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

Status: Draft 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.; David E. Cohen, M.D.; Curtis D. Klaassen, Ph.D.; Allan E. Rettie, Ph.D.; David Ross, Ph.D.; Thomas J. Slaga, Ph.D.; Paul W. Snyder, D.V.M., Ph.D.; and Susan C. Tilton, Ph.D. The Cosmetic Ingredient Review (CIR) Executive Director is Bart Heldreth, Ph.D., and the Senior Director is Monice Fiume. This safety assessment was prepared by Priya Cherian, M.S., Senior Scientific Analyst/Writer, CIR.

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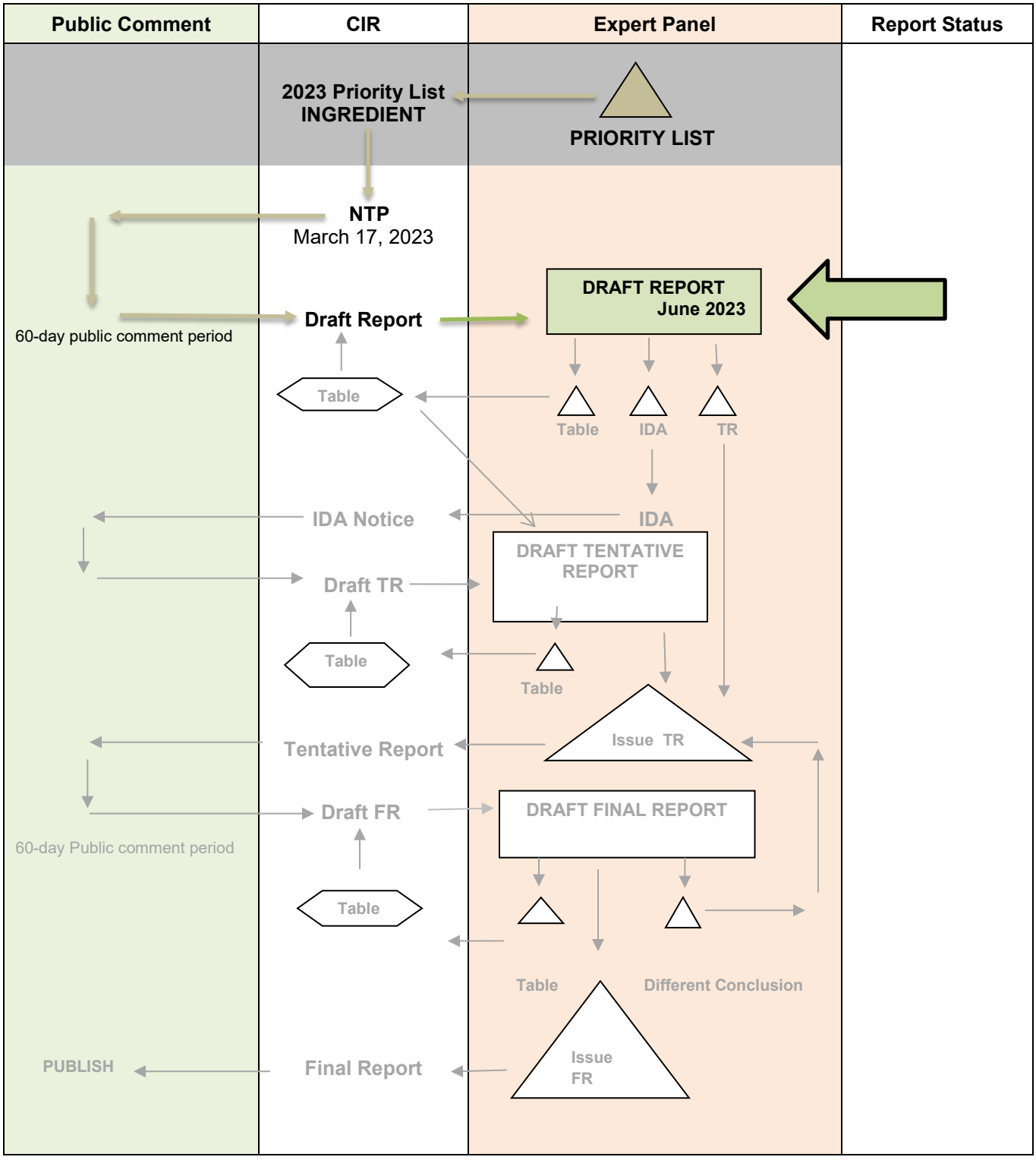
SAFETY ASSESSMENT FLOW CHART

INGREDIENT/FAMILY Prostaglandin Analogs

MEETING June 2023



Cosmetic Ingredient Review

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Memorandum

To: Expert Panel for Cosmetic Ingredient Safety Members and Liaisons
From: Priya Cherian, M.S., Senior Scientific Analyst/Writer, CIR
Date: May 19, 2023
Subject: Safety Assessment of Ethyl Tafluprostamide and Isopropyl Cloprostenate as Used in Cosmetics

Enclosed is the Draft Report of the Safety Assessment of Ethyl Tafluprostamide and Isopropyl Cloprostenate (identified in the pdf as *report_ProstaglandinAnalogues_062023*). Due to a lack of relevant published data, a Scientific Literature Review Notice to Proceed (NTP) was issued for these ingredients on March 17, 2023. Since the issuing of the NTP, several in vitro and in vivo ocular irritation studies, as well as HRIPTs performed using products containing Isopropyl Cloprostenate, have been received (summarized below). The majority of studies yielded negative results (or predictions of negative results).

- HET-CAM assay performed on an eyelash serum containing 0.005% Isopropyl Cloprostenate (*data1_ProstaglandinAnalogues_062023*)
- HET-CAM assay performed on 0.1% Isopropyl Cloprostenate (*data2_ProstaglandinAnalogues_062023*)
- HET-CAM assay performed on an eyelash serum containing 0.005% Isopropyl Cloprostenate (*data3_ProstaglandinAnalogues_062023*)
- HET-CAM assay performed on an eyelash serum containing 0.0044% Isopropyl Cloprostenate (*data4_ProstaglandinAnalogues_062023*)
- HET-CAM assay performed on an eyelash serum containing 0.0044% Isopropyl Cloprostenate (*data5_ProstaglandinAnalogues_062023*)
- HET-CAM assay performed on eyelash serum containing 0.005% Isopropyl Cloprostenate (*data6_ProstaglandinAnalogues_062023*
- Ocular irritation assay performed in 32 subjects using an eyelash serum containing 0.005% Isopropyl Cloprostenate (*data7_ProstaglandinAnalogues_062023*)
- In-use study performed in 30 subjects using an eyelash and eyebrow serum containing 0.005% Isopropyl Cloprostenate (*data8_ProstaglandinAnalogues_062023)*
- HRIPT performed in 50 subjects using an eyelash serum containing 0.005% Isopropyl Cloprostenate (*data9_ProstaglandinAnalogues_062023*)
- HRIPT performed in 53 subjects using an eyelash serum containing 0.005% Isopropyl Cloprostenate (*data10_ProstaglandinAnalogues_062023*)
- HRIPT performed in 56 subjects using an eyelash serum containing 0.0044% Isopropyl Cloprostenate (*data11_ProstaglandinAnalogues_062023*)
- HRIPT performed in 53 subjects using an eyelash serum containing 0.0044% Isopropyl Cloprostenate (*data12_ProstaglandinAnalogues_062023*)
- Ocular irritation study (12 wk application) using an eyelash serum containing 0.0044% Isopropyl Cloprostenate (*data13_ProstaglandinAnalogues_062023*)
- Ocular irritation studies (6-7 wk applications) performed using eyelash or eyebrow serums containing 0.0044% Isopropyl Cloprostenate (*data13_ProstaglandinAnalogues_062023*)
- Data on several endpoints regarding Ethyl Tafluprostamide (only relevant data from this submission were included in the CIR report; the full data submission can be found herein as *data14_ProstaglandinAnalogues_062023*)

The Summary of Key Points in *data14_ProstaglandinAnalogues_062023*, presented on pages 32-35, also provides some perspectives from several external pharmacokinetic and toxicological experts regarding the safety evaluation of Ethyl

Tafluprostamide as used in one specific eyelash product. For example: *According to two experts, systemic exposure to DDDE is highly unlikely*; *Dr. Wilding concluded that there is "negligible risk" of DDDE having a physiological effect on the eyelid skin or on the eye*. Furthermore, on page 32, a safety margin of 4286 was calculated based on an NOAEL of 0.03 mg/kg bw derived from a comparable Tafluprost study (because the NOAEL for Ethyl Tafluprostamide was not available). The Panel is expected to review such assessment and to determine whether it is valid to be included in the report.

In addition, relevant data were included in the CIR report from an SCCS opinion on prostaglandins and prostaglandin-analogues (including Ethyl Tafluprostamide and Isopropyl Cloprostenate) used in cosmetic products. The SCCS was not able to conclude on the safety of Isopropyl Cloprostenate and Ethyl Tafluprostamide due to a lack of data on these ingredients. Although data were available for cloprostenol and R-cloprostenol, the SCCS determined that drawing conclusions on the toxicokinetics profile of Isopropyl Cloprostenate from the toxicokinetics data on cloprostenol and R-cloprostenol would not be appropriate, as the systemic uptake and bioavailability/distribution would differ between Isopropyl Cloprostenate and cloprostenol/R-cloprostenol. ***Does the Panel agree that data on cloprostenol are not appropriate for inclusion in the report, because the data cannot be read across to Isopropyl Cloprostenate*?**

Three uses (all of which are "other eye makeup preparations") are reported for Isopropyl Cloprostenate, according to 2023 FDA VCRP data. (Frequency of use Ethyl Tafluprostamide were not reported in the VCRP.) Concentrations of use were not received for either Ethyl Tafluprostamide or Isopropyl Cloprostenate in response to a survey initiated by the Council in 2022 (*concentration_ProstaglandinAnalogues_062023*). However, unpublished data reporting calculations of the concentration of Isopropyl Cloprostenate in two eyelash serums were received and are included herein as *data15_ProstaglandinAnalogues_062023*; these serums were reported to contain 0.0044% and 0.0048% Isopropyl Cloprostenate, respectively. (It was not specified as to whether these formulations are marketed.) In addition, unpublished data on Ethyl Tafluprostamide indicate that this ingredient is used in products intended for use on eyelashes, eyebrows, or scalp hair, at concentrations ranging from 0.012 to 0.2% (*data14_ProstaglandinAnalogues_062023*).

The following documents are also included in this packet:

- transcripts regarding the inclusion of these ingredients on the 2023 priority list (*transcripts_ProstaglandinAnalogues_032023*)
- report history (*history_ProstaglandinAnalogues_062023*)
- data profile (*dataprofile_ProstaglandinAnalogues_062023*)
- search strategy (*search_ProstaglandinAnalogues_062023*)
- flow chart (*flow_ProstaglandinAnalogues_062023*)

After reviewing these documents, if the available data are deemed sufficient to make a determination of safety, the Panel should issue a Tentative Report with a safe as used, safe with qualifications, unsafe, or split conclusion, and Discussion items should be identified. If the available data are insufficient, the Panel should issue an Insufficient Data Announcement (IDA), specifying the data needs therein.

Prostaglandin Analogues – History

March 2023

NTP issued

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, and dermal sensitization data

Data received on Ethyl Tafluprostamide (several endpoints)

June 2023

Panel reviews Draft Report

Prostaglandin Analogues Data Profile – June 2023 – Writer, Priya Cherian

				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	Animal	Human	Phototoxicity	In Vitro	Animal	Retrospective/ Multicenter	Case Reports
Ethyl Tafluprostamide				X	X													X					X											
Isopropyl Cloprostenate	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				

Search Strategy

Search terms below searched in all listed links

Typical Search Terms (this is informational – not for inclusion for search strategy that goes to the Panel)

- INCI names
- CAS numbers
- chemical/technical names

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/

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 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 17, 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?

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

Status: Draft Report for Panel Review
Release Date: May 19, 2023
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The Expert Panel for Cosmetic Ingredient Safety members are: Chair, Wilma F. Bergfeld, M.D., F.A.C.P.; Donald V. Belsito, M.D.; David E. Cohen, M.D.; Curtis D. Klaassen, Ph.D.; Allan E. Rettie, Ph.D.; David Ross, Ph.D.; Thomas J. Slaga, Ph.D.; Paul W. Snyder, D.V.M., Ph.D.; and Susan C. Tilton, Ph.D. The Cosmetic Ingredient Review (CIR) Executive Director is Bart Heldreth, Ph.D., and the Senior Director is Monice Fiume. This safety assessment was prepared by Priya Cherian, M.S., Senior Scientific Analyst/Writer, CIR.

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ABBREVIATIONS

ADME	absorption, distribution, metabolism, and excretion
CAS	Chemical Abstracts Service
CIR	Cosmetic Ingredient Review
CLP	classification, labeling, and packaging
Council	Personal Care Products Council
CPSC	Consumer Product Safety Commission
ECHA	European Chemicals Agency
ED_5	median effective dose
EU	European Union
FDA	Food and Drug Administration
HET-CAM	hen's egg test chorioallantoic membrane
HRIPT	human repeat insult patch test
log K_{ow}	n-octanol/water partition coefficient
MOS	margin of safety
NR	none reported
NTP	Notice to Proceed
OECD	Organisation for Economic Cooperation and Development
Panel	Expert Panel for Cosmetic Ingredient Safety
$PGF_{2\alpha}$	prostaglandin $F_{2\alpha}$
PoD	point of departure
QSAR	quantitative structure-activity relationship
SCCS	Scientific Committee on Consumer Safety
SED	systemic exposure dosage
TG	test guideline
TSV	toxicological screening value
VCRP	Voluntary Cosmetic Registration Program
wINCI; *Dictionary*	web-based *International Cosmetic Ingredient Dictionary and Handbook*

INTRODUCTION

This assessment reviews the safety of Ethyl Tafluprostamide and Isopropyl Cloprostenate as used in cosmetic formulations. According to the web-based *International Cosmetic Ingredient Dictionary and Handbook (*wINCI; *Dictionary)*, both Ethyl Tafluprostamide and Isopropyl Cloprostenate are reported to function in cosmetics as hair conditioning agents.[1] Ethyl Tafluprostamide is also reported to function in cosmetics as a nail conditioning agent (Table 1).

These ingredients are being grouped together due to structural similarities as synthetic prostaglandin analogues. In March 2023, a Scientific Literature Review Notice to Proceed (NTP) on this ingredient group was issued due to a lack of relevant published data, and toxicological data were requested. Data on several endpoints have been provided since the issuing of the NTP.

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 March 2023. A listing of the search engines and websites that are used and the sources that are typically explored, as well as the endpoints that the Expert Panel for Cosmetic Ingredient Safety (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.[2] Please note that the SCCS website provide 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.[3] However, both of these ingredients are synthetic analogues. The definitions of these ingredients are provided in Table 1.

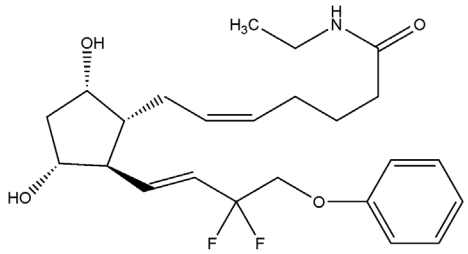

Figure 1. Ethyl Tafluprostamide

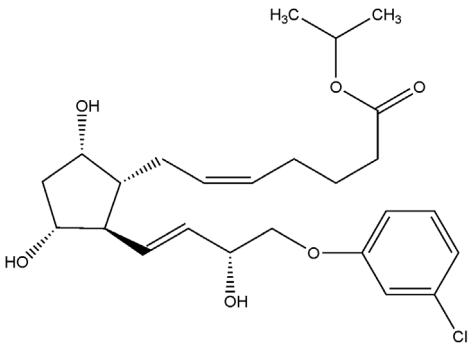

Figure 2. Isopropyl Cloprostenate

Chemical Properties

The ingredients reviewed in this report are hydrophobic, water-insoluble substances. [2] For example, Ethyl Tafluprostamide is a colorless to pale yellow solution, with a reported water solubility of 0.09 mg/l (at 25° C), and a high octanol/water partition coefficient (log K_{ow}; 5.03).[2] Other physical and chemical properties of Ethyl Tafluprostamide and Isopropyl Cloprostenate can be found in Table 2.

Method of Manufacture

Method of manufacture data were not found in the published literature, and unpublished data were not submitted.

Composition and Impurities

Ethyl Tafluprostamide

According to the SCCS and an unpublished data submission, Ethyl Tafluprostamide has a purity of no less than 99%.[2,4] In addition, according to the unpublished data submission, Ethyl Tafluprostamide should not contain more than 1% impurities.

Isopropyl Cloprostenate

The SCCS also reported that Isopropyl Cloprostenate has a purity level no less than 99.4%.[2] Impurities and accompanying contaminants in this ingredient include 15-epimer (0.25%), ethyl acetate (0.2%), and water (0.15%).

USE

Cosmetic

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

According to the 2023 VCRP survey data, Isopropyl Cloprostenate is reported to be used in 3 formulations, all of which are reported to be "other eye makeup preparations" (Table 3).[5] No uses were reported for Ethyl Tafluprostamide. No concentrations of use were reported for either Ethyl Tafluprostamide or Isopropyl in response to a survey initiated by the Council in 2022 (and for which results were submitted in 2023).[6] However, according to data submitted by industry as a submission separate from the concentration of use survey, the average concentration of Isopropyl Cloprostenate in two eyelash serums (unknown if these are marketed serums) were determined to be 0.0044 and 0.0048%, respectively (corresponding to a weight of 8.4 and 13 mg Isopropyl Cloprostenate, per usage of each serum, respectively).[7]

In addition, according to a different unpublished data submission, products intended for use on eyelashes, eyebrows, or scalp hair contain Ethyl Tafluprostamide in concentrations ranging from 0.012 – 0.02% (unknown if these are marketed products).[4] The amount of an eyelash product containing 0.018% Ethyl Tafluprostamide applied per brush stroke was evaluated to be, on average, 2.4 mg (maximum amount of 4 mg per brush stroke). The average amount of Ethyl Tafluprostamide applied per brush stroke was calculated to be 0.432 µg (maximum amount of 0.72 µg per brush stroke).

Although products containing these ingredients may be marketed for use with airbrush delivery systems, this information is not available from the VCRP or the Council survey. Without information regarding the frequency and concentrations of use of these ingredients (and without consumer habits and practices data or particle size data related to this use technology), the data are insufficient to evaluate the exposure resulting from cosmetics applied via airbrush delivery systems.

The ingredients named in the report are not restricted from use in any way under the rules governing cosmetic products in the European Union.[8] The SCCS is not able to conclude on the safety of Ethyl Tafluprostamide and Isopropyl Cloprostenate when used up to the intended use concentrations (0.018% for Ethyl Tafluprostamide and 0.006% and 0.007% for Isopropyl Cloprostenate).[2] The SCCS noted concerns about the safety of Ethyl Tafluprostamide and Isopropyl Cloprostenate when used in cosmetic products, particularly those used near the eye, as these are pharmacologically active substances that may have effects at low concentrations.

Non-Cosmetic

No FDA-approved prescription or over-the-counter drug uses for these ingredients were found in the literature. Aside from cosmetics, no other types of industrial uses were found for these ingredients.

TOXICOKINETIC STUDIES

Dermal Absorption

Ethyl Tafluprostamide

According to unpublished data, the estimated maximum amount of Ethyl Tafluprostamide that would be dermally absorbed was determined to be 0.144 µg.[4] This calculation was based on a conservative dermal absorption rate of 20% and maximum single brush stroke application of an eyelash product containing 0.018% Ethyl Tafluprostamide (corresponding to maximum amount of 0.72 µg Ethyl Tafluprostamide, per brush stroke).

Isopropyl Cloprostenate

Dermal absorption of Isopropyl Cloprostenate was estimated using a quantitative structure-activity relationship (QSAR) model.[2] The estimated dermal absorption was determined to be 10% (based on a molecular weight of 476 g/mol and a log K_{ow} of 5.15 for Isopropyl Cloprostenate).

TOXICOLOGICAL STUDIES

Acute Toxicity Studies

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 dimethyl sulfoxide and water) via intraperitoneal injection, and observed for 14 d.[9] Two control groups were treated with physiological solution or dimethyl sulfoxide and water. No adverse effects regarding clinical parameters, mortality, or body weight were observed.

Short-Term Toxicity Studies

Parenteral

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.[9] Control groups received a solution of dimethyl sulfoxide and water. Parameters evaluated include red blood cell count, hemoglobin, hematocrit, and red/white cell indices. Two hours after the last administration, animals were 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.[2] No other details were provided.

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.[10] Intraperitoneal injections of the test substance were given to mice in a dose of 25 µg/kg bw/d, and to rats in doses of either 25 or 100 µg/kg bw/d. Control groups of mice and rats were left untreated. Animals were killed at different time intervals (7, 14, and 28 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, affected spermatogenesis, and nuclear condensation of the testicular cells. Macrophages, decreased spermatozoa, and affected spermatogenesis were observed in treated mice.

A similar study was performed in male mice (12 mice/group; strain of mice not specified).[11] 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

In Silico

Isopropyl Cloprostenate

A QSAR model and a statistical-based model of an Ames test on Isopropyl Cloprostenate predicted no genotoxicity.[2] No details were provided.

CARCINOGENICITY STUDIES

In Silico

Ethyl Tafluprostamide and Isopropyl Cloprostenate

An in silico analysis of Ethyl Tafluprostamide and Isopropyl Cloprostenate was performed by the SCCS to predict carcinogenicity.[2] Although these ingredients are predicted to be non-mutagenic and non-genotoxic, 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.

OTHER RELEVANT STUDIES

Characterization of Prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) Receptors in Human Eyelids

The following study has been included in this report as it may provide insight regarding the potential sites of toxicity of Isopropyl Cloprostenate.

Isopropyl Cloprostenate

The distribution and presence of $PGF_{2\alpha}$ receptors in human hair follicles was evaluated in excised lower eyelid specimens.[12] Analysis was performed on 37 samples examining 17 eyes of 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.

Evaluation of Conjunctival Hyperemia

Isopropyl Cloprostenate

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

Pupil Constriction

Isopropyl Cloprostenate

The effect of Isopropyl Cloprostenate on the constriction of pupils was evaluated in cats.[13] 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 (or median effective dose). The ED_5 for Isopropyl Cloprostenate was determined to be 0.013 µg. No other details were provided in this study.

Intra-Ocular Pressure

Ethyl Tafluprostamide

The effect of an eyelash product containing 0.018% Ethyl Tafluprostamide on intra-ocular pressure was evaluated in 19 subjects.[4] Subjects were instructed to use the product for 28 d, and were evaluated at baseline and on day 28. No changes in intra-ocular pressure were observed in subjects after 28 d of product use. The within-eye differences in intra-ocular pressure from the beginning to the end of the study were not statistically significant ($t > 0.05$). The results of the ocular irritation evaluation performed during this study can be found in the Ocular Irritation section of this report.

Isopropyl Cloprostenate

The intra-ocular pressure lowering efficacy of Isopropyl Cloprostenate was evaluated in conscious ocular hypertensive cynomolgus monkeys.[13] 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.

DERMAL IRRITATION AND SENSITIZATION STUDIES

Details on the dermal sensitization studies summarized below can be found in Table 4.

An eyelash product containing 0.018% Ethyl Tafluprostamide (n = 51; tested neat) and 7.5% Ethyl Tafluprostamide in phenoxyethanol (n = 54) were considered to be non-sensitizing in human repeat insult patch tests (HRIPTs).[4] HRIPTs were also performed using eyelash serums containing Isopropyl Cloprostenate (0.0044% and 0.005%; tested neat; n = 50-56).[14-17] The majority of assays were performed under semi-occlusive conditions. The serums tested were considered to be non-irritating and non-sensitizing in all assays.

OCULAR IRRITATION STUDIES

Details on the in vitro and human ocular irritation studies summarized below can be found in Table 5.

An eyelash product containing 0.018% Ethyl Tafluprostamide was not predicted to be an ocular irritant in a hen's egg chorioallantoic membrane (HET-CAM) assay (tested concentration not stated).[4] 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).[18-22] All test substances were predicted to be slightly or non-irritating. Similarly, Isopropyl Cloprostenate (0.1%) was predicted to be non-irritating in a HET-CAM assay (tested at a 50% dilution resulting in an actual test concentration of 0.05% Isopropyl Cloprostenate).[23]

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).[4] 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.[24] 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.[25,26] 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.[2]

CLINICAL STUDIES

Clinical Trial

Isopropyl Cloprostenate

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

Case Report

Isopropyl Cloprostenate

A 32-yr-old woman presented to an outpatient department due to periocular discoloration for 4 mo.[27] 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 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 Isopropyl Cloprostenate were also observed in a 35-yr-old woman who reported use of the product for 10 mo.[28] 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% over the course of 2 yr (2011 – 2013).[4] The number of reported undesirable effects for this product, during this time period, was 0.00717% of the number of sold units. 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

Isopropyl Cloprostenate

A margin of safety (MOS; calculated as the ratio between a point of departure (PoD) and systemic exposure dosage (SED)) calculation was performed on Isopropyl Cloprostenate.[2] The MOS was determined to be 2.5 (with an estimated combined SED of 0.00000084 mg/kg bw/d from eyelash and eyebrow products). In the calculation, the toxicological screening value (TSV) was used as the point of departure (PoD; detailed numerical value was not available due to confidentiality issue), thus a MOS ≥ 1 was considered to be protective.[29]

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 2023 VCRP data, Isopropyl Cloprostenate is used in 3 "other eye makeup preparation" formulations, and no uses were reported to Ethyl Tafluprostamide. 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, according to data submitted by industry as a submission separate from the concentration of use survey, two eyelash serums were determined to contain 0.0044% and 0.0048% Isopropyl Cloprostenate, respectively. 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%.

According to unpublished data, the estimated maximum amount of Ethyl Tafluprostamide that would be dermally absorbed was determined to be 0.144 µg (based on maximum use of a product containing 0.018% Ethyl Tafluprostamide and dermal absorption rate of 20%). An estimated dermal absorption of Isopropyl Cloprostenate was determined to be 10%, according to a QSAR model. This value was based on a molecular weight of 476 g/mol and a log K_{ow} of 5.15.

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

A hematological analysis was performed in rats given Isopropyl Cloprostenate (15 mg/kg bw/d), via intraperitoneal injection, for 7 d. No hematological abnormalities were observed.

Based on an in silico analysis, the SCCS flagged Ethyl Tafluprostamide and Isopropyl Cloprostenate as potential reproductive/developmental toxicants. The effect of Isopropyl Cloprostenate (25 or 100 µg/kg bw/d) on gonads and testicular cells was evaluated in mice and rats. In these assays, animals were treated for 28 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.

A QSAR model and a statistical-based model of an Ames test on Isopropyl Cloprostenate predicted no genotoxicity. Although these ingredients are predicted to be non-mutagenic and non-genotoxic, the SCCS flagged Ethyl Tafluprostamide and Isopropyl Cloprostenate for potential carcinogenicity based on an in silico analysis.

The distribution and presence of $PGF_{2\alpha}$ receptors in human hair follicles was evaluated using excised lower eyelid samples. Receptors were only 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.

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.

No statistically-significant changes in intra-ocular pressure were observed in 19 subjects after a 28-d use period of a product containing 0.018% Ethyl Tafluprostamide. A 39% reduction in intraocular pressure was observed in ocular hypertensive monkeys treated with 1 µg Isopropyl Cloprostenate (in lasered right eyes).

An eyelash product containing 0.018% Ethyl Tafluprostamide (tested neat) and 7.5% Ethyl Tafluprostamide in phenoxyethanol 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.

An eyelash product containing 0.018% Ethyl Tafluprostamide was not predicted to be an ocular irritant in a HET-CAM 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. Similarly, Isopropyl Cloprostenate (0.1% (tested at a 50% dilution, resulting in an actual test concentration of 0.05%)) was predicted to be non-irritating in a HET-CAM assay.

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). 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. 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 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% 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 sold units.

The MOS for Isopropyl Cloprostenate was calculated to be 2.5, based on the ratio of TSV (as PoD) over SED in eyelash and eyebrow products.

DISCUSSION

To be developed

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	[2]
Color	colorless to pale yellow	[2]
Molecular Weight (g/mol)	437.5	[2]
Water Solubility (mg/l @ 25ºC)	0.09	[2]
log K_{ow}	5.03	[2]
Isopropyl Cloprostenate		
Molecular Weight (g/mol)	467	[2]
Water Solubility (mg/l @ 25ºC)	0.047	[2]
log K_{ow}	5.15	[2]

Table 3. 2023 Frequency and concentration of use by product category[4-7]

	Isopropyl Cloprostenate		Ethyl Tafluprostamide	
	# of Uses	Max Conc of Use (%)	# of Uses	Max Conc of Use (%)
Eye Makeup Preparations				
Other Eye Makeup Preparations	3	NR[a]; 0.0044 - 0.0048[b]	NR	0.012 – 0.02[c]
Hair Preparations (non-coloring)				
Other Hair Preparations	NR	NR	NR	0.012 – 0.02[c]

NR = not reported

[a]none reported in response to a concentration of use survey performed by Council in 2022

[b]average concentration of Isopropyl Cloprostenate in two eyelash serums according a separate data submission

[c]concentration of Ethyl Tafluprostamide in products used on eyelashes, eyebrows, and scalp hair according to a separate data submission

Table 4. HRIPTs on Isopropyl Cloprostenate

Test Article	Vehicle	Concentration/Dose	Test Population	Procedure	Results	Reference
Eyelash product containing 0.018% Ethyl Tafluprostamide	NR	applied neat (dose not stated)	51	HRIPT; level of occlusion not stated; nine 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	[4]
7.5% Ethyl Tafluprostamide in phenoxyethanol	NR	applied neat (dose not stated)	54	HRIPT; same procedure as stated above	Non-irritating; non-sensitizing	[4]
Eyelash serum containing 0.0044% Isopropyl Cloprostenate	NR	0.2 ml; applied neat	53	HRIPT; semi-occlusive conditions; nine 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	[17]
Eyelash serum containing 0.0044% Isopropyl Cloprostenate	NR	100%; applied neat	56	HRIPT; semi-occlusive conditions; nine 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	[16]
Eyelash serum containing 0.005% Isopropyl Cloprostenate	NR	0.2 ml; applied neat	50	HRIPT; occlusive conditions to the infrascapular region of the back; nine 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	[14]
Eyelash serum containing 0.005% Isopropyl Cloprostenate	NR	100%; applied neat	53	HRIPT; semi-occlusive conditions; nine 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	[15]

HRIPT = human repeat insult patch test

Table 5. Ocular irritation studies

Test Article	Vehicle	Concentration/Dose	Test Population	Procedure	Results	Reference
IN VITRO						
Eyelash product containing 0.018% Ethyl Tafluprostamide	NR	NR	4 samples	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 an irritation potential of practically none) Reference test articles have historically been shown to be practically non-irritating. Study author concluded the test substance would have practically no ocular irritation potential in vivo	[4]
Eyelash serum containing 0.0044% Isopropyl Cloprostenate	Saline	0.3 ml; 10%	6 samples	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	[20]
Eyelash serum containing 0.0044% Isopropyl Cloprostenate	NR	0.3 ml; 50%*	4 samples	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 an irritation potential of practically none) Reference test articles have historically been shown to be practically non-irritating. Study author concluded that the test substance, at 100%, would have practically no ocular irritation in vivo.	[21]
Eyelash serum containing 0.005% Isopropyl Cloprostenate	NR	0.3 ml; 50%*	4 samples	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 an irritation potential of practically none) Reference test articles have historically been shown to be practically non-irritating. Study author concluded that the test substance, at 100%, would have practically no ocular irritation in vivo.	[22]
Eyelash serum containing 0.005% Isopropyl Cloprostenate	Saline	0.3 ml; 10%	6 samples	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	[18]

Table 5. Ocular irritation studies

Test Article	Vehicle	Concentration/Dose	Test Population	Procedure	Results	Reference
Eyelash serum containing 0.005% Isopropyl Cloprostenate	Saline	0.3 ml; 10%	6 samples	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	[19]
0.1% Isopropyl Cloprostenate	NR	0.3 ml; 50%	4 samples	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 an irritation potential of practically none) Reference test articles have historically been shown to be practically non-irritating Study author concluded that 0.1% Isopropyl Cloprostenate would have practically no ocular irritation potential in vivo	[23]
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.	[4]
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	[24]
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	[24]

Table 5. Ocular irritation studies

Test Article	Vehicle	Concentration/Dose	Test Population	Procedure	Results	Reference
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	[25]
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	[26]
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	[2]

HET-CAM = hen's egg test chorioallantoic membrane; NR = not reported

*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

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MB Research Labs

Study Title

Hen's Egg Test Chorioallantoic Membrane (HET-CAM) For Non-Opaque Materials

Test Article

[redacted], Eyelash Formula lash serum containing 0.005% Isopropyl Cloprostenate

Author

[redacted], Study Director

Study Completed On

10 Aug 2016

Performing Laboratory

MB Research Laboratories

[redacted]

MB Research Project

[redacted]

MB Research Protocol

[redacted]

Sponsor

[redacted]

MB Research Labs

Study Title : **HET-CAM**
Project # : [redacted]

KEY PERSONNEL

[redacted] B.S., MPH, DABT	Director of Toxicology
[redacted], B.S.	Study Director
[redacted], AAS, CVT, LATG	Senior Lab Technician
[redacted], B.A., RLAT	Technician

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MB Research Labs

Study Title : HET-CAM
Project # : [redacted]

GOOD LABORATORY PRACTICES COMPLIANCE STATEMENT

This study was conducted in accordance with applicable Good Laboratory Practices regulations of the FDA, 21 CFR Part 58, with the following exceptions:

Test article characterization information, provided by the Sponsor, was not complete. See Appendix A for information that was provided. The effect of the lack of full test article characterization information cannot be fully assessed.

Test article characterization, provided by the Sponsor, was not conducted according to the Good Laboratory Practices; however, it was conducted according to the Good Manufacturing Practices. This is not expected to have an impact on the outcome of the study.

Analysis of the test article and positive control in mixtures was not performed. The mixtures were prepared fresh daily. Although no adverse effect is expected, the lack of analysis cannot be fully assessed.

STUDY DIRECTOR: [redacted] 04AUG2016

[redacted] B.S. Date
MB RESEARCH LABORATORIES

MB Research Labs

Study Title : **HET-CAM**
Project # : [redacted]

QUALITY ASSURANCE EVALUATION

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

Date of Inspection	Phase	Performed By	Date Inspection Results Reported	
			Study Director	Management
20 Jun 2016	Sample Preparation	[redacted]	20 Jun 2016	20 Jun 2016
23 Jun 2016	Raw data audit		23 Jun 2016	28 Jun 2016
15 Jul 2016	Draft report audit		15 Jul 2016	09 Aug 2016
09 Aug 2016	Final report audit		09 Aug 2016	09 Aug 2016

[redacted] 09 Aug 2016
[redacted] Date
Quality Assurance Unit

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TABLE OF CONTENTS

MB Research Labs

PROJECT NUMBER : [redacted]
TEST ARTICLE : [redacted] Eyelash Formula
SPONSOR : [redacted]
TITLE : Hen's Egg Test Chorioallantoic Membrane (HET-CAM) For Non-Opaque Materials
PROTOCOL # : [redacted]

ABSTRACT

Objective: To determine the potential irritancy using an alternative to the Draize methodology. The methodology was based on that described in INVITTOX. 1992. Protocol No. 47: HET-CAM Test.

Method Synopsis: The chorioallantoic membrane (CAM) of twenty-four White Leghorn eggs, incubated for 10 days, was dosed with 300 µl of the test substance as listed below. The eggs were observed continuously for five minutes immediately following dosing for the appearance of lysis (sec L), hemorrhage (sec H) and/or coagulation (sec C). In addition, the eggs were scored for severity at 1 and 5 minutes postdose. The irritating potential of the test article was classified based on the irritation score (IS) and the threshold concentration (TH).

Summary:

Test Substance	# of Eggs Dosed
0.1 N Sodium Hydroxide (NaOH)	6
1% Sodium Dodecyl Sulfate (SDS)	6
0.9% Sodium Chloride Irrigation (saline)	6
10% [redacted], Eyelash Formula	6

The mean IS for 0.1 N NaOH and 1% SDS were 17.40 and 10.33, respectively. The vehicle control, 0.9% saline, had no adverse effects on the CAM. At 10% (v/v) in 0.9% saline, the IS of [redacted] Eyelash Formula, was 0.00. The threshold concentration for this test article was greater than 10%.

Conclusion: Based on the threshold concentration of greater than 10% and the $IS_{10\%}$ of 0.00, the irritating potential of [redacted], Eyelash Formula, was determined to be none to slight.

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OBJECTIVE

To determine the potential irritancy using an alternative to the Draize methodology. The methodology was based on that described in INVITTOX. 1992. Protocol No. 47: HET-CAM Test.

TEST ARTICLE

Identity	:	[redacted] Eyelash Formula
Test Article Characterization	:	See Appendix A for Test Article Characterization
Supplied by	:	[redacted]
Date Received	:	08 Jun 2016
Storage	:	Room temperature and humidity
Description	:	Clear colorless liquid
Sample Preparation	:	1.0 ml of test article was brought to a total volume of 10 ml with 0.9% saline to yield a 10% concentration.

POSITIVE CONTROLS

Identity	:	0.1 N Sodium Hydroxide (NaOH), Lot# SHBG0127V
Supplied By	:	[redacted]
Date Received	:	15 Jun 2016
Expiration Date	:	Feb 2018
Storage	:	Room temperature and humidity
Description	:	Clear colorless liquid
Sample Preparation	:	Used as received.

Identity	:	Sodium Dodecyl Sulfate (SDS), Lot# 134136
Supplied By	:	[redacted]
Date Received	:	02 Jan 2014
Expiration Date	:	Jul 2018
Storage	:	Room temperature and humidity
Description	:	White powder
Sample Preparation	:	0.1 g of SDS was brought to a total volume of 10 ml with distilled water to yield a 1% concentration.

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VEHICLE FOR POSITIVE CONTROL

Identity	:	Distilled water
Supplied by	:	[redacted]
Date Received	:	19 Feb 2016
Expiration Date	:	01 Mar 2017
Storage	:	Room temperature and humidity
Description	:	Clear colorless liquid

VEHICLE

Identity	:	0.9% Sodium Chloride Irrigation (saline), [redacted]
Supplied By	:	[redacted]
Date Received	:	06 Jun 2014
Expiration Date	:	01 Feb 2017
Storage	:	Room temperature and humidity
Description	:	Clear colorless liquid
Sample Preparation	:	Used as received

TEST DATES

Study Initiation	(date protocol signed)	:	17 Jun 2016
Experimental Start Date	(1st exposure to test substance)	:	20 Jun 2016
Experimental Term Date	(last date data collected)	:	20 Jun 2016
Draft Report Submitted	(if applicable)	:	18 Jul 2016
Final Report Signed	(study completion)	:	10 Aug 2016

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EXPERIMENTAL DESIGN

Test System

Fertile, White Leghorn eggs (twenty-four) received from Moyer's Chicks, Quakertown, PA were selected for use from a larger group and incubated on 10 Jun 2016. The eggs were kept in incubators at 99 (±2)°F for 10 days.

Pre-dose Procedures

The eggs were marked on one side with an "X" and on the other side with an "O", and placed horizontally in the incubator trays. The eggs were rotated once daily during the first nine days of incubation to ensure even atmospheric exposure.

On Day 9 of incubation, the eggs were rotated and turned up in the incubator with the large end upwards containing the air sac to facilitate access to the CAM.

On Day 10 of development, the eggs were removed from the incubator and candled to determine the viability of the embryo. A rectangular window was removed from the shell directly over the air sac using a rotating Dremel® drill with a diamond wheel bit. The egg membrane was carefully moistened with 2-3 ml of 0.9% saline and returned to the incubator. Eggs were examined for any abnormalities. All abnormal eggs were discarded.

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EXPERIMENTAL DESIGN (continued)

Dosing

The eggs were dosed within 30 minutes of opening. The excess saline solution was gently poured off of the egg membrane which was then removed, and the CAM exposed. The eggs were numbered and 300 µl of the 10% mixture of the test article, positive controls (0.1 N Sodium Hydroxide [NaOH] and 1% Sodium Dodecyl Sulfate [SDS]) or vehicle control (0.9% Sodium Chloride Irrigation (saline)) was pipetted onto the CAM.

Type and Frequency of Observations

The eggs were observed continuously for 5 minutes and the appearance of lysis (sec L), hemorrhage (sec H) and/or coagulation (sec C) was documented. If no reaction was observed, a value of 301 seconds was recorded. In addition, the eggs were scored for severity at 1 and 5 minutes postdose.

The severity of each reaction after 1 and 5 minutes were recorded as follows:

0 = no reaction
1 = slight reaction
2 = moderate reaction
3 = severe reaction

Analysis of Data

The severity score was used to determine the threshold concentration (TH).

Irritation potential was classified by a scheme which depended on two components. The first was the calculated irritation score (IS). The IS was based on the time until adverse reactions (hemorrhage, vessel lysis and coagulation) were first observed. The second component of irritation potential was a determination of the severity (slight, moderate or severe) of adverse reactions after 1 and 5 minutes. The threshold was defined as the lowest concentration at which slight reactions occur.

Calculations: The irritation score (IS) was calculated as follows:

$$IS = \frac{[(301 - \text{sec H}) * 5]}{300} + \frac{[(301 - \text{sec L}) * 7]}{300} + \frac{[(301 - \text{sec C}) * 9]}{300}$$

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EXPERIMENTAL DESIGN (continued)

ANALYSIS OF DATA (continued)

Interpretation

The HET-CAM method is intended as an alternative to the Draize eye irritation evaluation in rabbits. Whenever possible, the conclusions of the HET-CAM will be related to those of the Draize (Draize, J.H. et al. 1944. J. Pharm. Exp. Ther., 82:377-90). Classification of the irritating potential will be according to the chart below. (INVITTOX. 1992. Protocol No. 47: HET-CAM Test.)

Classification of Irritating Potential

Threshold Concentration (TH%)	Irritation Score (10%)	Severity	Classification
TH < 1	-	-	severe / corrosive
1.0 < TH < 2.5	> 16	-	severe / corrosive
2.5 < TH < 10.0	< 16	severe reaction after 1 min	severe / corrosive
1.0 < TH < 2.5	< 16	-	irritant
2.5 < TH < 10.0	> 16	-	irritant
2.5 < TH < 10.0	< 16	severe reaction after 5 min	irritant
2.5 < TH < 10.0	< 16	weak or no reaction	moderate
10.0 < TH	> 16	-	moderate
10.0 < TH	< 16	severe reaction	moderate
10.0 < TH	< 10	-	none/slight

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EXPERIMENTAL DESIGN (continued)

Retention of Data

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

All data generated during the conduct of this study will be archived at MB Research for at least 10 years from the date of the final report. The Sponsor will then be contacted in writing to determine final disposition of the records. If the Sponsor fails to respond within 90 days, the archived items will be properly discarded.

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

Amendment to the Protocol

See Appendix B for protocol in its entirety.

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MB Research Labs

Study Title : HET-CAM
Project # : [redacted]

RESULTS

Egg #	Hemorrhage (H) (sec)	Vessel Lysis (L) (sec)	Coagulation (C) (sec)	Score @ 1min	Score @ 5min	IS Score	Mean	SD
1% Sodium Dodecyl Sulfate								
1	68	38	301	1	2	10.02	10.33	0.315
2	59	34	301	1	2	10.26		
3	40	20	301	1	2	10.91		
4	53	32	301	1	2	10.41		
5	65	30	301	1	2	10.26		
6	59	41	301	1	2	10.10		
0.1N Sodium Hydroxide								
7	24	56	83	2	3	16.87	17.40	0.630
8	32	58	76	2	3	16.90		
9	26	54	78	2	3	17.04		
10	29	51	61	2	3	17.57		
11	20	48	72	2	3	17.46		
12	17	32	50	2	3	18.54		
0.9% Saline								
13	301	301	301	0	0	0.00	0.00	0.000
14	301	301	301	0	0	0.00		
15	301	301	301	0	0	0.00		
16	301	301	301	0	0	0.00		
17	301	301	301	0	0	0.00		
18	301	301	301	0	0	0.00		
10% [redacted], Eyelash Formula								
25	301	301	301	0	0	0.00	0.00	0.000
26	301	301	301	0	0	0.00		
27	301	301	301	0	0	0.00		
28	301	301	301	0	0	0.00		
29	301	301	301	0	0	0.00		
30	301	301	301	0	0	0.00		

MB Research Labs

Study Title : **HET-CAM**
Project # : [redacted]

DISCUSSION

The mean IS for 0.1 N NaOH and 1% SDS were 17.40 and 10.33, respectively. The vehicle control, 0.9% saline, had no adverse effects on the CAM. At 10% (v/v) in 0.9% saline, the IS of [redacted], Eyelash Formula, was 0.00. The threshold concentration for this test article was greater than 10%.

CONCLUSION

Based on the threshold concentration of greater than 10% and the $IS_{10\%}$ of 0.00, the irritating potential of [redacted], Eyelash Formula, was determined to be none to slight.

FINAL REPORT

Approved by: [redacted] 1 AUG 2016
[redacted] B.S. Date
Study Director

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CONSUMER PRODUCT TESTING COMPANY
cptclabs.com

FINAL REPORT

CLIENT: [redacted]

ATTENTION: [redacted]

TEST: The Hen's Egg Test - Utilizing the Chorioallantoic Membrane (HET-CAM)
Protocol: [redacted]
Protocol Date: 2/10/22

TEST ARTICLE: [redacted] isopropyl ester, Item: [redacted] Lot# [redacted]
Isopropyl Cloprostenate at 0.1%

EXPERIMENT REFERENCE NO.: [redacted]

[redacted] 10/12/22
Vice President
Laboratory Director

FDA Registration# 1000151293
DEA Registration# RC0199744 Schedule I-V
US EPA/NJ DEP Registration# NJD982726648
ISO/IEC 17025:2017 Accredited

[redacted]

Clinical • Photobiology • Analytical Chemistry • Microbiology • In-Vitro Safety • Consulting

[redacted]

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CONSUMER PRODUCT TESTING™ COMPANY
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FDA Registration# 1000151293
DEA Registration# RC0199744 Schedule I-V
US EPA/NJ DEP Registration# NJ0582726648
ISO/IEC 17025:2017 Accreditation # 80071

QUALITY ASSURANCE UNIT STATEMENT

CPT Study Number: [redacted]

The objective of the Quality Assurance Unit (QAU) is to monitor the conduct and reporting of nonclinical laboratory studies. This study has been performed under Good Laboratory Practice principles to the extent applicable, and in accordance with CPT Standard Operating Procedures and applicable Standard Protocols. The QAU has reviewed and approved this study on the date indicated below.

Approved by CPT Quality Assurance Unit:

[redacted] 10-13-22

Signature/[redacted]

[redacted]

Distributed for Comment Only -- Do Not Cite or Quote

Objective:

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

Introduction:

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

Test Article: [redacted] isopropyl ester, Item: [redacted], Lot# [redacted]

Reference Articles[3]: [redacted] One Coat Mascara
[redacted] Waterproof Ultra Eyeliner

Date of Assay Initiation & Completion: October 5, 2022

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

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

[3]Historical *in vivo* and *in vitro* data referenced.

Method:

Fresh, fertile, White Leghorn eggs were obtained from a suitably licensed/approved vendor. The eggs were stored at this facility for two days, at approximately 12° - 16° C, after arrival at this facility. For incubation Days 0 – 10, the eggs were placed in a Kuhl, humidified incubator so that the acutely angled ends face down. The incubator is such that the eggs are automatically rotated approximately once every hour. The temperature was maintained at 37° C (± 2° C) for the incubation period.

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

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

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

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

Results:

Test Article (%)	CAM #	Scores @ 0.5 min.	2 min.	5 min.	Total
[redacted]isopropyl ester, Item: [redacted] Lot# [redacted] (0.05%)	1	0	3	0	3
	2	0	0	1	1
	3	0	0	1	1
	4	0	0	1	1
				Average:	**1.50**

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

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

Each article was then classified as indicated in the following:

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

Discussion:

Previous studies have shown that the CAM of the hen's egg is more sensitive to liquid irritants than is the rabbit eye. Therefore, 50% dilutions of the liquid reference articles were used to approximate their 100% irritation potential. The Sponsor requested the irritation potential of their article at 0.1%. Therefore, a 0.05% dilution of the liquid test article wea used to approximate its 0.1% irritation potential.

Historical *In Vivo* Results:

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

Conclusion:

Under the conditions of this test, the results indicate that the sponsor-submitted product, [redacted] isopropyl ester, Item: [redacted], Lot# [redacted], at 0.1%, would have practically no ocular irritation potential *in vivo*.

Record Retention:

All records and documents pertaining to the conduct of testing shall be retained in the CPTC archives for a period of ten (10) years. At any time prior to the completion of the tenth archival year, a Sponsor may submit a written request to the CPTC QA Department to obtain custody of study records once the CPTC archive period has been completed. This transfer shall be performed at the Sponsor's expense. In the absence of a written request, study-related records shall be destroyed at the end of the CPTC archive period, with no further notice, in a manner that renders them useless.

