotch Magic 3M stripped on human skin.
Storage: ambient temperature

Matrix: Skin: 1 cm² (dermis and epidermis not separated for blank matrix) corresponding to the application area for dynamic Bronaugh cells
Skin: Dermatomed skin (300-400 µm)
Species: Human
Supplier: Private hospitals
Storage: Target temperature -20°C

Matrix: **Receptor fluid**
One of the receptor fluid below: RF1: PBS 0.01M pH7.4
RF2: 5BSA in PBS 0.01 M* pH* 7.4
Storage: Target temperature +4°C

APPENDIX D

CERTIFICATES OF ANALYSIS

CERTIFICATE of ANALYSIS

[redacted]

(+)-Cloprostenol isopropyl ester

(+)-9α,11α,15R-trihydroxy-16-(3-chlorophenoxy)-17,18,19,20-tetranor-prosta-5Z,13E-dien-1-oic acid, isopropyl ester
Item No. 10010016 • Batch No. 0694748

Purity Specification: ≥98%
Molecular Formula : C25H35ClO6
CAS Number: 157283-66-4 Formula Weight: 467 Expiry date: 01NOV2025

Overview

Tests	Results
HPLC	Purity: 100.0 %
IR	Conforms
Mass spec	M-H+FA: 511.0 M+Cl: 501.1
TLC	Purity: 100 %

Reviewed and approved by: [redacted]

[redacted]

CERTIFICATE of ANALYSIS

[redacted]

(+)-Cloprostenol

(+)-9α,11α,15R-trihydroxy-16-(3-chlorophenoxy)-17,18,19,20-tetranor-prosta-5Z,13E-dien-1-oic acid

Item No. 16765 • Batch No. 0412491

Purity Specification: >97%
Molecular Formula : C22H29ClO6
CAS Number: 54276-21-0 Formula Weight: 424.9 Expiry date 28AUG2025

Overview

Tests	Results
HPLC	Purity: 99.2 %
IR	Conforms
Mass spec	M-H+: 423.3 -ClC6H4OH: 295.1 -ClC6H4OH, H2O: 277.2 -ClC6H4OH, 2H2O: 259.2
TLC	Purity: 100 %
UV	λ max: 220, 275, 282 nm

Reviewed and approved by: [redacted]

[redacted]

REPORT

Title	Distribution and penetration study of one formulation containing Isopropyl cloprostenate and its metabolite.
Eurofins|ADME BIOANALYSES reference	24-0055B
Name of product	Isopropyl cloprostenate and its metabolite
Test Facility	Eurofins|ADME BIOANALYSES 75A, Avenue de Pascalet 30310 Vergèze France
Sponsor	Eurofins Product Testing US Inc. 11822 N Creek Pkwy #110, Bothell, WA 98011, USA
Date of the experiment	Protocol signature: May 17th, 2024 Start of the experiments (first application): May 23rd, 2024 Completion of the experiments: September 06th, 2024
Document status	Final
Date of version	November 07th, 2024

APPROVAL PAGE

This report has been approved by the Analytical Site. The following signatures document those approvals:

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Eurofins|ADME BIOANALYSES
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Deputy Analytical Site Management

Date and signature

[redacted]

Principal Scientist

[redacted]

Date and signature

[redacted]

SUMMARY

OBJECTIVE:

The objective of this study was to investigate the *in vitro* dermal absorption of a formulation containing Isopropyl cloprostenate through fresh healthy human skin and to analyse Isopropyl cloprostenate and its metabolite Cloprostenol (analysis of washing, *stratum corneum*, epidermis, dermis, receptor fluid and rinsing of donor compartment).

ANALYTICAL METHOD:

The qualification of an analytical method referenced as PKH/MOA/1745 (detailed in 24-0055A report), using LC-MS/MS techniques, for Isopropyl cloprostenate detection was performed in washing, strips (*stratum corneum*), skin and receptor fluid.

The results obtained during this qualification demonstrated that the analytical method can be used to measure Isopropyl cloprostenate and Cloprostenol concentrations from calibration ranges were:

for washing:
- ✓ 1 - 100 ng/mL for Isopropyl cloprostenate (LLOQ: 1 ng/mL)
- ✓ 0.5 - 50 ng/mL for Cloprostenol (metabolite) (LLOQ: 0.5 ng/mL)

for Strips:
- ✓ 0.1 - 50 ng/mL for Isopropyl cloprostenate (LLOQ: 0.1 ng/mL)
- ✓ 0.25 – 125 ng/mL for Cloprostenol (metabolite) (LLOQ: 0.25 ng/mL)

for Epidermis and Dermis:
- ✓ 0.05 - 25 ng/mL for Isopropyl cloprostenate (LLOQ: 0.05 ng/mL)
- ✓ 1 - 500 ng/mL for Cloprostenol (metabolite) (LLOQ: 1 ng/mL)

for Receptor fluid:
- ✓ 1 to 500 ng/mL for Isopropyl cloprostenate and Cloprostenol (metabolite) (LLOQ: 1 ng/mL)

The qualification was conducted and documented so that the qualified method can be used in support of dermal absorption.

EXPERIMENTAL DESIGN:

The formulation was tested on 3 replicates on 4 different donors. A total of 12 cells was performed.

Compound	Isopropyl cloprostenate
Formulation name	[redacted]
Theoretical compound concentration in % W/W	0.005% Isopropyl Cloprostenate, 0% Cloprostenol
Formulation applied per cell (1 cm^2)	10 µL
Theoretical amount applied	0.5 µg
Thickness of the skin	300 - 400 µm
Number of cell per donor	3
Number of donor per formulation	4
Total cells	12
Receptor fluid	PBS 0.01M pH7.4
Sampling of receptor fluid	6 timespoints: 0.5h, 1h, 2h, 4h, 6h, 24h
Washing solution	3 half cotton buds wetted with water containing 10% of Tween 80 in H_2O and 2 dry half cotton buds
Washing of the formulation	24h
Strips	20 strips were performed on each skin and were pooled by 5 (1-5, 6-10, 11-15,16-20).
Separation of epidermis and dermis	Yes

*The number of strips is detailed in Table 6.

APPLICATION RESULTS:

Results (Mean ± SD) of Isopropyl cloprostenate cutaneous distribution expressed as % of applied dose and in µg/cm² (cm² of skin surface) are presented in following tables:

Distribution in all compartments in % of the applied dose

Compound	Isopropyl cloprostenate		Cloprostenol	
Formulation name	[redacted]		[redacted]	
Number of cells	n=12		n=12	
Theoretical compound concentration in % W/W	0.005% Isopropyl Cloprostenate, 0% Cloprostenol			
	Mean	SD	Mean	SD
Strips	0.91	0.61	0.32	0.12
Epidermis	1.67	1.12	0.89	0.04
Dermis	0.05	0.03	0.89	0.04
Receptor fluid	4.61	0.30	4.61	0.30
Epidermis + Dermis + receptor fluid	6.34	1.20	6.40	0.37
Skin excess	109.27	15.72	2.90	0.13
Total Recovery	116.52	14.63	9.62	0.53

Skin excess = washing + rinsing of donor compartment + remaining skin
NC: Not Calculated
Values in bold front were BLQ. The equivalent mass value at the LLOQ is presented.
The Cloprostenol percentages are calculated relative to the applied amount of Isopropyl Cloprostenate.

Distribution in all compartments in µg/cm² of skin

Compound	Isopropyl cloprostenate		Cloprostenol	
Formulation name	[redacted]		[redacted]	
Number of cells	n=12		n=12	
Theoretical compound concentration in % W/W	0.005% Isopropyl Cloprostenate, 0% Cloprostenol			
	Mean	SD	Mean	SD
Applied amount of Isopropyl cloprostenate	0.56	0.02	0.56	0.02
Strips	0.01	0.003	0.002	0.001
Epidermis	0.01	0.01	0.01	0.00
Dermis	0.0003	0.0002	0.01	0.00
Receptor fluid	0.03	0.001	0.03	0.001
Epidermis + Dermis + receptor fluid	0.04	0.01	0.04	0.001
Skin excess	0.61	0.10	0.02	0.00

Skin excess = washing + rinsing of donor compartment + remaining skin
NC: Not Calculated
Values in bold front were BLQ. The equivalent mass value at the LLOQ is presented.
BLQ: Below the lower limit of quantification, replaced by the Lower Limit of Quantification value for the calculation.

CONCLUSION:

The objective of this study was to evaluate the skin absorption and distribution of Isopropyl cloprostenate from one formulation [redacted], containing Isopropyl cloprostenate at 0.005% w/w applied in vitro on freshly-excised human skin during 24h.

The theoretical Isopropyl cloprostenate concentration was used to perform calculations.

The analytical method using LC-MS/MS and referenced as PKH/MOA/1745 for the quantification of Isopropyl cloprostenate in the different generated samples was qualified in study 24-0055A. Values inferior to the Lower Limit of Quantification (BLQ) were replaced by the LLOQ value for calculations after discussion with the Sponsor.

For each cell, a mass-balance was calculated. The individual total recoveries were between 88.76% and 139.15%. The applications were performed on different occasions according to fresh human skin availability. For the last 3 cells applied (P, Q, R), the individual calculated recoveries were higher than 120%, which may be explained by the following hypothesis: the same tube of formulation was used for the applications of cells G to R. Consequently, the last 3 cells were applied with the last remaining of formulation in this tube. Isopropyl cloprostenate may have slightly concentrated because of precipitation or volatility of the other solvent.

The mean mass-balance value obtained (116.52% ± 14.63) was within the acceptance criteria, 100 ± 20% validating the experimentation.

The amount of compound in the epidermis + dermis + receptor fluid compartments in µg/cm² is estimated to:

- 0.04 ± 0.01 µg/cm² for [redacted] Isopropyl cloprostenate
- 0.04 ± 0.01 µg/cm² for [redacted] Cloprostenol

Isopropyl cloprostenate was mainly detected in the washing of the formulation (skin excess, mean 109.27 ± 15.72%) and poorly detected in epidermis (mean 1.67 ± 1.12%) and dermis (mean 0.05 ± 0.03%).

Isopropyl cloprostenate was not detected in the receptor fluid. Only two values were observed in the receptor fluid (cell H, Receptor fluid, 1 hour and cell M, Receptor fluid, 24 hours). In these samples, it should be taken into account that the measured concentrations were close to the lower limit of quantification of 1 ng/mL (1.1887 ng/mL and 1.0101 ng/mL respectively). The values presented in the result tables take into account the volume of receptor fluid collected at an interval of time. Both data (cell H, Receptor fluid, 1 hour and cell M, Receptor fluid, 24 hours) are given for information only, taking into account the analytical variability and the limit of quantification.

Cloprostenol was not detected in the samples, suggesting poor metabolism of Isopropyl cloprostenate into Cloprostenol or metabolism into Cloprostenol at levels not detectable due to the sensitivity of the analytical method. This may be correlated with the low skin penetration of Isopropyl cloprostenate.

Taken together, the results suggest that Isopropyl cloprostenate in [redacted] formulation poorly penetrated the skin, which is also consistent with no detectable level of Cloprostenol.

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GLOSSARY

BLQ	:	Below the Limit of Quantification
Cmean	:	Mean concentration
Cmeas	:	Measured or back calculated concentration
Cn	:	Nominal concentration
IS	:	Internal standard
LLOQ	:	Lower Limit Of Quantification
MeOH	:	Methanol
MRM	:	Multiple Reaction Monitoring
n	:	Number of determinations or replicates
PBS	:	Phosphate Buffered Saline
QC	:	Quality Control samples
RT	:	Retention Time
S0	:	Blank matrix sample
S0SI	:	Blank matrix spiked with internal standard
SD	:	Standard Deviation
Series	:	Analytical batch or run
SOP	:	Standard Operating Procedure
ULOQ	:	Upper Limit Of Quantification
Deviation %	:	Deviation (%) = $\frac{Cmeas - Cn}{Cn} x100$
Precision%	:	Precision (%) = $\frac{SD}{Cmean} x100$

1. OBJECTIVE

The objective of this study was to investigate the *in vitro* dermal absorption of a formulation containing Isopropyl cloprostenate through fresh healthy human skin and to analyse Isopropyl cloprostenate and its metabolite Cloprostenol (analysis of washing, *stratum corneum*, epidermis, dermis, receptor fluid and rinsing of donor compartment).

2. CONTRACT LABORATORY AND LOCATION

This study was conducted at Eurofins|ADME BIOANALYSES – 75A, Avenue de Pascalet – 30310 Vergèze – France under the responsibility of [redacted]

The study was performed according to:

- Eurofins|ADME BIOANALYSES Standard Operating Procedures,
- this protocol and its amendment referenced 24-0055B.

Good Laboratory Practices are not required for this study.

3. MODIFICATION AND DEVIATION OF THE FINAL PROTOCOL

The following minor deviation to the protocol occurred with minor impact on the study outcome:

-Protocol Paragraph 7.2, Preparation of fresh human skin: “Fresh human skin obtained from abdominal surgery will be used. Skin will be transferred to the laboratory within 4 hours of excision, stored at 4°C until use, and used within 24 hours of excision”. Skins from donor 1648, 1630 and 1649 arrived to the laboratory at 4h05, 5h10 and 6h35 after surgery respectively. Taking into consideration that the storage conditions were respected from surgery and during the shipment, and that the study experiments were started within 24 hours following the skin sampling, the time of transfer of these skins is considered to have no impact on the metabolism. This was verified with the results showing comparable results for these donors when compared to the donors transferred within 4 hours from surgery.

-Protocol Paragraphs 8.1 Calibration curves and 8.2 Quality control samples: the acceptance criteria were extended to ± 25% of the nominal value. The tests and acceptance criteria are detailed in section 6.Tests and acceptance criteria of current report. The runs G120724-RF, G190724-RFand G300824-RF for Isopropyl cloprostenate were also accepted with more than 25% of calibration standards excluded or with less than 67% of QC samples accepted. Taking into account that the targeted sensitivity was reached and that the concentrations were below the lower limit of quantification, the extension of the acceptance criteria is not considered to have an impact on the measured concentrations.

