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

Status:	Revised Draft Tentative Report for Panel Review
Release Date:	May 10, 2024
Panel Meeting Date:	June 3 – 4, 2024

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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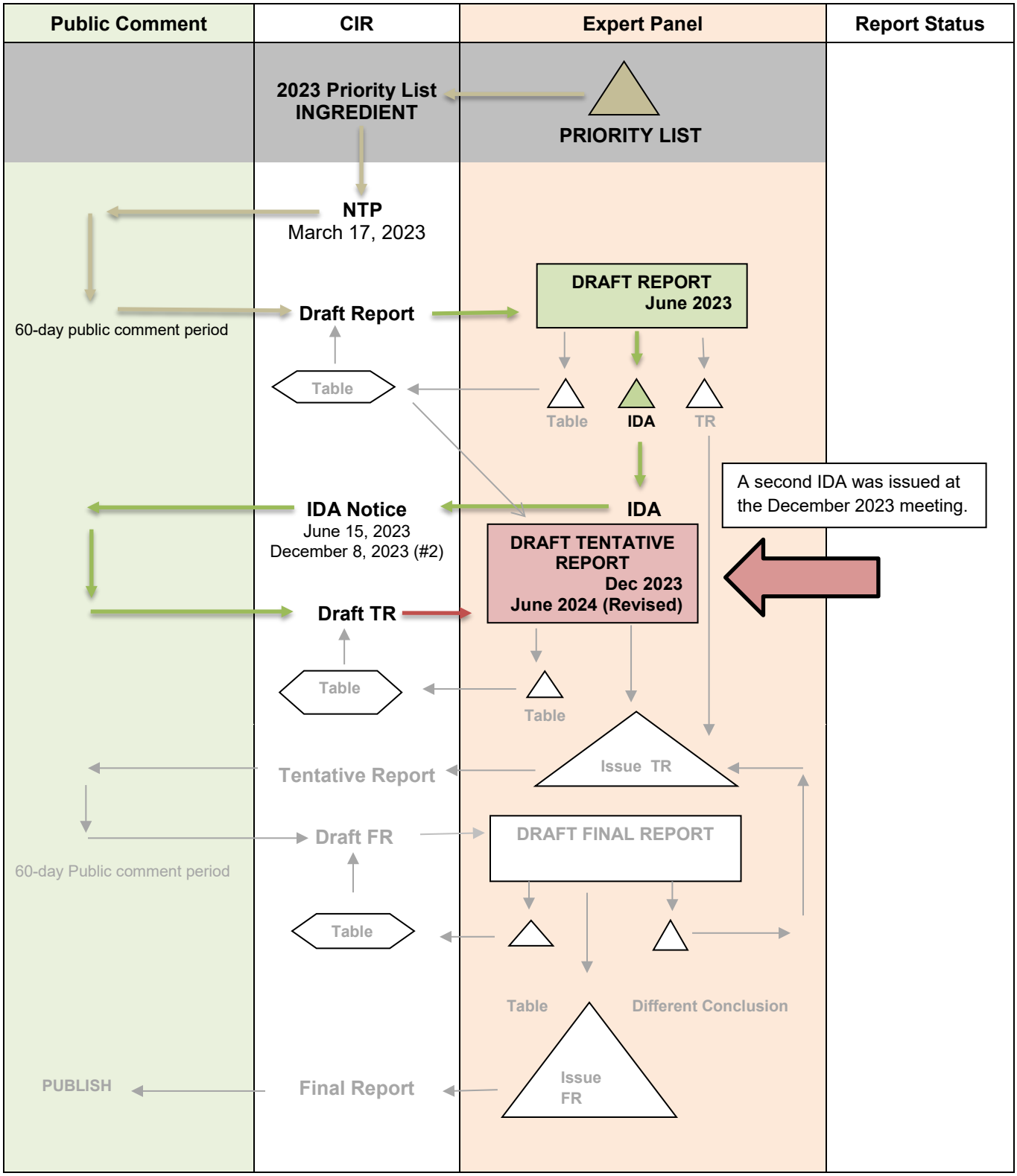
SAFETY ASSESSMENT FLOW CHART

INGREDIENT/FAMILY Ethyl Tafluprostamide and Isopropyl Cloprostenate

MEETING June 2024



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 10, 2024
Subject: Safety Assessment of Ethyl Tafluprostamide and Isopropyl Cloprostenate

Enclosed is the Revised Draft Tentative Report of the Safety Assessment of Ethyl Tafluprostamide and Isopropyl Cloprostenate as Used in Cosmetics (*report_ProstaglandinAnalogues_062024*). At the December 2023 meeting, the Panel issued a second insufficient data announcement (IDA) for these ingredients, and requested the following data:

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

Also at the December 2023 meeting, data were provided on potential read-across ingredients including tafluprost, travoprost, and cloprostenol. The use of cloprostenol as a read-across ingredient was previously rejected by the Panel; however, the Panel requested confirmatory data (e.g., receptor interaction studies and downstream profiles of adverse events) to determine if the use of tafluprost and travoprost are appropriate for use in this report. In April 2024, additional submissions were received providing further justification for the use of travoprost and cloprostenol as surrogates for Isopropyl Cloprostenate. Read-across justification tables have been prepared on these potential read-across substances (tafluprost, travoprost, and cloprostenol), and will be reviewed by the Read-Across Working-Group (RAWG). These tables will be presented to the Panel as Wave 2 following analysis by the RAWG.

Since the issuing of the second IDA, the following submissions have been received.

- *data1_ProstaglandinAnalogues_062024*: Roadmap to Safety Assessment for Isopropyl Cloprostenate. Submission to Cosmetic Ingredient Review.
  - summary of information on Isopropyl Cloprostenate at 0.0075% that will be submitted in the future; this information includes:
    - QSAR assessment
    - HET-CAM assay
    - EpiOcular assay
    - HRIPT
    - 8-wk clinical assay
- *data2_ProstaglandinAnalogues_062024*: Additional Data Supporting the Safe Use of Isopropyl Cloprostenate (up to 0.005%) in Cosmetics

    - summary of testing submitted previously using Isopropyl Cloprostenate at up to 0.005%
    - new data (which have been incorporated into the report in highlighted text):
        - Ames assay
        - In vitro micronucleus assay
    - summary of testing to be submitted in the future:
        - dermal metabolism and penetration assay
        - updated toxicological safety assessment with further substantiated read-across methodology
    - table presenting IDA requests along with request status/substantiation
- *data3_ProstaglandinAnalogues_062024*: Roadmap for Ethyl Tafluprostamide/DDDE and request for extension to respond to the IDA
    - summary of information on Ethyl Tafluprostamide that will be submitted in the future; this information includes:
        - receptor binding potency studies
        - in vitro neutral red uptake assay
        - ReproTracker assay
        - Toxprofiler assay
        - in silico endocrine receptor/activation predictions
        - literature research of endocrine receptor activation by analogues
        - analysis of differences in metabolism due to germinal fluorines in the suitability of analogues in read-across analyses
        - report on read-across analyses

At the previous meeting, several presentations were made on this ingredient group. Links to these presentations are provided below:

- **PRESENTATION: Safety assessment of Ethyl Tafluprostamide as used in in cosmetic products - Petry & Mishra**
  > Download PDF (1.08MB, 24PP)

- **PRESENTATION: New Studies Support Safety of Isopropyl Cloprostenate in Cosmetics - Abramowitz & Weiss**
  > Download PDF (602.78kB, 13PP)

Also included in this package for your review are a flow chart (*flow_ProstaglandinAnalogues_062024*), literature search strategy (*search_ProstaglandinAnalogues_062024*), ingredient data profile (*dataprofile_ProstaglandinAnalogues_062024*), ingredient history (*history_ProstaglandinAnalogues_062024*), and transcripts from previous meetings (*transcripts_ProstaglandinAnalogues_062024*).

At the December 2023 meeting, the Panel reviewed the Margin of Safety (MoS) calculations that were performed using systemic points of departure (POD) derived from chemicals similar to Ethyl Tafluprostamide and Isopropyl Cloprostenate (i.e., an NOAEL at 0.0003 mg/kg bw/d for tafluprost and an LOAEL at 0.00012 mg/kg bw/d for travoprost, respectively). The Panel requested an adjustment factor of 3 be applied for the extrapolation from LOAEL to NOAEL for travoprost. Consequently, the MoS for Isopropyl Cloprostenate was recalculated using the derived NOAEL of 0.00004 mg/kg bw/d for travoprost.

The Panel should carefully consider and discuss the data (or lack thereof), and the draft Abstract and draft Discussion presented in this report. A Tentative Report with a safe as used, safe with qualifications, insufficient, split, or unsafe conclusion should then be issued. Alternatively, after review of the received documents, the panel may wish to table this report. If this is the case, the Panel should set a firm deadline for when this report will return for their review.

Prostaglandin Analogues – History

March 2022

NTP issued

April 2022

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

May 2022

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

Data received on Ethyl Tafluprostamide (several endpoints)

June 2022

Panel reviews Draft Report

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

October 2022

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

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

November 2022

Instructions for use of a product containing Isopropyl Cloprostenate received

Concentration of use received from Isopropyl Cloprostenate

Comments on Tentative Report received from Council

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

June 2023

Panel reviews Draft Tentative Report and issues a second IDA with the following needs:

- for Ethyl Tafluprostamide:
    - acute toxicity data
    - repeated dose toxicity data
    - developmental and reproductive toxicity data
    - in vivo genotoxicity data

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

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

April 2024

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

June 2024

Panel reviewed Revised Draft Tentative Report

Prostaglandin Analogues Data Profile – June 2024 – 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												X	X				X	X			X		X		X			
Isopropyl Cloprostenate	X		X	X	X					X				X			X	X	X				X			X			X		X	X	X	X

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

Prostaglandin analogues

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

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– June 16, 2022

Prostaglandins not discussed in this team.

Full Panel – June 17, 2022

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

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

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

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

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

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

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

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

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

Dr. Bergfeld - Ok. *(inaudiable)

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

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

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

Dr. Belsito - Yeah.

Dr. Heldreth (CIR) - Yes.

Dr. Cohen - Yes, yes.

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

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

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

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

Dr. Bergfeld - Is there a second?

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

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

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

Dr. Belsito - OK too, yeah.

Dr. Bergfeld - 2.

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

Dr. Liebler - Yeah, all the prosteenoid structures.

Dr. Bergfeld - Yeah.

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

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

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

Dr. Belsito - Table 2 and 3.

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

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

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

Dr. Bergfeld - OK.

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

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

Dr. Belsito - Yes.

Dr. Ross - Correct.

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

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

Dr. Cohen - Yes.

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

Dr. Rettie - Yeah.

Dr. Heldreth (CIR) - Works for me.

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

SEPTEMBER 2022 PANEL MEETING – PRIORITY LIST DISCUSSION

Cohen Team – September 26, 2022

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

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

Dr. Bergfeld - Yeah.

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

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

Dr. Cohen - Good to, Wilma

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

Dr. Ross - We don't have.

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

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

Dr. Cohen - Yeah.

Belsito Team – September 26, 2022

Minutes not available.

Full Panel – September 27, 2022

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

Dr. Cohen - Someone just raised their hand.

Dr. Bailey (ECG) - OK.

Dr. Belsito - Yeah, it's John.

Dr. Bergfeld - John Bailey.

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

Dr. Belsito - Thanks John.

Dr. Bailey (ECG) - Yeah.

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

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

Dr. Belsito - Yes.

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

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

Dr. Bergfeld - OK.

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

Dr. Heldreth (CIR) - Correct.

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

Dr. Belsito - Yes.

Dr. Bergfeld - Yeah.

Dr. Cohen - Yeah.

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

JUNE 2023 PANEL MEETING – DRAFT REPORT

Cohen Team – June 12, 2023

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

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

DR. BERGFELD: This is before lunch though.

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

DR. ROSS: Let's do it.

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

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

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

DR. COHEN: That we cannot read across?

DR. ROSS: You cannot read across, yes.

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

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

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

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

DR. BAILEY: That's a mistake.

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

DR. COHEN: Yeah.

DR. TILTON: Okay.

DR. ANSELL: Or nanograms, not milligrams.

DR. COHEN: Okay.

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

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

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

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

DR. COHEN: Sure.

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

DR. BAILEY: Hi. John Bailey.

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

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

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

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

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

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

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

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

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

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

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

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

DR. ROSS: I think we need some direction.

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

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

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

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

DR. BAILEY: Pardon?

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

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

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

DR. ROSS: 180.

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

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

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

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

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

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

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

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

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

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

DR. BAILEY: Not quite.

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

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

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

DR. ROSS: Yeah.

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

DR. COHEN: Yeah.

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

DR. COHEN: Are you talking about Latisse?

DR. BERGFELD: Um-hmm.

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

DR. BERGFELD: No.

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

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

DR. ROSS: I see.

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

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

DR. BAILEY: Yes.

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

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

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

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

DR. COHEN: Tom?

DR. SLAGA: Tabling it would be fine.

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

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

DR. ROSS: Okay.

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

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

DR. COHEN: We have impurities.

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

DR. BERGFELD: Can't hear you.

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

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

DR. COHEN: On both?

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

DR. BERGFELD: Isopropyl (inaudible).

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

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

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

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

DR. COHEN: It's page 180?

DR. TILTON: Yeah.

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

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

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

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

DR. ANSELL: Right.

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

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

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

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

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

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

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

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

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

DR. BAILEY: They do. Yeah.

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

DR. BAILEY: Right.

DR. COHEN: The steroids.

DR. ROSS: Yeah.

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

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

DR. COHEN: Yeah.

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

DR. BAILEY: Pardon? Can't --

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

DR. BAILEY: Not specifically at this point.

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

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

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

DR. BAILEY: Thank you very much.

DR. COHEN: Thank you.

DR. ROSS: Thank you.

DR. HELDRETH: Thanks.

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

DR. BAILEY: Yes.

DR. BERGFELD: Okay, John, thank you.

DR. COHEN: What do you want to do?

DR. ANSELL: It's 11:52.

DR. SLAGA: Break for lunch and come back.

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

DR. SLAGA: Quarter to.

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

DR. ROSS: No, I'm fine.

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

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

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

DR. BERGFELD: That's nice especially.

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

Belsito Team – June 12, 2023

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

MS. CHERIAN: Monice.

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

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

DR. BELSITO: Curt, Paul?

DR. KLAASSEN: Fine.

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

DR. SNYDER: What page?

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

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

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

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

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

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

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

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

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

DR. BELSITO: Absorption, use --

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

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

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

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

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

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

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

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

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

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

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

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

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

DR. SNYDER: PC comments on the report.

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

DR. BAILEY: Dr. Belsito?

DR. BELSITO: Yes.

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

DR. BELSITO: Sure.

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

DR. RETTIE: Yeah.

DR. BELSITO: The KIs that you found for?

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

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

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

DR. BAILEY: Right.

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

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

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

DR. RETTIE: If it's out there.

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

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

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

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

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

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

DR. BAILEY: Not -- not --

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

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

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

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

MS. FIUME: PDF page 180 has a list.

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

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

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

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

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

DR. BELSITO: Okay.

DR. SNYDER: Don't you think?

DR. BELSITO: Yeah.

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

DR. BELSITO: Could be.

DR. SNYDER: Could be.

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

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

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

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

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

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

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

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

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

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

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

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

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

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

DR. BELSITO: Okay.

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

DR. BELSITO: Other comments? Paul?

DR. SNYDER: Mm-uhm.

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

DR. BELSITO: Yeah.

MS. FIUME: Okay. So, yeah.

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

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

MS. CHERIAN: Yeah.

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

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

DR. BAILEY: We can clarify that.

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

MS. FIUME: I just wanted to clarify that.

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

MS. FIUME: They were use studies.

DR. BELSITO: Yeah.

MS. FIUME: Yeah.

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

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

DR. SNYDER: Oh, okay.

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

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

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

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

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

DR. BELSITO: I'm sorry?

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

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

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

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

MS. FIUME: Yes.

DR. BELSITO: We've done this before.

DR. SNYDER: Okay.

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

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

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

DR. SNYDER: Okay.

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

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

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

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

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

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

DR. BELSITO: Anything else?

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

DR. BELSITO: What PDF are you on?

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

DR. SNYDER: Two and a half.

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

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

DR. KLAASSEN: What page is that?

DR. SNYDER: 23.

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

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

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

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

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

DR. BELSITO: Yeah.

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

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

DR. KLAASSEN: Yes.

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

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

DR. KLAASSEN: Yeah.

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

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

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

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

MS. FIUME: That's fine. Yes.

Full Panel – June 13, 2023

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

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

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

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

DR. BELSITO: Yes.

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

DR. BELSITO: Sure.

DR. BERGFELD: Any other discussion? David?

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

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

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

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

DR. BELSITO: Right.

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

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

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

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

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

DR. COHEN: With a reasonable model certainty.

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

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

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

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

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

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

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

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

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

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

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

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

DECEMBER 2023 PANEL MEETING – DRAFT TENTATIVE REPORT

Belsito Team – December 4, 2023

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

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

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

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

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

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

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

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

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

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

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

DR. RETTIE: That's right.

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

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

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

DR. BELSITO: Yes.

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

DR. HELDRETH: Let me see what we have.

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

DR. MISHRA: Yeah. Yes.

DR. BELSITO: Right.

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

DR. BELSITO: Okay.

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

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

DR. RETTIE: I think so.

DR. KLAASSEN: Yes.

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

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

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

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

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

DR. SNYDER: I agree.

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

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

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

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

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

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

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

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

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

DR. BELSITO: Right.

MS. CHERIAN: Okay.

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

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

DR. BAILEY: Okay.

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

DR. SNYDER: Yes. Yes, I agree.

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

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

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

DR. BELSITO: Pregnant women.

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

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

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

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

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

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

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

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

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

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

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

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

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

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

DR. RETTIE: Yeah.

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

DR. ATOR: There really isn't.

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

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

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

DR. RETTIE: Yep.

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

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

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

DR. SNYDER: Correct.

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

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

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

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

DR. BAILEY: Okay.

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

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

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

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

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

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

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

Now if you have a chemical --

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

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

DR. ATOR: That requires the assumption.

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

DR. ATOR: They do.

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

DR. MISHRA: Yes.

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

DR. BELSITO: According to the SCCS.

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

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

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

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

DR. BELSITO: Right.

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

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

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

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

DR. HELDRETH: We'll make that change.

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

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

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

DR. MISHRA: Company.

DR. PETRY: -- with our client.

DR. MISHRA: Yeah.

DR. BELSITO: Numbers I think are helpful here.

DR. PETRY: I know. We understand.

DR. MISHRA: Yeah.

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

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

DR. BELSITO: Okay.

DR. KLAASSEN: I'm fine.

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

So, has that been addressed?

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

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

DR. SNYDER: In the absence of read across.

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

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

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

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

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

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

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

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

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

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

DR. RETTIE: I thought they did.

DR. BELSITO: Pardon?

DR. RETTIE: I thought they did.

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

DR. MISHRA: Yep. We have data --

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

DR. KLAASSEN: Yeah.

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

DR. BELSITO: Yeah.

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

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

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

DR. MISHRA: Yes.

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

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

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

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

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

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

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

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

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

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

DR. KLAASSEN: Yes.

DR. RETTIE: I think so.

DR. SNYDER: I agree.

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

DR. PETRY: All right, thank you.

DR. MISHRA: Thank you.

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

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

DR. BERGFELD: Oh, lucky us.

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

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

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

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

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

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

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

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

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

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

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

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

DR. COHEN: I'm looking --

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

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

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

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

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

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

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

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

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

DR. ROSS: Was I?

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

DR. BERGFELD: You had a list.

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

DR. COHEN: Okay. Tom?

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

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

DR. SLAGA: Oh, okay.

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

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

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

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

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

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

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

DR. ATOR: All right.

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

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

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

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

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

DR. ATOR: Yeah.

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

DR. ATOR: Yes.

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

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

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

DR. ATOR: That was my understanding as well.

DR. COHEN: Any product or these?

MR. ABRAMOWITZ: Any product.

DR. ATOR: Right. Because the main --

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

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

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

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

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

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

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

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

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

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

DR. COHEN: Any other comments?