Professional personnel involved:

[redacted]

- Vice President
  Laboratory Director
  (Study Director)
- Senior Analyst
- Quality Assurance Supervisor
- Quality Assurance Auditor

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CONSUMER PRODUCT TESTING COMPANY
cptclabs.com

FINAL REPORT

CLIENT: [redacted]

ATTENTION: [redacted]

TEST: The Hen's Egg Test - Utilizing the Chorioallantoic Membrane (HET-CAM)
Protocol: [redacted]
Protocol Date: 2/10/22

TEST ARTICLE: [redacted]
lash serum containing 0.005% Isopropyl Cloprostenate

EXPERIMENT REFERENCE NO.: [redacted]

[redacted] 10/12/22
[redacted]
Vice President
Laboratory Director

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CONSUMER PRODUCT TESTING™ COMPANY
cptclabs.com

FDA Registration# 1000151293
DEA Registration# RCD199744 Schedule I-V
US EPA/NJ DEP Registration# NJD982729648
ISO/IEC 17025:2017 Accreditation # 80071

QUALITY ASSURANCE UNIT STATEMENT

CPT Study Number: [redacted]

The objective of the Quality Assurance Unit (QAU) is to monitor the conduct and reporting of nonclinical laboratory studies. This study has been performed under Good Laboratory Practice principles to the extent applicable, and in accordance with CPT Standard Operating Procedures and applicable Standard Protocols. The QAU has reviewed and approved this study on the date indicated below.

Approved by CPT Quality Assurance Unit:

[redacted] 10-13-22

Signature/Date

[redacted]

Objective:

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

Introduction:

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

Test Article: [redacted]

Reference Articles[3]**:** [redacted] One Coat Mascara
[redacted] Waterproof Ultra Eyeliner

Date of Assay Initiation & Completion: October 5, 2022

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

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

[3]Historical *in vivo* and *in vitro* data referenced.

Method:

Fresh, fertile, White Leghorn eggs were obtained from a suitably licensed/approved vendor. The eggs were stored at this facility for two days, at approximately 12° - 16° C, after arrival at this facility. For incubation Days 0 – 10, the eggs were placed in a Kuhl, humidified incubator so that the acutely angled ends face down. The incubator is such that the eggs are automatically rotated approximately once every hour. The temperature was maintained at 37° C (± 2° C) for the incubation period.

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

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

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

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

Results:

Test Article (%)	CAM #	Scores @ 0.5 min.	2 min.	5 min.	Total
[redacted] [redacted](50%)	1	0	3	0	3
	2	0	3	0	3
	3	0	3	0	3
	4	0	0	1	1
				Average:	**2.50**

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

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

Each article was then classified as indicated in the following:

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

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Discussion:

Previous studies have shown that the CAM of the hen's egg is more sensitive to liquid irritants than is the rabbit eye. Therefore, 50% dilutions of the liquid test and reference articles were used to approximate their 100% irritation potential.

Historical *In Vivo* Results:

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

Conclusion:

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

Record Retention:

All records and documents pertaining to the conduct of testing shall be retained in the CPTC archives for a period of ten (10) years. At any time prior to the completion of the tenth archival year, a Sponsor may submit a written request to the CPTC QA Department to obtain custody of study records once the CPTC archive period has been completed. This transfer shall be performed at the Sponsor's expense. In the absence of a written request, study-related records shall be destroyed at the end of the CPTC archive period, with no further notice, in a manner that renders them useless.

Professional personnel involved:

[redacted]

- Vice President
  Laboratory Director
  (Study Director)
- Senior Analyst
- Quality Assurance Supervisor
- Quality Assurance Auditor

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CONSUMER PRODUCT TESTING COMPANY
cptclabs.com

FINAL REPORT

CLIENT: [redacted]

ATTENTION: [redacted]

TEST: The Hen's Egg Test - Utilizing the Chorioallantoic Membrane (HET-CAM)
Protocol: [redacted]
Protocol Date: 2/10/22

TEST ARTICLE: [redacted]
lash serum containing 0.0044% Isopropyl Cloprostenate

EXPERIMENT REFERENCE NO.: [redacted]

[redacted] 10/12/22
[redacted]
Vice President
Laboratory Director

CONSUMER PRODUCT TESTING COMPANY
cptclabs.com

FDA Registration# 1000151293
DEA Registration# RC0193744 Schedule I-V
US EPA/NJ DEP Registration# NJD582726648
ISO/IEC 17025:2017 Accreditation # 80071

QUALITY ASSURANCE UNIT STATEMENT

CPT Study Number: [redacted]

The objective of the Quality Assurance Unit (QAU) is to monitor the conduct and reporting of nonclinical laboratory studies. This study has been performed under Good Laboratory Practice principles to the extent applicable, and in accordance with CPT Standard Operating Procedures and applicable Standard Protocols. The QAU has reviewed and approved this study on the date indicated below.

Approved by CPT Quality Assurance Unit:

[redacted] 10-13-22

Signature/Date

[redacted]

Objective:

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

Introduction:

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

Test Article: [redacted]

Reference Articles[3]: [redacted] One Coat Mascara
[redacted] Waterproof Ultra Eyeliner

Date of Assay Initiation & Completion: October 5, 2022

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

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

[3]Historical *in vivo* and *in vitro* data referenced.

Method:

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

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

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

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

Results:

Test Article (%)	CAM #	Scores @ 0.5 min.	2 min.	5 min.	Total
[redacted] (50%)	1	0	0	1	1
	2	0	0	0	0
	3	0	3	0	3
	4	0	0	1	1
				Average:	**1.25**

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

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

Each article was then classified as indicated in the following:

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

[redacted]

Discussion:

Previous studies have shown that the CAM of the hen's egg is more sensitive to liquid irritants than is the rabbit eye. Therefore, 50% dilutions of the liquid test and reference articles were used to approximate their 100% irritation potential.

Historical *In Vivo* Results:

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

Conclusion:

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

Record Retention:

All records and documents pertaining to the conduct of testing shall be retained in the CPTC archives for a period of ten (10) years. At any time prior to the completion of the tenth archival year, a Sponsor may submit a written request to the CPTC QA Department to obtain custody of study records once the CPTC archive period has been completed. This transfer shall be performed at the Sponsor's expense. In the absence of a written request, study-related records shall be destroyed at the end of the CPTC archive period, with no further notice, in a manner that renders them useless.

Professional personnel involved:

[redacted]

- Vice President
  Laboratory Director
  (Study Director)
- Senior Analyst
- Quality Assurance Supervisor
- Quality Assurance Auditor

MB Research Labs

Study Title

Hen's Egg Test Chorioallantoic Membrane (HET-CAM) For Non-Opaque Materials

Test Article

[redacted] lash serum containing 0.0044% Isopropyl Cloprostenate.

Author

[redacted] B.S., Study Director

Study Completed On

07 May 2019

Performing Laboratory

MB Research Laboratories

[redacted]

MB Research Project No.

[redacted]

MB Research Protocol No.

[redacted]

Sponsor

[redacted]

MB Research Labs

Study Title : HET-CAM
Project No. : [redacted]

GOOD LABORATORY PRACTICES COMPLIANCE STATEMENT

This study was conducted in accordance with applicable Good Laboratory Practices regulations of the FDA, 21 CFR Part 58, with the following exceptions:

The test article characterization information, provided by the Sponsor, did not include all GLP required parameters. See Appendix A for information that was provided. The effect of the lack of full test article characterization information cannot be fully assessed.

Test article characterization, provided by the Sponsor, was not conducted according to the Good Laboratory Practices. However, it was conducted according to the Good Laboratory Practices. This is not expected to have an impact on the outcome of the study.

Analysis of the test article or control article in the vehicle was not performed. The mixture was prepared fresh daily. Although no adverse effect is expected, the lack of analysis cannot be fully assessed.

STUDY DIRECTOR: [redacted] 07 May 2019
[redacted], B.S. Date
MB RESEARCH LABORATORIES

MB Research Labs

Study Title : HET-CAM
Project No. : [redacted]

QUALITY ASSURANCE EVALUATION

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

Date of Inspection	Phase	Performed By	Date Inspection Results Reported	
			Study Director	Management
10 Dec 2018	Sample Preparation	[redacted]	10 Dec 2018	12 Dec 2018
18 Dec 2018	Raw data audit		18 Dec 2018	20 Dec 2018
10 Jan 2019	Draft report audit		10 Jan 2019	06 May 2019
06 May 2019	Final report audit		06 May 2019	06 May 2019

[redacted] 06 May 19
Quality Assurance Unit Date

MB Research Labs

Study Title : HET-CAM
Project No. : [redacted]

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MB Research Labs

Study Title : HET-CAM
Project No. : [redacted]

KEY PERSONNEL

[redacted], B.S., MPH, DABT	Director of Toxicology
[redacted], B.S.	Study Director
[redacted], AAS, CVT, LATG	Senior Lab Technician
[redacted], RALAT	Technician
[redacted], B.A., RLATG	Technician

MB Research Labs

PROJECT No. : [redacted]
TEST ARTICLE : [redacted]
SPONSOR : [redacted]
TITLE : **Hen's Egg Test Chorioallantoic Membrane (HET-CAM) For Non-Opaque Materials**
PROTOCOL No. : [redacted]

ABSTRACT

Objective
To determine the potential irritancy using an alternative to the Draize methodology. The methodology is based on that described in INVITTOX, 1992. Protocol No. 47: HET-CAM Test.

Method Synopsis
The chorioallantoic membranes (CAM) of twenty-four 10-day-old White Leghorn eggs (six eggs per treatment) were dosed with 300 µl of a test substance, as listed below. Immediately following dose administration, each egg was observed continuously for five minutes for the first appearance of lysis, hemorrhage, and/or coagulation. Additionally, eggs were scored for severity of responses to the CAM at 1 and 5 minutes post-dosing. The ocular irritation potential of the test article was classified based on the irritation score and the threshold concentration.

Summary

Treatment	Identity	Irritation Score	Threshold Concentration
Positive Control	1% Sodium Dodecyl Sulfate (SDS)	9.8	-
Positive Control	0.1 N Sodium Hydroxide (NaOH)	17.8	-
Vehicle Control	0.9 Sodium Chloride Irrigation, USP (saline)	0.0	-
10% Test Article Formulation	[redacted]	0.0*	Greater than 10%

* Irritation score at 10% test article formulation (v/v) in saline

The vehicle control did not produce adverse effects on the chorioallantoic membranes of treated eggs. The positive control irritation scores were within acceptable ranges; therefore, this test is considered valid.

Conclusion
Based on the calculated irritation score (at 10%) and the threshold concentration, [redacted] is considered to have none-to-slight irritation potential according to the classification criteria set forth in INVITTOX Protocol No. 47 (1992).

MB Research Labs

Study Title : **HET-CAM**
Project No. : [redacted]

OBJECTIVE

To determine the potential irritancy using an alternative to the Draize methodology. The methodology was based on that described in INVITTOX. 1992. Protocol No. 47: HET-CAM Test.

TEST ARTICLE

Identity	:	[redacted]
Test Article Characterization	:	See Appendix A for Test Article Characterization
Supplied by	:	[redacted]
Date Received	:	05 Dec 2018
Storage	:	Room temperature and humidity
Description	:	Clear colorless liquid
Sample Preparation	:	1.0 ml of the test article and 9.0 ml of saline were mixed to yield a 10% solution.

VEHICLE FOR THE TEST ARTICLE

Identity	:	0.9 Sodium Chloride Irrigation, USP (saline), [redacted] (See Appendix B for certificate of Analysis)
Supplied By	:	[redacted]
Date Received	:	19 Feb 2018
Expiration Date	:	Aug 2020
Storage	:	Room temperature and humidity
Description	:	Clear colorless liquid
Sample Preparation	:	Used as received

MB Research Labs

Study Title : HET-CAM
Project No. : [redacted]

POSITIVE CONTROLS

Identity	:	Sodium Dodecyl Sulfate (SDS), [redacted] (See Appendix B for control Certificate of Analysis)
Supplied By	:	[redacted]
Date Received	:	02 Feb 2018
Expiration Date	:	Oct 2022
Storage	:	Room temperature and humidity
Description	:	White powder
Sample Preparation	:	0.100 g of SDS was brought to a total volume of 10 ml with distilled water to yield a 1% concentration.
Identity	:	0.1 N Sodium Hydroxide Solution (NaOH), Lot No. 175108 (See Appendix B for control Certificate of Analysis)
Supplied By	:	[redacted]
Date Received	:	26 Jan 2018
Expiration Date	:	Jul 2019
Storage	:	Room temperature and humidity
Description	:	Clear colorless liquid
Sample Preparation	:	Used as received

VEHICLE FOR THE SDS POSITIVE CONTROL

Identity	:	Distilled water
Supplied by	:	[redacted]
Date Received	:	17 Aug 2018
Expiration Date	:	25 Sep 2019
Storage	:	Room temperature and humidity
Description	:	Clear colorless liquid
Sample Preparation	:	Used as received

MB Research Labs

Study Title : HET-CAM
Project No. : [redacted]

TEST DATES

Study Initiation	(date protocol signed)	:	06 Dec 2018
Experimental Start Date	(1st exposure to test substance)	:	10 Dec 2018
Experimental Term Date	(last date data collected)	:	10 Dec 2018
Draft Report Submitted	(if applicable)	:	21 Jan 2019
Final Report Signed	(study completion)	:	07 May 2019

EXPERIMENTAL DESIGN

Test System

Fertile, White Leghorn eggs (twenty-four) received from [redacted], were selected for use from a larger group and incubated on 30 Nov 2018. The eggs were kept in incubators at 99 (±2)°F for 10 days.

Pre-dose Procedures

The eggs were marked on one side with an "X" and on the other side with an "O", and placed horizontally in the incubator trays. The eggs were rotated once daily during the first nine days of incubation to ensure even atmospheric exposure.

On Day 9 of incubation, the eggs were rotated and turned up in the incubator with the large end upwards containing the air sac to facilitate access to the CAM.

On Day 10 of development, the eggs were removed from the incubator and candled to determine the viability of the embryo. A rectangular window was removed from the shell directly over the air sac using a rotating Dremel® drill with a diamond wheel bit. The egg membrane was carefully moistened with 2-3 ml of 0.9% saline and returned to the incubator. Eggs were examined for any abnormalities. All abnormal eggs were discarded.

Dosing

The eggs were dosed within 30 minutes of opening. The excess saline solution was gently poured off of the egg membrane which was then removed, and the CAM exposed. The eggs were numbered and 300 µl of the 10% mixture of the test article, positive controls (0.1 N NaOH and 1% SDS) or vehicle control (saline) were pipetted onto the CAM.

MB Research Labs

Study Title : HET-CAM
Project No. : [redacted]

EXPERIMENTAL DESIGN (continued)

Type and Frequency of Observations

The eggs were observed continuously for 5 minutes and the appearance of hemorrhage (sec H), lysis (sec L) and/or coagulation (sec C) was documented (see RESULTS, page 12). If no reaction was observed, a value of 301 seconds was recorded. Additionally, the eggs were scored for severity of responses to the CAM at 1 and 5 minutes post-dosing. The severity of each reaction after 1 and 5 minutes were recorded as follows:

0 = no reaction
1 = slight reaction
2 = moderate reaction
3 = severe reaction

Analysis of Data

The severity score was used to determine the threshold concentration (TH).

Irritation potential was classified by a scheme which depended on two components. The first was the calculated Irritation Score (IS). The IS was based on the time until adverse reactions (hemorrhage, vessel lysis and coagulation) were first observed. The second component of irritation potential was a determination of the severity (slight, moderate or severe) of adverse reactions after 1 and 5 minutes. The threshold was defined as the lowest concentration at which slight reactions occur.

Calculations: The irritation score (IS) was calculated as follows:

$$IS = \left[\left(\frac{301 - \text{sec H}}{300}\right) \times 5\right] + \left[\left(\frac{301 - \text{sec H}}{300}\right) \times 7\right] + \left[\left(\frac{301 - \text{sec H}}{300}\right) \times 9\right]$$

Interpretation

The HET-CAM method is intended as an alternative to the Draize eye irritation evaluation in rabbits. Whenever possible, the conclusions of the HET-CAM will be related to those of the Draize (Draize, J.H. et al. 1944. J. Pharm. Exp. Ther., 82:377-90). Classification of the irritating potential will be according to the chart below. (INVITTOX. 1992. Protocol No. 47: HET-CAM Test.)

MB Research Labs

Study Title : HET-CAM
Project No. : [redacted]

EXPERIMENTAL DESIGN (continued)

Classification of Irritating Potential

Threshold Concentration (TH%)	Irritation Score (10%)	Severity	Classification
TH < 1	-	-	severe / corrosive
1.0 < TH < 2.5	> 16	-	severe / corrosive
2.5 < TH < 10.0	< 16	severe reaction after 1 min	severe / corrosive
1.0 < TH < 2.5	< 16	-	irritant
2.5 < TH < 10.0	> 16	-	irritant
2.5 < TH < 10.0	< 16	severe reaction after 5 min	irritant
2.5 < TH < 10.0	< 16	weak or no reaction	moderate
10.0 < TH	> 16	-	moderate
10.0 < TH	< 16	severe reaction	moderate
10.0 < TH	< 10	-	none/slight

Retention of Data

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

All data generated during the conduct of this study will be archived at MB Research for at least 10 years from the date of the final report. The Sponsor will then be contacted in writing to determine final disposition of the records. If the Sponsor fails to respond within 90 days, the archived items will be properly discarded.

Any remaining test article will be returned to the Sponsor following submission of the report.

Amendment to the Protocol

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

MB Research Labs

Study Title : HET-CAM
Project No. : [redacted]

RESULTS

Treatment	Egg ID	End Points[1]			Severity Score		Irritation Score (IS)	Mean IS	St. Dev.
		H	L	C	1-min.	5-min.			
1% Sodium Dodecyl Sulfate	1	74	48	301	1	2	9.7	9.8	0.44
	2	104	54	301	1	2	9.0		
	3	82	40	301	1	2	9.7		
	4	61	37	301	1	2	10.2		
	5	58	41	301	1	2	10.1		
	6	65	33	301	1	2	10.2		
0.1N Sodium Hydroxide	7	17	29	68	2	3	18.1	17.8	0.34
	8	18	32	62	2	3	18.2		
	9	23	42	74	2	3	17.5		
	10	22	32	67	2	3	17.9		
	11	18	38	61	2	3	18.1		
	12	31	46	71	2	3	17.4		
Saline	13	301	301	301	0	0	0.0	0.0	0.00
	14	301	301	301	0	0	0.0		
	15	301	301	301	0	0	0.0		
	16	301	301	301	0	0	0.0		
	17	301	301	301	0	0	0.0		
	18	301	301	301	0	0	0.0		
10% Test Article Formulation	19	301	301	301	0	0	0.0	0.0	0.00
	20	301	301	301	0	0	0.0		
	21	301	301	301	0	0	0.0		
	22	301	301	301	0	0	0.0		
	23	301	301	301	0	0	0.0		
	24	301	301	301	0	0	0.0		

1 = Time (in seconds) until the first appearance of the end points: hemorrhage (H), lysis (L), and coagulation (C). A value of 301 seconds indicates that none of the end points were observed.

MB Research Labs

Study Title : HET-CAM
Project No. : [redacted]

DISCUSSION

Treatment	Identity	Irritation Score	Threshold Concentration
Positive Control	1% Sodium Dodecyl Sulfate (SDS)	9.8	-
Positive Control	0.1 N Sodium Hydroxide (NaOH)	17.8	-
Vehicle Control	0.9 Sodium Chloride Irrigation, USP (saline)	0.0	-
10% Test Article Formulation	[redacted]	0.0*	Greater than 10%

* Irritation score at 10% test article formulation (v/v) in saline

The vehicle control did not produce adverse effects on the chorioallantoic membranes of treated eggs. The positive control irritation scores were within acceptable ranges; therefore, this test is considered valid.

CONCLUSION

Based on the calculated irritation score (at 10%) and the threshold concentration, [redacted] is considered to have none-to-slight irritation potential according to the classification criteria set forth in INVITTOX Protocol No. 47 (1992).

FINAL REPORT

Approved by: [redacted] 07 May 2019
[redacted] B.S. Date
Study Director

MB Research Labs

Study Title

Hen's Egg Test Chorioallantoic Membrane (HET-CAM)
for Non-Opaque Materials

Test Article

[redacted] lash serum containing
0.005% Isopropyl Cloprostenate

Author

[redacted], B.S., Study Director

Study Completed On

18 Jan 2018

Performing Laboratory

MB Research Laboratories
[redacted]

MB Research Project No.

[redacted]

MB Research Protocol No.

[redacted]

Sponsor

[redacted]

MB Research Labs

Study Title : HET-CAM
Project No. : [redacted]

GOOD LABORATORY PRACTICES COMPLIANCE STATEMENT

This study was conducted in accordance with applicable Good Laboratory Practices regulations of the FDA, 21 CFR Part 58, with the following exceptions:

Test article characterization information, provided by the Sponsor, was not complete. See Appendix A for information that was provided. The effect of the lack of full test article characterization information cannot be fully assessed.

Test article characterization, provided by the Sponsor, was not conducted according to the Good Laboratory Practices. However, it was performed according to the Good Manufacturing Practices. This is not expected to have an impact on the outcome of the study.

Analysis of the test article and SDS positive control in the mixture was not performed. The mixtures were prepared fresh daily. Although no adverse effect is expected, the lack of analysis cannot be fully assessed.

STUDY DIRECTOR: [redacted] 18JAN08

[redacted], B.S. Date
MB RESEARCH LABORATORIES

MB Research Labs

Study Title : HET-CAM
Project No. : [redacted]

QUALITY ASSURANCE EVALUATION

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

Date of Inspection	Phase	Performed By	Date Inspection Results Reported	
			Study Director	Management
11 Dec 2017	Sample Preparation	[redacted]	11 Dec 2017	11 Dec 2017
02 Jan 2018	Raw data audit	[redacted]	02 Jan 2018	03 Jan 2018
10 Jan 2018	Draft report audit	[redacted]	10 Jan 2018	17 Jan 2018
17 Jan 2018	Final report audit	[redacted]	17 Jan 2018	17 Jan 2018

[redacted] 17 Jan 2018
Quality Assurance Unit Date

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TABLE OF CONTENTS

MB Research Labs

Study Title : **HET-CAM**
Project No. : [redacted]

KEY PERSONNEL

[redacted], B.S., MPH, DABT	Director of Toxicology
[redacted], B.S.	Study Director
[redacted], B.A., RLATG	Technician

MB Research Labs

PROJECT No. : [redacted]
TEST ARTICLE : [redacted]
SPONSOR : [redacted]
TITLE : **Hen's Egg Test Chorioallantoic Membrane (HET-CAM) For Non-Opaque Materials**
PROTOCOL No. : [redacted]

ABSTRACT

Objective
To determine the potential irritancy using an alternative to the Draize methodology. The methodology is based on that described in INVITTOX, 1992. Protocol No. 47: HET-CAM Test.

Method Synopsis
The chorioallantoic membranes (CAM) of twetny-four 10-day-old White Leghorn eggs (six eggs per treatment) were dosed with 300 µl of a test substance, as listed below. Immediately following dose administration, each egg was observed continuously for five minutes for the first appearance of lysis, hemorrhage, and/or coagulation. Additionally, eggs were scored for severity of responses to the CAM at 1 and 5 minutes post-dosing. The ocular irritation potential of the test article was classified based on the irritation score and the threshold concentration.

Summary

Treatment	Identity	Irritation Score	Threshold Concentration
Positive Control	1% Sodium Dodecyl Sulfate (SDS)	10.1	
Positive Control	0.1 N Sodium Hydroxide (NaOH)	17.6	
Vehicle Control	0.9% Sodium Chloride Irrigation, USP (saline)	0.0	
10% Test Article Formulation	[redacted]	2.6*	Greater than 10%

* Irritation score at 10% test article formulation (v/v) in saline

The vehicle control did not produce adverse effects on the chorioallantoic membranes of treated eggs. The positive control irritation scores were within acceptable ranges; therefore, this test is considered valid.

Conclusion
Based on the calculated irritation score (at 10%) and the threshold concentration, [redacted] Formula is considered to have none-to-slight irritation potential according to the classification criteria set forth in INVITTOX Protocol No. 47 (1992).

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OBJECTIVE

To determine the potential irritancy using an alternative to the Draize methodology. The methodology was based on that described in INVITTOX. 1992. Protocol No. 47: HET-CAM Test.

TEST ARTICLE

Identity	:	[redacted]
Test Article Characterization	:	See Appendix A for Test Article Characterization
Supplied by	:	[redacted]
Date Received	:	06 Dec 2017
Storage	:	Room temperature and humidity
Description	:	Clear colorless liquid
Sample Preparation	:	1.0 ml of the test article was added to 9.0 ml of saline and mixed.

POSITIVE CONTROLS

Identity	:	Sodium Dodecyl Sulfate (SDS), [redacted]
Supplied By	:	[redacted]
Date Received	:	02 Jan 2014
Expiration Date	:	Jul 2018
Storage	:	Room temperature and humidity
Description	:	White powder
Sample Preparation	:	0.100 g of SDS was brought to a total volume of 10 ml with distilled water to yield a 1% concentration.

Identity	:	0.1 N Sodium Hydroxide Solution (NaOH), [redacted]
Supplied By	:	[redacted]
Date Received	:	15 Jun 2016
Expiration Date	:	Feb 2018
Storage	:	Room temperature and humidity
Description	:	Clear colorless liquid
Sample Preparation	:	Used as received

MB Research Labs

Study Title : **HET-CAM**
Project No. : [redacted]

VEHICLE FOR THE SDS POSITIVE CONTROL

Identity	:	Distilled water
Supplied by	:	[redacted]
Date Received	:	01 Sep 2017
Expiration Date	:	27 Oct 2018
Storage	:	Room temperature and humidity
Description	:	Clear colorless liquid

VEHICLE

Identity	:	0.9% Sodium Chloride Irrigation, USP (saline), Lot No. [redacted]
Supplied By	:	[redacted]
Date Received	:	18 Apr 2017
Expiration Date	:	Dec 2019
Storage	:	Room temperature and humidity
Description	:	Clear colorless liquid
Sample Preparation	:	Used as received

TEST DATES

Study Initiation	(date protocol signed)	:	08 Dec 2017
Experimental Start Date	(1st exposure to test substance)	:	11 Dec 2017
Experimental Term Date	(last date data collected)	:	11 Dec 2017
Draft Report Submitted	(if applicable)	:	11 Jan 2018
Final Report Signed	(study completion)	:	18 Jan 2018

MB Research Labs

Study Title : **HET-CAM**
Project No. : [redacted]

EXPERIMENTAL DESIGN

Test System

Fertile, White Leghorn eggs (twelny-four) received from [redacted] were selected for use from a larger group and incubated on 01 Dec 2017. The eggs were kept in incubators at 99 (±2)°F for 10 days.

Pre-dose Procedures

The eggs were marked on one side with an "X" and on the other side with an "O", and placed horizontally in the incubator trays. The eggs were rotated once daily during the first nine days of incubation to ensure even atmospheric exposure.

On Day 9 of incubation, the eggs were rotated and turned up in the incubator with the large end upwards containing the air sac to facilitate access to the CAM.

On Day 10 of development, the eggs were removed from the incubator and candled to determine the viability of the embryo. A rectangular window was removed from the shell directly over the air sac using a rotating Dremel® drill with a diamond wheel bit. The egg membrane was carefully moistened with 2-3 ml of 0.9% saline and returned to the incubator. Eggs were examined for any abnormalities. All abnormal eggs were discarded.

Dosing

The eggs were dosed within 30 minutes of opening. The excess saline solution was gently poured off of the egg membrane which was then removed, and the CAM exposed. The eggs were numbered and 300 µl of the 10% mixture of the test article, positive controls (0.1 N NaOH and 1% SDS) or vehicle control (saline) was pipetted onto the CAM.

Type and Frequency of Observations

The eggs were observed continuously for 5 minutes and the appearance of hemorrhage (sec H), lysis (sec L) and/or coagulation (sec C) was documented (see RESULTS, page 12). If no reaction was observed, a value of 301 seconds was recorded. Additionally, the eggs were scored for severity of responses to the CAM at 1 and 5 minutes post-dosing.

The severity of each reaction after 1 and 5 minutes were recorded as follows:

0 = no reaction
1 = slight reaction
2 = moderate reaction
3 = severe reaction

MB Research Labs

Study Title : HET-CAM
Project No. : [redacted]

EXPERIMENTAL DESIGN (continued)

Analysis of Data

The severity score was used to determine the threshold concentration (TH).

Irritation potential was classified by a scheme which depended on two components. The first was the calculated Irritation Score (IS). The IS was based on the time until adverse reactions (hemorrhage, vessel lysis and coagulation) were first observed. The second component of irritation potential was a determination of the severity (slight, moderate or severe) of adverse reactions after 1 and 5 minutes. The threshold was defined as the lowest concentration at which slight reactions occur.

Calculations: The irritation score (IS) was calculated as follows:

$$IS = \left[\left(\frac{301 - \text{sec H}}{300}\right) \times 5\right] + \left[\left(\frac{301 - \text{sec H}}{300}\right) \times 7\right] + \left[\left(\frac{301 - \text{sec H}}{300}\right) \times 9\right]$$

Interpretation

The HET-CAM method is intended as an alternative to the Draize eye irritation evaluation in rabbits. Whenever possible, the conclusions of the HET-CAM will be related to those of the Draize (Draize, J.H. et al. 1944. J. Pharm. Exp. Ther., 82:377-90). Classification of the irritating potential will be according to the chart below. (INVITTOX. 1992. Protocol No. 47: HET-CAM Test.)

Classification of Irritating Potential

Threshold Concentration (TH%)	Irritation Score (10%)	Severity	Classification
TH < 1	-	-	severe / corrosive
1.0 < TH < 2.5	> 16	-	severe / corrosive
2.5 < TH < 10.0	< 16	severe reaction after 1 min	severe / corrosive
1.0 < TH < 2.5	< 16	-	irritant
2.5 < TH < 10.0	> 16	-	irritant
2.5 < TH < 10.0	< 16	severe reaction after 5 min	irritant
2.5 < TH < 10.0	< 16	weak or no reaction	moderate
10.0 < TH	> 16	-	moderate
10.0 < TH	< 16	severe reaction	moderate
10.0 < TH	< 10	-	none/slight

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EXPERIMENTAL DESIGN (continued)

Retention of Data

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

All data generated during the conduct of this study will be archived at MB Research for at least 10 years from the date of the final report. The Sponsor will then be contacted in writing to determine final disposition of the records. If the Sponsor fails to respond within 90 days, the archived items will be properly discarded.

Any remaining test article will be returned to the Sponsor following submission of the report.

Amendment to the Protocol

See Appendix B for protocol in its entirety.

MB Research Labs

Study Title : HET-CAM
Project No. : [redacted]

RESULTS

Treatment	Egg ID	End Points[1]			Severity Score		Irritation Score (IS)	Mean IS	St. Dev.
		H	L	C	1-min.	5-min.			
1% Sodium Dodecyl Sulfate	1	113	34	301	1	2	9.4	10.1	0.44
	2	61	20	301	1	2	10.6		
	3	48	30	301	1	2	10.5		
	4	75	34	301	1	2	10.0		
	5	72	26	301	1	2	10.2		
	6	62	42	301	1	2	10.0		
0.1N Sodium Hydroxide	7	12	33	72	2	3	17.9	17.6	0.80
	8	15	24	51	2	3	18.7		
	9	20	41	74	2	3	17.6		
	10	27	52	101	2	3	16.4		
	11	32	49	78	2	3	17.1		
	12	21	47	64	2	3	17.7		
0.9% Saline	13	301	301	301	0	0	0.0	0.0	0.00
	14	301	301	301	0	0	0.0		
	15	301	301	301	0	0	0.0		
	16	301	301	301	0	0	0.0		
	17	301	301	301	0	0	0.0		
	18	301	301	301	0	0	0.0		
10% Test Article Formulation	25	301	55	301	1	1	5.7	2.6	2.91
	26	301	114	301	0	1	4.4		
	27	301	301	301	0	0	0.0		
	28	301	61	301	0	1	5.6		
	29	301	301	301	0	0	0.0		
	30	301	301	301	0	0	0.0		

1 = Time (in seconds) until the first appearance of the end points: hemorrhage (H), lysis (L), and coagulation (C). A value of 301 seconds indicates that none of the end points were observed.

MB Research Labs

Study Title : **HET-CAM**
Project No. : [redacted]

DISCUSSION

Treatment	Identity	Irritation Score	Threshold Concentration
Positive Control	1% Sodium Dodecyl Sulfate (SDS)	10.1	
Positive Control	0.1 N Sodium Hydroxide (NaOH)	17.6	
Vehicle Control	0.9% Sodium Chloride Irrigation, USP (saline)	0.0	
10% Test Article Formulation	[redacted]	2.6*	Greater than 10%

* Irritation score at 10% test article formulation (v/v) in saline

The vehicle control did not produce adverse effects on the chorioallantoic membranes of treated eggs. The positive control irritation scores were within acceptable ranges; therefore, this test is considered valid.

CONCLUSION

Based on the calculated irritation score (at 10%) and the threshold concentration, [redacted] is considered to have none-to-slight irritation potential according to the classification criteria set forth in INVITTOX Protocol No. 47 (1992).

FINAL REPORT

Approved by: [redacted] 18JAN2018

[redacted], B.S. Date
Study Director

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eurofins | CRL

CLINICAL STUDY REPORT

Report Status	Final
Report Date	22 June 2021
CRL Study Number	[redacted]
Protocol Number	[redacted]
Study Title	A Clinical Study to Evaluate the Ophthalmic Safety and Consumer Perception of a Test Material and to Obtain Photographs
Sponsor	[redacted]
Sponsor Representative	[redacted]
Test Material	[redacted]
Principal Investigator	[redacted] MD Diplomate, American Board of Ophthalmology
Sub-Investigator	[redacted] Medical Research Scientist
Investigating Laboratory	Eurofins | CRL, Inc. [redacted]
Study Initiation Date	19 January 2021
Study Completion Date	13 April 2021

lash serum containing
0.005% Isopropyl Cloprostenate

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PRINCIPAL INVESTIGATOR SIGNATURE

Study Title: A Clinical Study to Evaluate the Ophthalmic Safety and Consumer Perception of a Test Material and to Obtain Photographs

I have read Clinical Study Report [redacted] and confirm that, to the best of my knowledge, the report accurately describes the conduct and results of the study.

[redacted] Digitally signed by [redacted]
[redacted] MD Date: 2021.06.24 07:15:43 -04'00'

.....

[redacted] MD Date

Diplomate, American Board of Ophthalmology
Principal Investigator

[redacted] Digitally signed by [redacted] Date: 2021.06.22 11:43:31 -04'00'

.....

[redacted] Date

Medical Research Scientist
Sub-Investigator

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Quality Assurance Audit Statement

Clinical Study Number: [redacted]

Start Date: 19 January 2021

Completion Date: 13 April 2021

Eurofins | CRL, Inc. (CRL) follows established, standardized procedures for clinical testing designed to ensure the well-being of clinical study subjects and the generation of reliable study data. The study was conducted in accordance with the study protocol and CRL Standard Operating Procedures (SOPs). In addition, the study was conducted following applicable ICH GCP standards to ensure reliability of data, subject safety and confidentiality. All data included in the report is accurately represented. The clinical study master file was reviewed by the Principal Investigator and the Quality Assurance representative.

[redacted] Digitally signed by [redacted]
Date: 2021.06.30 11:35:12 -04'00'

Signature of QA Auditor and Date

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1.0 ETHICS

1.1. ETHICAL CONDUCT OF THE STUDY

Eurofins | CRL, Inc. (CRL) followed established, standardized procedures for clinical testing designed to ensure the wellbeing of clinical study subjects and the generation of reliable study data. The Study Sponsor was responsible for ensuring the study complied with applicable Drug, Cosmetic, or Medical Device regulations, which vary by product.

1.2. INFORMED CONSENT

Each subject was given a copy of the Informed Consent Form (ICF) and Photograph Release Form and had the nature and the purpose of the study explained to them by CRL personnel. Prior to entry into the study, the subject gave voluntary written consent to participate by signing the ICF. The Principal Investigator retains the original signed Informed Consent Form and signed Photograph Release Form in the subject's file and gave a copy of the Informed Consent Form and Photograph Release Form to the subject.

1.3. SUBJECT CONFIDENTIALITY

The Principal Investigator ensured that the research subject's confidentiality was maintained. Subjects were identified by a study ID number only. Documents were kept in strict confidence by the Principal Investigator. Any use of personally identifiable data or private health information was justified by the Principal Investigator.

2.0 OBJECTIVE

The objective of this clinical study was to evaluate the ophthalmic irritation potential and consumer perception of a test material, and to obtain photographs.

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3.0 STUDY DESIGN

Thirty-five subjects were enrolled in this clinical study to evaluate the ophthalmic irritation potential and consumer perception of a test material, and to obtain photographs. Study evaluations included ophthalmic evaluations, lifestyle photography, and consumer perception questionnaires.

A study schedule appears below.

Study Procedures	Baseline	Immediately Post-Application	1 Month	2 Months	3 Months
Informed Consent Obtained	X				
Inclusion and Exclusion Criteria Verified	X				
Test Material, Use Instructions and Daily Diary Distributed	X				
Test Material Application	X				
Ophthalmic Evaluations	X		X	X	X
Lifestyle Photography	X		X	X	X
Consumer Perception Questionnaire		X	X	X	X
Test Material Collected					X
Daily Diary Reviewed for Compliance and Collected					X

4.0 TEST MATERIALS AND RECORD RETENTION

4.1. IDENTIFICATION

The test material was identified by CRL study, panel and subject numbers, in accordance with distribution. Test material identification was as follows:

Sponsor Identification	CRL Identification Number
[redacted]	[redacted]

The Sponsor assumed responsibility for the purity, stability, characterization, and adequate preservation of the test materials. The Sponsor provided assurance that the test materials submitted were determined to be safe for use in humans.

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4.0 TEST MATERIALS AND RECORD RETENTION (CONTINUED)

4.2. STORAGE AND RETENTION

Prior to study start, the test materials were stored at room temperature and humidity. All unused test materials will be retained by CRL for a minimum of 6 months, in accordance with CRL Standard Operating Procedures.

All original forms of this study will be retained by CRL as specified in CRL Standard Operating Procedures.

4.3. PRODUCT USE INSTRUCTIONS

Application:

- Remove makeup, cleanse, and dry your face. Dry eyelids and lashes completely. Apply Serum using a single stroke on your eyelid just above your upper lash line on both eyes. Use once daily in the evening before bed. Allow one to two minutes for the serum to dry.

Tips:

- You're applying to the skin closest to the lashes (the lash root), not the actual lashes.
- You don't need multiple applications or double dips, using more than instructed will not yield quicker results. One dip into the bottle is enough for both eyes.

5.0 RANDOMIZATION

No randomization was required for this study.

6.0 BLINDING

Subjects were blinded to the name of the test material. The investigatory staff was not blinded. Test materials were labeled with unique CRL study identification and panel codes and subject numbers upon test material receipt by CRL.

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7.0 COMPLIANCE OF PRODUCT APPLICATION

Daily Diaries were reviewed by clinic staff to confirm study compliance.

8.0 SUBJECT SELECTION

A total of 35 female subjects, ranging in age from 20 to 70 years, who met all of the inclusion criteria and none of the exclusion criteria as outlined in the clinical study protocol, were selected for study participation (Appendix I).

9.0 TEST METHOD

This study was conducted according to clinical study protocol [redacted]

10.0 PROTOCOL DEVIATIONS

No protocol deviations occurred over the duration of the study.

11.0 ADVERSE EVENTS

No adverse events were reported over the duration of the study.

12.0 TEST RESULTS

12.1. COMPLETED AND DISCONTINUED SUBJECTS

A total of 32 subjects completed the study. Two subjects (#02 and #33) were lost to follow up. One subject (#21) withdrew from the study for personal reasons.

12.2. OPHTHALMIC EVALUATIONS

Individual Ophthalmic evaluations appear in Table I.

12.3. LIFESTYLE PHOTOGRAPHY

Lifestyle photography photos were sent to the Sponsor on 03 May 2021.

12.4. CONSUMER PERCEPTION QUESTIONNAIRE

Questionnaire results appear in Appendix II.

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13.0 CONCLUSION

Under the conditions of this study and in this test population, [redacted] did not demonstrate a potential for eliciting ocular irritation.

Under the conditions of this study and in this test population, [redacted] exhibited a favorable consumer perception in 8 out of 8 questions following immediate test material use.

Under the conditions of this study and in this test population, [redacted] exhibited a favorable consumer perception in 2 out of 2 questions following 1 month of test material use.

Under the conditions of this study and in this test population, [redacted] exhibited a favorable consumer perception in 2 out of 2 questions following 2 months of test material use.

Under the conditions of this study and in this test population, [redacted] exhibited a favorable consumer perception in 8 out of 8 questions following 3 months of test material use.

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Table I - Ophthalmic Evaluations

Subjective and Objective Ocular Evaluation

Maximum Increase from Baseline to 1 Month

Subject Number	Eye Type	Subjective Irritation		Lacrimation		Eyelid Irritation (Upper/ Lower)		Palpebral Conjunctival Irritation (Upper/ Lower)		Bulbar Conjunctival Irritation		Cornea		Contact Lens Changes	
		R	L	R	L	R	L	R	L	R	L	R	L	R	L
01	N	0	0	0	0	0/0	0/0	0/0	0/0	0	0	0	0	NA	NA
02	Discontinued														
03	N	0	0	0	0	0/0	0/0	0/0	0/0	0	0	0	0	NA	NA
04	N	0	0	0	0	0/0	0/0	0/0	0/0	0	0	0	0	NA	NA
05	N	0	0	0	0	0/0	0/0	0/0	0/0	0	0	0	0	NA	NA
06	N	0	0	0	0	0/0	0/0	0/0	0/0	0	0	0	0	NA	NA
07	N	0	0	0	0	0/0	0/0	0/0	0/0	0	0	0	0	NA	NA
08	N	0	0	0	0	0/0	0/0	1/1	1/1	0	0	0	0	NA	NA
09	N	0	0	0	0	0/0	0/0	0/0	0/0	0	0	0	0	NA	NA
10	N	0	0	0	0	0/0	0/0	1/1	1/1	1	1	0	0	NA	NA
11	N	0	0	0	0	0/0	0/0	0/0	0/0	0	0	0	0	NA	NA
12	N	0	0	0	0	0/0	0/0	0/0	0/0	0	0	0	0	NA	NA
13	N	0	0	0	0	0/0	0/0	0/0	0/0	0	0	0	0	NA	NA
14	N	0	0	0	0	0/0	0/0	0/0	0/0	0	0	0	0	NA	NA
15	N	0	0	0	0	0/0	0/0	0/0	0/0	0	0	0	0	NA	NA
16	N	0	0	0	0	0/0	0/0	0/0	0/0	0	0	0	0	NA	NA
17	N	0	0	0	0	0/0	0/0	0/0	0/0	0	0	0	0	NA	NA
18	N	0	0	0	0	0/0	0/0	1/1	1/1	0	0	0	0	NA	NA
19	N	0	0	0	0	0/0	0/0	0/0	0/0	0	0	0	0	NA	NA
20	N	0	0	0	0	0/0	0/0	0/0	0/0	0	0	0	0	NA	NA
21	Discontinued														
22	N	0	0	0	0	0/0	0/0	0/0	0/0	0	0	0	0	NA	NA
23	N	0	0	0	0	0/0	0/0	0/0	0/0	0	0	0	0	NA	NA
24	N	0	0	0	0	0/0	0/0	0/0	0/0	0	0	0	0	NA	NA
25	N	0	0	0	0	0/0	0/0	0/0	0/0	0	0	0	0	NA	NA
26	N	0	0	0	0	0/0	0/0	0/0	0/0	0	0	0	0	NA	NA
27	N	0	0	0	0	0/0	0/0	0/0	0/0	0	0	0	0	NA	NA
28	N	0	0	0	0	0/0	0/0	0/0	0/0	0	0	0	0	NA	NA
29	N	0	0	0	0	0/0	0/0	0/0	0/0	0	0	0	0	NA	NA
30	N	0	0	0	0	0/0	0/0	0/0	0/0	0	0	0	0	NA	NA
31	N	0	0	0	0	0/0	0/0	0/0	0/0	0	0	0	0	NA	NA
32	N	0	0	0	0	0/0	0/0	0/0	0/0	0	0	0	0	NA	NA
33	Discontinued														
34	N	0	0	0	0	0/0	0/0	0/0	0/0	0	0	0	0	NA	NA
35	N	0	0	0	0	0/0	0/0	0/0	0/0	0	0	0	0	NA	NA

N = Non-Contact Lens Wearer

Table I – Ophthalmic Evaluations (Continued)

Subjective and Objective Ocular Evaluation

Maximum Increase from Baseline to 2 Months

Subject Number	Eye Type	Subjective Irritation		Lacrimation		Eyelid Irritation (Upper/ Lower)		Palpebral Conjunctival Irritation (Upper/ Lower)		Bulbar Conjunctival Irritation		Cornea		Contact Lens Changes	
		R	L	R	L	R	L	R	L	R	L	R	L	R	L
01	N	0	0	0	0	0/0	0/0	0/0	0/0	0	0	0	0	NA	NA
02	Discontinued														
03	N	0	0	0	0	0/0	0/0	0/0	0/0	0	0	0	0	NA	NA
04	N	0	0	0	0	0/0	0/0	0/0	0/0	0	0	0	0	NA	NA
05	N	0	0	0	0	0/0	0/0	0/0	0/0	0	0	0	0	NA	NA
06	N	0	0	0	0	0/0	0/0	0/0	0/0	0	0	0	0	NA	NA
07	N	0	0	0	0	0/0	0/0	0/0	0/0	0	0	0	0	NA	NA
08	N	0	0	0	0	0/0	0/0	0/0	0/0	0	0	0	0	NA	NA
09	N	0	0	0	0	0/0	0/0	0/0	0/0	0	0	0	0	NA	NA
10	N	0	0	0	0	0/0	0/0	1/1	1/1	0	0	0	0	NA	NA
11	N	0	0	0	0	0/0	0/0	0/0	0/0	0	0	0	0	NA	NA
12	N	0	0	0	0	0/0	0/0	0/0	0/0	0	0	0	0	NA	NA
13	N	0	0	0	0	0/0	0/0	0/0	0/0	0	0	0	0	NA	NA
14	N	0	0	0	0	0/0	0/0	0/0	0/0	0	0	0	0	NA	NA
15	N	0	0	0	0	0/0	0/0	0/0	0/0	0	0	0	0	NA	NA
16	N	0	0	0	0	0/0	0/0	0/0	0/0	0	0	0	0	NA	NA
17	N	0	0	0	0	0/0	0/0	0/0	0/0	0	0	0	0	NA	NA
18	N	0	0	0	0	0/0	0/0	0/0	0/0	0	0	0	0	NA	NA
19	N	0	0	0	0	0/0	0/0	0/0	0/0	0	0	0	0	NA	NA
20	N	0	0	0	0	0/0	0/0	0/0	0/0	0	0	0	0	NA	NA
21	Discontinued														
22	N	0	0	0	0	0/0	0/0	0/0	0/0	0	0	0	0	NA	NA
23	N	0	0	0	0	0/0	0/0	0/0	0/0	0	0	0	0	NA	NA
24	N	0	0	0	0	0/0	0/0	0/0	0/0	0	0	0	0	NA	NA
25	N	0	0	0	0	0/0	0/0	0/0	0/0	0	0	0	0	NA	NA
26	N	0	0	0	0	0/0	0/0	0/0	0/0	0	0	0	0	NA	NA
27	N	0	0	0	0	0/0	0/0	0/0	0/0	0	0	0	0	NA	NA
28	N	0	0	0	0	0/0	0/0	0/0	0/0	0	0	0	0	NA	NA
29	N	0	0	0	0	0/0	0/0	0/0	0/0	0	0	0	0	NA	NA
30	N	0	0	0	0	0/0	0/0	0/0	0/0	0	0	0	0	NA	NA
31	N	0	0	0	0	0/0	0/0	0/0	0/0	0	0	0	0	NA	NA
32	N	0	0	0	0	0/0	0/0	0/0	0/0	0	0	0	0	NA	NA
33	Discontinued														
34	N	0	0	0	0	0/0	0/0	0/0	0/0	0	0	0	0	NA	NA
35	N	0	0	0	0	0/0	0/0	0/0	0/0	0	0	0	0	NA	NA

N = Non-Contact Lens Wearer

Table I – Ophthalmic Evaluations (Continued)

Subjective and Objective Ocular Evaluation

Maximum Increase from Baseline to 3 Months/Final

Subject Number	Eye Type	Subjective Irritation		Lacrimation		Eyelid Irritation (Upper/ Lower)		Palpebral Conjunctival Irritation (Upper/ Lower)		Bulbar Conjunctival Irritation		Cornea		Contact Lens Changes	
		R	L	R	L	R	L	R	L	R	L	R	L	R	L
01	N	0	0	0	0	0/0	0/0	0/0	0/0	0	0	0	0	NA	NA
02	Discontinued														
03	N	0	0	0	0	0/0	0/0	0/0	0/0	0	0	0	0	NA	NA
04	N	0	0	0	0	0/0	0/0	0/0	0/0	0	0	0	0	NA	NA
05	N	0	0	0	0	0/0	0/0	0/0	0/0	0	0	0	0	NA	NA
06	N	0	0	0	0	0/0	0/0	0/0	0/0	0	0	0	0	NA	NA
07	N	0	0	0	0	0/0	0/0	0/0	0/0	0	0	0	0	NA	NA
08	N	0	0	0	0	0/0	0/0	0/0	0/0	0	0	0	0	NA	NA
09	N	0	0	0	0	0/0	0/0	0/0	0/0	0	0	0	0	NA	NA
10	N	0	0	0	0	0/0	0/0	0/0	0/0	0	0	0	0	NA	NA
11	N	0	0	0	0	0/0	0/0	0/0	0/0	0	0	0	0	NA	NA
12	N	0	0	0	0	0/0	0/0	0/0	0/0	0	0	0	0	NA	NA
13	N	0	0	0	0	0/0	0/0	0/0	0/0	0	0	0	0	NA	NA
14	N	0	0	0	0	0/0	0/0	0/0	0/0	0	0	0	0	NA	NA
15	N	0	0	0	0	0/0	0/0	0/0	0/0	0	0	0	0	NA	NA
16	N	0	0	0	0	0/0	0/0	0/0	0/0	0	0	0	0	NA	NA
17	N	0	0	0	0	0/0	0/0	0/0	0/0	0	0	0	0	NA	NA
18	N	0	0	0	0	0/0	0/0	0/0	0/0	0	0	0	0	NA	NA
19	N	0	0	0	0	0/0	0/0	0/0	0/0	0	0	0	0	NA	NA
20	N	0	0	0	0	0/0	0/0	0/0	0/0	0	0	0	0	NA	NA
21	Discontinued														
22	N	0	0	0	0	0/0	0/0	0/0	0/0	0	0	0	0	NA	NA
23	N	0	0	0	0	0/0	0/0	0/0	0/0	0	0	0	0	NA	NA
24	N	0	0	0	0	0/0	0/0	0/0	0/0	0	0	0	0	NA	NA
25	N	0	0	0	0	0/0	0/0	0/0	0/0	0	0	0	0	NA	NA
26	N	0	0	0	0	0/0	0/0	0/0	0/0	0	0	0	0	NA	NA
27	N	0	0	0	0	0/0	0/0	0/0	0/0	0	0	0	0	NA	NA
28	N	0	0	0	0	0/0	0/0	0/0	0/0	0	0	0	0	NA	NA
29	N	0	0	0	0	0/0	0/0	0/0	0/0	0	0	0	0	NA	NA
30	N	0	0	0	0	0/0	0/0	0/0	0/0	0	0	0	0	NA	NA
31	N	0	0	0	0	0/0	0/0	0/0	0/0	0	0	0	0	NA	NA
32	N	0	0	0	0	0/0	0/0	0/0	0/0	0	0	0	0	NA	NA
33	Discontinued														
34	N	0	0	0	0	0/0	0/0	0/0	0/0	0	0	0	0	NA	NA
35	N	0	0	0	0	0/0	0/0	0/0	0/0	0	0	0	0	NA	NA

N = Non-Contact Lens Wearer

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Appendix I - Subject Demographics

Subject Number	Age	Eye Type	Sex
01	20	NCLW	F
02	59	NCLW	F
03	41	NCLW	F
04	64	NCLW	F
05	47	NCLW	F
06	69	NCLW	F
07	44	NCLW	F
08	69	NCLW	F
09	44	NCLW	F
10	65	NCLW	F
11	64	NCLW	F
12	66	NCLW	F
13	68	NCLW	F
14	69	NCLW	F
15	69	NCLW	F
16	66	NCLW	F
17	36	NCLW	F
18	70	NCLW	F
19	59	NCLW	F
20	45	NCLW	F
21	66	NCLW	F
22	27	NCLW	F
23	66	NCLW	F
24	67	NCLW	F
25	45	NCLW	F
26	61	NCLW	F
27	39	NCLW	F
28	65	NCLW	F
29	28	NCLW	F
30	70	NCLW	F
31	28	NCLW	F
32	57	NCLW	F
33	63	NCLW	F
34	42	NCLW	F
35	53	NCLW	F

Eye Type: NCLW = Non-Contact Lens Wearer

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AN IN-USE, EYE ASSESSMENT, STUDY IN 30 HEALTHY FEMALE VOLUNTEERS TO INVESTIGATE THE IRRITATION POTENTIAL OF TWO TEST ARTICLES.

lash serum and brow serum containing 0.005% Isopropyl Cloprostenate

Prepared for:

[redacted]

Prepared by:

PCR Corp

[redacted]

Draft Report v1: 2nd March 2018
Final Report: 7th March 2018

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AN IN-USE, EYE ASSESSMENT, STUDY IN 30 HEALTHY FEMALE VOLUNTEERS TO INVESTIGATE THE IRRITATION POTENTIAL OF TWO TEST ARTICLES.