4. REFERENCE ITEM AND BLANK MATRICES

	Reference item		Internal Standard
Name	Isopropyl cloprostenate	Cloprostenol	Prostaglandin F2Alpha-d4
Batch number	0694748-3	0412491-8	0482283
Supplier	Bertin Technologies	Bertin Technologies	Bertin Technologies
Molecular weight (g/mol)	467	424.9	358.5
Corrective factor	1	1.008	1
Purity	100	99.2	100
Storage conditions	Target temperature -20°C	Target temperature -20°C	Target temperature -20°C
Retest or expiring date	01 11 25	28 08 25	07 05 25
Traceability	Details of the reference item used were fully described in the raw data and final report. The certificates of analysis are reproduced in APPENDIX C.		
Handling	The analytical reference item was handled according to the SOP EER/GES/002P in use at Eurofins|ADME BIOANALYSES. All safety precautions were taken to prevent any accidental injection, swallowing or inhalation of the tested compounds. The pure standard compounds were handled while wearing a lab-coat, glasses, mask and gloves.		
Responsibility	All safety information relative to the manipulation of reference item were documented by the suppliers. Reference item was sent with the corresponding information (certificate of analysis, labeling, purity, water content, expiry date). Compliance with this information was the responsibility of the suppliers.		

5. TEST SYSTEMS

The following matrix were used:

Matrix: **Washing**
3 half cotton buds wetted with water containing 10% of Tween 80 in H2O
2 dry half cotton buds
Storage: Ambient temperature

Matrix: **Strips on human skin**
Scotch: 5 Scotch Magic 3M stripped on human skin.
Storage: Ambient temperature

Matrix: **Skin: 1 cm²** (dermis and epidermis not separated for blank matrix) corresponding to the application area for dynamic Bronaugh cells
Skin: Frozen Dermatomed skin (300-400 µm)
Species: Human
Supplier: Tissue banks or private hospitals
Storage: Target temperature -20°C

Matrix: **Receptor fluid**
RF: PBS 0.01M pH7.4
Storage: Target temperature 4°C

6. SUMMARY OF THE ANALYTICAL RUNS

The summary of the analytical runs is presented in Table 3.

7. IN VITRO ABSORPTION

7.1. Formulation

Formulation name	[redacted]
Formulation batch number	12575*
Nominal concentration of reference item (w/w)	0.005% Isopropyl Cloprostenate, 0% Cloprostenol
Reference item	Isopropyl cloprostenate
Supplier	[redacted]
Storage conditions	Target temperature +20°C
Manufacture date	26/02/2024
Expiry date	02/2026

The formulation was provided by the Sponsor ready to use and the certificate of analysis was provided by the Sponsor.

In the absence of detection of Isopropyl Cloprostenate in the first batch of formulation provided by the Sponsor (batch number 11989) and in the study samples following the first application, a new batch of formulation (batch number 12575) was provided by the Sponsor. Consequently, the first application performed with previous batch 11989 was not taken into account in current study 24-0055B. The 24-0055B study results presented in this report were obtained from the application of the formulation batch 12575.

Certificate of analysis are presented in APPENDIX C.

7.2. Experimental design

The formulation was tested on 3 replicates on 4 different donors. A total of 12 cells was performed.

Compound	Isopropyl cloprostenate
Formulation name	[redacted]
Theoretical compound concentration in % W/W	0.005% Isopropyl Cloprostenate, 0% Cloprostenol
Formulation applied per cell (1 cm^2)	10 µL
Theoretical amount applied	0.5 µg
Thickness of the skin	300 - 400 µm
Number of cell per donor	3
Number of donor per formulation	4
Total cells	12
Receptor fluid	PBS 0.01M pH7.4
Sampling of receptor fluid	6 timespoints: 0.5h, 1h, 2h, 4h, 6h, 24h
Washing solution	3 half cotton buds wetted with water containing 10% of Tween 80 in H_2O and 2 dry half cotton buds
Washing of the formulation	24h
Strips	20 strips were performed on each skin and were pooled by 5 (1-5, 6-10, 11-15,16-20).
Separation of epidermis and dermis	Yes

*The number of strips is detailed in Table 6.

7.3. Measurement before the application

The receipt, storage and destruction of human skin samples were performed in accordance with Eurofins ADME Bioanalyses SOP ETU/PEN/001P.

Before application, the following parameters were measured and the acceptance criteria are specified in the following table.

	Acceptance criteria	Measures
Room temperature	Between 20 and 25°C	Between 21.1 and 22.20°C
Relative humidity	Between 30 and 70%	Between 55.5 and 64.7%
TEWL for abdomen skin	Between 0.5 and 13 g/m²/h	Between 6.56 and 11.94 g/m²/h
Cutaneous temperature	32 ± 1°C	Between 31.7 and 32.8 °C
Mean full thickness of each skin sample (µm)	-	Between 356 - 396 µm

The results are presented in Table 4.

The skin samples for all cells were in the acceptance criteria.

Skin characteristics are provided in Table 5. Fresh human skin obtained from abdominal surgery were used. Skins were provided by private hospital.

Skins were transferred to the laboratory within 4 hours of excision (when possible), stored at 4°C until use, and used within 24 hours of excision. Under these conditions, metabolism is considered to be maintained. Exceptions were referenced as deviations and detailed in section 3 of the current report.

7.4. Preparation of diffusion cells

Application area: 1cm²

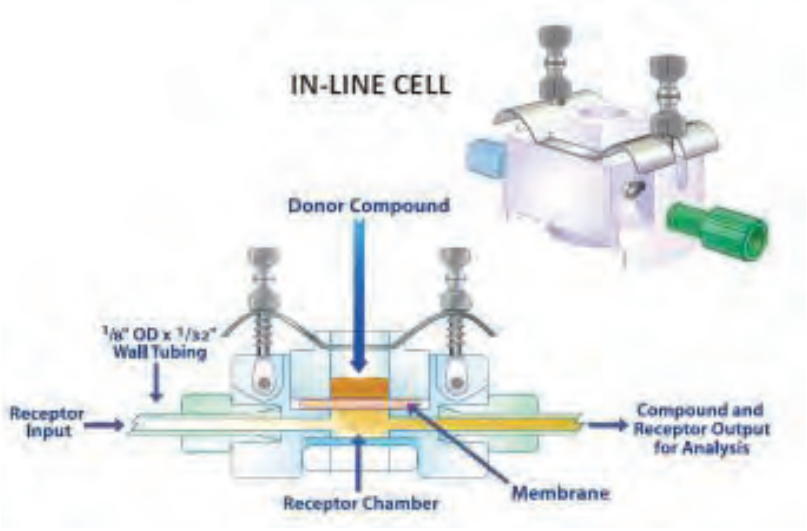
Flow: 1 mL/h.

A flow-through diffusion cell system (developed by Bronaugh), also called dynamic cells or in line cells, was used for the study.

Flow-through cells are characterized by a continuously replaced receptor fluid. Receptor fluid was continually replaced using tubes and a suitable pump (at the flow rate specified above).

The dynamic cells used corresponded to an application area equal to 1 cm² with donor chamber diameter of 11.28 mm.

Each apparatus of in-line (dynamic) cells was composed of seven cells. Cells were identified by a letter.



The skin samples were placed on the receptor compartment and the donor compartment were placed onto the skin samples. A clamp was placed to seal both compartments and the cell was tilted before being placed in the chamber to evacuate any air bubbles in the system.

The skin surface temperature was maintained at 32°C ± 1°C, using a static water bath integrated as part of the dynamic system.

After at least 30 minutes of stabilization, the integrity of the skin was verified using the TEWL method.

The donor compartment was not closed (non-occlusive system).

7.5. Start of the experiment

The experiment was start immediately after the application of the formulation on the skin surface.

The experiment was stopped around 24 hours after application experimental start.

Time was noted in the raw data.

7.6. Application of the formulation

The formulation was homogeneously applied on each skin at a rate of 10 µL/cell.

The application was performed by using a positive displacement pipette and the exact amount applied was determined by weight then recorded.

Before application, the positive displacement pipette containing the volume to be applied was weighed (P1). Then the empty device was weighed after application (P2). The applied amount (Px) corresponded to: Px = P1 – P2.

7.7. Sampling of receptor fluid

The receptor fluid was collected at 6 timepoints: 0.5h, 1h, 2h, 4h, 6h and 24h after application.

Receptor fluid samples were precisely weighed and were stored at -20°C before and after the analysis.

7.8. Washing and Dismantling of cells

24 hours after the start of experiment, the formulation excess on the skin was removed and cells were dismantled.

- Washing of the application site:

3 half cotton buds wetted with water containing 10% of Tween 80,

2 dry half cotton buds, were used to remove the excess of formulation at the surface of the skin.

Cells and the different compartments were then dismantled.

The washing samples were placed in one vial and should be stored at -20°C before and after the analysis according to the analytical method PKH/MOA/1745.

- Washing of the donor compartment (RCD)

The donor compartment and the upper seal were placed in a flask and target 25 mL of Methanol was added.

The closed flask was shaken at least 2h at room temperature and stored at -20°C before and after the analysis according to the analytical method for Strips in the analytical method PKH/MOA/1745.

Lower seal was discarded.

- Strips treatment (ST)

The skin was taken with tweezers and each skin sample was placed between seal and aluminum foil.

The *stratum corneum* was taken off from the skin specimen using Scotch Magic 3M by stripping. In order to standardize stripping, a weight of at least 50 g/cm^2 was placed on top of the Scotch tape for at least 10 s before taking off.

A maximum of 20 strips was performed on each skin and were pooled by 5 (1-5, 6-10, 11-15,16-20).

If a sample contains less than 5 strips, the sample were completed with blank adhesive tape until the number of 5 strips is reached.

The strips samples were stored with Methanol at -20°C before and after the analysis according to the analytical method PKH/MOA/1745.

- Epidermis (E), Dermis (D), remaining skin (RS) treatment

Using the scalpel blade, the skin corresponding to the application area was separated from the remaining (surrounding) skin (corresponding to the non-application area). The epidermis and partial dermis were separated using the scalpel blade and then placed in the corresponding vials.

The remaining skin (RS) was cut in four parts.

The Epidermis, Dermis and Remaining skin flasks were stored at -20°C with Methanol according to the analytical method PKH/MOA/1745.

The samples were analyzed with the analytical method qualified for Skin.

- Receptor fluid (RF)

The receptor fluid was collected at 6 timepoints after application, as specified above in section §8.2 Experimental Design.

Receptor fluid samples were precisely weighed and were stored at -20°C before and after the analysis according to the analytical method PKH/MOA/1745.

All the samples generated during the study will be destroyed after the approval of the final report.

8. Analysis of samples

Samples analysis were performed with the method PKH/MOA/1745, qualified in study 24-0055A..

The final extract was analysed by LC-MS/MS.

One stock solution of Isopropyl Cloprostenate and one stock solution of Cloprostenol were prepared for calibration standards and QCs.

8.1. Calibration curves

8 calibration standards were freshly prepared for the quantification according to corresponding analytical method.

All results were used for deviations calculation except those rejected for defined analytical problem.

Fresh calibration standards, prepared in Methanol solvent, were used for the quantification.

The ranges were (in accordance with the analytical method PKH/MOA/1745) :

- **Washing**: For Isopropyl cloprostenate: 1 - 100 ng/mL
  Corresponding to target 2 - 200% of the applied dose of Isopropyl cloprostenate for the formulation at 0.005% w/w.

  For Cloprostenol (metabolite): 0.5 - 50 ng/mL
  Expected extraction volume = 10 mL

- **Strips**: For Isopropyl cloprostenate: 0.1 - 50 ng/mL
  Corresponding to target 0.1% - 50% of the applied dose for the formulation at 0.005% w/w.
  Expected extraction volume = 5 mL

  For Cloprostenol (metabolite): 0.25 - 125 ng/mL

- **Epidermis and Dermis**: For Isopropyl cloprostenate: 0.05 - 25 ng/mL
  Expected extraction volume = 5 mL
  Corresponding to target 0.05% - 25% of the applied dose for the formulation at 0.005% w/w.

  For Cloprostenol (metabolite): 1 - 500 ng/mL

- **Receptor fluid:** For Isopropyl cloprostenate and Cloprostenol (metabolite): 1 to 500 ng/mL
  Expected Bronaugh cells volume collected around 24 mL (flow rate of 1 mL/h)
  Corresponding to target 4.8 - 2404.8% of the applied dose for the formulation at 0.005% w/w.

The **Epidermis, Dermis and Remaining Skin** were analysed with the analytical method qualified for Skin.

The **RCD** (donor compartment washing) were analysed with the analytical method qualified for Strips.

Acceptance criteria:

- *deviation for the calibration standards were ranged between ± 25.00% of the nominal value for all concentrations (See section 3 of current report).*
- *LLOQ and ULOQ were included in the calibration curve, but if the LLOQ or the ULOQ were outside the acceptance criteria, the calibration standard after the LLOQ became the new LLOQ and the calibration standard just before the ULOQ became the new ULOQ.*

– *25% calibration standards can be excluded from the final calibration curve if the back calculated deviation is not comprised within ± 20.00% for all concentrations.*

Detailed results

The results obtained for regression parameters and calibration standards are presented in:

- Table 9 for washing solution,
- Table 11 for skin,
- Table 13 for strips,
- Table 15 for receptor fluid.

8.2. Quality control samples

In order to check the quality of the method during the sample assays, quality control samples were included in the run.

The nominal value was used to determine the difference with the measured value of the QC.

The quality control samples were prepared in duplicate at 3 LLOQ, 0.5 ULOQ and 0.8 ULOQ

Acceptance criteria:

– *Deviation of QCs samples were calculated and had to be within ± 25.00% (See section 3 of current report).*

– *at least 67% of QC samples should meet the criteria above.*

Detailed results

All the QC samples were prepared in the corresponding matrix.

The QC results are presented in:

- Table 10 for washing solution,
- Table 12 for skin,
- Table 14 for strips,
- Table 16 for receptor fluid.

The QC samples for all series were in the acceptance criteria validating the concentration results.

8.3. Assay organization

Series of assay consisted of:

- 1 Blank matrix not spiked (S0).
- 8 calibration standards different from zero prepared on the same day in the matrix.
- In case of carry-over determinate during qualification study: at least 1 Blank matrix not spiked (S0).
- 2 Quality Controls.
- The samples to be assayed.
- 2 Quality Controls.
- The samples to be assayed.
- 2 Quality Controls.
- 1 Blank matrix not spiked (S0) (was determined upon completion of the qualification study)
- At least 3 calibration standards different from zero prepared on the same day in the matrix.