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

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

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

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

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

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

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

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

MR. ABRAMOWITZ: Correct.

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

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

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

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

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

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

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

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

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

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

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

DR. COHEN: We did notice that.

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

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

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

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

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

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

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

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

DR. COHEN: Any comments, David?

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

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

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

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

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

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

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

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

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

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

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

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

DR. COHEN: Well, again --

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

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

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

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

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

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

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

DR. COHEN: And the ocular effect.

DR. TILTON: Yeah.

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

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

DR. COHEN: Yeah. Okay.

DR. ROSS: And that's --

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

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

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

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

DR. BERGFELD: Shebang. Yeah.

DR. ROSS: Yeah.

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

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

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

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

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

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

DR. COHEN: (Audio skip).

DR. BAILEY: That's largely because of the challenge of using animals to do the 28-day dermal.

DR. COHEN: Okay.

DR. ROSS: But you would certainly need irritation and sensitization. Every piece of data we have is on the 0.048 percent. It's not on the 0.0075 percent.

DR. COHEN: Okay. And for ET?

DR. ROSS: We need to sort out the read across issue.

DR. COHEN: Well, what is -- we have to articulate our data needs.

DR. ROSS: Well, it's the read across issue if it's the conclusion of the whole panel, the read across is not going to work then you are going to need additional data to fill in for the lack of read across.

DR. COHEN: So, can you outline that?

DR. ROSS: Well, it was beautifully outlined in Dr. Petry's presentation. I'll just go back to your dossier. What you were using for read across, I think there was some local tox, but DART as well for --

DR. COHEN: We may be able to go to the very nice chart that's in front of all of our reports, right?

DR. ROSS: I like the one that Dr. Petry -- that submission was -- even though, as I said, I disagreed with some of it -- it was a beautiful submission and really helped.

DR. PETRY: I can quickly try to get it out here.

DR. ROSS: I've got it --

DR. PETRY: Is this the chart you mean?

DR. COHEN: Is this it?

DR. ROSS: I don't see --

DR. COHEN: Acute dermal tox or 28-day dermal tox?

DR. ROSS: There's acute toxicity read across to tafluprost, repeat dose toxicities read across to tafluprost, and repro and developmental is read across to tafluprost.

DR. TILTON: And the alternative to that is additional data to support the read across.

DR. ROSS: Correct, Susan. Just going to go there, yeah.

DR. COHEN: So, Suan, these aren't the terms we'd necessarily use in our IDA's. Can we outline what we want in our IDA if we don't have additional information for the read across, what are our data needs? So, we need dermal --

DR. TILTON: It may be easier to request data to support a read across.

DR. COHEN: Well, we just may not get what we want. So, we need the whole acute tox profile, repeated tox profile. I mean, there's a lot of boxes there, guys.

DR. ROSS: There's a lot of boxes and --

DR. COHEN: I know. But I want to make sure we're asking for what we really need to help this along.

DR. ROSS: But I think if you approach it from the sense that you may need more data to decide on read across or not and if the data that might help support that read across would be -- I think, Dr. Petry made two additional suggestions this evening. Receptor interaction studies of amides and carboxylic acids and downstream profiling of effects of these two prostaglandin types (audio skip) and that could support the read across. If the read across is still not supported, then you need to fill up these gaps that the read across was previously filling.

DR. COHEN: Okay. I think I have --

DR. PETRY: If I may add. I think it depends a little bit if we, let's say, even if we have -- if the receptor interaction studies show there is receptor interaction I would still look if there's a downstream consequence to it.

DR. ROSS: That comment --

DR. COHEN: Yes. It's an and, not an or. Right?

DR. ROSS: Yeah.

DR. PETRY: Yep.

DR. COHEN: Any other comments on that? I think I have my work cut out trying to summarize this into an intelligible discussion tomorrow.

DR. ROSS: And David --

DR. BERGFELD: It needs it.

DR. ROSS: -- can we make sure we have something about the cautionary statements on both of these?

DR. COHEN: I believe I have that already written but I'll -- okay. Okay.

MS. FIUME: David, can I clarify that? And then I see that there is a hand up. So, the plan is to issue a second IDA even though some of the requests are the same as what you had issued before because there wasn't a concentration limit in those original requests. It was maximum concentration of use. So, some of them will be the same but the reason for the second IDA is because you do have additional data requests that weren't included in the original IDA. Is that correct?

DR. COHEN: Well, I think that's partially correct, right? I mean, the original IDA was predicated on a max concentration of use which has been updated, right? And then there's this discussion about a failure to read across or potential failure to read across which is creating additional insufficient data needs.

MS. FIUME: Okay. No, I was just curious because the original IDA didn't specify what that max concentration of use was, it just stated at max concentration of use.

DR. COHEN: Now, I don't remember, but did we know it was 0.075 in that original report?

DR. ROSS: No.

MS. FIUME: So, we did, but then as part of the IDA it had -- no we did not know that. That was the lower, I believe. But we said at max concentration of use. If max concentrations of use are higher than what's used already in the report. So, that dermal irritation and sensitization included a caveat in case there was a higher concentration.

DR. COHEN: What you're saying is that the original IDAs weren't met?

MS. FIUME: Part of them were, but the reason for the second IDA is because you have additional needs, correct, compared to what was in the original IDA? Okay.

DR. COHEN: Yes, yes. Yeah, I think I understand your question and I think you've articulated what we're asking for.

MS. FIUME: Okay. So, I guess my point was that if you knew that there was a higher concentration of use, but you didn't have those data, typically you would go forward then maybe stating it's okay at one concentration but not the other because of the data you've received. But now that there are additional needs, that predicates the second IDA and then anything from that first IDA can be updated. Does that make sense what I'm trying to say?

DR. COHEN: Yes, yes.

MS. FIUME: Okay.

DR. COHEN: Let me just come back. I don't know where I went here. Okay.

MS. FIUME: We can see you.

DR. COHEN: I can't see anybody. Let me just --

MS. FIUME: Oh.

DR. COHEN: -- Paul came in and then just knocked me off the meeting.

DR. ROSS: We can still see you. And hear you.

DR. COHEN: I'm sorry for that.

DR. ROSS: I think he's trying to say he's on vacation right now. Now he's frozen.

MS. FIUME: Now he's frozen. He may have been going out and coming back in.

DR. ROSS: He's frozen smiling.

DR. BERGFELD: Well, that's a nice freeze. David, are you with us or are you frozen in all ways?

MS. FIUME: I have a feeling he might be trying to come back in even though it doesn't show that he left the meeting.

DR. BERGFELD: Do you want to send him a chat that we can't see him or hear him?

MS. FIUME: I'll see if I can send a text because I think the chat is disabled.

DR. BERGFELD: Okay.

DR. ROSS: I'm just thinking how well we've done on the technology.

MS. CHERIAN: It looks like Dr. Cohen needs to be made a presenter. He's listed as --

MS. FIUME: Okay. So, he must've come back in. Thank you.

MS. CHERIAN: You're welcome.

DR. COHEN: Yes. I was waving my hands, but I could not get in.

MS. FIUME: Sorry.

DR. COHEN: So, Monice, I think I understand what you're saying but I'm not sure what I'm supposed to do with this statement, to be honest with you.

MS. FIUME: So, I guess it was just more for understanding procedure as just for the purpose of this meeting and future meetings that originally some of the list that was given were the same IDA requirements from the last meeting that didn't state at a concentration of 0.005. so even though you would've received a different concentration we still could've gone forward -- the panel still could've gone forward with the information that was in the report to decide if it covered that higher concentration or not.

But since there were additional data needs that predicates going forward with the second IDA because if you had -- if the data needs weren't any different and it was just based on a concentration typically the panel doesn't issue a

second insufficient data announcement especially since the first one said at maximum concentrations of use even if they're higher. Give us what you have. It was just a matter of procedure.

DR. COHEN: Yeah, yeah. No, no. I get that. So, I mean, one way to look at it is we don't have that data at max use.

MS. FIUME: Right. So typically, if that's the case, then you go insufficient as a conclusion not asking for a second IDA.

DR. COHEN: I get it. So, I und- --

MS. FIUME: But you have additional needs so then that does predicate a second IDA because they weren't included the first time around.

DR. COHEN: Well, what we have is, we have an IDA for ET, but we have insufficient data for ethyl. For isopropyl, I mean. Right?

MS. FIUME: Right. So, since it's going out anyway, then to combine it all and ask again makes total sense.

DR. COHEN: Right. It's not technically an IDA. It's insufficient to the original IDA.

MS. FIUME: Well, it would be a second. Right, you'd be issuing a second IDA. If you didn't have additional data needs, then you would probably -- according to procedure -- you would typically go forward with an insufficient conclusion which someone could comment on. But since you have additional needs then it's an insufficient data announcement.

DR. COHEN: Yes. I think for the purposes of giving everyone a chance, I think I'd prefer an IDA here because it gives people another go around with this as opposed to getting it closer to a final report that's not going to work.

MS. FIUME: Right. And, as I said, you have needs that weren't in the previous one so it would have to go IDA regardless because you can't go insufficient conclusion for something that you hadn't asked for.

DR. COHEN: Right, right. No, I get that. Okay. Any other business on the prostaglandins?

MR. ABRAMOWITZ: Just before we go, I'm going to try to understand. Is there no way that the panel can issue a safe for use up to 0.005 percent while issuing an IDA on 0.0075 percent since all the data has been submitted and that the panel has done that before where it's issued to a certain concentration when it has a full data set on that particular concentration? We're just trying to understand because, obviously, it's a product that's not represented here that's simply been an anonymous submission and we don't have a basis for understanding whether that data will ever come in.

DR. COHEN: Well, we don't know that but sometimes we'll get reports on lots of uses. I don't know who they are from. Most of the time, we don't have representatives advocating for a specific product, we have 80 uses and maybe a couple dozen concentration reports. So, this is routine for us to get different concentrations of use.

DR. BERGFELD: I think that maybe what you're really asking is to go forward faster than we would like. It doesn't mean at the next meeting we won't do exactly what you suggested.

MR. ABRAMOWITZ: Okay. I mean, I think that's our concern is that we share that concern, they'll never be any data on that particular concentration.

DR. COHEN: We get it.

DR. BERGFELD: I think that we're going to make another try and then we're going to make a decision. Yeah.

DR. ROSS: There were some insufficiencies even in the IC reports even though I'm not expecting any surprises there but there was no genotox in that report. For example, I pointed that out earlier. So that's probably needed. But yeah. I think we got the gist of it.

DR. BERGFELD: Okay.

DR. COHEN: One second. The genotox, David, was that in our original IDA?

DR. ROSS: No, I don't think so. And someone can correct me if I missed it. I don't think I missed it. It was there for ET, but I didn't see it for IC.

UNKNOWN MALE: Did we have any genotox?

MR. ABRAMOWITZ: We have read across data for genotox for IC. There's not a lot of direct IC genotox because of the animal testing ban.

DR. KOWCZ: They want the dermal tox.

MS. FIUME: Dr. Cohen, in response genotox was not in the original IDA so that would need to be added.

DR. ROSS: Well, I mean, yeah. The point is that you've got read across here. So, they're reading across from travoprost and if that read across is okay then --

MR. ABRAMOWITZ: And cloprostenol, both of them, genotox from travoprost and cloprostenol.

DR. ROSS: Yeah, we already looked at cloprostenol as not read across. But anyway, that's a different discussion.

DR. COHEN: Okay. I think we've had a good conversation here.

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DR. COHEN: That's a lot of time on this one. Yes, so there was quite a robust conversation both in the morning and in the afternoons about the Prostaglandins. This assessment is for Ethyl Tafluprostamide and Isopropyl Cloprostenate. At the June 2023 meeting the Panel issued an Insufficient Data Announcement for these ingredients and requested the following information: concentration of use, information on packaging of products and directions for consumer use, 28-day dermal tox data and if absorbed further systemic tox, dermal sensitization and irritation data at maximum concentration of use (if maximum concentration of use are higher than the concentration used in dermal irritation and sensitization studies already present in the report) -- that was a provision -- intraocular pressure data on eyelash preparation containing Isopropyl Cloprostenate, and potency/inhibition constant binding affinity data on Ethyl Tafluprostamide and Isopropyl Cloprostenate as compared to bimatoprost (an FDA-approved prostaglandin analog for ocular hypertension/glaucoma as well as eyelash lengthening)

Since the issuing of this IDA, new data has been received on both chemicals. Amongst the several discussions we had, our group was generally uncomfortable with read-across and this was pointedly expressed by Dr. Klaassen yesterday. We have new max use of Isopropyl Cloprostenate at 0.0075 percent. We had some issues in the calculations of margin of safety for Ethyl Tafluprostamide as it relates to retention factors in the use of LOAEL instead of NOAEL.

Our motion is as follows, since there's new max concentration of use we're issuing an IDA for Isopropyl Cloprostenate with the following needs recognizing that some of these are previously insufficient from the initial IDA, however, our motion is for a new IDA; our needs are irritation and sensitization at max use, local ocular effects including pressure and eye risk color change at max use, acute tox, repeated dose tox, DART, we still need genotox, and we're requesting an independent ophthalmology evaluation to assess color imagery data regarding iris color change data.

For Ethyl Tafluprostamide we have an IDA for the following needs; acute tox, repeat dose tox, DART or if there are confirmatory data on read-across that would suffice such as receptor interaction studies and downstream profiles of adverse effects. Lastly, we have a need for eyelash product information or consumer use instructions to include cautionary statements for pregnancy, lactation, pediatric use and those who are sensitive individuals such as those undergoing chemotherapy or treatment of eye disease. That's the completion of my motion.

DR. BELSITO: So for Ethyl you did not want genotox? You didn't mention that.

DR. COHEN: Let me look back, Don, once second.

DR. SNYDER: I wrote down that he mentioned it.

DR. BELSITO: No, for Isopropyl he mentioned genotox.

DR. BERGFELD: I didn't hear it either.

DR. COHEN: Genotox.

DR. BERGFELD: You're adding genotox?

DR. COHEN: Yeah.

DR. BERGFELD: Okay.

DR. BELSITO: I'll second it. You want way more than we asked for, but it would go out as an IDA anyway so we can just go out with all of your requests.

DR. COHEN: Okay.

DR. BELSITO: The only other thing, if they want, read-across would be to give us more robust information on all the possible targets and mechanism that would come out.

DR. BERGFELD: Any other comments for the IDA, the second one?

DR. COHEN: Priya?

MS. CHERIAN: We had genotoxicity data for Ethyl. That's probably why it wasn't in your IDA mention.

DR. ROSS: Was that read-across?

MS. CHERIAN: It was in vitro data.

DR. ROSS: Okay.

DR. BELSITO: But we need in vivo data.

MS. CHERIAN: Okay.

DR. BELSITO: It's on PDF Page 36. Oh, we do have a micronucleus assay as well.

DR. COHEN: Yeah.

DR. HELDRETH: We have a couple hands up from attendees, if this is a good time to address those.

DR. BERGFELD: Can you call them? I can't see them.

DR. HELDRETH: Yeah, I'm bringing them in. Okay, so we have Mr. Abramowitz and John Bailey.

MR. BAILEY: I'll let David go first and then I'll follow. And I think Tom Petry has also raised his hand to address the IDA element. David, won't you go ahead.

MR. ABRAMOWITZ: Thank you, John. And thank you to the Panel. After hearing the needs on ICN we understand the max use issue as opposed to the .005 percent concentration issue, which is where you've received most of your data. I do want to ask some questions about for example the genotox request. We are certainly -- we started our safety program long before this commission and Panel started looking at IC, and so we've been continuously working towards establishing the safety of IC at .005 percent or less. So, could you please, if you can tell me, what exact genotox studies you'd like to see, so we can move forward with that progress, we'd appreciate it. Genotox is a big category.

DR. BERGFELD: Curt, or Tom, or David?

DR. SLAGA: Well, actually we'd prefer to see several in vitro and in vivo tests.

MR. ABRAMOWITZ: Which in vitro test would you like to see, AMES?

DR. SLAGA: In vitro would be AMES, primarily.

MR. ABRAMOWITZ: And given the animal testing done, what in vivo test are you looking for?

DR. KLAASSEN: Micronucleus test, isn't that what you're asking for, Tom?

DR. SLAGA: Come again?

DR. ROSS: Yeah. It's usually micronucleus.

DR. KLAASSEN: The micronucleus test we would like.

DR. SLAGA: Yes, micronucleus test would be one.

MR. ABRAMOWITZ: Okay. And we're trying to outline here so we can make sure the Panel has everything they need on .005 percent or less. In addition, you mentioned DART. Now, obviously we can't go back in time and run DART studies on mice or rats, or rabbits. Is there an in vitro test you're looking for on DART, or other information? I know we have provided read-across both on travoprost and on the primary metabolite of IC, cloprostenol. We'd appreciate it, if you don't have any suggestions for DART, if the Panel would go back and reexamine that particular read-across considering that cloprostenol, for example, is the primary metabolite of IC in the body. So it should provide a robust system for looking at IC.

DR. BERGFELD: Comment? Curt?

DR. COHEN: I think we discussed the read-across quite a bit yesterday.

DR. BERGFELD: Yeah.

DR. KLAASSEN: There aren't a large number of in vitro DART studies unfortunately. So what we usually hope to see is some in vivo studies. It's about the only way we have for looking at teratogenicity and other reproductive problems.

MR. ABRAMOWITZ: Thank you, we appreciate that. And we would like the Panel to continue to consider that the read-across for IC. It's different than a read-across for ET. And our methodology is different since we're using the primary metabolite as a bases as well as a more similar chemical.

I don't know exactly how to deal with your DART request. In addition to that individual ophthalmology assessment, we have photos from our clinical study, if that's something that the panel would like us to submit. We can submit something like eight gigabytes worth of photos for the ophthalmology study, if the Panel wishes to seek an ophthalmologist to assist it.

DR. COHEN: I think that would be useful to get an independent person to look at it, and look at the colorimetric data.

DR. BERGFELD: I think it would be important if it's someone who's very knowledgeable of that kind of technique and interpretation. Any other comments?

MR. BAILEY: I just want to thank the Panel for your comprehensive deliberations. And I think the IDA that you're issuing is extremely helpful. We need the details to understand how we need to fill the data gaps between now and the next meeting. You know I keep emphasizing read across, but it's extremely important to be able to fill these data gaps. And what I think I heard is that you're willing to consider more in-depth justifications for applications for the IC and the ET. So, we'll certainly work to provide that.

And one final thing, yesterday there was a lot of discussion back and forth, and I just wanted to reemphasize that we're really talking about cosmetic uses here, not drug uses, not drug endpoints, but cosmetic safety. And this was affirmed by FDA early on. So I just want to make sure that we keep that in mind, we're looking at cosmetic safety endpoints, going forward. So, with that, I think we have a pretty good idea of what we're going to need. And, again, appreciate the Panel's deliberations.

DR. BERGFELD: Thank you, John. Dr. Petry, did you want to say something? I heard that your hand was up.

DR. PETRY: Yeah, first of all, again also to thank the Panel for the good discussions we had on the read-across justification. I certainly understood what you're specifically looking for. And I think we have some good ideas on what we can do and hopefully we can provide the data for the next review.

There was one question I do have with regard to genotoxicity for ET. Because you included now in the IDC, we have a base set of the (inaudible) tox in vitro data, which is the AMES test, the mammalian mutagenicity test and also the micronucleus test in vitro. Is there anything in addition that you're looking for?

DR. BELSITO: We usually ask -- yeah, I think you're fine. It was my mistake.

DR. BERGFELD: So, you're withdrawing the need for genotox?

DR. BELSITO: For ET.

DR. BERGFELD: For ET.

DR. KLAASSEN: In regard to the micronucleus, you said you did that but it was in vitro, or in vivo?

DR. PETRY: It was in vitro.

DR. BELSITO: It was in vitro.

DR. KLAASSEN: Yeah, well, we would much -- we usually look at in vivo micronucleus. That would make us much happier; let me put it that way.

DR. PETRY: Well, maybe we can bring you that next time again. Because if we can establish a good read-across, we know that for Tafluprost there is in vivo micronucleus data as well.

DR. KLAASSEN: Great.