PCR CORP REPORT NO: [redacted]

I declare that the following report constitutes a true and faithful account of the procedures adopted and the results obtained in the performance of this study. The aspects of the study conducted by PCR Corp were performed, where relevant, in accordance with the principles of Good Clinical Research Practice.

[redacted]
(Principal Investigator)

..... [redacted]

Date 8th March 2018

I have reviewed this report and concur with its contents.

[redacted] BSc.MB BS.DO(Lond),MRC Ophth.
(Consultant Ophthalmologist)

..... [redacted]

Date 7-3-2018

QUALITY ASSURANCE STATEMENT

This report has been audited and is considered to be an accurate description of the methods used and an accurate presentation of the data obtained during the conduct of the study.

[redacted]
(Quality Assurance)

... [redacted]

Date 8th March 2018

CONTENTS

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SUMMARY

1. This was a single-blind, within-subject comparison, single-centre study, to investigate the human eye irritation potential of two test articles.

2. Subjects had a slit-lamp examination and assessment of bulbar conjunctival irritation, palpebral conjunctival irritation and lid disease prior to application. Each subject received two test articles, one applied to the left eye area and the second applied to the right eye area. Subjects had a second slit-lamp examination and assessment of bulbar conjunctival irritation, palpebral conjunctival irritation and lid disease - 8 hours after application.

3. Individual and mean data for bulbar conjunctival irritation, palpebral conjunctival irritation and lid disease are presented in this report for the 30 subjects who completed the study.

4. Test article 1 - [redacted] serum [redacted]) elicited the same or lower scores for palpebral conjunctival irritation and lid disease when compared to baseline at the 8-hour time-point. Slight bulbar conjunctival irritation was observed at the 8-hour time-point. Test article 2 - [redacted] serum [redacted] elicited the same or lower scores for all assessed parameters compared to baseline at the 8-hour time-point.

5. It can be concluded that under the conditions of this study, the following claims can be supported for the test articles:

   Test article 1 - [redacted] serum [redacted]:

   Ophthalmologist Tested

   Approved Suitable for Contact Lens Wearers

   Safe for use with Lash Extensions

6. Test article 2 - [redacted] serum [redacted]:

   Ophthalmologist Tested

   Approved Suitable for Contact Lens Wearers

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KEY STUDY PERSONNEL AND RESPONSIBILITIES

Key Personnel	General Responsibilities
Principal Investigator (PI) [redacted] PCR Corp [redacted]	The Principal Investigator (PI) is responsible for ensuring sufficient resources are available to conduct the study and is responsible for the study design, review of the study protocol and report, and ensuring that they concur with the study findings report.
Project Supervisor (PS) [redacted] PCR Corp [redacted]	The Project Supervisor (PS) will be responsible for the conduct of the study on a daily basis.
Project Manager (PM) [redacted] PCR Corp [redacted]	The Project Manager (PM) will be involved with the study design, compilation of study results, and writing the study protocol and report.
Project Coordinator (PC) [redacted]	The Project Coordinator (PC) will be the primary point of contact on behalf of the Sponsor of this study and will represent the Sponsor ([redacted]) of this study.

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INTRODUCTION AND OBJECTIVE

The objective of this study was to assess the human eye irritation potential of two test articles to substantiate the following claims:

Objective for Test article 1 - [redacted] serum ([redacted]): Substantiate the claims of:

1. Ophthalmologist Tested
2. Approved Suitable for Contact Lens Wearers
3. Safe for use with Lash Extensions

Objective for Test article 2 - [redacted] serum ([redacted]): Substantiate the claims of:

1. Ophthalmologist Tested
2. Approved Suitable for Contact Lens Wearers

MATERIALS AND METHODS

1. STUDY DESIGN

The study was a single blind, in-use, at a single centre.

A total of 30 subjects applied with the test articles.

2. SELECTION OF SUBJECTS

2.1. SCREENING

Thirty subjects were recruited into the study. Subjects satisfied the following inclusion and exclusion criteria, were prepared to accept the prohibitions and restrictions and gave written informed consent (Appendix 1).

The suitability of each potential subject was confirmed before their acceptance by review of a study specific pre-treatment questionnaire (Appendix 2).

2.2. INCLUSION CRITERIA

a. Subject is a healthy female, aged 18 years or older (50% to be regular users of contact lenses).

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b. Zero scores for ocular bulbar conjunctival irritation, palpebral conjunctival irritation and lid disease, at examination prior to issue of test article.

a.

b. Subject has signed a written Informed Consent.

2.3. Exclusion Criteria

a. Subject was pregnant, nursing, or planning to become pregnant.
b. A current ocular disease or condition, other than simple refraction error, unless considered irrelevant by the examining ophthalmologist.
c. Heavy alcohol consumption (i.e. more than 21 units per week or 8 units a day for men, more than 14 units per week or 4 units a day for women).
d. Current use or history of repeated use of street drugs.
e. Treatment or medication containing sympathomimetics, antihistamines or corticosteroids in the seven days prior to study start.
f. Hayfever.
g. Use of false eyelashes, any topical prescription or cosmetic products on the eyes, eyelashes or the periorbital areas of the face on the day of the study.

2.4. Prohibitions and Restrictions

a. Subject agreed not to use any products in, or around, their eyes on the study day.

3. MATERIALS

3.1. Test Articles

The test articles were supplied by the Sponsor and labelled as follows:

1. [redacted] serum ([redacted])
2. [redacted] serum ([redacted])

The test articles were used as supplied by the Sponsor.

The Sponsor provided the ingredient listings (Appendix 3) and certifies that the products supplied to PCR Corp for the clinical trial had been manufactured/formulated with ingredients that are safe and suitable for the product's stated purpose. The control product is currently marketed.

It was the responsibility of the Sponsor to determine, for each batch of test article, the identity, strength, purity, composition, and other characteristics which appropriately define the test article before its use in the study. The determination of its stability and documentation of methods of synthesis and derivation were also the Sponsor's responsibility.

It was the responsibility of the Sponsor that the test article met all necessary transport regulations, particularly those regulations involving the carriage of hazardous goods and the import/export of goods, and that any costs including tax/duty are fully met by the Sponsor prior to receipt of the test article at PCR Corp. No liability with regard to safe receipt or costs involved in carriage of goods to any PCR Corp site was accepted.

On study completion, any remaining unused test articles were disposed of, unless otherwise requested by the Sponsor, after issuance of the final report or 28 days after study completion, whichever came first. Sponsors requesting the return of products were liable for any costs incurred.

4. METHOD

4.1. OPHTHALMOLOGICAL EXAMINATIONS

The Consultant Ophthalmologist assessed the eye health of the subjects at baseline on Day 1, before application of the test articles, and at 8 hours, at the conclusion of the study. The following was assessed and a score recorded:

Bulbar Conjunctival Irritation

0	=	Within normal limits
1	=	Mildly pink
2*	=	Moderately pink
3*	=	Intense red vessels, dilated

Palpebral Conjunctival Irritation

0	=	Within normal limits
1	=	Mildly pink
2*	=	Moderately pink
3*	=	Cherry to deep red

Lid Disease

0 = No disease

1 = Disease and the study have no mutually adverse effect i.e. neither the disease is likely to get aggravated nor result of the study is likely to be affected.

2 = Disease is likely to get worse or it can affect the study.

4.2. Issue and Use of Test Article

Following the subjects eye examinations at baseline on Day 1 of the study (see Section 4.1), the test articles were applied. They were instructed to not use any other eye products except for the test articles issued to them, for the duration of the study.

Subjects returned to the Test Centre after 8 hours with any unused test article. They then underwent a second, and final, eye examination by the Consultant Ophthalmologist (see Section 4.1).

4.3. Test Article Application

The test articles were applied to all 30 subjects. Each subject had the first test article applied to their left eye lash, and the second test article applied to their right eye brow.

4.4. Evaluation of Results

Baseline and 8-hour visual acuity scores were compared. Providing there is no significant difference between them the test articles will be considered as safe to use and claims such as "Ophthalmologist Tested/Approved" and "Suitable for Contact Lens Wearers" will be supported.

5. STUDY ETHICS

5.1. Declaration of Helsinki

The study conformed to the requirements of the 1964 Declaration of Helsinki and its subsequent amendments (World Medical Association; 2013).

5.2. INDEMNITY PROVISION

The Sponsor shall be responsible, without regard to legal liability, and shall indemnify PCR Corp, or any of their respective officers or employees in the event of claims for compensation from subjects suffering injury or other deterioration in health or well-being as a result of participation in this study, except and insofar as such claims arise as a result of any negligent act or omission on the part of PCR Corp employees or any persons undertaking or involved in the study by arrangement with PCR Corp.

6. QUALITY ASSURANCE

The study will be carried out within the spirit of the ICH Guidelines on Good Clinical Practice, 1996 (1) and other recognised guidelines. An audit of the final report will be completed, for accuracy and completeness of presentation. Additionally, the study may be subject to the following Quality Assurance procedures:

- Review of protocol and protocol amendments for completeness, clarity and adequacy.
- Inspection and/or audit of critical phases of study conduct for compliance with protocol and PCR Corp procedures.

PCR Corp Quality Assurance, will inform PCR Corp management of any findings that may affect the integrity of the study.

7. RETENTION OF DATA

All raw data generated by PCR Corp during the course of the study, and including protocol and final report, will be retained in the PCR Corp Archive for a minimum period of five years from study completion. In the event of original data being transferred to the Sponsor at their request, exact copies will be so retained. At no time will archived data be destroyed without prior written approval of the Sponsor. All study data will be available at any time, by appointment, for inspection by the Sponsor or their authorised representative.

8. REFERENCES

1. International Conference on Harmonisation of Technical Requirements for the Registration of Pharmaceuticals for Human Use. Note for Guidance on Good Clinical Practice, Consolidated Guideline. Step 4, Consolidated Guideline, 1/5/96. CPMP/ICH/135/95.

2. *World Medical Association (2013). "Declaration of Helsinki: Ethical Principles for Medical Research Involving Human Subjects". JAMA* ***310*** *(20): 2191–2194. doi:10.1001/jama.2013.281053*

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RESULTS

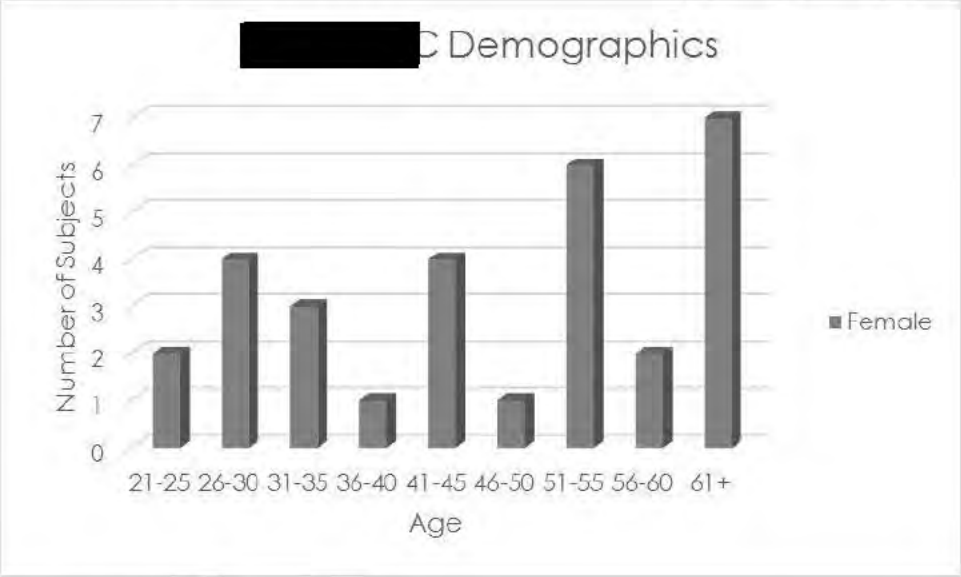
1 LOCATION AND DATES OF THE STUDY

The study was performed at PCR Corp, on 2nd February 2018.

2 SUBJECTS

30 female subjects were recruited into, and completed the study.

Figure 1: [redacted]C Demographics



3 ADVERSE EVENTS, ADVERSE REACTIONS AND SUBJECTS NOT COMPLETING THE STUDY

No adverse events or reactions were reported, no subjects withdrew. The study was completed by all 30 subjects.

4 ASSESSMENTS – BULBAR AND PALPEBRAL CONJUNCTIVAL IRRITATION AND LID DISEASE

Test article 1 - [redacted] serum ([redacted]) elicited the same or lower scores for palpebral conjunctival irritation and lid disease when compared to baseline at the 8-hour time-point. Slight bulbar conjunctival irritation was observed at the 8-hour time-point. Test article 2 - [redacted] serum ([redacted]) elicited the same or lower scores for all assessed parameters compared to baseline at the 8-hour time-point.

TABLE 1 – INDIVIDUAL SCORES FOR TEST ARTICLE 1 - [redacted] SERUM ([redacted]) (LEFT EYE) AND TEST ARTICLE 2 - [redacted] SERUM ([redacted]) (RIGHT EYE) AT BASELINE AND 8 HOURS

BASELINE

Subject	Right eye			Left eye		
Number	B	P	L	B	P	L
1	0	0	0	0	0	0
2	0	0	0	0	0	0
3	0	0	0	0	0	0
4	0	0	0	0	0	0
5	0	0	0	0	0	0
6	0	0	0	0	0	0
7	0	0	0	0	0	0
8	0	0	0	0	0	0
9	0	0	0	0	0	0
10	0	0	0	0	0	0
11	0	0	0	0	0	0
12	0	0	0	0	0	0
13	0	0	0	0	0	0
14	0	0	0	0	0	0
15	0	0	0	0	0	0
16	0	0	0	0	0	0
17	0	0	0	0	0	0
18	0	0	0	0	0	0
19	0	0	0	0	0	0
20	0	0	0	0	0	0
21	0	0	0	0	0	0
22	0	0	0	0	0	0
23	0	0	0	0	0	0
24	0	0	0	0	0	0
25	0	0	0	0	0	0
26	0	0	0	0	0	0
27	0	0	0	0	0	0
28	0	0	0	0	0	0
29	0	0	0	0	0	0
30	0	0	0	0	0	0
MEAN	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**

8 HOURS

Subject	Right eye			Left eye		
Number	B	P	L	B	P	L
1	0	0	0	0	0	0
2	0	0	0	0	0	0
3	0	0	0	0	0	0
4	0	0	0	0	0	0
5	0	0	0	1	0	0
6	0	0	0	1	0	0
7	0	0	0	0	0	0
8	0	0	0	1	0	0
9	0	0	0	1	0	0
10	0	0	0	2	0	0
11	0	0	0	1	0	0
12	0	0	0	1	0	0
13	0	0	0	0	0	0
14	0	0	0	1	0	0
15	0	0	0	1	0	0
16	0	0	0	1	0	0
17	0	0	0	0	0	0
18	0	0	0	0	0	0
19	0	0	0	0	0	0
20	0	0	0	0	0	0
21	0	0	0	0	0	0
22	0	0	0	0	0	0
23	0	0	0	0	0	0
24	0	0	0	0	0	0
25	0	0	0	0	0	0
26	0	0	0	0	0	0
27	0	0	0	0	0	0
28	0	0	0	0	0	0
29	0	0	0	0	0	0
30	0	0	0	0	0	0
MEAN	**0.0**	**0.0**	**0.0**	**0.4**	**0.0**	**0.0**

B = Bulbar Conjunctival Irritation P = Palpebral Conjunctival Irritation L = Lid disease

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CONCLUSIONS

It can be concluded that under the conditions of this study, the following claims can be supported for the test articles:

Test article 1 - [redacted] serum ([redacted]):

1. Ophthalmologist Tested
2. Approved Suitable for Contact Lens Wearers
3. Safe for use with Lash Extensions

Test article 2 - [redacted] serum ([redacted]):

1. Ophthalmologist Tested
2. Approved Suitable for Contact Lens Wearers

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APPENDIX 1: INFORMED CONSENT

Study Code: [redacted] Subject #: _____

INTRODUCTION
You are being asked to participate in a research study. Prior to giving your consent to be a subject, it is important that you take the time to read and understand what your participation would involve. This consent form may contain technical language which you may not understand. If you do not understand any of this consent form, please ask the clinical staff any questions you may have.

You will be provided with a signed copy of this consent form and any other necessary written information prior to the start of the study.

OBJECTIVE
The objective of the study is to assess the comparative eye irritation potential of two test articles to substantiate the claims of, "Ophthalmologist Tested/Approved" and "Suitable for Contact Lens Wearers".

TEST ARTICLES
The two test articles applied to your lashes/brows may include make-up, personal care products etc.

STUDY PROCEDURES
You will be one of approximately 30 subjects enrolled onto this study. Your participation in this study will last 1 day and will include two visits to the testing facility.

Visit 1 (Study day 1): Prior to acceptance on the study, you will be consented and screened for eligibility to participate on the study. Following verification of your acceptance, you will undergo an eye examination by our Ophthalmologist and providing your eyes are judged to be healthy you will have two test articles, one to the left eye lash/brow and the second to the right eye lash/brow, applied.

Visit 2 (Study day 1): After 8 hours, you will return to the test centre. You will undergo a second and final eye examination by our Ophthalmologist. You will then be compensated for your time and inconvenience.

RISKS
To the best of our knowledge, these products are not expected to induce an allergic reaction. While the potential for irritation or other reactions during this study are minimal, it is possible for a reaction to occur. Expected reactions for these test article categories are mild in nature and may include the following: redness, itching, stinging or burning. In addition to the risks described, there may be other risks that are currently unforeseeable.

No significant adverse reactions are expected to occur. However, if you develop an adverse reaction or complication as a result of your participation in this study, medical treatment will be provided by clinical staff nurses at PCR Corp or you will be referred for appropriate treatment at no cost to you, as long as you have followed the study instructions. Provisions of such medical care is not an admission of legal responsibility. You will be followed by PCR Corp until the adverse reaction has resolved. No additional compensation will be available to you. Neither the sponsoring company nor the investigating company will be held responsible for any future medical expenses.

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BENEFITS
While it is likely that you will not receive any direct benefit from your participation in the study, the study results may have the potential to increase scientific knowledge about skincare products and may allow for new and improved products to be marketed.

CONFIDENTIALITY
Information concerning you that is obtained in connection with this study will be kept confidential by PCR Corp, except that the sponsoring company whose product is being tested will receive a copy of the study records. The records will be uniquely coded to protect your identity. In addition, third party regulatory authorities, including the U.S. Food and Drug Administration (FDA), may inspect the records of the study. In all cases, your confidentiality will be maintained and your identity will remain private.

Your signature on the Informed Consent provides your permission for these agencies to view your personal information and the study data.

NEW FINDINGS
Any new information that is discovered during the study and which may influence your willingness to continue in the study will be made available to you.

MEDICAL TREATMENT
In the event of an emergency, dial 999. If you receive any medical care during the course of the study, inform medical personnel that you are participating in a research study. Please contact PCR Corp staff as soon as possible to inform them of your condition.

WHO TO CONTACT
If you have any questions about this study or in the case of an emergency, contact [redacted] on [redacted] during normal business hours.

VOLUNTARY PARTICIPATION/WITHDRAWAL
Your participation in this research study is strictly voluntary. You may refuse to participate or may discontinue participation at any time during the study without penalty or loss of benefits to which you are otherwise entitled. However, you must contact the test facility and inform a clinical staff member of your decision to withdraw from the study.

If you agree to participate in the study, you are also agreeing to provide PCR Corp with accurate information and to follow study instructions as given to you. If you fail to follow study instructions, you may be asked to discontinue participation.

Your participation in the study may be discontinued at any time without your consent by PCR Corp, regulatory agencies, or the sponsoring company for reasons of but not limited to a severe side effect and accompanying illness, or if you do not follow study instructions.

NON-DISCLOSURE
As a condition to your participation in the study you are asked not to discuss any information regarding the products that you are testing, your experiences with the products, or your opinion of the products with anyone outside of the testing facility. By your signature on the Consent you are agreeing to abide by this condition of participation.

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COMPENSATION
If you agree to participate in this study, you will be paid £ upon completion of the study.

PHOTOGRAPHY AUTHORIZATION
As an additional part of this study, study staff may take photographs or videotape during the study. These photos or videos may be used for the following purposes: training of PCR materials, PCR advertising, documentation of study procedures/results or upon request of the sponsor. By signing this consent form you are giving your authorization for PCR to take, use, reproduce, and distribute these photographs/videotapes taken during your participation in this study.

CONSENT TO PARTICIPATE
I know that my participation in this study is voluntary and that I have the right to refuse to participate. I know that I may withdraw from the study at any time without penalty or loss of benefits to which I am otherwise entitled. If, at the discretion of the Investigator, it is best to discontinue my participation for reasons other than a failure to obey the directions of the study, I will be paid in full or for the portion of the study I have completed once the study is over.

CONSENT
I have read all of the pages of this consent form and have been given an opportunity to ask questions about this study. Answers to such questions (if any) were satisfactory. I am at least eighteen years old and without reservation give my consent to serve as a subject in this study. By signing this form, I have not given up any of my legal rights as a research subject. I will receive a copy of this signed consent document.

You are making a decision whether or not to participate. Your signature indicates that you have decided to participate, having read the information provided above.

Subject's Name Printed: First	**Middle Initial**	**Last**
Subject's Signature		**Date**
Signature of Person Conducting Consent Discussion		**Date**
Subject Number		

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APPENDIX 2: PRE-TREATMENT QUESTIONNAIRE

FOR OFFICE USE ONLY		
Subject's Initials		
Subject's DOB: ____ Subject's Age: ____		
MALE/FEMALE		

Study Code: [redacted]

[redacted]

Inclusion Criteria		Yes	No
1.	Subject is a healthy female, aged 18 years or older.	☐	☐
2.	Zero scores for ocular bulbar conjunctival irritation, palpebral conjunctival irritation and lid disease, at examination prior to issue of test article.	☐	☐
3.	Subject has signed a written Informed Consent.	☐	☐
Exclusion Criteria		**Yes**	**No**
1.	Subject is pregnant, nursing, or planning to become pregnant.	☐	☐
2.	A current ocular disease or condition, other than simple refraction error, unless considered irrelevant by the examining ophthalmologist.	☐	☐
3.	Heavy alcohol consumption (i.e. more than 21 units per week or 8 units a day for men, more than 14 units per week or 4 units a day for women).	☐	☐
4.	Current use or history of repeated use of street drugs.	☐	☐
5.	Treatment or medication containing sympathomimetics, antihistamines or corticosteroids in the seven days prior to study start.	☐	☐
6.	Hayfever.	☐	☐
7.	Use of false eyelashes, any topical prescription or cosmetic products on the eyes, eyelashes or the periorbital areas of the face on the day of the study.	☐	☐
Prohibitions and Restrictions		**Yes**	**No**
1.	Subject agrees not to use any products in, or around, their eyes on the study day.	☐	☐

Have you ever had any skin problems related to the use of any of the following types of material?

Material	Yes	No	When? – Which products? – What happens?
Eye Lash Products			
Eye Brow Products			
Serum Products			
Other Personal Care Products – please specify			

Questionnaire checked and confirmed by:

_____ _____

Signature Date

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APPENDIX 3: INCI LISTS

TEST ARTICLE 1 – [redacted] serum ([redacted])

[redacted]

TEST ARTICLE 2 – [redacted] serum ([redacted])

[redacted]

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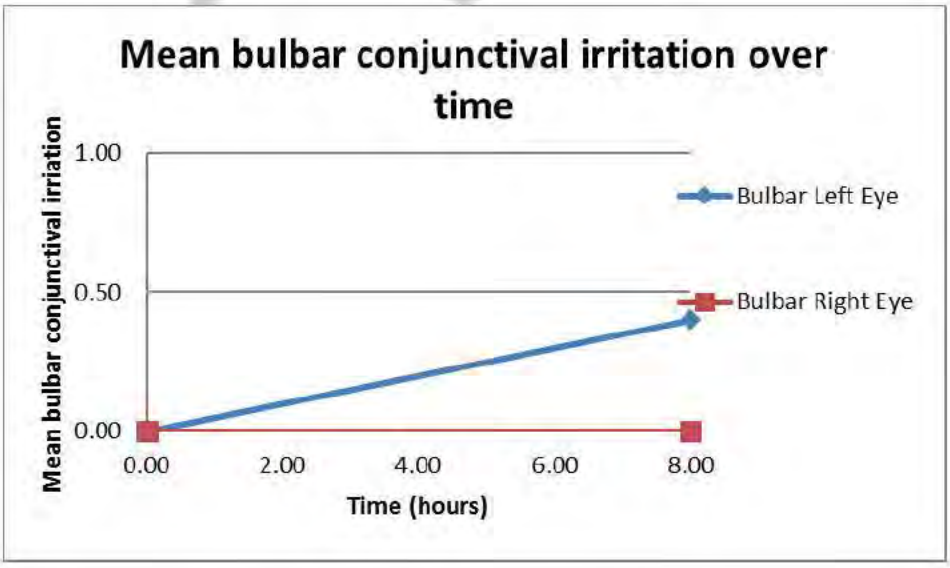
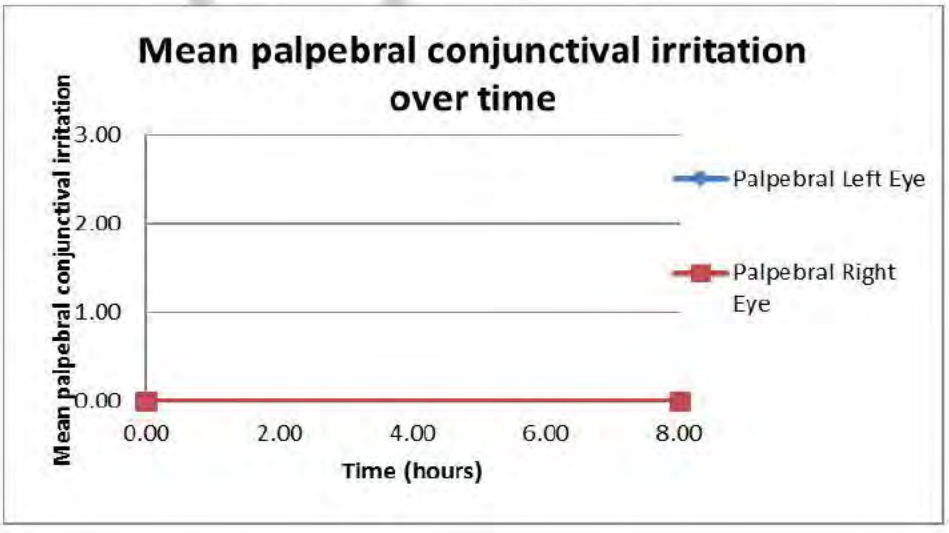
APPENDIX 4: Individual & Mean Bulbar Conjunctival Irritation Scores

	Left Eye		Right Eye	
Subject	Baseline	8 hours	Baseline	8 hours
Number	L	L	L	L
1	0	0	0	0
2	0	0	0	0
3	0	0	0	0
4	0	0	0	0
5	0	1	0	0
6	0	1	0	0
7	0	0	0	0
8	0	1	0	0
9	0	1	0	0
10	0	2	0	0
11	0	1	0	0
12	0	1	0	0
13	0	0	0	0
14	0	1	0	0
15	0	1	0	0
16	0	1	0	0
17	0	0	0	0
18	0	0	0	0
19	0	0	0	0
20	0	0	0	0
21	0	0	0	0
22	0	0	0	0
23	0	0	0	0
24	0	0	0	0
25	0	0	0	0
26	0	0	0	0
27	0	0	0	0
28	0	0	0	0
29	0	0	0	0
30	0	0	0	0
MEAN	**0.0**	**0.4**	**0.0**	**0.0**



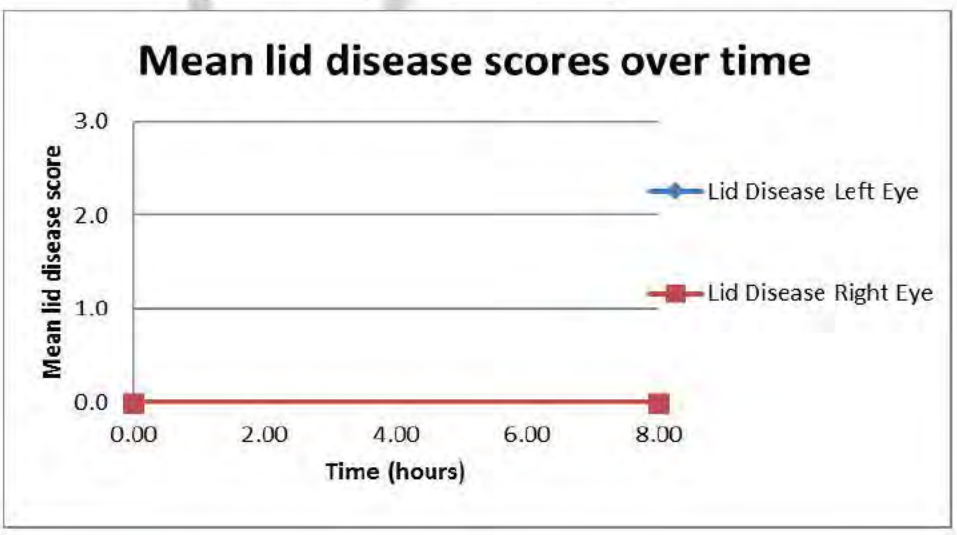
APPENDIX 5: Individual & Mean Palpebral Conjunctival Irritation Scores

	Left Eye		Right Eye	
Subject	Baseline	8 hours	Baseline	8 hours
Number	L	L	L	L
1	0	0	0	0
2	0	0	0	0
3	0	0	0	0
4	0	0	0	0
5	0	0	0	0
6	0	0	0	0
7	0	0	0	0
8	0	0	0	0
9	0	0	0	0
10	0	0	0	0
11	0	0	0	0
12	0	0	0	0
13	0	0	0	0
14	0	0	0	0
15	0	0	0	0
16	0	0	0	0
17	0	0	0	0
18	0	0	0	0
19	0	0	0	0
20	0	0	0	0
21	0	0	0	0
22	0	0	0	0
23	0	0	0	0
24	0	0	0	0
25	0	0	0	0
26	0	0	0	0
27	0	0	0	0
28	0	0	0	0
29	0	0	0	0
30	0	0	0	0
MEAN	**0.0**	**0.0**	**0.0**	**0.0**



APPENDIX 6: Individual & Mean Lid Disease Scores

	Left Eye			Right Eye	
Subject	Baseline	8 hours		Baseline	8 hours
Number	L	L		L	L
1	0	0		0	0
2	0	0		0	0
3	0	0		0	0
4	0	0		0	0
5	0	0		0	0
6	0	0		0	0
7	0	0		0	0
8	0	0		0	0
9	0	0		0	0
10	0	0		0	0
11	0	0		0	0
12	0	0		0	0
13	0	0		0	0
14	0	0		0	0
15	0	0		0	0
16	0	0		0	0
17	0	0		0	0
18	0	0		0	0
19	0	0		0	0
20	0	0		0	0
21	0	0		0	0
22	0	0		0	0
23	0	0		0	0
24	0	0		0	0
25	0	0		0	0
26	0	0		0	0
27	0	0		0	0
28	0	0		0	0
29	0	0		0	0
30	0	0		0	0
MEAN	**0.0**	**0.0**		**0.0**	**0.0**



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AMA LABORATORIES, INC. [redacted]

50 HUMAN SUBJECT REPEAT INSULT PATCH TEST
SKIN IRRITATION/SENSITIZATION EVALUATION
(Occlusive Patch)

AMA Ref. No.: [redacted]

Date: July 14, 2016

Sponsor: [redacted]

1.0 Objective:

Consumer products or raw materials designed for consistent reapplication to areas of the skin may, under proper conditions, prove to be contact sensitizers or irritants in certain individuals. It is the intention of a Repeat Insult Patch Test (RIPT) to provide a basis for evaluation of this irritation/sensitization potential if such exists.

2.0 Test Material: lash serum containing 0.005% Isopropyl Cloprostenate

2.1 Test Material Description:

On June 7, 2016 one test sample labeled [redacted] Eyelash Solution-[redacted] was received from [redacted] and assigned AMA Lab [redacted].

2.2 Handling:

Upon arrival at AMA Laboratories, Inc., the test material is assigned a unique laboratory code number and entered into a daily log identifying the lot number, sample description, sponsor, date received and tests requested.

Samples are retained for a period of three months beyond submission of final report unless otherwise specified by the sponsor or, if sample is known to be in support of governmental applications, representative retained samples are kept two years beyond final report submission.

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

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2.3 Test Material Evaluation Prerequisite:

Prior to induction of a human test panel, toxicology, microbiology or in-vitro performance spectra may be required to assess the feasibility of commencement as dictated by an Institutional Review Board (IRB) described in Section 3.0.

Sponsor purports that prior to sample submission the following tests were conducted with no adverse results and that the test data are on file on their premises and have not been made available to AMA personnel:

- USP or CTFA Preservative Efficacy Test or equivalent
- 90 Day Accelerated Stability and Container Compatibility Study

3.0 Institutional Review Board:

Reference: CFR Title 21 Part 56, Subparts A, B, C, and D. The IRB of AMA Laboratories, Inc., consists of five or more individuals, chosen from within the company for technical expertise and from the local community for lay interaction. The list of IRB members is kept on file at AMA Laboratories, Inc. and is available for inspection during the hours of operation.

4.0 Panel Selection:

4.1 Standards for Inclusion in a Study:

- Individuals who are not currently under a doctor's care.
- Individuals free of any dermatological or systemic disorder which would interfere with the results, at the discretion of the Investigator.
- Individuals free of any acute or chronic disease that might interfere with or increase the risk of study participation.
- Individuals who will complete a preliminary medical history form mandated by AMA Laboratories, Inc. and are in general good health.
- Individuals, who will read, understand and sign an informed consent document relating to the specific type of study they are subscribing. Consent forms are kept on file and are available for examination on the premises of AMA Laboratories, Inc. only.
- Individuals able to cooperate with the Investigator and research staff, willing to have test materials applied according to the protocol, and complete the full course of the study.

4.2 Standards for Exclusion from a Study:

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

4.3 Recruitment:

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

4.4 Informed Consent and Medical History Forms:

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

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

5.0 Population Demographics:

Number of subjects enrolled		52
Number of subjects completing study		50
Age Range		23-68
Sex	Male	10
	Female	42
Race	Caucasian	43
	Hispanic	8
	Asian	1

6.0 Equipment:

- Patch Description: Parke-Davis Hypoallergenic Readi Bandages or the equivalent.
- 1ml volumetric syringe without a needle.

7.0 Procedure:

- Subjects are requested to bathe or wash as usual before arrival at the facility.
- 0.2 ml of the test material is dispensed onto the occlusive, hypoallergenic patch.
- The patch is then applied directly to the skin of the infrascapular regions of the back, to the right or left of the midline and the subject is dismissed with instructions not to wet or expose the test area to direct sunlight.
- After 24 hours the patch is removed by the panelist at home.
- This procedure is repeated until a series of nine consecutive 24 hour exposures have been made for every Monday, Wednesday, and Friday for three consecutive weeks.
- In the event of an adverse reaction, the area of erythema and edema is measured. The edema is estimated by the evaluation of the skin with respect to the contour of the unaffected normal skin. Reactions are scored just before applications two through nine and the next test date following application nine. In most instances this is approximately 24 hours after patch removal. Clients are notified immediately in the case of adverse reaction and determination is made as to treatment program if necessary.
- Subjects are then given a 10 - 14 day rest period after which a challenge or retest dose is applied once to a previously unexposed test site. The retest dose is equivalent to any one of the original nine exposures. Reactions are scored 24 and 48 hours after application.
- Comparison is made between the nine inductive responses and the retest dose.
- At the conclusion of the study, the consulting Dermatologist reviewed this data and confirmed the stated conclusions.

8.0 Results:

Please refer to attached Table.

9.0 Observations:

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

10.0 Archiving:

All original samples, raw data sheets, technician's notebooks, correspondence files and copies of final reports and remaining specimens are maintained on premises of AMA Laboratories, Inc. in limited access storage files marked "Archive". A duplicate disk copy of final reports is separately archived in a bank safe deposit vault.

11.0 Reference:

Appraisal of the Safety of Chemicals in Food, Drugs and Cosmetics, published by The Association of Food and Drug Officials of The United States, 1965 (modified).

12.0 Security Label Disclosure:

To prevent loss of and protect intellectual property, original, certified documents issued by AMA Laboratories Inc. can be identified by a proprietary, tamper evident security hologram affixed to all Conclusion/Signature pages on final reports. Any attempt to remove the hologram will irreversibly damage the label and leave an immediate trace, thus invalidating the document.

Only reports containing the AMA LABS, INC. hologram intact will be recognized by AMA Laboratories Inc. as a certified original.

13.0 Conclusions:

The test material (AMA Lab. No.: [redacted]; Client No.: [redacted] Eyelash Solution-[redacted]) when tested under occlusion as described herein, may be considered: a **NON-PRIMARY IRRITANT** and **NON-PRIMARY SENSITIZER** to the skin according to the reference.

[redacted] M.D.
Study Director

[redacted], M.D.
Dermatologist

[redacted] B.S.
Technician

[redacted] B.S.
Technician

[redacted], Medical Assistant
NCCT Certified
Technician

[redacted] B.S.
Technical Director

7/14/16
Date

[redacted]

TABLE
SUMMARY OF RESULTS
(Occlusive Patch)

AMA Lab No.: [redacted]
Client No.: [redacted] Eyelash Solution [redacted]

No.	Subject ID	RACE	SEX	Response 1	2	3	4	5	6	7	8	9	Chall. 24 HR	Chall. 48 HR	Score
1	40 0533	C	F	0	0	0	0	0	0	0	0	0	0	0	0.0
2	42 8272	C	F	0	0	0	0	0	0	0	0	0	0	0	0.0
3	44 2748	C	F	0	0	0	0	0	0	0	0	0	0	0	0.0
4	44 3503	C	F	0	0	0	0	0	0	0	0	0	0	0	0.0
5	44 7118	C	F	0	0	0	0	0	0	0	0	0	0	0	0.0
6	44 7255	C	F	0	0	0	0	0	0	0	0	0	0	0	0.0
7	46 1633	C	F	0	0	0	0	0	0	0	0	0	0	0	0.0
8	46 7866	C	F	0	0	0	0	0	0	0	0	0	0	0	0.0
9	48 0946	C	F	0	0	0	0	0	0	0	0	0	0	0	0.0
10	48 4004	H	F	0	0	0	0	0	0	0	0	0	0	0	0.0
11	48 6153	H	M	0	0	0	0	0	0	0	0	0	0	0	0.0
12	52 4442	H	M	0	0	0	0	0	0	0	0	0	0	0	0.0
13	54 3239	C	F	0	0	0	0	0	0	0	0	0	0	0	0.0
14	54 9679	C	F	0	0	0	0	0	0	0	0	0	0	0	0.0
15	56 1236	C	F	0	0	0	0	0	0	0	0	0	0	0	0.0
16	56 3141	H	F	0	0	0	0	0	0	0	0	0	0	0	0.0
17	56 3379	C	F	0	0	0	0	0	0	0	0	0	0	0	0.0
18	56 3465	H	F	0	0	0	0	0	0	0	0	0	0	0	0.0
19	56 4584	C	M	0	0	0	0	0	0	0	0	0	0	0	0.0
20	56 5529	C	F	0	0	0	0	0	0	0	0	0	0	0	0.0
21	56 9114	C	F	0	0	0	0	0	0	0	0	0	0	0	0.0
22	58 3068	C	F	0	0	0	0	0	0	0	0	0	0	0	0.0
23	60 3225	C	F	0	0	0	0	0	0	0	0	0	0	0	0.0
24	60 7979	C	F	0	0	0	0	0	0	0	0	0	0	0	0.0
25	60 8701	C	F	0	0	0	0	0	0	0	0	0	0	0	0.0
26	60 9466	C	F	0	0	0	0	0	0	0	0	0	0	0	0.0
27	64 0383	H	F	0	0	0	0	0	0	0	0	0	0	0	0.0
28	64 5779	C	F	0	0	0	0	0	0	0	0	0	0	0	0.0
29	64 6653	H	F	0	0	0	0	0	0	0	0	0	0	0	0.0

TABLE (CONT'D)
SUMMARY OF RESULTS
(Occlusive Patch)

AMA Lab No.: [redacted]
Client No.: [redacted] Eyelash Solution-[redacted]

No.	Subject ID	RACE	SEX	Response 1	2	3	4	5	6	7	8	9	Chall. 24 HR	48 HR	Score
30	64 9034	C	M	0	0	0	0	0	0	0	0	0	0	0	0.0
31	66 1101	C	F	0	0	0	0	0	0	0	0	0	0	0	0.0
32	66 1649	C	F	0	0	0	0	0	0	0	0	0	0	0	0.0
33	66 6606	H	M	0	0	Dc	Dc	Dc	Dc	Dc	Dc	Dc	Dc	Dc	N/A
34	68 0458	C	M	0	0	0	0	0	0	0	0	0	0	0	0.0
35	68 7601	C	F	0	0	0	0	0	0	0	0	0	0	0	0.0
36	70 5391	C	F	0	0	0	0	0	0	0	0	0	0	0	0.0
37	72 3555	C	F	0	0	0	0	0	0	0	0	0	0	0	0.0
38	74 0600	C	F	0	0	0	0	0	0	0	0	0	0	0	0.0
39	74 8531	C	M	0	0	0	0	0	0	0	0	0	0	0	0.0
40	76 1298	C	F	0	0	0	0	0	0	0	0	0	0	0	0.0
41	76 2719	C	F	0	0	0	0	0	0	0	0	0	0	0	0.0
42	76 7056	C	F	0	0	0	0	0	0	0	0	0	0	0	0.0
43	76 8434	C	F	0	0	0	0	0	0	0	0	0	0	0	0.0
44	78 5272	C	M	0	0	0	0	0	0	0	0	0	0	0	0.0
45	80 0080	C	M	0	0	0	0	0	Dc	Dc	Dc	Dc	Dc	Dc	N/A
46	80 0847	C	F	0	0	0	0	0	0	0	0	0	0	0	0.0
47	80 3313	C	F	0	0	0	0	0	0	0	0	0	0	0	0.0
48	80 7035	A	M	0	0	0	0	0	0	0	0	0	0	0	0.0
49	82 4670	C	F	0	0	0	0	0	0	0	0	0	0	0	0.0
50	82 7228	C	F	0	0	0	0	0	0	0	0	0	0	0	0.0
51	90 5388	C	F	0	0	0	0	0	0	0	0	0	0	0	0.0
52	92 5874	C	F	0	0	0	0	0	0	0	0	0	0	0	0.0

Evaluation Period:

This study was conducted from June 8, 2016 through July 13, 2016.

Scoring Scale and Definition of Symbols Shown in Table:

0 - No evidence of any effect

? - (Barely perceptible) minimal faint (light pink) uniform or spotty erythema

1 - (Mild) pink uniform erythema covering most of contact site

2 - (Moderate) pink\red erythema visibly uniform in entire contact area

3 - (Marked) bright red erythema with accompanying edema, petechiae or papules

4 - (Severe) deep red erythema with vesiculation or weeping with or without edema

D - Patch eliminated due to reaction

Dc - Discontinued due to absence of subject on application date

M - Patch applied to an adjacent site after strong test reaction

N/A - Score is not calculated for subjects discontinued before challenge

S - Skin stained from pigment in product

T - Tan

NOTE: All technical employees of AMA LABORATORIES, INC. are required to take and pass a visual discrimination examination conducted by a Board Certified Ophthalmologist using the Farnsworth-Munsell 100 Hue Test as published; which determines a person's ability to discern color against a black background. This test was additionally modified to include a flesh tone background more nearly approaching actual use conditions, wherein erythematous skin is graded according to intensity.

14.0 Quality Assurance Statement:

This study was inspected in accordance with the Standard Operating Procedures of AMA Laboratories, Inc. To assure compliance with the study protocol, the Quality Assurance Unit completed an audit of the study records and report.

Report reviewed by:

[redacted]

[redacted] B.S.
Quality Assurance Supervisor

7/14/16
Date

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CLINICAL RESEARCH LABORATORIES

CLINICAL STUDY REPORT

Report Status:	Final
Report Date:	15 January 2018
CRL Study Number:	[redacted]
CRL Protocol Number:	[redacted]
Study Dates:	15 November 2017 - 22 December 2017
Study Title:	Repeated Insult Patch Test (RIPT) lash serum containing 0.005% Isopropyl Cloprostenate
Test Material:	[redacted]
Sponsor:	[redacted]
Sponsor Representative:	[redacted]
Principal Investigator:	[redacted] M.D. Dermatologist

APPROVAL SIGNATURE:

[redacted]

M.D.

Digitally signed by [redacted] M.D.
[redacted]
Date: 2018.01.15 11:59:42 -05'00'

Principal Investigator Signature/Date

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CLINICAL RESEARCH LABORATORIES

Good Clinical Practice
Quality Assurance Audit Statement

Clinical Study Number: [redacted]

Start Date: 15 November 2017

Completion Date: 22 December 2017

The clinical study listed above was conducted in accordance with the clinical study protocol, Clinical Research Laboratories, LLC Standard Operating Procedures (SOPs), which incorporate the principles of Good Clinical Practice (GCP) defined by applicable guidelines and regulations established by the International Council for Harmonization (ICH) and U.S. Regulatory Agencies. The clinical study master file was reviewed for compliance with the clinical study protocol, CRL SOPs, and applicable guidelines and regulations by the Principal Investigator and the Quality Assurance representative.

[redacted] Digitally signed by [redacted]
Date: 2018.01.15 07:47:24 -05'00'

Signature of QA Auditor and Date

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CLINICAL STUDY REPORT

Repeated Insult Patch Test (RIPT)

1.0 OBJECTIVES

The objectives of this study were to determine the potential of a test material to elicit dermal irritation or induce sensitization following repeated patch applications.

2.0 PRINCIPAL INVESTIGATOR AND INVESTIGATIVE SITE

[redacted], M.D.
Dermatologist

Clinical Research Laboratories, LLC
[redacted]

3.0 SPONSOR REPRESENTATIVE AND SPONSOR SITE

[redacted]

[redacted]

4.0 TEST MATERIAL

The following test material was provided by [redacted] and was received by Clinical Research Laboratories, LLC on 9 November 2017.