9. DETERMINATION OF THE CONCENTRATIONS

Concentrations were calculated using Watson® 7.5 SP1 (HF1) directly from peak area obtained after automatic integration of chromatograms by LabSolutions V6.60 or Analyst 1.6.3.

Concentrations of the QC samples were calculated by interpolation with the weighted calibration curves prepared in the same conditions and assessed daily after automatic integration. Results of concentrations, once accepted, are expressed in “ng/mL”.

Concentrations were reported with 5 significant figures.

10. RESULTS

10.1. Absorption

The absorption was equal to: Receptor fluid + Skin (Dermis and Epidermis without *stratum corneum*).

For each cell, the absorption of Isopropyl cloprostenate in ng/cm² and in % of the applied dose were calculated.

Those results were presented with at least 2 decimals.

Results below the limit of quantitation (BLQ) were replaced by the lower limit of quantification (LLOQ) values in the results tables, following Sponsor’s request.

Detailed results Individual results are presented in Table 7 for [redacted] formulation.

10.2. Recovery

For each experiment cell, a mass-balance was calculated.

The mean mass balance value for each formulation were in the acceptance criteria 100±20%.

Results (Mean ± SD) of Isopropyl cloprostenate cutaneous distribution expressed as % of applied dose and in µg/cm^2 (cm² of skin surface) are presented in following tables:

Table 1. Distribution in all compartments in % of the applied dose

Compound	**Isopropyl cloprostenate**		**Cloprostenol**	
Formulation name	[redacted]		[redacted]	
Number of cells	**n=12**		**n=12**	
Theoretical compound concentration in % W/W	0.005% Isopropyl Cloprostenate, 0% Cloprostenol			
	Mean	**SD**	**Mean**	**SD**
Strips	0.91	0.61	0.32	0.12
Epidermis	1.67	1.12	0.89	0.04
Dermis	0.05	0.03	0.89	0.04
Receptor fluid	4.61	0.30	4.61	0.30
Epidermis + Dermis + receptor fluid	6.34	1.20	6.40	0.37
Skin excess	109.27	15.72	2.90	0.13
Total Recovery	116.52	14.63	9.62	0.53

Skin excess = washing + rinsing of donor compartment + remaining skin
NC: Not Calculated
Values in bold front were BLQ. The equivalent mass value at the LLOQ is presented.
The Cloprostenol percentages are calculated relative to the applied amount of Isopropyl Cloprostenate.

Table 2. Distribution in all compartments in µg/cm² of skin

Compound	**Isopropyl cloprostenate**		**Cloprostenol**	
Formulation name	[redacted]		[redacted]	
Number of cells	**n=12**		**n=12**	
Theoretical compound concentration in % W/W	0.005% Isopropyl Cloprostenate, 0% Cloprostenol			
	Mean	**SD**	**Mean**	**SD**
Applied amount of Isopropyl cloprostenate	0.56	0.02	0.56	0.02
Strips	0.01	0.003	0.002	0.001
Epidermis	0.01	0.01	0.01	0.00
Dermis	0.0003	0.0002	0.01	0.00
Receptor fluid	0.03	0.001	0.03	0.001
Epidermis + Dermis + receptor fluid	0.04	0.01	0.04	0.001
Skin excess	0.61	0.10	0.02	0.00

Skin excess = washing + rinsing of donor compartment + remaining skin
NC: Not Calculated
Values in bold front were BLQ. The equivalent mass value at the LLOQ is presented.
BLQ: Below the lower limit of quantification, replaced by the Lower Limit of Quantification value for the calculation.

11. CONCLUSION

The objective of this study was to evaluate the skin absorption and distribution of Isopropyl cloprostenate from one formulation [redacted], containing Isopropyl cloprostenate at 0.005% w/w applied in vitro on freshly-excised human skin during 24h.

The theoretical Isopropyl cloprostenate concentration was used to perform calculations.

The analytical method using LC-MS/MS and referenced as PKH/MOA/1745 for the quantification of Isopropyl cloprostenate in the different generated samples was qualified in study 24-0055A. Values inferior to the Lower Limit of Quantification (BLQ) were replaced by the LLOQ value for calculations after discussion with the Sponsor.

For each cell, a mass-balance was calculated. The individual total recoveries were between 88.76% and 139.15%. The applications were performed on different occasions according to fresh human skin availability. For the last 3 cells applied (P, Q, R), the individual calculated recoveries were higher than 120%, which may be explained by the following hypothesis: the same tube of formulation was used for the applications of cells G to R. Consequently, the last 3 cells were applied with the last remaining of formulation in this tube. Isopropyl cloprostenate may have slightly concentrated because of precipitation or volatility of the other solvent.

The mean mass-balance value obtained (116.52% ± 14.63) was within the acceptance criteria, 100 ± 20% validating the experimentation.

The amount of compound in the epidermis + dermis + receptor fluid compartments in µg/cm² is estimated to:

- 0.04 ± 0.01 µg/cm² for [redacted] Isopropyl cloprostenate
- 0.04 ± 0.01 µg/cm² for [redacted] Cloprostenol

Isopropyl cloprostenate was mainly detected in the washing of the formulation (skin excess, mean 109.27 ± 15.72%) and poorly detected in epidermis (mean 1.67 ± 1.12%) and dermis (mean 0.05 ± 0.03%).

Isopropyl cloprostenate was not detected in the receptor fluid. Only two values were observed in the receptor fluid (cell H, Receptor fluid, 1 hour and cell M, Receptor fluid, 24 hours). In these samples, it should be taken into account that the measured concentrations were close to the lower limit of quantification of 1 ng/mL (1.1887 ng/mL and 1.0101 ng/mL respectively). The values presented in the result tables take into account the volume of receptor fluid collected at an interval of time. Both data (cell H, Receptor fluid, 1 hour and cell M, Receptor fluid, 24 hours) are given for information only, taking into account the analytical variability and the limit of quantification.

Cloprostenol was not detected in the samples, suggesting poor metabolism of Isopropyl cloprostenate into Cloprostenol or metabolism into Cloprostenol at levels not detectable due to the sensitivity of the analytical method. This may be correlated with the low skin penetration of Isopropyl cloprostenate.

Taken together, the results suggest that Isopropyl cloprostenate in [redacted] formulation poorly penetrated the skin, which is also consistent with no detectable level of Cloprostenol.

12. ARCHIVING PROCEDURE

All the documentation relative to this study will be archived as described in the Protocol (APPENDIX B).

APPENDIX A

TABLES OF RESULTS

A summary of accepted series included in the study is presented below:

Table 3. Summary of analytical runs

Calibration curve	Watson run ID	Biological Matrix	Tests performed	Results
G120724-W-CLO	44	Washing	Assay Washing	Accepted
G120724-LR-CLO	45	Receptor fluid	Assay LR	Accepted
G120724-W-ISOCLO	46	Washing	Assay Washing	Accepted
G120724-LR-ISOCLO	47	Receptor fluid	Assay LR	Accepted
G150724-RCD-CLO	48	Strips	Assay Strip	Accepted
G150724-RCD-ISOCLO	49	Strips	Assay Strip	Accepted
G150724-ST-ISOCLO	50	Strips	Assay Strip	Accepted
G150724-ST-CLO	51	Strips	Assay Strip	Accepted
G150724-Skin-CLO	52	Skin	Assay Skin	Accepted
G150724-Skin-ISOCLO	53	Skin	Assay Skin	Accepted
G190727-RF-CLO	56	Receptor fluid	Assay LR	Accepted
G190727-RF-IC	57	Receptor fluid	Assay LR	Accepted
G190724-W-CLO	58	Washing	Assay Washing	Accepted
G190724-W-IC	59	Washing	Assay Washing	Accepted
G220724-Skin-CLO	60	Skin	Assay Skin	Accepted
G220724-Skin-ISOCLO	61	Skin	Assay Skin	Accepted
G220724-ST-CLO	62	Strips	Assay Strip	Accepted
G220724-ST-ISOCLO	63	Strips	Assay Strip	Accepted
G220724-RCD-CLO	64	Strips	Assay Strip	Accepted
G220724-RCD-ISOCLO	65	Strips	Assay Strip	Accepted
G300824-RF-CLO	66	Receptor fluid	Assay LR	Accepted
G300824-RF-ISOCLO	67	Receptor fluid	Assay LR	Accepted
G300824-W-CLO	68	Washing	Assay Washing	Accepted
G300824-W-ISOCLO	69	Washing	Assay Washing	Accepted
G020924-RCD-CLO	70	Strips	Assay Strip	Accepted
G020924-RCD-ISOCLO	71	Strips	Assay Strip	Accepted
G020924-ST-CLO	72	Strips	Assay Strip	Accepted
G020924-ST-ISOCLO	73	Strips	Assay Strip	Accepted
G020924-Skin-CLO	74	Skin	Assay Skin	Accepted
G020924-Skin-ISOCLO	75	Skin	Assay Skin	Accepted

Rejected or failed runs, not included in the study results, are not presented in the table above.

Table 4. Thickness, TEWL and skin surface temperature for application of [redacted] formulation

Formulation	[redacted]											
Matrix	Dermatomed Human Skin											
Cells	G	H	I	J	K	L	M	N	O	P	Q	R
Room Temperature (°C)	appli 1: min 21.10°C - max 21.20°C / appli 2: min 21.1°C - max 21.5°C / appli 3: min 22.10°C - max 22.20°C											
Relative Humidity (%)	appli 1: min 63.4% - max 64.70% / appli 2: min 55.5% - max 56% / appli 3: min 57.10% - max 57.80%											
TEWL (g/m²/h)	11.94	11.04	9.57	6.83	6.73	6.56	10.35	7.46	8.00	7.87	8.44	6.95
Mean	8.48											
SD	1.83											
Cutaneous temperature (°C)	31.8	32.8	32.3	31.7	31.9	32.1	32.4	32.2	32.3	32.4	32.3	32.3
Mean	32.21											
SD	0.30											
Thickness µm	396	372	366	382	396	356	394	382	386	396	390	376
Mean	382.67											
SD	13.05											

Table 5. Human skin donor's identification

HUMAN DONOR IDENTIFICATION	ORIGIN	DATE OF REMOVAL	AGE (Year)	GENDER	ANATOMICAL SITE	Supplier
1641	Caucasian	10/07/2024	37	F	Abdominal	Private hospital
1642	Non-Caucasian	17/07/2024	47	F	Abdominal	Private hospital
1648	Caucasian	28/08/2024	31	F	Abdominal	Private hospital
1649	Caucasian	28/08/2024	54	F	Abdominal	Private hospital

Table 6. Number of strips performed

Formulation	Cell	Number of strips
[redacted]	G	4
	H	4
	I	1
	J	8
	K	7
	L	7
	M	6
	N	4
	O	2
	P	6
	Q	4
	R	2

Table 7. Individual results obtained after Isopropyl cloprostenate application on human skin with [redacted]