DR. COHEN: So, Don, we could keep your suggestion in my undated motion.

DR. BELSITO: Thank you.

DR. BERGFELD: And that suggestion is keeping the genotox need in there?

DR. COHEN: Yes.

DR. BERGFELD: Okay.

DR. ROSS: Yeah, the weight of evidence usually goes down on the in vivo genotoxicity, so, Dr. Petry, that would be good to have.

DR. PETRY: Well, on ET it's obviously for animal testing reasons we cannot do it. But let's hope that we can provide an improvement of the read-across justification. And then we should have also the link to the in vivo data which we have for Tafluprost.

DR. BELSITO: And just a comment. In terms of using the major metabolite of IC, one of the issues as we have no information on absorption distribution of metabolism. So we don't really know how long IC sits un-metabolized on the skin, and to Curt's point about the uniqueness of chemical interaction with receptor.

DR. ROSS: Yeah, and Dr. Petry's point about Tafluprost. We had almost exhaustive and certainly exhausting discussions on read-across yesterday. And I think we came down that Tafluprost, certainly in our meeting, wasn't going to cut that. I don't want to reopen that issue, but you should be aware of that.

DR. BERGFELD: All right. We have any other discussion, because we're ready to vote on the second IDA.

DR. ATOR: Can I just ask a quick question regarding genotoxicity testing?

DR. BERGFELD: Okay. Yes.

DR. HELDRETH: Can you please state your name and affiliation on the record, please?

DR. ATOR: This is Jennifer Ator. I wrote the tox report for the IC.

DR. HELDRETH: Thank you.

DR. ATOR: We are concerned that doing an in vivo micronucleus assay is not feasible because of the animal test guideline band. With that in mind I want to ask the Panel if maybe a different approach would be acceptable. For example, if we perform a battery of in vitro tests that covers all the relevant assays. And they are all negative, would that address the Panel's concern. And secondarily, if the answer is no, would and in vitro micronucleus assay address the concern.

DR. BERGFELD: Curt, Tom, David?

DR. KLAASSEN: Well, I think I'll just start off and then let Tom follow up. I guess it kind of depends on the whole package. And to give you an absolute yes or an absolute no, I think is impossible at this time. I think this group is always willing to look at the whole package, and how you describe it to us, what you have, you know, which chemicals you're looking at, which read-across, is there any in vitro that you can kind of use like Dr. Petry was just mentioning. So, put your best package together. We realize that there is a problem in this day and age with getting the ideal information. So that's what I would say. Tom?

DR. SLAGA: I agree with Curt. I think we'd like to see whatever package with genotoxicity you bring forward. And, if possible, the read-across data it's acceptable for eliminating doing an in vivo genotox. We would like to look at that and make a decision from it.

DR. ATOR: Okay, great, thank you very much.

DR. BERGFELD: All right, are we ready to vote on this? So this is a vote on an IDA, seconded, on the Prostaglandins. All those opposed? Abstaining? Unanimously voted on to approve to go forward with the second IDA. All right, at last we're going on to Yeast, Dr. Belsito.

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

Status: Revised Draft Tentative Report for Panel Review
Release Date: May 10, 2024
Panel Meeting Date: June 3 – 4, 2024

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
DDDE	dechloro dihydroxy difluoro ethylcloprostenolamide
DMSO	dimethyl sulfoxide
DPRA	direct peptide reactivity assay
ECHA	European Chemicals Agency
ED_5	dose estimated to produce a 5 unit area (mm*h) in a graph of the different in pupil diameter in the dosed eye versus time
$E_{product}$	estimated daily exposure to a cosmetic product per kg bw
EU	European Union
FDA	Food and Drug Administration
HET-CAM	hen's egg test chorioallantoic membrane
HRIPT	human repeated insult patch test
IC_{30}	30% inhibitory concentration
IC_{50}	half maximal inhibitory concentration
log K_{ow}	n-octanol/water partition coefficient
MoS	margin of safety
NR	none reported
NTP	Notice to Proceed
OECD	Organisation for Economic Co-operation 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
SAR	structure-activity relationship
SCCS	Scientific Committee on Consumer Safety
SED	systemic exposure dosage
TG	test guideline
TSV	toxicological screening value
US	United States
VCRP	Voluntary Cosmetic Registration Program
Dictionary	web-based *International Cosmetic Ingredient Dictionary and Handbook* (wINCI)

DRAFT ABSTRACT

The Expert Panel for Cosmetic Ingredient Safety (Panel) assessed the safety of the synthetic prostaglandin analogues Ethyl Tafluprostamide and Isopropyl Cloprostenate, which are reported to be used as hair conditioning agents in cosmetics. Ethyl Tafluprostamide is also reported to function in cosmetics as a nail conditioning agent. The Panel reviewed all relevant data and concluded that Ethyl Tafluprostamide and Isopropyl Cloprostenate… [to be determined].

INTRODUCTION

This assessment reviews the safety of Ethyl Tafluprostamide and Isopropyl Cloprostenate as used in cosmetic formulations. According to the 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] Additionally, Ethyl Tafluprostamide is reported to function in cosmetics as a nail conditioning agent (Table 1). Ethyl Tafluprostamide is also known as dechloro dihydroxy difluoro ethylcloprostenolamide (DDDE).

Some prostaglandin analogues (e.g., bimatoprost) are US Food and Drug Administration (FDA)-approved drugs.[2] However, the FDA has determined that the uses of the prostaglandin analogues reviewed in this report (i.e., Ethyl Tafluprostamide and Isopropyl Cloprostenate) are considered to be cosmetic uses (and are not considered to be drug uses). Thus, these uses are within the purview of the Panel and the safety of these cosmetic ingredients is reviewed herein.

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 April 2024. A listing of the search engines and websites that are used and the sources that are typically explored, as well as the endpoints that the Panel typically evaluates, is provided on the Cosmetic Ingredient Review (CIR) website (https://www.cir-safety.org/supplementaldoc/preliminary-search-engines-and-websites; https://www.cir-safety.org/supplementaldoc/cir-report-format-outline). Unpublished data are provided by the cosmetics industry, as well as by other interested parties.

Much of the data included in this safety assessment were found on the Scientific Committee on Consumer Safety (SCCS) website.[3] 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.[4] However, both of these ingredients are synthetic analogues. The definitions of these ingredients are provided in Table 1.

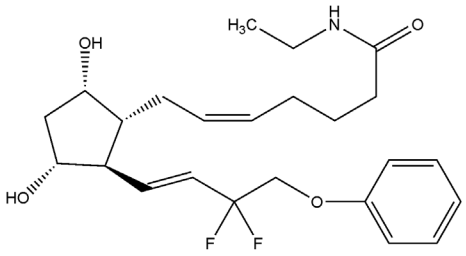

Figure 1. Ethyl Tafluprostamide

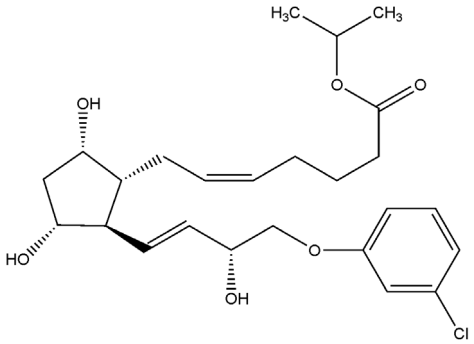

Figure 2. Isopropyl Cloprostenate

Chemical Properties

The ingredients reviewed in this report are hydrophobic, water-insoluble substances. [3] Ethyl Tafluprostamide is a colorless to pale yellow solution, with a reported water solubility of 1.05 g/l (at 20° C), and a high octanol/water partition coefficient (log K_{ow}: 2.74 ± < 0.01; 5.03).[3,5,6] 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%.[3,7] 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%.[3] 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 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 included herein were obtained in 2023 from 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) that was initiated in 2022. The data were provided by cosmetic product categories, based at that time on 21CFR Part 720. For most cosmetic product categories, 21CFR Part 720 does not indicate type of application and, therefore, airbrush application is not considered. Airbrush delivery systems are within the purview of the US Consumer Product Safety Commission (CPSC), while ingredients, as used in airbrush delivery systems, are within the jurisdiction of the FDA. Airbrush delivery system use for cosmetic application has not been evaluated by the CPSC, nor has the use of cosmetic ingredients in airbrush technology been evaluated by the FDA. Moreover, no consumer habits and practices data or particle size data are publicly available to evaluate the exposure associated with this use type, thereby preempting the ability to evaluate risk or safety.

According to 2023 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).[8] No uses were reported in the VCRP for 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 (and for which results were submitted in 2023).[9] However, according to data submitted by industry as a submission separate from the concentration of use survey, the average concentrations of Isopropyl Cloprostenate in two eyelash serums were determined to be 0.0044 and 0.0048%, respectively (corresponding to a weight of 8.4 and 13 ng Isopropyl Cloprostenate per usage of each serum, respectively); it is unknown if these are marketed serums.[10] Another separate unpublished data submission (specifically stating concentration of use) reported that an eyelash serum contained 0.0075% Isopropyl Cloprostenate (corresponding to 21 ng Isopropyl Cloprostenate per usage of each serum; calculation can be found in the Toxicokinetics Studies section of this report).[11,12]

In addition, according to another unpublished data submission, products intended for use on eyelashes, eyebrows, or scalp hair contain Ethyl Tafluprostamide in concentrations ranging from 0.012 – 0.02%.[7] The amount of an eyelash product containing 0.018% Ethyl Tafluprostamide applied per brush stroke was evaluated to be, on average, 2.4 mg of the product

(maximum amount of 4 mg per brush stroke).[13] Accordingly, the average amount of Ethyl Tafluprostamide applied per brush stroke was calculated to be 0.432 µg, with a 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.[14] 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).[3] 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.

Eyelash Product Information/Consumer Use Instructions

Ethyl Tafluprostamide

An eyelash product containing 0.018% Ethyl Tafluprostamide is reported to be a thickened solution provided in an aluminum, tube-like container.[7] A multi-use applicator wand is attached to the container's screw-on cap. The tip of the applicator consists of a very fine brush that is designed to optimize precise application of a small amount of the product to the eyelashes. The tube neck removes excess solution from the applicator when the applicator is removed from the container.

This product is to be used once per day, directly to the eyelashes, near the base, above the eyelash line, and should be dried completely prior to the application of other products. This product includes caution statements that inform consumers to avoid contact with the eye, rinse eyes if eye contact occurs, reduce and/or discontinue frequency of product use if irritation occurs, and to keep out of reach of children. The caution statement also informs users of potential skin discoloration of the eyelash base following use (predominantly excessive use) of this product, and to discontinue use of the product if this discoloration is of concern to the consumer.

Isopropyl Cloprostenate

Eyelash serums containing 0.004 and 0.005% Isopropyl Cloprostenate are to be applied once a day, as a thin line on the eyelid, just above the upper lash line.[15,16] These serums are reported to be packaged similarly to the eyelash product containing 0.018% Ethyl Tafluprostamide described above. Serums should be applied to a clean, dry lash line, using a single stroke (similar to application of liquid eyeliner). Users are instructed to use one dip into the bottle for both eyes, and to allow 1 - 2 min for the serum to dry. Applications should occur nightly for a duration of approximately 3 mo. After 3 mo, users should apply every other day or 2 - 3 times per week to maintain benefits. Caution statements on these products inform users to rinse eyes with cold water if eye contact occurs, and to discontinue use if irritation occurs. Statements also suggest certain populations avoid use of the product (e.g., those who are pregnant, under the age of 18, undergoing chemotherapy, or with previous history of eye disorders or illnesses).

Non-Cosmetic

No FDA-approved prescription or over-the-counter drug uses for these ingredients were found in the literature. However, it should be noted that while these prostaglandin analogues are not reported to be used in FDA-approved drug formulations, other prostaglandin analogues are used in FDA-approved pharmaceuticals to treat glaucoma (e.g., bimatoprost, latanoprost, travoprost).[2] Bimatoprost is also used at a concentration of 0.03% to treat hypotrichosis of the eyelashes by increasing their length, thickness, and darkness.[17] Aside from cosmetics, no other types of industrial uses were found for Ethyl Tafluprostamide or Isopropyl Cloprostenate.

TOXICOKINETIC STUDIES

Dermal Absorption

In Vitro

A percutaneous absorption study performed according to Organisation for Economic Co-operation and Development (OECD) test guidelines (TG) 428, using human skin samples (n = 3 replicates/dose), was performed using different eyelash products containing radiolabeled Ethyl Tafluprostamide ([^{3}H]Ethyl Tafluprostamide) at concentrations of 0.012, 0.018, 0.020, and 0.024%.[18] The test formulations were applied to skin samples (approximately 1 µCi at 10 µg/cell) for 24 h. Following application of test substances containing 0.012, 0.018, 0.020, and 0.024% Ethyl Tafluprostamide, the absorbed fraction was reported to be 6.44 ± 2.14, 6.51 ± 2.16. 9.12 ± 7.23, and 10.68 ± 7.18% of the applied dose, respectively.

Computational

Ethyl Tafluprostamide

According to unpublished data, the estimated maximum amount of Ethyl Tafluprostamide that would be dermally absorbed from an eyelash product containing 0.018% Ethyl Tafluprostamide was determined to be 0.144 µg per use.[7] This calculation was based on a conservative dermal absorption of 20% and maximum single brush stroke application of the product (corresponding to maximum amount of 0.72 µg Ethyl Tafluprostamide, per brush stroke).

Isopropyl Cloprostenate

Dermal absorption of Isopropyl Cloprostenate was estimated using a quantitative structure-activity relationship (QSAR) model.[3] 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; no other information provided). The daily exposure to cosmetic eyelash serum is estimated at 0.28 mg,[12] and unpublished data submissions indicate that the highest concentration of Isopropyl Cloprostenate in eyelash serum is 0.0075%.[11] This results in a daily exposure of 21 ng of Isopropyl Cloprostenate per each use of the lash serum (each use consists of one application to the upper lash line of both eyes).

Absorption, Distribution, Metabolism, and Excretion (ADME)

In Vitro

Ethyl Tafluprostamide

An in vitro percutaneous metabolism study was performed according to OECD TG 428 using human skin samples (n = 3 replicates/dose; 0.5 cm^2 skin area).[19] Ethyl Tafluprostamide (6.0 µg/cm^2 diluted in 50% ethanol) was applied to skin samples for up to 24 h on a static transwell system. Identification and quantification of Ethyl Tafluprostamide, its metabolite tafluprost (free acid), and the reference substance (caffeine) were evaluated using an ultra-high-performance liquid chromatography system. Ethyl Tafluprostamide was found to be extensively metabolized into the free acid (tafluprost; 68.5 ± 2.7%) after 24 h. Bioavailability of Ethyl Tafluprostamide in the skin model was 12.3 ± 2.2 and 42.4 ± 23.1% after 4 and 24 h, respectively. Penetration of the marker compound (caffeine) was comparable with existing data from the literature.

Human

Oral

Ethyl Tafluprostamide

No oral toxicokinetic studies on Ethyl Tafluprostamide were found in the literature; however, based on reported physical and chemical properties, Ethyl Tafluprostamide is estimated to have a moderate oral absorption potential.[13] This estimation is based on a molecular weight of 452.5 g/mol, water solubility of 1.05 g/l, and a log K_{ow} of 2.74. Of note, however, the molecular weight of Ethyl Tafluprostamide is not 452.5 g/mol, but is 437.5 g/mol. Tafluprost, a chemical used by the study authors as a read-across source to target Ethyl Tafluprostamide, has a molecular weight of 452.5 g/mol.

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 (DMSO) and water) via intraperitoneal injection, and observed for 14 d.[20] Two control groups were treated with physiological solution or DMSO and water. No adverse effects regarding mortality, body weight, or hematological parameters were observed.

Short-Term Toxicity Studies

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

DEVELOPMENTAL AND REPRODUCTIVE TOXICITY STUDIES

In Silico

Ethyl Tafluprostamide and Isopropyl Cloprostenate

The SCCS flagged both Ethyl Tafluprostamide and Isopropyl Cloprostenate as potential reproductive/developmental toxicants with a reasonable model certainty, based on an in silico assessment.[3] The systems used included QSAR-based systems (VEGA-QSAR and US EPA-TEST) and read-across (TOXREAD). 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.[21] Intraperitoneal injections of the test substance were given to mice in a dose of 25 µg/kg bw/d, and to rats in doses of either 25 or 100 µg/kg bw/d. Control groups of mice and rats were left untreated. Animals were killed at different time intervals (after 7, 14, and 28 d of treatment), and histological examinations of the gonads were performed. Normal structures of the testicular cells were observed in control groups. In rats treated with 100 µg/kg bw/d, enlarged blood vessels were noted. Blood vessel diameter increased in a time-dependent manner. This effect was also noted in rats treated with 25 µg/kg bw/d; however, the increase in blood vessel diameter was smaller. After 14 and 28 d of treatment, hyaline-like material was observed in the interstitial space surrounding the seminiferous tubules in rats treated with 100 µg/kg bw/d. Also observed in this group was accumulation of polymorphonuclear neutrophils and macrophages, reduced spermatozoa numbers, decreased spermatogenesis, and nuclear condensation of the testicular cells. Macrophages, decreased spermatozoa numbers, and decreased spermatogenesis were observed in treated mice.

A similar study was performed in male mice (12 mice/group; strain of mice not specified).[22] Mice were treated with Isopropyl Cloprostenate (25 µg/kg bw/d) for 28 d via intraperitoneal injection. A control group of mice was left untreated. After 7, 14, or 28 d, animals were killed and effects on the gonads were examined. Results revealed swollen endothelial cells, macrophages with residual bodies, a large number of fibroblasts in interstices, lysosome-like dense bodies in the cytoplasm of Sertoli cells, clumped erythrocytes in capillaries, spermatocytes with condensed cytoplasm, and nuclei with a high chromatin condensation.

GENOTOXICITY STUDIES

Details on the genotoxicity assays summarized below can be found in Table 4. A 2-part Ames assay was performed using Ethyl Tafluprostamide (at concentrations up to 5000 µg/plate; with and without metabolic activation; using *Salmonella typhimurium* and *Escherichia coli* strains).[23] The test substance was considered to be non-mutagenic. Similarly, no mutagenicity was observed in a 2-part micronucleus assay performed on human lymphocytes using Ethyl Tafluprostamide (up to 350 µg/ml; with and without metabolic activation).[24] No mutagenicity was observed in an Ames assay using Isopropyl Cloprostenate (up to 5000 µg/plate; with and without metabolic activation; using *S. typhimurium* strains) or in a 2-part micronucleus assay evaluating the potential genotoxicity of 10% Isopropyl Cloprostenate in ethanol (tested at concentrations up to 750 µg/ml; with and without metabolic activation; using Chinese hamster ovary cells).[25] A QSAR model and a statistical-based model of an Ames test on Isopropyl Cloprostenate predicted no genotoxicity.[3]

CARCINOGENICITY STUDIES

In Silico

Ethyl Tafluprostamide and Isopropyl Cloprostenate

According to a structure-activity relationship (SAR) analysis conducting using OECD QSAR Toolbox v.4.6 and Derek Nexus v.6.2.1, no structural alerts were found on Ethyl Tafluprostamide indicating a potential for carcinogenicity.[13] However, according to an in silico analysis of Ethyl Tafluprostamide and Isopropyl Cloprostenate performed by the SCCS, both Ethyl Tafluprostamide and Isopropyl Cloprostenate were flagged for potential carcinogenicity with a reasonable model certainty, raising the concern that these ingredients may be non-genotoxic carcinogens.[3] QSAR analysis conducted using the VEGA v.1.2 and liver specific cancer (rat/mouse in vivo) Danish QSAR model platforms gave mixed results (which included negative, positive, and inconclusive predictions of carcinogenicity for Ethyl Tafluprostamide).[13] However, predictions by these platforms were outside the applicability domain.

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.