Test Material	Test Condition	Patch Type
[redacted]	Neat	Semi-occlusive*

The test material was coded with the following CRL identification number:

[redacted]

5.0 STUDY DATES

This study was initiated on 15 November 2017 and was completed on 22 December 2017.

* Semi-occlusive Strip ([redacted])

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6.0 SUBJECT SELECTION

Each subject was assigned a permanent CRL identification number. All subjects signed an Informed Consent Form in compliance with 21 CFR Part 50: "Protection of Human Subjects" and a HIPAA Authorization Form in compliance with 45 CFR Parts 160 and 164. All subjects completed a Subject Profile provided by Clinical Research Laboratories, LLC prior to the study (Subject Demographics - Appendix I). Subjects who met the following Inclusion Criteria and none of the Exclusion Criteria were impaneled:

6.1 INCLUSION CRITERIA

a. Subject is male or female between the ages of 18 and 70 years;

b. Female subjects who are sexually active are using adequate method of birth control;

c. Subject does not exhibit any skin diseases which might be confused with a skin reaction from the test material;

d. Subject agrees to avoid exposure of the test sites to the sun and to refrain from visits to tanning salons during the course of this study;

e. Subject agrees to refrain from getting patches wet and from scrubbing or washing the test area with soap or applying powder, lotions or personal care products to the area during the course of the study;

f. Subject has signed an Informed Consent in conformance with 21CFR Part 50: "Protection of Human Subjects;"

g. Subject has completed a HIPAA Authorization Form in conformance with 45CFR Parts 160 and 164;

h. Subject is in generally good health and has a current Subject Profile on file;

i. Subject is dependable and able to follow directions as outlined in the protocol.

6.2 EXCLUSION CRITERIA

a. Subject is pregnant, nursing, or planning to become pregnant, or not using adequate birth control;

b. Subject is currently using any systemic or topical corticosteroids, anti-inflammatory drugs, or antihistamines on a regular basis;

c. Subject reports allergies to cosmetics, toiletries, or personal care products;

d. Subject exhibits any skin disorders, sunburn, scars, excessive tattoos, etc. in the test area;

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6.0 SUBJECT SELECTION (CONTINUED)

8.2. EXCLUSION CRITERIA (CONTINUED)

e. Subject has scheduled, or is planning to undergo, any medical or surgical procedures during the 6-week course of the study.

7.0 STUDY EVALUATIONS

The following Dermal Scoring System will be used:

Dermal Score	Description
0	No visible skin reaction
±	Barely perceptible erythema
1+	Mild erythema
2+	Well defined erythema
3+	Severe erythema and edema
4+	Erythema and edema with vesiculation

Letter Codes

e = Edema
P = Peeling
S = Spreading of reaction beyond patch site.
Sc = Scabbing
d = Dryness/scaling
D = Oozing, crusting, and/or superficial erosions
I = Itching
F = Follicular irritation with or without pustule formation (folliculitis)
Hr = Hyperpigmentation
Ho = Hypopigmentation
X = Subject Absent
NP = No patching
Pa = Papules
C = Changed site
--- = No reading

8.0 TEST METHOD SUMMARY

8.1 SUBJECT IDENTIFICATION

All subjects were initially identified by a permanent identification number. Subjects who met the qualification criteria were assigned a study subject number. This subject number was assigned in sequence as subjects were enrolled in the study. A master roster was kept of the permanent identification number and the corresponding study subject number.

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8.0 TEST METHOD SUMMARY (CONTINUED)

8.2. INDUCTION PHASE

Informed Consent was obtained, Inclusion/Exclusion criteria verified and qualified subjects were enrolled. The test site was cleansed with 70% isopropyl alcohol. The test material was applied to the upper back, between the scapulae and the waist, to either side of the spinal midline. The test material was applied to the same site three times each week, usually Monday, Wednesday, and Friday, for a total of nine applications. However, the schedule may have been modified to accommodate inclement weather, holidays, or missed applications. At the discretion of the Principal Investigator, the test material may have been applied on two consecutive days during the Induction Phase or a makeup day may have been added at the end of the Induction Phase.

The test product was applied nine times over the approximate three-week induction phase. Subjects had no fewer than eight subsequent evaluations unless requested by the Sponsor.

The test site was marked with a gentian violet surgical marker to ensure the continuity of patch application. The subjects were instructed to remove the patch after 24 hours of exposure. An evaluation of the site was made just prior to the application of the next patch for signs of dermal reactions, according to the scoring scale listed in Section 7.0.

Rest periods consisted of 24 hours following Tuesday and Thursday patch removal and 48 hours following the Saturday patch removal, except when the schedule was modified as described above.

If at any time during the Induction Phase of the study, a test material elicited a score of 2+ or greater, the application of that test material was moved to an adjacent virgin site. At the discretion of the Principal Investigator or designee, a test material eliciting a score less than a 2+ may have been moved to an adjacent site. The site may also have been changed if the subject exhibited abrasion, maceration of the skin or tape reaction around the site.

If a 2+ reaction or greater occurred on the changed site, the application of the test material may have been discontinued for the remainder of the Induction Phase, but may have been challenged on the appropriate day of the study.

At the discretion of the Principal Investigator or designee, subjects exhibiting a significant reaction at the beginning of the Induction Phase may have been considered "pre-sensitized" to an ingredient(s) of the test product and may have been discontinued from the patching of that test material for the remainder of the study.

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8.0 TEST METHOD SUMMARY (CONTINUED)

8.3. CHALLENGE PHASE

Approximately 10 to 21 days after the Induction Phase, a challenge patch was applied to a virgin site on the lower back, following the same procedure described for the Induction Phase. After 24 hours, the patch was removed by a study technician and the site was evaluated for dermal irritation.

Additional dermal evaluations were performed 48 and 72 hours after application. If a reaction with a dermal score of greater than "±" persists at the 72-hour reading, the site may have been evaluated at 96 hours. A site exhibiting a dermal score of "±" or greater for the first time at the 72-hour reading was evaluated at 96 hours when possible.

If a subject missed a visit during the Challenge Phase, he/she was asked to return to the laboratory for a 96-hour reading to ensure three data points were collected. Subjects having only 2 challenge phase data points may have been included in the analysis if the final visit was at the 72 or 96-hour time point. Dermal Irritation was graded using the scoring scale listed in Section 7.0.

9.0 ADVERSE EVENTS

No adverse events were reported during the study.

10.0 RESULTS

This study was initiated with 56 subjects. Three subjects discontinued study participation for reasons unrelated to the test material. A total of 53 subjects completed the study.

Individual dermal scores recorded during the Induction and Challenge Phases appear in Table I.

11.0 CONCLUSION

Based on the test population of 53 subjects and under the conditions of this study, the test material identified as [redacted] did not demonstrate a potential for eliciting dermal irritation or inducing sensitization.

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12.0 RETENTION

Test materials and all original forms of this study will be retained by Clinical Research Laboratories, LLC as specified in CRL Standard Operating Procedures 30.6 and 30.6C, unless designated otherwise by the Sponsor.

13.0 REFERENCES

Draize, J. H. (1959). Dermal toxicity. Appraisal of the safety of chemicals in foods, drugs and cosmetics, 46-59.

Shelanski, H. A., & Shelanski, M. V. (1953, May). A new technique of human patch tests. In Proceedings of the Scientific Section of the Toilet Goods Association (Vol. 19, No. 46, pp. 4-7).

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Table I - Summary of Dermal Scores

Test Material: [redacted]

Subject Number	Induction Scores									Challenge Scores		
	1	2	3	4	5	6	7	8	9	24 Hour	48 Hour	72 Hour
1	0	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0	0	0	0
4	0	0	0	0	0	0	0	0	0	X	0	0*
5	0	0	0	0	0	0	0	0	0	0	0	0
6	0	0	0	0	0	0	0	0	0	0	0	0
7	0	0	0	0	0	0	0	0	0	0	0	0
8	0	0	0	0	0	0	0	0	0	0	0	0
9	0	0	0	0	0	0	0	0	0	0	0	0
10	0	0	0	0	0	0	0	0	0	0	0	0
11	0	0	0	0	0	0	0	0	0	0	0	0
12	0	0	0	0	0	0	0	0	0	0	0	0
13	0	0	0	0	0	0	0	0	0	0	0	0
14	0	0	0	0	0	0	0	0	0	0	0	0
15	0	0	0	0	0	0	0	0	0	0	0	0
16	0	0	0	0	0	0	Discontinued					
17	0	0	0	0	0	0	0	0	0	0	0	0
18	0	0	0	0	0	0	0	0	0	0	0	0
19	0	0	0	0	0	0	0	0	0	0	0	0
20	0	0	0	0	0	0	0	0	0	0	0	0
21	0	0	0	0	0	0	0	0	0	0	0	0
22	0	0	0	0	0	0	0	0	0	0	0	0
23	0	0	0	0	0	0	0	0	0	0	0	0
24	0	0	0	0	0	0	0	0	0	0	0	0
25	0	0	0	0	0	0	0	0	0	0	0	0
26	0	0	0	0	0	0	0	0	0	0	0	0
27	0	0	0	0	0	0	0	0	0	0	0	0
28	0	0	0	0	0	0	0	0	0	0	0	0

*No reaction was observed at the 96 hour evaluation

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Table I - Summary of Dermal Scores (continued)

Test Material: [redacted]

Subject Number	Induction Scores									Challenge Scores		
	1	2	3	4	5	6	7	8	9	24 Hour	48 Hour	72 Hour
29	0	0	0	0	0	0	0	0	0	Discontinued		
30	0	Discontinued										
31	0	0	0	0	0	0	0	0	0	0	0	0
32	0	0	0	0	0	0	0	0	0	0	0	0
33	0	0	0	0	0	0	0	0	0	0	0	0
34	0	0	0	0	0	0	0	0	0	0	0	0
35	0	0	0	0	0	0	0	0	0	0	0	0
36	0	0	0	0	0	0	0	0	0	0	0	0
37	0	0	0	0	0	0	0	0	0	0	0	0
38	0	0	0	0	0	0	0	0	0	0	0	0
39	0	0	0	0	0	0	0	0	0	0	0	0
40	0	0	0	0	0	0	0	0	0	0	0	0
41	0	0	0	0	0	0	0	0	0	0	0	0
42	0	0	0	0	0	0	0	0	0	0	0	0
43	0	0	0	0	0	0	0	0	0	0	0	0
44	0	0	0	0	0	0	0	0	0	0	0	0
45	0	0	0	0	0	0	0	0	0	0	0	0
46	0	0	0	0	0	0	0	0	0	0	0	0
47	0	0	0	0	0	0	0	0	0	0	0	0
48	0	0	0	0	0	0	0	0	0	0	0	0
49	0	0	0	0	0	0	0	0	0	0	0	0
50	0	0	0	0	0	0	0	0	0	0	0	0
51	0	0	0	0	0	0	0	0	0	0	0	0
52	0	0	0	0	0	0	0	0	0	0	0	0
53	0	0	0	0	0	0	0	0	0	0	0	0
54	0	0	0	0	0	0	0	0	X	0	0	0
55	0	0	0	0	0	0	0	0	0	X	0	0*
56	0	0	0	0	0	0	0	0	0	0	0	0

*No reaction was observed at the 96 hour evaluation

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Appendix I - Subject Demographics

Subject Number	Subject Initials	Age	Sex
1	[redacted]	68	F
2		49	F
3		41	F
4		62	F
5		43	F
6		60	F
7		42	F
8		42	F
9		51	M
10		35	M
11		45	F
12		52	F
13		60	F
14		38	M
15		53	F
16		29	F
17		25	F
18		56	M
19		65	F
20		39	F
21		28	M
22		60	F
23		41	F
24		45	M
25		46	M
26		52	F
27		38	F
28		45	F

Subject Number	Subject Initials	Age	Sex
29	[redacted]	63	F
30		55	F
31		27	F
32		32	M
33		70	F
34		38	F
35		57	F
36		18	F
37		25	F
38		24	M
39		52	F
40		18	F
41		61	F
42		52	F
43	[redacted]	39	F
44		50	F
45		22	M
46		44	F
47		28	F
48		59	F
49		33	F
50		58	F
51		60	F
52		57	F
53		39	F
54		50	M
55		41	M
56		33	F

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CLINICAL STUDY REPORT

Report Status:	Final
Report Date:	07 February 2019
CRL Study Number:	[redacted]
CRL Protocol Number:	[redacted]
Study Title:	Repeated Insult Patch Test (RIPT)
Test Material:	[redacted] lash serum containing 0.0044% Isopropyl Cloprostenate
Sponsor:	[redacted]
Sponsor Representative:	[redacted]
Investigating Laboratory:	Eurofins CRL, Inc. [redacted]
Principal Investigator:	[redacted] M.D.* Dermatologist
Study Initiation Date:	17 December 2018
Study Completion Date:	25 January 2019

*[redacted] MD is no longer employed at Eurofins CRL, Inc. The report will be signed by [redacted] MD (Board Certified Dermatologist).

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PRINCIPAL INVESTIGATOR SIGNATURE

Study Title: Repeated Insult Patch Test

I have read Clinical Study Report [redacted] *and confirm that to the best of my knowledge it accurately describes the conduct and results of the study.*

[redacted] Digitally signed by [redacted] M.D.
[redacted]
M.D. Date: 2019.02.08 08:46:40 -05'00'

Principal Investigator Signature/Date

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Quality Assurance Audit Statement

Clinical Study Number: [redacted]

Start Date: 17 December 2018

Completion Date: 25 January 2019

Eurofins CRL, Inc. follows established, standardized procedures for clinical testing designed to ensure the well-being of clinical study subjects and the generation of reliable study data. The study was conducted in accordance with the study protocol and Eurofins CRL, Inc. Standard Operating Procedures. All data included in the report is accurately represented. The clinical study master file was reviewed by the Principal Investigator and the Quality Assurance representative.

[redacted] Digitally signed by [redacted]
Date: 2019.02.07 13:07:14 -05'00'

Signature of QA Auditor and Date

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CLINICAL STUDY REPORT

Repeated Insult Patch Test (RIPT)

1.0 OBJECTIVE

The objective of this study was to determine the potential of a test material to elicit dermal irritation and/or induce sensitization following repeated patch applications.

2.0 PRINCIPAL INVESTIGATOR AND INVESTIGATIVE SITE

[redacted], M.D.
Dermatologist

Eurofins CRL, Inc.
[redacted]

3.0 SPONSOR REPRESENTATIVE AND SPONSOR SITE

[redacted]

[redacted]

4.0 TEST MATERIAL

The following test material was provided by [redacted] and was received by Eurofins CRL, Inc. on 10 December 2018.

Test Material	Test Condition	Patch Type
[redacted]	Neat	Semi-occlusive*

The test material was coded with the following CRL identification number:

[redacted]

5.0 STUDY DATES

This study was initiated on 17 December 2018 and was completed on 25 January 2019.

* Semi-occlusive Strip ([redacted])

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6.0 SUBJECT SELECTION

A total of 60 male and female subjects, ranging in age from 18 to 70 years and in generally good health, were selected for the study (Subject Demographics – Appendix I). Subjects who met all of the inclusion criteria and none of the exclusion criteria listed in the study protocol were enrolled for participation.

6.1 INCLUSION CRITERIA

a. Subject is male or female between the ages of 18 and 70 years;

b. Female subjects who are sexually active are using adequate method of birth control;

c. Subject does not exhibit any skin diseases which might be confused with a skin reaction from the test material;

d. Subject agrees to avoid exposure of the test sites to the sun and to refrain from visits to tanning salons during the course of this study;

e. Subject agrees to refrain from getting patches wet and from scrubbing or washing the test area with soap or applying powder, lotions or personal care products to the area during the course of the study;

f. Subject has signed an Informed Consent in conformance with 21CFR Part 50: "Protection of Human Subjects;"

g. Subject has completed a HIPAA Authorization Form in conformance with 45CFR Parts 160 and 164;

h. Subject is in generally good health and has a current Subject Profile on file;

i. Subject is dependable and able to follow directions as outlined in the protocol.

6.2. EXCLUSION CRITERIA

a. Subject is pregnant, nursing, or planning to become pregnant, or not using adequate birth control;

b. Subject is currently using any systemic or topical corticosteroids, anti-inflammatory drugs, or antihistamines on a regular basis;

c. Subject reports allergies to cosmetics, toiletries, or personal care products;

d. Subject exhibits any skin disorders, sunburn, scars, excessive tattoos, etc. in the test area;

e. Subject has scheduled, or is planning to undergo, any medical or surgical procedures during the 6-week course of the study.

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7.0 STUDY EVALUATIONS

The following Dermal Scoring System was used:

Dermal Score	Description
0	No visible skin reaction
±	Barely perceptible erythema
1+	Mild erythema
2+	Well defined erythema
3+	Severe erythema and edema
4+	Erythema and edema with vesiculation

Letter Codes

e = Edema
P = Peeling
S = Spreading of reaction beyond patch site.
Sc = Scabbing
d = Dryness/scaling
D = Oozing, crusting, and/or superficial erosions
I = Itching
F = Follicular irritation with or without pustule formation (folliculitis)
Hr = Hyperpigmentation
Ho = Hypopigmentation
X = Subject Absent
NP = No patching
Pa = Papules
C = Changed site
--- = No reading

8.0 TEST METHOD SUMMARY

8.1 SUBJECT IDENTIFICATION

All subjects were initially identified by a permanent identification number. Subjects who met the qualification criteria were assigned a study subject number. This subject number was assigned in sequence as subjects were enrolled in the study. A master roster was kept of the permanent identification number and the corresponding study subject number.

8.2. INDUCTION PHASE

Informed Consent was obtained, Inclusion/Exclusion criteria verified and qualified subjects were enrolled. The test site was cleansed with 70% isopropyl alcohol. The test material was applied to the upper back, between the scapulae and the waist, to either side of the spinal midline. The test material was applied to the same site three times each week, usually Monday, Wednesday, and Friday, for a total of nine applications. However, the schedule may have been modified to accommodate inclement weather, holidays, or missed applications. At the discretion of the Principal Investigator, the test material may have been applied on two consecutive days during the Induction Phase or a makeup day may have been added at the end of the Induction Phase.

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8.0 TEST METHOD SUMMARY (CONTINUED)

The test product was applied nine times over the approximate three-week induction phase. Subjects had no fewer than eight subsequent evaluations unless requested by the Sponsor.

The test site was marked with a gentian violet surgical marker to ensure the continuity of patch application. The subjects were instructed to remove the patch after 24 hours of exposure. An evaluation of the site was made just prior to the application of the next patch for signs of dermal reactions, according to the scoring scale listed in Section 7.0.

Rest periods consisted of 24 hours following Tuesday and Thursday patch removal and 48 hours following the Saturday patch removal, except when the schedule was modified as described above.

If at any time during the Induction Phase of the study, a test material elicited a score of 2+ or greater, the application of that test material was moved to an adjacent virgin site. At the discretion of the Principal Investigator or designee, a test material eliciting a score less than a 2+ may have been moved to an adjacent site. The site may also have been changed if the subject exhibited abrasion, maceration of the skin or tape reaction around the site.

If a 2+ reaction or greater occurred on the changed site, the application of the test material may have been discontinued for the remainder of the Induction Phase, but may have been challenged on the appropriate day of the study.

At the discretion of the Principal Investigator or designee, subjects exhibiting a significant reaction at the beginning of the Induction Phase may have been considered "pre-sensitized" to an ingredient(s) of the test product and may have been discontinued from the patching of that test material for the remainder of the study.

8.3. CHALLENGE PHASE

Approximately 10 to 21 days after the Induction Phase, a challenge patch was applied to a virgin site on the lower back, following the same procedure described for the Induction Phase. After 24 hours, the patch was removed by a study technician and the site was evaluated for dermal irritation.

Additional dermal evaluations were performed 48 and 72 hours after application. If a reaction with a dermal score of greater than "±" persists at the 72-hour reading, the site may have been evaluated at 96 hours. A site exhibiting a dermal score of "±" or greater for the first time at the 72-hour reading was evaluated at 96 hours when possible.

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8.0 TEST METHOD SUMMARY (CONTINUED)

If a subject missed a visit during the Challenge Phase, he/she was asked to return to the laboratory for a 96-hour reading to ensure three data points were collected. Subjects having only 2 challenge phase data points may have been included in the analysis if the final visit was at the 72 or 96-hour time point. Dermal Irritation was graded using the scoring scale listed in Section 7.0.

9.0 STUDY RESULTS

9.1. COMPLETED AND DISCONTINUED SUBJECTS

This study was initiated with 60 subjects. Four subjects discontinued study participation for reasons unrelated to the test material. A total of 56 subjects completed the study.

9.2. DERMAL EVALUATIONS

Individual dermal scores recorded during the Induction and Challenge Phases appear in Table I.

9.3. ADVERSE EVENTS

No adverse events were reported during the study.

10.0 CONCLUSION

Based on the test population of 56 subjects and under the conditions of this study, the test material identified as [redacted] did not demonstrate a potential for eliciting dermal irritation or inducing sensitization.

11.0 TEST MATERIAL AND DOCUMENT RETENTION

Test materials and all original forms of this study will be retained by Eurofins CRL, Inc. in accordance with Eurofins CRL, Inc. Standard Operating Procedures.

12.0 REFERENCES

Draize, J. H. (1959). Dermal toxicity. Appraisal of the safety of chemicals in foods, drugs and cosmetics, 46-59.

Shelanski, H. A., & Shelanski, M. V. (1953, May). A new technique of human patch tests. In Proceedings of the Scientific Section of the Toilet Goods Association (Vol. 19, No. 46, pp. 4-7).

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Table I - Summary of Dermal Scores (continued)

Test Material:	[redacted]

Subject Number	Induction Scores									Challenge Scores		
	1	2	3	4	5	6	7	8	9	24 Hour	48 Hour	72 Hour
61	0	0	0	0	0	0	0	0	0	0	0	0
62	0	0	0	0	0	0	0	0	0	0	0	0
63	0	0	0	0	0	0	0	0	0	0	0	0
64	0	0	0	0	0	0	0	0	0	0	0	0
65	0	0	0	0	0	0	0	0	0	0	0	0
66	0	0	0	0	0	0	0	0	0	0	0	0
67	0	0	0	0	0	0	0	0	0	0	0	0
68	0	0	0	0	0	0	0	0	0	0	0	0
69	0	0	0	0	0	0	0	0	0	0	0	0
70	0	0	0	0	0	0	0	0	0	0	0	0
71	0	0	0	0	0	0	0	0	0	0	0	0
72	0	0	0	0	0	0	0	0	0	0	0	0
73	Discontinued											
74	0	0	0	0	0	0	0	0	0	0	0	0
75	0	0	0	0	0	0	0	0	0	0	0	0
76	0	0	0	0	0	0	0	0	0	0	0	0
77	0	0	0	0	0	0	0	0	0	0	0	0
78	0	0	0	0	0	0	0	0	0	0	0	0
79	0	0	0	0	0	0	0	0	0	0	0	0
80	0	0	0	0	0	0	0	0	0	0	0	0
81	0	0	0	0	0	0	0	0	0	0	0	X*
82	0	0	0	0	0	0	0	0	0	0	0	0
83	0	0	0	0	0	0	0	0	0	0	0	0
84	0	0	0	0	0	0	0	0	0	0	0	0
85	0	0	0	0	0	0	0	0	0	0	0	0
86	0	0	0	0	0	0	0	0	0	0	0	0
87	0	0	0	0	0	0	0	0	0	0	0	0
88	0	Discontinued										
89	0	0	0	0	0	0	0	0	0	0	0	0
90	0	0	0	0	0	0	0	0	0	0	0	0

*No reaction was observed at the 96 hour evaluation.

Table 1 - Summary of Dermal Scores (continued)

Test Material:	[redacted]

Subject Number	Induction Scores									Challenge Scores		
	1	2	3	4	5	6	7	8	9	24 Hour	48 Hour	72 Hour
91	0	0	0	0	0	0	0	0	0	0	0	0
92	0	0	Discontinued									
93	0	0	0	0	0	0	0	0	0	0	0	0
94	0	0	0	0	0	0	0	0	0	0	0	0
95	0	0	0	0	0	0	0	0	0	0	0	0
96	0	0	0	0	0	0	0	0	0	0	0	0
97	0	0	0	0	0	0	0	0	0	0	0	0
98	0	0	0	0	0	0	0	0	0	0	0	0
99	0	0	0	0	0	0	0	0	0	0	0	0
100	0	0	0	0	0	0	0	0	0	0	0	0
101	0	0	0	0	0	0	0	0	0	0	0	0
102	0	0	0	0	0	0	0	0	0	0	0	0
103	0	0	0	0	0	0	0	0	0	0	0	0
104	0	0	0	0	0	0	0	0	0	0	0	0
105	0	0	0	0	0	0	0	0	0	0	0	0
106	0	0	0	0	0	0	0	0	0	0	0	0
107	0	0	0	0	0	0	0	0	0	0	0	0
108	0	0	0	0	0	0	0	0	0	0	0	0
109	0	0	0	0	0	0	0	0	0	0	0	0
110	Discontinued											
111	0	0	0	0	0	0	0	0	0	0	0	0
112	0	0	0	0	0	0	0	0	0	0	0	0
113	0	0	0	0	0	0	0	0	0	0	0	0
114	0	0	0	0	0	0	0	0	0	0	0	0
115	0	0	0	0	0	0	0	0	0	0	0	0
116	0	0	0	0	0	0	0	0	0	0	0	0
117	0	0	0	0	0	0	0	0	0	0	0	0
118	0	0	0	0	0	0	0	0	0	0	0	0
119	0	0	0	0	0	0	0	0	0	0	0	0
120	0	0	0	0	0	0	0	0	0	0	0	0

Appendix I - Subject Demographics

Subject Number	Subject Initials	Age	Sex
61	[redacted]	61	F
62		46	M
63		47	F
64		60	M
65		55	F
66		59	F
67		70	F
68		57	F
69		52	F
70		19	F
71		62	F
72		43	F
73		38	F
74		49	F
75		38	F
76		58	F
77		18	F
78		66	M
79		55	F
80		54	M
81		70	F
82		49	F
83		65	F
84		66	F
85		49	F
86		48	F
87		47	F
88		57	F
89		55	F
90		68	F

Subject Number	Subject Initials	Age	Sex
91	[redacted]	55	M
92		44	F
93		22	F
94		60	F
95		54	F
96		54	F
97		56	F
98		51	F
99		46	F
100		37	F
101		64	F
102		37	F
103		67	F
104		37	F
105		57	M
106		52	F
107		28	F
108		49	F
109	[redacted]	64	F
110		27	F
111		56	F
112		32	F
113		56	F
114		21	F
115	[redacted]	47	M
116		42	F
117		24	F
118		59	F
119		60	F
120		63	F

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CONSUMER PRODUCT TESTING COMPANY
cptclabs.com

FINAL REPORT

CLIENT: [redacted]

ATTENTION: [redacted]

TEST: Repeated Insult Patch Test
Protocol No.: [redacted]
Protocol Date: 06/29/20

TEST MATERIAL: [redacted]
lash serum containing 0.0044% Isopropyl Cloprostenate

STUDY NUMBER: [redacted]

Reviewed by: [redacted] M.D.
Medical Director
Board Certified Dermatologist

Approved by: [redacted] R.N.
Executive Vice President, Clinical Evaluations

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CONSUMER PRODUCT TESTING™ COMPANY
cptclabs.com

FDA Registration# 1000151293
DEA Registration# RC0109744 Schedule I-V
US EPA/NJ DEP Registration# NJD982726648
ISO/IEC 17025:2017 Accreditation # 40071

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 ten (10) years. At any time prior to the completion of the tenth archival year, a Sponsor may submit a written request to the CPTC 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

12-30-21
Date

[redacted]

Objective: To determine by repetitive epidermal contact the potential of a test material to induce primary or cumulative irritation and/or allergic contact sensitization.

Subjects: Fifty-six (56) qualified subjects, male and female, ranging in age from 18 to 77 years, were selected for this evaluation. Fifty-three (53) subjects completed this clinical trial. The remaining subjects discontinued their participation for various reasons, none of which were related to the application of the test material.

Inclusion Criteria:

1. Subjects must have read, signed, and dated an Informed Consent Form that included a HIPAA statement;
2. Subjects who were male or female, aged 16-79 years, inclusive;
3. Subjects who were considered reliable and capable of understanding and following directions; and
4. Subjects aged 16 or 17 years must have read, signed, and dated an Adolescent Assent Form after their parent or legal guardian had read, signed, and dated an Informed Consent Form.

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, that, in the opinion of the Investigator, could have influenced the purpose, integrity, or outcome of the trial;
3. Subjects who had used any prescribed or OTC anti-inflammatory, antihistamine, corticosteroid, immunosuppressant, or antibiotic drug within 7 days prior to initiation of the trial or during their participation on this trial;
4. Female subjects who were pregnant, planning to become pregnant, or lactating during the trial;
5. Subjects with any visible disease, sunburn, scars, excessive tattoos, etc., that might have been confused with a skin reaction to the test material or, as determined by the Principal Investigator, might have interfered with the evaluation;
6. Subjects who had a history of adverse reactions to cosmetics, adhesive tapes, OTC drugs, or other personal care products; or
7. Subjects who introduced the use of any new cosmetic, toiletry, or personal care products during the trial.

Test Material: [redacted]

Trial Schedule:

Panel #	Initiation Date	Completion Date
[redacted]	November 3, 2021	December 17, 2021

Methodology:

The informed consent process fully apprised 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 subject executed the Informed Consent Form (ICF) after which the potential subject entered the clinical trial as a subject. Staff who conducted the informed consent process also executed the form. 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 ICF. Subjects completed a Medical History Form to determine initial qualification.

The upper back between the scapulae served as the treatment area. Approximately 0.2 ml of the test material, or an amount sufficient to cover the contact surface, was applied to the 1 in^2 absorbent pad portion of a clear, adhesive dressing. This was then applied to the appropriate treatment site to form a semi-occlusive patch.

Induction Phase:

Patches were applied three (3) times per week (e.g., Monday, Wednesday, and Friday) for a total of nine (9) applications. The site was marked to ensure the continuity of patch application. Following supervised removal and scoring of the first Induction patch, participants were instructed to remove all subsequent Induction patches at home, one day after application. The evaluation of this site was made again just prior to re-application. If a participant was unable to report for an assigned test day, one (1) makeup day was permitted. This day was added to the Induction period.

With the exception of the first supervised Induction Patch reading, if any test site exhibited a moderate (2-level) reaction during the Induction Phase, application was moved to an adjacent area. Applications were discontinued for the remainder of this test phase if a moderate (2-level) reaction was observed on this new test site. Applications would also be discontinued if marked (3-level) or severe (4-level) reactivity was noted.

Rest periods consisted of one day following each Tuesday and Thursday removal, and two days following each Saturday removal.

Methodology (continued):

Challenge Phase:

At least 10 days following the final Induction Phase patch removal, a Challenge patch was applied to a virgin test site adjacent to the original Induction patch site, following the same procedure described for Induction. The patch was removed and the site scored at the clinic Day 1 and Day 3 post-application.

Evaluation Criteria (Erythema and additional Dermal Sequelae):

0	=	**No visible skin reaction**	**E**	=	**Edema**
0.5	=	**Barely perceptible**	**D**	=	**Dryness**
1	=	**Mild**	**S**	=	**Staining**
2	=	**Moderate**	**P**	=	**Papules**
3	=	**Marked**	**V**	=	**Vesicles**
4	=	**Severe**	**B**	=	**Bullae**
			U	=	**Ulceration**
			Sp	=	**Spreading**

Erythema was scored numerically according to this key. If present, additional Dermal Sequelae were indicated by the appropriate letter code and a numerical value for severity.

Adverse Events: There were no adverse events.

Amendments: There were no amendments.

Deviations: There were no deviations.

Results: The results of each participant are appended (Table 1).

Observations remained negative throughout the test interval.

Subject demographics are presented in Table 2.

Summary: Under the conditions of this clinical trial, test material, [redacted] indicated no potential for dermal irritation or allergic contact sensitization.

Table 1
Panel [redacted]

Individual Results

[redacted]

Subject Number	Day1*	Induction Phase 1	2	3	4	5	6	7	8	9	Virgin Challenge Site Day 1*	Day 3
1	0	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0	0	0	0
4	0	0	0	0	0	0	0	0	0	0	0	0
5	0	0	0	0	0	0	0	0	0	0	0	0
6	0	0	0	0	0	0	0	0	0	0	0	0
7	0	0	0	0	0	0	0	0	0	0	0	0
8	0	0	0	0	0	0	0	0	0	0	0	0
9	0	0	0	0	0	0	0	0	0	0	0	0
10	0	0	0	0	0	0	0	0	0	0	0	0
11	0	0	0	0	0	0	0	0	0	0	0	0
12	0	0	0	0	0	0	0	0	0	0	0	0
13	0	0	0	0	0	0	0	0	0	0	0	0
14	0	0	0	0	0	0	0	0	---WITHDREW CONSENT---			
15	0	0	0	0	0	0	0	0	0	0	0	0
16	0	0	0	0	0	0	0	0	0	0	0	0
17	0	0	0	0	0	0	0	0	0	0	0	0
18	0	0	0	0	0	0	0	0	0	0	0	0
19	0	0	0	0	0	0	0	0	0	0	0	0
20	0	0	0	0	0	0	0	0	0	0	0	0
21	0	0	0	0	0	0	0	0	0	0	0	0
22	0	0	0	0	0	0	0	0	0	0	0	0
23	0	0	0	0	0	0	0	0	0	0	0	0
24	0	0	0	0	0	0	0	0	0	0	0	0
25	0	0	0	0	0	0	0	0	0	0	0	0
26	0	0	0	0	0	0	0	0	0	0	0	0
27	0	0	0	0	0	0	0	0	0	0	0	0
28	0	0	0	0	0	0	0	0	0	0	0	0
29	0	0	0	0	0	----------WITHDREW CONSENT----------						

Day 1* = Supervised removal

Table 1
(continued)
Panel [redacted]

Individual Results

[redacted]

Subject Number	Day1*	Induction Phase 1	2	3	4	5	6	7	8	9	Virgin Challenge Site Day 1*	Day 3
30	0	0	0	0	0	WITHDREW CONSENT						
31	0	0	0	0	0	0	0	0	0	0	0	0
32	0	0	0	0	0	0	0	0	0	0	0	0
33	0	0	0	0	0	0	0	0	0	0	0	0
34	0	0	0	0	0	0	0	0	0	0	0	0
35	0	0	0	0	0	0	0	0	0	0	0	0
36	0	0	0	0	0	0	0	0	0	0	0	0
37	0	0	0	0	0	0	0	0	0	0	0	0
38	0	0	0	0	0	0	0	0	0	0	0	0
39	0	0	0	0	0	0	0	0	0	0	0	0
40	0	0	0	0	0	0	0	0	0	0	0	0
41	0	0	0	0	0	0	0	0	0	0	0	0
42	0	0	0	0	0	0	0	0	0	0	0	0
43	0	0	0	0	0	0	0	0	0	0	0	0
44	0	0	0	0	0	0	0	0	0	0	0	0
45	0	0	0	0	0	0	0	0	0	0	0	0
46	0	0	0	0	0	0	0	0	0	0	0	0
47	0	0	0	0	0	0	0	0	0	0	0	0
48	0	0	0	0	0	0	0	0	0	0	0	0
49	0	0	0	0	0	0	0	0	0	0	0	0
50	0	0	0	0	0	0	0	0	0	0	0	0
51	0	0	0	0	0	0	0	0	0	0	0	0
52	0	0	0	0	0	0	0	0	0	0	0	0
53	0	0	0	0	0	0	0	0	0	0	0	0
54	0	0	0	0	0	0	0	0	0	0	0	0
55	0	0	0	0	0	0	0	0	0	0	0	0
56	0	0	0	0	0	0	0	0	0	0	0	0

Day 1* = Supervised removal

Table 2
Panel [redacted]

Subject Demographics

Subject Number	ID#	Age	Gender
1	84433	40	M
2	3653	55	F
3	85558	58	F
4	84422	18	M
5	83151	37	F
6	89262	58	F
7	18531	53	F
8	11872	75	F
9	3626	72	M
10	28045	44	M
11	3777	75	F
12	82560	23	F
13	57164	39	F
14	84404	50	F
15	91374	20	F
16	56003	61	F
17	62476	20	F
18	91549	24	F
19	79872	67	F
20	90354	72	F
21	91169	50	F
22	45295	55	F
23	66610	64	M
24	91394	59	F
25	79343	32	F
26	60209	46	F
27	60204	72	F
28	51839	71	F
29	84754	59	F

Table 2
(continued)
Panel [redacted]

Subject Demographics

Subject Number	ID#	Age	Gender
30	76822	26	F
31	81115	40	F
32	22464	73	F
33	32358	65	M
34	87574	76	F
35	37275	62	F
36	72846	54	F
37	84134	23	M
38	73274	26	F
39	8068	58	M
40	85316	45	M
41	57310	67	F
42	15787	34	F
43	51221	28	F
44	88094	29	M
45	68681	18	M
46	23109	68	M
47	87469	41	M
48	87718	36	F
49	90422	54	F
50	79171	77	F
51	57148	51	F
52	72643	56	M
53	14341	73	F
54	57299	60	M
55	83605	51	F
56	44617	59	F

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Personal Care Products Council
Committed to Safety, Quality & Innovation

Memorandum

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

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

DATE: May 12, 2023

SUBJECT: Isopropyl Cloprostenate

Toxicology Services. 2023. Review of two clinical studies performed on lash or lash and brow serums containing 0.0044% Isopropyl Cloprostenate.

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TOXSERVICES
TOXICOLOGY RISK ASSESSMENT CONSULTING

TO: [redacted]

FROM: [redacted] **M.S.**
Ph.D., M.H.S., D.A.B.T.
[redacted] **Ph.D., M.P.H., C.Biol., F.R.S.B., E.R.T., D.A.B.T.**

DATE: **May 11, 2023**

RE: **Review of Two Clinical Studies Performed on [redacted] Formulations**

The purpose of this memorandum is to summarize two clinical studies, with a specific focus on safety and adverse events, if applicable, as requested by [redacted] The products evaluated in the clinical studies and reviewed in this memo are the following:

- [redacted]
- [redacted]
- [redacted]

Each clinical study is discussed below.

lash serum containing 0.0044% isopropyl cloprostenate

[redacted] **Home Use Study (PCR 2019)**

Princeton Consumer Research Corp. (PCR) performed a 12-week home use study with the [redacted] formulation with healthy female subjects utilizing professional photography, subjective questionnaires, and VISIA-CR® (the standard used in facial imaging) to assess efficacy and acceptability of the product. Subjects were chosen using the following inclusion criteria:

- Healthy non-pregnant, non-nursing female between 18 and 50 years of age;
- No current skin disease or ocular disease or history of diabetes, allergies to similar cosmetic products, or general sensitivity to products used around the eyes;
- Written informed consent;
- Willing to use the lash enhancer as directed (once daily before bed) and complete all study-related requirements; and
- Want to improve the appearance of their eyelashes.

A total of 37 women were screened and 36 were enrolled; 32 subjects completed the study. The five subjects who discontinued participation in the study did so for reasons unrelated to the test article. After an initial visit to perform a baseline screening and initial assessment, the study subjects were issued the test article and the instructions for use for 12 weeks. Six weeks following the beginning of the study, subjects completed questionnaires and after 12 weeks, they returned to the testing facility for their second visit for a final assessment. There were no adverse events or safety-related concerns reported in the "comments" section of the 6- and 12-week subjective questionnaires.

[redacted]

There were two adverse events reported during the study: subject 11 reported slight transient stinging on both eyes, and subject 33 reported itching on both eyes for the first two weeks of the study. There were no severe adverse events reported. Details of the two adverse events are presented below.

Subject 11

This subject reported that three or four times during the home use study, she experienced slight stinging in both eyes if the product was applied too close to the corner of the eye; however, in each instance, the stinging had resolved by the following morning. Investigators characterized the reaction as intermittent and mild in severity, with no further action required. Although no eye irritation was observed by the PCR clinical staff during the final visit at 12 weeks, investigators determined that the irritation was possibly related to the use of the product.

Subject 33

This subject reported that 20 minutes after the application of the product, her upper eyelids on both eyes were itchy (the medical term for this symptom is ocular pruritis); the itching occurred intermittently after an unspecified number of applications for the following two weeks. At the end of that 2-week period, the itching stopped and did not recur for the remainder of the study. Investigators characterized the reaction as mild in severity, with no further action required. The investigators concluded that the ocular pruritis was possibly related to the use of the product.

Conclusion

Overall, there were two adverse events associated with the use of [redacted] that were mild and transient in nature. This home use study indicates that regular use of [redacted] for durations up to four months is likely to be well tolerated by consumers, with at most mild effects that are short-term and reversible in nature.

[redacted] and [redacted] Home Use Study (PCR 2020)

lash and brow serum containing 0.0044% Isopropyl Cloprostenate

PCR performed a six-week (eye lash) / seven-week (eye brow) home use study with [redacted] and [redacted] with healthy female subjects, utilizing professional photography and subjective questionnaires to assess efficacy and acceptability of the products. The study was performed in compliance with International Council for Harmonization (ICH) good clinical practice (GCP) guidelines.

Subjects were chosen using the following inclusion criteria:

- Healthy non-pregnant, non-nursing female between 18 and 55 years of age;
- No current skin disease or ocular disease, no history of diabetes or other serious health conditions, and no allergies to similar cosmetic products;
- Written informed consent;
- Willing to use the lash enhancer and eyebrow enhancer as directed and complete all study-related requirements;

- Willing to be photographed and signed a photograph release form
- Agreed to use an acceptable method of birth control or had a bilateral tubal ligation, hysterectomy, bilateral oophorectomy, was post-menopausal for at least one year, or their male partner had a vasectomy; and
- Has internet capability to complete online self-perception questionnaires at indicated timepoints.

A total of 32 subjects were screened and 31 were enrolled; 29 subjects completed the study. The two subjects who did not complete the study discontinued their participation for reasons unrelated to the test materials. After an initial visit to perform a baseline screening, the study subjects were issued the test articles and the instructions for use for six or seven weeks. Specifically, investigators instructed the subjects to apply the lash serum to the top lash line once daily and to apply the brow serum once daily to the entire brow area, avoiding eye contact for both products. The subjects used the eyebrow product for the entire seven weeks, and the eyelash product for the first six weeks. Subjects 19 and 21 each missed one day of product use, but the principal investigator (PI) determined that this had no effect on the integrity of the study. Each subject completed questionnaires either online or at the test facility after two, four, and five weeks of eyelash serum use and after six and seven weeks of eyebrow serum use. Additionally, professional photographs were taken at baseline, after four weeks of eyelash serum use, and after 6 weeks of eyebrow serum use.

There were seven adverse events reported, all reported by subject 6. There were no severe adverse events reported among any subject.

Subject 6

The subject was in a car accident during the home study and reported the following adverse events:

- Bruised right arm
- Bruised left arm
- Upper back pain
- Lower back pain
- Facial bruising
- Bruising of the right knee
- Bruising of the left knee

These events were monitored on the visits during weeks four, six, and seven. During the final visit, the subject was healing and did not require medical attention. The PI determined that the adverse events were moderate in nature and not related to the use of either product.

Conclusion

The reported adverse events experienced by one subject were unrelated to use of either [redacted] product, and there were no reported adverse events associated with use of the [redacted] or the [redacted]. This home use study indicates that regular use of [redacted] or the [redacted] for durations up to six and seven weeks, respectively, is likely to be well tolerated by consumers.

[redacted]

REFERENCES

Princeton Consumer Research Corp. (PCR). 2019. A home use study in 30 healthy females between ages 18-50, to evaluate the efficacy of a lash enhancer by utilizing professional photography, subjective questionnaires, and VISIA-CR. Report Number [redacted]. Dated September 9, 2019.

Princeton Consumer Research Corp. (PCR). 2020. A home use study in 30 healthy females ages 18-55 to evaluate the efficacy of an eye lash enhancer and eyebrow enhancer by utilizing professional photography and subjective questionnaires. Report Number [redacted]. Dated September 18, 2020.

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Personal Care Products Council
Committed to Safety, Quality & Innovation

Memorandum

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

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

DATE: May 16, 2023

SUBJECT: Ethyl Tafluprostamide

Bailey, J. 2023. Data Demonstrating the Safe Use of Ethyl Tafluoprostamide in Cosmetics.

Data Demonstrating the Safe Use of Ethyl Tafluprostamide in Cosmetics

Submitted By:

John Bailey, PhD
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Submitted On Behalf Of:

COMPANY

Submitted To:

Bart Heldreth, PhD
Executive Director
Cosmetic Ingredient Review
Washington, DC
heldrethb@cir-safety.org

May 16, 2023

Submission To: CIR	Submitted By: COMPANY via EAS Consulting Group
May 16, 2023	Data Demonstrating the Safe Use of **Ethyl Tafluprostamide** / **DDDE** in Cosmetics

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May 16, 2023	Data Demonstrating the Safe Use of **Ethyl Tafluprostamide** / **DDDE** in Cosmetics

1. CIR CALL FOR DATA

1.1 CIR review of Prostaglandin Analogs

At its September 27, 2022 meeting, the CIR included in its 2023 priority list a review of two prostaglandin analogs ("PGA s"), Isopropyl Cloprostenate and Ethyl Tafluprostamide

1.2 Scientific Literature Review (SLR) Notice to Proceed (NTP)

On March 17, 2023, the CIR issued a Scientific Literature Review (SLR) Notice to Proceed (NTP). As stated in the NTP:

> According to 2023 FDA VCRP data, Isopropyl Cloprostenate is used in 3 total formulations of the product category "other eye makeup preparations."[1] There are no reported uses for Ethyl Tafluprostamide. A concentration of use survey is currently underway for these two ingredients.
>
> Although use information has been reported for Isopropyl Cloprostenate, an intensive search of the published information on this ingredient, as well as Ethyl Tafluprostamide, resulted in insufficient information to justify preparation of a formal SLR. CIR, therefore, is issuing this SLR Notice to Proceed (NTP) to alert interested parties that a safety assessment is being prepared and significant data needs remain.

The SLR NTP invited all interested persons "to submit comments and/or published or unpublished data" to the CIR by May 16, 2023. Data provided in response to the SLR NTP will be incorporated into CIR's draft report, which will be reviewed by the Expert Panel for Cosmetic Ingredient Safety.

1.3 Scope of Relevant Data

According to the SLR NTP, CIR is seeking information on a wide range of areas, including:

- Chemistry information, including composition and structure, method of manufacture, and impurity data;
- Toxicokinetics data relevant to routes of exposure expected with cosmetic use;
- General toxicity data;
- Developmental and reproductive toxicity data;
- Genotoxicity data;
- Carcinogenicity data;
- Dermal irritation and sensitization data;
- Ocular toxicity/irritation data
- Inhalation toxicity data; and
- Any other relevant safety information that may be available

2. INTRODUCTION

2.1 Ingredient

This report provides data regarding the safe use of **Ethyl Tafluprostamide** in cosmetics. As explained below, COMPANY has retained use of the original INCI name for this molecule.

2.1.1 Dechloro Dihydroxy Difluoro Ethylcloprostenolamide / Ethyl Tafluprostamide

In June 2010, the Personal Care products Council assigned the INCI name of Dechloro Dihydroxy Difluoro Ethylcloprostenolamide (DDDE) to the PGA ingredient COMPANY uses in some of its cosmetic products. This ingredient is also known as Ethyl Tafluprostamide. In this report, we refer to this ingredient as **DDDE** to be consistent with the safety studies conducted by COMPANY on its products.

DDDE is not an ingredient in any product that has been approved by the FDA or any governmental regulatory authority for use as a drug.

2.1.2 TEA / Ethyl Tafluprostamide

Prior to the assignment of the INCI name to this ingredient in June 2010, COMPANY referred to the molecule as "**TEA**". Some safety tests, described below, that were conducted by COMPANY prior to June 2010, refer to "TEA". TEA and DDDE are the same molecule as Ethyl Tafluprostamide.

2.2 Submitter

The submitter, referred to herein as COMPANY, is a U.S.-based international cosmetics company whose product portfolio focuses on products intended to enhance the beauty and health of ***hair***, including eyelashes, eyebrows and scalp hair. All of COMPANY's products are marketed exclusively as cosmetics with marketing claims that are consistent with cosmetic product intended uses, generally enhancing the appearance of hair.

Some of COMPANY's products include the ingredient, Dechloro Dihydroxy Difluoro Ethylcloprostenolamide (DDDE), *aka* Ethyl Tafluprostamide, which is a PGA.

COMPANY was founded over fifteen years ago. From its inception, COMPANY has made product safety a high priority and has diligently used scientific testing to confirm the safety of its cosmetic products under the conditions of use prescribed in the product labeling.

2.3 Use of DDDE in COMPANY's Products

COMPANY uses DDDE as an ingredient in products intended for use on eyelashes, eyebrows or scalp hair. The concentration of DDDE in these products ranges from 0.012% - 0.020%.

2.3.1 Cosmetic Uses

All of COMPANY's products, including those that contain DDDE, are marketed exclusively as cosmetics with intended uses that are limited to effects ***on hair*** (not the body) and are consistent with cosmetic use generally as a hair conditioner.

Products that claim effects on the structure or function of hair (or nails) only are regulated as cosmetics, not drugs, under well-established FDA laws and regulations.[1] The statutory

[1] Peter Barton Hutt, "*The Legal Distinction in the United States Between a Cosmetic and a Drug*", chapter in Cosmeceuticals: Drugs vs. Cosmetics, 2001, ("Products that are represented only to change the structure or function of the hair or nails are regarded as cosmetics and not drugs. For example, permanent waves and cuticle removers are cosmetics, not drugs (citation removed). Products that are represented to affect the hair or nails

definition of ”cosmetic” was not changed in the Modernization of Cosmetics Regulation Act of 2022 (MoCRA)[2].