Skin donor identification	1641	1641	1641	1642	1642	1642	1648	1648	1648	1649	1649	1649			
Application area (cm²)	1	1	1	1	1	1	1	1	1	1	1	1			
Cell	G	H	I	J	K	L	M	N	O	P	Q	R			
Amount applied (mg)	10.90	11.10	11.20	10.60	10.90	10.90	11.60	12.00	11.50	12.00	11.30	10.50	11.21	0.49	4.39
Amount applied (mg/cm²)	10.90	11.10	11.20	10.60	10.90	10.90	11.60	12.00	11.50	12.00	11.30	10.50	11.21	0.49	4.39
Test substance applied (ng)	545.00	555.00	560.00	530.00	545.00	545.00	580.00	600.00	575.00	600.00	565.00	525.00	560.42	24.63	4.39
Test substance applied (µg/cm²)	0.54	0.55	0.56	0.53	0.54	0.54	0.58	0.60	0.57	0.60	0.56	0.53	0.56	0.02	4.39
Strips (1) (ng)	6.83	14.74	2.89	3.06	2.99	1.82	6.24	5.72	3.06	3.08	4.02	2.46	4.74	3.53	74.33
Strips (2) (ng)	NS	NS	NS	1.35	1.07	0.83	0.50	NS	NS	0.50	NS	NS	0.85	0.37	43.42
Strips (3) (ng)	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NC	NC	NC
Strips (4) (ng)	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NC	NC	NC
Strips total (ng)	6.8280	14.7420	2.8948	4.4133	4.0641	2.6486	6.7400	5.7200	3.0633	3.5756	4.0237	2.4559	5.0974	3.3854	66.41
Strips (µg/cm²)	0.01	0.01	0.003	0.004	0.004	0.003	0.01	0.01	0.003	0.004	0.004	0.002	0.01	0.003	66.41
Strips (%)	1.25	2.66	0.52	0.83	0.75	0.49	1.16	0.95	0.53	0.60	0.71	0.47	0.91	0.61	66.92
Epidermis (ng)	14.9810	10.0400	25.4645	3.7082	3.6149	4.1826	13.8960	8.1965	8.5460	4.4416	7.6370	7.8870	9.3829	6.2891	67.03
Epidermis (µg/cm²)	0.01	0.01	0.03	0.00	0.00	0.00	0.01	0.01	0.01	0.00	0.01	0.01	0.01	0.01	67.03
Epidermis (%)	2.75	1.81	4.55	0.70	0.66	0.77	2.40	1.37	1.49	0.74	1.35	1.50	1.67	1.12	67.13
Dermis (ng)	0.25	0.25	0.93	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.31	0.20	63.85
Dermis (µg/cm²)	**0.0003**	**0.0003**	0.0009	**0.0003**	**0.0003**	**0.0003**	**0.0003**	**0.0003**	**0.0003**	**0.0003**	**0.0003**	**0.0003**	0.0003	0.0002	63.85
Dermis (%)	**0.05**	**0.05**	0.17	**0.05**	**0.05**	**0.05**	**0.04**	**0.04**	**0.04**	**0.04**	**0.04**	**0.05**	0.05	0.03	63.87
Epidermis + Dermis (µg/cm²)	0.02	0.01	0.03	0.00	0.00	0.00	0.01	0.01	0.01	0.00	0.01	0.01	0.01	0.01	66.54
Epidermis + Dermis (%)	2.79	1.85	4.71	0.75	0.71	0.81	2.44	1.41	1.53	0.78	1.40	1.55	1.73	1.15	66.64
Receptor fluid (ng) 0.5	**0.51**	**0.52**	**0.52**	**0.55**	**0.52**	**0.53**	**0.56**	**0.53**	**0.51**	**0.48**	**0.52**	**0.52**	0.52	0.02	3.79
Receptor fluid (%) 0.5	**0.09**	**0.09**	**0.09**	**0.10**	**0.09**	**0.10**	**0.10**	**0.09**	**0.09**	**0.08**	**0.09**	**0.10**	0.09	0.01	6.28
Receptor fluid (ng) 1	**0.49**	0.56	**0.48**	**0.52**	**0.53**	**0.54**	**0.53**	**0.53**	**0.54**	**0.50**	**0.52**	**0.53**	0.52	0.02	4.52
Receptor fluid (%) 1	**0.09**	0.10	**0.09**	**0.10**	**0.10**	**0.10**	**0.09**	**0.09**	**0.09**	**0.08**	**0.09**	**0.10**	0.09	0.01	6.52
Receptor fluid (ng) 2	**1.08**	**1.06**	**1.08**	**1.11**	**1.07**	**1.12**	**0.96**	**0.90**	**0.95**	**0.89**	**0.93**	**0.93**	1.01	0.09	8.48
Receptor fluid (%) 2	**0.20**	**0.19**	**0.19**	**0.21**	**0.20**	**0.20**	**0.17**	**0.15**	**0.17**	**0.15**	**0.17**	**0.18**	0.18	0.02	11.62
Receptor fluid (ng) 4	**2.21**	**2.14**	**2.19**	**2.27**	**2.16**	**2.29**	**2.07**	**2.00**	**2.08**	**1.93**	**2.06**	**2.03**	2.12	0.11	5.18
Receptor fluid (%) 4	**0.41**	**0.39**	**0.39**	**0.43**	**0.40**	**0.42**	**0.36**	**0.33**	**0.36**	**0.32**	**0.36**	**0.39**	0.38	0.03	8.63
Receptor fluid (ng) 6	**2.16**	**2.07**	**2.14**	**2.17**	**2.06**	**2.00**	**2.08**	**2.03**	**2.07**	**1.96**	**2.07**	**2.03**	2.07	0.06	2.99
Receptor fluid (%) 6	**0.40**	**0.37**	**0.38**	**0.41**	**0.38**	**0.37**	**0.36**	**0.34**	**0.36**	**0.33**	**0.37**	**0.39**	0.37	0.02	6.22
Receptor fluid (ng) 24	**20.34**	**19.22**	**19.41**	**20.37**	**19.10**	**20.06**	19.92	**18.98**	**19.75**	**18.60**	**19.44**	**19.32**	19.54	0.55	2.82
Receptor fluid (%) 24	**3.73**	**3.46**	**3.47**	**3.84**	**3.51**	**3.68**	3.43	**3.16**	**3.44**	**3.10**	**3.44**	**3.68**	3.50	0.22	6.25
Receptor fluid total (ng)	**26.785**	25.582	**25.824**	**26.981**	**25.439**	**26.537**	**26.114**	**24.976**	**25.896**	**24.365**	**25.537**	**25.369**	25.7838	0.7498	2.91
Receptor fluid (µg/cm²)	**0.03**	**0.03**	**0.03**	**0.03**	**0.03**	**0.03**	**0.03**	**0.02**	**0.03**	**0.02**	**0.03**	**0.03**	0.03	0.001	2.91
Receptor fluid (%)	**4.91**	4.61	**4.61**	**5.09**	**4.67**	**4.87**	**4.50**	**4.16**	**4.50**	**4.06**	**4.52**	**4.83**	4.61	0.30	6.46
Epidermis + dermis + receptor fluid (ng)	42.016	35.872	52.216	30.9396	29.3040	30.9693	40.2603	33.4228	34.6917	29.0567	33.4238	33.5056	35.4732	6.6001	18.61
Epidermis + dermis + receptor fluid (µg/cm²)	0.04	0.04	0.05	0.031	0.029	0.031	0.04	0.03	0.03	0.03	0.03	0.03	0.04	0.01	18.61
Epidermis + dermis + receptor fluid (%)	7.71	6.46	9.32	5.84	5.38	5.68	6.94	5.57	6.03	4.84	5.92	6.38	6.34	1.20	18.95
Strip + Epidermis + Dermis + Receptor Fluid (ng)	48.844	50.614	55.111	35.3528	33.3681	33.6179	47.000	39.143	37.755	32.6323	37.448	35.962	40.571	7.708	19.00
Strip + Epidermis + Dermis + Receptor Fluid (µg/cm²)	0.05	0.05	0.06	0.04	0.03	0.03	0.05	0.04	0.04	0.03	0.04	0.04	0.04	0.01	19.00
Strip + Epidermis + Dermis + Receptor Fluid (%)	8.96	9.12	9.84	6.67	6.12	6.17	8.10	6.52	6.57	5.44	6.63	6.85	7.25	1.40	19.27
Washing (ng)	491.05	439.24	486.91	576.67	618.48	591.69	677.88	686.68	639.59	745.78	673.51	691.84	609.94	95.59	15.67
Donor Compartment Rinsing (ng)	**2.50**	**2.50**	**2.50**	**2.50**	**2.50**	**2.50**	**2.50**	**2.50**	**2.50**	**2.50**	5.10	**2.50**	2.72	0.75	27.61
Remaining Skin (ng)	0.29	**0.25**	**0.25**	**0.25**	**0.25**	**0.25**	**0.25**	**0.25**	**0.25**	**0.25**	**0.25**	**0.25**	0.25	0.01	4.44
Skin Excess (ng)	493.84	441.99	489.66	579.42	621.23	594.44	680.63	689.43	642.34	748.53	678.86	694.59	612.91	95.74	15.62
Skin Excess (µg/cm²)	0.49	0.44	0.49	0.58	0.62	0.59	0.68	0.69	0.64	0.75	0.68	0.69	0.61	0.10	15.62
Skin Excess (%)	90.61	79.64	87.44	109.32	113.99	109.07	117.35	114.90	111.71	124.75	120.15	132.30	109.27	15.72	14.38
Recovery (%)	99.57	88.76	97.28	115.99	120.11	115.24	125.45	121.43	118.28	130.19	126.78	139.15	116.52	14.63	12.56

NS: No Sample
NC: Not Calculated
BLQ (Below the lower limit of quantification) values replaced by Lower Limit of Quantification values (in bold).

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Table 8. Individual results obtained after Cloprostenol application on human skin with [redacted]

Skin donor identification	1641	1641	1641	1642	1642	1642	1648	1648	1648	1649	1649	1649			
Application area (cm²)	1	1	1	1	1	1	1	1	1	1	1	1			
Cell	G	H	I	J	K	L	M	N	O	P	Q	R			
Amount applied (mg)	10.90	11.10	11.20	10.60	10.90	10.90	11.60	12.00	11.50	12.00	11.30	10.50	11.21	0.49	4.39
Amount applied (mg/cm²)	10.90	11.10	11.20	10.60	10.90	10.90	11.60	12.00	11.50	12.00	11.30	10.50	11.21	0.49	4.39
Test substance applied (ng)	545.00	555.00	560.00	530.00	545.00	545.00	580.00	600.00	575.00	600.00	565.00	525.00	560.42	24.63	4.39
Test substance applied (µg/cm²)	0.54	0.55	0.56	0.53	0.54	0.54	0.58	0.60	0.57	0.60	0.56	0.53	0.56	0.02	4.39
Strips (1) (ng)	**1.25**	**1.25**	**1.25**	**1.25**	**1.25**	**1.25**	**1.25**	**1.25**	**1.25**	**1.25**	**1.25**	**1.25**	**1.25**	NC	NC
Strips (2) (ng)	NS	NS	NS	1.25	1.25	1.25	1.25	NS	NS	1.25	NS	NS	1.25	NC	NC
Strips (3) (ng)	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NC	NC	NC
Strips (4) (ng)	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NC	NC	NC
Strips total (ng)	**1.25**	**1.25**	**1.25**	**2.50**	**2.50**	**2.50**	**2.50**	**1.25**	**1.25**	**2.50**	**1.25**	**1.25**	**1.77**	0.64	36.35
Strips (µg/cm²)	**0.001**	**0.001**	**0.001**	**0.003**	**0.003**	**0.003**	**0.003**	**0.001**	**0.001**	**0.003**	**0.001**	**0.001**	**0.002**	0.001	36.35
Strips (%)	**0.23**	**0.23**	**0.22**	**0.47**	**0.46**	**0.46**	**0.43**	**0.21**	**0.22**	**0.42**	**0.22**	**0.24**	**0.32**	0.12	36.76
Epidermis (ng)	**5.00**	**5.00**	**5.00**	**5.00**	**5.00**	**5.00**	**5.00**	**5.00**	**5.00**	**5.00**	**5.00**	**5.00**	**5.00**	NC	NC
Epidermis (µg/cm²)	**0.01**	**0.01**	**0.01**	**0.01**	**0.01**	**0.01**	**0.01**	**0.01**	**0.01**	**0.01**	**0.01**	**0.01**	**0.01**	0.00	0.00
Epidermis (%)	**0.92**	**0.90**	**0.89**	**0.94**	**0.92**	**0.92**	**0.86**	**0.83**	**0.87**	**0.83**	**0.88**	**0.95**	**0.89**	0.04	4.35
Dermis (ng)	**5.00**	**5.00**	**5.00**	**5.00**	**5.00**	**5.00**	**5.00**	**5.00**	**5.00**	**5.00**	**5.00**	**5.00**	**5.00**	NC	NC
Dermis (µg/cm²)	**0.005**	**0.005**	**0.005**	**0.005**	**0.005**	**0.005**	**0.005**	**0.005**	**0.005**	**0.005**	**0.005**	**0.005**	**0.01**	0.00	0.00
Dermis (%)	**0.92**	**0.90**	**0.89**	**0.94**	**0.92**	**0.92**	**0.86**	**0.83**	**0.87**	**0.83**	**0.88**	**0.95**	**0.89**	0.04	4.35
Epidermis + Dermis (µg/cm²)	**0.01**	**0.01**	**0.01**	**0.01**	**0.01**	**0.01**	**0.01**	**0.01**	**0.01**	**0.01**	**0.01**	**0.01**	**0.01**	0.00	0.00
Epidermis + Dermis (%)	**1.83**	**1.80**	**1.79**	**1.89**	**1.83**	**1.83**	**1.72**	**1.67**	**1.74**	**1.67**	**1.77**	**1.90**	**1.79**	0.08	4.35
Receptor fluid (ng) 0.5	**0.51**	**0.52**	**0.52**	**0.55**	**0.52**	**0.53**	**0.56**	**0.53**	**0.51**	**0.48**	**0.52**	**0.52**	**0.52**	0.02	3.79
Receptor fluid (%) 0.5	**0.09**	**0.09**	**0.09**	**0.10**	**0.09**	**0.10**	**0.10**	**0.09**	**0.09**	**0.08**	**0.09**	**0.10**	**0.09**	0.01	6.28
Receptor fluid (ng) 1	**0.49**	**0.47**	**0.48**	**0.52**	**0.53**	**0.54**	**0.53**	**0.53**	**0.54**	**0.50**	**0.52**	**0.53**	**0.52**	0.02	4.72
Receptor fluid (%) 1	**0.09**	**0.09**	**0.09**	**0.10**	**0.10**	**0.10**	**0.09**	**0.09**	**0.09**	**0.08**	**0.09**	**0.10**	**0.09**	0.01	6.51
Receptor fluid (ng) 2	**1.08**	**1.06**	**1.08**	**1.11**	**1.07**	**1.12**	**0.96**	**0.90**	**0.95**	**0.89**	**0.93**	**0.93**	**1.01**	0.09	8.48
Receptor fluid (%) 2	**0.20**	**0.19**	**0.19**	**0.21**	**0.20**	**0.20**	**0.17**	**0.15**	**0.17**	**0.15**	**0.17**	**0.18**	**0.18**	0.02	11.62
Receptor fluid (ng) 4	**2.21**	**2.14**	**2.19**	**2.27**	**2.16**	**2.29**	**2.07**	**2.00**	**2.08**	**1.93**	**2.06**	**2.03**	**2.12**	0.11	5.18
Receptor fluid (%) 4	**0.41**	**0.39**	**0.39**	**0.43**	**0.40**	**0.42**	**0.36**	**0.33**	**0.36**	**0.32**	**0.36**	**0.39**	**0.38**	0.03	8.63
Receptor fluid (ng) 6	**2.16**	**2.07**	**2.14**	**2.17**	**2.06**	**2.00**	**2.08**	**2.03**	**2.07**	**1.96**	**2.07**	**2.03**	**2.07**	0.06	2.99
Receptor fluid (%) 6	**0.40**	**0.37**	**0.38**	**0.41**	**0.38**	**0.37**	**0.36**	**0.34**	**0.36**	**0.33**	**0.37**	**0.39**	**0.37**	0.02	6.22
Receptor fluid (ng) 24	**20.34**	**19.22**	**19.41**	**20.37**	**19.10**	**20.06**	**19.72**	**18.98**	**19.75**	**18.60**	**19.44**	**19.32**	**19.53**	0.54	2.77
Receptor fluid (%) 24	**3.73**	**3.46**	**3.47**	**3.84**	**3.51**	**3.68**	**3.40**	**3.16**	**3.44**	**3.10**	**3.44**	**3.68**	**3.49**	0.22	6.28
Receptor fluid total (ng)	**26.79**	**25.49**	**25.82**	**26.98**	**25.44**	**26.54**	**25.92**	**24.98**	**25.90**	**24.37**	**25.54**	**25.37**	**25.76**	0.75	2.90
Receptor fluid (µg/cm²)	**0.03**	**0.03**	**0.03**	**0.03**	**0.03**	**0.03**	**0.03**	**0.02**	**0.03**	**0.02**	**0.03**	**0.03**	**0.03**	0.00	2.90
Receptor fluid (%)	**4.91**	**4.59**	**4.61**	**5.09**	**4.67**	**4.87**	**4.47**	**4.16**	**4.50**	**4.06**	**4.52**	**4.83**	**4.61**	0.30	6.50
Epidermis + dermis + receptor fluid (ng)	**36.79**	**35.49**	**35.82**	**36.98**	**35.44**	**36.54**	**35.92**	**34.98**	**35.90**	**34.37**	**35.54**	**35.37**	**35.76**	0.75	2.09
Epidermis + dermis + receptor fluid (µg/cm²)	**0.04**	**0.04**	**0.04**	**0.04**	**0.04**	**0.04**	**0.04**	**0.03**	**0.04**	**0.03**	**0.04**	**0.04**	**0.04**	0.001	2.09
Epidermis + dermis + receptor fluid (%)	**6.750**	**6.395**	**6.397**	**6.978**	**6.503**	**6.704**	**6.192**	**5.829**	**6.243**	**5.728**	**6.290**	**6.737**	**6.40**	0.37	5.83
Strip + Epidermis + Dermis + Receptor Fluid (ng)	**38.04**	**36.74**	**37.07**	**39.48**	**37.94**	**39.04**	**38.42**	**36.23**	**37.15**	**36.87**	**36.79**	**36.62**	**37.53**	1.04	2.76
Strip + Epidermis + Dermis + Receptor Fluid (µg/cm²)	**0.04**	**0.04**	**0.04**	**0.04**	**0.04**	**0.04**	**0.04**	**0.04**	**0.04**	**0.04**	**0.04**	**0.04**	**0.04**	0.00	2.76
Strip + Epidermis + Dermis + Receptor Fluid (%)	**6.98**	**6.62**	**6.62**	**7.45**	**6.96**	**7.16**	**6.62**	**6.04**	**6.46**	**6.14**	**6.51**	**6.97**	**6.71**	0.41	6.12
Washing (ng)	**5.00**	**5.00**	**5.00**	**5.00**	**5.00**	**5.00**	**5.00**	**5.00**	**5.00**	**5.00**	**5.00**	**5.00**	**5.00**	NC	NC
Donor Compartment Rinsing (ng)	**6.25**	**6.25**	**6.25**	**6.25**	**6.25**	**6.25**	**6.25**	**6.25**	**6.25**	**6.25**	**6.25**	**6.25**	**6.25**	NC	NC
Remaining Skin (ng)	**5.00**	**5.00**	**5.00**	**5.00**	**5.00**	**5.00**	**5.00**	**5.00**	**5.00**	**5.00**	**5.00**	**5.00**	**5.00**	NC	NC
Skin Excess (ng)	**16.25**	**16.25**	**16.25**	**16.25**	**16.25**	**16.25**	**16.25**	**16.25**	**16.25**	**16.25**	**16.25**	**16.25**	**16.25**	NC	NC
Skin Excess (µg/cm²)	**0.02**	**0.02**	**0.02**	**0.02**	**0.02**	**0.02**	**0.02**	**0.02**	**0.02**	**0.02**	**0.02**	**0.02**	**0.02**	0.00	0.00
Skin Excess (%)	**2.98**	**2.93**	**2.90**	**3.07**	**2.98**	**2.98**	**2.80**	**2.71**	**2.83**	**2.71**	**2.88**	**3.10**	**2.90**	0.13	4.35
Recovery (%)	**9.96**	**9.55**	**9.52**	**10.52**	**9.94**	**10.14**	**9.43**	**8.75**	**9.29**	**8.85**	**9.39**	**10.07**	**9.62**	0.53	5.49