The distribution and presence of $PGF_{2\alpha}$ receptors in human hair follicles was evaluated in excised lower eyelid specimens.[26] Analysis was performed on 37 samples examining 17 eyes using 15 patients. Samples were stained with hematoxylin and eosin prior to analysis. All specimens contained hair follicles in the anagen phase, while only 4 samples had

specimens in the catagen phase, and staining was only present in hair follicles on the anagen stage. Among the four parts of the hair follicle (bulb, stem/suprabulbar, isthmus, and infundibulum), only the bulb and stem/suprabulbar areas displayed positive staining for $PGF_{2\alpha}$ receptors. In the bulb, the strongest staining occurred in the matricular cells and in the inner sheath layer. Within the inner sheath of the bulb (consisting of Henley, Huxley, and cuticle layers), the presence of $PGF_{2\alpha}$ receptors was observed mainly in the Huxley layer. Generally, when staining was apparent, it occurred predominantly in the cytoplasm of cells with slight membranous staining.

Evaluation of Conjunctival Hyperemia

The following studies on conjunctival hyperemia, pupil constriction, intraocular pressure, ocular pigmentation, and periorbital volume have been included in this report as they may provide insight on ocular effects following exposure to prostaglandin analogues.

Isopropyl Cloprostenate

Conjunctival hyperemia was evaluated in New Zealand albino rabbits.[27] The dose of Isopropyl Cloprostenate estimated to produce conjunctival hyperemia in 15% of the tested rabbits over a 4 h period was 0.3 µg. No other details were provided for this study.

Pupil Constriction

Isopropyl Cloprostenate

The effect of Isopropyl Cloprostenate on the constriction of pupils was evaluated in cats.[27] Potency was expressed as an ED_5 value which represents the dose estimated to produce a 5 unit area (mm*h) in a graph of the difference in pupil diameter in the dosed eye versus time. The ED_5 for Isopropyl Cloprostenate was determined to be 0.013 µg. No other details were provided in this study.

Intraocular Pressure

Ethyl Tafluprostamide

The effect of an eyelash product containing 0.018% Ethyl Tafluprostamide on intraocular pressure was evaluated in 19 subjects.[7] Subjects were instructed to use the product for 28 d, and were evaluated at baseline and on day 28. No changes in intraocular pressure were observed in subjects after 28 d of product use. The within-eye differences in intraocular pressure from the beginning to the end of the study were not statistically significant ($t > 0.05$). A similar assay was performed in 19 subjects using an eyelash product containing 0.025% Ethyl Tafluprostamide.[13,28] Subjects applied the product to eyelashes, once per day for 28 d, with evaluations occurring at baseline and day 28. No statistically significant reduction in intraocular pressure was observed over the 28-d study. The results of the ocular irritation evaluation performed during these studies can be found in the Ocular Irritation section of this report.

Isopropyl Cloprostenate

The intraocular pressure lowering efficacy of Isopropyl Cloprostenate was evaluated in conscious ocular-hypertensive cynomolgus monkeys.[27] A 39% reduction in intraocular pressure was observed following application of Isopropyl Cloprostenate (1 µg) to lasered right eyes. No other details were provided for this study.

The potential for an eyelash serum containing 0.005% Isopropyl Cloprostenate to affect intraocular pressure was evaluated in a 28-d study on 21 subjects.[29] Subjects were instructed to apply the serum to the eyelashes of both eyes, nightly. Intraocular pressure measurements were taken at baseline and at day 28. No statistically significant differences in intraocular pressure was observed in either the left or right eyes after 28 d of use.

Ocular Pigmentation, Periorbital Volume, and Adverse Effects

Isopropyl Cloprostenate

The effect of an eyelash serum containing 0.0044% Isopropyl Cloprostenate on ocular pigmentation and periorbital volume was evaluated in 114 subjects.[15,30] Subjects were instructed to apply the serum, once daily, to the clean, dry upper lash line of both eyes, using a single stroke on the eyelid, for 8 mo. Imaging was performed at baseline and at 1, 2, 4, and 8 mo intervals to measure the potential change in ocular pigmentation and periorbital volume. For the left iris, there were no statistically-significant differences in red color, green color, or blue color values after all time points of test material use. However, for the right iris, a statistically-significant decrease in green color and blue color was observed with 4 mo of test material use (no differences observed at different time points, or with red color values). When compared to baseline, there was a statistically-significant increase in overall color change of the left and right iris at all time points. Statistically-significant increases in redness values were observed after 8 mo of test material use in the left iris (compared to baseline) and after 4 and 8 mo of test material use in the right iris (compared to baseline). In addition, statistically-significant increases in yellowness values were observed in the left iris after 4 and 8 mo of test substance use (compared to baseline) and after 8 mo of test substance use in the right iris (compared to baseline). When compared to baseline values for the left orbital side, there was a statistically-significant decrease in periorbital volume after 1 mo of use (no difference noted after 2, 4, and 8 mo of use). Similarly, when compared to baseline values for the right orbital side, there was a statistically-significant difference in the right orbital volume after 2 and 8 mo of test material use. Adverse effects observed throughout study include Meibomian

gland dysfunction, erythema, corneal epithelial erosion, lid chalazion, conjunctivitis, and tarsal follicles. Ocular irritation evaluated in this study can be viewed in the Ocular Irritation section of this report.

Endocrine Effects

Ethyl Tafluprostamide

The endocrine activity potential of Ethyl Tafluprostamide was evaluated using several in silico tools (OECD QSAR Toolbox v.4.6, Derek Nexus version 6.2.1, Danish QSAR models, VEGA v.1.2.3, Endocrine Disruptome).[13] Mixed results were obtained, indicating that Ethyl Tafluprostamide may have some endocrine disruption activity (parameters evaluated include various hormonal pathways including estrogen, androgen, thyroid functions, and steroidogenesis).

DERMAL IRRITATION AND SENSITIZATION STUDIES

Details on the dermal irritation and sensitization studies summarized below can be found in Table 5.

Ethyl Tafluprostamide (98.5% purity; tested neat) was determined to be non-irritating in one EpiDerm™ assay (1-h exposure);[31] however, Ethyl Tafluprostamide (99.78% purity; tested neat) was determined to be irritating in a different EpiDerm™ assay (15-min exposure).[32] A negative prediction for sensitization was determined in a direct peptide reactivity assay (DPRA) using Ethyl Tafluprostamide (98.5% purity) in acetonitrile (100 mM; cysteine peptides only used in assay).[33] In a DPRA performed using Ethyl Tafluprostamide (99.78% purity) in acetonitrile (100 mM; cysteine and lysine peptides used in assay), a negative prediction for sensitization was determined according to the cysteine 1:10/lysine 1:50 prediction model; however, precipitation was observed in the lysine-peptide assay (conclusion of lack of reactivity could not be drawn with sufficient confidence).[34] Ethyl Tafluprostamide (98.5% purity; up to 2000 μM) in DMSO was not predicted to induce sensitization in a KeratinoSens™ assay.[35] However, inconclusive results were obtained in a KeratinoSensTM assay with Ethyl Tafluprostamide (99.78% purity; luciferase induction evaluation only performed at concentrations ≤ 250 μM) due to no clear dose-dependent results (increase in luciferase induction at 250 μM only; all lower test concentrations showed induction values in range of solvent control).[36] An eyelash product containing 0.018% Ethyl Tafluprostamide (n = 51; tested neat),[7] an eyelash conditioner containing 0.025% Ethyl Tafluprostamide (n = 51; tested neat),[37] and 7.5% Ethyl Tafluprostamide in phenoxyethanol (n = 54; final test concentration of 0.267% Ethyl Tafluprostamide) were considered to be non-sensitizing in human repeat insult patch tests (HRIPTs).[7,37,38] HRIPTs were also performed using eyelash serums containing Isopropyl Cloprostenate (0.0044% and 0.005%; tested neat; n = 50-56).[39-42] Three of the four assays were performed under semi-occlusive conditions. The serums tested were considered to be non-irritating and non-sensitizing in all assays.

Phototoxicity

Ethyl Tafluprostamide

Although no photo-induced toxicity studies were available in the literature on these ingredients, an ultraviolet-visible study with Ethyl Tafluprostamide (neat oil) performed in accordance with OECD TG 101 revealed an absorption band in the range of 210 - 240 nm, with maximum absorption at 226 nm, and an absorption band in the range of 250 - 285 nm, with three maxima at 265, 258, and 276 nm.[43] Molar extinction coefficients for these three maxima were within the range of 1046.2 – 1306.1 l/(mol * cm). Because these maxima are above the cut-off limit (> 1000 l/(mol * cm), photoreactivity cannot be ruled out.

OCULAR IRRITATION STUDIES

Details on the in vitro and human ocular irritation studies summarized below can be found in Table 6.

An eyelash product containing 0.018% Ethyl Tafluprostamide (tested neat),[7] an eyelash product containing 0.025% Ethyl Tafluprostamide (tested neat),[44] and Ethyl Tafluprostamide (99.78% purity; tested neat)[45] were not predicted to be an ocular irritant in a hen's egg chorioallantoic membrane (HET-CAM) assays. Eyelash serums containing Isopropyl Cloprostenate (0.0044[46,47] and 0.005%[48-50]) 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%) 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).[51]

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).[7] Similar results were observed in a use study performed in 19 subjects using an eyelash product containing 0.025% Ethyl Tafluprostamide for 28 d.[13,28] 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.[52] Reversible ocular irritation was observed in 2 subjects in a 12-wk assay in which 32 subjects applied an eyelash serum containing 0.0044% Isopropyl Cloprostenate. Slight, transient ocular irritation was observed in an 8-mo use study performed in 114 subjects using an eyelash serum containing 0.0044% Isopropyl Cloprostenate.[30] No ocular irritation, other than slight bulbar conjunctival irritation in one assay, was observed in two ocular irritation assays performed in humans (n = 30; 32) using eyelash and eyebrow serums

containing 0.005% Isopropyl Cloprostenate.[53,54] 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.[3]

CLINICAL STUDIES

Clinical Trial

Isopropyl Cloprostenate

The effect of an eyewash containing Isopropyl Cloprostenate (0.01%) in a phosphate-buffered saline was evaluated in 23 patients with glaucoma.[3] The eye wash was applied to the eyes once daily for 3 mo. Mild hyperemia of the bulbar conjunctiva was observed; however, this was reported to disappear after 2-3 d of treatment. No reactions relating to appearance of the optic disc, visual acuity, visual field, appearance of papilla, or intraocular pressure were observed.

Case Report

Isopropyl Cloprostenate

A 32-yr-old woman presented to an outpatient department due to periocular discoloration for 4 mo.[55] 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.[56] During use period, the patient reported hollowing, thinning, wrinkling, and darkening of the skin of the periorbital region. Six months after discontinued use, the patient reported extensive improvement of symptoms.

Adverse Event Reports

Ethyl Tafluprostamide

According to an unpublished data submission, a company evaluated undesirable effects that were reported by consumers of an eyelash product containing 0.018% Ethyl Tafluprostamide over the course of 2 yr (2011 – 2013).[7] The number of reported undesirable effects for this product, during this time period, was 0.00717% of the number of sold units (number of sold units not stated). The reported adverse effects were described as typical in nature to those associated with cosmetic products near the eyes, specifically mascara and eyeliner.

RISK ASSESSMENT

True margins of safety for these ingredients could not be calculated as systemic toxicity data on these ingredients are not available. However, margin of safety (MoS) calculations have been performed using systemic points of departure (PoD) derived from chemicals similar to Ethyl Tafluprostamide and Isopropyl Cloprostenate (tafluprost and travoprost, respectively). The MoS for an eyelash product containing 0.018% Ethyl Tafluprostamide was calculated to be 481 when the average amount of product is applied, and 288 for when the maximum amount of product is applied.[13] This calculation was based on a dermal absorption rate at 8.67%, a dermal retention factor of 0.5, and an NOAEL at 0.0003 mg/kg bw/d for tafluprost from a prenatal developmental toxicity study in rats following intravenous administration.

An MoS of an eyelash serum containing 0.0075% Isopropyl Cloprostenate was calculated to be 228.[12] This calculation was based on a default dermal absorption rate at 50%, a dermal retention factor of 1 and an LOAEL at 0.00012 mg/kg bw/d for travoprost from a 3-generation study in rats by subcutaneous injection, with an assessment factor of 3 for extrapolation from LOAEL to NOAEL. Each of these MoS values is considered to be protective. Explanations of the parameters used for these calculations can be found in Table 7.

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, unpublished data submitted separately from the survey state that Isopropyl Cloprostenate is used at up to 0.0075% in eyelash serums. In addition, an unpublished data submission indicated products used on eyelashes, eyebrows, or scalp hair contain Ethyl Tafluprostamide in concentrations ranging from 0.012% - 0.020%.

User instructions on an eyelash product containing 0.018% Ethyl Tafluprostamide state that the product is to be applied once per day, directly to the eyelashes, near the base, above the eyelash line, and should be dried completely prior to the use of other products. Eyelash serums containing 0.004 and 0.005% Isopropyl Cloprostenate are also to be applied once per day; however, these products are applied in a thin line on the eyelash line (similar to application of liquid eyeliner). Caution statements are provided on these products informing users to rinse eyes and discontinue use if irritation occurs.

According to unpublished data, the estimated maximum amount of Ethyl Tafluprostamide that would be dermally absorbed was calculated to be 0.144 µg (based on maximum use of a product containing 0.018% Ethyl Tafluprostamide and dermal absorption rate of 20%). The estimated maximum amount of Isopropyl Cloprostenate that would be dermally absorbed was calculated to be 0.02 µg (based on estimated maximum use of a product containing 0.005% Isopropyl Cloprostenate and a dermal absorption rate of 10%). In an in vitro percutaneous absorption study, the absorbed fraction of an eyelash product containing 0.018% Ethyl Tafluprostamide and an eyelash product containing 0.024% Ethyl Tafluprostamide was determined to be 6.51 ± 2.16 and 10.68 ± 7.18% of the applied dose, respectively (after a 24 h exposure period). An estimated dermal absorption of Isopropyl Cloprostenate was determined to be 10%, according to a QSAR model. In an in vitro percutaneous metabolism study, Ethyl Tafluprostamide (50% in ethanol) was found to be extensively metabolized into tafluprost (i.e., free acid) after 24 h. Ethyl Tafluprostamide was estimated to have a moderate oral absorption potential based on the reported physical and chemical properties of this ingredient.

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

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

Based on an in silico analysis, the SCCS flagged Ethyl Tafluprostamide and Isopropyl Cloprostenate as potential reproductive/developmental toxicants. The effect of Isopropyl Cloprostenate (25 or 100 µg/kg bw/d) on gonads and testicular cells was evaluated in male mice and rats. In these assays, animals were treated for 28 d, and killed at different time intervals prior to evaluation. Time- and dose-dependent adverse effects (e.g., enlarged blood vessels, macrophages, reduced spermatozoa, reduced spermatogenesis, dense bodies in cytoplasm of Sertoli cells, clumped erythrocytes) were observed in treated animals.

Ethyl Tafluprostamide was determined to be non-mutagenic in a 2-part Ames assay (concentrations up to 5000 µg/plate; with and without metabolic activation) and in a 2-part micronucleus assay performed on human lymphocytes (up to 350 µg/ml; with and without metabolic activation). Similarly, no genotoxicity was observed in an Ames assay using Isopropyl Cloprostenate (up to 5000 µg/plate; with and without metabolic activation or in a 2-part micronucleus assay evaluating 10% Isopropyl Cloprostenate (tested at concentrations up to 750 µg/ml; with and without metabolic activation). A QSAR model and a statistical-based model of an Ames test on Isopropyl Cloprostenate predicted no genotoxicity.

No structural alerts were observed for Ethyl Tafluprostamide according to SAR analyses performed using OECD QSAR Toolbox v.4.6 and Derek Nexus v.6.2.1. However, both Ethyl Tafluprostamide and Isopropyl Cloprostenate were flagged for potential carcinogenicity by the SCCS due to in silico analyses. QSAR analysis conducted using the Vega v.1.2 and liver specific cancer (rat/mouse in vivo) Danish QSAR model platforms gave mixed results (negative, positive or inconclusive predictions of carcinogenicity for Ethyl Tafluprostamide).

The distribution and presence of $PGF_{2\alpha}$ receptors in human hair follicles was evaluated using excised lower eyelid samples. Receptors were found in hair follicles in the anagen stage and were primarily present in the matricular cells of the bulb and inner sheath layer of the hair follicle.

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 intraocular pressure were observed in 19 subjects after a 28-d use period of an eyelash product containing 0.018% Ethyl Tafluprostamide or an eyelash product containing 0.025% Ethyl Tafluprostamide. A 39% reduction in intraocular pressure was observed in ocular hypertensive monkeys treated with 1 µg Isopropyl Cloprostenate (in lasered right eyes). No statistically-significant changes in intraocular pressure were observed in a 28-d study in which 21 subjects applied an eyelash serum containing 0.005% Isopropyl Cloprostenate nightly.

The potential for an eyelash serum containing 0.0044% Isopropyl Cloprostenate to cause changes in ocular pigmentation and periorbital volume was evaluated in an 8-mo study involving 114 subjects. Statistically-significant increases in overall ocular pigmentation were observed in both left and right iris at all observed time points (1, 2, 4, and 8 mo), compared to baseline. In addition, statistically-significantly decreased periorbital volume was observed 1 mo after test substance use in the left orbital side, and after 2 and 8 mo of use in the right orbital side (compared to baseline values).

Mixed results were observed in several in silico models evaluating the potential endocrine disruption activity of Ethyl Tafluprostamide. This suggests that this ingredient may have some endocrine disruption activity.

Ethyl Tafluprostamide (98.5% purity; tested neat) was determined to be non-irritating in one EpiDerm™ assay (1-h exposure); however, Ethyl Tafluprostamide (99.78%) was determined to be non-irritating in a different EpiDerm™ assay (15-min exposure). A negative prediction for sensitization was determined in a DPRA using Ethyl Tafluprostamide (98.5% purity) in acetonitrile (100 mM); however, precipitation was observed in the lysine-peptide assay of a different DPRA using Ethyl Tafluprostamide (99.78% purity) in acetonitrile (100 mM). Ethyl Tafluprostamide (98.5% purity; up to 2000 µM) in DMSO was not predicted to induce sensitization in a KeratinoSens™ assay. However, inconclusive results were obtained in a KeratinoSens™ assay with Ethyl Tafluprostamide (99.78% purity; luciferase induction evaluation only performed at concentrations ≤ 250 µM) due to no clear dose-dependent results (increase in luciferase induction at 250 µM only; all lower test concentrations showed induction values in range of solvent control). An eyelash product containing 0.018% Ethyl Tafluprostamide (tested neat), an eyelash conditioner containing 0.025% Ethyl Tafluprostamide (tested neat), and 7.5% Ethyl Tafluprostamide in phenoxyethanol (final test concentration of 0.267% Ethyl Tafluprostamide) were considered to be non-sensitizing in HRIPTs. HRIPTs were performed using serums containing Isopropyl Cloprostenate (0.0044% and 0.005%; tested neat). The serums tested were considered to be non-irritating and non-sensitizing in all assays.

No phototoxicity studies on these ingredients were found in the literature; however, according to an ultraviolet-visible study with Ethyl Tafluprostamide, photoreactivity could not be ruled out as calculated molar extinction coefficients were above the cut-off limit. Because the maximum absorbance wavelength was below 313 nm, no further in vitro toxicity testing is required, according to SCCS notes of guidance.

An eyelash product containing 0.018% Ethyl Tafluprostamide (tested neat), an eyelash product containing 0.025% Ethyl Tafluprostamide, and Ethyl Tafluprostamide (99.78% purity; tested neat), were not predicted to be ocular irritants in HET-CAM assays. 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.

The majority of subjects displayed no signs of irritation in two use assays in which eyelash products containing either 0.018% Ethyl Tafluprostamide or 0.025% Ethyl Tafluprostamide were applied daily for 28 d (n =19 in both assays). No ocular irritation was observed in 29 subjects after use of an eyelash serum containing 0.0044% Isopropyl Cloprostenate for 6 wk and of an eyebrow serum containing 0.0044% Isopropyl Cloprostenate for 7 wk. Slight ocular irritation was observed in an 8-mo use study in which 120 subjects used an eyelash serum containing 0.0044% Isopropyl Cloprostenate. Reversible ocular irritation was observed in 2 subjects in a 12-wk assay in which 32 subjects applied an eyelash serum containing 0.0044% Isopropyl Cloprostenate. No ocular irritation, other than slight bulbar conjunctival irritation in one assay, was observed in ocular irritation assays performed in humans (n = 30; 32) using eyelash and eyebrow serums containing 0.005% Isopropyl Cloprostenate. No 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 an eyelash product containing 0.018% Ethyl Tafluprostamide was calculated to be 288 based on a maximum daily amount of 8 mg of the product. An MoS of an eyelash serum containing 0.0075% Isopropyl Cloprostenate was calculated to be 228.