The following are representative marketing claims:

- Enhances shine of hair
- Adds curl to lashes
- Enhances body/volume of hair
- Enhances appearance of vitality of hair
- Enhances flexibility/curl of hair
- Strengthens hair and helps protect against breakage
- Improves appearance of lashes
- Healthier-looking hair/lashes

None of COMPANY’s products, including those that contain DDDE, make any claims indicating an intended use for therapeutic or medical purposes or to affect the structure or function *of the body*. The intended use of COMPANY’s products that contain DDDE clearly fall within the statutory definition of a cosmetic in the U.S.[3]

2.4 Scope of Data Included in This Report

2.4.1 Eyelash Product

This report focuses on safety data obtained in tests of COMPANY’s marquee ***eyelash*** product (referred to herein as ”**Product A**”). While DDDE also is an ingredient in COMPANY’s products applied to eyebrows or scalp hair, because Product A is applied near eyes it has been the most extensively tested to ensure that its safety is scientifically substantiated. In addition to industry-standard safety tests to ensure that Product A is safe to ***skin***, Product A has also has been tested in multiple studies to ensure that it is safe to ***eyes***.

All of COMPANY’s products, regardless of whether they contain DDDE, are tested using industry-standard safety tests for cosmetic products. Any differences in test results obtained on the eyebrow or scalp hair products from Product A will be noted in this report.

2.4.2 Product-Based Data

The data provided in this report are ***product-based*** data, meaning they were obtained using the 19-ingredient formulation sold as Product A. The safety tests conducted include industry-standard *in vitro* tests and human use studies. Summaries of the study results are provided in this report. COMPANY will provide redacted copies of the full study reports to CIR upon request.

As explained in the next section, COMPANY is currently conducting additional safety testing on DDDE itself separate from its products. Most of this testing is in progress and the results will be

systemically, on the other hand, are regarded as drugs.”) Mr Hutt was Chief Counsel of the FDA from 1971 – 1975.

[2] FDA website, Modernization of Cosmetics Regulation Act of 2022 (MoCRA), https://www.fda.gov/cosmetics/cosmetics-laws-regulations/modernization-cosmetics-regulation-act-2022.

[3] Federal Food, Drug & Cosmetic Act (FDC&A), section 201(i).

provided to CIR in a Supplemental Report as described below. A skin irritiation/sensitization test on DDDE has been completed and those results are included in this report (see section 6.1.2, below).

2.5 Supplemental Report To Be Submitted to CIR

COMPANY is an international cosmetics company that sells its cosmetic products worldwide, including Europe. As the CIR is aware, the European Commission is also engaged in a safety assessment of the use of PGA s in cosmetics. The European Commission initiated this safety assessment in response to concerns raised primarily by Germany and Austria that eyelash products that contain a PGA could potentially have the same effects on eyes as eyedrop medications with PGA ingredients that are approved to treat glaucoma by lowering intraocular pressure (IOP).

In June 2020, the European Commission issued a Call for Data on the use of PGA s in cosmetics. COMPANY submitted extensive safety data on the use of DDDE in Product A and two other companies submitted data on the use of Isopropyl Cloprostenate in their eyelash products to the European Commission. These data were provided to the Scientific Committee on Consumer Safety (SCCS) to conduct a safety assessment on the use of PGA s in cosmetics, as requested by the European Commission.

In February 2022, the SCCS issued an opinion[4] ("SCCS PGA Opinion") in which it said that it did not have the data it needed to complete a risk assessment of PGAs in cosmetics. The SCCS stated that the *product*-based data that had been submitted were not relevant and that ***ingredient***-based data were needed for it to do a risk assessment.

Following the publication of the SCCS PGA Opinion, COMPANY informed the European Commission that it intended to obtain the ingredient-based safety data requested by the SCCS. COMPANY has contracted with a Europe-based toxicology firm, ToxMinds, to oversee completion of an extensive portfolio of *in vitro* tests under applicable OECD guidelines and to prepare a complete safety dossier on DDDE that will include a toxicology analysis and risk assessment. The safety dossier will be submitted to the SCCS.

Note that because animal model testing is prohibited in the EU for cosmetic products and ingredients, *in vitro* tests are the only avenue for obtaining the data requested by the SCCS. Use of animal testing for assessing the safety of cosmetic products is also disfavored in the United States and "should be phased out" under MoCRA.[5]

COMPANY has maintained close communication with the European Commission regarding its testing plan and the projected timeline for submission of a safety dossier on DDDE. ToxMinds

[4] SCCS Opinion on Prostaglandins and prostaglandin-analogues used in cosmetic product, SCCS/1635/21, February 3, 2022, available at: https://health.ec.europa.eu/publications/prostaglandins-and-prostaglandin-analogues-used-cosmetic-products_en.

[5] MoCRA, FDC&A, Sec. 807, *"It is the sense of the Senate that animal testing should not be used for the purposes of safety testing on cosmetic products and should be phased out with the exception of appropriate allowances."*

estimates that the safety testing will be completed by late July/early August 2023. When the ***ingredient***-based data and ToxMinds' analyses are available, COMPANY will submit a supplemental report to CIR. We estimate submitting a supplemental report to CIR in August/September 2023.

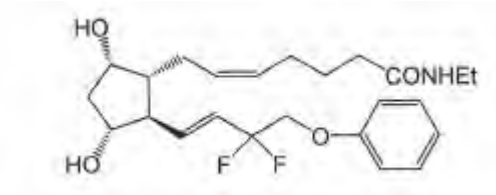
Below is a 'roadmap' of testing on DDDE that is in progress. Data have been collected for all but the MNT and UV absorption studies. Estimated dates of completion of the tests or final study reports and preparation of a safety dossier on DDDE are provided.

Test/Activity	Status / Projected Completion Date
In vitro **skin penetration** of radiolabeled test item on healthy human skin (OECD 428, GLP)	May 2023
In vitro skin sensitization - Direct Peptide Reactivity Assay (**DPRA**) (OECD 442C, GLP)	May-June 2023
Human Subject Repeat Insult Patch Test (**HRIPT**) for Skin Irritation and Skin Sensitization Evaluation	Completed
In vitro skin sensitization – **KeratinoSens** test, (ARE-Nrf2 Luciferase KeratinoSensTM OR ARE-Nrf2 Luciferase LuSens Test Method), (OECD 442D, GLP)	May-June 2023
In vitro Bacterial Reverse Mutation Test (**AMES** Test) (OECD 471, GLP)	June 2023
In vitro mammalian cell micronucleus (**MNT**) Assay (OECD 487, GLP)	June-July 2023
In vitro skin irritation – **EpiDerm**™ Test (Reconstructed human Epidermis (RhE) Test) (OECD 439, GLP)	May-June 2023
In-vitro eye irritation – **EpiOcular**™ Reconstructed Human Cornea-like Epithelium Test (RhCE-Test) (OECD 492, GLP)	May-June 2023
UV-VIS Absorption Spectra (OECD 101, GLP)	June 2023
Preparation of safety dossier by ToxMinds with complete toxicology analysis and risk assessment	July – August 2023
Submission of a Supplemental Report to CIR on *in vitro* test results of DDDE	July – August 2023

3. CHEMISTRY AND PHYSICAL PROPERTIES OF DDDE

3.1 Chemical Structure and Definition

a) The chemical structure of DDDE is shown below. (Annex 1[1])



b) Molecular Formula: $C_{24}H_{33}F_2NO_4$ (Annex 1)

c) Molecular Weight: 437.52 (Annex 1)

d) CAS: 1185851-52-8 (Annex 1)

e) INCI Name: ***Dechloro dihydroxy difluoro ethylcloprostenolamide*** (DDDE), assigned in June 2010 by the Personal Care Products Council. DDDE is the same molecule as Ethyl Tafluprostamide. (Annex 2[2])

3.2 Physical Properties

DDDE is a colorless to pale yellow solution. (Annex 1)

3.2.1 Purity

NLT 99.00%. (Annex 1)

3.2.2 Impurities

NMT 1.00% (Annex 1)

4. COMPOSITION AND PROPERTIES OF PRODUCT A

4.1 General Description of Product A

Product A is a preserved and thickened aqueous-based mixture of mainly emollient, skin conditioning and humectant agents used in formulating an eyelash conditioning solution. The solution is provided in an aluminum tube-like container, similar to a typical mascara container.

Similar to mascara products, Product A includes a multi-use applicator wand that is attached to the container's screw-on cap. The tip of the applicator is similar to applicators used with eyeliners and consists of a very fine brush that is designed to optimize precise application of a small amount of the solution to the eyelashes, as directed by the Directions For Use. The tube neck includes a wiper designed to remove excess solution from the applicator when the applicator is removed from the container.

4.2 Application of Product A

Product A is intended to be applied once each day with a fine brush applicator as a thin line directly to the ***eyelashes***. Below are the Directions for Use and Caution Statements provided in the packaging for Product A.

Directions For Use	Caution Statements
Once a day, apply a thin line of [Product A] directly to eyelashes, above the lash line. Let dry completely before applying additional beauty products.	Do not get in eye. Rinse immediately with water if eye contact occurs. If irritation develops, reduce frequency of use until irritation resolves. If irritation persists or is excessive, discontinue use and consult a physician. Some users have reported a faint darkening of the eyelash base (primarily with excessive use); if this is of concern, do not use. Keep out of reach of children.

4.3 Composition of Product A

There are 19 ingredients in Product A. Table 1 below lists the 19 ingredients and their function.

Table 1. Composition of Product A

Ingredient	Function
Water	Solvent
Glycerin	Conditioning Agent; Humectant
Biotin	Conditioning Agent
Cellulose Gum	Viscosity Increasing Agent
Phenoxyethanol	Preservative
Chlorphenesin	Preservative
Disodium Phosphate	Buffering Agent
Phosphoric Acid	pH Adjustor
Dechloro Dihydroxy Difluoro Ethylcloprostenolamide	Conditioning Agent
Butylene Glycol	Solvent
Calendula Officinalis Flower Extract	Conditioning Agent
Panax Ginseng Root Extract	Conditioning Agent
Serenoa Serrulata Fruit Extract	Conditioning Agent
Camellia Sinensis Leaf Extract	Emollient
Triticum Vulgare (Wheat) Protein	Conditioning Agent
Pentylene Glycol	Humectant
Swertia Japonica Extract	Conditioning Agent
Biotinoyl Tripeptide-1	Conditioning Agent
Octapeptide-2	Conditioning Agent

With regards to the safety of Product A, it is important to note the inclusion of two ***preservatives*** (phenoxyethanol and chlorphenesin[6]) and a ***thickener*** (cellulose gum). The preservatives allow for safe repeated applications with the same applicator brush, similar to other cosmetic products applied near eyes – mascara and eyeliner. As discussed in more detail below, the thickener in Product A increases the viscosity to ensure the solution remains on the eyelashes where it is applied and does not migrate to contact the fluid or membranes surrounding the eye. Product A was purposefully designed to have an effect on eyelashes and not come in contact with eyes.

4.4 Concentration of DDDE in Product A

The concentration of DDDE in Product A, COMPANY's marquee eyelash product, is 0.018%.

[6] The concentration of each preservative is below the maximum allowable concentrations.

4.5 Amount of Product A Applied Per Application

4.5.1 Normal Application to Human Eyelashes

The amount of Product A applied per brushstroke to the upper eyelashes was determined by weighing the brush applicator that is part of the Product A container after the applicator was removed from the tube container (pre-application) and again immediately after it was used to apply Product A to the upper eyelashes, above the lash line (e.g., before and after a single brushstroke to the eyelashes). (Annex 3[3]) Ten different applicator brushes were used in the study with the same person applying Product A.

The range of Product A applied per brush stroke was 1 – 4 mg. On average, **2.4 mg** of Product A was applied to the upper eyelashes with each brush stroke.

4.5.2 Application to Mink Hair Samples

Recently, COMPANY conducted additional in-house tests to determine the amount of Product A that was applied to commercially available mink hair samples using the brush applicator. The results are presented in Annex 4[4] and summarized here.

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 Product A applied per brushstroke was calculated as the difference between the applicator after it was removed from the tube container (pre-application) and after the applicator brush applied Product A across the mink hair sample (post-application). There were 10 replications of each procedure/insertion method.

The range of averages was a low of **1.287 mg** (Procedure 2, Insertion Method A) to a high of **2.422 mg** (Procedure 1, Insertion Method B). The highest average (2.422 mg) is almost identical to the average amount of Product A applied (**2.42 mg**) per brushstroke to human upper eyelashes (see section 4.5.1, above).

4.6 Amount of DDDE Applied Per Application

A very small amount of DDDE is applied per application to the eyelashes. Calculations of the average and maximum amounts of DDDE applied per application to the upper eyelashes are presented in Annex 3.

Average: The measured average amount of Product A applied per brush stroke is 2.4 mg. The concentration of DDDE in Product A is 0.018%. The *average* amount of DDDE applied per brush stroke is 0.018% DDDE x 2.4 mg Product A = 0.000432 mg or **0.432 µg**.

Maximum: The maximum amount of Product A applied per application is 4 mg. The *maximum* amount of DDDE applied per brush stroke is 0.018% DDDE x 4.0 mg Product A = 0.00072 mg or **0.72 µg**.

5. POTENTIAL EXPOSURE: DERMAL, OCULAR AND SYSTEMIC

5.1 Exposure Controlled By Viscosity

Product A was purposefully formulated to ensure that the solution remains where it is applied on the eyelashes and does not migrate to the eyelid skin or the eye. The presence of a ***thickener*** in Product A plays a critical role in controlling the exposure of skin and eyes to Product A (and DDDE), which in turn, is a critical factor in assessing the safety of Product A. Data presented in this report substantiates that Product A is safe to skin and eyes.

5.2 Impact of Site and Method of Application – Exposure Comparisons With Mascara and Eyeliner

The exposure of the body to any ingredient in a cosmetic depends critically on multiple factors: where the product is applied; how much product is applied; the amount of ingredient applied; the likely exposure of areas of the body to the product/ingredients; and properties of the product that can affect exposure. The impact of these factors on exposure estimates is illustrated by comparing the site and method of application of Product A with other cosmetics that are applied near eyes, mascara and eyeliner.

Like mascara, Product A is intended to be applied *directly to eyelashes*. But unlike mascara, which is applied along the full length of eyelashes, Product A is applied as a fine line to the eyelashes near their base, above the lash line. Thus, Product A is applied more like eyeliner but applied to eyelashes rather than skin.

The differences in the site and method of application of Product A, mascara and eyeliner are significant factors in the exposure of skin or eyes to the products. Mascara is applied directly to eyelashes but is applied to a greater surface area because it is applied along the full length of the eyelashes. Also, the applicator brush is a dense web of bristles designed to hold and apply a substantial amount of product. It is likely, therefore, that more mascara product is applied per application than eyeliner or Product A, which are applied as a single fine line applied with a fine brush applicator. In addition to the amount of mascara that is applied (and often applied multiple times each day) another factor that affects dermal and ocular exposure is the likelihood that some product falls off the upper eyelashes with frequent blinking. It is not surprising, therefore, that allergic reactions to mascara are common.

In contrast to mascara, eyeliner is applied as a fine line and directly *to the skin*. Thus, while a smaller amount may be applied per application, there is direct dermal exposure.

Because of Product A's site and method of application ***it poses less risk of potential exposure to skin and eyes than mascara or eyeliner.*** Unlike eyeliner, Product A is applied directly to eyelashes and the thickener in Product A reduces the likelihood of migration to the skin. Unlike mascara, the surface area of Product A application is much smaller (a fine line across the eyelashes rather than coverage along the full length of eyelashes) and blinking is unlikely to cause Product A to fall off the eyelashes (especially since many consumers apply Product A before going to bed).

An *in vitro* test of ocular irritation of Product A by an independent laboratory actually showed that Product A had a ***lower*** ocular irritation potential than mascara and eyeliner. A more detailed summary of this test is provided in section 6.2.1, below. Briefly, the hen's egg chorioallantoic membrane (CAM) of the chick embryo was used to test the ocular irritation potential of Product A and a commercially available mascara and eyeliner. Product A's scores indicated *no ocular irritation potential*. Both the mascara and eyeliner products scored in the *mild ocular irritation* range.

5.3 Exposure to DDDE

The assessment of the safety of prostaglandin analogs in cosmetics must take into account the properties and intended use of the product in which the PGA ingredient is used. The discussion in section 5.2, above, makes it apparent that where and how a product is applied, as well as the properties of the applied product, are significant factors that need to be considered when assessing the safety of any ingredient in a cosmetic, including a prostaglandin analog. This is because these factors substantially affect the exposure of the body to each of the ingredients in the applied product.

As explained in section 5.2 above, there is a ***significantly reduced risk of exposure*** to skin and eyes with Product A than mascara or eyeliner, both well-established cosmetic products. In other words, based on these factors, Product A can be expected to be *at least as*, *if not more*, safe than mascara and eyeliner.

The essential question then is whether the presence of a very small amount of DDDE in Product A changes that safety profile? Stated differently, is Product A less safe than mascara or eyeliner (well-accepted cosmetics that are used near eyes), despite having a lower risk of exposure to skin and eyes, because it contains a very small amount of DDDE?

COMPANY will present two lines of evidence demonstrating that DDDE is a safe ingredient in Product A. In this report we present data from *product-based* studies showing that Product A is a safe cosmetic. If use of Product A is safe, then it follows that the ingredients in Product A are safe as formulated and under intended condition of use as prescribed in product labeling. Next, in a supplemental report, we will present data from *ingredient-based* studies on DDDE itself, along with a complete toxicological analysis and risk assessment.

5.4 Dermal Exposure

In this report we present data showing that Product A is non-irrritating to human skin. (See section 6.1, below) Currently, dermal absorption and skin irritation tests on DDDE are in progress (see section 2.5, above) and COMPANY will provide a full report on these data to CIR in our Supplemental Report.

5.5 Ocular Exposure

As with all cosmetic products, a small percentage of consumers experience allergic reactions to common cosmetics (mascara and eyeliner) that are applied in proximity of eyes. Product A was formulated with a thickener to minimize ocular irritation by keeping the product away from ocular fluid and membranes.

In addition to classic concerns regarding ocular irritation associated with any cosmetic applied near eyes, safety concerns have been raised about using PGA ingredients in cosmetic eyelash products. A small number (4-5) of prostaglandin analogs have been approved as active ingredients in drugs intended to treat glaucoma by lowering IOP. These IOP-lowering medications are applied as eyedrops *directly to the eye* and, because of the multiple sturdy membrane barriers that protect the eye, contain relatively large amounts of prostaglandin analog. Generally, these ophthalmic drugs have excellent safety profiles. Nonetheless, the concern is that a PGA used as an ingredient in a cosmetic eyelash product could have unwanted (good or bad) effects on eyes.

This is a valid concern and one that COMPANY has treated very seriously since it first launched cosmetic eyelash products over fifteen years ago. COMPANY has taken a two-pronged approach to ensuring its eyelash products are safe to eyes. First, as stated previously, COMPANY ***designed*** Product A (and its precursors) to provide cosmetic benefits on *eyelashes* without having an effect on eyes. Product A is formulated with a *thickener* to ensure it remains on the eyelashes where it is applied. If Product A does not come in contact with eyes then no ingredient in Product A, including DDDE, can have a physiological effect (good or bad) on eyes. Second, COMPANY conducted the studies described in the following sections to assess the ocular exposure to Product A/DDDE and confirm that neither contacts the eye with normal application of Product A.

5.5.1 Photographic Evidence that Product A Does Not Contact the Eye

A practicing research ophthalmologist on the faculty at the UCLA School of Medicine, Paul Donzis, MD, used an ophthalmic slit lamp microscope to assess photographically if COMPANY's eyelash products that contain a PGA migrate from where the product is applied to contact the fluid surrounding the eye. The study was conducted in 2008 on two earlier formulations of Product A that contained different prostaglandin analogs than DDDE but contained cellulose gum and had substantially the same the viscosity as Product A. Therefore, the results of this study are applicable to the current product, Product A.

A small amount of ophthalmic fluorescein dye was added to the eyelash product solution ("Product") before applying it to the female test subjects. The dye was applied to the tip of the applicator brush and then the brush was dipped into a tube of product. The Product solution + dye was then applied to the eyelash margin[7] of the test subject and the subject was instructed to blink normally. The green fluorescein dye allowed the distribution of the Product to be documented photographically using a cobalt blue light.

As shown in the slit lamp photographs provided below, the Product + dye remained locally on the eyelash margin where it had been applied immediately (Figure 1) and 2 minutes (Figure 2) after application. Even after 5 minutes after application (Figure 3) there was still no dripping or migration of the Product + dye into the eye. None of the Product + dye migrated into the eye

[7] Note the site of application used for this test is slightly different (upper eyelash margin) than for Product A (eyelashes, above the lash line) but was even closer to the eye than the current site of application. The fact that no dye was seen in the fluid surrounding the eye indicates there is even less likelihood that Product A will seep into the eye fluid.

despite normal blinking by the subject and the fact that the presence of the dye slightly decreased the viscosity of the product solution.

Figures: Slit lamp photographs of eyelash product + fluorescent dye

Fig. 1. Slit lamp photo: Product + dye (fluorescent green) immediately after application.

Fig. 2. Slit lamp photo: Product + dye, **2 minutes** post-application. The Product + dye is still confined to the base of the upper eyelashes. None has migrated into the eye.

Fig. 3. Slit lamp photo: Product + dye, **5 minutes** post-application.

Dr. Donzis concluded that ”both formulations of [redacted] are of sufficient viscosity that the product remains where applied on the eyelid even with normal blinking by the subjects. [redacted] [COMPANY’s] estimate of no more than *de minimis* direct ocular exposure was substantiated by my tests*. In fact, I found no migration into the eye or exposure to conjunctiva, sclera, or cornea.”*

5.5.2 Physiological Evidence that DDDE Does Not Have an Effect on Eyes With Normal Use

A commercial laboratory, Evalulab, Inc. conducted a 28-day study, ”*Determination of the Ocular Safety and Irritation Potential of an Eyelash Conditioner*,” involving 19 female subjects to assess the ocular safety and tolerability of Product A. The study was reviewed and approved by an Institutional Review Board. The subjects applied Product A as directed in the package insert. Ocular safety was assessed by a certified ophthalmologist who examined each subject at the beginning of the study and 28 days later at the end of the study. The ophthalmic examinations included slit lamp examination of the eyelids and the components of the eye and measurement of the intraocular pressure (IOP) in each eye.

No ocular irritation was observed in the study (“*Under the conditions in the procedure referenced above, the test product referenced above did not produce any signs of ocular*

irritation or hypersensitivity ….") Also, there was **no change in IOP** in subjects after 28 days of regular use of Product A. (The within-eye differences in IOP from the beginning to the end of the study were not statistically significant ($t > 0.05$)).

The results of the IOP tests in this human use study are very important in the scientific assessment of the safe use of DDDE in Product A. As discussed above, a valid safety concern is whether prostaglandin analogs in cosmetic eyelash products could have the same pharmacological effect on eyes (good and bad) as prostaglandin analogs that are ingredients in glaucoma drugs (referred to herein as 'ophthalmic prostaglandins'). Although DDDE is not an active ingredient in an approved drug to treat glaucoma, some have argued that no PGA should be used in cosmetic eyelash products because all have the potential to have a physiological effect on the eye. The results of this study refute that position. This study demonstrated that normal, sustained use of Product A does not, in fact, have an effect on IOP.

The fact that IOP did not change over the 28-day study provides functional evidence that DDDE, when used as an ingredient in Product A, does not contact the eye and does not have a physiological effect (good or bad) on the eye. As will be discussed in the next section, below, this is in contrast to an eyelash product that is commercially available in the U.S. *as a drug* to grow eyelashes and does contain an ophthalmic prostaglandin.

5.5.3 Ocular Exposure: Product A vs. Latisse

Latisse is an approved drug in the U.S. for treating alopecia. Latisse contains the prostaglandin, bimatoprost. Importantly, the solution in Latisse is identical to the solution in Lumigan, an approved drug for treating glaucoma. Lumigan is a watery solution that is administered as an eyedrop directly into the eye. Lumigan was approved first by the FDA. Subsequently, the manufacturer of Lumigan obtained FDA approval for a new indication (growing eyelashes) of the same bimatoprost ophthalmic solution.

In contrast to Product A, Latisse is applied directly to eyelid *skin* with single-use applicators (necessary because of Latisse's formulation). The product insert for Latisse states: "Apply nightly directly to the *skin* of the upper eyelid margin at the base of the eyelashes using the accompanying applicators." Latisse's site of application optimizes contact with eyelash hair follicles, which is consistent with its intended use to grow eyelashes for people with inadequate eyelashes.

Importantly, because the solution in Latisse is the same solution used in Lumigan, Latisse does not have a thickening agent. The lack of a thickening agent in the original bimatoprost solution (Lumigan) results in a watery solution that is very appropriate as an eye drop but when applied to the eyelid can allow drips to contact the eye.

There is clinical evidence that Latisse does, in fact, contact eyes with normal use. In the pivotal clinical trial of Latisse there was a statistically significant ***decrease in IOP*** over the course of the study. The product label[8] for Latisse states, "In clinical trials, in patients with or without elevated IOP, Latisse lowered IOP, however the magnitude of the reduction was not cause for

[8] Latisse (bimatoprost ophthalmic) solution label, available at: https://www.accessdata.fda.gov/drugsatfda_docs/label/2012/022369s005lbl.pdf

clinical concern." (Latisse label, Section 5.1) Because of the effect on IOP, the FDA required a warning that "Latisse® may lower intraocular pressure although not to a level that will cause clinical harm. In patients using Lumigan® or other prostaglandin analogs for the treatment of elevated intraocular pressure, the concomitant use of Latisse® may interfere with the desired reduction in IOP. Patients using prostaglandin analogs for IOP reduction should only use Latisse® after consulting with their physician." (Latisse label, Section 17.3)

The fact that Latisse caused a statistically significant decrease in IOP in its clinical trial shows that enough of the product can migrate into the eye fluid to have a significant physiological effect on the eye.

In summary, ***Latisse decreased IOP whereas Product A did not change IOP in human use studies.*** The differences in the effect of Latisse and Product A on eyes can be attributed to different sites of application (eyelashes vs. eyelid skin) and viscosity (Product A has a thickener and Latisse does not). These differences stem from the fact that Product A was designed and formulated to be used ***as a cosmetic***. In contrast, the bimatoprost ophthalmic solution used in Latisse was developed for use as an eyedrop medication to treat glaucoma. The comparison between Product A and Latisse illustrates that product design (formulation, site and method of application, cosmetic vs. drug intended uses) is a critical factor in assessing the safety of using PGA ingredients in eyelash products.

5.5.4 Ocular Exposure: Product A vs. FDA Approved Drugs

As discussed previously, a concern about the use of PGA ingredients in cosmetic products applied near the eye is whether the PGA could have pharmaceutical effects on eyes. This concern stems from the fact that four prostaglandin analogs have been approved by the FDA as active ingredients in eye drop drugs intended for use to treat glaucoma by lowering IOP. While this is a valid concern in the abstract, the facts presented in this report collectively show that this concern *is not scientifically substantiated.*

Assessment of the potential for DDDE, as used in Product A, to have a pharmaceutical effect on the eye requires consideration of multiple factors, including: the quantities of PGA applied per topical application, where on the body the PGA is topically applied, the potential for the PGA to come in contact with the fluid or membranes of the eye either via migration from the application site and/or dermal absorption, and the amount of PGA that could potentially contact the eye. As discussed above in the comparison of the effect of Latisse vs. Product A on eyes (specifically, IOP), *where* the product is applied and the *product's properties* that affect potential migration (e.g. viscosity) significantly impact the product's effect on ocular physiology.

Below, data are presented comparing the *amount* of PGA that is applied per application in Product A vs. FDA approved drugs that contain a prostaglandin analog.

The data in Table 2, below, compares the ocular exposure to prostaglandin analogs that are ingredients in products that are approved as drugs in the United States with DDDE in Product A. Information about the concentration and quantities for the drug products in Table 2 was obtained from public sources, such as the package inserts. A fully annotated version of Table 2 is provided in Annex 7[5].

Table 2. Average amounts of prostaglandin or PGA applied per application in five FDA-approved drugs vs. the cosmetic, Product A.

	Latanoprost (Xalatan®)	Travoprost (Travatan-Z®)	Tafluprost (Zioptan®)	Bimatoprost (Lumigan®)	Bimatoprost (Latisse®)	DDDE (Product A)
Concentration (%)	0.005%	0.004%	0.0015%	0.03%	0.03%	0.018%
Concentration (mg/ml)	0.05	0.04	0.0150	0.30	0.30	0.180
Drops/mL	29.5	34.6	n/a	33.3	n/a	n/a
Quantity of *solution* applied / application	0.0338 mL	0.0289 mL	0.15 mL	0.03 mL	0.0015 mL	2.4 mg
Quantity of *prostaglandin* analog applied per application	1.7 µg	1.16 µg	2.25 µg	9.0 µg	0.45 µg	0.432 µg
Intended Use	Treat Glaucoma	Treat Glaucoma	Treat Glaucoma	Treat Glaucoma	Treat Alopecia (Grow Eyelashes)	Eyelash Conditioner
Site of application	Eye	Eye	Eye	Eye	***Skin*** at base of upper eyelashes	***Eyelashes***
Method of application	Eye drop	Eye drop	Eye drop	Eye drop	Fine Brush Applicator	Fine Brush Applicator
Contains Thickener	No	No	No	No	No	**Yes**
Effect of product on IOP	Decreases	Decreases	Decreases	Decreases	**Decreases**	**None**

The range of PGA applied *directly to the eye* in each eyedrop medication to treat glaucoma is **1.16 µg – 9.0 µg**. In comparison, on average **0.432 µg of DDDE** is applied per brushstroke to *eyelashes*.

The amount of bimatoprost applied per application of Latisse is 0.45 µg. This quantity is similar to the amount of DDDE applied per brushstroke. Recall, however, that in human use studies Latisse was shown to lower IOP whereas Product A did not. These data indicate that a sufficient amount of Latisse contacts eyes to have a pharmaceutical effect, which may be due to the watery nature of the Latisse solution and its application on the eyelid.

5.5.5 Assessment of Potential Ocular Exposure to DDDE With Normal Use of Product A

In addition to diligently collecting data to assess potential ocular exposure to Product A and DDDE, COMPANY asked a pharmacokinetics expert, Ian Wilding, PhD, to evaluate the likely migration of Product A to eyelid skin or eye, the possible effect of DDDE on the eye and the potential for systemic exposure to DDDE. Dr. Wilding's curriculum vitae is provided as Annex 5[6]. Dr. Wilding's redacted report is provided as Annex 6[7]. For the convenience of the reader, below are questions posed to Dr. Wilding that relate to likely exposure of the body to DDDE and his conclusions. The excerpts presented below have been redacted, substituting "COMPANY" and "Product A" where needed.

a. What is the likely exposure of the eyelid skin or the eye to DDDE with normal use of Product A applied in accordance with the instructions for use?

Due to the site of application ("*a thin line of Product A directly to the **eyelashes***"), the presence of cellulose gum which acts as a "viscosity increasing agent," and that "at most, 0.72 µg of DDDE (4 mg of Product A x 0.00018 = 0.00072 mg (or 0.72 µg[9])) is applied per brushstroke," Dr. Wilding concluded "*it is **highly unlikely** any of the very small amount of DDDE that is applied to the eyelashes with normal use would be transferred onto the eyelid or into the eye*." (Annex 6, section 4.1.(b)(i), emphasis added.)

b. If Product A comes in contact with the eyelid skin, describe the likely absorption by the skin. Is there any likelihood that DDDE could migrate through all of the layers of the eyelid skin to contact the outer surface of the eye?

Because of the viscosity increasing agent (cellulose gum) and since "*no more than 0.72 µg of DDDE is applied per application to the eyelashes, the quantity of DDDE that is likely to migrate to the eyelid and be available for skin penetration is **negligible***." (Annex 6, section 4.2.(a)(i), emphasis added.)

c. If Product A migrates to the outer surface of the eye, describe the likely absorption by the eye.

The anatomy of the eye poses very effective barriers to absorption of topically applied solutions. "*Upon administration to the surface of the eye, precorneal factors and anatomical barriers negatively affect the bioavailability of topical formulations*." "*Considering all the precorneal factors, contact time with absorptive membranes is low, which is considered to be the primary reason for **less than 5% of the applied dose** of an eye drop reaching intraocular tissues*." Annex 6, section 4.2.(b)(i) (emphasis added). Experiments using hydroxyethylcellulose formulations found that "*over **75%** of the product was … **cleared within circa 60 seconds*****." Annex 6, section 4.2.(b)(vi) (emphasis added).

The fluorescent dye study photographs *"provide graphical and very clear support to the conclusion that little, if any, of the product contacted the eye*. It is even less likely that Product A, which is applied to the eyelashes, migrates into the eye." (Annex 6, section 4.2.(a)(ix), emphasis added.) Even *"under a 'worst case' scenario only **0.72 µg** [of DDDE] could be administered into the eye assuming it was inadvertently applied to the surface of the eye*." Based on toxicokinetic data on ophthalmic solutions Dr. Wilding stated that "***circa 75%** of that dose [75% of 0.72 µg = 0.54 µg] **would be cleared within 60 seconds** thereby minimizing any possibility of tissue penetration for pharmacological effect*." (Annex 6, section 4.2(a)(viii), emphasis added.)

Dr. Wilding concluded, *"In summary, due to the increased viscosity of Product A and the barriers to penetration posed by the anatomical layers of the eye, it is **highly unlikely** for Product A to have a pharmacological effect on the eye* when applied as directed to the eyelashes. Similarly, inadvertent contact of Product A with the surface of the eye (e.g. a

[9] Dr. Wilding was referring to the *maximum* amount of Product A applied per brushstroke. The *average* is 0.432 µg (see Table 2).

wayward brush stroke) is unlikely to have a pharmacological effect due to the small amount of Product A involved and the clearance properties of the eye." (Annex 6, section 4.2(a)(x), emphasis added.)

d. Based on answers to previous questions, describe the likely physiological effects of DDDE on the eye (outer surface of the eye and intraocular).

Because "little, if any, Product A contacts the eye *... it is unlikely that a sufficient amount of DDDE permeates the cornea to have a pharmacological effect*." (Annex 6, section 4.3(a)(i), emphasis added.) Dr. Wilding said "*my analysis is supported by data* [from a human use study] *showing that* ***normal use of Product A has no effect on intraocular pressure (IOP)."*** (Annex 6, section 4.3(a)(ii), emphasis added.)

Dr. Wilding distinguished Product A from Latisse with regards to their physiological effect on eyes. In contrast to Product A, normal use of Latisse in a clinical study led to a *lowering of IOP*, indicating sufficient inadvertent migration of Latisse from the eyelid margin into the eye to have a pharmacological effect. Because "*the amount of DDDE delivered to the cornea following inadvertent administration of Product A via poor user technique is likely to be much more limited than for Latisse in view of the incorporation of a thickener in Product A, which is not present in Latisse.* ***It is therefore hard to imagine on a scientific basis how Product A could have an effect on IOP at all***." (Annex 6, section 4.3(a)(iii), emphasis added.)

e. Compare the effects of DDDE on the eye to other cosmetic products used near the eye (mascara and eyeliner).

Dr. Wilding reviewed the results of an *in vitro* toxicology study of the ocular irritation potential using the chorioallantoic membrane (CAM) of a chick embryo on Product A and a commercially available mascara and eyeliner. (The details of this "HET-CAM" study are presented in section 3.4.2.1, below.) Dr. Wilding noted that "[t]he results showed that all three products had ***no potential*** to cause in vivo ocular irritation." (Annex 6, section 4.4(a)(i), emphasis added

f. Compare the effects of DDDE (in Product A) on the eye with prostaglandin analogues that are ingredients in glaucoma drugs.

Answer: *"[I]t is* ***unlikely*** *that sufficient amounts of DDDE contact the surface of the eye or permeate to intraocular layers of the eye to have a pharmacological effect."* (Annex 6, section 4.5(a)(i), emphasis added.)

g. What is the likelihood that the DDDE in Product A has <u>systemic</u> effects?

Answer:, *"It is my view that* ***there is a negligible likelihood that the DDDE in Product A has systemic effects*** *irrespective of whether it is administered in accordance with the package insert (on the eyelashes) or used incorrectly e.g. applied directly to the eyelid or cornea."* (Annex 6, section 4.6(a)(i), emphasis added.)

5.5.6 Expert Assessment of Potential Pharmacological Effect of Product A on Eyes

The data presented above in sections 5.5.2 and 5.5.4, above, do not support statements made by the SCCS in the SCCS PGA Opinion (see footnote 4). On page 40 of the SCCS PGA Opinion, the SCCS said the maximum amount of DDDE that "theoretically" could contact eyes with use of Product A was "in the same order of magnitude" as the range of doses used in PGA -contaning drugs intended to treat glaucoma. For the reasons provided below, this statement, and the conclusion drawn from it, are factually inaccurate.

Dr. Ian Wilding was asked to give an expert opinion on the factual accuracy of the SCSS' statement referenced above. The SCCS statement in full is:

> "*For pharmacological treatment of intra-ocular pressure, a daily dose of one drop with a PGA is prescribed. This implies, depending on the type of analogue, a dose of 0.75 – 2.5 µg per eye per day. In the absence of data on skin absorption from the application of an eyelash growth formulation, assuming a dermal absorption of 50% and full transfer from the eye-lid conjunctiva to the eye, a maximum exposure of the eye of 0.36 µg DDDE and 2.5 µg isopropyl cloprostenate can theoretically be estimated. These doses are in the same order of magnitude as those used for the epi-ocular pharmacological treatment of intra-ocular pressure.*"[10]

Dr. Wilding's complete analysis of the SCCS statement is provided in Annex 8[8] (see Third Question, p. 6 of Annex 8). For the convenience of the reader, key elements of Dr. Wilding's analysis are provided here.

a. The dose range of drugs used to treat glaucoma is not correct. The SCCS presented the range as 0.75 µg – 2.5 µg per day. The actual range is 1.16 µg – 9.0 µg. (Annex 8, para. (k)).

b. The SCCS estimate of maximum ocular exposure to DDDE with normal use of Product A is based on a flawed assumption that 50% of the DDDE applied to eyelashes could reasonably be expected to contact the eye. (Annex 8, para.(h)).

c. Because of the thickener in Product A it is "*highly unlikely that any of the very small amount of DDDE that is applied to the eyelashes with normal use woud be transferred onto the eyelid or into the eye – therefore, the quantity of DDDE that is likely to be misapplied to or migrate to the eyelid and be available for dermal absorption with correct use of the product is negligible*." (Annex 8, para.(c)).

d. Based on data actually cited by the SCCS, a 10% dermal absorption rate is a more reasonable estimate for DDDE than 50%. (Annex 8, para.(h)).

e. Using a conservative assumption that as much as 20% of DDDE could be absorbed, the maximum amount of DDDE that would be absorbed is **0.144 µg**.[11] (Annex 8,

[10] SCCS PGA Opinion, page 40.

[11] The actual rate of dermal absorption rate of DDDE is substantially less than the assumed 20% rate Dr. Wilding used for his calculation. Recent *in vitro* tests of dermal absorption of DDDE showed that the average dermal absorption rate is well under 10%.

para.(j)). (The maximum amount of Product A applied per application is 4 mg and the maximum amount of DDDE applied is 0.72 µg.[12] 20% x 0.72 µg = 0.144 µg.)

f. More accurately, the maximum potential ocular exposure to DDDE with normal use of Product A of **0.144 µg** is substantially ***lower*** than the range of doses for PGA - containing glaucoma drugs – **1.16 µg – 9.0 µg**. Annex 8, para.(n)).

g. Dr. Wilding concludes, "*The SCCS states that the maximum exposure of DDDE in the eye is "in the same order of magnitude as those used for pharmacological treatment of intra-ocular pressure (IOP)". **I disagree with this assertion**."* Annex 8, para.(m), emphasis added).

In summary, a key conclusion in the SCCS PGA Opinion is factually inaccurate. There is no factual support for the hypothetical assumption that DDDE would have a pharmaceutical effect with normal use of Product A. In fact, there is no evidence that DDDE would have an effect on IOP if the eye was exposed to larger quantities of DDDE. Even generously assuming that 20% of the DDDE applied per brushstroke is absorbed by the eyelid skin or migrates to the eye (which is unlikely given the viscosity of Product A) the maximum amount of ocular exposure to DDDE (0.144 µg) is orders of magnitude ***smaller*** than the smallest dose (1.16 µg) of the PGA drugs used to treat glaucoma. It is highly unlikely that this amount of DDDE would have a pharmaceutical effect on the eye. This conclusion is consistent with the fact that normal, sustained use of Product A was shown not to have an effect on IOP.

5.6 Systemic Exposure

COMPANY asked two experts to evaluate the potential systemic exposure of DDDE with normal use of Product A. The relevant portions of their expert opinions are provided below. One expert is an ophthalmologist and the other is an expert in pharmacokinetics. ***Both experts concluded that systemic exposure to DDDE is very unlikely***.

5.6.1 Expert Assessment by Ophthalmologist Expert

Dr. Paul Donzis, a clinical ophthalmologist who conducted the flourescent dye study presented above (section 5.5.1) assessed the potential for ocular and systemic exposure to DDDE with normal use of Product A. His expert opinion is priovided as Annex 9[9]. Key elements of Dr. Donzis' opinion are provided below.

As a practicing ophthalmologist, Dr. Donzis is very familiar with prostaglandin/PGA-containing drugs approved to treat glaucoma. Dr. Donzis refers to these drugs as "ophthalmic prostaglandin analogs." At the time of his assessment, Dr. Donzis had performed about 1500 glaucoma patient examinations each year for 15 years of medical practice.

Dr. Donzis explained that about **80%** of a topically applied ophthalmic prostaglandin analog eyedrop solution is absorbed through the nasolacrimal duct and is ***systemically absorbed***.

> *"The ophthalmic prostaglandin analogs, which are applied as eye drops, have ocular effects resulting from direct absorption by ocular membranes but also potentially*

[12] See sections 4.5 and 4.6, above.

have systemic effects resulting from absorption through the nasolacrimal duct. After topical administration of eye drops, ***80%*** *of the volume drains through the nasolacrimal duct and* ***is absorbed systemically****, bypassing hepatic metabolism.*" (Annex 9, p. 2, emphasis added)

Based on his analysis of the product properties and the very small amount of DDDE in Product A, Dr. Donzis concluded that ocular exposure to DDDE and absorption of DDDE through ocular membranes "is highly unlikely."

"Based on my review of scientific data, including the amount of DDDE present in each application of [Product A], there would not be any expected ocular exposure associated with the use of [Product A] in accordance with the package instructions and, therefore, any absorption of DDDE through the ocular membranes is highly unlikely ...." (Annex 9, p. 5)

Dr. Donzis concluded that systemic exposure to DDDE is not likely since it is for DDDE to come in contact with ocular membranes or the nasolacrimal duct.

"Thus, no expected systemic absorption from the ocular surface or nasolacrimal duct would be expected." (Annex 9, p. 5)

Dr. Donzis also noted that his review of consumer complaints related to Product A indicated only local effects and **no systemic effects** and do "***not raise concerns about ocular safety***."

"In reviewing the log of consumer complaints supplied to me … the majority of complaints relate to allergic irritation and sensitivity to [Product A], which can occur with any cosmetic product. None of the complaints indicate systemic absorption of [Product A]. The paucity of complaints also speaks to the overall high safety and tolerance of [Product A]. The profile of consumer complaints does not raise concerns about ocular safety." (Annex 9, p. 5)

5.6.2 Expert Assessment by Pharmacokinetics Expert

In his expert assessment of potential ocular and systemic exposure to DDDE with normal use of product A, Dr. Ian Wilding also concluded that systemic exposure to DDDE is unlikely.

Question: What is the likelihood that the DDDE in Product A has systemic effects?

Answer:, *"It is my view that* ***there is a negligible likelihood that the DDDE in Product A has systemic effects*** *irrespective of whether it is administered in accordance with the package insert (on the eyelashes) or used incorrectly e.g. applied directly to the eyelid or cornea."* (Annex 6, section 4.6(a)(i), emphasis added.)

6. SKIN AND OCULAR IRRITATION TEST RESULTS

6.1 Skin Irritation Evaluation

6.1.1 Test of Product A

A "Human Subject Repeat Insult Patch Test For Skin Irritation and Skin Sensitization Evaluation" was performed on Product A by BioScreen Testing Services in 2009. The objective of the study was to determine the skin irritation and sensitization (contact allergy) potential of

Product A after repeated application via a patch to the skin of human subjects. The study was reviewed and approved by an Institutional Review Board.

Patches containing the test product were affixed to the skin on the back (intrascapular area) of 51 human subjects (ages 18 – 59 years) for 24 hours, 3 times/week for 3 consecutive weeks (total of 9, 24-hour exposures) and two retest/challenges 10-14 days later applied to a previously unexposed test site (48 and 96 hour exposure). Test sites were evaluated by trained laboratory personnel. Each evaluation was scored using the International Contact Dermatitis Research Group Scoring Scale: 0 = no reaction (negative), 1 = erythema throughout at least ¾ of patch area, 2 = erythema and induration throughout at least ¾ of patch area, 3 = erythema, induration and vesicles, 4 = erythema, induration and bullae.

Results: Two of the total 561 evaluations were scored "1", all other evaluations were scored "0". The study report stated, "*No adverse reactions of any kind were reported during the course of this study*."

Conclusion: The study report stated, "*Under conditions of the study, there were no identifiable signs or symptoms of sensitization (contact allergy) noted for [Product A]*."

6.1.2 Test of DDDE

A "Human Subject Repeat Insult Patch Test For Skin Irritation and Skin Sensitization Evaluation" was performed on DDDE by BioScreen Testing Services in 2022. The objective of the study was to determine the skin irritation and sensitization (contact allergy) potential of DDDE after repeated application via a patch to the skin of human subjects. The study was reviewed and approved by an Institutional Review Board.

Patches containing the test material (7.5% DDDE in phenoxyethanol) were affixed to the skin on the back (intrascapular area) of 54 human subjects (ages 18 – 64 years) for 24 hours, 3 times/week for 3 consecutive weeks (total of 8-9, 24-hour exposures) and two retest/challenges 10-14 days later applied to a previously unexposed test site (48 and 96 hour exposure). Test sites were evaluated by trained laboratory personnel. Each evaluation was scored using the International Contact Dermatitis Research Group Scoring Scale: 0 = no reaction (negative), 1 = erythema throughout at least ¾ of patch area, 2 = erythema and induration throughout at least ¾ of patch area, 3 = erythema, induration and vesicles, 4 = erythema, induration and bullae.

Results: All of the 486 total evaluations were scored "0". The study report stated, "*No adverse reactions of any kind were reported during the course of this study*."

Conclusion: The study report stated, "*The test product was dermatologist tested and under the conditions of the study, there was no indication of a potential to elicit dermal irritation or sensitization (contact allergy) noted for [DDDE]*."

6.2 Ocular Irritation Evaluation

6.2.1 *In vitro* Hen's Egg Test-Chorio Allantoic Membrane (HET-CAM) Assay

The hen's egg chorioallantoic membrane (CAM) of the chick embryo was used to test the ocular irritation potential of Product A. The CAM is a complete tissue that is used extensively in toxicology tests and is accepted as an alternative to animal testing. The chorionic epithelium is

ectodermal and the allantoic epithelium is endodermal. The CAM responds to injury with a complete inflammatory reaction comparable to that induced in the rabbit eye test but has the advantage, as an *in vitro* test, of avoiding exposing nerves to painful stimuli. Published studies have shown that the hen's egg CAM is more sensitive to liquid irritants than the rabbit eye.

The Consumer Product Testing Co. used the HET-CAM assay to assess the irritation potential of Product A and, for reference, two other cosmetic products that are used in the vicinity of the eye, a commercially available mascara and eyeliner.

Each test CAM was examined and scored for irritant effects. The scoring classification is shown below.

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

As shown in the excerpt below from the final report, Product A had CAM **scores of 0** (zero) at *all* test points (30 seconds, 2 and 5 minutes after exposure), showing that Product A had ***no irritant effect*** on the CAM.

Test Article (%)	CAM #	Scores @ 0.5 min.	2 min.	5 min.	Total
Eyelash Conditioner [redacted]	1	0	0	0	0
	2	0	0	0	0
	3	0	0	0	0
	4	0	0	0	0
				Average:	**0.00**

The reference test articles, a **mascara** and an **eyeliner**, had a ***greater irritant effect on the CAM than Product A***. As shown in the excerpt below from the final report, the mascara product had a CAM score of 0.50 and the eyeliner product had a CAM score of 0.75. While these are acceptably low scores for cosmetic products, it is significant for the safety assessment of the use of DDDE in Product A that **Product A was *objectively shown in this in vitro assay to have less ocular irritation potential than representative mascara and eyeliner products***.

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

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

Conclusion: The study report stated, "[T]he sponsor-submitted product [Product A] … would have ***practically no ocular irritation potential in vivo***."

6.2.2 Assessment of ocular irritation in humans

Ocular irritation was evaluated in a 28-day study involving 19 adult human subjects conducted by Evalulab. In this study, the volunteer subjects applied Product A daily to the upper eyelashes in accordance with the directions for use. The study was conducted under the supervision of an ophthalmologist and included measurement of IOP in each eye at the beginning and end of the study. The IOP results are discussed in section 5.5.2, above.

Ocular irritation was assessed by the supervising ophthalmologist who queried each subject about adverse reactions and also conducted an ophthalmic exam on Day 0, at the beginning of the study, and on Day 28, at the end of the study. The ophthalmic exam was performed with a slit lamp and included the subject's eyelids, cornea, conjunctive, anterior chambers, papillary reactions, and visual acuity. The ophthalmologist scored any observed intolerance to Product A as 0 (none), 1 (slight), 2 (moderate) and 3 (high). Most of the entries are zeros, there was one 2 and no 3's. Four subjects reported minor adverse reactions consistent with allergic reactions.