NS: No Sample
NC: Not Calculated
BLQ (Below the lower limit of quantification) values replaced by Lower Limit of Quantification values (in bold).

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CALIBRATION CURVES AND QC RESULTS OBTAINED DURING THE APPLICATION

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Table 9. Isopropyl cloprostenate regression parameters and back calculated concentrations (ng/mL) for washing

Run Date	Curve Number	Slope	Intercept	R-Squared
12-Jul-2024	46	0.002820	0.0007503	0.9827
19-Jul-2024	59	0.004429	0.0005926	0.9801
30-Aug-2024	69	0.002625	-0.0001947	0.9845

Run Date	Curve Number	S0.5/1 1.0000 ng/mL	%Deviation	S1/2 2.0000 ng/mL	%Deviation	S2.5/5 5.0000 ng/mL	%Deviation	S5/10 10.000 ng/mL	%Deviation	S10/20 20.000 ng/mL	%Deviation	S25/50 50.000 ng/mL	%Deviation	S45/90 90.000 ng/mL	%Deviation	S50/100 100.00 ng/mL	%Deviation
12-Jul-2024	46	1.0313	3.13	2.0327 1.9387	1.64 -3.07	4.7345	-5.31	8.8458 8.9348	-11.54 -10.65	23.210	16.05	46.463	-7.07	103.00 104.00	14.44 15.56	86.821	-13.18
19-Jul-2024	59	1.0608	6.08	1.8890 1.7504	-5.55 -12.48	5.1390	2.78	10.700 11.288	7.00 12.88	23.526	17.63	40.612	-18.78	81.045 80.857	-9.95 -10.16	110.55	10.55
30-Aug-2024	69	1.0179	1.79	1.6648 2.2671	-16.76 13.36	4.9556	-0.89	9.1072 11.468	-8.93 14.68	17.811	-10.95	49.565	-0.87	90.102 **120.76	0.11	108.46	8.46

Reasons Deactivated

* Chromatographic profile not acceptable

** Out of acceptance criteria

Table 10. Isopropyl cloprostenate QC sample values for washing

Run Date	Curve Number	L 3.0000 ng/mL	%Deviation	M 50.000 ng/mL	%Deviation	H 80.000 ng/mL	%Deviation
12-Jul-2024	46	3.4095 ~3.9573	13.65 31.91	59.021 58.332	18.04 16.66	87.257 85.060	9.07 6.33
19-Jul-2024	59	~5.1891 2.6818	72.97 -10.61	53.893 51.532	7.79 3.06	93.402 64.262	16.75 -19.67
30-Aug-2024	69	~3.6980 3.4759	23.27 15.86	51.585 58.836	3.17 17.67	83.281 86.647	4.10 8.31

~ > 20%Deviation

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Table 11. Isopropyl cloprostenate regression parameters and back calculated concentrations (ng/mL) for skin

Run Date	Curve Number	Slope	Intercept	R-Squared
15-Jul-2024	53	53820	8317	0.9974
22-Jul-2024	61	73830	6717	0.9962
02-Sep-2024	75	165500	12730	0.9975

Run Date	Curve Number	S1/0.05 0.050000 ng/mL	%Deviation	S2/0.1 0.10000 ng/mL	%Deviation	S10/0.5 0.50000 ng/mL	%Deviation	S50/2.5 2.5000 ng/mL	%Deviation	S100/5 5.0000 ng/mL	%Deviation	S250/12.5 12.500 ng/mL	%Deviation	S450/22.5 22.500 ng/mL	%Deviation	S500/25 25.000 ng/mL	%Deviation
15-Jul-2024	53	0.050331	0.66	*0.33295		0.45594	-8.81	2.5789	3.16	5.0075	0.15	12.096	-3.23	21.854	-2.87	25.953	3.81
				*0.28193				2.7142	8.57			12.321	-1.43				
22-Jul-2024	61	0.050253	0.51	0.090110	-9.89	0.48041	-3.92	2.6463	5.85	5.2295	4.59	12.539	0.31	22.898	1.77	24.599	-1.60
				0.10943	9.43			2.4676	-1.30			11.782	-5.74				
02-Sep-2024	75	0.047738	-4.52	0.099890	-0.11	0.48446	-3.11	2.5236	0.94	4.8951	-2.10	12.371	-1.03	21.121	-6.13	26.348	5.39
				0.10973	9.73			2.5433	1.73			12.401	-0.79				

Reason Deactivated

* Out of acceptance criteria

Table 12. Isopropyl cloprostenate QC sample values for skin

Run Date	Curve Number	L 0.15000 ng/mL	%Deviation	M 12.500 ng/mL	%Deviation	H 20.000 ng/mL	%Deviation
15-Jul-2024	53	~1.1823	688.20	12.315	-1.48	18.096	-9.52
		~1.1788	685.87	12.782	2.26	20.021	0.11
22-Jul-2024	61	0.13884	-7.44	14.015	12.12	20.359	1.80
		0.13389	-10.74	13.209	5.67	21.158	5.79
02-Sep-2024	75	0.16960	13.07	12.721	1.77	16.457	-17.72
		0.13751	-8.33	10.686	-14.51	20.077	0.39

~ > 25%Deviation

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Table 13. Isopropyl cloprostenate regression parameters and back calculated concentrations (ng/mL) for strips and RCD

Run Date	Curve Number	Slope	Intercept	R-Squared
15-Jul-2024	49	51000	84330	0.9950
15-Jul-2024	50	51140	3856	0.9918
22-Jul-2024	63	72570	4458	0.9971
22-Jul-2024	65	77490	6228	0.9969
02-Sep-2024	71	3595	354.4	0.9876
02-Sep-2024	73	168200	12540	0.9853

Run Date	Curve Number	S0.25/0.1 0.10000 ng/mL	%Deviation	S0.2/0.5 0.20000 ng/mL	%Deviation	S0.5/0.2 0.20000 ng/mL	%Deviation	S5/2 2.0000 ng/mL	%Deviation	S25/10 10.000 ng/mL	%Deviation	S50/20 20.000 ng/mL	%Deviation	S62.5/25 25.000 ng/mL	%Deviation	S112.5/45 45.000 ng/mL	%Deviation	S125/50 50.000 ng/mL	%Deviation
15-Jul-2024	49	*-1.4581				0.22223	11.12	2.0110	0.55	9.2244	-7.76	20.863	4.32	24.927	-0.29	46.870	4.16	51.520	3.04
						0.17803	-10.99			9.4245	-5.76			25.402	1.61				
15-Jul-2024	50	0.10303	3.03			0.18932	-5.34	1.7745	-11.28	11.297	12.97	22.113	10.57	24.936	-0.26	44.697	-0.67	43.120	-13.76
						*0.13148				10.520	5.20			24.884	-0.46				
22-Jul-2024	63	0.10044	0.44			0.18122	-9.39	2.0214	1.07	10.062	0.62	19.895	-0.53	24.276	-2.90	46.585	3.52	47.282	-5.44
						0.21665	8.33			10.517	5.17			24.775	-0.90				
22-Jul-2024	65	0.10362	3.62			0.19336	-3.32	2.1145	5.73	10.179	1.79	20.614	3.07	23.510	-5.96	45.649	1.44	53.016	6.03
						0.19102	-4.49			10.139	1.39			22.675	-9.30				
02-Sep-2024	71	*0.10527				*0.32794		1.8136	-9.32	*19.280		*39.530		23.235	-7.06	40.413	-10.19	49.877	-0.25
						0.20114	0.57			12.123	21.23			26.253	5.01				
02-Sep-2024	73	0.094398	-5.60			0.18090	-9.55	1.8484	-7.58	9.5316	-4.68	18.977	-5.12	24.450	-2.20	42.219	-6.18	45.605	-8.79
						0.24266	21.33			11.471	14.71			28.414	13.66				

Reason Deactivated

* Out of acceptance criteria

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Table 14. Isopropyl cloprostenate QC sample values for strips and RCD

Run Date	Curve Number	L 0.30000 ng/mL	%Deviation	M 25.000 ng/mL	%Deviation	H 40.000 ng/mL	%Deviation
15-Jul-2024	49	~-1.2611	-520.37	23.193	-7.23	37.502	-6.25
		~-1.2555	-518.50	22.589	-9.64	39.271	-1.82
15-Jul-2024	50	0.32580	8.60	26.624	6.50	40.430	1.08
		0.31770	5.90	26.494	5.98	39.732	-0.67
22-Jul-2024	63	0.26963	-10.12	23.657	-5.37	35.860	-10.35
		0.29885	-0.38	23.338	-6.65	40.481	1.20
22-Jul-2024	65	0.30208	0.69	24.407	-2.37	38.158	-4.61
		0.29722	-0.93	23.784	-4.86	39.173	-2.07
02-Sep-2024	71	~0.43154	43.85	27.883	11.53	44.824	12.06
		~0.37999	26.66	25.162	0.65	43.033	7.58
02-Sep-2024	73	0.31222	4.07	23.438	-6.25	38.971	-2.57
		0.26286	-12.38	24.175	-3.30	40.246	0.62

~ > 25%Deviation

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Table 15. Isopropyl cloprostenate regression parameters and back calculated concentrations (ng/mL) for receptor fluid

Run Date	Curve Number	Slope	Intercept	R-Squared
12-Jul-2024	47	2603	952.5	0.9794
19-Jul-2024	57	1026	546.9	0.9526
30-Aug-2024	67	2455	-1085	0.9924

Run Date	Curve Number	S1 1.0000 ng/mL	%Deviation	S2 2.0000 ng/mL	%Deviation	S10 10.000 ng/mL	%Deviation	S50 50.000 ng/mL	%Deviation	S100 100.00 ng/mL	%Deviation	S250 250.00 ng/mL	%Deviation	S450 450.00 ng/mL	%Deviation	S500 500.00 ng/mL	%Deviation
12-Jul-2024	47	1.1066	10.66	1.7874	-10.63	*18.137		48.442	-3.12	86.813	-13.19	289.27	15.71	*595.55		*876.15	
				1.7898	-10.51	*15.629						277.69	11.08				
19-Jul-2024	57	1.1567	15.67	1.6395	-18.03	7.7576	-22.42	61.386	22.77	113.56	13.56	301.76	20.70	*697.04		*934.76	
				1.8947	-5.27	7.5503	-24.50					243.74	-2.50				
30-Aug-2024	67	1.0130	1.30	2.1348	6.74	9.0063	-9.94	49.760	-0.48	*76.984		255.75	2.30	*293.39		572.83	14.57
				1.8748	-6.26	9.4342	-5.66					243.57	-2.57				