DRAFT DISCUSSION

[Note: This Discussion is in the draft form, and changes will be made following the Panel meeting.]

This assessment reviews the safety of 2 prostaglandin analogues, Ethyl Tafluprostamide and Isopropyl Cloprostenate, as used in cosmetic formulations. The Panel concluded [TBD].

The Panel's respiratory exposure resource document (https://www.cir-safety.org/cir-findings) notes that airbrush technology presents a potential safety concern, and that no data are available for consumer habits and practices thereof. As a result of deficiencies in these critical data needs, the safety of cosmetic ingredients applied by airbrush delivery systems cannot be determined by the Panel. Therefore, the Panel has concluded the data are insufficient to support the safe use of cosmetic ingredients applied via an airbrush delivery system.

CONCLUSION

To be determined.

TABLES

Table 1. Definitions, structures, and reported functions[1, CIR STAFF]

Ingredient (CAS No.)	Definition	Function
Ethyl Tafluprostamide (1185851-52-8)	Ethyl Tafluprostamide is a synthetic analogue of a prostaglandin. It conforms to the structure in Figure 1.	hair conditioning agents; nail conditioning agent
Isopropyl Cloprostenate (157283-66-4)	Isopropyl Cloprostenate is a synthetic analogue of a prostaglandin. It conforms to the structure in Figure 2.	hair conditioning agent

Table 2. Chemical properties

Property	Value	Reference
Ethyl Tafluprostamide		
Physical Form	liquid	[3]
Color	colorless to pale yellow	[3]
Molecular Weight (g/mol)	437.5	[3]
Density (g/ml)	1.21	[13]
Vapor pressure (Pa at 25 ºC)	1.25×10^{-13}	[13]
Melting Point (°C)	95.08	[13]
Boiling Point (°C)	503.76	[13]
Water Solubility (g/l @ 20ºC)	1.05	[6]
log K_{ow} (@ 25° C)	2.74 ± < 0.01	[5]
log K_{ow}	5.03	[3]
UV Absorption (nm; symmetric peak)	226 - 276	[43]
Isopropyl Cloprostenate		
Molecular Weight (g/mol)	467	[3]
Water Solubility (mg/l @ 25ºC)	0.047	[3]
log K_{ow}	5.15	[3]

Table 3. Frequency and concentration of use (2023) by product category[7-11]

	Isopropyl Cloprostenate		Ethyl Tafluprostamide	
	# of Uses	Max Conc of Use (%)	# of Uses	Max Conc of Use (%)
Eye Makeup Preparations				
Other Eye Makeup Preparations	3	0.0044 – 0.0048[a]; 0.0075[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]average concentration of Isopropyl Cloprostenate in eyelash serums according to unpublished data sources[10]

[b]concentration of Isopropyl Cloprostenate in eyelash serum according to an unpublished data submission[11]

[c]concentration of Ethyl Tafluprostamide in products used on eyelashes, eyebrows, and scalp hair, according to an unpublished data submission[7]

Table 4. Genotoxicity studies

Test Article	Vehicle	Concentration/Dose	Test System	Procedure	Results	Reference
In Vitro						
Ethyl Tafluprostamide (purity: 99.78%)	DMSO	Experiment 1: 3.16, 10.0, 31.6, 100, 316, 1000, 2500 and 5000 µg/plate (TA100) 31.6, 100, 316, 1000, 2500, and 5000 µg/plate (TA98, TA1535, TA1537, and E. coli WP2 uvrA (PKM101)) Experiment 2: 3.16, 10.0, 31.6, 100, 316, 1000, 2500 and 5000 µg/plate (TA100, TA1535) 31.6, 100, 316, 1000, 2500, and 5000 µg/plate (TA98, TA1535, TA1537, and E. coli WP2 uvrA (PKM101))	*S. typhimurium* strains TA98, TA100, TA1535, TA1537, and *E. coli* WP2 uvrA (pKM101)	2-part Ames assay; OECD TG 471; both parts performed with and without metabolic activation; negative controls: distilled water; positive controls: 4-nitro-o-phenylene-diamine, methylmethanesulfonate; 2-aminoanthracene	Non-mutagenic; controls gave expected results	[23]
Ethyl Tafluprostamide (purity: 99.78%)	DMSO	Experiment 1: without metabolic activation: 250, 325, and 350 µg/ml with metabolic activation: 100, 250, and 300 µg/ml Experiment 2: without metabolic activation: 25, 50, and 100 µg/ml	human lymphocytes	2-part micronucleus assay; OECD TG 487; experiment 1 performed with and without metabolic activation for 4 h; experiment 2 performed without metabolic activation for 44 h; negative controls: culture medium, DMSO; positive controls: methylmethanesulfonate; colchicine, cyclophosphamide	Non-mutagenic; controls gave expected results	[24]
Isopropyl Cloprostenate	sterile deionized water	50, 100, 500, 1000, and 5000 µg/plate	*S. typhimurium* strains TA 97a, TA98, TA100, TA 102, and TA 1535	Ames assay; performed with and without metabolic activation; negative control: sterile deionized water; positive controls: sodium azide, daunomycin, mitomycin C, ICR 191 acridine, 2-aminoanthracene	Non-mutagenic; controls gave expected results	[25]
10% Isopropyl Cloprostenate	ethanol	Experiment 1: 2.9, 5.9, 12, 23, 47, 94, 190, 380, and 750 µg/ml Experiment 2: 0.23, 0.47, 0.94, 1.9, 3.8, 7.5, 15, and 30 µg/ml	Chinese hamster ovary cells	2-part mammalian cell micronucleus assay; OECD 487; in experiment 1, test item tested for 4 h in the absence and presence of metabolic activation and for 23 h in the absence of metabolic activation; in experiment 2, test item tested for 23 h in absence of metabolic activation; negative control: ethanol; positive controls: colchicine, cyclophosphamide, mitomycin C	Non-mutagenic; controls gave expected results	[25]
Computational						
Isopropyl Cloprostenate	-	-	NR	QSAR model ToxTree v. 3.1.0-1851 and US EPA T.E.S.T v. 4.2.1	No prediction of genotoxicity; Cramer class III toxicity (substance with chemical structure that permit no strong initial impression of safety or may even suggest a significant toxicity)	[3,57]

DMSO: dimethyl sulfoxide; NR = not reported; OECD = Organisation for Economic Co-Operation and Development TG = test guideline

Table 5. Dermal irritation/sensitization

Test Article	Vehicle	Concentration/Dose	Test Population	Procedure	Results	Reference
IRRITATION						
In Vitro						
Ethyl Tafluprostamide (purity: 98.5%)	NR	100%; 30 µl	3 samples	EpiDerm™ assay; reconstructed human epidermis; OECD TG 439; 1h exposure period; negative control: phosphate-buffered saline; positive control: 5% sodium dodecyl sulfate	Non-irritating Tissue viability in 3 replicates was 100, 106, and 82% at end of test Control substances gave expected results	31
Ethyl Tafluprostamide (purity: 99.78%)	NR	100%; 30 µl	3 samples	EpiDerm™ assay; reconstructed human epidermis; OECD TG 439; 15-min exposure period; negative control: phosphate-buffered saline; positive control: 5% sodium dodecyl sulfate	Irritating Tissue viability in 3 replicates determined to be 3, 17.2 and 27.7% at end of test. The mean value of relative tissue viability was 16.0% after the treatment. This value is below the threshold for skin irritation (50 %). Thus, the test item is considered to be an irritant to skin. Control substances gave expected results.	32
SENSITIZATION						
In Chemico/In Vitro						
Ethyl Tafluprostamide (purity: 98.5%)	acetonitrile	100 mM; 50 µl	cysteine peptides	Direct peptide reactivity assay; OECD TG 442C; cys- peptides assay; solvent used as negative control; positive control: cinnamic aldehyde	Negative prediction for sensitization Negative and positive controls gave expected results	33
Ethyl Tafluprostamide (purity: 99.78%)	acetonitrile	100 mM; 50 µl	lysine and cysteine peptides	Direct peptide reactivity assay; OECD TG 442C; cys- and lys- peptides assay; solvent used as negative control; positive control: cinnamic aldehyde	Negative prediction for sensitization according to cysteine 1:10/lysine 1:50 prediction model; however, observed precipitation in lys-peptide assay; conclusion on lack of reactivity could not be drawn from conditions of this study Mean peptide depletion in the cys-peptide assay, which showed no precipitation for test item was 3.2%, suggesting no or minimal reactivity Control substances gave expected results	34

Table 5. Dermal irritation/sensitization

Test Article	Vehicle	Concentration/Dose	Test Population	Procedure	Results	Reference
Ethyl Tafluprostamide (purity: 98.5%)	DMSO	Test 1: 0.98, 1.95, 3.91, 7.81, 15.63, 31.25, 62.5, 125, 250, 500, 1000, and 2000 µM Test 2: 55.80, 72.54, 94.30, 122.59, 159.37, 207.18, 269.33, 350.13, 455.17, 591.72, 769.23, and 1000 µM Test 3: 67.29, 80.75, 96.90, 116.28, 139.54, 167.45, 200.94, 241.13, 289.35, 347.22, 416.67, and 500 µM All concentrations tested at a dose volume of 50 µl	KeratinoSens™ cell line	KeratinoSens™ assay; OECD 442D; solvent used as negative control; positive control: trans-cinnamaldehyde; test 2 and 3 performed to determine IC_{30} and IC_{50} values more precisely since strong cytotoxicity was observed at high concentrations	Negative prediction for sensitization Control substances gave expected results	[35]
Ethyl Tafluprostamide (purity: 99.78%)	DMSO	0.98, 1.95, 3.91, 7.81, 15.63, 31.25, 62.5, 125, 250, 500, 1000, and 2000 µM; 50 µl In both repetitions, a cytotoxic effect was observed at concentrations above 500 µM; therefore, the three highest test item concentrations were excluded from the evaluation of the luciferase induction in both repetitions	KeratinoSens™ cell line	KeratinoSens™ assay; OECD 442D; solvent used as negative control; positive control: cinnamic aldehyde; experiment repeated due to lack of dose-response in first experiment	Inconclusive results In experiment 1, a statistically significant increase in luciferase induction >1.5-fold was observed at 250 µm; all lower concentrations showed induction values in the range of the solvent control In experiment 2, a statistically significant increase in luciferase induction to exactly 1.5-fold was observed at 250 µM; induction values at lower concentrations were all in range of solvent control No clear dose-dependent results were observed – result was considered inconclusive Precipitation of test article not visible in any of the repetitions Control substances gave expected results	[36]

Table 5. Dermal irritation/sensitization

Test Article	Vehicle	Concentration/Dose	Test Population	Procedure	Results	Reference
			Human			
eyelash product containing 0.018% Ethyl Tafluprostamide	NR	100%; dose not stated	51	HRIPT; level of occlusion not stated; 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	[7]
eyelash conditioner containing 0.025% Ethyl Tafluprostamide	NR	100%; 0.02 – 0.05 ml	51	HRIPT; occlusive conditions; nine applications to the upper back over a 3-wk period for induction (1st patch 24-h exposure; remaining patches 48-h exposures); test challenge patch after a 10 - 14 d rest period; challenge patch applied to a previously untreated site adjacent to the test site (48- and 96-h exposures)	Non-irritating; non-sensitizing	[37]
7.5% Ethyl Tafluprostamide in phenoxyethanol (final test concentration of 0.267% Ethyl Tafluprostamide)	deionized water	3.55%; 0.02 – 0.05 ml	54	HRIPT; semi-occlusive conditions; eight to nine applications to the upper back over a 3-wk period for induction (1st patch 24-h exposure; remaining patches 48-h exposures); test challenge patch after a 10 - 14 d rest period; challenge patch applied to a previously untreated site adjacent to the test site (48- and 96-h exposures)	Non-irritating; non-sensitizing	[7,38]
eyelash serum containing 0.0044% Isopropyl Cloprostenate	NR	100%; 0.2 ml	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	[39]
eyelash serum containing 0.0044% Isopropyl Cloprostenate	NR	100%; dose volume not stated	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	[40]
eyelash serum containing 0.005% Isopropyl Cloprostenate	NR	100%; 0.2 ml	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	[41]

Table 5. Dermal irritation/sensitization

Test Article	Vehicle	Concentration/Dose	Test Population	Procedure	Results	Reference
eyelash serum containing 0.005% Isopropyl Cloprostenate	NR	100%; dose not stated	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	[42]

DMSO = dimethyl sulfoxide; HRIPT = human repeated insult patch test; IC_{30} = 30% inhibitory concentration; IC_{50} = half maximal inhibitory concentration; OECD = Organisation for Economic Co-Operation and Development TG = test guideline

Table 6. Ocular irritation studies

Test Article	Vehicle	Concentration/Dose	Test Population	Procedure	Results	Reference
IN VITRO						
Eyelash product containing 0.018% Ethyl Tafluprostamide	NR	100%	hen's egg chorioallantoic membranes (n = 4)	HET-CAM assay; reference test articles include a one-coat mascara and waterproof eyeliner (details regarding these substances not stated); evaluations performed 0.5, 2, and 5 min after test article exposure	Irritation potential score: 0.0 (mean scores of 0.0 - 4.9 indicate minimal irritation potential) Reference test articles have historically been shown to be minimally irritating. Study author concluded the test substance would have minimal ocular irritation potential in vivo	7
Eyelash product containing 0.025% Ethyl Tafluprostamide	NR	100%; 0.3 ml	hen's egg chorioallantoic membranes (n = 4)	HET-CAM assay; reference test articles include a one-coat mascara and waterproof eyeliner (details regarding these substances not stated); evaluations performed 0.5, 2, and 5 min after test article exposure	Non-irritating Mean irritation score of 0.0 at all test points Reference test articles have historically been shown to be minimally irritating.	44
Ethyl Tafluprostamide (purity: 99.78%)	NR	100%; 0.03 ml	reconstructed human corneal epithelium (n = 2)	EpiOcular™ assay; OECD TG 492: negative control: phosphate-buffered saline; positive control: sodium dodecyl sulfate	Non-irritating Control substances gave expected results	45
Eyelash serum containing 0.0044% Isopropyl Cloprostenate	saline	10%: 0.3 ml	hen's egg chorioallantoic membranes (n = 6)	HET-CAM assay; vehicle control: saline; positive controls: sodium hydroxide and sodium dodecyl sulfate	Irritation potential score: 0.0 Threshold concentration (lowest concentration at which slight reactions occur) for this test substance was greater than 10% Control substances gave expected results Study author concluded that the irritation potential of the test substance was determined to be none to slight.	46
Eyelash serum containing 0.0044% Isopropyl Cloprostenate	NR	50%*; 0.3 ml	hen's egg chorioallantoic membranes (n = 4)	HET-CAM assay; reference test articles include a one-coat mascara and waterproof eyeliner (details regarding these substances not stated); evaluations performed 0.5, 2, and 5 min after test article exposure	Irritation potential score for eyelash serum: 1.25 (mean scores of 0.0 - 4.9 indicate minimal ocular irritation) Reference test articles have historically been shown to be minimally irritating, Study author concluded that the test substance, at 100%, would have minimal ocular irritation in vivo.	47
Eyelash serum containing 0.005% Isopropyl Cloprostenate	NR	50%*; 0.3 ml	hen's egg chorioallantoic membranes (n = 4)	HET-CAM assay; reference test articles include a one coat mascara and waterproof eyeliner (details regarding these substances not stated); evaluations performed 0.5, 2, and 5 min after test article exposure	Irritation potential score for eyelash serum: 2.50 (mean scores of 0.0 - 4.9 indicate minimal irritation potential) Reference test articles have historically been shown to be minimally irritating. Study author concluded that the test substance, at 100%, would have minimally irritating in vivo.	48

Table 6. Ocular irritation studies

Test Article	Vehicle	Concentration/Dose	Test Population	Procedure	Results	Reference
Eyelash serum containing 0.005% Isopropyl Cloprostenate	saline	10%: 0.3 ml	hen's egg chorioallantoic membranes (n = 6)	HET-CAM assay; vehicle control: saline; positive controls: sodium hydroxide and sodium dodecyl sulfate	Irritation potential score: 0.0 Threshold concentration (lowest concentration at which slight reactions occur) for this test substance was greater than 10% Control substances gave expected results Study author concluded that the irritation potential of the test substance was determined to be none to slight	[49]
Eyelash serum containing 0.005% Isopropyl Cloprostenate	saline	10%; 0.3 ml	hen's egg chorioallantoic membranes (n = 6)	HET-CAM assay; vehicle control: saline; positive controls: sodium hydroxide and sodium dodecyl sulfate	Irritation potential score: 2.6 Threshold concentration (lowest concentration at which slight reactions occur) for this test substance was greater than 10% Control substances gave expected results Study author concluded that the irritation potential of the test substance was determined to be none to slight	[50]
0.1% Isopropyl Cloprostenate	NR	50%*; 0.3 ml	hen's egg chorioallantoic membranes (n = 6)	HET-CAM assay; reference test articles include a one coat mascara and waterproof eyeliner (details regarding these substances not stated); evaluations performed 0.5, 2, and 5 min after test article exposure	Irritation potential score for eyelash serum: 1.50 (mean scores of 0.0 - 4.9 indicate minimal irritation potential) Reference test articles have historically been shown to be minimally irritating, Study author concluded that 0.1% Isopropyl Cloprostenate would have minimal ocular irritation potential in vivo	[51]
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.	[7]
Eyelash product containing 0.025% Ethyl Tafluprostamide	NR	100%	19 subjects	Home use study. Subjects applied product to eyelashes for 28 d. Intraocular pressure was measured in each eye of each subject at beginning and end of study. Eyes were assessed by ophthalmologist at baseline and on day 28 (slit-lamp examinations).	Minor ocular effects self-reported by 4/19 volunteers (slight dryness, slight itching, slight stinging, slight watering and redness, moderate to high burning) Study authors determined that the formulation did not produce an eye irritation or hypersensitivity of clinical magnitude.	[13,28]

Table 6. Ocular irritation studies

Test Article	Vehicle	Concentration/Dose	Test Population	Procedure	Results	Reference
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	52
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	52
Eyelash serum containing 0.0044% Isopropyl Cloprostenate	NR	100%	114 subjects	Home use study. Subjects applied eyelash serum daily for 8 mo. Slit-lamp evaluations occurred at baseline, 1 mo, 2 mo, 4 mo, and 8 mo intervals.	Slight transient ophthalmological irritation observed. The serum was determined to be safe for use by both contact lens and non-contact lens wearers.	30
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	53
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	54
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	3

HET-CAM = hen's egg test chorioallantoic membrane; NR = not reported; OECD TG = Organisation for Economic Co-Operation and Development Test Guidelines

*study author states that a 50% dilution of the test and reference articles may be used to approximate in vivo irritation potential at 100%, as the hen's egg is more sensitive to liquid irritants than the rabbit eye

Table 7. Margin of safety calculation parameters

	Ethyl Tafluprostamide[13]	**Isopropyl Cloprostenate[12]**
estimated daily exposure to eyelash products	0.04 mg/kg bw/d (average) 0.067 mg/kg bw/d (maximum)*	0.28 mg/d (or 0.0047 mg/kg bw/d, for lash serum only)
concentration of ingredient	0.018%	0.0075%
dermal absorption	8.67% (based on in vitro percutaneous study provided in this report[18]; the mean absorbed mean absorbed fraction (6.51 ± 2.16%) plus 1 standard deviation was used)	50%
dermal retention factor	0.5	1
body weight	60 kg	60 kg
systemic exposure dose	6.24×10^{-7} mg/kg/d (average) 1.04×10^{-6} mg/kg/d (max)	1.75×10^{-7} mg/kg/d
systemic point of departure	NOAEL: 0.0003 mg/kg bw/d (derived from a prenatal developmental toxicity study on tafluprost (rats, intravenous))[58]	NOAEL: 0.00004 mg/kg/d (derived from a systemic toxicity assay on travoprost: an LOAEL at 0.00012 mg/kg/d from a 3-generation study (rats, subcutaneous) with an assessment factor of 3 for extrapolation from LOAEL to NOAEL)[59]
margin of safety	481 (based on average daily exposure) 288 (based on maximum daily exposure)	228

*** On average, 2.4 mg of cosmetic eyelash product was applied to the upper eyelashes with each brush stroke; considering once daily application to both eyes, the “average amount” of 4.8 mg/d and the “maximum amount” of 8 mg/d were used for the MoS calculations.[13]

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Roadmap to Safety Assessment for Isopropyl Cloprostenate

Submission to Cosmetic Ingredient Review

This report provides a summary of data and other information that will be provided to support the safety of isopropyl cloprostenate ("IPC") in cosmetic lash serums at a 0.0075% use concentration, and is respectfully submitted in response to the Cosmetic Ingredient Review ("CIR") Expert Panel's Insufficient Data Announcement dated December 8, 2023. CIR already carried out an extensive review of the toxicology and safety assessment for IPC in a serum lash formulation containing 0.005%. Accordingly, this roadmap is intended to identify additional data and information that will be provided to supplement that earlier evaluation, and to highlight why this additional data and information will support the safety of IPC at the slightly higher use concentration of 0.0075%. A roadmap outlining a future submission is provided in lieu of a complete submission at this time because additional data are currently in development and under review, and this submission respectfully requests additional time to review and analyze that data and other information before submitting it to CIR. The additional data and information that will be provided is summarized below.