Conclusion: The study report stated, "*The test product did not produce an ocular irritation or hypersensitivity of clinical magnitude, in the totality of the test panel (19 volunteers). Therefore, **the test product may be considered safe for use as an eyelash conditioner**"* (emphasis added).

7. ADVERSE EVENTS AND SERIOUS ADVERSE EVENTS

7.1 COMPANY Records and Data

COMPANY markets its cosmetics globally in over 70 countries. Legal requirements for defining, recording and reporting adverse reactions reported by consumers varies by country. For example, the EU has a well-established legal framework that defines "undesirable effects" (UEs) and "serious undesirable effects" (SUEs) and requires reporting of SUEs related to cosmetics to regulatory authorities, sharing of information about SUEs with the competent authorities of all Member States and with the manufacturer of the cosmetic product (if the SUE report did not come from the manufacturer). In contrast, until the enactment of the Modernization of Cosmetic Regulation Act of 2022 (MoCRA)[13], there was no definition of adverse event (AE) or serious adverse event (SAE) and no requirements for tracking, maintaining records or reporting SAEs for cosmetics in the U.S.

Because the EU's legal requirements are (pre-MoCRA) among the most rigorous internationally, COMPANY has used the EU framework for its AE/SAE systems and procedures globally. Thus, for many years COMPANY's market surveillance of its products has *exceeded* the legal requirements under FDA laws and regulations. COMPANY's systems and procedures

[13] MoCRA, Available at: https://www.fda.gov/cosmetics/cosmetics-laws-regulations/modernization-cosmetics-regulation-act-2022.

will be modified to comply with MoCRA's requirements[14] and will be implemented by December 2023.

7.2 EU Requirements

7.2.1 Definitions

"Undesirable effects" (UEs) are defined in the Cosmetics Regulation as "adverse reactions for human health attributable to the normal or reasonably foreseeable use of a cosmetic product."[15]

"Serious undesirable effects" (SUEs) are defined as "undesirable effects which result in temporary or permanent functional incapacity, disability, hospitalization, congenital anomalies or an immediate vital risk or death."[16]

7.2.2 Requirements for Reporting UEs and SUEs

a. UEs

Records of reported UEs must be maintained and available statistical data on reported UEs must be included in the Cosmetic Product Safety Report.[17]

b. SUEs

Regulation (EC) No. 1223/2009 on cosmetics products (the "Cosmetics Regulation") created a framework for the uniform management of SUEs reported to responsible persons[18], distributors or competent authorities. Responsible persons must be located in the EU and act as local representatives of non-EU based companies. SUEs that are reported to responsible persons or distributors must be reported without delay to the competent authority of the Member State in which the event occurred.[19] Data on SUEs reported to a competent authority must be made available to the public.[20]

If a competent authority of a Member State receives a report of an SUE directly from a health professional or an end user, and if the reported event meets the seriousness criterion of the definition of an SUE, the competent authority must immediately transmit information about the

[14] Under MoCRA, "**adverse event**" is defined as any health-related event associated with the use of a cosmetic product that is adverse" (FDCA sec. 604 (1)) and "**serious adverse event**" is defined as "as an adverse event that (A) results in death; a life-threatening experience; impatient hospitalization; a persistent or significant disability or incapacity; a congenital anomaly or birth defect; or significant disfigurement (including serious and persistent rashes or infections, second- or third-degree burns, significant hair loss, or permanent or significant alteration of appearance), other than as intended, under conditions of use that are customary or usual; or (B) requires, based on reasonable medical judgment, a medical or surgical intervention to prevent an outcome described in subparagraph (A)." (FDCA sec. 604(5)).

[15] Article 2.1(o) of Cosmetics Regulation 1223/2009.

[16] Article 2.1(p) of Cosmetics Regulation 1223/2009.

[17] EC Guidelines to Annex I of Cosmetic Regulation.

[18] Each cosmetic product sold in the EU must be linked to a responsible person established in the EU. Article 11 of Cosmetics Regulation. 1223/2009.

[19] Article 23(4) of Regulation (EC) no. 1223/2009 of the European Parliament and of the Council, of 30 November 2009 on cosmetics.

[20] Article 21 of Cosmetics Regulation 1223/2009.

SUE to the responsible person for the manufacturer of the cosmetic product and to other competent authorities of the Member States.

Records of reported UEs must be maintained and available statistical data on reported SUEs must be included in the Cosmetic Product Safety Report.

Importantly, under the Cosmetics Regulation and the SUE Reporting Guidelines there is a standardized process for ensuring that information about reported SUEs is quickly shared among entities associated with the product (responsible persons and distributors) and the competent authorities of EU Member States. As such, COMPANY must be informed of any reported SUE associated with use of any product sold by COMPANY by an end user residing in an EU member State.

7.3 UEs / AEs Associated With Product A

COMPANY uses the terms adverse event (AE) and undesirable effect (UE) interchangeably. The definition of ”adverse event” under MoCRA is not substantially different from the EU’s definition of undesirable effect and, therefore, is not expected to impact the AE/UE profile for Product A.

7.3.1 Statistical Data

The rate of reported AEs/UEs for Product A have consistently been extremely low. From April 2022 – April 2023, the number of reported AEs domestically was 0.154% of total units of Product A.

7.3.2 Reported AEs/UEs for Product A are are Typical of *Cosmetics*

Three independent experts have evaluated reported AEs/UEs associated with the use of Product A and all concluded that they are consistent with other cosmetic products used in the vicinity of the eye, namely mascara and eyeliner.

Dr. Wilding, in his expert pharmacokinetic assessment of Product A, evaluated consumer complaints that had been reported by consumers. Dr. Wilding concluded that ”*All complaints are **minor and transient in nature**, and do not indicate an intraocular effect.”* (Annex 6, section 4.4(a)(ii))

Dr. Donzis, an ophthalmologist, in his expert opinion on the potential ocular or systemic exposure to DDDE with normal use of Product A, evaluated consumer complaints (AEs/UEs) related to Product A. Dr. Donzis concluded that the “***the majority of complaints relate to allergic irritation and sensitivity to [Product A], which can occur with any cosmetic product***.”

> ”In reviewing the log of consumer complaints supplied to me … the majority of complaints relate to allergic irritation and sensitivity to [Product A], which can occur with any cosmetic product. None of the complaints indicate systemic absorption of [Product A]. The paucity of complaints also speaks to the overall high safety and tolerance of [Product A]. The profile of consumer complaints does not raise concerns about ocular safety.” (Annex 9, p. 5)

In a 2014 safety assessment of Product A for Product A's Product Information File (PIF) in Europe, Intertek evaluated UEs that had been reported by Product A EU consumers over the course of two years (2011 - 2013) to a COMPANY EU distributor. During that time, the number of reported UEs for Product A was **0.00717%** of the number of sold units. Intertek's assessment of these reported UEs was that they "suggest that some sensitive individuals may adversely react to this product." Intertek concluded, *"Under normal or reasonably foreseeable conditions of use, a product made to this formulation is unlikely to produce an abnormally high number of adverse reactions. The product gives users the level of safety they can reasonably expect when used as directed."*

In summary, the AEs/UEs that have been reported by users of Product A are ***typical*** in nature to those associated with other cosmetic products used in the vicinity of the eyes, specifically ***mascara and eyeliner***. This is consistent with the fact that Product A demonstrated ***less*** ocular irritation than representative mascara and eyeliner products in the *in vitro* HET-CAM assay (see section 6.2.1, above).

7.4 SAEs / SUEs Associated With Product A

7.4.1 SAEs

COMPANY has not received any reports of substantiated SAEs associated with Product A. Product A has been sold in the U.S. since 2011. Under industry standards and COMPANY's procedures, a substantiated SAE requires, at a minimum, an identified reporter (necessary for follow-up) and medical documentation of allleged symptoms.

7.4.2 SUEs

Neither COMPANY nor its responsible person has been notified by any person (end user, healthcare provider) or competent authority of any EU Member State of any SUEs associated with Product A. Product A has been sold in the EU since 2011.

The absence of any reported SUE associated with Product A is particularly noteworthy because of the reporting requirements in the EU. As explained above, under the Cosmetics Regulation and SUE Reporting Guidelines, if a competent authority was notified of an SUE associated with one of COMPANY's products, either directly by end users or health professionals or indirectly by COMPANY's responsible person or distributor, unless causality is excluded, the competent authority ***is required*** to notify COMPANY's responsible person, who would then inform COMPANY.

8. EXPERT SAFETY ASSESSMENT OF PRODUCT A

COMPANY sells Product A and other cosmetics in the EU and maintains a Product Information File (PIF) on its products in Brussels, as required under EU laws and regulations for cosmetic products. Included in the PIF is a safety assessment of the product by expert assessors. Attached as Annex 10[10] is the most recent safety assessment of Product A, prepared by an independent expert in chemistry, Veit Nitsche, PhD. Annex 10 includes Dr. Nitsche's CV. The safety assessment covered all of the ingredients in Product A. Below are excerpts related to DDDE.

8.1 Toxicolological Profile

"Ethyl Tafluprostamide: Ethyl Tafluprostamide is a prostaglandin derivative with fatsoluble properties. It is used as a conditioner in hair products applied to the hair. It is not irritating to skin. A sensitizing property is not known. A study of transdermal penetration of a topically applied prostaglandin reported penetration limited to the outer layer of the epidermis, which is devoid of blood vessels, and insignificant penetration to the vascularized dermis. (Transdermal delivery of prostaglandins, C.J.N. Oognejisofar, 1996, University of Saskatchwan.). Transdermal penetration is proportional to the concentration of prostaglandin in the solution. (Oognejisofar, 1996). In the 1996 study, a 0.05% of PGE1 had a skin penetration rate of 1% over a period of 24 hours. Because the concentration of ethyl tafluprostamide used in this product is lower (0.018 %) and any incidental skin exposure to the conditioner would be brief, the 1% transdermal penetration rate is an upper limit. A NOAEL for Ethyl Tafluprostamide is not available. A NOAEL for tafluprost from a carcinogenicity study is given as **0.03 mg/kg** body weight (Center For Drug Evaluation And Research 2011, Pharmacology/Toxicology NDA Review And Evaluation Of Tafluprost. Application Number: 202514orig1s000)."[21]

8.2 Determination of Systemic Exposure

Dr. Nitsche used the tafluprost NOAEL of 0.03 mg/kg body weight to calculate a Margin of Safety (MoS) for DDDE as an ingredient in Product A.

> "Ethyl Tafluprostamide: Ethyl Tafluprostamide is used in this product in a concentration of 0.018 %. If 0,0024 g Eyelash Conditioner is used, a systemic concentration of 0,000007 mg/kg could be achieved taking into account a skin penetration rate of 100 %. A **safety margin of 4286** is calculated from a NOAEL of 0.03 mg/kg body weight (NOAEL is derived from a comparable Tafluprost study). *A risk to human health can thus be excluded and this substance can be assessed as safe in the given concentration*."[22] (emphasis added)

The MoS calculated for DDDE by Dr. Nitsche was **4,286** mg/kg bw/d. This MoS was calculated assuming a 100% dermal absorption rate for DDDE (which Dr. Nitsche considered a theoretical upper limit). Even with that very generous skin penetration rate, the MoS for DDDE far exceeds the safety threshold for a safe cosmetic ingredient of 100.

8.3 Expert Conclusion

Dr. Nitsche concluded, "***A risk to human health can thus be excluded and this substance can be assessed as safe in the given concentration***."

9. SUMMARY OF KEY POINTS

1. This report presents data and other information relevant to the CIR's safety assessment of the use of the prostaglandin analog (PGA), Ethyl Tafluprostamide, as an ingredient in

[21] Annex 7, pages 5-6.

[22] Annex 7, page 9.

cosmetics. Another name for Ethyl Tafluprostamide is Dechloro Dihydroxy Difluoro Ethylcloprostenolamide (DDDE).

2. COMPANY, is a U.S.-based international cosmetics company whose product portfolio focuses on products intended to enhance the beauty and health of ***hair***, including eyelashes, eyebrows and scalp hair. Some of COMPANY's cosmetic products include small amounts of DDDE.

3. DDDE is not an active ingredient in any approved drug or medicinal product and is not synthesized from any prostaglandin that is an active ingredient in an approved drug/medicinal product.

4. All of COMPANY's products, including those that contain DDDE, are marketed exclusively as cosmetics with intended uses that are limited to effects ***on hair*** (not the body) and are consistent with cosmetic use generally as a hair conditioner. None of COMPANY's products, including those that contain DDDE, make any claims indicating an intended use for therapeutic or medical purposes or to affect the structure or function *of the body*. The intended use of COMPANY's products that contain DDDE clearly fall within the statutory definition of a cosmetic in the U.S.

5. Four prostaglandins or PGAs are active ingredients in FDA approved drugs intended for use to treat glaucoma by lowering intraocular pressure (IOP). These PGAs are referred to herein as "ophthalmic PGAs." The ophthalmic PGAs are applied directly to eyes as eye drops. DDDE is not a derivative of any ophthalmic PGA and there is no evidence that DDDE lowers IOP or has any other effect on ocular physiology.

6. This report focuses on safety data obtained in tests of COMPANY's ***eyelash*** product (referred to herein as "**Product A**"). While DDDE is an ingredient in COMPANY's products applied to eyebrows or scalp hair, because Product A is applied near eyes it has been the most extensively tested to ensure that its safety is scientifically substantiated.

7. The data provided in this report are ***product-based*** data, meaning they were obtained using the 19-ingredient formulation sold as Product A. COMPANY currently is working with multiple commercial laboratories to conduct *in vitro* safety tests on DDDE itself that will be used by a toxicology firm, ToxMinds, to perform a comprehensive toxicological analysis and prepare a safety dossier. The safety dossier on DDDE will be provided to the SCCS (in Europe) for its safety assessment as well as to the CIR as a supplemental report to this submission. COMPANY expects to submit the supplemental report to CIR in August-September 2023. A roadmap of tests currently in progress and estimated timelines is provided in section 2.5 of this report.

8. Product A is an eyelash conditioner that is applied with a multi-use fine brush applicator as a thin line directly to ***eyelashes*** (primarily the upper eyelashes) above the lash line. Product A is formulated with a ***thickener*** (cellulose gum) to ensure that the product stays on the eyelashes where it is applied and does not migrate to contact the fluid or membranes surrounding the eye. Product A was designed to affect the appearance of eyelashes and NOT to come in contact with eyes.

9. A highly qualified pharmacokinetic expert, Dr. Ian Wilding, assessed the likely local (eyelid) and systemic exposure to DDDE with normal and even errant use (misapplication) of Product A. According to Dr. Wilding, "[b]ecause of the cellulose gum in the product, which acts to increase viscosity, minimal amounts of Product A, if any, are likely to migrate" to the eyelid skin or the eye. Dr. Wilding concluded that there is "***negligible risk***" of DDDE having a physiological effect on the eyelid skin or on the eye

10. Photographic tests conducted by an independent ophthalmologist researcher documented that Product A stays where it is applied and does not migrate to contact eyes. Fuorescent dye was added to Product A and slit-lamp photography was used to photograph the distribution of Product A + dye after normal application by human volunteers. ***Product A remained where it was applied and none entered the fluid around the eye.***

11. Potential dermal and/or ocular exposure are important factors in evaluating the safe use of any ingredient in cosmetics. When compared to other cosmetics applied near eyes, mascara and eyeliner, there is actually ***less*** potential for dermal or ocular exposure with Product A. Product A and mascara are both applied directly to ***eyelashes***, which decreases the risk of dermal exposure. However, the risk of ocular exposure is *greater* with mascara than Product A because of how they are applied. A fairly large amount of mascara is applied along the full length of eyelashes, whereas a relatively small amount of Product A is applied as a fine line to the eyelashes above the lash line. Because eyeliner is applied to eyelid ***skin*** it has the greatest risk of dermal absorption.

12. Industry standard tests for cosmetic products have shown individually and collectively that Product A is ***safe to the skin***. These tests include tests of antimicrobial effectiveness, mutagenic potential and skin irritation.

13. Product A was shown to be ***safe to the eyes*** and has ***less ocular irritation potential than mascara and eyeliner*** *in* the *in vitro* HET-CAM Membrane Assay, which determined that Product A has *"practically no potential for ocular irritation in humans*." Further, Product A was less irritating than the mascara and eyeliner products that were tested at the same time.

14. Product A also was shown to be ***safe to eyes*** in human use studies conducted under the supervision of an ophthalmologist who examined the eyes and measured IOP at the beginning and end of the 28-day study. The ophthalmologist reported that Product A is safe and non-irritant to the eyes. In addition, ***there was no statistically significant change in IOP after 28 days of Product A use.*** These data show there is no pharmaceutical effect on eyes with normal use of Product A.

15. In contrast to Product A, in a clinical study of Latisse, an FDA approved drug for growing eyelashes, there was a *statistically significant decrease in IOP* over the course of the study. The different effect of Product A and Latisse on IOP is consistent with the fact that Product A contains a *thickener* to keep it where it is applied and Latisse does not. Latisse is a relabeled glaucoma eye drop that is watery, which increases the potential for dripping into the eye. Another difference is that Latisse is applied to the eyelid whereas Product A is applied to eyelashes. The differences between Product A and Latisse on IOP illustrate that evaluation of

the effect of any ingredient (including a PGA) in an eyelash product must take into account product properties and how/where the product is applied.

16. The potential ocular exposure to DDDE with normal use of Product A is *de minimis* and well below the dose range of the ophthalmic PGAs. The dose range of PGA applied *directly to the eye* in each ophthalmic PGA is **1.16 µg – 9.0 µg**. In comparison, on average **0.432 µg of DDDE** is applied per brushstroke to *eyelashes.* Even the maximum amount of DDDE applied *to eyelashes*, **0.72 µg**, could not expose the eye to even the lowest end (**1.16 µg**) of the pharmaceutical dose range of the ophthalmic PGAs, which are applied directly to *eyes*. Multiple lines of evidence show that Product A does not migrate from eyelashes to contact eyes, but even if some of the DDDE applied did migrate to the eyes or reached the eyes through dermal absorption it would be a *de minimis* amount that is well below the pharmaceutical range of the ophthalmic PGAs.
17. According to two experts, systemic exposure to DDDE is highly unlikely.
18. Consumer experience with Product A also supports that it is a safe cosmetic. The rate of adverse event reports is very low (0.154%, April 2022 – April 2023). Adverse reactions related to Product A are typical of other cosmetic products applied in the vicinity of the eyes (e.g., mascara and eyeliner) and mostly involve allergic reactions that resolve with ceasing use of the product. Independent experts, including an ophthalmologist and a pharmacokinetics expert, who evaluated consumer complaints involving Product A concluded that none of the reported adverse reactions involved intraocular effects.
19. No substantiated serious unexpected effects (SUEs) or serious adverse effects (SAEs) related to Product A use have been reported to COMPANY.

10. CONCLUSIONS

Collectively, the data presented in this report demonstrate that Product A is safe to skin and eyes and is *at least as safe as mascara and eyeliner*.

The data presented in this report also demonstrate that products that contain a PGA ingredient can be designed to be used safely as cosmetics. Such products need to contain reasonable concentrations of PGA ingredients, be appropriately formulated to have sufficient viscosity to ensure the product does not migrate into the eye and have adequate directions for safe use. CIR could consider providing guidance to the industry on the formulation of safe cosmetics that contain a PGA and recommendations for adequate safety testing of such products.

As has been demonstrated with Product A, the safety of cosmetic eyelash products that contain a PGA can be demonstrated with objective scientific tests of skin and ocular irritation potential, as well as human studies demonstrating the lack of physiological effects of the product on eyes (e.g., lowering IOP) with normal use. In addition, going forward, under MoCRA data on AEs and SAEs will be available for all cosmetic products, including those with PGA ingredients. These data will provide important information that can be used to assess the safety of PGAs in cosmetic products.

In summary, the data provided in this report demonstrate that Product A is a safe cosmetic. While this report presented mostly product-based data, it follows that if Product A was shown to be safe to skin and to eyes when used under intended conditions of use as prescribed in product labeling, no ingredient in Product A has a harmful effect. Nonetheless, additional testing on DDDE itself is in progress so a complete toxicological analysis can be performed. These data and the safety assessment based on these data will be provided to CIR as a supplemental report, most likely in August-September 2023.

11. ANNEXES

[1] Annex 1: DDDE COA

[2] Annex 2: DDDE Safety Data Sheet

[3] Annex 3: Quantity of Product A and DDDE Applied per Brushstroke to Human Eyelashes

[4] Annex 4: Quantity of Product A Applied Per Brushstroke to Mink Hair Samples

[5] Annex 5: Curriculum Vitae of pharmacokinetics expert, Ian Wilding, PhD

[6] Annex 6: Pharmacokinetic Assessment of Product A by Ian Wilding, PhD

[7] Annex 7: Comparison of Amount of PGA Applied per Application – Product A vs. FDA Approved Drugs

[8] Annex 8: Expert Opinion of Factual Accuracy of SCCS PGA Opinion by Ian Wilding, PhD.

[9] Annex 9: Expert Assessment by Paul Donzis, MD, of Potential Ocular and Systemic Exposure to DDDE With Use of Product A

[10] Annex 10: Safety Assessment of Product A by Veit Nitsche, PhD (for EU Product Information File)

Annex 1
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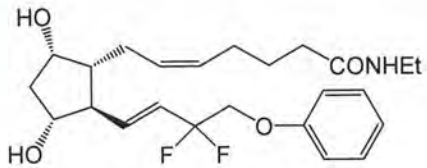
[redacted]

[redacted]

CERTIFICATE OF ANALYSIS

COA No.: TAFEA-F-019-001

Product: Dechloro Dihydroxy Difluoro Ethylcloprostenolamide (7.5% by weight in 2-phenoxyethanol)
Chemical Structure:



CAS: 1185851-52-8
MF: $C_{24}H_{33}F_2NO_4$
MW: 437.52

Manufacturing Date: December 2018
Release Date: 28-JAN-2019
Re-Test Date: 28-JAN-2023

Batch/Lot: TAFEA-F-1218-01
Storage: 2-8°C, well-closed containers

Test for	*Specification*	*Result*
Description	Colorless to pale yellow solution	Conforms
Identity 01	NMR, IR, or HPLC	Conforms
Identity 02	LCMS M+1 = 438.3	Conforms
Purity: **(including isomers NMT 2.5%)**	NLT 99.00%	99.84%
Other Impurities	NMT 1.00%	0.16%

Result: Product Conforms to Specifications.

Quality Control: [redacted] *Date:* 28 Jan 2019

Approved: [redacted] *Date:* 29 JAN 2019

Quality Control Laboratory
[redacted]

[redacted]

Annex 2

Safety Data Sheet

Dechloro Dihydroxy Difluoro Ethylcloprostenolamide

10% in Ethyl Alcohol

Revision Date: 26-OCT-2017

Section 1: Product Name and Company Identification

1.1 Product Identifier

Product Name:	Dechloro Dihydroxy Difluoro Ethylcloprostenolamide
Chemical Family:	Prostaglandins
Synonyms:	N-ethyl-9alpha, 11alpha-dihydroxy-15,15-difluoro-16-phenoxy-17,18,19,20 tetranor-prosta-5Z,13E-dien-1-amide Ethyl Tafluprostamide 3D
CAS #:	1185851-52-8

1.2 Relevant identified uses of the substance or mixture and uses advised against

Identified Uses: Laboratory chemicals, Manufacture of substances

1.3 Details of the supplier of the safety data sheet

Company Name: [redacted]

Emergency Contact: Industrial Environmental Contracting (IEC)
Phone: 732-662-7222

Section 2: Hazards Identification

2.1 Classification of the substance or mixture

GHS Classification in accordance with 29 CFR 1910 (OSHA HCS)
Flammable liquids, (Category 2)
Eye irritation (Category 2A), H319
Acute toxicity, Oral (Category 4), H302
Toxic to reproduction, (Category 1B), H360
Target organ systemic toxicity, (Category 3)

2.2 GHS Label elements, including precautionary statements

Pictogram:

Signal word: Warning

Hazard statements:

H225	Highly flammable liquid and vapor.
H302	Harmful if swallowed.
H319	Causes serious eye irritation.
H360	May damage fertility or the unborn child.

Prevention statements:

P201	Obtain special instructions before use.
P210	No smoking. Keep away from heat, sparks, open flames, and hot surfaces.
P264	Wash skin thoroughly after handling.
P270	Do not eat, drink or smoke when using this product.
P280	Wear protective gloves/ eye protection/ face protection.

Response statements:

P301, P312	If swallowed, call a Poison Center or doctor if you feel unwell.
P308, P313	If exposed or concerned, get medical attention/advice.
P330	Rinse mouth.
P305, P351, P338	If case of contact with eyes, flush eyes with plenty of water. After initial flushing, remove contact lenses, if present. Continue rinsing for several minutes.
P337, P313	If eye irritation persists, get medical advice/ attention.
370, P378	In case of fire: Use dry sand, dry chemical or alcohol-resistant foam to extinguish.
P405, P233, P235	Store in well-ventilated place. Keep container tightly closed. Keep cool.
P501	Dispose of contents/ container to an approved waste disposal plant.

Section 3: Composition/Information on Ingredients

3.1 Hazardous Components

CAS #	Chemical Name	Composition
1185851-52-8	Dechloro Dihydroxy Difluoro Ethylcloprostenolamide (3D)	10.0%
64-17-5	Ethyl alcohol	90.0%

For the full text of the H-Statements, see Section 16

Section 4: First Aid Measures

4.1 General Advice:

Consult a physician. Show this safety data sheet to the doctor. Move out of dangerous area.

If Inhaled:
Move person into fresh air. If not breathing, give artificial respiration. Consult a physician.
In case of skin contact:
Wash off with soap and plenty of water. Remove contaminated clothing/ shoes, and consult a physician if symptoms occur. Wash clothing before reuse.
In case of eye contact:
Rinse eyes thoroughly with plenty of water for at least 15 minutes. Have eyes examined by medical personnel.
If swallowed:
Never give anything by mouth to an unconscious person. Rinse mouth with water. Consult a physician. Do NOT induce vomiting unless directed to do so by medical personnel.

4.2 Most important symptoms and effects, both acute and delayed

The most important symptoms and effects are described in the labelling (see section 2.2) and/or in section 11. Exposure can cause: diarrhea, dizziness, fever, flushing, headache, hypotension, nausea, shivering, vomiting. May cause anemia, cough, CNS depression, drowsiness, headache, heart damage, lassitude, liver damage, narcosis, reproductive effects, and/or teratogenic effects.

4.3 Indication of any immediate medical attention and special treatment needed

No data available.

Section 5: Fire Fighting Measures

5.1 Extinguishing media

Suitable media: Use water spray, alcohol-resistant foam, dry chemical or carbon dioxide.
Unsuitable media: A solid water stream may be insufficient.

5.2 Flammable properties and hazards

Can release vapors that form explosive mixtures at temperatures at or above the flashpoint.
Container explosion may occur under fire conditions.
Emits toxic fumes under fire conditions.
Sensitive to static discharge.
Vapors can travel to a source of ignition and flash back.
Hazardous decomposition products (carbon oxides) formed under fire conditions.
Flash point: 14°C (closed cup)
Autoignition: 393°C
Explosive limits: LEL: 3.3% at 25°C UEL: 19.0% at 25°C

5.3 Advice for firefighters

Wear self-contained breathing apparatus for firefighting if necessary. Wear full protective gear to prevent contact with skin and eyes. Material is flammable as it is diluted in ethyl alcohol.

5.4 Further Information

Use water spray to cool unopened, fire-exposed containers.

Section 6: Accidental Release Measures

6.1 Personal precautions, protective equipment and emergency procedures

Use personal protective equipment. Avoid breathing vapors, mist or gas. Ensure adequate ventilation. Remove all sources of ignition. Evacuate personnel to safe location. Beware of vapors accumulating to form explosive concentrations. Vapors can accumulate in low areas. For personal protection see section 8.

6.2 Environmental precautions

Do not let product enter drains.

6.3 Methods and materials for containment and cleaning up

Soak up with inert absorbent material and dispose of as hazardous waste. Keep in suitable, closed containers for disposal. Dispose of according to local regulations.

Section 7: Handling and Storage

7.1 Precautions for safe handling

Avoid contact with skin and eyes. Avoid inhalation of vapor or mist.
Use explosion-proof equipment. Keep away from sources of ignition - No smoking.
Take measures to prevent the buildup of electrostatic charge.
For precautions see section 2.2.

7.2 Conditions for safe storage, including any incompatibilities

Keep container tightly closed in a cool, dry and well-ventilated place.
Keep away from heat, sparks, and flame.
Recommended storage temperature 2-8°C.
Store under inert gas.
Hygroscopic.

7.3 Specific end use(s)

Apart from the uses mentioned in section 1.2 no other specific uses are stipulated.

Section 8: Exposure Controls/Personal Protection

8.1 Control parameters

Components with workplace control parameters

CAS #	Chemical Name	Value	Control Parameters	Basis
1185851-52-8	Dechloro Dihydroxy Difluoro Ethylcloprostenolamide (3D)	No data.	No data.	No data.
64-17-5	Ethyl alcohol	TWA STEL	PEL 1,000 ppm	USA. ACGIH Threshold Limit Values (TLV)

64-17-5	Ethyl alcohol (continued)	TWA	1,000 ppm 1,900 mg/m^3	USA. Occupational Exposure Limits (OSHA) - Table Z-1 Limits for Air Contaminants
64-17-5	Ethyl alcohol (continued)	TWA	1,000 ppm 1,900 mg/m^3	USA. NIOSH Recommended Exposure Limits

8.2 Exposure controls

Appropriate engineering controls

Handle in accordance with good industrial hygiene and safety practice. Wash hands before breaks and at the end of workday. Use process enclosures, local exhaust ventilation, or other engineering controls to control airborne levels.

Personal protective equipment

Eye/face protection

Use equipment for eye protection tested and approved under appropriate government standards such as NIOSH (US) or EN 166(EU).

Skin protection

Handle with compatible chemical-resistant gloves. Gloves must be inspected prior to use. Use proper glove removal technique (without touching glove's outer surface) to avoid skin contact with this product. Dispose of contaminated gloves after use in accordance with applicable laws and good laboratory practices. Wash and dry hands.

Body Protection

Wear flame retardant and antistatic lab coat/protective clothing. The type of protective equipment must be selected per concentration and amount of dangerous substance at the specific workplace.

Respiratory protection

Where risk assessment shows air-purifying respirators are appropriate use a full-face respirator with multi- purpose combination (US) or type ABEK (EN 14387) respirator cartridges as a backup to engineering controls. If the respirator is the sole means of protection, use a full-face supplied air respirator. Use respirators and components tested and approved under appropriate government standards such as NIOSH (US) or CEN (EU).

Control of environmental exposure

Do not let product enter drains.
Do not release to the environment.

Section 9: Physical and Chemical Properties

9.1 Information on basic physical and chemical properties

Appearance	Form: Liquid solution
Odor	No data available.

Melting point	No data available.
Boiling point	No data available.
Flash point	14°C (57.2°F) - closed cup
Evaporation rate	No data available.
Flammability (solid, gas)	No data available.
Upper/lower flammability or explosive limits	Upper explosion limit: 19% (V) at 25°C. Lower explosion limit: 3.3% (V) at 25°C.
Vapor pressure	43 mm Hg at 20°C.
Vapor density	No data available.
Relative density	No data available.
Water solubility	No data available.
Autoignition temperature	363°C (685.4 °F).
Viscosity	No data available.
Explosive properties	No data available.
Oxidizing properties	No data available.

Section 10: Stability and Reactivity

10.1 Reactivity

No data available.

10.2 Stability

Stable under recommended storage conditions.

10.3 Possibility of hazardous reactions

No data available.

10.4 Conditions to avoid

Heat, flames, sparks.

10.5 Incompatible materials

Alkali metals, strong oxidizing agents, peroxides, ammonia, and bases.

10.6 Hazardous decomposition products

Carbon oxides (CO, CO_2)

Other decomposition products - No data available.

In the event of fire: see Section 5.

Section 11: Toxicological Information

11.1 The toxicological effects of this compound have not been thoroughly studied.

Routes of entry

Eye contact, inhalation, ingestion.

Toxicity to animals

Ethyl alcohol: Acute oral toxicity (LD50): 10,470 mg/kg (Rat).
Acute dermal toxicity (LD50): 15,800 mg/kg (Rabbit).
Acute inhalation toxicity (LC50): 30,000 mg/L – 4 hour (Rat).

Irritation/corrosion

Ethyl alcohol: Skin irritation (rabbit): moderate, 20mg (24 hr).
Eye irritation (rabbit): moderate (OECD Test Guideline 405)

Chronic effects on humans

Ethyl alcohol - Investigated as a mutagen, reproductive effector, and tumorigen.

Carcinogenicity – Ethyl alcohol

Carcinogenicity - Mouse - Oral
Tumorigenic: Equivocal tumorigenic agent by RTECS criteria. Liver: Tumors. Blood: Lymphomas including Hodgkin's disease.
Ethyl alcohol RTECS number: KQ6300000. See RTECS entry for complete information.
IARC: No component of this product present at levels greater than or equal to 0.1% is identified as probable, possible or confirmed human carcinogen by IARC.
NTP: No component of this product present at levels greater than or equal to 0.1% is identified as a known or anticipated carcinogen by NTP.
OSHA: No component of this product present at levels greater than or equal to 0.1% is identified as a carcinogen or potential carcinogen by OSHA.

Further toxicological information

The toxicological effects of this product have not been thoroughly studied.

Section 12: Ecological Information

12.1 Toxicity

Avoid release into the environment. Runoff from fire control or dilution water may cause pollution.

Ethyl alcohol:

Toxicity to fish:	(LC50) - Pimephales promelas (fathead minnow) - 14,200 mg/l - 96 h
Toxicity to daphnia and other aquatic invertebrates:	(LC50) - Ceriodaphnia dubia (water flea) - 5,012 mg/l - 48 h
	NOEC - Daphnia magna (Water flea) - 9.6 mg/l - 9 d
Toxicity to algae:	(EC50) - Chlorella vulgaris (Fresh water algae) - 275 mg/l - 72 h (OECD Test Guideline 201)

12.2 Persistance and degradability

Ethyl alcohol: Biodegradability - Result: 95 % - Readily biodegradable.

Section 13: Disposal Considerations

13.1 Waste disposal method

Dispose in accordance with local, state and federal regulations.

Section 14: Transportation Information

14.1 DOT (US) – Land transport

Proper shipping name: Ethyl alcohol solution
Hazard class: 3 – FLAMMABLE LIQUID
UN/NA Number: 1170
Packing Group: II
Labels:

14.2 IMDG – Sea transport

Proper shipping name: Ethyl alcohol solution
Hazard class: 3 – FLAMMABLE LIQUID
UN Number: 1170
Packing Group: II
EMS-No: F-E, S-D

14.3 IATA/ICAO – Air transport

Proper shipping name: Ethyl alcohol solution
Hazard class: 3 – FLAMMABLE LIQUID
UN Number: 1170
Packing Group: II
IATA Classification: 3

14.4 ADR/RID (Europe) – Land transport

Proper shipping name: Ethyl alcohol solution
Hazard class: 3 – FLAMMABLE LIQUID
UN Number: 1170
Packing Group: II

14.5 Additional transport information

Transport in accordance with local, state, and federal regulations.

Section 15: Regulatory Information

SARA 302 Components

No chemicals in this material are subject to the reporting requirements of SARA Title III, Section 302.

SARA 313 Components

This material does not contain any chemical components with known CAS numbers that exceed the threshold (De Minimis) reporting levels established by SARA Title III, Section 313.

SARA 311/312 Hazards

Fire Hazard, Acute Health Hazard, Chronic Health Hazard

Massachusetts/Pennsylvania/New Jersey Right To Know Components

Ethyl Alcohol CAS: 64-17-5 Revision Date: 2007-03-01

California Prop. 65 Components

This product does not contain any chemicals known to State of California to cause cancer, birth defects, or any other reproductive harm.

Section 16: Other Information

Full text of H-Statements referred to under sections 2 and 3

Acute Tox.	Acute toxicity
Eye Irrit.	Eye irritation
Flam Liq.	Flammable liquids
H225	Highly flammable liquid and vapor.
H302	Harmful if swallowed.
H319	Causes serious eye irritation.

HMIS Rating

Health Hazard:	2
Chronic Health Hazard:	*
Flammability:	3
Physical Hazard:	0

NFPA Rating

Health Hazard:	2
Fire Hazard:	3
Reactivity Hazard:	0

Company Policy or Disclaimer

DISCLAIMER: This information is believed to be accurate and represents the best information currently available to us. However, we make no warranty of merchantability or any other warranty, express or implied, with respect to such information, and we assume no liability resulting from its use. Users should make their own investigations to determine the suitability of the information for their purposes.

Revision Date: 26-OCT-2017

ANNEX 3

Amount of Product A Applied to Eyelashes Per Brushstroke

	A	B	C	D	E
Sample	**Applicator - Dry** Weight of Applicator Without Product A	**Applicator – Wet** Weight of Applicator With Product A	**Amount of Product A On Applicator** [B – A]	**Applicator – Post-Application (Single Brush Stroke)**	**Amount of Product A Applied to Eyelashes** [C – D]
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	**3838.2 mg**	**3844.5 mg**	**6.3 mg**	**3842.1 mg**	**2.4 mg**

Explanation of measurements: The fine brush applicator that is part of the Product A container was weighed dry (without any product on the brush) [Column A] and after the brush was dipped into the vial to coat the brush with Product A solution [Column B]. All measurements are in milligrams (mg). Ten separate brushes were used in the study ["Sample" Column]. The amount of Product A on each brush before application to the eyelashes is shown in Column C [Col. B – Col. A]. Each brush was weighed again immediately after it was used to apply Product A to the upper eyelashes, as directed by the package instructions for use. The amount of Product A applied to the upper eyelashes by each brush stroke is shown in Column E [Col. D – Col. B].

Amount of <u>Product A</u> applied to the eyelashes: The range of Product A that was applied per brushstroke to the upper eyelashes was **1-4 mg** and the <u>average</u> was **2.4 mg**. The <u>maximum</u> amount of Product A applied per brushstroke was **0.072 mg**.

Amount of <u>DDDE</u> applied to eyelashes per brushstroke: The concentration of DDDE in Product A is 0.018%. Therefore, the amount of DDDE that was applied to the eyelashes per brushstroke is:

Average: 0.018% x 2.4 mg = 0.00018 x 2.4 mg = 0.000432 mg (or **0.432 µg**)

Range: 0.00018 mg - 0.00072 mg (or **0.18 µg - 0.72 µg)**

Procedure 1:

In Procedure 1, the same tube of product was used with different applicators. Ten applicators were used; each applicator was used for 2 applications.

Insertion Method A: the applicator was inserted into the tube, applicator was twisted on all the way, removed, weighed, swiped across a measured length of mink eyelashes, and reweighed.

Insertion Method B: 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 reweighed.

Procedure 2:

In Procedure 2, the same applicator was used with different tubes of product. Each tube was used for 2 applications.

Insertion Method A: the applicator was inserted into the tube, applicator was twisted on all the way, removed, weighed, swiped across measure length of mink eyelashes, and reweighed.

Insertion Method B: 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 reweighed.

Measurements (in mg):

Procedure 1	**Average Amount Applied (mg)**
Insertion Method A	1.999
Insertion Method B	2.422
Procedure 2	
Insertion Method A	1.287
Insertion Method B	1.725

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CURRICULUM VITAE

CURRENT POSITIONS

Jan 15 - Non-executive Director, Locate Bio

Locate Bio is an innovative orthobiologics company with a proprietary, regenerative medicine pipeline, delivering exciting orthobiologics products that have great disruptive potential.

Jan 05 - President, Ian Wilding Associates Limited (IWAL)

IWAL is a company established to provide my consultancy services in early drug development, product lifecycle management, commercial /strategic management and due diligence. Through the Company, I am currently working with 30 pharmaceutical and biotech drug development companies in Europe, USA and Japan – over 85% of consultancy revenues arise from collaborations with non-UK companies.

Oct 01 - Special Professor, School of Pharmacy, Nottingham University

The School of Pharmacy at Nottingham is ranked 5th in the world in the 2021 QS World Rankings for pharmacy and pharmacology. The School came joint 1st in the UK on quality of research for Pharmacy Schools in the 2014 Research Excellence Framework and is the only School of Pharmacy to have 100% of research at 4* in the 'Impact on Society' category.

PREVIOUS ROLES

1990- 2004 Founder and Chief Executive, Pharmaceutical Profiles Limited (PP)

- PP was established as a spinout company from Nottingham University in 1990 to commercialise the use of radionuclide imaging to visualise drug delivery in the gastrointestinal and respiratory tract of healthy volunteers and patients.
- I exited from the company via a 3i backed MBO in 2005 and by that time I had developed the company into a niche exploratory clinical trials business employing 110 people and with a turnover of 10million GBP.
- In 2004, PP received Frost & Sullivan's prestigious Excellence in Technology Award for its pioneering efforts to enhance the adoption of innovative technologies in early phase drug development.
- As well as having management responsibility for PP, I also worked extensively in a business development role and via "peer to peer" selling interfaced with project teams in pharmaceutical and biotech companies in the US, Europe and Japan.
- In 1999, VC investment was obtained to fund the development of a remote controlled capsule (Enterion™) which enabled site specific delivery within the human intestine to evaluate drug absorption and therapeutic properties. The patent protected capsule, of which I was the key inventor, has now been dosed to several thousand subjects in over 250 clinical studies for worldwide pharma and biotech companies.

2002 - 2009 Non-Executive and Founding Director, BioCity Nottingham

BioCity Nottingham is the largest bioscience innovation & incubation centre in Europe and was established via a unique collaboration between Nottingham Trent University, the University of Nottingham and the East Midlands Development Agency.

2004 - 2013 Board Advisor, Molecular Profiles Limited

Molecular Profiles (now Juniper Pharma Services) is a contract research organisation providing global clients pharmaceutical development services (formulation & analytical development, clinical trial manufacturing up to Phase IIa), advanced analytical support and expert consultation for intellectual property issues. The Company was acquired by Columbia Labs in September 2013.

2006 - 2010 Non-Executive Chairman and Investor Director, R5 Pharmaceuticals Limited (R5)

R5 Pharmaceuticals was established in 2006 to provide formulation development, analytical chemistry and GMP services to the global biotech and pharmaceutical industry. The company was acquired by Aesica in June 2010 and is widely acknowledged as one of the leading providers of pharmaceutical dosage form development across Europe. Shortly after exit in 2010, we received the 'Venture Capital Backed Team of the Year' award from the British Venture Capital Association for our work at R5.

2007 - 2012 Non-executive Director, Photopharmica Limited (PPA)

PPA was a product development company focused on infection control and wound healing using photodynamic therapy. In October 2011, the Company announced that in a phase IIb study its lead product had produced a substantial and significant reduction in the bacterial load of chronic leg ulcers.

2007 - 2014 Director and Scientific Advisor, Modern Biosciences plc (MBS)

MBS is a drug development company that sources late-stage discovery projects from academia and spin-out companies, conducts early proof-of-principle clinical studies and subsequently out-licenses the resulting programs to the pharmaceutical industry.

2007 – 2020 Director and VP Development, Zysis Limited

Zysis was a specialty pharma company working on the reformulation, development and commercialisation of CNS products in the psychiatry and neurology arena. An oral aripiprazole once-weekly maintenance product intended to improve adherence and therefore clinical outcomes in schizophrenia and bipolar disorder therapy will shortly enter phase II development.

2009 - 2013 Board Advisor, Heron Evidence Development Limited

HERON was one of the largest independent providers of evidence-based strategy, research and communication services to global biopharmaceutical clients prior to its sale to Parexel. With expertise and methodologies that align evidence development and economic evaluation with pricing, reimbursement, and market access planning, the Company works with clients to quantify and communicate product value and commercial opportunity on a global basis.

2010 - 2013 Member of the Board of Directors at Bend Research

Based in Oregon USA, Bend Research specialises in the advancement, development and commercialisation of pharmaceutical & health science technologies. The Company focuses on developing a deep scientific understanding of clients' challenging drug development problems and by applying novel solutions advances difficult compounds to market. The Company was acquired by Capsugel in September 2013.

QUALIFICATIONS

2015	Doctor of Laws *honoris causa*, Monash University, Melbourne, Australia
2013	Fellowship of the Academy of Pharmaceutical Sciences of Great Britain
1985- 1988	Ph.D., Department of Pharmaceutical Sciences, Nottingham University
1984 -1985	MRPharmS, Royal Pharmaceutical Society of Great Britain
1981-1984	B.Pharm First Class, Department of Pharmaceutical Sciences, Nottingham University

FDA INTERACTION

1997 – 1998	Expert scientist for the FDA in the area of Food Effects on Drug Bioavailability
1999 onwards	Frequent speaker at internal FDA training meetings
2002	FDA Expert scientist at Advisory Committee on Food Effect Guidelines and BCS Development

PREVIOUS INNOVATION ROLES

2006 – 2008	FP7 Advisory Committee on SMEs, European Commission
2005 – 2006	Governance Board, Nottingham Science City
April 2004	EU Informal Competitiveness Council, Dromoland Castle (Irish Presidency)
2004 – 2006	Innovation EM (Science and Industry Council, East Midlands)
2003 – 2007	University of Nottingham Institute for Enterprise and Innovation (UNIEI) Advisory and Strategy Board
2002 - 2006	CBI Technology and Innovation Committee

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EXPERT REPORT OF DR IAN WILDING ON [redacted]

DATED: 14 AUGUST 2013

1. INTRODUCTION

1.1 I have worked as a pharmaceutical scientist for over 25 years. I obtained my PhD in 1988 from the Department of Pharmaceutical Sciences at Nottingham University and was the principal founder of Pharmaceutical Profiles, a phase 1 clinical research organisation, to commercialise the use of nuclear medicine imaging to visualise drug delivery in humans in 1990. I grew the company to over 100 people with an annual turnover of 10 million GBP before selling the business in 2005. During that time, I also co-supervised several PhD students on a variety of research topics within the field of pharmaceuticals. I received the Career Achievement Award in Oral Drug Delivery from the Controlled Release Society in 2005 and became a Special Professor in the School of Pharmacy at the University of Nottingham in 2001. In addition, I have just been made an Eminent Fellow of the Academy of Pharmaceutical Sciences of Great Britain

1.2 My curriculum vitae and a brief resumé are at **Exhibit IW1**.

1.3 Since leaving Pharmaceutical Profiles, I have consulted widely for pharmaceutical and biotechnology companies on formulation strategy for drug development, pharmacokinetic interpretation of clinical data and design of early stage exploratory clinical studies.

1.4 I have co-authored and published in excess of 250 scientific papers, abstracts and patents, many of them relating to the field of drug delivery and pharmacokinetics. A list of my publications is at **Exhibit IW2**.

1.5 I have been asked by [redacted] solicitors for [redacted] Inc. ("[redacted]"), to provide a report relating to [redacted] product [redacted] ("[redacted]"). I understand that this report will be submitted to the Medical Products Agency to assist them in their evaluation of eyelash products containing prostaglandin analogues ("**PGAs**"). I understand that, in the case of [redacted] product, the PGA is dechloro dihydroxy difluoro ethylcloprostenolamide ("**DDDE**").

1.6 I have previously provided several expert reports on Intellectual Property issues and regulatory matters around the use of sustained release formulations. In 1997 I was appointed as an expert scientist for the US Food and Drug Administration (USFDA) in the area of food effects on oral bioavailability. In addition, I was a frequent visitor to the USFDA giving several seminars and presentations on the use of imaging in drug development for a variety of dosage routes.

1.7 I confirm that I understand my duty as an expert and that I have complied with, and will continue to comply with, that duty.

2. INSTRUCTIONS

2.1 I have been asked to give my opinion on the following issues:

(a) The likely absorption by the skin or eye if [redacted] comes in contact with the eyelid skin or migrates to contact the outer surface of the eye.

(b) The likely physiological effects of DDDE on the eye (outer surface of the eye and intraocular).

(c) The likely exposure of the eyelid skin or the eye to DDDE with normal use of [redacted].

(d) The effects of DDDE on the eye as compared to other cosmetic products (such as mascara and eyeliner).

(e) The effects of DDDE (in [redacted]) on the eye as compared with the effects of ophthalmic PGAs used to treat glaucoma.

(f) The likelihood that the DDDE in [redacted] has systemic effects.

(g) Whether the current warning for pregnant and breast feeding women is necessary.

A full copy of the questions provided to me by [redacted] is at **Exhibit IW3** to this report.

2.2 For the purpose of this report, I have made reference to the following:

(a) a document bundle provided to me by [redacted] – the index for this document bundle is at **Exhibit IW4** to my report;

(b) a packaged sample of [redacted] provided to me by [redacted];

(c) European Medicines Agency "Clinical Investigation of Corticosteroids intended for use on the skin" (Guidance to Directive 75/318/EEC) 1987;

(d) GW Bean and CB Camras "Commercially Available Prostaglandin Analogs for the Reduction of Intraocular Pressure: Similarities and Differences" Survey of Ophthalmology 53 S1 (2008): S69 – S84;

(e) JL Cohen "Enhancing the Growth of Natural Eyelashes: The Mechanism of Bimatoprost-Induced Eyelash Growth" Dermatologic Surgery 36 (2010): 1361-1371 (I note that this paper was written by a consultant and clinical trial participant for Allergan, Inc);

(f) IM Haeck, TJ Rouwen, L Timmer-de-Mik, MS Bruin-Weller and CA Bruijnzeel-Koomen "Topical corticosteroids in atopic dermatitis and the risk of glaucoma and cataracts" J Am Acad Dermatol 64 (2011): 275-281;

(g) M-H Tan, M Lebwohl, AC Esser & H Wei "The penetration of 0.005% fluticasone propionate ointment in eyelid skin" J Am Acad Dermatol 45 (2001): 392-396;

(h) A Urtti "Challenges and obstacles of ocular pharmacokinetics and drug delivery" Adv Drug Delivery Rev 58 (2006): 1131–1135; and

(i) CG Wilson "Topical drug delivery in the eye" Experimental Eye Research 78 (2004): 737-743.