Reason Deactivated

* Out of acceptance criteria

Table 16. Isopropyl cloprostenate QC sample values for receptor fluid

Run Date	Curve Number	L 3.0000 ng/mL	%Deviation	M 250.00 ng/mL	%Deviation	H 400.00 ng/mL	%Deviation
12-Jul-2024	47	~2.0222	-32.59	282.04	12.82	~790.61	97.65
		~4.1360	37.87	231.00	-7.60	~686.95	71.74
19-Jul-2024	57	~2.3076	-23.08	280.05	12.02	~672.34	68.09
		~1.8303	-38.99	~393.64	57.46	~858.52	114.63
30-Aug-2024	67	3.4464	14.88	~133.71	-46.52	~204.10	-48.98
		~4.2117	40.39	~137.83	-44.87	~213.41	-46.65

~ > 20%Deviation

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Table 17. Cloprostenol regression parameters and back calculated concentrations (ng/mL) for washing

Run Date	Curve Number	Slope	Intercept	R-Squared
12-Jul-2024	44	0.01230	0.0006507	0.9837
19-Jul-2024	58	0.01214	-0.00007191	0.9778
30-Aug-2024	68	0.01400	0.0004006	0.9898

Run Date	Curve Number	S0.5/1 0.50000 ng/mL	%Deviation	S1/2 1.0000 ng/mL	%Deviation	S2.5/5 2.5000 ng/mL	%Deviation	S5/10 5.0000 ng/mL	%Deviation	S10/20 10.000 ng/mL	%Deviation	S25/50 25.000 ng/mL	%Deviation	S45/90 45.000 ng/mL	%Deviation	S50/100 50.000 ng/mL	%Deviation
12-Jul-2024	44	0.51274	2.55	0.96759	-3.24	2.6442	5.77	4.3304	-13.39	11.290	12.90	22.937	-8.25	51.504	14.45	43.338	-13.32
				0.99319	-0.68			4.4688	-10.62					51.230	13.84		
19-Jul-2024	58	0.55672	11.34	0.84950	-15.05	2.4290	-2.84	6.0952	21.90	9.5814	-4.19	21.886	-12.46	44.665	-0.74	48.924	-2.15
				0.86243	-13.76			5.9710	19.42					44.334	-1.48		
30-Aug-2024	68	0.51570	3.14	0.88412	-11.59	2.4605	-1.58	4.5310	-9.38	9.2900	-7.10	23.723	-5.11	46.107	2.46	55.593	11.19
				1.0607	6.07			5.5949	11.90					**56.447			

Reasons Deactivated

* Chromatography not acceptable

** Out of acceptance criteria

Table 18. Cloprostenol QC sample values for washing

Run Date	Curve Number	L 1.5000 ng/mL	%Deviation	M 25.000 ng/mL	%Deviation	H 40.000 ng/mL	%Deviation
12-Jul-2024	44	1.6214	8.09	28.928	15.71	42.184	5.46
		~1.8051	20.34	28.176	12.70	44.500	11.25
19-Jul-2024	58	1.5455	3.03	~31.293	25.17	~49.982	24.96
		1.4280	-4.80	29.076	16.30	32.032	-19.92
30-Aug-2024	68	1.7060	13.73	26.761	7.04	43.181	7.95
		1.5385	2.57	~30.342	21.37	43.155	7.89

~ > 20%Deviation

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Table 19. Cloprostenol regression parameters and back calculated concentrations (ng/mL) for skin

Run Date	Curve Number	Slope	Intercept	R-Squared
15-Jul-2024	52	0.02831	0.0005667	0.9984
22-Jul-2024	60	0.02993	-0.0003429	0.9981
02-Sep-2024	74	0.03356	0.004212	0.9964

Run Date	Curve Number	S1/0.05 1.0000 ng/mL	%Deviation	S2/0.1 2.0000 ng/mL	%Deviation	S10/0.5 10.000 ng/mL	%Deviation	S50/2.5 50.000 ng/mL	%Deviation	S100/5 100.00 ng/mL	%Deviation	S250/12.5 250.00 ng/mL	%Deviation	S450/22.5 450.00 ng/mL	%Deviation	S500/25 500.00 ng/mL	%Deviation
15-Jul-2024	52	0.98966	-1.03	2.0047 2.0170	0.24 0.85	10.382	3.82	51.487 52.250	2.97 4.50	100.23	0.23	239.17 242.23	-4.33 -3.11	418.82	-6.93	513.98	2.80
22-Jul-2024	60	0.95982	-4.02	2.0357 2.1080	1.79 5.40	10.453	4.53	49.515 49.931	-0.97 -0.14	102.13	2.13	244.36 245.49	-2.26 -1.80	463.83	3.07	461.34	-7.73
02-Sep-2024	74	0.94501	-5.50	2.0923 2.1081	4.62 5.41	10.540	5.40	52.281 48.923	4.56 -2.15	93.324	-6.68	246.33 226.66	-1.47 -9.34	456.53	1.45	518.49	3.70

Table 20. Cloprostenol QC sample values for skin

Run Date	Curve Number	L 3.0000 ng/mL	%Deviation	M 250.00 ng/mL	%Deviation	H 400.00 ng/mL	%Deviation
15-Jul-2024	52	~25.348 ~25.425	744.93 747.50	240.87 260.82	-3.65 4.33	387.72 395.38	-3.07 -1.16
22-Jul-2024	60	3.3995 3.3580	13.32 11.93	253.19 253.53	1.28 1.41	404.85 411.59	1.21 2.90
02-Sep-2024	74	3.3674 2.9716	12.25 -0.95	257.83 245.60	3.13 -1.76	372.93 371.99	-6.77 -7.00

~ > 25%Deviation

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Table 21. Cloprostenol regression parameters and back calculated concentrations (ng/mL) for strips

Run Date	Curve Number	Slope	Intercept	R-Squared
15-Jul-2024	48	0.02773	0.0004189	0.9990
15-Jul-2024	51	0.02655	0.0001735	0.9981
22-Jul-2024	62	0.02986	0.0004300	0.9987
22-Jul-2024	64	0.03201	-0.00003535	0.9994
02-Sep-2024	70	0.03203	0.004165	0.9842
03-Sep-2024	72	0.03366	0.005409	0.9923

Run Date	Curve Number	S0.25/0.1 0.25000 ng/mL	%Deviation	S0.2/0.5 0.50000 ng/mL	%Deviation	S0.5/0.2 0.50000 ng/mL	%Deviation	S5/2 5.0000 ng/mL	%Deviation	S25/10 25.000 ng/mL	%Deviation	S50/20 50.000 ng/mL	%Deviation	S62.5/25 62.500 ng/mL	%Deviation	S112.5/45 112.50 ng/mL	%Deviation	S125/50 125.00 ng/mL	%Deviation
15-Jul-2024	48	0.24262	-2.95			0.50857 0.51990	1.71 3.98	5.0826	1.65	25.569 25.462	2.28 1.85	51.393	2.79	60.504 61.044	-3.19 -2.33	107.49	-4.45	123.34	-1.33
15-Jul-2024	51	0.25838	3.35			0.47908 0.48684	-4.18 -2.63	4.9632	-0.74	26.092 26.263	4.37 5.05	53.137	6.27	60.721 61.168	-2.85 -2.13	107.46	-4.48	122.45	-2.04
22-Jul-2024	62	0.24789	-0.84			0.52428 0.48329	4.86 -3.34	5.1308	2.62	25.227 23.916	0.91 -4.34	50.106	0.21	61.931 59.539	-0.91 -4.74	114.39	1.68	129.88	3.90
22-Jul-2024	64	0.25195	0.78			0.49482 0.49851	-1.04 -0.30	4.8531	-2.94	25.374 25.387	1.50 1.55	51.540	3.08	62.018 61.875	-0.77 -1.00	108.13	-3.88	128.78	3.02
02-Sep-2024	70	0.29548	18.19			0.39465 0.41829	-21.07 -16.34	5.3595	7.19	27.883 26.525	11.53 6.10	50.530	1.06	62.034 59.017	-0.75 -5.57	110.33	-1.93	126.98	1.58
03-Sep-2024	72	*0.48410				0.41898 0.58095	-16.20 16.19	5.0310	0.62	23.318 25.784	-6.73 3.14	49.471	-1.06	63.215 64.817	1.14 3.71	111.74	-0.68	124.83	-0.14

Reason Deactivated

* Out of acceptance criteria

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Table 22. Cloprostenol QC sample values for strips

Run Date	Curve Number	L 0.75000 ng/mL	%Deviation	M 62.500 ng/mL	%Deviation	H 100.00 ng/mL	%Deviation
15-Jul-2024	48	0.78783	5.04	60.962	-2.46	99.189	-0.81
		0.77658	3.54	60.607	-3.03	98.671	-1.33
15-Jul-2024	51	0.77873	3.83	64.246	2.79	100.95	0.95
		0.79564	6.09	63.737	1.98	99.543	-0.46
22-Jul-2024	62	0.81451	8.60	64.156	2.65	108.07	8.07
		0.85249	13.67	61.910	-0.94	102.36	2.36
22-Jul-2024	64	0.76915	2.55	61.956	-0.87	94.910	-5.09
		0.74469	-0.71	59.541	-4.73	98.801	-1.20
02-Sep-2024	70	0.64554	-13.93	61.347	-1.84	107.92	7.92
		0.66137	-11.82	62.699	0.32	109.04	9.04
03-Sep-2024	72	0.69295	-7.61	63.330	1.33	97.797	-2.20
		0.58178	-22.43	63.750	2.00	98.815	-1.19

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Table 23. Cloprostenol regression parameters and back calculated concentrations (ng/mL) for receptor fluid

Run Date	Curve Number	Slope	Intercept	R-Squared
12-Jul-2024	45	0.03228	0.006772	0.9810
19-Jul-2024	56	0.03248	-0.001729	0.9971
30-Aug-2024	66	0.01537	-0.003111	0.9926

Run Date	Curve Number	S1 1.0000 ng/mL	%Deviation	S2 2.0000 ng/mL	%Deviation	S10 10.000 ng/mL	%Deviation	S50 50.000 ng/mL	%Deviation	S100 100.00 ng/mL	%Deviation	S250 250.00 ng/mL	%Deviation	S450 450.00 ng/mL	%Deviation	S500 500.00 ng/mL	%Deviation
12-Jul-2024	45	0.91578	-8.42	2.0086	0.43	11.717	17.17	54.145	8.29	107.00	7.00	231.93	-7.23	367.06	-18.43	421.60	-15.68
				2.1973	9.87	11.453	14.53					231.18	-7.53				
19-Jul-2024	56	0.98216	-1.78	1.9305	-3.48	9.3884	-6.12	50.083	0.17	98.932	-1.07	243.88	-2.45	439.20	-2.40	481.50	-3.70
				2.1324	6.62	10.829	8.29					264.78	5.91				
30-Aug-2024	66	0.99908	-0.09	1.9994	-0.03	8.9199	-10.80	46.075	-7.85	92.636	-7.36	247.67	-0.93	448.82	-0.26	507.70	1.54
				*2.5359		11.369	13.69					280.24	12.10				

Reason Deactivated

* Out of acceptance criteria

Table 24. Cloprostenol QC sample values for receptor fluid

Run Date	Curve Number	L 3.0000 ng/mL	%Deviation	M 250.00 ng/mL	%Deviation	H 400.00 ng/mL	%Deviation
12-Jul-2024	45	3.1081	3.60	223.68	-10.53	360.80	-9.80
		3.5629	18.76	231.62	-7.35	342.41	-14.40
19-Jul-2024	56	2.9317	-2.28	237.38	-5.05	404.86	1.22
		2.9343	-2.19	263.74	5.50	447.94	11.99
30-Aug-2024	66	3.4300	14.33	246.69	-1.32	434.91	8.73
		3.5243	17.48	278.55	11.42	466.01	16.50

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APPENDIX B

FINAL PROTOCOL

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PROTOCOL

Title	Distribution and penetration study of one formulation containing Isopropyl cloprostenate and its metabolite.
Eurofins|ADME BIOANALYSES reference	24-0055-B
Name of product	Isopropyl cloprostenate and its metabolite
Test Facility	Eurofins|ADME BIOANALYSES 75A, Avenue de Pascalet 30310 Vergèze France
Sponsor Monitor	Eurofins Product Testing US Inc. 11822 N Creek Pkwy #110, Bothell, WA 98011, USA
Document status	Final
Date of version	May 17th 2024

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Confidentiality level: high

APPROVAL PAGE

This protocol has been approved by the Analytical Site. The following signatures document those approvals:

Analytical Site

Eurofins|ADME BIOANALYSES
75A, Avenue de Pascalet
30310 Vergèze
France

Analytical Site Management

Date and signature

[redacted]

Principal Scientist

Date and signature

[redacted]

Deputy Principal Scientist

Date and signature

[redacted]

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APPROVAL PAGE (continued)

This protocol has been approved by the Sponsor. The following signatures document those approvals:

Sponsor

Date and signature [redacted] ....

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GLOSSARY

BLQ	:	Below the Limit of Quantification
Cmean	:	Mean concentration
Cmeas	:	Measured or back calculated concentration
Cn	:	Nominal concentration
LLOQ	:	Lower Limit Of Quantification
MRM	:	Multiple Reaction Monitoring
n	:	Number of determinations or replicates
PBS	:	Phosphate Buffered Saline
QC	:	Quality Control samples
RT	:	Retention Time
S0	:	Blank matrix sample
SD	:	Standard Deviation
Series	:	Analytical batch or run
SOP	:	Standard Operating Procedure
ULOQ	:	Upper Limit Of Quantification
Deviation %	:	Deviation (%) = $\frac{Cmeas - Cn}{Cn} x100$
Precision%	:	Precision (%) = $\frac{SD}{Cmean} x100$

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1. OBJECTIVES

The objective of this study is to investigate the *in vitro* dermal absorption of a formulation containing Isopropyl cloprostenate through fresh healthy human skin and to analyse Isopropyl cloprostenate and its metabolite Cloprostenol (analysis of washing, *stratum corneum*, epidermis, dermis, receptor fluid and rinsing of donor compartment).

2. CONTRACT LABORATORY AND LOCATION

This study will be conducted at Eurofins|ADME BIOANALYSES – 75A, Avenue de Pascalet – 30310 Vergèze – France under the responsibility of [redacted]

The study will be performed according to:

- Eurofins|ADME BIOANALYSES Standard Operating Procedures
- this protocol referenced 24-0055-B and its eventual amendment.