First, a comprehensive assessment of the Quantitative Structure-Activity Relationship (QSAR) of IPC will be submitted. Both Travoprost and Latanoprost will be used in the QSAR assessment as surrogate chemicals. The QSAR assessment will utilize the Organization for Economic Cooperation and Development (OECD) Toolbox and ChemMines to fill in any data outstanding data gaps, as well as identify and address some of the key concerns presented in the European Union's (EU) Scientific Committee on Consumer Safety (SCCS) Opinion on "Prostaglandins and Prostaglandin-Analogues used in Cosmetic Products."[1] This approach will specifically address any outstanding concerns for carcinogenicity, reproductive/developmental toxicity, and dermal sensitization, as well as positive response in an *in vitro* human lymphocyte assay.

In addition, new data will be presented on similar formulations for cosmetic applications that contain IPC at the slightly greater amount (0.0075%, a small increase of 0.0025%), which further demonstrate that IPC produces similar (if not identical) results to those toxicological studies already presented to CIR on IPC. These new data include, but are not limited to:

- **HET-CAM:** Nitka, S. The Hen's Egg Test – Utilizing the Chloriallantoic Membrane (HET-CAM); Lash-Brow Serum. Consumer Product Testing Co. Fairfield, New Jersey, Experiment Reference No. V15-2816.
- **EpiOcular Test**: Troese, M. EpiOcular Eye Irritation Test (EIT). MB Research Laboratory, Spinners town, PA. MB Research Project No. 20-27939.19.
- **HRIPT:** Shoshani, L. 100 Subject Human Repeat Insult Patch Test for Skin Irritation and Skin Sensitization Evaluation. BioScreen Testing Services, Inc. Torrance, CA. Laboroatory Study No. 21-527A& 21-528A.
- **8-Week Clinical Study: In-Use Eyelash & Eyebrows:** Jiand, L., Stephens, M.T., and Acevedo, S. A Single-Center Clinical Study to Evaluate the Safety and Efficacy of Lash Boost when Used on Eyelashes and Eyebrows. Thomas J. Stephens & Associates, Inc., Stephens Study No. C18-D094; Sponsors Unpublished Study No. 2018TSA025.

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

We respectfully submit that the forthcoming submission on the use of IPC in lash serums at a level of 0.0075% will demonstrate that IPC may be considered safe to a reasonable certainty, and is not injurious to users under the conditions of use prescribed in a cosmetic's labeling, or under such conditions of use as are customary or usual, in accordance with 21 U.S.C. § 364d(1)-(2).

Additional Data Supporting the Safe Use of Isopropyl Cloprostenate (up to 0.005%) in Cosmetics

Submitted By:
John Bailey

Submitted on Behalf of:

Company 1
(markets cosmetic lash serum containing 0.0044% isopropyl cloprostenate)

&

Company 2
(markets cosmetic lash serum containing 0.005% isopropyl cloprostenate)

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

April 26, 2024

This report provides additional data supporting the safe use of Isopropyl Cloprostenate ("IC") in the concentration contained in Company 1's cosmetic lash serum (0.0044% IC) and Company 2's cosmetic lash serum (0.005% IC).

I. Testing on IC (up to 0.005%)

a. Testing Submitted *Previously*

In response to the Expert Panel's Scientific Literature Notice to Proceed, dated March 17, 2023, the Companies submitted 6 HET-CAMs, 4 HRIPTs, 3 Ocular Irritation Studies, 1 In-Use Eye Assessment, 1 Ocular Irritation Assay, 2 IC Assays and 2 Average Weight Usage Tests.

In response to the Expert Panel's Insufficient Data Announcement ("IDA"), dated June 15, 2023, the Companies submitted a report, dated October 27, 2023, entitled Additional Data Supporting the Safe Use of IC in Cosmetics (the "October 2023 Submission"), an 8-month Ophthalmological In-Use Safety Evaluation on 120 female subjects (the "8-Month Clinical Study"), a 28-day clinical trial on 24 female participants to evaluate potential impact on intraocular pressure (the "Intraocular Pressure Assay") and a toxicological safety assessment prepared by ToxServices LLC (the "Toxicological Safety Assessment").

b. Testing Submitted *Now*

In response to the Expert Panel's IDA, dated December 8, 2023, the Companies are now submitting this report (this "April 2024 Submission"), an OECD Guideline 471 Bacterial Reverse Mutation Test (the "AMES Test") finding that IC is not mutagenic and a draft report OECD Guideline 487 In Vitro Micronucleus Test (the "Micronucleus Test") finding that IC does not cause structural or numerical chromosomal aberrations. The AMES Test is attached is Annex 1. The Micronucleus Test is attached as Annex 2. We expect the final report of the Micronucleus Test to be issued imminently and will send under separate cover once available.

c. Testing to be Submitted *in Future*

The Companies are currently conducting an OECD Guideline 428 Dermal Metabolism and Penetration Test (the "Dermal Metabolism and Penetration Test"). IC, also known as cloprostenol isopropyl ester, is hydrolyzed to cloprostenol under physiologic conditions. The Dermal Metabolism and Penetration Test will provide critical data identifying the extent of absorption of IC itself as well as the degree to which IC is converted to cloprostenol, which impacts systemic exposure.

The Companies intend to incorporate the results of the Dermal Metabolism and Penetration Test into an updated Toxicological Safety Assessment with a further substantiated read-across methodology (the "Supplemental Toxicological Safety Assessment") and submit those materials to the Panel when available, likely by September 2024.

II. Read-Across Methodology for IC (up to 0.005%)

Read-across is applied globally as an effective tool for utilizing available data to ensure the safety of ingredients under use conditions in cosmetic products as defined and regulated under the Federal Food, Drug and Cosmetic Act and the Modernization of Cosmetics Regulation Act of 2022 (MoCRA). Published read-across frameworks are available, such as OECD's Guidance on the Grouping of Chemicals[1] and EHCA's Read-Across Assessment Framework.[2] Reliance on read-across is even more important considering that bans on the testing of cosmetic ingredients in animals preclude performance of new in vivo toxicology assays to fill data gaps.

In the previously submitted Toxicological Safety Assessment, we leveraged the PGF2α analogue chemical class to identify surrogates for IC. We systematically assessed the suitability of each potential surrogate within this chemical class using the OECD and ECHA read-across frameworks, which consider structural, functional, and mechanistic similarities. Read-across identified travoprost and cloprostenol as appropriate surrogates based on structural similarity (MCS Tanimoto score and functional groups), commonality of effects (reduction in IOP, hyperemia), and similarity with respect to the critical toxicological effect (reproductive/developmental). The read-across methodology set forth in the Toxicological Safety Assessment is particularly valid and appropriate in this case because, as noted above, IC is hydrolyzed to cloprostenol under physiologic conditions.

However, differences in the physicochemical properties that govern absorption, such as the partition coefficient, suggest that IC and cloprostenol are likely to have different systemic exposure potentials when applied topically. Absorbed IC and cloprostenol are also likely to display different tissue distribution patterns. This emphasizes the importance of the Dermal Metabolism and Penetration Test in confirming the read-across methodology: if the results of the Dermal Metabolism and Penetration Test show a high degree of conversion into cloprostenol, cloprostenol will be a uniquely appropriate read-across surrogate. The results will also further inform whether travoprost or cloprostenol is the best surrogate for any intact IC that is absorbed. In any event, any systematically available IC or cloprostenol is unlikely to result in adverse systemic health effects given the *de minimis* exposure. See the two previously submitted Average Weight Usage Tests demonstrating that, on average 0.0000084 mg of IC is applied to the upper lash line of both eyes with each application of Company 1's product, while 0.000013 mg of IC is applied to the upper lash line of both eyes with each application of Company 2's product.

The read-across approach will be further expanded to include additional tools for the identification of potential analogues, for the assessment of similarity (e.g., structural alerts and receptor binding), and for the prediction of health effects in an endpoint-specific manner, increasing confidence in the reliability of modeling results and in the conclusions of the safety assessment. The process will be captured in a data matrix for each endpoint for ease of analysis. We will also include an expanded discussion of the role of read-across in the safety evaluation of cosmetic ingredients.

III. IDA for IC (up to 0.005%)

The Companies understand that the first 2 data requests for IC from the December 8, 2023 IDA (dermal irritation and sensitization data at current maximum concentration of use and data on local

[1] https://one.oecd.org/document/env/jm/mono(2014)4/en/pdf
[2] https://echa.europa.eu/documents/10162/17221/raaf_en.pdf/614e5d61-891d-4154-8a47-87efebd1851a

ocular effects at current maximum concentration of use) have already been fulfilled for IC up to 0.005% and are targeted to the maximum concentration of use of IC up to 0.0075%.

The table below lists the other data requests for IC and provides an overview of the relevant testing and data that has been or will be provided for IC up to 0.005%.

IDA Request	Substantiation / Status
Acute toxicity data	1. Read-across methodology set forth in the Toxicological Safety Assessment: Cloprostenol has been characterized as having low acute toxicity potential; one oral LD_{50} of >25 mg/kg in rats is available. (**submitted October 2023**) 2. The results of the Dermal Metabolism and Penetration Test will strengthen the analogue selection process for this endpoint. (**to be submitted September 2024)** 3. In an expanded weight of evidence approach, the Supplemental Toxicological Safety Assessment will include QSAR-based acute oral toxicity estimates for both IC and surrogate(s) and a discussion of the basis for using in silico methods to predict acute oral toxicity potential. (**to be submitted September 2024**)
Repeated dose toxicity data	1. The 8-Month Clinical Study confirms the repeated-dose safety of IC at up to 0.005% for its intended cosmetic use. (**submitted October 2023**) 2. Multiple robust repeated-dose toxicity studies are available for the surrogates identified using the read-across methodology set forth in the Toxicological Safety Assessment, including subacute, subchronic, and chronic studies utilizing oral and parenteral exposures. These studies collectively revealed little evidence of adverse systemic effects on non-reproductive tissues. For this reason, the margin of safety (MOS) calculation for IC was based on the most sensitive/critical reproductive/developmental effect, as explained below. (**submitted October 2023**)
Developmental and reproductive toxicity data	1. Read-across data were included in the Toxicological Safety Assessment for the identified surrogates. We selected the lowest LOAEL (reduced litter size from a 3-generation study with travoprost) from all available read-across reproductive and developmental toxicity studies as the basis for the MOS calculation. We applied a safety factor of 3 to this LOAEL to account for use of a LOAEL. A sufficient MOS of 343, obtained via read-across methodology, confirms that the intended cosmetic use of IC poses no significant risk of adverse systemic effects. (**submitted October 2023**)

	2. The results of the Dermal Metabolism and Penetration Test will strengthen the analogue selection process for this endpoint. (**to be submitted September 2024**) 3. The Supplemental Toxicological Safety Assessment will include any available data of sufficient quality for other chemicals identified as adequate structural surrogates on the basis of a sufficient similarity score, such as a Tanimoto score >0.7, as there are no validated in silico or QSAR methodologies that can adequately evaluate these endpoints. (**to be submitted September 2024**)
In vitro and in vivo genotoxicity data	1. As expected based on data for cloprostenol, an OECD Guideline 471 Ames Test confirms that IC is not mutagenic. (**submitted April 2024**) 2. Also as expected based on data for cloprostenol, an OECD Guideline 487 In Vitro Micronucleus Test confirms that IC does not cause structural or numerical chromosomal aberrations. (**submitted April 2024**) 3. These findings are supported by read-across methodology set forth in the Toxicological Safety Assessment: cloprostenol tested negative in multiple genotoxicity assays, including an Ames test, *in vitro* tests in mammalian cells for mutagenicity and chromosomal aberrations, and an *in vivo* mouse micronucleus assay. (**submitted October 2023**)
Confirmatory data (e.g., receptor interaction studies and downstream profiles of adverse effects) to determine if the use of the proposed read across sources is appropriate.	1. The 8-Month Clinical Study confirms that there are no adverse downstream/phenotypic effects regardless of receptor interaction status. (**submitted October 2023**) 2. The Intraocular Pressure Test confirms that the intended cosmetic use of IC does not affect IOP, indicating that PGFα receptor activation, if any, as well as activation of other receptors or downstream signaling pathways, is not sufficient to result in the drug effect of concern. (**submitted October 2023**) The molecular mode(s) of action or adverse outcome pathway(s) mediating potential adverse events associated with the use of prostaglandin analogues in the ocular region has not been fully characterized. Instead, numerous contradictory multi-step pathways have been theorized with no conclusive evidence in the scientific literature establishing whether any particular receptor or pathway is responsible. The Companies have submitted clinical studies directly examining relevant endpoints for potential adverse events (changes in IOP, irritation, iris pigmentation) and demonstrate no evidence of such adverse events associated with the intended cosmetic use of IC at levels up to 0.005%.

	As noted in the October 2023 submission and above, *de minimis* exposure to IC and potential metabolites, including cloprostenol, reinforces evidence of safe use in cosmetic products. Moreover, specific use instructions and warnings associated with avoiding ocular exposure and use in potentially sensitive populations (pregnancy, breast-feeding, specific cancer and ocular diseases) also reinforces the safety of IC for its intended cosmetic use at concentrations up to 0.005%.
Information on possible targets and mechanisms	1. See directly above in Confirmatory Data section.

Annex 1
(AMES Test)

CONSUMER PRODUCT TESTING COMPANY
cptclabs.com

BACTERIAL REVERSE MUTATION ASSAY

GLP FINAL REPORT

REPORT NUMBER: M24-0224

SPONSOR: [redacted]

SPONSOR'S REPRESENTATIVE: [redacted]

TESTING FACILITY: Consumer Product Testing Company, Inc.
70 New Dutch Lane
Fairfield, NJ 07004
[redacted]

STUDY DIRECTOR: [redacted] Ph.D.
Director, Microbiology

REGULATORY CLASSIFICATION GLP

STUDY INITIATION: February 07, 2024

STUDY COMPLETION: February 21, 2024

APPROVED: [redacted] Ph.D.
Study Director
02/22/2024
Date

REVIEWED BY [redacted]
Quality Assurance
2/22/24
Date

Quality Assurance Unit Statement

Study No.: M24-0224

The objective of the Quality Assurance Unit (QAU) is to monitor the conduct and accurate reporting of non-clinical laboratory studies. These studies have been performed with strict adherence to the Good Laboratory Practice Act (21 CFR 58) and in accordance with standard operating procedures and applicable standard protocols. The QAU maintains copies of the study protocols and standard operating procedures and has inspected this study on the date(s) listed below. The findings of these inspections have been reported to management and to the study Director.

Dates of inspections:

02/08/2024
02/14/2024

Dates Findings Reported to Management and the Study Director:

02/09/2024
02/15/2024
02/16/2024

[redacted]
Quality Assurance

2/22/24
Date

[redacted]

CONSUMER PRODUCT TESTING™ COMPANY
cptclabs.com

FDA Registration# 100015293
DEA Registration# RC0199744 Schedule I-V
US EPA/NJ DEP Registration# NJ0982726648
ISO/IEC 17025:2017 Accreditation # 80071

Good Laboratory Practice Statement

This is to certify that Study # M24-0224 Bacterial Reverse Mutation Assay (Test Article Name: Cloprostenol isopropyl ester) was conducted in accordance with the Good Laboratory Practice Regulations, 21 CFR Part 58.

Study Director: [redacted] Ph.D.
Director, Microbiological Services
Consumer Product Testing Company

02/22/2024
Date

[redacted]

TABLE OF CONTENTS

1.0 STUDY PURPOSE

The purpose of this study was to evaluate if the test article would induce a mutagenic response in five different strains of *Salmonella typhimurium*, namely TA97a, TA98, TA 100, TA 102, and TA 1535. The test article was screened at different dose levels by plating them with the tester strains both with and without PB/BNF induced rat liver microsomes (S9). The test article was considered mutagenic if it caused an increase in revertant colonies above the spontaneous background (i.e. no test article) level.

2.0 TEST ARTICLE

The test article below was received from the sponsor and assigned the test article number M24-0224.01. It was stored as indicated by the client-supplied storage conditions until testing commenced. Test article derivation, characterization and stability was the responsibility of the sponsor.

Name: Cloprostenol isopropyl ester
Lot Number: 0695607-4
Sample Storage Conditions: Ambient
CPTC ID No.: M24-0224.01

3.0 TEST SYSTEM:

The test systems used for the Bacterial Reverse Mutation Assay were:

Salmonella typhimurium TA 97a
Salmonella typhimurium TA 98
Salmonella typhimurium TA 100
Salmonella typhimurium TA 102
Salmonella typhimurium TA 1535

4.0 TEST SYSTEM JUSTIFICATION:

The Bacterial Reverse Mutation Assay is widely used to evaluate the mutagenic properties of chemicals. The test is based on the work of Dr. Bruce Ames and his coworkers and is commonly referred to as the Ames Test. Their studies involved the development of select histidine auxotrophs of *S. typhimurium* that are normally growth arrested due to mutations in a gene needed to produce the essential amino acid Histidine. In the absence of an external histidine source, the cells cannot grow to form colonies unless a reversion of the mutation occurs which allows the production of histidine to be resumed. As might be expected, spontaneous reversions occur with each of the strains. However, chemical agents can induce a mutagenic response so that the number of revertant colonies is substantially higher than the spontaneous background reversion level. The test involves the analysis of the number of revertant colonies that are obtained with each strain in the presence and absence of the test article. Since the mutagenic response of a formulation could vary with the concentration, test articles are routinely dosed over an appropriate concentration range. In this study, a complete set of positive and negative controls was included with each assay and was plated routinely with all of the tester strains. PB/BNF induced rat liver microsomes were included to mimic the *in vivo* activity of the liver enzymes in activating some pro-mutagens to mutagenic status.

5.0 PROCEDURE:

All testing was conducted in accordance with Protocol M24-0224 (See Appendix A).

5.1 SOLUBILITY

The test article was found to be completely soluble in Sterile Deionized Water. This was the solvent used to dissolve the test article in this study.

5.2 BACTERIAL REVERSE MUTATION (AMES MUTAGENICITY) ASSAY

The bacterial reverse mutation assay was used to evaluate the mutagenic potential of the test article at five (5) concentrations of the test article per plate: 5.0, 1.0, 0.5, 0.1, and 0.05 milligrams.