3. SUMMARY

3.1 Briefly, and for the reasons that I develop more fully in this report, my overall conclusion is that it is highly unlikely that the DDDE in [redacted] when applied as directed to the eyelashes, has a significant physiological effect on eyes or the rest of the body for that matter. Irrespective of whether [redacted] is applied to the eyelashes in accordance with the package insert directions or used incorrectly (e.g. applied directly to the eyelid or cornea), the quantity of DDDE that could be delivered to the eye is too low to exert any significant effect on eye physiology. In addition, due to the use of a "viscosity increasing agent" (thickener) in the [redacted] formulation, coupled with the small amount of DDDE applied to the eyelashes per brushstroke, DDDE is highly unlikely to penetrate the eyelid skin to any significant degree and therefore the chance of imparting a significant physiological effect on the body is negligible.

4. OPINION

4.1 First Question - Exposure of skin or eye to DDDE:

(a) *What is the likely exposure of the eyelid skin or the eye to DDDE with normal use of* [redacted] *applied in accordance with the instructions for use? Please consider the composition of* [redacted] *(e.g. thickener), the method and site of application and the amount of DDDE that potentially could contact the skin or the eye.*

(i) The instructions for product use are explained clearly in the [redacted] package insert (**Exhibit IW5**) and instruct the subject to apply a thin line of [redacted] *directly to the eyelashes*. According to "in use" studies provided in the document bundle (**Exhibit IW6**), typically 1 to 4mg of [redacted] is applied to the eyelashes per brush stroke. The concentration of DDDE in [redacted] is 0.018%. Therefore, at most, 0.72µg of DDDE (4mg of [redacted] x 0.00018 = 0.00072 mg (or 0.72 µg)) is applied to the eyelashes per brushstroke. [redacted] contains cellulose gum to act as a "viscosity increasing agent". While no data on the actual viscosity of [redacted] are available, based on my personal observation, the [redacted] formulation holds its form as an applied thin line along the eyelashes without propensity to drip. Therefore, it is highly unlikely any of the very small amount of DDDE that is applied to the eyelashes with normal use would be transferred onto the eyelid or into the eye.

4.2 **Second Question - Local absorption of DDDE:**

(a) *Skin: If* [redacted] *comes in contact with the eyelid skin, describe the likely absorption by the skin. Describe the layers of the skin and absorption by sequential layers. Describe the location of blood vessels in the skin layers and the likelihood that DDDE would penetrate to that layer and have access to blood vessels. Is there any likelihood that DDDE could migrate through all of the layers of the eyelid skin to contact the outer surface of the eye?*

(i) As a starting point, potential absorption of DDDE by the eyelid skin is dependent on the amount of DDDE that is likely to come in contact with the eyelid skin with normal use of [redacted]. As discussed above in 4.1(a)(i), [redacted] is applied directly to the eyelashes. Because of the cellulose gum in the product, which acts to increase viscosity, minimal amounts of [redacted] if any, are likely to migrate to the eyelid skin other than through misapplication. Since no more than 0.72µg of DDDE is applied per application to the eyelashes, the quantity of DDDE that is likely to migrate to the eyelid and be available for skin penetration is negligible.

(ii) The skin is the largest organ of the body, with a total area of about 20 square feet. It consists of three layers: epidermis (the outermost layer of skin); the dermis (beneath the epidermis which contains tough connective tissue, blood vessels, hair follicles, and sweat glands) and finally the deeper subcutaneous tissue (hypodermis) which is made of fat and connective tissue. The major barrier to permeation within the skin is the nonviable stratum corneum, the outermost cornified layer of the epidermis, usually 15– 20 cells thick and consisting of cells (corneocytes) that have lost their nucleus and all capacity for metabolic activity. A thick stratum corneum is responsible for the weak drug penetration in areas such as the palms of the hands and the soles of the feet. However, it is widely accepted the stratum corneum is at its thinnest on the eyelid (approximately 0.05 cm) and therefore rapid absorption could potentially occur.

(iii) For many years there was anecdotal evidence that the long-term application of topical corticosteroids to the periorbital region and eyelid in treatment of atopic dermatitis was associated with glaucoma[1], posterior subcapsular cataracts[2] and amaurosis[3]. Steroid penetration

[1] Glaucoma is a disease characterized by elevated intraocular pressure, which causes optic nerve damage and subsequent peripheral vision loss.

[2] A posterior subcapsular cataract occurs at the back of the lens. People with diabetes or those taking high doses of steroid medications have a greater risk of developing such a condition.

[3] Amaurosis is a condition of partial or total blindness, caused by a disease of the optic nerve.

through the eyelids was viewed as a possible explanation for the onset of these ocular complications. To address this issue, studies were undertaken to evaluate the penetration of fluticasone propionate ointment (0.005%) through human eyelid skin using modified Franz diffusion cells (see *Tan et al* J Am Acad Dermatol 2001 at **Exhibit IW7**). Only very small amounts of steroid were found to penetrate the eyelid skin (range 0.62% to 1.47%).

(iv) In 2011 *Haeck et a*l (J Am Acad Dermatol) reported the results of a large usage study which confirmed that topical application of steroids to the eyelids and periorbital skin is not associated with a significant risk for glaucoma or cataracts, even in cases of chronic, habitual use (**Exhibit IW8**).

(v) Therefore in situations where a drug containing corticosteroids is applied directly to the eyelid for treatment of topical disease there is (a) extremely limited skin penetration despite the thinness of the stratum corneum and (b) there are no resulting clinical sequelae even from long term chronic administration.

(vi) This is also consistent with the findings for bimatoprost in a review article from Cohen (see Dermatologic Surgery 2010 at **Exhibit IW9**) in which it is stated that low ocular levels occur when drug solution is applied topically *to the eyelid* margin and that the barrier formed by the skin, ensures that absorption of active drug across the cutaneous surface into ocular tissues is minimal.

(b) *Eye: If* [redacted] *migrates to contact the outer surface of the eye, describe the likely absorption by the eye. Describe the layers of the eye, including the tear film surrounding the eye, and absorption by the various layers. Describe the location of blood vessels in the layers of the eye. Describe which layers DDDE would need to access to have an intraocular effect (e.g. decrease in IOP).*

(i) For most of the drugs topically applied to the eye, the site of action is usually different layers of the cornea, conjunctiva, sclera, and the other tissues of the anterior segment such as the iris and ciliary body (anterior uvea). Upon administration to the surface of the eye, precorneal factors and anatomical barriers negatively affect the bioavailability of topical formulations. Precorneal factors include solution drainage, blinking, tear film, tear turnover and induced lacrimation. Tear film, of which composition and amount are determinants of a healthy ocular surface, offers the first resistance due to its high turnover rate. Mucin is a glycoprotein present in the tear film and plays a protective role by forming a hydrophilic layer that moves over the glycocalyx of the ocular surface and clears debris and pathogens. Human tear volume is estimated to be 7 µl, and the inferior conjunctival cul-de-sac can transiently contain around 30 µl of the administered eye drop. However,

tear film displays a rapid restoration time of only 2 to 3 min and most of the topically administered solutions are washed away within just 15 to 30 seconds after instillation. Considering all the precorneal factors, contact time with the absorptive membranes is low, which is considered to be the primary reason for less than 5% of the applied dose of an eye drop reaching the intraocular tissues (Urtti "Challenges and obstacles of ocular pharmacokinetics and drug delivery" at **Exhibit IW10**).

(ii) In addition to the tear film, various layers of the cornea, conjunctiva, and sclera have an important effect on drug permeation. The cornea, the anterior-most layer of the eye, is a mechanical barrier which limits the entry of exogenous substances into the eye and protects the ocular tissues. It can be divided into the epithelium, stroma and endothelium. Each layer offers a different polarity and a potential rate-limiting structure for drug permeation. The corneal epithelium is lipoidal in nature – it contains 90% of the total cells in the cornea and poses a significant resistance for permeation of topically administered hydrophilic drugs, which do not permeate well through lipoidal tissue. The stroma, which comprises 90% of the corneal thickness, poses a significant mechanical barrier to permeation of lipophilic drug molecules due to its thickness. Even though the endothelium is a separating barrier between the stroma and aqueous humour, it helps maintain the aqueous humour and corneal transparency due to its selective carrier-mediated transport and secretory function and, as a result, it also acts as an important barrier to penetration of substances into the eye.

(iii) Despite the selective barriers to corneal penetration outlined above, some drug absorption is possible across the cornea. By contrast conjunctival drug absorption is considered to be non-productive due to the presence of conjunctival blood capillaries and lymphatics, which can cause drug loss into the systemic circulation thereby lowering ocular bioavailability.

(iv) The sclera mainly consists of collagen fibres and proteoglycans embedded in an extracellular matrix. Permeability through the sclera is considered to be comparable to that of the corneal stroma (i.e. it poses a significant barrier to permeation of lipophilic drug molecules).

(v) Over the years, drug delivery scientists have designed and evaluated many formulation strategies to overcome these natural barriers to penetration and change the eye clearance kinetics to increase residence time and the potential for drug penetration. The most commonly used non-invasive approaches have involved the use of polymers to increase viscosity of the tear drops or the use of gelling materials to increase adhesion to the mucin layer covering the glycocalyx.

(vi) Human product visualization studies using the non-invasive imaging technique of gamma scintigraphy have been used for many years to assess the *in vivo* behaviour of ophthalmic formulations. Such investigations with hydroxyethylcellulose (HEC) formulations (0 to 0.5% w/v) of increasing viscosity (1 to 105 centipoise, respectively) showed both a significantly increased corneal residence time and slowed presentation to the nasolacrimal duct with increasing viscosity (Wilson; Experimental Eye Research 2004 – see **Exhibit IW11**). However, over 75% of the product was still cleared from the corneal region within *circa* 60 seconds following dosing with the 0.3% HEC formulation suggesting it is only possible to prolong the retention of a significant minority of the formulation using these polymer approaches.

(vii) As already mentioned [redacted] contains cellulose gum as a "viscosity increasing agent". This helps the formulation remain localised to the site of administration (i.e. on the eyelashes) after usage. I have considered whether this increased viscosity of [redacted] would reduce ocular clearance kinetics from the eye, should [redacted] be inadvertently administered by a user directly to the eye. To the naked eye the flow characteristics of the [redacted] formulation are not consistent with a high viscosity preparation. As a consequence, it is likely that the ocular clearance kinetics of [redacted], inadvertently administered in the eye, would be cleared from the eye at least at the same rate as the HEC formulations discussed earlier.

(viii) "In use" studies provided in the document bundle (**Exhibit IW6**) suggest that typically 1 to 4mg of [redacted] is applied to eyelash per brush stroke. The concentration of DDDE in [redacted] is 0.018% and therefore even under "worst case" scenario only 0.72µg (4mg of [redacted] could be administered into the eye assuming it was inadvertently applied to the surface of the eye. Based on the HEC experience *circa* 75% of that dose would be cleared within 60 seconds thereby minimizing any possibility of tissue penetration for pharmacological effect.

(ix) I have also been provided with a report from a study carried out by Dr Paul Donzis (**Exhibit IW12**). Dr Donzis used an ophthalmic slit lamp microscope to look for signs of migration of an earlier formulation of the product (that I am told had the same viscosity as [redacted]) from the eyelash margin into the eye itself. The photographs from this study (see **Exhibit IW13**) provide very graphical and clear support to the conclusion that little, if any, of the product contacted the eye. It is even less likely that [redacted], which is applied to the eyelashes, migrates into the eye.

(x) In summary, due to the increased viscosity of [redacted] and the barriers to permeation posed by the anatomical layers of the eye, it is highly unlikely for [redacted] to have a pharmacological effect on the eye when

applied as directed to the eyelashes. Similarly, inadvertent contact of [redacted] with the surface of the eye (e.g. a wayward brush stoke) is unlikely to have a pharmacological effect due to the small amount of [redacted] involved and the clearance properties of the eye.

4.3 **Third Question - Physiological effects of DDDE on the eye:**

(a) *Based on answers to (2) above, please describe the likely physiological effects of DDDE on the eye (outer surface of the eye and intraocular).*

(i) An analysis of the physiological effect of DDDE on the eye must first take into account where the product ([redacted]) is applied (to the eyelashes) and the likely exposure of the eye to DDDE. As discussed in 4.2, little, if any, [redacted] contacts the eye and, therefore, it is unlikely that a sufficient amount of DDDE permeates the cornea to have a pharmacological effect.

(ii) My analysis is supported by data showing that normal use of [redacted] has no effect on intraocular pressure (IOP). I have been provided with a report of a human study carried out by [redacted] Inc. in which the safety and tolerability of a product called '[redacted] 0.025%" (a previous formulation of [redacted]) was evaluated (**Exhibit IW14**). I have been informed by [redacted] that "TEA" is the same molecule as "DDDE", which is in the current formulation of [redacted]. I note that the concentration of TEA in the tested product in this study is slightly greater (0.025%) than the concentration of DDDE (0.018%) in [redacted]. The study involved 20 female volunteers who self-administered the preparation daily after being given instructions to apply product along the upper eyelashes in the same way they would apply eyeliner for a period of four weeks. I note that the instructions on how to apply [redacted] have since changed so that [redacted] is now applied *to the eyelashes themselves*, rather than as eyeliner (**Exhibit IW5**). A certified ophthalmologist measured the IOP in each eye of each user on day 0 and day 28. The results of this "in-use" study, using user controlled administration, demonstrate DDDE does not cause a significant reduction in IOP.

(iii) Topical ocular prostaglandin agonists are used to treat elevated IOP by improving the drainage of ocular aqueous humour from the anterior chamber angle. In a review article from Cohen (Dermatologic Surgery 2010) (**Exhibit IW9**), it is estimated that using the supplied applicator a single application of bimatoprost (0.03%) solution to the upper eyelid margin, as advised in the patient information leaflet for Latisse (**Exhibit IW15**), delivers approximately 5% of the dose (by weight) to the eye compared to an eye drop for the treatment of glaucoma. This routine, chronic inadvertent daily delivery of low amounts of bimatoprost to the eye (when used for the treatment of hypotrichosis of the eyelashes) led

to a lowering of IOP but the magnitude of any IOP lowering was not cause for any clinical concern. As discussed in 4.2, the amount of DDDE delivered to the cornea following inadvertent administration of RLA via poor user technique is likely to be much more limited than for Latisse in view of the incorporation of a thickener in [redacted] which is not present in Latisse. It is therefore hard to imagine on a scientific basis how [redacted] could have any effect on IOP at all.

4.4 **Fourth Question – Comparison to other cosmetic products used near the eye:**

(a) *Please provide your observations on the effects of DDDE on the eye as compared to other cosmetic products (such as mascara and eyeliner).*

(i) I have been provided with a toxicology report testing the irritation potential of the product by testing it *in vitro* on the chorioallantoic membrane (CAM) of a chick embryo, conducted by [redacted] and dated 2 October 2009 (**Exhibit IW16**). The CAM model has been shown over many years to be a qualitative method of assessing the potential irritancy of chemicals. It responds to injury with an inflammatory process similar to that observed in the conjunctival tissue of a rabbit's eye and its well-developed vascularization provides an ideal model for ocular irritation studies. The study undertaken for [redacted] compared ocular irritation potential for TEA versus two routinely used commercially cosmetic products (Almay One Mascara and Maybelline Waterproof Eyeliner). The results showed that all three products had no potential to cause *in vivo* ocular irritation.

(ii) This is consistent with my review of the customer complaints reported to [redacted] from 25 October 2011 to 10 January 2013 related to use of [redacted] (**Exhibit IW17**). All of the complaints are minor and transient in nature, and do not indicate an intraocular effect.

4.5 **Fifth Question – Comparison with ophthalmic PGAs:**

(a) *Four ophthalmic PGAs are ingredients in commercially available prescription drugs used to treat glaucoma: Lumigan (bimatoprost); Zioptan (tafluprost); (Travatan) travoprost; and Xalatan (latanoprost). Patient information leaflets for these drugs (and for Latisse, which is the same formulation as Lumigan, but is not sold for treatment of glaucoma, rather as a prescription eyelash treatment available in the US) have been provided to you. The manufacturers of these PGAs recommend them for reducing intraocular pressure ("IOP"). Please provide your observations on the effects of DDDE (in [redacted]) on the eye as compared with the effects of the ophthalmic PGAs.*

(i) As discussed above, it is unlikely that sufficient amounts of DDDE contact the surface of the eye or permeate to intraocular layers of the eye to have a pharmacological effect.

4.6 **Sixth Question - Systemic effects of DDDE:**

(a) *What is the likelihood that the DDDE in [redacted] has systemic effects?*

(i) It is my view that there is a negligible likelihood that the DDDE in [redacted] has systemic effects irrespective of whether it is administered in accordance with the package insert (on the eyelashes) or used incorrectly e.g. applied directly to the eyelid or cornea.

4.7 **Seventh Question - Label warning for pregnant or breast feeding women:**

(a) *Based on the answer to (6) above, in your opinion, are the current label warnings necessary?*

(i) As the product has not been tested under all possible conditions, [redacted] have taken the prudent approach of including a warning on the package insert for pregnant women and nursing mothers not to use [redacted]. However, the negligible likelihood that the DDDE in [redacted] has systemic effects warrants a further discussion on the necessity of this labelling from a safety perspective.

STATEMENT OF TRUTH

I confirm that insofar as the facts stated in my report are within my own knowledge I have made clear which they are and I believe them to be true, and that the opinions I have expressed represent my true and complete professional opinion.

Signed I. Wilding

Dr Ian Wilding

Date: 14th August 2013

LIST OF EXHIBITS

Exhibit IW1	Curriculum vitae and brief resumé for Dr Ian Wilding
Exhibit IW2	Publications by Dr Ian Wilding
Exhibit IW3	Questions posed by [redacted] Inc., April 2013
Exhibit IW4	Index for bundle of documents supplied for review with April 2013 questions
Exhibit IW5	[redacted] Product Information Leaflet
Exhibit IW6	Report by [redacted] to determine the average amount of [redacted] applied to the upper eyelashes per application, dated 11 January 2013
Exhibit IW7	M-H Tan, M Lebwohl, AC Esser & H Wei "The penetration of 0.005% fluticasone propionate ointment in eyelid skin" J Am Acad Dermatol 45 (2001): 392-396
Exhibit IW8	IM Haeck, TJ Rouwen, L Timmer-de-Mik, MS Bruin-Weller and CA Bruijnzeel-Koomen "Topical corticosteroids in atopic dermatitis and the risk of glaucoma and cataracts" J Am Acad Dermatol 64 (2011): 275-281
Exhibit IW9	JL Cohen "Enhancing the Growth of Natural Eyelashes: The Mechanism of Bimatoprost-Induced Eyelash Growth" Dermatologic Surgery 36 (2010): 1361-1371 (written by a consultant and clinical trial participant for Allergan, Inc)
Exhibit IW10	Urtti A "Challenges and obstacles of ocular pharmacokinetics and drug delivery" Adv Drug Delivery Rev 58 (2006): 1131–1135
Exhibit IW11	CG Wilson "Topical drug delivery in the eye" Experimental Eye Research 78 (2004):737-743
Exhibit IW12	Study by Dr Paul Donzis, dated 10 March 2009
Exhibit IW13	Photographs accompanying Study by Dr Paul Donzis dated 10 March 2009
Exhibit IW14	Controlled Human Use Test ([redacted]), dated 8 January 2010
Exhibit IW15	Latisse Patient Information Leaflet - revised June 2012

Exhibit IW16	Toxicology report performed by [redacted] dated 21 October 2009
Exhibit IW17	Customer Complaints received by [redacted] for period 25 October 2011 to 10 January 2013

	Latanoprost (Xalatan®)	Travoprost (Travatan-Z®)	Tafluprost (Zioptan®)	Bimatoprost (Lumigan®)	Bimatoprost (Latisse®)	DDDE (Product A)
Concentration (%)	0.005%[1]	0.004%[2]	0.0015%[3]	0.03%[4]	0.03%[5]	0.018%
Concentration (mg/ml)	0.05[6]	0.04	0.0150	0.30[7]	0.30[8]	0.180
Drops/mL[9]	29.5	34.6	n/a[10]	33.3	n/a	n/a
Quantity of *solution* applied / application	0.0338 mL[11]	0.0289 mL[12]	0.15 mL[13]	0.03 mL[14]	0.0015 mL[15]	2.4 mg
Quantity of *prostaglandin* analog applied per application	0.001695 mg[16] 1.7 µg	0.00116 mg[17] 1.16 µg	0.00225 mg[18] 2.25 µg	0.009 mg[19] 9.0 µg	0.00045 mg[20] 0.45 µg	0.000432 mg 0.432 µg
Intended Use	Treat Glaucoma	Treat Glaucoma	Treat Glaucoma	Treat Glaucoma	Treat Alopecia (Grow Eyelashes)	Eyelash Conditioner
Site of application	Eye	Eye	Eye	Eye	***Skin*** at base of upper eyelashes	***Eyelashes***
Method of application	Eye drop	Eye drop	Eye drop	Eye drop	Fine Brush Applicator	Fine Brush Applicator
Contains Thickener	No	No	No	No	No	**Yes**
Effect of product on IOP	Decreases	Decreases	Decreases	Decreases	**Decreases**	**None**

1 Xalatan package insert, http://www.xalatan.com/content/prescribing-information.aspx

[2] Travatan-Z package insert, http://www.alcon.com/en/alcon-products/glaucoma.aspx.

[3] Zioptan package insert, http://www.merck.com/product/usa/pi_circulars/z/zioptan/zioptan_pi.pdf.

[4] Lumigan package insert, http://www.lumigan.com/

[5] Latisee package insert, http://www.lumigan.com/, https://media.allergan.com/actavis/actavis/media/allergan-pdf-documents/product-prescribing/20170829-LATISSE-USPI-72303US17.pdf.

[6] Concentration expressed as a percentage represents the number of grams per 100 mL of solution for wt/vol concentrations. (H. Vance, PharmD, JD; http://en.wikipedia.org/wiki/Mass_concentration_(chemistry)#Usage_in_biology). 0.005% = 0.005 mg/100 mL = 0.05 mg/mL.

[7] Lumigan package insert,

[8] Lumigan package insert.

[9] VHA Drug Class Review: Ophthalmic Prostaglandin Analogs, Pharmacy Benefits Management Strategic Healthcare Group, Table 12 (June 2011)

[10] Unlike the other ophthalmic prostaglandin analogs, Zioptan is *preservative free*. (See Therapeutic Class overview: Ophthalmic Prostaglandin Analogues, UMass Medical School (May 2012), https://www.medicaid.nv.gov/Downloads/provider/Ophthalmic%20Prostaglandin%20Analogues.pdf. It is supplied as a sterile solution in translucent low density polyethylene *single-use containers* packaged in foil pouches (10 single-use containers per pouch). Each single-use container has 0.3 mL solution corresponding to 0.0045 mg tafluprost. Each container has enough solution to treat both eyes, so 0.00225 mg (0.0045 mg/2 = 0.00225 mg) would be applied to each eye.

[11] 1 mL / 29.5 drops/mL = 0.0338 mL/drop.

[12] 1 mL / 34.6 drops/mL = 0.0289 mL/drop.

[13] Zioptan package insert, Section 2; Zioptan website, ("Each single-use container contains enough solution to treat both eyes.") http://www.zioptan.com/zioptan/hcp/dosing-information.html.

[14] There are 33.3 drops/mL. Each drop = 1 mL / 33.3 drops = 0.03 mL.

[15] According to the manufacturer (Allergan), the amount of bimatoprost solution applied per application of Latisse is approximately 5% of the amount of bimatoprost solution applied as eye drop of Lumigan. (Allergan report to FDA Advisory Committee on Latisse clinical study, p. 5.)

[16] 1 mL = 0.05 mg. 1 mL = 29.5 drops. Each drop = 0.05 mg/mL / 29.56 drops = 0.001695 mg/drop.

[17] 1 mL = 0.04 mg. 1 mL = 34.6 drops. Each drop = 0.04 mg/mL / 34.6 drops = 0.00116 mg/drop.

[18] Each container contains 0.0045 mg tafluprost and enough solution to treat both eyes. (See Note 10, above.) 50% of 0.0045 mg = 0.0025 mg.

[19] 1 mL = 0.30 mg. 1 mL = 33.3 drops. Each drop = 0.30 mg/mL / 33.3 drops = 0.009 mg/drop.

[20] 5% of Lumigan.

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Annex 8

SECOND EXPERT REPORT OF DR IAN WILDING ON [redacted]

DATED 21ST NOVEMBER 2021

1 Introduction

1.1 I have worked as a pharmaceutical scientist for over 30 years. I obtained my PhD in 1988 from the Department of Pharmaceutical Sciences at Nottingham University and was the principal founder of Pharmaceutical Profiles, a phase 1 clinical research organisation, to commercialise the use of nuclear medicine imaging to visualise drug delivery in humans in 1990. I grew the company to over 100 people with an annual turnover of 10 million GBP before selling the business in 2005. During that time, I also co-supervised several PhD students on a variety of research topics within the field of pharmaceuticals. I received the Career Achievement Award in Oral Drug Delivery from the Controlled Release Society in 2005 and became a Special Professor in the School of Pharmacy at the University of Nottingham in 2001. In 2015, I received an honorary doctorate from Monash University in recognition of my outstanding contribution as a scientific innovator and leader in drug development. I am also an Eminent Fellow of both the Academy of Pharmaceutical Sciences of Great Britain and the Controlled Release Society.

1.2 My up-to-date summary curriculum vitae is at **Exhibit IW18**.

1.3 Since leaving Pharmaceutical Profiles, I have consulted widely for pharmaceutical and biotechnology companies on formulation strategy for drug development, pharmacokinetic interpretation of clinical data, design of early-stage exploratory clinical studies and CMC regulatory strategy. In particular, my expertise has been relied upon by the US FDA (Food & Drug Administration), the UK MHRA (Medicines & Healthcare products Regulatory Agency) and various European health regulatory authorities.

1.4 I have co-authored and published in excess of 250 scientific papers, abstracts and patents, many of them relating to the field of drug delivery and pharmacokinetics. An updated list of my publications is at **Exhibit IW19**.

1.5 I have been asked by [redacted], solicitors for [redacted] ("[redacted]"), to review an opinion issued by the Scientific Committee on Consumer Safety (**SCCS**) dated 27 September 2021 on "Prostaglandins and prostaglandin-analogues used in cosmetic products", which sought to review two prostaglandin analogues ("**PGAs**") used in eyelash conditioners, namely Isopropyl Cloprostenate (CAS 7 157283-66-4) (which I will refer to as "**ICP**" for brevity) and Ethyl Tafluprostamide or dechloro dihydroxy difluoro ethylcloprostenolamide, abbreviated commonly to "**DDDE**" (CAS 1185851-52-8) (the "**SCCS Report**"). DDDE is the PGA used in [redacted] ("[redacted]"). I understand that this report will be submitted to the SCCS in [redacted] follow up to the SCCS report to assist the SCCS in their evaluation of the safety of DDDE for use in eyelash products.

1.6 I have previously provided an expert report on the safety of the cosmetic product [redacted] in a report dated 14 August 2013. I understand that this was submitted as Annex 6 to [redacted] original submission to the European Commission

call for data on prostaglandins and their analogues used in cosmetic products, that submission dated 20 October 2020 ("[redacted] **2020 Submission**").

1.7 I confirm that I understand my duty as an expert and that I have complied with, and will continue to comply with, that duty.

2 Instructions

2.1 I have been asked to give my opinion on the following issues raised in the SCCS Report:

(a) At page 21 of the SCCS Report, it states that "*Within the remit of the SCCS, safety assessment are based on assessment of the ingredients and not cosmetic formulations. Test results relating to cosmetic formulations have therefore not been taken into consideration in this Opinion.*"

(i) **First question:** If an ingredient is only presented in a final formulation and experimental data indicates that final formulation is safe for cosmetic use, is it reasonable to conclude that the ingredient itself is safe at the effective dilution level for that particular formulation?

(ii) **Second question:** In this instance, with the final formulation for [redacted], is it reasonable to rely on the data submitted to the SCCS to assess whether DDDE is safe up to a concentration level of 0.018%? If so, why is that? If not, why not?

(b) **Third question:** At page 40 of the SCCS Report in Annex 1, the SCCS concludes, under the sub-title "*Human data*": "*For pharmacological treatment of intra-ocular pressure, a daily dose of one drop with a PGA is prescribed. This implies, depending on the type of analogue, a dose of 0.75 – 2.5 µg per eye per day. In the absence of data on skin absorption from the application of an eyelash growth formulation, assuming a dermal absorption of 50% and full transfer from the eye-lid conjunctiva to the eye, a maximum exposure of the eye of 0.36 µg DDDE and 2.5 µg isopropyl cloprostenate can theoretically be estimated. These doses are in the same order of magnitude as those used for the epi-ocular pharmacological treatment of intra-ocular pressure.*"

(i) Is this calculation of a maximum exposure of the eye of 0.36 µg correct?

(ii) Can you comment on this conclusion?

(c) **Fourth question:** At page 40 of the SCCS Report in Annex 1, under the sub-title "*Human data*", the SCCS concludes: "*The SCCS review of the open literature has indicated that PGAs caused serious adverse effects in ocular and periocular tissues in some glaucoma patients after direct eye applications (Nakakura et al., 2015, Shah et al., 2013; Wang et al., 2014). These data indicate a concern for the manifestation of serious and irreversible histological changes after consumer exposure to the PGAs in cosmetic products.*"

(i) Do you share that concern?

(ii) If so, why? If not, why not?

(d) **Fifth question:** Throughout the SCCS Report, there are references to PGAs having the potential to cause effects at very low concentrations and the intended use in the proximity of the eye (see, for example, page 3, lines 29-31 of the SCCS Report). What do you understand "very low concentrations" to mean?

2.2 For the purpose of this report, I have made reference to the following documents:

(a) the SCCS Report

(b) [redacted] 2020 Submission

(c) "*A Review of the Main Considerations for Formulation Development in Preclinical Toxicology Studies*", Saunders, International Journal of Toxicology 2021, Vol. 40(6) 551–556 ("Saunders, 2021") (**Exhibit IW20**)

(d) "*Tolerable Levels of Nonclinical Vehicles and Formulations Used in Studies by Multiple Routes in Multiple Species With Notes on Methods to Improve Utility*", Gad et al, International Journal of Toxicology 2016, Vol. 35(2) 95-178, (**Exhibit IW21**)

(e) "*Amount of* [redacted] *and DDDE Applied Per Brush Stroke to Eyelashes*", (**Exhibit IW22**) (also at page 102 of [redacted] 2020 Submission)

(f) "The SCCS Notes of Guidance for the Testing of Cosmetic Ingredients and their Safety Evaluation, 11th Revision", 30-31 March 2021, ("**SCCS/1628/21**") (**Exhibit IW23**)

(g) ECHA "*Guidance on information requirements and chemical safety assessment Chapter R.7c: Endpoint specific guidance*", published in June 2017 (**Exhibit IW24**)

(h) "*Modeling the human skin barrier — Towards a better understanding of dermal absorption*", O.G. Jepps et al, Advanced Drug Delivery Reviews 65 (2013) 152–168 at 156, column 1, lines 5-8 (**Exhibit IW25**)

(i) "*Enhancing the Growth of Natural Eyelashes: The Mechanism of Bimatoprost-Induced Eyelash Growth*", Cohen, Dermatological Surgery 2010 Sep;36(9):1361-71 at (**Exhibit IW9** to my first report)

(j) [redacted] Evaluation of Ocular Safety (**Exhibit IW14** to my first report)

(k) Package insert for [redacted] (also at Annex 7 at page 84 of [redacted] 2020 Submission) (**Exhibit IW26**)

(l) "*A new look at the safety and tolerability of prostaglandin analogue eyedrops in glaucoma and ocular hypertension*", Katsanos et al, Expert Opinion on Drug Safety, 16 June 2021 (https://www.tandfonline.com/doi/full/10.1080/14740338.2022.1996560) (**Exhibit IW27**)

3 Summary

3.1 Briefly, and for the reasons that I develop more fully in this report, my overall conclusion is that, based on the totality of the available evidence, it would not have been scientifically unreasonable for the SCCS to reach a conclusion as to the safety of DDDE when used at a concentration of 0.018% in [redacted]. I understand the concern raised by the SCCS around the impact of ingredients applied close to the eyes however users of [redacted] are provided clear instructions to apply the product directly to

the eyelashes. Therefore, with correct usage, the quantity of DDDE that is likely to migrate to the eyelid and be available for dermal absorption (and thereby lead to local adverse effects) is negligible. In addition, I don't share the concern posed by the SCCS that in the rare situation that consumers use the product entirely incorrectly and apply 100% of the dose to the eyelid, the resulting eye exposure would be comparable to that of a 9µg dose of Bimatoprost (the closest predicate PGA to DDDE) dosed correctly directly to the eye for glaucoma treatment. Finally, there is compelling evidence of excellent safety (local & systemic) for a range of topically applied PGAs applied directly into the eye in drops and used for multiple months of treatment in glaucoma. I accept that with the chronic long-term administration of PGAs for therapeutic purposes, there is an increased risk of topical adverse effects. However, all the available "long-term safety and tolerability evidence" for [redacted] demonstrate that there are no reports of such topical adverse effects which is consistent with the extremely limited risk of any eye exposure from [redacted] containing 0.018% DDDE used correctly via application to the eyelashes or via dermal penetration of product applied incorrectly to the eyelids.

4 Opinion

4.1 ***First question:*** *If an ingredient is only presented in a final formulation and experimental data indicates that final formulation is safe for cosmetic use, is it reasonable to conclude that the ingredient itself is safe at the effective dilution level for that particular formulation?*

(a) In the toxicological safety assessment of an active pharmaceutical ingredient (**API**) in drug development, it is rarely possible to administer neat API to any preclinical animal species by most dosage routes. Therefore, in the majority of cases, it is necessary to give the test item as part of a formulation which may vary between simple solutions or suspensions to complex drug delivery systems depending on the API biopharmaceutical properties e.g. solubility and proposed route of administration e.g. oral, topical.

(b) It therefore seems a logical corollary that if the final product for cosmetic use is at the simple end of the spectrum of possible formulation strategies that the safety of the ingredient, within specific concentration ranges, could be extrapolated from the prior human experience of the finished product.

(c) In [redacted] 2020 Submission, [redacted] was commonly referred to as "*a preserved and thickened aqueous-based mixture of mainly emollient, skin conditioning and humectant agents used in formulating an eyelash conditioning solution*". Similar language is also used in the Intertek Toxicology Assessment provided in the same submission at Annex 5 (page 57). However, it appears that nobody has drilled down further on the broader role and quantities of excipient contained in [redacted]. To my mind that assessment is important in considering the suitability of [redacted] as a useful surrogate for a DDDE safety assessment formulation and therefore the potential of the entire [redacted] package to help assuage possible safety concerns around use of DDDE in a cosmetic setting.

(d) As a skilled practitioner in the design of simple formulations for toxicology safety assessment of APIs, I bracketed the main [redacted] excipients into differing functional categories:

(i) ***Solvents:*** Water is the primary solvent ([redacted]). Glycerol (present at [redacted]), whilst not selected for this purpose in [redacted], is commonly used as a

cosolvent in formulations so, in a broader deconstruction assessment of the [redacted] product, it could be considered in this context such that the overall solvent % is 98.337%.

(ii) ***Buffers:*** It is common for a solution/suspension formulation to be buffered. Disodium phosphate ([redacted]) and phosphoric acid ([redacted]) are incorporated for this purpose, i.e. 0.172% in total.

(iii) ***Preservatives:*** For a simple multi-dose toxicology formulation, preservatives would be a consideration and, in this case, phenoxyethanol ([redacted]) and chlorphenesin ([redacted]) are incorporated i.e. 0.601% in total.

(iv) **Suspending or thickening agent:** Cellulose derivatives are commonly used for suspension formulations to ensure content uniformity - hydroxyethyl cellulose could fulfil such a role and is present in [redacted] at [redacted].

(e) This means that, in addition to the concentration of the ingredient of interest, DDDE (0.018%), the sub-total of all the drug and traditional safety assessment excipients suitable for a simple safety assessment formulation amount to 99.488% of the RLA product.

(f) My perspective is reinforced by a couple of recent review articles. Saunders, 2021, (Exhibit IW20) explores the main considerations for the development of a formulation for preclinical safety assessment testing detailing both classes of excipients and their function specifically listing buffers and cosolvents. In the introduction at page 551, Saunders says:

"…*it is necessary that any formulation is shown to be stable and homogeneous under the conditions of the preclinical study and will need to be demonstrated by subsampling of the formulations during every GLP study. This is not just for reasons of quality alone but is also for ethical reasons. If a study is conducted and the formulation is found to be unstable, for example, the test item has precipitated out of solution prior to or during the dosing of the animals, this will affect the dosage that the animal received and require a repeat of that study"*

(g) An authoritative review by Gad et al, 2016, Exhibit IW21) identifies tolerable levels of nonclinical vehicles and formulations used in studies by multiple routes in multiple species and specifically references the majority of the [redacted] excipients described above in table 4 at pages 100-109.

(h) Therefore, 99.5% of [redacted] can scientifically be argued to reflect a simple buffered, preserved solution/suspension formulation which would be designed to evaluate safety, tolerability etc for an API. The alternative construct is that only 0.5% of the excipients in the [redacted] product "prevent" the formulation from being considered as a simple presentation of DDDE for safety assessment purposes.

(i) In the report reference is often made that the SCCS remit requires that "*safety assessments are based on assessment of ingredients and not cosmetic formulations*". However, as argued above, presentation via a formulation provides the basis for such an assessment and whilst [redacted] per se is not designed for such a purpose only 0.5% of the product excipients would not be standard for that goal. It therefore seems a strange scientific decision to completely disregard a wide body of existing toxicological and human investigations in considering safety of DDDE at exactly the concentration utilised in [redacted] especially when 99.5% of the product is consistent with the principles of a formulation developed for such a safety assessment.

(j) It is therefore my contention that the data from a 99.5% "standard" formulation presentation could have been considered as body of evidence by the SCCS when assessing possible safety concerns for DDDE without materially diluting its remit to assess ingredients only.

4.2 **Second question:** In this instance, with the final formulation for [redacted], is it reasonable to rely on the data submitted to the SCCS to assess whether DDDE is safe up to a concentration level of 0.018%? If so, why is that? If not, why not?

(a) As discussed at length in response to Q1, I don't believe that it is unreasonable to consider the entire set of available data to address the narrowly drafted question posed to the SCCS about DDDE. In fact, I would argue that it is scientifically perverse to ignore such an extensive data set which includes human testing and substantial market safety data. I would have much more sympathy with the position of SCCS if the question had not been qualified by concentration and had been more generically posed around safety of DDDE at any concentration, which is not generally realistic or appropriate and is not what is asked here.

(b) I note that the final remark from the SCCS in the report is they will be ready to assess any evidence provided to support the safe use of PGAs in cosmetic products. I hope that now the SCCS has further evidence on the formulation principles delineated in response to Q1 above that would be required to test DDDE at all, that the Committee will be willing to consider [redacted] as a sufficiently simple formulation for it to act as surrogate for an ingredient safety assessment for DDDE in concentrations up to 0.018% (i.e. the maximum concentration at which all the testing has been done across all the tests (with one test at a higher concentration of 0.025%) and in a formulation supplied to consumers). This is especially important given the necessity to give the test item (DDDE) as part of such a formulation to make such an evaluation in the first instance

4.3 **Third question:** At page 40 of the SCCS Report in Annex 1, the SCCS concludes, under the sub-title "Human data": "*For pharmacological treatment of intra-ocular pressure, a daily dose of one drop with a PGA is prescribed. This implies, depending on the type of analogue, a dose of 0.75 - 2.5 µg per eye per day. In the absence of data on skin absorption from the application of an eyelash growth formulation, assuming a dermal absorption of 50% and full transfer from the eye-lid conjunctiva to the eye, a maximum exposure of the eye of 0.36 µg DDDE and 2.5 µg isopropyl cloprostenate can theoretically be estimated. These doses are in the same order of magnitude as those used for the epi-ocular pharmacological treatment of intra-ocular pressure.*" Is this calculation of a maximum exposure of the eye of 0.36 *µ*g correct? Can you comment on this conclusion?

(a) My PhD was on the use of modelling and simulation ("**M&S**") to predict the in vivo behaviour of drug delivery systems based on a range of inputs. In the absence of actual in vivo data, M&S (however simple) can play an invaluable role in helping to establish understanding and qualify risk, so I support the idea of scenario mapping as envisaged by the SCCS. However, the credibility of any simulation/ projection/ prediction is critically dependent on the quality of the assumptions and the rigour in their application. With that sentiment, I am struggling to understand and justify the approach used by the SCCS to assert a maximum exposure to the eye of 0.36 *µ*g DDDE following use of [redacted].

(b) The context of this assessment in Annex 1 of the SCCS report suggests it was one of the important factors considered by the Committee in reaching their decision to

conclude that there is a basis for serious concern around the use of DDDE (as a prostaglandin analogue) in cosmetic products. As a consequence, I feel it is important to review the assumptions and context around this assessment in detail and evaluate the validity of the conclusion.

(c) As a reminder, the instructions for [redacted] use are to apply a thin line of product directly to the eyelashes. According to "in use" studies (Exhibit IW22), typically 1 to 4mg of [redacted] is applied to the eyelashes per brush stroke. The concentration of DDDE in [redacted] is 0.018%. Therefore, at most, 0.72 µg of DDDE (4mg of [redacted] x 0.00018 = 0.00072 mg (or 0.72 µg)) is applied to the eyelashes per brushstroke. [redacted] contains cellulose gum to act as a "viscosity increasing agent". While no data on the actual viscosity of [redacted] are available, based on my personal observation, the [redacted] formulation holds its form as an applied thin line along the eyelashes without propensity to drip. Therefore, it is highly unlikely any of the very small amount of DDDE that is applied to the eyelashes with normal use would be transferred onto the eyelid or into the eye – therefore the quantity of DDDE that is likely to be misapplied to or migrate to the eyelid and be available for dermal absorption with correct use of the product is negligible.

(d) However, in the rare situation that consumers use the product entirely incorrectly and apply 100% of the dose (0.72 µg) to the eyelid and none to the eyelashes what is the likely outcome? The SCCS reference in their report an excerpt from the ICP ingredient dossier in which the calculated dermal absorption (via QSAR, Episuite 1.0) was 10% on the basis of a molecular weight of 476D and a partition coefficient of 5.15. However, for the purposes of the estimate of maximum exposure, the SCCS choose to ignore the calculated value of 10% for ICP and utilise 50% for both ICP and DDDE as the default figure according to SCCS/168/21 (Exhibit IW23) where "*only inadequate dermal absorption data are available*".

(e) The most recent version of this ECHA "*Guidance on information requirements and chemical safety assessment Chapter R.7c: Endpoint specific guidance*" was published in June 2017 ("**ECHA Guidance**") (Exhibit IW24). That guidance states at page 228 that "*Percutaneous absorption through intact skin is highly dependent on the physico-chemical properties of substances, and in particular of molecular weight and lipophilicity. Molecules above a certain molecular weight are unlikely to cross intact skin, and substances which are either too lipophilic or too hydrophilic have a low skin penetration. Cut off points at a molecular weight of 500 and log P values below -1 or above 4 have been used to set a conservative default absorption factor at 10% cutaneous absorption*".

(f) A further decision tree is provided in the ECHA Guidance, Figure R.7.12-5 on the broader interpretation of dermal absorption risk which allows for latitude when the ingredient doesn't meet the absolute criteria around molecular weight and lipophilicity.

(g) Both ICP or DDDE meet the criteria of log P above 4 (5.15 and 5.03, respectively) but are smaller than the 500Da molecular weight cut-off. However, as reported above, the EPIsuite software calculated a 10% dermal absorption for ICP which is presumably due to the very high lipophilicity of this PG analogue, despite not meeting the molecular weight cut-off. This is consistent with established transdermal science that for "*highly hydrophilic drugs… penetration is limited primarily by the [stratum corneum], while for increasingly lipophilic drugs the effects of the aqueous layer become more significant "choking" the flux of such drugs into the skin…*" (O.G. Jepps et al, Advanced Drug Delivery Reviews 65 (2013) 152–168 at 156, column 1, lines 5-8 (Exhibit IW25)).

(h) Moving back to the current SCCS report, I believe the decision to select a default 50% dermal absorption for both ICP and DDDE is overly simplistic. In terms of ICP, it would

have been scientifically sensible to consider the 10% calculated dermal absorption value which would lower the estimate of maximal exposure from 2.5µg to 0.05µg. Overall the biopharmaceutics properties of ICP and DDDE are similar with nearly identical lipophilicity (and thereby choking effect on penetration). As a consequence, it would not be unreasonable to assume a 10% dermal absorption for DDDE in the absence of the corresponding calculated value. However, taking a highly conservative approach, I have assumed that the dermal penetration for DDDE was double IPC at 20%.

(i) The low suggested dermal penetration for DDDE is also consistent with the findings for Bimatoprost which, as a prostamide analogue, is in the same structural class as DDDE. In a review article from Cohen (see Dermatological Surgery 2010 Sep;36(9):1361-71 at **Exhibit IW9** to my first report) it is stated that low ocular levels occur when drug solution is applied topically to the eyelid margin and that the barrier formed by the skin, ensures that absorption of active drug across the cutaneous surface into ocular tissues is minimal.

(j) Leaving other reasonable scientific assumptions aside, including that [redacted] is not applied to the eyelids other than in error in minimal quantities, based on a 20% dermal penetration estimate of the entire amount that is applied, the maximum exposure of the eye to DDDE from [redacted] applied inadvertently to the eyelid would be 0.144 µg.

(k) The SCCS state in Annex 1 of the report that "*For pharmacological treatment of intra-ocular pressure, a daily dose of one drop with a PGA is prescribed. This implies, depending on the type of analogue, a dose of 0.75 - 2.5 µg per eye per day*". I am struggling with this interpretation of current clinical practice in IOP treatment by the SCCS. It is my understanding from the [redacted] 2020 Submission that Latanoprost, Travoprost, Tafluprost and Bimatoprost are used at the following dose per application 1.7µg, 1.16µg, 2.25µg and 9µg so a range of 1.16 - 9µg not 0.75 - 2.5 µg.

(l) Bimatoprost is often grouped with the other prostaglandin analogues but as a prostamide, it is structurally diverse from the others. As already discussed, DDDE is structurally similar to Bimatoprost and structurally diverse from Latanoprost, Travoprost and Tafluprost.

(m) The SCCS state that the maximum exposure of DDDE in the eye is "*in the same order of magnitude as those used for pharmacological treatment of intra-ocular pressure (IOP)*". I disagree with this assertion.

(n) Based on the analysis above the comparison of relevance is 0.144 µg for DDDE using a conservative 20% dermal penetration from [redacted] applied incorrectly to the eyelid compared with the 9µg dose for Bimatoprost dosed correctly to the eye as a topical drop for direct delivery. I don't view these two values as being comparable.

(o) My analysis is supported by data showing that normal use of [redacted] has no effect on IOP. I have been provided with a report of a human study carried out by [redacted] in which the safety and tolerability of a product called "[redacted] 0.025%" (a previous formulation of [redacted]) was evaluated (**Exhibit IW14** to my first report, also at Annex 11 at page 132 of [redacted] 2020 Submission). I have been informed by [redacted] that "TEA" is the same molecule as "DDDE", which is in the current formulation of [redacted]. I note that the concentration of TEA in the tested product in this study is greater (0.025%) than the concentration of DDDE (0.018%) in [redacted]. The study involved 20 female volunteers who self-administered the preparation daily after being given instructions to apply product along the upper eyelashes in the same way they would apply eyeliner for a period of four weeks. I note that the instructions on how to apply

[redacted] differ in that [redacted] is applied to the eyelashes themselves, rather than as eyeliner (current instructions in package insert for [redacted] at **Exhibit IW26** (also at Annex 7 at page 84 of [redacted] 2020 Submission)). A certified ophthalmologist measured the IOP in each eye of each user on day 0 and day 28. The results of this "in-use" study, using user-controlled administration, demonstrate DDDE does not cause a significant reduction in IOP, even where a large proportion (if not 100%) is applied to the eyelid itself.

4.4 **Fourth question:** At page 40 of the SCCS Report in Annex 1, under the sub-title "*Human data*", the SCCS concludes: "*The SCCS review of the open literature has indicated that PGAs caused serious adverse effects in ocular and periocular tissues in some glaucoma patients after direct eye applications (Nakakura et al., 2015, Shah et al., 2013; Wang et al., 2014). These data indicate a concern for the manifestation of serious and irreversible histological changes after consumer exposure to the PGAs in cosmetic products.*" Do you share that concern? If so, why? If not, why not?

(a) I am not a clinical practitioner skilled in the treatment of glaucoma so cannot comment on the nuances of the long-term safety of PGAs in treatment of ophthalmic disease and its subsequent impact on consumer use of cosmetic PGA products. However, I am a skilled drug development scientist, and I am clear that the risk/benefit analysis for any specific drug therapy requires significantly more rigour that a general, non-specific search of the open literature. In my own search, I found a very recent authoritative, broad-ranging review (October 2021) published in "Expert Opinion on Drug Safety" on the "*safety and tolerability of prostaglandin analogue eyedrops in glaucoma and ocular hypertension*" (Katsanos et al 2021 (Exhibit IW27)). The review critically examines key evidence, and identifies gaps in current knowledge, on the safety and tolerability of available and emerging IOP-lowering PGAs.