Good Laboratory Practices are not required for this study.

3. MODIFICATION AND DEVIATION TO THE FINAL PROTOCOL

All unplanned deviation(s) from the study plan that may occur during the progression of the experiment will be noted in the paragraph "deviation(s) to the study plan" in the final report.

In case of deviation impacting the results, the Sponsor will be informed during the study.

4. EXPECTED SCHEDULE

Proposed start of the dermal application:	After completion of method qualification, 2024
Expected delivery of the results:	Two weeks after the end of experiments, 2024
Expected draft report:	Four weeks after the end of experiments
Expected final report:	1 month after receipt of finalization approval

Any modification to this schedule will not require an amendment to the final protocol.

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5. REFERENCE ITEM AND BLANK MATRICES

	Reference item		Internal Standard
Name	Isopropyl cloprostenate	Cloprostenol	Prostaglandin F2Alpha-d4
Batch number	0694748-3	0412491-8	0482283
Supplier	Bertin Technologies	Bertin Technologies	Bertin Technologies
Molecular weight (g/mol)	467	424.9	358.5
Corrective factor	1	1.008	1
Purity	100	99.2	100
Storage conditions	Target temperature -20°C	Target temperature -20°C	Target temperature -20°C
Retest or expiring date	01 11 25	28 08 25	07 05 25

Traceability	Details of the reference item used will be fully described in the raw data and final report. The certificates of analysis will be reproduced in the final report.
Handling	The analytical reference item will be handled according to the SOP EER/GES/002P in use at Eurofins|ADME BIOANALYSES. All safety precautions will be taken to prevent any accidental injection, swallowing or inhalation of the tested compounds. The pure standard compounds will be handled while wearing a lab-coat, glasses, mask and gloves.
Responsibility	All safety information relative to the manipulation of reference item will be documented by the suppliers. Reference item will be sent with the corresponding information (certificate of analysis, labeling, purity, water content, expiry date). Compliance with this information will be the responsibility of the suppliers.

The certificates of analysis will be presented in the report.

According to the availability of reference item, the batch number, and/or purity and corrective factor could be modified. New reference or batch of reference item could be ordered during the study, if necessary.

Matrix: **Washing**
3 half cotton buds wetted with water containing 10% of Tween 80 in H2O
2 dry half cotton buds
Storage: Ambient temperature

Matrix: **Strips on human skin**
Scotch: 5 Scotch Magic 3M stripped on human skin.
Storage: Ambient temperature

Matrix: **Skin: 1 cm²** (dermis and epidermis not separated for blank matrix) corresponding to the application area for dynamic Bronaugh cells
Skin: Frozen Dermatomed skin (300-400 µm)
Species: Human
Supplier: Tissue banks or private hospitals
Storage: Target temperature -20°C

Matrix: **Receptor fluid**
One of the receptor fluid below:
RF1: PBS 0.01M pH7.4
RF2: 5% BSA in PBS 0.01 M* pH* 7.4
Storage: Target temperature 4°C or ambient temperature

6. TEST ITEM

The formulations was provided by the Sponsor.

Formulation name	[redacted]
Formulation batch number	11989
Nominal concentration of reference item (w/w)	0.005 %
Reference item	Isopropyl cloprostenate
Supplier	[redacted]
Storage conditions	Target temperature +20°C
Manufacture date	26/02/2024
Expiry date	02/2026

The formulation is provided by the Sponsor ready to use and the certificate of analysis should be provided by the Sponsor.

7. *IN VITRO* SKIN ABSORPTION

7.1. Experimental design

The experimental design is summarized in the table below:

Compound	Isopropyl cloprostenate
Formulation name	[redacted]
Theoretical compound concentration in % W/W	0.005%
Formulation applied per cell (1 cm^2)	10 µL
Theoretical amount applied	0.5 µg
Thickness of the skin	300 - 400 µm
Number of cell per donor	3
Number of donor per formulation	4
Total cells	12
Receptor fluid	Depending on solubility test results in receptor fluids
Sampling of receptor fluid	6 timespoints : 0.5h, 1h, 2h, 4h, 6h, 24h
Washing solution	3 half cotton buds wetted with water containing 10% of Tween 80 in H2O and 2 dry half cotton buds
Washing of the formulation	24h
Strips	20 strips will be performed on each skin and will be pooled by 5 (1-5, 6-10, 11-15,16-20).
Separation of epidermis and dermis	Yes

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7.2. Preparation of fresh human skin

Fresh human skin obtained from abdominal surgery will be used. Skin will be transferred to the laboratory within 4 hours of excision, stored at 4°C until use, and used within 24 hours of excision. Under these conditions, metabolism is considered to be maintained.

Excess subcutaneous fat will be removed, if necessary. Human skins will be provided by the bank of tissue or private hospitals. Information relative to the donors will be specified in the final report.

Four different donors will be tested.

7.3. Preparation of fresh human dermatomed skin

Fresh skin samples will be dermatomed between 300 and 400 µm and the skin will be excised and cut into pieces of around 1.8 cm x 1.8 cm. Thickness will be measured using Oditest calipers.

7.4. Measurement of *stratum corneum* integrity

Room temperature should be maintained between 20 and 25°C and the relative humidity between 30 and 70%.

Before application, the *stratum corneum* integrity will be evaluated for each skin by measuring the TransEpidermal Water Loss (TEWL) using Tewameter. There should be no water on the skin and in the cell donor compartment and the measurement will be taken away from any heating source and air stream.

The human skin will be included in the study if the TEWL is in the range of 0.5 – 13 g/m²/h (open chamber).

If the TEWL of the skin is not in the acceptance criteria, the skin will not be used and will be replaced by another piece of skin. For the condition with stripping and burning there will be no criteria of TEWL.

7.5. Measurement of temperature of skin surface

The passive diffusion of chemicals (and therefore their dermal absorption) is affected by temperature. The diffusion chamber and skin will be maintained at a constant temperature of 32 ± 1°C. The temperature will be measured for each cell using a contact thermometer.

7.6. Preparation of diffusion cells

Application area: 1cm²

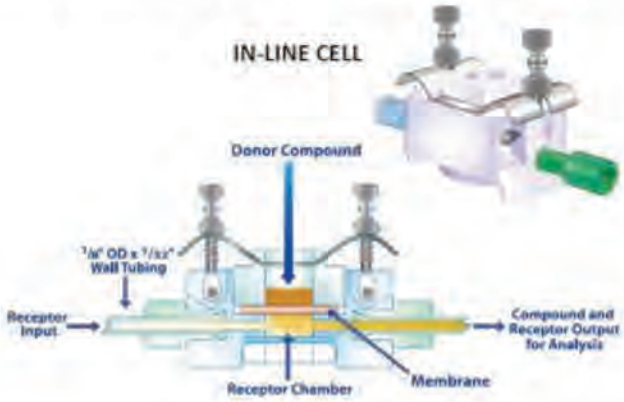
Flow: 1 mL/h.

A flow-through diffusion cell system (developed by Bronaugh), also called dynamic cells or in line cells, will be used for the study.

Flow-through cells are characterized by a continuously replaced receptor fluid. Receptor fluid is continually replaced using tubes and a suitable pump (at the flow rate specified above).

The dynamic cells used will correspond to an application area equal to 1 cm² with donor chamber diameter of 11.28 mm.

Each apparatus of in-line (dynamic) cells is composed of seven cells. Cells will be identified by a letter.



The skin samples will be placed on the receptor compartment and the donor compartment will be placed onto the skin samples. A clamp will be placed to seal both compartments and the cell will be tilted before being placed in the chamber to evacuate any air bubbles in the system.

The skin surface temperature will be maintained at 32°C ± 1°C, using a static water bath integrated as part of the dynamic system.
After at least 30 minutes of stabilization, the integrity of the skin will be verified using the TEWL method.
The donor compartment will not be closed (non-occlusive system).

7.7. Application of the formulation

The formulation will be applied homogeneously (spreading with glass rod or spatula but no massage) on each skin at a rate of 10 µL/cell.

The application will be performed by using a positive displacement pipette (or spatula, depending on the viscosity of the formulation) and the exact amount applied will be determined by weight then recorded.

Before application, the positive displacement pipette (or spatula and/or glass rod) containing the volume to be applied will be weighed (P1). Then the empty device will be weighed after application (P2). The applied amount (Px) will correspond to: Px = P1 – P2.

7.8. Start of the experiment

The experiment will start immediately after the application of the formulation on the skin surface.

The experiment will be stopped around 24 hours after application experimental start.

Start and end of experiment will be noted in the raw data.

7.9. Sampling of receptor fluid

The receptor fluid will be collected at 6 timepoints after application, as specified above in section §8.2 Experimental Design.

Receptor fluid samples will be precisely weighed and should be stored at -20°C before and after the analysis.

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7.10. Washing and Dismantling of cells

24 hours after the start of experiment, the formulation excess on the skin will be removed and cells will be dismantled.

- Washing of the application site:

3 half cotton buds wetted with water containing 10% of Tween 80,

2 dry half cotton buds,

will be used to remove the excess of formulation at the surface of the skin.

Cells and the different compartments will then be dismantled.

The washing samples will be placed in one vial and should be stored at -20°C before and after the analysis (according to the stability period defined during the Qualification of Method PKH/MOA/1745).

- Washing of the donor compartment (RCD)

The donor compartment and the upper seal will be placed in a flask and target 25 mL of appropriate solvent will be added.

The closed flask will be shaken at least 2h at room temperature and stored at -20°C before and after the analysis according to the analytical method for Strips (according to the stability period defined during the Qualification of Method PKH/MOA/1745).

Lower seal will be discarded.

- Strips treatment (ST)

The skin will be taken with tweezers and each skin sample will be placed between seal and aluminum foil.

The stratum corneum will be taken off from the skin specimen using Scotch Magic 3M by stripping. In order to standardize stripping, a weight of at least 50 g/cm^2 will be placed on top of the Scotch tape for at least 10 s before taking off.

A maximum of 20 strips will be performed on each skin and will be pooled by 5 (1-5, 6-10, 11-15,16-20).
If a sample contains less than 5 strips, the sample will be completed with blank adhesive tape until the number of 5 strips is reached.

The strips samples will be stored with appropriate solvent at -20°C before and after the analysis (according to the stability period defined during the Qualification of Method PKH/MOA/1745).

- Epidermis (E), Dermis (D), remaining skin (RS) treatment

Using the scalpel blade, the skin corresponding to the application area will be separated from the remaining (surrounding) skin (corresponding to the non-application area), the epidermis and partial dermis will be separated and then placed in the corresponding vials.

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The remaining skin (RS) will be cut in four parts.

The Epidermis, Dermis and Remaining skin flasks will be stored at -20°C with appropriate solvent (according to the stability period defined during the Qualification of Method PKH/MOA/1745).

The samples will be analyzed with the analytical method qualified for Skin.

- Receptor fluid (RF)

The receptor fluid will be collected at 6 timepoints after application, as specified above in section §8.2 Experimental Design.

Receptor fluid samples will be precisely weighed and should be stored at -20°C before and after the analysis (according to the stability period defined during the Qualification of Method PKH/MOA/1745).

All the samples generated during the study will be destroyed after the approval of the final report.

8. Analysis of samples

Samples analysis will be performed with the previously qualified method PKH/MOA/1745.

The final extract will be analysed by LC-MS/MS.

One stock solution of Isopropyl Cloprostenate and one stock solution of Cloprostenol will be prepared for calibration standards and QCs.

The formulation will not be analysed.

8.1. Calibration curves

At least 8 calibration standards will be freshly prepared for the quantification according to corresponding analytical method (PKH/MOA/1745)

The ranges will be (in accordance with PKH/MOA/1745)

- **Washing**: For Isopropyl cloprostenate: 1 - 100 ng/mL
  Corresponding to target 0.4 – 40 % of the applied dose of Isopropyl cloprostenate for the formulation at 0.005% w/w.

  For Cloprostenol (metabolite): 0.5 - 50 ng/mL
  Expected extraction volume = 10 mL

- **Strips**: For Isopropyl cloprostenate: 0.1 - 50 ng/mL
  Corresponding to target 0.04% - 10% of the applied dose for the formulation at 0.005% w/w.
  Expected extraction volume = 5 mL

  For Cloprostenol (metabolite): 0.25 – 125 ng/mL

- **Epidermis and Dermis**: For Isopropyl cloprostenate: 0.05 - 25 ng/mL
  Expected extraction volume = 5 mL
  Corresponding to target.0.02% - 10% of the applied dose for the formulation at 0.005% w/w.

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For Cloprostenol (metabolite): 1 - 500 ng/mL

- **Receptor fluid:** For Isopropyl cloprostenate and Cloprostenol (metabolite): 1 to 500 ng/mL
  Expected Bronaugh cells volume collected around 24 mL (flow rate of 1 mL/h)
  Corresponding to target 0.4 - 20% of the applied dose for the formulation at 0.005% w/w.

The **Epidermis, Dermis and Remaining Skin** will be analysed with the analytical method qualified for Skin.

The **RCD** (donor compartment washing) will be analysed with the analytical method qualified for Strips.

Acceptance criteria:

- *Deviation for the calibration standards will be ranged between ± 20.00% of the nominal value for all concentrations,*
- *LLOQ and ULOQ will be included in the calibration curve, but if the LLOQ or the ULOQ will be outside the acceptance criteria, the calibration standard after the LLOQ will became the new LLOQ and the calibration standard just before the ULOQ will became the new ULOQ.*
- *25% calibration standards can be excluded from the final calibration curve if the back calculated deviation is not comprised within ± 20.00% for all concentrations.*

8.2. Quality control samples

In order to check the quality of the method during the sample assays, quality control samples will be included in the run.

The nominal value will be used to determine the difference with the measured value of the QC.

The quality control samples will be prepared in duplicate at different concentrations: 3 LLOQ, 0.5 ULOQ and 0.8 ULOQ.