Testing was done with the appropriate solvent control and positive cultures were plated with overnight cultures of the test systems (TA97a, TA98, TA 100, TA 102, and TA 1535) on selective minimal glucose agar in the presence and absence of PB/BNF-induced rat liver S9. All dose levels of the test article, solvent control and positive controls were plated in triplicate. (Refer to Appendix A: Protocol M24-0224 for the detailed test procedure).

6.0 RESULTS

Results for the mutagenicity test for test material M24-0224.01 are present in the following Tables:

Table 1: Ames Mutagenicity (w/o S9 Activation) for test article M24-0224.01

Table 2: Ames Mutagenicity (w/ S9 Activation) for test article M24-0224.01

Ames Mutagenicity Test Results.

Table # 1: Number of revertants without S-9 activation.

Sponsor: [redacted]
Sample: Cloprostenol isopropyl ester

Study # M24-0224.01
Lot# 0695607-4

Concentration tested at : 5.0, 1.0, 0.5, 0.1 and 0.05 mg/plate.
Solvent used = Sterile Deionized Water

Test Strain #		Solvent Control	Positive Control Est. #	5.0 mg sample	1.0 mg sample	0.5 mg sample	0.1 mg sample	0.05 mg sample
TA 97a	1-	76	1368	81	90	90	76	71
	2-	80	1370	72	82	79	94	74
	3-	86	1380	97	86	82	80	99
Average =		81	1373	83	86	84	83	81
Std. Deviation =		5.03	6.43	12.66	4.00	5.69	9.45	15.37

Test Strain #		Solvent Control	Positive Control Est. #	5.0 mg sample	1.0 mg sample	0.5 mg sample	0.1 mg sample	0.05 mg sample
TA 98	1-	56	1342	43	49	50	48	44
	2-	43	1351	41	52	47	40	55
	3-	52	1336	43	51	43	56	46
Average =		50	1343	42	51	47	48	48
Std. Deviation =		6.66	7.55	1.15	1.53	3.51	8.00	5.86

Test Strain #		Solvent Control	Positive Control Est. #	5.0 mg sample	1.0 mg sample	0.5 mg sample	0.1 mg sample	0.05 mg sample
TA 100	1-	97	1311	100	102	103	121	102
	2-	103	1380	104	96	96	123	106
	3-	113	1340	107	102	97	109	118
Average =		104	1344	104	100	99	118	109
Std. Deviation =		8.08	34.65	3.51	3.46	3.79	7.57	8.33

Test Strain #		Solvent Control	Positive Control Est. #	5.0 mg sample	1.0 mg sample	0.5 mg sample	0.1 mg sample	0.05 mg sample
TA 102	1-	198	1311	205	202	220	209	215
	2-	202	1358	221	211	206	214	230
	3-	230	1322	213	210	218	210	201
Average =		210	1330	213	208	215	211	215
Std. Deviation =		17.44	24.58	8.00	4.93	7.57	2.65	14.50

Test Strain #		Solvent Control	Positive Control Est. #	5.0 mg sample	1.0 mg sample	0.5 mg sample	0.1 mg sample	0.05 mg sample
TA 1535	1-	18	512	13	26	20	25	21
	2-	16	530	17	20	24	23	28
	3-	20	528	19	13	40	13	13
Average =		18	523	16	20	28	20	21
Std. Deviation =		2.00	9.87	3.06	6.51	10.58	6.43	7.51

[redacted]

Ames Mutagenicity Test Results.

Table # 2: Number of revertants with S-9 activation.

Sponsor: [redacted]
Sample: Cloprostenol isopropyl ester

Study # M24-0224.01
Lot# 0695607-4

Concentration tested at : 5.0, 1.0, 0.5, 0.1 and 0.05 mg/plate.
Solvent used = Sterile Deionized Water

Test Strain #		Solvent Control	Positive Control Est. #	5.0 mg sample	1.0 mg sample	0.5 mg sample	0.1 mg sample	0.05 mg sample
TA 97a	1-	85	1396	70	76	90	93	87
	2-	90	1368	85	90	78	84	100
	3-	75	1353	92	104	101	81	98
Average =		83	1372	82	90	90	86	95
Std. Deviation =		7.64	21.83	11.24	14.00	11.50	6.24	7.00

Test Strain #		Solvent Control	Positive Control Est. #	5.0 mg sample	1.0 mg sample	0.5 mg sample	0.1 mg sample	0.05 mg sample
TA 98	1-	56	1386	54	39	47	54	50
	2-	40	1369	45	41	47	40	37
	3-	41	1396	38	50	38	39	48
Average =		46	1384	46	43	44	44	45
Std. Deviation =		8.96	13.65	8.02	5.86	5.20	8.39	7.00

Test Strain #		Solvent Control	Positive Control Est. #	5.0 mg sample	1.0 mg sample	0.5 mg sample	0.1 mg sample	0.05 mg sample
TA 100	1-	112	1339	106	107	112	97	96
	2-	105	1350	113	98	98	104	109
	3-	109	1335	101	119	105	99	95
Average =		109	1341	107	108	105	100	100
Std. Deviation =		3.51	7.77	6.03	10.54	7.00	3.61	7.81

Test Strain #		Solvent Control	Positive Control Est. #	5.0 mg sample	1.0 mg sample	0.5 mg sample	0.1 mg sample	0.05 mg sample
TA 102	1-	250	1333	207	230	207	229	208
	2-	212	1339	215	206	227	223	219
	3-	222	1313	200	219	211	210	214
Average =		228	1328	207	218	215	221	214
Std. Deviation =		19.70	13.61	7.51	12.01	10.58	9.71	5.51

Test Strain #		Solvent Control	Positive Control Est. #	5.0 mg sample	1.0 mg sample	0.5 mg sample	0.1 mg sample	0.05 mg sample
TA 1535	1-	12	525	19	13	16	12	20
	2-	19	516	9	15	20	11	18
	3-	20	530	8	9	21	14	22
Average =		17	524	12	12	19	12	20
Std. Deviation =		4.36	7.09	6.08	3.06	2.65	1.53	2.00

[redacted]

7.0 STATISTICAL ANALYSIS

The mean and standard deviations were calculated at each dose level of test article for each test organism.

8.0 PROTOCOL AMENDMENTS/DEVIATIONS

There were no protocol amendments or deviations for this study.

9.0 CONCLUSION/DISCUSSION

The results in Tables 1 and 2 show that the test strains are sensitive to the positive control mutagens and showed the appropriate mutagenic response (i.e. positive control counts were greater than 2.5 times the negative solvent control) The spontaneous reversion rate was well within the accepted values of each strain, indicating that under the test conditions, the strains were sensitive to the detection of potentially genotoxic agents. The data in Tables 1 and 2 shows that the test article was not cytotoxic to the test system at all five (5) dose levels. There was no precipitation of the test article noted at any test concentration either with or without S-9 for the test system.

The metabolic activation using the S9 activation mixture shows an active microsomal preparation.

Using the same test conditions, there was no detectable genotoxic activity at the concentration shown above (i.e. the test article did not show a 2.5 fold increase in counts over the negative solvent control) associated with M24-0224.01) neither in the absence (Table 1) or presence (Table 2) of the S9 enzyme activation.

10.0 PROFESSIONAL PERSONNEL

[redacted]

Vice-President, Quality Assurance and Regulatory Affairs
Director, Microbiology
Supervisor, Microbiology
Senior Microbiologist
Auditor, Quality Assurance
Auditor, Quality Assurance

11.0 RECORDS AND RETENTION

All records and documents pertaining to the conduct of this study shall be retained in the CPTC archives for a minimum of five (5) years. At any time prior to the completion of the fifth archival year, a Sponsor may submit a written request to the CPTC 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.

Appendix A

Protocol

(8 pages)

CONSUMER PRODUCT TESTING COMPANY
cptclabs.com

GLP Protocol: BACTERIAL REVERSE MUTATION ASSAY

STUDY NUMBER: M24-0224

SPONSOR: [redacted]

SPONSOR'S REPRESENTATIVE: [redacted]

TESTING FACILITY: Consumer Product Testing Company, Inc.
70 New Dutch Lane
Fairfield, NJ 07004
[redacted]

STUDY DIRECTOR: [redacted] Ph.D.
Director of Microbiology

PROPOSED STARTING DATE: Week of 01/29/2024

PROPOSED COMPLETION DATE: Week of 03/04/2024

APPROVALS/REVIEWS: I have reviewed and accepted the protocol to be used in this study.

Approved by.: [redacted] 1/7/2024
[redacted] Ph.D. Date
Study Director
Consumer Product Testing Company

Approved by: [redacted] 1-22-2024
Date
Sponsor Representative

Reviewed by: [redacted] 1-22-2024
[redacted] Vice President Date
Quality Assurance and Regulatory Affairs
Consumer Product Testing Company

BACTERIAL REVERSE MUTATION ASSAY

1.0 PURPOSE

The purpose of this protocol is to evaluate the ability of a chemical, formulation or extract to induce a mutagenic response in five different strains of *Salmonella typhimurium*, namely TA 97a, TA 98, TA 100, TA 102, and TA 1535. Test articles are screened at different dose levels by plating them with the tester strains both with and without Phenobarbital/ß-Naphthoflavone (PB/BNF) induced rat liver microsomes (S9). Articles are considered mutagenic if they cause an increase in revertant colonies above the spontaneous background (i.e., no test article) level.

This protocol is compliant with OECD 471 Guideline for Testing of Chemicals: Bacterial Reverse Mutation Assay.

2.0 STUDY DIRECTOR

The Study Director will be [redacted] Ph.D. The Study Director will have responsibility for all aspects of the study and will serve as the primary contact to the Sponsor's Project Leader. The Study Director's *curriculum vita* is available upon request by the Sponsor's Representative.

3.0 TEST ARTICLE:

CPTC ID No:	Name:	Lot Number:	Sample Storage Conditions:
M24-0224.01	Cloprostenol isopropyl ester	0695607-4	Ambient

4.0 TEST SYSTEM:

Salmonella typhimurium TA97a
S. typhimurium TA 98
S. typhimurium TA100
S. typhimurium TA102
S. typhimurium TA1535

5.0 TEST SYSTEM JUSTIFICATION:

The Bacterial Reverse Mutation Assay is widely used to evaluate the mutagenic properties of chemicals. The test is based on the work of Dr. Bruce Ames and his coworkers and is commonly referred to as the Ames Test. Their studies involved the development of select histidine auxotrophs of *S. typhimurium* that are normally growth arrested due to mutations in a gene needed to produce the essential amino acid Histidine. In the absence of an external histidine source, the cells cannot grow to form colonies unless a reversion of the mutation occurs which allows the production of histidine to be resumed. As might be expected, spontaneous reversions occur with each of the strains. However, chemical agents can induce a mutagenic response so that the number of revertant colonies is substantially higher than the spontaneous background reversion level. The test involves the analysis of the number of revertant colonies that are obtained with each strain in the presence and absence of the test chemical. Since the mutagenic response of a formulation could vary with the concentration, test articles are routinely dosed over an appropriate concentration range. In this protocol, a complete set of positive and negative controls is included with each assay and is plated routinely with all the tester strains. Phenobarbital/ß-Naphthoflavone (PB/BNF) induced rat liver microsomes are included to mimic the *in vivo* activity of the liver enzymes in activating some pro-mutagens to mutagenic status.

6.0 RECOMMENDED EQUIPMENT AND SUPPLIES

Media and Reagents and Supplies
Nutrient Broth, Oxoid No. 2
Nutrient Agar
Histidine/Biotin Top Agar 0.05 mM (for use with *Salmonella* strains)
Minimal Glucose Agar Plates
NADPH REGENSYS™ A and B
ST Quad PC™ Plates
ControlChem™ Mutagens (As specified in this protocol for each bacterial tester strain)
PB/BNF Induced Male Sprague Dawley rat liver S9, in 0.15M KCL.
Dimethyl Sulfoxide (DMSO)
Ampicillin (preferably an 8 mg/ml stock in 0.02 M NaOH)
Tetracycline (preferably a 0.8 mg/d stock in 0.02 M HC1)
Sterile water
Sterile forceps (optional)
Sterile flasks or containers
Sterile inoculating loops
Sterile swabs (optional)
100 and 1000 µl sterile pipette tips
Ice and Ice bucket
1 ml and 5 ml sterile pipets
Pipet-aid or rubber bulb
Latex gloves
Test tube rack
13 x 100 mm sterile disposable test tubes
Various other laboratory supplies including glassware, forceps, and pipettes

Equipment
37°C incubator with shaker
45°C heating block
Automatic colony counter or magnifying counter
Sterilizer
Vortex Mixer
Positive displacement pipette aid (e.g., Pipette-man, Eppendorf)

7.0 TEST PROCEDURE

The sections that follow describe the procedures for the conduct of the basic five strain mutagenicity assay. The methods described are based on Maron & Ames (ibid.).

A. Pre-test

1. Before setting up the assay determine the solubility of the test article(s) in available compatible solvents (see p. 200, Maron & Ames for listing). Solubility will be tested to determine the permitted preparation of the highest soluble or workable stock concentration, up to 50 mg/mL.
2. Determine the doses to be tested. In general, the upper dose should not exceed 5 mg/plate (50 mg/ml assuming a 100 µl /plate dosing volume). Select 5 doses separated by half logs for the mutagenicity assay: 5.0, 1.0, 0.5, 0.1, and 0.05 mg/plate.
3. Assemble the supplies and equipment needed to perform the test on the day before.
   a. Remove the QUAD plates from the refrigerator, cut off the plastic sleeve and allow to dry upright at room temperature overnight.
   b. Label Minimal Glucose Agar plates and Top Agar tubes with strain number, test identification and dose, S9 *(+/-)* and date of test. Be sure to include the diagnostic positive and negative controls.
   c. Adjust the temperature of your water bath or dry block heater to approximately 45°C.

B. One Day Prior to the Assay

1. Label sterile flasks with the strain number (e.g., TA97a, TA98, TA100, TA102, TA1535). Using aseptic technique, carefully decant approximately 16-25 ml of Oxoid #2 nutrient broth into Erlenmeyer flasks. Add ampicillin to all flasks for each strain except for TA1535 (no ampicillin) to final concentration of 25 µg/ml (to 16-25 ml nutrient broth, up to 78 µl of an 8 mg/ml solution). Also, to TA102, add up to 62.5uL of Tetracycline at a 0.8mg/mL solution.
2. Remove the STDisc™ vials from the refrigerator and warm to room temperature before opening.
3. Remove the vial closure.
4. Using a sterile loop/needle, pick up one or more discs and drop them into the appropriately labeled flask containing nutrient broth.
5. After the flasks are inoculated, place them on the shaker and incubate at 37°C with the shaker operating at approximately 150 rpm. Incubation must not exceed 16 hours.
6. Note: In some cases, it may be desirable to grow your cells on the day of the assay. To do this, increase the size of the initial inoculum; if you inoculate with 6-7 discs the culture should reach an absorbance of 1.0 to 1.2 (@ 660 nm) within approximately 5-6 hours. It is important that your culture is not overgrown.
7. After incubation (e.g., on the morning of the assay) remove the flask cultures and place them in the refrigerator until needed.

C. The Day of the Assay

1. Melt the top agar. After melting, place the top agar bottles into a 45°C water bath - allow at least 45 minutes for temperature equilibration.
2. Cultures and the S9 must be placed on ice prior to use and kept on ice throughout the assay.
3. Prepare the positive controls:
   Note: *Latex or vinyl plastic gloves must be worn when handling these chemicals.*
4. Add one ml of the appropriate solvent to each of the CONTROLCHEM tubes.

Mutagen	Strain(s)
ICR 191 Acridine	TA97a
Daunomycin	TA98
Sodium Azide	TA100 & TA1535
Mitomycin C	TA102
2-Aminoanthracene (w/S9)	All

5. Perform the dilutions of your test article.
6. Load the 45°C heating block with sterile 13 x 100 mm tubes with closures equal to the number of labeled minimal glucose agar plates. Pipette 2 ml of molten, 45°C, top agar into each tube (top agar containing histidine for all *Salmonella* strains). Arrange your previously labeled Minimal Glucose Agar plates by strain and condition (e.g., controls, +/- S9, etc.).
7. Decide which strain you are going to begin with. In the scheme shown below it is assumed that TA97a will be used first in a triplicate plate, + and - S9 assay.
8. Assay your Test Article:

WITHOUT S9

a. Begin with the solvent control; add 100 µl of water or DMSO (or other solvent used to solubilize your test article) to the first three tubes pre-heated to 37±2°C. Then, in ascending sequence, add 100 µl of each test article dilution to each additional trio of pre-heated tubes.
b. Add 100 µl of the TA97a culture to the first three tubes (solvent control tubes).
c. Without delay, gently mix the tube contents using a vortex mixer. Decant the mixture onto the surface of the appropriately labeled Minimal Glucose Agar plate.

Do one tube at a time. Immediately upon decantation, gently tilt the plate and rotate to obtain an even distribution of the plating mixture over the surface of the bottom agar. Place onto a level surface and allow to harden.

d. Repeat steps 8b. and 8c. for each dose of the test article.

WITH S9

e. Repeat step 8a.

f. Add 500 µl of the previously prepared S9 mix to the first three tubes (solvent control tubes).

g. Without delay, add 100 µl of the TA97a culture as described in step 8b through 8d (above).

h. Repeat steps 8f. and 8g. for each of the test article doses.

REPEAT THE ABOVE PROCEDURES FOR EACH STRAIN

D. Positive Control, Phenotypic Characterization, Test Organism Titer

1. Treat the Positive Control Cultures:
   a. Set up 6 tubes for each strain. Three tubes will be used for the - S9 diagnostic control and 3 will be used for the + S9 positive control.

   b. Add 2 ml of molten agar to each tube as before. Add 100 µl of the positive control solutions according to the following scheme:

Chemical	Strain
Sodium Azide	TA100 & 1535
Daunomycin	TA98
Mitomycin C	TA102
ICR 191 Acridine	TA97a
2-Aminoanthracene	All (+S9)

2. Following the methods described in Section 8, add 100 µl of the appropriate strain and decant, spread, and set aside. For 2-Aminoanthracene, add 500 µl of the S9 mix and strains as was previously described.
3. Inoculate the Quad Plates with the Salmonella strains
   a. Using a sterile loop or swab, wet with the appropriate culture and inoculate each of the four sectors of a Quad PCTM plate using a "Z" inoculation pattern.
   b. Repeat for each strain. After all plates are inoculated, open the vial containing the crystal violet discs and using forceps or an inoculating loop, place a single disc on the agar surface in Sector II of each of the Quad plates.
4. Determine the Titer of the Strain Cultures
   a. Arrange sets of 3 sterile tubes with closures for each strain. Pipette 4.95 ml sterile water into each tube.
   b. Using your positive displacement pipette, inoculate the first tube with 50 µl of the appropriate strain culture. Mix thoroughly - use a vortex mixer at low speed or mix by use of a 5 ml pipette. This tube contains 1:100 dilution of the sampled culture. Add 50 µl of the 1:100 dilution to the second tube containing 4.95 ml sterile water - mix as before. The second dilution is 1:10,000. Complete the dilutions by adding 50 µl of the 1:10,000 dilution to the third 4.95 ml tube, mix. The final dilution is 1:1,000,000.
   c. Arrange sets of 2 sterile tubes with closures for each strain and place in 45°C water bath. Add 2 ml of molten top agar to each tube.
   d. Using the positive displacement pipette, inoculate the top agar-containing tubes with 50 µl of the 1:10,000 and 1:1,000,000 dilutions. Mix and pour onto the appropriately labeled Nutrient Agar plates. The plated volumes result in final dilutions of 5×10^{-6} and 5×10^{-8} for the 1:10,000 and 1:1,000,000 dilutions in water, respectively.