(b) The authors state "*As a drug class, PGAs demonstrate several advantages compared to the other available IOP-lowering medications: they are presently the most efficacious class of medications for 24-hour IOP lowering, they possess an excellent systemic safety profile, and they are conveniently dosed once daily. These advantages explain why PGAs currently comprise the first line and first choice therapy in managing glaucoma. However, despite the availability of a significant body of evidence concerning their efficacy, the long-term safety and tolerability profiles of these as well as other available IOP-lowering drugs, have attracted less attention. In part, the scarcity of long-term safety and tolerability evidence with PGAs may reflect regulatory requirements that focus mostly on short-term efficacy outcomes. Indeed, registration trials are principally designed to detect differences (or lack thereof) in IOP lowering over a period of a few months and this is the standard time period of safety and tolerability monitoring of the various topical glaucoma medications.*". The authors go on to state that the *"treatment of glaucoma is lifelong and can last for decades in many patients. Consequently, the true safety and tolerability profile of a given agent may only become evident from long-term post-marketing studies and cumulative clinical experience following years of administering therapy*".

(c) I interpret the Katsanos et al review paper as providing evidence of excellent safety (local or systemic) for a range of topically applied PGAs applied directly into the eye in drops and used for multiple months of treatment. However, on chronic long-term administration, there is an increased risk of topical adverse effects, such as

conjunctival hyperaemia, pigmentation of the iris and/or periocular skin, hypertrichosis, periorbital fat atrophy, and ocular surface irritation.

(d) The authors conclude that "*for the reasons delineated in this review PGAs have evolved into an indispensable therapeutic option in the current glaucoma armamentarium. Indeed, no other IOP-lowering drug class appears to offer similar advantages as yet. Their versatility has enabled clinicians worldwide to employ PGAs in several clinical algorithms. Consequently, PGAs have successfully been employed in stepwise glaucoma therapy as fixed, or unfixed options. Thus, it is reasonable to assume that PGAs will continue to play a key role in glaucoma therapy of the future.*"

(e) This suggests that the limited risk of local adverse effects with topical PGA therapy is vastly outweighed by the clinical benefit of their effectiveness in treatment of glaucoma and is a perception that is unlikely to change in the near future.

(f) I accept the risk/benefit analysis will be different for the use of PGAs in consumer cosmetic products. However, crucially the already low risk of these topical adverse effects with IOP PGA therapeutics is further reduced with a 0.018% DDDE product such as RLA in the consumer setting. The increased viscosity of [redacted] and the barriers to permeation posed by the anatomical layers of the eye, ensure it is highly unlikely [redacted] will have a pharmacological effect on the eye when applied as directed to the eyelashes.

(g) Finally, Intertek evaluated Undesirable effects (UEs) that had been reported by [redacted] users over the course of two years (2011 - 2013) to an EU distributor. During that time, 125,409 units of [redacted] had been sold by the distributor. A total of 9 self-reported, but unconfirmed, UEs were reported to the distributor (and no SUEs – I understand from [redacted] that none have ever been reported to date). That is 0.00717% of the number of sold units. Intertek's assessment of these reported UEs was that they "*suggest that some sensitive individuals may adversely react to this product*." However, Intertek went on to conclude that, "*Under normal or reasonably foreseeable conditions of use, a product made to this formulation is unlikely to produce an abnormally high number of adverse reactions. The product gives users the level of safety they can reasonably expect when used as directed.*"

(h) In [redacted] 2020 Submission, [redacted] summarised these UE observations as follows: "*the adverse reactions/UEs that have been reported by users of [redacted] are typical in nature to those associated with other cosmetic products used in the vicinity of the eyes, specifically mascara and eyeliner.*"

(i) It therefore appears from the "long-term safety and tolerability evidence" for [redacted] that there are no reports of the local adverse effects that have been reported with chronic long-term treatment of glaucoma following direct instillation of pharmacological relevant doses into the eye.

(j) Therefore, for the reasons cited in response to this question (and others in this report), I don't concur with the SCCS that these data indicate a concern for the manifestation of serious and irreversible histological changes after consumer exposure of DDDE in [redacted] as a cosmetic product.

4.5 **Fifth question:** Throughout the SCCS Report, there are references to PGAs having the potential to cause effects at very low concentrations and the intended use in the proximity of the eye (see, for example, page 3, lines 29-31 of the SCCS Report). What do you understand "very low concentrations" to mean?

(a) In considering this question, I went back to the SCCS report to assess in further detail the approach to addressing concentrations. This is especially important when the starting concentration of DDDE in [redacted] is only 0.018% which would be considered by most pharmaceutical scientists to already be a "very low" concentration.

(b) As discussed above topical prostaglandin analogues for the treatment of glaucoma include Latanoprost, Travoprost, Tafluprost and Bimatoprost and the starting concentration of each in the commercial products is 0.005%, 0.004%, 00015% and 0.03%, respectively. These would again be considered very low concentrations by pharmaceutical scientists which identifies a problem with "qualitative" use of language around concentrations. This is because if the starting concentration of the API in the product is very low, the delivered dose per application has to also be very low and therefore for these products intended for treatment of IOP by direct instillation in the eye the effective concentrations are therefore by default "very low".

(c) Interestingly, there are a couple of instances in the report where the SCCS use the phrase "very high" concentrations and that is when discussing the outcome of the in vitro chromosomal aberration test in human lymphocytes, in which an increased frequency of aberrations were observed at "very high concentrations (2320 μg/mL)". Importantly when utilising a qualitative term, the Committee took the opportunity to qualify the concentration, so the reader has a benchmark to understand the language and thereby assess relative risk.

(d) In contrast the phrase "very low concentration" has no such reference point and as explained above has no meaning in this context as the starting concentration and subsequent amounts entering the eye from inadvertent application of [redacted] to the eyelid would have to be considered to be "very low".

STATEMENT OF TRUTH

I confirm that insofar as the facts stated in my report are within my own knowledge, I have made clear which they are and I believe them to be true, and that the opinions I have expressed represent my true and complete professional opinion.

Signed by Dr Ian Wilding

Ian Wilding Digitally signed by Ian Wilding Date: 2021.11.21 15:16:32Z

Date 21st November 2021

Distributed for Comment Only -- Do Not Cite or Quote

Annex 9

EYE INSTITUTE
Paul B. Donzis, M.D., M.B.A., Esq.
Cataract and Refractive Surgery
Diplomate, American Board of Ophthalmology

[redacted]

July 23, 2015

[redacted]

Re: [redacted]

Expert Opinion on [redacted] Warning Label re: Use During Ocular Disorder

Dear [redacted]:

I have been asked to provide an expert opinion on the present warning label for [redacted], which states, "IF YOUR ARE BEING TREATED FOR ANY OCULAR DISORDER (INCLUDING GLAUCOMA...USE ONLY UNDER THE SUPERVISION OF YOUR PHYSICIAN OR OPHTHALMOLOGIST" (the "Warning"). Specifically I have been asked if that particular warning is necessary or appropriate based on available information regarding the physiological effect of [redacted] when used as directed.

To help formulate my opinion, I have been provided with and have reviewed a binder of documents, a list of which is attached as Exhibit 1 to this expert opinion report. In addition, I have reviewed pertinent literature, and rely on clinical experience from my medical practice and those of my colleagues as reported at ophthalmology meetings.

[redacted] is a cosmetic product that is applied directly to the upper eyelashes with a fine applicator brush. [redacted] is a multi-ingredient product consisting of ingredients that are commonly used in cosmetic products and a novel, proprietary ingredient developed by [redacted], dechloro dihydroxy difluoro ethylcloprostenol-amide (DDDE). All of the ingredients used in [redacted] have been assigned an INCI (International Nomenclature of Cosmetic Ingredients) name (#1 in Exhibit 1).

Because DDDE is a novel, proprietary ingredient, I will focus my analysis in this report on DDDE. DDDE is a prostaglandin F2α analog. Several different prostaglandin F2α analogs have been used as topical glaucoma medications (referred to collectively herein as "ophthalmic prostaglandin analogs") for almost two decades. There is an extensive published literature on the safety of ophthalmic prostaglandin analogs. Although the ophthalmic prostaglandin analogs are different

molecules from DDDE, the safety literature on the ophthalmic prostaglandin analogs is relevant to my analysis of the safety of DDDE in [redacted] but must be considered in view of significant differences between [redacted] and the ophthalmic prostaglandin analogs. The ophthalmic prostaglandin analogs are applied directly to the eye as an eye drop, whereas [redacted] is applied directly to the upper eyelashes with a fine applicator brush. A thickener in [redacted] functions to prevent migration of [redacted] from the eyelashes into the eye, thereby preventing ocular surface exposure to DDDE.

Understanding these differences, I have reviewed the literature on ophthalmic prostaglandin analogs, as described below. In addition, as a practicing ophthalmologist, I have relied on my extensive experience in the clinical treatment of glaucoma, including use of ophthalmic prostaglandin analogs. For your convenience, I have attached my Curriculum Vitae to this report as Exhibit 2.

OPHTHALMIC PROSTAGLANDIN ANALOGS

Xalatan®, which has the active prostaglandin analog, Latanoprost, was first approved by the FDA in 1996 as a glaucoma eye drop medication. The FDA approved Bimatoprost, in 2001 for use in the glaucoma eye drop medication Lumigan®. A third medication, Travatan®, which contains Travoprost, was also approved by the FDA for the treatment of glaucoma in 2001.

The ophthalmic prostaglandin analogs, which are applied as eye drops, have ocular effects resulting from direct absorption by ocular membranes but also potentially have systemic effects resulting from absorption through the nasolacrimal duct. After topical administration of eye drops, 80% of the volume drains through the nasolacrimal duct and is absorbed systemically, bypassing hepatic metabolism. As an example, the use of ophthalmic timolol, a beta blocker (*not* an ophthalmic prostaglandin analog), has been associated with serious respiratory and cardiac events attributable to systemic absorption.

Scientific evidence indicates that all of the ophthalmic prostaglandin analogs have a similar and excellent safety profile.[1] This is consistent with my extensive clinical experience in treating patients with ophthalmic prostaglandin analogs. Even though the ophthalmic prostaglandin analogs differ from DDDE in that they are applied directly to the eye as an eye drop (and DDDE is applied directly to the upper eyelashes with a fine applicator brush) and are different molecules, their clinical safety profile is relevant to my analysis for two reasons. First, their safety profile represents a 'worst case scenario' of ocular exposure to prostaglandin F2α analogs. Second, there is extensive safety data available on the ophthalmic prostaglandin analogs from their history of widespread clinical use.

[1] Alm A, et. al., Side Effects Associated with Prostaglandin Analog Therapy, Surv Ophthal.53:S93-S105, Nov.2008.

LITERATURE REVIEW

Search Terms

Glaucoma accounts for over 10 million visits to physicians per year in the United States.[2] A significant amount of these patients are being treated with ophthalmic prostaglandin analogs. Additionally, bimatoprost, the active ingredient in the ophthalmic prostaglandin analog, Lumigan®, is also present in the product, Latisse®, which has been approved by the FDA to grow eyelashes. Latisse® is applied to the skin at the base of the upper eyelashes. To determine if the Warning is necessary or appropriate I first performed a pubmed search looking for adverse effects from the use of ophthalmic prostaglandin analogs. I combined the search terms side effects, ocular disorders, cataract, and macula with each of lantanoprost, bimatoprost, travaprost, Latisse, DDDE (the full chemical name), as well as prostaglandin. Pertinent articles (along with their pertinent references and related citations) were then reviewed to determine any safety concerns and side effects regarding the use of ophthalmic prostaglandin analogs in patients with ocular disorders.

Review of Relevant Literature

The literature search revealed an excellent review article entitled, "**Side Effects Associated with Prostaglandin Analog Therapy.**"[3] The article notes that many ocular and periocular side effects such as periocular pigmentation, conjuctival hyperemia, and iris darkening are associated with the use of prostaglandin analog eye drops. Except for the iris darkening these were typically benign side effects, however, it should be noted that in a patient with iris melanoma or nevus, induced darkening of the iris could make evaluating the lesion more difficult. Additional ocular disease including cystoid macular edema (CME), anterior uveitis, and reactivation of herpes simplex have been noted to have an association with the use of ophthalmic prostaglandin analog eye drops, but no proof of a causal relationship was determined.[4] Thus, there is no absolute contraindication for the use of ophthalmic prostaglandin analog eye drops in patients with a risk of developing these disorders but the clinician must be aware of the risks in patients who have had complicated cataract surgery (and are at higher risk of developing CME), prior anterior uveitis, or prior herpes keratitis.

The mechanism of action with regards to these ocular side effects is thought to be due to the absorption of the ophthalmic prostaglandin analog directly into the eye when applied to the ocular surface as an eye drop. As explained above, topical ocular medications applied as eye drops have the potential for systemic

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[2] Center for Disease Control and Prevention/National Center for Health Stati stics, 2010 & 1995

[3] Alm A, et. al., Side Effects Associated with Prostaglandin Analog Therapy, Surv Ophthal.53:S93-S105, Nov.2008.

[4] Ibid. at p. S102.

absorption. However, since the side effects noted above only occurred in the eye receiving the eye drop, systemic absorption did not contribute to the side effect

CLINCAL EXPERIENCE

I perform about 1500 glaucoma patient examinations per year and have done so now for at least the past 15 years. The majority of these patients who take topical medications are using or have used one of the ophthalmic prostaglandin analog drugs. Most of these patients are over 50 and have concurrent ocular disease, such as blepharitis, cataracts, macular disease, or dry eye. My clinical experience is similar to that described in the literature cited above. I have never been required to stop use in my patients of ophthalmic prostaglandin analogs due to any interaction with a specific ocular disease process except in a few patients with risk factors, such as CME noted above. In addition, pre-existing lid disease, such as blepharitis has not worsened with prostaglandin analog eye drops in my clinical experience, even though eye drops would migrate directly onto the lid margin with blinking.

I have had to discontinue use of ophthalmic prostaglandin analogs in my glaucoma patients due to local allergy or adverse cosmetic effects such as conjunctival hyperemia. But all of these patients were using eye drops which are absorbed into the eye and are also placed directly on the surface of the eye, unlike [redacted] Eye drops, due to their absorption into the eye and placement on the conjunctiva and cornea would be expected to have an occasional adverse interaction such as the CME. Because [redacted] is applied to the upper eyelashes and contains a thickener to prevent migration from the site of application, as explained below, side effects associated with the ophthalmic prostaglandin analogs would not be expected to be associated with DDDE in [redacted]

ASSESSSMENT OF POTENTIAL PHYSIOLOGICAL EFFECTS OF [redacted]

The potential physiological effect of [redacted] is largely determined by where it is applied and the properties of the product. [redacted] is distinctly different from the ophthalmic prostaglandin analog products both in where and how it is applied and in its viscous formulation. [redacted] is applied directly to the upper eyelashes with a fine applicator brush. In contrast, the ophthalmic prostaglandin analogs are applied as eye drops directly to the surface of the eye. Also, [redacted] contains cellulose gum, which provides sufficient viscosity to prevent migration from the eyelashes to the ocular surface (conjunctiva and cornea).

<u>Potential Ocular Absorption</u>

In my previous study (#13 in Exhibit 1), I noted that [redacted] did not migrate from the site of application into the eye. Further, review of the Evalulab study provided to me (#5 and #6 in Exhibit 1) indicates no statistical change in the intraocular pressure (IOP) in subjects using [redacted] for 4 weeks. By contrast, Latisse®,

which is the same solution as the ophthalmic prostaglandin analog, Lumigan® but approved by the FDA to grow eyelashes when applied to the eyelid at the base of the upper eyelashes, does not contain a viscosity agent and did produce a statistically significant reduction in IOP.[5]

Based on my review of scientific data, including the amount of DDDE present in each application of [redacted] there would not be any expected ocular exposure associated with the use of [redacted] in accordance with the package instructions and, therefore, any absorption of DDDE through the ocular membranes is highly unlikely (see below).

Potential Systemic Absorption

As noted above, the mechanism of systemic absorption for the topical ocular medications is through the nasolacrimal duct. In contrast to topical ophthalmic prostaglandin analogs, [redacted] is not applied to the ocular surface as an eye drop or in any other form. The directions clearly state that it is to be applied in a thin line ***directly to the upper eyelashes*** above the level of the skin. As noted in the section above on ocular exposure to [redacted] due to the viscous nature of [redacted] and the placement upon the eyelashes, no migration into the eye would be expected. Thus, no expected systemic absorption from the ocular surface or nasolacrimal duct would be expected.

This would leave possible skin absorption as another route for potential systemic effects. Dr. Ian Wilding's report (#12 in Exhibit 1) noted that the skin is an effective barrier to systemic absorption. Based on review of his report and the supporting literature, I would not expect any clinically significant systemic amount of DDDE to occur via skin absorption, both due to the effective barrier of the skin and the minimal amount of potential migration of [redacted] from the lashes to the eyelid margin and skin.

CONSUMER COMPLAINTS

In reviewing the log of consumer complaints supplied to me (#10 in exhibit 1), the majority of complaints relate to allergic irritation and sensitivity to [redacted] which can occur with any cosmetic product. None of the complaints indicate systemic absorption of [redacted] The paucity of complaints also speaks to the overall high safety and tolerance of [redacted] The profile of consumer complaints does not raise concerns about ocular safety.

CONCLUSION

For all the foregoing reasons, I do not see any scientific basis for the requirement of the cosmetic product, [redacted] being used under the supervision of a physician or ophthalmologist in patients being treated for ocular disorders, including glaucoma.

[5] *See* Latisse® Prescribing Information.

Since the product is not placed directly on the ocular surface and no migration onto the surface would be expected, [redacted] should not exacerbate or interfere with the treatment of any ocular disorder. At most, occasional irritation or allergy as noted in the consumer logs, would occur as with any cosmetic product.

If you have further questions please do not hesitate to contact me.

Sincerely,

Paul B. Donzis, M.D., M.B.A., Esq.

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Annex 10

EXPERT REPORT

Safety assessment of cosmetic products according regulation (EG) 1223/2009

Product: [redacted]

Author of expert report: V. NITSCHE, PhD
Prinz Eugen Straße 6
1040 Wien
authorized expert
according § 73 LMSVG

KOS 3593E 1120

This expert report consists of pages 1 - 16

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SAFETY ASSESSMENT

according the regulation (EG) 1223/2009

Product: [redacted]

Preparation: **031012-43, information of manufacturer of 10.03.2013**

Manufacturer: [redacted] **EA Amsterdam, The Netherlands**

Distribution: **EU:** [redacted] **EA Amstedam, The Netherlands**
UK: [redacted]
Cardiff, Wales, CF10 2HH

The assessment is based on

a) the toxicological profile of the ingredients and raw materials and their available toxicological/dermatologic documentation, the tests on the finished product including microbiological tests, the safety data leaflets, the legal regulation and market observations.

b) the chemical structure including the composition, the test procedures of the finished product and the raw materials and physical/chemical tests as well.

c) the rate of exposure resulting from the typical intended use as indicated in the instruction for use. For the protection of consumers, the voluntary and statutory warnings and other information are verified, which are listed in labeling, instructions for use and application instructions.

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Name of product:

[redacted]. With this designation, the type of application as a eyelash conditioner is clearly defined.

Product:

The product is a clear, colorless liquid with an characteristic odor.

Packaging:

The product is offered in 3.5 ml aluminum tubes with an applicator. The materials used are food-safe and may be used safely for the packaging of cosmetics.

Elements of labeling:

The elements defined in the regulation (EG) 1223/2009, Article 19 for the labeling of cosmetic products are met.

Composition of the product:

[redacted] contains

Aqua, Cellulose Gum, Chlorphenesin,Biotin, Glycerin, Phenoxyethanol, Swertia Japonica Extract, Serenoa Serrulata Fruit Extract, Camellia Sinensis Leaf Extract, Panax Ginseng Root Extract, Triticum Vulgare Germ Protein,Pentylene Glycol, Calendula Officinalis Flower Extract, Butylene Glycol, Octapeptide-2, Biotinoyl Tripeptide-1, Ethyl Taflupros-tamide, Phosphoric Acid, Disodium Phosphate

The CAS-numbers of the ingredients are listed in annex 1.

The ingredients are indicated according the INCI nomenclature if listed.

Further predictible application:

Another forseeable application of the [redacted] is not given because of the clearly instructions of use.

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Toxicological profile of single ingredients:

Aqua Most of the cosmetic products contain water as a main component. From a toxicological point of view water is harmless. The water used in this product is purified, which means an adequate quality for cosmetics.

Cellulose Gum: Cellulose Gum is a whitish powder with a characteristic odor that is used in cosmetics as a thickener. It is not irritating to the skin or eyes. A sensitizing effect is not known. The acute oral toxicity in the rat is 27,000 mg/kg (MSDS). The average molecular weight is 90,000 daltons. The ADI is indicated as "not specified". Cellulose Gum is rated as safe in cosmetic products by a toxicology expert panel (CIR, Cosmetic Ingredient Review, 20012) if the concentration does not exceed 20%.

Chlorphenesin: Chlorphenesin is a white, crystalline substance that is used in cosmetics as a preservative. It is slightly irritating to the skin and very irritating to the eyes. A sensitizing effect is possible. According to the Cosmetics Ordinance, chlorphenesin may be used as a preservative up to a concentration of 0.3%.

Biotin: Biotin is a substance from the vitamin B complex and is used in cosmetics as a caring component. It is not irritating to the skin or eyes. A sensitizing effect is not known. Biotin has been assessed by a panel of toxicology experts (CIR, Cosmetic Ingredient Review, 2015) as safe in cosmetic products if a concentration of 1% is not exceeded.

Glycerin: Glycerin, a clear and visvous liquid, is obtained by saponification of animal or vegetable fats. The glycerin used in this product is of vegetable origin. The substance is slightly irritating to skin and eyes. A sensitizing effect is unknown (OECD SIDS, March 2002). Its acute, oral toxicity in the rat is 12600 mg/kg (MSDS). A NOAEL of 2200 mg/kg from human Data is reported (CIR 2015).

Phenoxyethanol: Phenoxyethanol is used in cosmetics as a solubilizer and preservative. According to the Cosmetics Regulation, phenoxyethanol may be used as a preservative up to a concentration of 1%. A NOAEL of 500 mg/kg body weight is given (ECHA, Registration Dossier).

Swertia Japonica Extract: Swertia Japonica Extract is an extract of the Japnese swamp star, which is used in Japanese folk medicine as a hairstrengthening agent. The plant belongs to the group of gentian plants with the ingredient swertiamarin, which is also found in the European gentian root. A panel of toxicology experts (Plants in Cosmetics, Volume II, Council of Europe, 2001) recommends a concentration of up to 7% for gentian root extracts as a safe use in cosmetic products.

Serenoa Serrulata Fruit Extract: Serenoa Serrulata Fruit Extract is an extract of the saw palmetto fruit, which is used in cosmetics as an invigorating component. The extract is not irritating to the skin and eyes. A sensitizing effect is not known. Saw palmetto extract contains up to 0.16 % of the phytosterol ß-sitosterol (Penugonda K., Nutrients, 2013, 5, 3617-3633). A NOAEL of 210 mg/kg is given for beta-sitosterol (Mattilsynet 2012).

Camellia Sinensis Leaf Extract: Camellia Sinensis Leaf Extract is a green tea extract with a characteristic smell that is used in cosmetics as a nourishing component. It does not irritate the eyes or skin and does not cause sensitization (MSDS). A toxicology expert panel (Plants in Cosmetics, Volume III, Council of Europe, 2006) gives a NOAEL of 450 mg/kg for the green tea ingredient methyl salicylate (fresh leaves: 20%). Dehydrolinalool (hotrienol) is contained in finished tea up to 70%. For dehydrolinalool, a NOAEL of 117

mg/kg body weight (ECHA, Registration Dossier Linalool) can be used analogously to linalool.

Panax Ginseng Root Extract: Panax Ginseng Root Extract is a brownish extract of the ginseng root, which is used in cosmetics as a toning component. It is not irritating to eyes or skin. A sensitizing property is not known. A European committee of toxicology experts (Plants in Cosmetics, Volume I, Council of Europe, 2002) allows Panax Ginseng Root Extract (glycolic) to be used safely in cosmetics up to a concentration of 5%.

Triticum Vulgare Germ Protein: Triticum Vulgare Germ Protein is a white, neutral smelling powder that is used in cosmetics as a hair conditioning component. It is not irritating to the eyes or skin and has no sensitizing properties. Wheat proteins are also ingested with food, so a toxic effect is not to be expected. Wheat proteins are judged to be safe in cosmetic products by a panel of toxicology experts (CIR, Cosmetic Ingredient Review, 2013) if the concentration does not exceed 1.7%.

Pentylene Glycol: Pentylene Glycol is a colorless liquid with a characteristic odor that is used in cosmetics as a conditioning and solubilizing component. It is hardly irritating to the skin or eyes. A sensitizing effect is not known. This substance can be used safely when used in dilution. The acute oral toxicity in the rat is 2000 mg/kg. In a comparative study with other diols, pentylene glycol was found to be safe for use in cosmetics (Sundberg J.J., Expert Opin Investig Drugs. 2008, Apr; 17 (4): 601-10). A NOEL of 1000 mg/kg body weight is reported (CIR, Safety Assessment of Alkane Diols, 2016).

Calendula Officinalis Flower Extract: Calendula Officinalis Flower Extract is a yellow liquid with a faint odor that is used in cosmetics as a nourishing component. It does not irritate the eyes or skin. A sensitizing effect is possible. A panel of toxicology experts (Plants in Cosmetics, Council of Europe, Volume 1, 2002) rated Calendula Officinalis Flower Extract as safe in cosmetic products up to a concentration of 10%. Calendula Officinalis Extract is judged to be safe in cosmetic products by a toxicology expert panel (CIR, Cosmetic Ingredient Review, 2015) if the concentration does not exceed 6%.

Butylene Glycol: Butylene Glycol is a clear liquid which is used in cosmetics as a solvent. It is not irritant for skin and eyes and has no sensitizing properties. An expert panel of toxicologists (CIR, Cosmetic Ingredient Review, 2015) confirms the safe use of Butylene Glycol in cosmetics in concentration up to 89%. A NOAEL of 200 mg/kg bodyweight is reported (CIR, Alkane Diols, 2016).

Octapeptide-2: Octapeptide-2 is a synthetic peptide that is used in cosmetics as a hair restorer. It is non-irritating to the skin and eyes and is non-sensitizing. For this peptide, a skin penetration of 0.01% (FDA) can be assumed, analogously to acetyl hexapeptide-8.

Biotinoyl Tripeptide-1: Biotinoyl Tripeptide-12 is a synthetic peptide that is used in cosmetics as a hair restorer. It is non-irritating to the skin and eyes and is non-sensitizing. For this peptide, a skin penetration of 0.01% (FDA) can be assumed, analogously to acetyl hexapeptide-8. Biotinoyl Tripeptide-1 is assessed by a toxicology expert panel (CIR, Cosmetic Ingredient Review, 2014) as safe in cosmetic products if the concentration does not exceed 1%.

Ethyl Tafluprostamide: Ethyl Tafluprostamide is a prostaglandin derivative with fatsoluble properties. It is used as a conditioner in hair products applied to the hair. It is not irritating to skin. A sensitizing property is not known. A study of transdermal penetration of a topically applied prostaglandin reported penetration limited to the outer layer of the epidermis, which is devoid of blood vessels, and insignificant penetration to the vascu-

larized dermis. (Transdermal delivery of prostaglandins, C.J.N. Oognejisofar, 1996, University of Saskatchwan.). Transdermal penetration is proportional to the concentration of prostaglandin in the solution. (Oognejisofar, 1996). In the 1996 study, a 0.05% of PGE1 had a skin penetration rate of 1% over a period of 24 hours. Because the concentration of ethyl tafluprostamide used in this product is lower (0.018 %) and any incidental skin exposure to the conditioner would be brief, the 1% transdermal penetration rate is an upper limit. A NOAEL for Ethyl Tafluprostamide is not available. A NOAEL for tafluprost from a carcinogenicity study is given as 0.03 mg/kg body weight (Center For Drug Evaluation And Research 2011, Pharmacology/Toxicology NDA Review And Evaluation Of Tafluprost. Application Number: 202514orig1s000).

Phosphoric Acid: Phosphoric Acid is a colorless liquid that is used in cosmetics as a buffering component. If undiluted, it has a strong irritant effect on the eyes and skin and does not show any sensitizing effects. This substance can be used without hesitation when used as a dilution. An MTDI of 70 mg/kg body weight is given (JECFA 1982).

Disodium Phosphate: Disodium Phosphate is a crystalline powder that is used in cosmetics as a buffer substance. It is slightly irritating to the skin and eyes. A sensitizing effect is not known. This substance can be used safely when used as a dilution. The acute oral toxicity in the rat is 17.5 g / kg (MSDS). The maximum tolerated daily dose (MTDI) is given as 70 mg/kg (JECFA, 2006).

Determination of systemic exposure:

The eyelash conditioner is used for eyelash care.

The eyelash conditioner is applied with an eyeliner brush directly to the eyelashes. The product can be classified as a leave-on product. A quantity of application of 0.0024 g per day can be assumed for the eyelash conditioner (result of [redacted] in-house investigations).

Aqua: For water the assessment of systemic exposure can be omitted.

Cellulose Gum: Cellulose gum is used in this product in a concentration of [redacted] If 0.0024 g eyelash conditioner is used, a maximum systemic concentration of 0.00014 mg/kg could be achieved with 100% absorption through the skin. Cellulose gum is assessed by a toxicology expert panel (CIR, Cosmetic Ingredient Review, 20012) as safe in cosmetic products if the concentration does not exceed 20%. This means that this substance can be classified as safe for human health in the given concentration.

Chlorphenesin: Chlorphenesin is used in this product in a concentration of [redacted] If 0,0024 g Eyelash Conditioner is used, a systemic concentration of 0,00012 mg/kg could be achieved with complete absorption through the skin. According to the EU Cosmetic Regulation, chlorphenesin may be used as a preservative up to a concentration of 0.3%. This means that this substance can be classified as safe for human health in the given concentration.

Biotin: Biotin is used in this product in a concentration of [redacted]. If 0,0024 g Eyelash Conditioner is used, a systemic concentration of 0,0002 mg/kg could be achieved with complete absorption through the skin. Biotin has been assessed by a toxicology expert panel (CIR, Cosmetic Ingredient Re-view, 2015) as safe in cosmetic products if a concentration of 1% is not exceeded. A risk to human health can thus be excluded and this substance can be assessed as safe in the given concentration.

Glycerin: Glycerin is used in this product in a concentration of [redacted] If 0,0024 g Eyelash Conditioner is used, a systemic concentration of 0,00024 mg/kg could be achieved with complete absorption through the skin. A NOAEL of 2200 mg/kg per day from human data is given (CIR 2015). This results in a safety margin of 9166667. A risk to human health can thus be ruled out and this substance can be assessed as safe in the given concentration.

Phenoxyethanol: Phenoxyethanol is used in this product in a concentration of [redacted] If 0,0024 g Eyelash Conditioner is used, a systemic concentration of 0,00012 mg/kg could be achieved with complete absorption through the skin. According to the Cosmetics Regulation, phenoxyethanol may be used as a preservative up to a concentration of 1%. A NOAEL of 500 mg/kg body weight is given. This results in a safety margin of 4166667. This means that this substance can be classified as safe for human health in the given concentration.

Swertia Japonica Extract: Swertia Japonica Extract is used in this product in a concentration of [redacted]. If 0,0024 g Eyelash Conditioner is used, a systemic concentration of 0,00000016 mg/kg could be achieved with complete absorption through the skin. A panel of toxicology experts (Plants in Cosmetics, Volume II, Council of Europe, 2001) recommends a concentration of up to 7% for gentian extracts as a safe use in cosmetic products. A risk to human health can thus be excluded and this substance can be assessed as safe in the given concentration.

Serenoa Serrulata Fruit Extract: Serenoa Serrulata Fruit Extract is used in this product in a concentration of [redacted]. If 0,0024 g Eyelash Conditioner is used, a systemic concentration of 0,00000032 mg/kg could be achieved with complete absorption through the skin. ß-Sitosterol is contained up to 0.16 %. A NOAEL of 210 mg/kg is given for beta-sitosterol. This results in a acceptable margin of safety for ß-sitosterol. A risk to human health can thus be excluded and this substance can be assessed as safe in the given concentration

Camellia Sinensis Leaf Extract: Camellia Sinensis Leaf is used in this product in a concentration of [redacted] If 0,0024 g Eyelash Conditioner is used, a systemic concentration of 0,00000024mg/kg could be achieved with complete absorption through the skin. A committee of toxicology experts (Plants in Cosme-tics, Volume III, Council of Europe, 2006) gives a NOAEL of 450 mg/kg for the green tea ingredient methyl salicylate (fresh leaves: 20%). Dehydrolinalool (hotrienol) is contained in finished tea up to 70%. For dehydrolinalool, a NOAEL of 117 mg/kg body weight (ECHA, Registration Dossier Linalool) can be used analogously to linalool. For methyl salicylate (up to 20%) with a NOAEL of 450 mg/kg, this results in a safety margin of 9375000000. This means that this extract can be classified as safe for human health at the given concentration.

Panax Ginseng Root Extract: Panax Ginseng Root Extract is used in this product in a concentration of [redacted]. After using 0,0024 g Eyelash Conditioner, a maximum systemic concentration of 0,0000004 mg/kg could be achieved. A European committee of toxicology experts (Plants in Cosmetics, Volume I, Council of Europe, 2002) allows Panax Ginseng Root Extract (glycolic) to be used safely in cosmetics up to a concentration of 5%. A risk to human health can thus be ruled out and this substance can be assessed as safe in the given concentration.

Triticum Vulgare Germ Protein: Triticum Vulgare Germ Protein is used in this product at a concentration of [redacted]. If 0.0024 g eyelash conditioner is used, a systemic concentration of 0.000000033 mg/kg could be achieved with complete absorption through the skin. Wheat proteins are also ingested with food, so a toxic effect is not to be expected. Wheat proteins are judged to be safe in cosmetic products by a panel of toxicology experts (CIR, Cosmetic Ingredient Review, 2013) if the concentration does not exceed 1.7%. A risk to human health can thus be ruled out and this substance can be assessed as safe in the given concentration.

Pentylene Glycol: Pentylene Glycol is used in this product in a concentration of [redacted]. If 0.0024 g of eyelash conditioner is applied, a systemic concentration of 0.000000033 mg/kg could be achieved with complete absorption through the skin. A NOEL of 1000 mg/kg body weight is reported. This results in a safety margin of 30303030303. This means that a risk to human health can be excluded and this substance can be assessed as safe in the given concentration.

Calendula Officinalis Flower Extract: Calendula Officinalis Flower Extract is used in this product in a concentration of [redacted]. If 0.0024 g eyelash conditioner is applied, a systemic concentration of 0.00000048 mg/kg could be achieved with complete absorption through the skin. A panel of toxicology experts (Plants in Cosmetics, Council of Europe, Volume 1, 2002) rated Calendula Officinalis Flower Extract as safe in cosmetic products up to a concentration of 10%. Calendula Officinalis Extract is judged to be safe in cosmetic products by a toxicology expert panel (CIR, Cosmetic Ingredient Review, 2015) if the concentration does not exceed 6%.

Butylene Glycol: Butylene Glycol is used in this product in a concentration of [redacted] If 0,0024 g Eyelash Conditioner is used, a systemic concentration of 0,0000032 mg/kg

could be achieved with complete absorption through the skin. Butylene Glycol is judged to be safe in cosmetic products by a panel of toxicology experts (CIR, Cosmetic Ingredient Review, 2015) if the concentration does not exceed 89%. A NOAEL of 200 mg/kg body weight is reported. This results in a safety margin of 62500000. This means that any risk to human health can be excluded and this substance can be assessed as safe in the given concentration.

Octapeptide-2: Octapeptide-2 is used in this product in a concentration of [redacted]. If 0.0024 g eyelash conditioner is used, a systemic concentration of 0.000000002 mg/kg could be achieved with complete absorption through the skin. No toxic effects can be ascribed to this concentration (TTC). This means that this substance can be classified as safe for human health in the given concentration.

Biotinoyl Tripeptide-1: Biotinoyl Tripeptide-1 is used in this product in a concentration of [redacted] If 0.0024 g eyelash conditioner is used, a systemic concentration of 0.000000064 mg/kg could be achieved with complete absorption through the skin. Biotinoyl Tripeptide-1 is assessed by a toxicology expert panel (CIR, Cosmetic Ingredient Review, 2014) as safe in cosmetic products if the concentration does not exceed 1%.

Ethyl Tafluprostamide: Ethyl Tafluprostamide is used in this product in a concentration of 0.018 %. If 0,0024 g Eyelash Conditioner is used, a systemic concentration of 0,000007 mg/kg could be achieved taking into account a skin penetration rate of 100 %. A safety margin of 4286 is calculated from a NOAEL of 0.03 mg/kg body weight (NOAEL is derived from a comparable Tafluprost study). A risk to human health can thus be excluded and this substance can be assessed as safe in the given concentration.

Phosphoric Acid: Phosphoric Acid is used in this product at a concentration of [redacted]. If 0.0024 g eyelash conditioner is used, a systemic concentration of 0.000012 mg/kg could be achieved with complete absorption through the skin. Phosphoric acid is bound in a buffer system and thus its acidic effect is limited. With an MTDI of 70 mg/kg, there is a safety margin of 583333333. At this use concentration, a risk to human health can be excluded and this substance can be assessed as safe in the given concentration.

Disodium Phosphate: Disodium Phosphate is used in this product in a concentration of [redacted] If 0,0024 g Eyelash Conditioner is used, a systemic concentration of 0,000056 mg/kg could be achieved with complete absorption through the skin.. With an MTDI of 70 mg/kg, analogous to the ADI, there is a safety margin of 125000000. This means that this substance can be classified as safe for human health at the given concentration.

Margin of Safety:

The exact margin of safety can not be estimated because of missing NOAEL-values for most of the single components. The margin of safety derived from NOAEL should not be below 100.

The classifications derived from toxicological expert reports (CIR, ADI, HSDB, OECD, TTC, JECFA, Plants in Cosmetics) are resulting in toxicologically safe levels.

Calculation of Systemic Exposure Dose (SED):

Calculation of SED was made in the chapter determination of systemic exposure. A listing of the calculations is given in annex 2.

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Stability of the cosmetic product:

Microbiological stability:

A micribiological test of the Eyelash Conditioner is performed. The product complies to the cosmetic regulation.

Physicochemical stability:

A shelftest during several months gives prove for the physicochemical stability of the product. The tested product showed no differences to initial values.

Conditions of application and warnings:

The Eyelash Conditioner should be used as an Eyelash Conditioner. Following warnings are indicated on the label:
„Do not get in the eye“
„Rinse immediately with water if eye contact occurs”
„If irritation develops, reduce frequency of use until the irritation resolves”
„If irritation persists or is excessive, discontinue use and consult a physician”
„Keep out of reach of children”.

Prove of effect:

No certain effect is praised that requires a proof of effectiveness. The good skin tolerance is confirmed by a dermatological examination (patch test). The ocular tolerance is confirmed by a ophtalmological examination.

Adverse effects:

No adverse effects are registered.

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Results of assessment:

All components of the product are documented with safety data leaflets or product specifications, confirming the harmlessness to the human health of the components in the given concentration and the typical intended use.

There are no ingredients which are not allowed [Regulation (EG) 1223/2009, Annex II] or which are used in concentrations not covered by the cosmetic regulation [(EG) 1223/2009, Annex III)].

No allergenic substances have to be indicated on the label.

Following warnings are indicated on the label:
„Do not get in the eye“
„Rinse immediately with water if eye contact occurs”
„If irritation develops, reduce frequency of use until the irritation resolves”
„If irritation persists or is excessive, discontinue use and consult a physician”
„Keep out of reach ofchildren”.

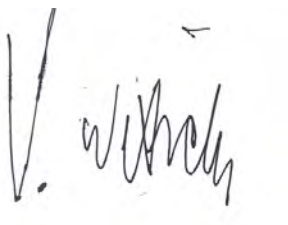
The good skin tolerance is confirmed by a dermatological examination (patch test).

The ocular tolerance is confirmed by a ophtalmological examination.

The shelf life of the product is stated to be more than 30 months. This makes it necessary to keep the open jar with the indication of the shelf life after opening with 12 months in the label. A batch designation, the filling quantity and the distribution are also given in the labeling.

The elements defined in the regulation (EG) 1223/2009, Article 19 for the labeling of cosmetic products are met.

Taking into account the entire exposure of the present product to the human body there is no risk concerning the human health and safety when the product is applied in a usual and anticipated way.



Vienna, 13 Nov 2020

date

signature
(approved expert according § 73 LMSVG)

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PERSONAL RECORD

Veit NITSCHE, PhD

1945: Born in Vienna

1963: Maturity, commencing university study of technical sciences

1972: Awarding of PhD of technical sciences (chemistry)

1973: Austrian Research Centre Seibersdorf (former SGAE), institutes of chemistry (labelled isotopes) and biology (biochemistry)

1974: Pharmakologische Untersuchungsgesellschaft, Vienna (pharmacology, toxicology, pharmacokinetics, biopharmaceutics)

1977: Head of Pharmakologische Untersuchungsgesellschaft in Vienna

Since 1984: Head of CRO Biokinet GmbH, Vienna, clinical studies on pharmacokinetics and biopharmaceutics

1982 Scientific award of the Austrian Society for Dermatology

1990 Member of the Sientific Board of the First European Congress for Biopharmaceutics and Pharmacokinetics (ECBP)

1997 Approved expert for cosmetics according § 50 LMG 1975 (now § 73 LMSVG 2006)

41 Publications

Annex 1

[redacted], 031012-43

CAS#	TRADE NAME	% BY WEIGHT	INCI NOMENCLATURE	% MATERIAL BREAKDOWN	FUNCTION
7732-18-5	Purfied Aqua	[redacted]	Aqua	100,000%	Solvent
9000-11-7 9004-32-4	Cellulose Gum CMC400SF	[redacted]	Cellulose Gum	100,000%	Viscosity Increa-sing Agent
104-29-0	Germazide C R10284	[redacted]	Chlorphenesin	100,000%	Preservative
58-85-5	Biotin/D-Biotin	[redacted]	Biotin	100,000%	Hair Conditioning Agent
56-81-5	Glycerin	[redacted]	Glycerin	100,000%	Hair Conditioning Agent; Humectant
122-99-6	Phenoxetol	[redacted]	Phenoxyethanol	100,000%	Preservative
56-81-5	DL Swertia Ja-ponica GL	[redacted]	Glycerin	79,900%	Hair Conditioning Agent; Humectant
94167-11-0			Swertia Japonica Extract	20,000%	Skin Conditioning Agent
7732-18-5	Actiphyte of Saw Palmetto GL 335812-13	[redacted]	Aqua	39,500%	Solvent
84604-15-9			Serenoa Serrulata Fruit Extract	20,000%	Skin Conditioning Agent
56-81-5			Glycerin	39,500%	Hair Conditioning Agent; Humectant
122-99-6			*Phenoxyethanol	1,000%	Preservative
7732-18-5	ABS Green Tea Extract WS	[redacted]	Aqua	79,000%	Solvent
84650-60-2			Camellia Sinensis Leaf Extract	20,000%	Emollient
122-99-6			*Phenoxyethanol	1,000%	Preservative
7732-18-5	Actiphyte of Ginseng GL 318120-13	[redacted]	Aqua	39,500%	Solvent
90045-38-8			Panax Ginseng Root Extract	20,000%	Skin Conditioning Agent
56-81-5			Glycerin	39,500%	Hair Conditioning Agent; Humectant
122-99-6			*Phenoxyethanol	1,000%	Preservative
7732-18-5	Tensine GR	[redacted]	Aqua	89,500%	Solvent
100684-25-1			Triticum Vulgare (Wheat) Protein	5,500%	Hair Conditioning Agent
5343-92-0			Pentylene Glycol	5,000%	Solvent

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7732-18-5	Actiphyte of Calendula GL 313640-13	[redacted]	Aqua	39,500%	Solvent
84776-23-8			Calendula Officinalis Flower Extract	20,000%	Skin Conditioning Agent
56-81-5			Glycerin	39,500%	Hair Conditioning Agent; Humectant
122-99-6			*Phenoxyethanol	1,000%	Preservative
7732-18-5	Octapeptide-2 Peptide Solution	[redacted]	Aqua	98,000%	Solvent
107-88-0			Butylene Glycol	1,950%	Solvent
Not Referenced			Octapeptide-2	0,050%	Skin Conditioning Agent
107-88-0	Biotinyl-GHK Solution	[redacted]	Butylene Glycol	49,950%	Solvent
7732-18-5			Aqua	49,950%	Solvent
Not Referenced			Biotinoyl Tripeptide-1	0,100%	Hair Conditioning Agent
Not Referenced	Dechloro Dihydroxy Difluoro Ethylcloprostenolamide	0,2400%	Ethyl Tafluprostamide	7,500%	Conditioning Agent
122-99-6			Phenoxyethanol	92,500%	Preservative
7664-38-2	Phosphoric Acid	[redacted]	Phosphoric Acid	100,000%	pH Adjustor
7558-79-4; 7782-85-6; 10140-65-5	Sodium Phosphate Dibasic	[redacted]	Disodium Phosphate	100,000%	Buffering Agent

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Annex 2

1 Exposure to the cosmetic product

Formula for the calculation of dermal exposure, Exp_{derm} (mg/kg/day):

$$Exp_{derm} = (G \times A) \times F \times R / K$$

Bodyweight **K** *(kg)*:	Adult 60 kg
Way of exposure:	dermal
Retention factor **R**:	Leave-on-products = 1; Rinse-off- products <1
Application area **A** (cm^2):	Corresponding to instructions of manufacturer
Amount of application **G** *(mg/cm^2)*:	
Frequency of Application **F** *(x/day)*:	

a) Application site:	Eyelashes
b) Surface of application (Application area, **A**) *[cm^2]*	
c) Quantity per application (G) (Applied quantity **G**) *[g]*	0,0024
d) Duration (based on product type, derived from it: Retention factor, **R**)	Leave on
e) Frequency of use (Frequency of Application, **F**) *[x per day]*	1x /day
f) Normal and reasonable forseeable expoisure route	
g) Target group of application: (Bodyweight, **K**) *[kg]*	adults 60 kg
h) Dermal exposure (Exp_{derm}) *[mg/kg/day]*	0,04 mg/kg/day
i) Oral exposure	
j) Inhalation exposure	

2 Exposure to the substances

$$SED_{Bestandteil\ X} = \frac{EXP_{derm} \cdot Konzentration_{Bestandteil\ X}\ [\%]}{100} \cdot \frac{Permeation_{Haut}\ [\%]}{100}$$

Substance	Concentration in finished product [%]	Skin penetration [%]	$SED_{substance}$ [mg/kg]
Cellulose Gum	[redacted]	[redacted]	[redacted]
Chlorphenesin	[redacted]	[redacted]	[redacted]
Biotin	[redacted]	[redacted]	[redacted]
Glycerin	[redacted]	[redacted]	[redacted]
Phenoxyethanol	[redacted]	[redacted]	[redacted]
Swertia Japonica Extract	[redacted]	[redacted]	[redacted]
Serenoa Serrulata Fruit Extract	[redacted]	[redacted]	[redacted]
Camellia Sinensis Leaf Extract	[redacted]	[redacted]	[redacted]
Panax Ginseng Root Extract	[redacted]	[redacted]	[redacted]
Triticum Vulgare Germ Protein	[redacted]	[redacted]	[redacted]
Pentylene Glycol	[redacted]	[redacted]	[redacted]
Calendula Officinalis Flower Extract	[redacted]	[redacted]	[redacted]
Butylene Glycol	[redacted]	[redacted]	[redacted]
Octapeptide-2	[redacted]	[redacted]	[redacted]
Biotinoyl Tripeptide-1	[redacted]	[redacted]	[redacted]
Ethyl Tafluprostamide	0,018	100	0,000007
Phosphoric Acid	[redacted]	[redacted]	[redacted]
Disodium Phosphate	[redacted]	[redacted]	[redacted]

Personal Care Products Council
Committed to Safety, Quality & Innovation

Memorandum

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

FROM: Carol Eisenmann, Ph.D.
Personal Care Products Council (PCPC)

DATE: April 17, 2023

SUBJECT: Concentration of Use by FDA Product Category: Prostaglandin Analogues

The listed prostaglandin analogues were included in a concentration of use survey. No uses were reported for any of these ingredients.

Isopropyl Cloprostenate
Dehydrolatanoprost
Ethyl Tafluprostamide
Ethyl Travoprostamide

Bimatoprost
Cyclopropylbimatoprost
Cloprostenol
Travoprost
Dihydroxypropyl Didehydrolatanoprostamide
Norbimatoprost
Nortafluprost
Trifluoromethyl Dehydrolatanoprost
Methyl Bimatoprost Acidate
Noralfaprostol
Travoprostamide
Isopropyl Dimethylnorcarboprostate

Personal Care Products Council
Committed to Safety, Quality & Innovation

Memorandum

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

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

DATE: May 10, 2023

SUBJECT: Isopropyl Cloprostenate

Consumer Product Testing Company. 2023. Use information for Isopropyl Cloprostenate in lash serum.

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]

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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
[redacted]
Manager,
Analytical Services

Approved By:
[redacted]
01/04/23
Quality Assurance

[redacted]

FDA Registration# 1000151293
DEA Registration# RC0199744 Schedule I-V
US EPA/NJ DEP Registration# NJD982726648
ISO/IEC 17025:2017 Accredited

[redacted]

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[redacted]

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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	

FDA Registration# 1000151293
DEA Registration# RC0199744 Schedule I-V
US EPA/NJ DEP Registration# NJD982726648
ISO/IEC 17025:2017 Accredited

[redacted]

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[redacted]

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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:
[redacted]
3/21/2023
[redacted]
Manager,
Analytical Services

Approved By:
[redacted]
03/21/23
Quality Assurance

[redacted]

FDA Registration# 1000151293
DEA Registration# RC0199744 Schedule I-V
US EPA/NJ DEP Registration# NJD982726648
ISO/IEC 17025:2017 Accredited

[redacted]

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[redacted]

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FDA Registration# 1001151293
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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CONSUMER PRODUCT TESTING™ COMPANY
cptclabs.com

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]