Acceptance criteria:

- *Deviation of QCs samples will be calculated and will have to be within ± 20.00%,*
- *at least 67% of QC samples should meet the criteria above*

8.3. Assay organization

Each series of assays will consist of:

- 1 Blank matrix not spiked (S0).
- 8 calibration standards different from zero prepared on the same day in the matrix.
- In case of carry-over determinate during qualification study: at least 1 Blank matrix not spiked (S0).
- 2 Quality Controls.
- The samples to be assayed.
- 2 Quality Controls.
- The samples to be assayed.
- 2 Quality Controls.
- 1 Blank matrix not spiked (S0) (will be determined upon completion of the qualification study)
- 8 calibration standards different from zero prepared on the same day in the matrix. (will be determined upon completion of the qualification study)

The assay organisation may be adjusted upon completion of the qualification study.

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9. DETERMINATION OF THE CONCENTRATIONS

Concentrations will be calculated using Watson® 7.5 SP1 (HF1) directly from peak area obtained after automatic integration of chromatograms by LabSolutions V6.60 or Analyst 1.6.3.

Concentrations of the QC samples will be calculated by interpolation with the weighted calibration curves prepared in the same conditions and assessed daily after automatic integration. Results of concentrations, once accepted, will be expressed in "ng/mL".

Concentrations will be reported with 5 significant figures in Excel® tables.

9.1. Repeat and confirmatory analysis

Re-assays will be done in the following cases:

- Concentration of a sample more than 20% higher than the highest nominal level of calibration curve. This sample could be re-assayed , after an appropriate dilution, according to the SOP ETU/PEN/012P, or the extract could be diluted and re-injected with the same calibration curve upon sponsor's decision.
- Series not conform to acceptance criteria.

In these cases, single re-assay will be performed if sample volume is sufficient.

Only the final result will be presented in the final report.

Re-assays and result selection could be done without approval of the Sponsor according to the SOP in use at Eurofins|ADME BIOANALYSES.

10. RESULTS

10.1. Absorption

The absorption will be equal to: Receptor fluid + Skin (Dermis and Epidermis without *stratum corneum*).

For each cell, the absorption of Isopropyl cloprostenate in ng/cm² and in % of the applied dose will be calculated.

Those results will be presented with at least 2 decimals.

Results below the limit of quantitation will be noted BLQ in result tables. All BLQ values will be considered equal to zero for calculation.

10.2. Recovery

For each experiment and each cell, a recovery balance will be calculated. The individual value will have to be 100 ±20%, otherwise results will have to be justified. Cell(s) outside the acceptance criteria (100 ± 20%) could be excluded. If cell(s) outside the acceptance criteria is not excluded, justification should be provided in the report.

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11. FINAL REPORT

The study will be presented in one final report written in English. A final report (in PDF format with Principal Scientist and Analytical Site Management approvals) will be sent to the Sponsor by FileXchange (secure server).

- Margins: right: 1.8 cm / left: 1.8 cm / top: 2.5 cm and bottom 2.5 cm.
- Pagination: page number/total number of pages
- Heading: On the top of the sheet: "Eurofins|ADME BIOANALYSES reference: 24-0055 -B - Type of document."

12. ARCHIVING PROCEDURE

All study data and associated study specific documentation will be retained in secure storage by Eurofins ADME BIOANALYSES for a period of 1 year from completion of the study (issue of final results' document or report) after which time it will be securely destroyed. The Sponsor will not be systematically informed in advance of this destruction. Extended storage may be requested by the Sponsor in advance but will be subject to additional cost agreements.
Contents are as follows:

- Signed protocol and its possible amendments
- All relevant correspondence
- Internal memoranda
- Analytical records
- Signed final report and its possible amendments
- List of staff involved
- Laboratory notebook(s)
- All raw data relevant to the study

APPENDIX C

CERTIFICATES OF ANALYSIS

CERTIFICATE of ANALYSIS

[redacted]

(+)-Cloprostenol isopropyl ester

(+)-9α,11α,15R-trihydroxy-16-(3-chlorophenoxy)-17,18,19,20-tetranor-prosta-5Z,13E-dien-1-oic acid, isopropyl ester
Item No. 10010016 • Batch No. 0694748

Purity Specification: ≥98%
Molecular Formula : C25H35ClO6
CAS Number: 157283-66-4 Formula Weight: 467 Expiry date: 01NOV2025

Overview

Tests	Results
HPLC	Purity: 100.0 %
IR	Conforms
Mass spec	M-H+FA: 511.0 M+Cl: 501.1
TLC	Purity: 100 %

Reviewed and approved by: [redacted]

[redacted]

CERTIFICATE of ANALYSIS

[redacted]

(+)-Cloprostenol

(+)-9α,11α,15R-trihydroxy-16-(3-chlorophenoxy)-17,18,19,20-tetranor-prosta-5Z,13E-dien-1-oic acid

Item No. 16765 • Batch No. 0412491

Purity Specification: >97%
Molecular Formula : C22H29ClO6
CAS Number: 54276-21-0 Formula Weight: 424.9 Expiry date 28AUG2025

Overview

Tests	Results
HPLC	Purity: 99.2 %
IR	Conforms
Mass spec	M-H+: 423.3 -ClC6H4OH: 295.1 -ClC6H4OH, H2O: 277.2 -ClC6H4OH, 2H2O: 259.2
TLC	Purity: 100 %
UV	λ max: 220, 275, 282 nm

Reviewed and approved by: [redacted]

[redacted]

[redacted]

[redacted]

CERTIFICATE OF ANALYSIS

PRODUCT:	EL-LG-2ML AMZSW	CUSTOMER:	[redacted]	BATCH NUMBER:	12575
LOT #:	5EKW87	DATE OF MFG:	03/19/2024	EXPIRATION DATE	03/19/2026
PO#	PO-EL936	FILL SIZE	2.0 mL	SKU	[redacted]

TEST	TEST METHOD	STANDARD	RESULTS	STATUS
Appearance	TM-Q-01-A	Liquid	Matches Std.	PASSED
Color	TM-Q-01-A	Characteristic	Matches Std.	PASSED
Odor	TM-Q-01-A	Characteristic	Matches Std.	PASSED
pH at 25°C		4.5 – 7.00	6.69	PASSED
Total Plate Count		<100 cfu/ml	<10 cfu/ml	PASSED
Total Mold Count		<100 cfu/ml	<10 cfu/ml	PASSED
Total Yeast Count		<100 cfu/ml	<10 cfu/ml	PASSED

This Certificate of Analysis for the product above shows compliance with Quality Standards for all products with batch number matching this certificate. The product is traceable back to original parent lot/batch of base ingredients for which certification has also been done and results are on file.

Certification of test results and statement of GMP compliance is made by the Manufacturer [redacted] and the following management representatives.

Quality Manager

[redacted] Date of Certification: 6/17/24

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November 14, 2025

Via email to:

CIR Expert Panel

Bart Heldreth, PhD
Executive Director
Cosmetic Ingredient Review
Washington, D.C.
heldrethb@cir-safety.org

RE: Written Submission for Discussion in December 2025 CIR Meeting − RAWG Position on Animal Testing related to Prostaglandin Analogs ("PGAs")

Dear Dr. Heldreth:

We submit this letter which contains our views related to the prostaglandin analogs ("PGAs") matter being reviewed by the Expert Panel at the December 2025 CIR meeting.[1]

We are writing to express our **serious concerns** with respect to the positions on **animal testing** expressed by the read-across working group ("RAWG") in its evaluation of two PGAs, Ethyl Tafluprostamide ("ET") and Isopropyl Cloprostenate ("ICP"), during the June 2025 CIR meeting.

As you know, **animal testing of cosmetic ingredients is now widely *prohibited* in many countries, including the EU, UK, Canada, and Australia among others**. In the United States, 12 states ban animal testing of cosmetic products/ingredients, and a **national ban has been proposed**. Moreover, the **PCPC** has been very active in supporting the federal Humane Cosmetics Act, which would prohibit animal testing for cosmetics nationally in the United States.

In the absence of animal test data, there has been a paradigm shift in the safety assessment of cosmetic ingredients to a multi-modality approach that is **more ethical and biologically-relevant** to human use of cosmetics.[2]

Consistent with this shift, at the beginning of the June 2025 CIR Meeting, the toxicology firms that prepared our clients' dossiers on ET and ICP presented their read-across data to the RAWG. After the two presentations, the RAWG discussed the submitted data. After complimenting the presenters for the "wealth

[1] This letter represents the views of the sponsor of submissions to the CIR on ET (the "ET Sponsor") represented by Carol Pratt, PhD, JD of Lee & Hayes, P.C. and the sponsor of submissions to the CIR on use of ICP at 0.005% concentration in cosmetics (the "0.005% ICP Sponsor") represented by Nathan Beaver, JD of Foley & Lardner LLP. "Our clients" in this letter refers to the ET Sponsor and the 0.005% ICP Sponsor.

In accordance with Section 38 of the CIR Procedures & Support to the Expert Panel for Cosmetic Ingredient Safety ("CIR Procedures"), **we request that this submission be distributed to each Expert Panel member and liaison representative prior to and during the December 2025 CIR meeting, and that the information herein be considered by the Expert Panel in its review of the PGA matter and included in the meeting's record**.

[2] Vaillancourt, E., Afdal Mohamed, A., Ansell, J., Ashikaga, T., Ayad, A., Ayman, H., Campos, M.V., Chien, H., Giusti, A., Hatao, M., Hill, F., Khattab, A., Kreiling, R., Lee, A.Y., Li, M., Lin, P., Lin, Y-C., Mencarelli, V., Milstein, S., Montemayor, B., Motter Catarino, C., Negm, R., Ou´edraogo, G., Park, E.J., Varçin, M., Yang, T-Y., Zang, Y. J., Dent, M., *Highlighting best practices to advance next-generation risk assessment of cosmetic ingredients*, NAM Journal 1 (2025), https://doi.org/10.1016/j.namjnl.2025.100020.

of data" and "heroic work", **the RAWG ultimately opined that *animal testing* was the only way to generate scientifically valid data to assess the safety of the ingredients.** This position is unacceptable given the intended purpose of the RAWG, current international and U.S. state laws, consumer expectations, and PCPC values and goals. As a result, we would request that CIR recognize the value and scientific validity of this read-across data.

We are aware that the RAWG has **previously accepted read-across data for other ingredients**. Recently, the RAWG accepted a read-across justification for a different group of ingredients, the alkylamphoacetates, in September. In the read-across document for alkylamphoacetates, the authors assumed that the target chemical and the analogues will be distributed similarly in the human body and will exhibit similar toxicological properties based on their structural and compositional similarities. **CIR accepted this read-across reasoning despite the fact that there are differences in the structures and physicochemical properties of their analogues compared to the target chemical**. It appears that the RAWG is **inconsistently** applying criteria for acceptable read-across data—structural differences, physicochemical differences, and a possible developmental toxicity concern in animal studies were **acceptable for the alkylamphoacetates but not acceptable for PGAs**. This inconsistency is concerning and makes it difficult for sponsors to scientifically, legally, and ethically comply with requirements and develop new products for a global economy that expects "cruelty-free" cosmetics.

We are concerned that the RAWG is inconsistent in its application and acceptance of read-across data and that **it has rejected scientifically valid read-across data in favor of animal testing**.

We appreciate your careful and timely consideration of our concerns. It is our goal that we can resolve this issue in a way that aligns with industry standards, consumer expectations, and good science.

Sincerely,



Nathan A. Beaver, JD
Representing the 0.005% ICP Sponsor

Foley & Lardner LLP
3000 K Street, N.W., Suite 600
Washington, D.C., 20007
nbeaver@foley.com



Carol Pratt, PhD, JD
Representing ET Sponsor

Lee & Hayes, P.C.
601 W. Riverside, Suite 1400
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Cc via email to:

Emily Manoso
Executive Vice President & General Counsel
Personal Care Products Council
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Thomas F. Myers
President & CEO
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November 17, 2025

TO: Bart Heldreth, PhD, Executive Director
Cosmetic Ingredient Review
heldrethb@cir-safety.org

FROM: Jay Ansell, PhD, DABT, ERT
EAS Consulting Group
Jay.Ansell@easconsultinggroup.com
On Behalf of 0.0044% IC Sponsor

DATE: November 17, 2025

RE: Safety Assessment of Ethyl Tafluprostamide and Isopropyl Cloprostenate as Used in Cosmetics

We respectfully submit the following comments on The Revised Draft Tentative Report for Panel Review of the Safety Assessment of Ethyl Tafluprostamide and Isopropyl Cloprostenate as Used in Cosmetics, dated November 10, 2025.

The submitter has included a track changes version of 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 for your consideration:

Isopropyl Cloprostenate

The effect of an eyelash serum containing 0.0044% Isopropyl Cloprostenate on ocular pigmentation and periorbital volume was evaluated in 114 subjects.[18,35] 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 (evaluations performed by board-certified ophthalmologist investigator). 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). When compared to baseline, there was a statistically-significant change in overall color of the left and right iris at all time points (this effect was not considered to be clinically relevant according to an independent panel of three board-certified ophthalmologists who unanimously found that 228 of the 228 paired baseline and 8-month photographs captured during the study were of equal stromal iris pigmentation and did not show a pattern of iris stromal pigmentation darkening characteristic of prostaglandin-induced iris color change).[36] 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 not considered clinically relevant. The board-certified ophthalmologist investigator reported no evidence of changes in iris color associated with product use as part of the subjects' physical exams over the course of 8 months. 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 statistically-significant difference noted after 2, 4, and 8 mo of use). Similarly, when compared to baseline values for the right orbital side, there was a statistically-significant decrease in the right orbital volume after 1 and 4 mo of test material use (no statistically-significant difference noted after 2 and 8 mo of use). There was no statistically-significant change in periorbital volume from baseline after 8 months of use. The board-certified ophthalmologist investigator reported no evidence of changes in periorbital volume associated with product use as part of the subjects' physical exams over the course of 8 months. Adverse events were observed as follows: 1 instance of 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 probably conjunctivitis with punctuate epitheliopathy following sand exposure (subject continued study, all symptoms resolved), and 1 instance of unilateral conjunctivitis (subject removed from study with all symptoms resolving). The board-certified ophthalmologist investigator determined that the test material was safe for contact lens and non-contact lens wearers. Ocular irritation evaluated in this study can be viewed in the Ocular Irritation section of this report.

Please include this information in Wave 2 for the CIR December 4- 5 meeting.