5. Incubate the Assay
   a. Invert the plates and arrange them in stacks corresponding to each experimental condition.
   b. Place in a 37°C incubator and continue incubation for approximately 48 h.
6. Read the Assay
   a. After the incubation period, remove the inverted plates and allow them to come to room temperature.
   b. Colony counting can be performed manually with the aid of a magnifying counter (e.g., "Quebec" counter) or with an automatic colony counter (e.g., Protocol). Depending on the activity of your test, large numbers of colonies may develop in certain dose groups. In some cases, it may be desirable to utilize sector-counting techniques ("estimated counts") rather than full plate counts.
   c. After counting and recording the results for the test treatments, the diagnostic positive control plates should be counted.
   d. Examine the cell titer (nutrient agar) plates. The 5×10^{-6} plates should be too numerous to count. In contrast, the 5×10^{-8} plates should contain approximately 25-100 colonies; such a result indicates that the initial population (the stock culture) was in the range of 1 to 2×10^{9} "viable" cells per ml. Very much lower or higher initial titers may result in reduced frequencies and background or increased backgrounds, respectively.
   e. The Quad plates (phenotypic confirmation media) should be examined, and the results compared to those described in the relevant strain QC sheets and the Maron & Ames paper.

8.0 RESULTS EVALUATION AND INTERPRETATION

A. Negative (solvent) Control Counts

The colonies that grew on the Minimal Glucose Agar plates developed from single cells that had regained their ability to grow in the absence of added histidine. The genetic reversion, from histidine auxotrophy to prototrophy, that enabled those cells to grow in the absence of exogenous histidine might have arisen spontaneously or as the result of a mutation induced by the treatments (see Maron & Ames, p. 181). It is important to realize that some of the colonies that arose in the positive control plates would have grown in the absence of treatment; they arose spontaneously. Accordingly, the negative (solvent) control colony counts constitute an important baseline in your evaluation of the test results. Unfortunately, the spontaneous reversion frequencies for the various tester strains can be quite variable - nevertheless, large deviations from the "normal" range of spontaneous reversion values may signal systematic problems with the assay.

B. Diagnostic Positive Control Counts

In general, the positive control frequencies (number of colonies per plate) should be at least 2.5 times the negative control counts (spontaneous frequency). Large deviations usually indicate problems with cell management, e.g., high spontaneous frequencies (due, perhaps to culture overgrowth) often are paralleled by low induced frequencies. Such eventualities reduce the resolving power of the assay and raise questions regarding the interpretation of the results of the test treatments.

C. Phenotypic Confirmation

The Quad plates are prepared with four different media that provide basic information concerning the genotypes of the strains provided in the kit (see the QC sheets for the specific strains). By sector, the results should be:

Quad Plate Sector	Observation	Genotype
I	No growth (all strains)	*his-*
II	Zonal inhibition around CV disc (all)	*rfa*
III	Profuse growth (all) except TA 1535 which shows no growth	pKM101
IV	No Growth all except TA 102 (shows growth)	pAQ 1

D. Test Article Results

In general, the 2 or 2.5 times over the background (spontaneous frequency) "rule-of-thumb" serves as a useful way of distinguishing active mutagens from non-mutagenic test articles. The presence of a dose response (not necessarily linear) is often used as an adjunct criterion for and interpretation of positive activity in the assay.

9.0 STATISTICAL ANALYSIS

The mean and standard deviations will be calculated at each dose level of test article for each test organism.

10.0 GOOD LABORATORY PRACTICE REGULATIONS

This protocol will be conducted according to FDA Good Laboratory Practice Regulations 21 CFR Part 58.

11.0 PROTOCOL AMENDMENTS/DEVIATIONS

If the study design for this protocol needs to be revised after being signed, a protocol amendment will be written describing the change. The amendment will be signed by the Study Director and sent to the Sponsor for an approval signature. Departures from the conduct of the protocol will be documented in a protocol deviation, signed by the Study Director, and sent to the Sponsor for signature. Changes or clarifications to approved protocols that do not affect study conduct/design, or final results (for example: typos, administrative changes, proposed start/end dates) will be documented in a file memo.

12.0 FINAL REPORT

A final report will be issued which will include the following information:

- Descriptions of the test article(s).
- Dates of study initiation and completion
- Any protocol deviation(s)/amendment(s).
- Study results and a summary/conclusion
- A copy of this protocol

13.0 MAINTENANCE OF RECORDS

All records and documents pertaining to the conduct of this study shall be retained in the CPTC archives indefinitely. At any time, a Sponsor may submit a written request to the CPTC QA Department to obtain custody of study records. This transfer shall be performed at the Sponsor's expense.

14.0 TEST ARTICLE CHARACTERIZATION AND STABILITY

The Sponsor assumes responsibility for test article derivation, characterization, and stability testing.

15.0 RETENTION OF TEST ARTICLES

At the completion of the study, the remaining test material will be retained for 2 months and destroyed at the end of this period unless otherwise directed. Test articles may be returned to Sponsor at their expense, if requested.

16.0 REFERENCES:

Ames, B.N., McCann, J. and Yamasaki, E. "Methods for Detecting Carcinogens and Mutagens with the *Salmonella* Mammalian Microsome mutagenicity test." Mut. Res. 31: (1997) p. 347-364.

Ames, B.N., Maron, D. "Revised methods for the *Salmonella* mutagenicity test". Mut. Res. 113: (1983) p. 173-215.

OECD 471 Guideline for Testing of Chemicals: Bacterial Reverse Mutation Assay

FDA 21 CFR Part 58 "Good Laboratory Practice for Nonclinical Laboratory Studies"

Annex 2
(Micronucleus Test)

[redacted]

NUCRO TECHNICS

DRAFT REPORT

IN VITRO MAMMALIAN CELL MICRONUCLEUS TEST OF (+)-CLOPROSTENOL ISOPROPYL ESTER IN CHINESE HAMSTER OVARY CELLS

Nucro-Technics' Study No.: 403393

FOR

Study Sponsor

[redacted]

REPORT APPROVAL

The *In Vitro* Mammalian Cell Micronucleus Test of (+)-Cloprostenol Isopropyl Ester (Clo-IPE) in Chinese Hamster Ovary Cells was conducted at Nucro-Technics in accordance with Nucro-Technics' Study Plan No. LAS/403393, Study Specific Supplement No. 1 and 2, applicable Nucro-Technics' Standard Operating Procedures, and in compliance with the Good Laboratory Practices of the Organization for Economic Co-operation and Development (1) and the United States Food and Drug Administration (2) with the exception that dose formulation analysis was not performed. Stability, characterization, identity, and verification of the test item were the responsibility of the study Sponsor.

NUCRO-TECHNICS

[redacted] _____ _____

Date

Study Director
Group Leader, Genetic Toxicology

[redacted] _____ _____

Date

President

QUALITY ASSURANCE STATEMENT

Inspections of Nucro-Technics' Study No. 403393 entitled "*In Vitro* Mammalian Cell Micronucleus Test of (+)-Cloprostenol Isopropyl Ester in Chinese Hamster Ovary Cells" were conducted on the following phases by the Quality Assurance Unit of Nucro-Technics. Inspection results were reported to the Study Director and Facility Management as indicated below.

Inspection Date	Phase Inspected	Date Submitted to Study Director and Facility Management
February 14, 2024	Test item formulations preparation and treatment	February 15, 2024
April 15 & 16, 2024	Raw data and draft report	April 16, 2024
	Final report	

The methods, results and data contained in this report accurately reflect the procedures followed and raw data collected.

_____ _____

[redacted] Date

Quality Assurance Associate

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SUMMARY

The test item, (+)-Cloprostenol Isopropyl Ester (Clo-IPE), was evaluated for its potential to induce micronuclei (clastogenic response) or hypodiploidy (aneugenic response) in cultured Chinese Hamster Ovary (CHO) cells in the absence and presence of metabolic activation (S9). The experimental design followed the OECD Guideline for the Testing of Chemicals – 487, *In Vitro* Mammalian Cell Micronucleus Test.

As the test item was provided as a solution in ethanol (10% w/w; 75 mg/mL), a solubility test was performed to evaluate its solubility when added to the test system. Dose-dependent test item precipitate was observed for the three highest concentrations in Growth Medium. Based on the results of the solubility test, ethanol was selected as the vehicle for the main study experiments.

In Experiment 1, the test item was tested at 750, 380, 190, 94, 47, 23, 12, 5.9, and 2.9 µg/mL using three treatment conditions, short-term treatments (4 hours) in the absence and presence of S9 and extended exposure (23 hours) in the absence of S9. Concurrent negative/vehicle and positive controls were included in each treatment condition to verify the test system.

In Experiment 1, dose-dependent test item precipitate was observed at 750, 380, and 190 µg/mL in the absence of S9, and at 750 and 380 µg/mL in the presence of S9. Relative Increase in Cell (Nuclear) Counts (RICC), which is based on the number of nuclei present in the treated samples versus the negative control samples, was used as a measure of cytotoxicity. Following treatment with Clo-IPE, RICC values ranged from 101 to -41% for all treatment conditions indicating there was dose-dependent test item-induced cytotoxicity. When the maximum test item concentration is based on cytotoxicity, the highest concentration for evaluation should aim to achieve an RICC of 45 ± 5%. At least three non-cytotoxic (RICC ≥ 40%) concentrations of the test item were available for the evaluation of micronuclei for the short-term treatments, and micronuclei were scored in at least 2000 nuclei test item concentration and control (≥ 15,806 per replicate). Therefore, Clo-IPE was tested with a sufficient number of concentrations up to the limit of cytotoxicity in accordance with OECD guidelines. Due to overt cytotoxicity (RICC < 40%) in the long-term treatment, only the two lowest concentrations of Clo-IPE were available for evaluation of micronuclei, therefore, this condition was repeated in Experiment 2 with a modified dose design.

In Experiment 2, the test item was tested at 30, 15, 7.5, 3.8, 1.9, 0.94, 0.47, and 0.23 µg/mL for 23 hours in the absence of S9. Concurrent negative/vehicle and positive controls were included to verify the test system. No test item precipitate was observed for any concentration when treated to the test system. Test item RICC values ranged from 102 to -25% indicating there was dose-dependent test item-induced cytotoxicity. At least three

non-cytotoxic concentrations of the test item were available for the evaluation of micronuclei, and micronuclei were scored in at least 2000 nuclei per test item concentration and control (≥ 18,349 per replicate). Therefore, Clo-IPE was tested with a sufficient number of concentrations up to the limit of cytotoxicity in accordance with OECD guidelines.

For both experiments, when compared to the concurrent negative control, no change in pH (indicated by a change in treatment media colour and/or measurement by pH meter) and osmolality (measurement by osmometer) was observed following treatment with Clo-IPE, where applicable.

In all treatment conditions, there were no biologically relevant increases in either the frequency of micronuclei (mean %MN) or in the frequency of hypodiploidy (mean %HD) compared to the concurrent negative control for any concentration of Clo-IPE. The mean %MN and %HD values were all < 3-fold or < 7-fold the concurrent negative controls, respectively, and within the laboratory historical negative control range. In the absence of any indication of a biologically relevant response, no statistical analysis was performed for any Clo-IPE concentration for any treatment condition. Clo-IPE was considered to be negative, i.e. the test item did not induce chromosome breaks and/or loss in cultured CHO cells.

The replication increases of the vehicle controls met the acceptance criteria of being ≥ 1.8 demonstrating that a substantial proportion of the negative control cells underwent cell division. The frequencies of MN and HD for the concurrent negative controls were within the historical negative control data. At least one concentration of the positive controls for each treatment condition produced increases in %MN that were ≥ 3-fold when compared with the concurrent negative controls and fell within the normal characteristic range of the historical positive control data. Consistent with its mode of action (aneugen), colchicine induced increases in %HD that were ≥ 7-fold when compared with the concurrent negative control and that fell within the normal characteristic range of the historical positive control data. Therefore, the study met all the acceptance criteria for a valid test.

In conclusion, the test item, Clo-IPE, did not induce micronuclei (via chromosome breaks and/or loss of whole chromosome(s)) or hypodiploidy (chromosome loss, sub-2n nuclei) in cultured CHO cells in the absence (4 and 23 hours treatment) and presence (4 hours treatment) of metabolic activation when tested up to the limit of cytotoxicity under the conditions of the test.

1. STUDY INFORMATION

1.1 Objective

The objective of this study was to evaluate the test item and any possible metabolites for their potential to induce micronuclei and hypodiploidy in cultured Chinese Hamster Ovary cells.

1.2 Nucro-Technics' Study No.

403393

1.3 Test Facility

Nucro-Technics
2000 Ellesmere Road, Unit #16
Scarborough, Ontario, Canada
M1H 2W4
[redacted]

Study Director	[redacted] Group Leader, Genetic Toxicology Address as cited for Test Facility [redacted]
Facility Management	[redacted] President Address as cited for Test Facility [redacted]
Quality Assurance	[redacted] Quality Assurance Associate [redacted]

1.4 Sponsor

[redacted]

Sponsor's Representative [redacted]

Study Monitor [redacted]

1.5 Study Dates

Study Initiation:	February 12, 2024
Experimental Start:	February 13, 2024
Experimental Completion:	March 7, 2024
Study Completion:	Date Study Director signs the final report

2. TEST AND CONTROL ITEMS

2.1 Test Item

Name:	(+)-Cloprostenol isopropyl ester (Clo-IPE)
Chemical Name:	(+)-9a,11a,15R-trihydroxy-16-(3-chlorophenoxy)-17,18,19,20-tetranor-prosta-5Z,13E-dien-1-oic acid, isopropyl ester
Molecular Formula:	C25H35ClO6
Formula Weight:	467 g/mol
CAS No.:	157283-66-4
Item No.:	10010016
Batch No.:	0695607-8
Expiry Date:	December 21, 2025
Color/Form:	Clear colorless liquid
Potency:	10% w/w in ethanol (~ 75 mg/mL)
Purity by HPLC:	100.0%
Storage Conditions:	Freezer (-10 to -25°C)
Handling Precautions:	Standard Laboratory Precautions
Manufacturer:	[redacted]

2.2 Test Item Characterization

Documentation of the identity, strength, purity, composition, and stability of the test item is on file with the Sponsor. A Certificate of Analysis is included in Appendix II.

The Sponsor has appropriate documentation on file concerning the method of synthesis, manufacture or derivation of the test item, and this information is available to the appropriate regulatory agencies should it be requested.

2.3 Test Item Inventory and Disposition

Records of the receipt, distribution and storage of the test item were maintained. The remainder of the test item was discarded after completion of all studies at Nucro-Technics.

2.4 Analysis of Test Item Preparations

Test item preparations in the vehicle were not collected or analyzed for formulation accuracy and stability to cover the dosing period, over the range of concentrations used in the study.

2.5 Control Items

2.5.1 Negative/Vehicle Control

Name:	Ethanol (Ethyl alcohol anhydrous) (100%)
Item Number:	P016EAAN
Lot Number:	036580
Expiry Date:	June 30, 2024
Color/Form:	Clear colorless liquid
Purity/Potency:	99.95%
Storage Conditions:	15 to 30°C
Supplier:	

2.5.2 Positive Controls

Name:	Colchicine (COL)
CAS Number:	64-86-8
Product Number:	C9754
Batch Number:	SLCP1631
Retest Date:	August 31, 2025
Color/Form:	White powder
Storage Conditions:	15 to 30°C
Supplier:	

Name:	Cyclophosphamide monohydrate (CP)
CAS Number:	6055-19-2
Product Number:	C0768
Batch Number:	MKCS5505
Retest Date:	December 31, 2025
Colour/Form:	White powder
Storage Conditions:	2 to 8°C
Supplier:	

Name:	Mitomycin C (MMC)
CAS Number:	50-07-7
Product Number:	M0503
Batch Number:	SLCH5866
Retest Date:	March 31, 2025
Color/Form:	Light grey powder
Storage Conditions:	2 to 8°C
Supplier:	

3. EXPERIMENTAL DESIGN

3.1 Method

The study design followed the current OECD Guideline for the Testing of Chemicals – 487, *In Vitro* Mammalian Cell Micronucleus Test (3). The study was conducted in accordance with Study Plan No. LAS/403393, and Study Specific Supplement No. 1 and 2, included in Appendix I.

3.2 Principle of Test Method

Micronuclei may originate from acentric chromosome fragments (i.e., lacking a centromere), or whole chromosomes that are unable to migrate to the poles during the anaphase stage of cell division (3). Hypodiploid cells are those that have lost entire chromosomes (have sub-2N nuclei). The *in vitro* micronucleus test detects hypodiploidy arising from aneugenic (chromosome loss) events, as well as micronuclei arising from clastogenic (chromosome breakage) or aneugenic events in cells that have undergone cell division during or after exposure to the test item (4). Cultures of Chinese Hamster Ovary (CHO) cells are exposed to the test item in the absence and presence of exogenous drug-metabolizing enzymes from rat liver. After 1.5-2.0 cell cycle lengths, the cells are sequentially stained to distinguish apoptotic/necrotic cells from viable cells, and to score hypodiploidy and micronuclei in viable cells by flow cytometry.

3.3 Materials

3.3.1 Cells

Chinese Hamster Ovary cell line (CHO-WB_L) was obtained from the Department of Pathobiology at the University of Guelph (Guelph, Ontario, Canada). The cells grow as an adherent monolayer, doubling approximately every 12 to 15 hours. Cell cultures were maintained in an exponential cell growth phase to expose the cells to the test item at different stages of the cell cycle.

3.3.2 Media and Culture Conditions

Growth Medium (GM) was comprised of McCoy's 5A Modified Medium containing 10% heat-inactivated fetal bovine serum (HIFBS), 1.5 mM L-glutamine, 25 mM HEPES, and 1× antibiotic/antimycotic (100 units penicillin, 0.1 mg streptomycin, and 0.25 µg amphotericin B). To initiate an experiment, a working frozen culture was thawed at approximately 37ºC and grown in tissue culture flasks in GM.

The cultures were incubated at 37 ± 2ºC in a humidified atmosphere containing approximately 5% CO_2. The term "incubate" throughout this report refers to these conditions.

When the cell monolayer reached approximately 50-90% confluency, cells were dislodged from the flask surface with 0.05% Trypsin. Cells were collected by centrifugation for 7 minutes at 200 × g and at 2 to 8ºC. The term "centrifuge" throughout this report refers to these conditions. Cells were resuspended in fresh medium and seeded into flasks. Each trypsinization was recorded as one passage.

The health status of the cultures was monitored by cell counting and observation under a microscope. The working frozen culture tested negative for mycoplasma contamination.

3.3.3 Metabolic Activation

Since CHO cells do not have endogenous metabolic capacity, an exogenous metabolizing system was used, i.e. a co-factor-supplemented post-mitochondrial fraction (S9) prepared from the livers of male Sprague-Dawley rats treated with an enzyme inducer (phenobarbital and β-naphthoflavone). S9 was purchased from Molecular Toxicology Inc. and was stored at -80 ± 10°C. Immediately prior to use, S9 stock was thawed and an S9 mix was prepared in serum-free medium containing the following components (final concentrations):

- 10 mM Glucose-6-phosphate
- 8 mM NADP
- 66 mM KCl / 16 mM MgCl2
- 20% Rat liver S9

3.3.4 Controls

Negative Controls: Ethanol (100%), used to prepare dosing solutions, treated alone in the treatment medium in the same way as the treatment cultures was used as the vehicle control. Because a routine vehicle that has been shown to have no deleterious or mutagenic effects was used as the vehicle control, untreated controls were not included in the study.

Positive Controls: Positive controls in the absence of S9 were:
- MMC (4-hour treatment): 0.20 and 0.25 µg/mL (clastogen)
- COL (23-hour treatment): 0.15 and 0.18 µg/mL (aneugen)

Positive control in the presence of S9 was:
- CP (4-hour treatment): 2.0 and 4.0 µg/mL (clastogen)

All positive controls were formulated in water.

3.4 Test Procedure

3.4.1 Preparation of Cultures

The day before treatment, the number of cells was counted using trypan blue exclusion. A cell suspension (passage number 22 and 8 for Experiments 1 and 2, respectively) of 3×10^4 cells per mL was prepared in GM. The suspension was seeded in flat-bottom 24-well plates at a density of 3×10^4 cells/well (1 mL per well) and incubated for approximately 24 hours.

3.4.2 Test Item Solubility

Solubility testing of Clo-IPE was performed outside the scope and directive of Study Plan LAS/403393 (Appendix I). As the test item was provided as a solution in ethanol (10% w/w; 75 mg/mL), this stock solution was serially diluted in ethanol and each dilution was added to GM at 10 µL/mL to evaluate its solubility in the test system. Extreme, moderate and slight precipitate was observed at 0.75, 0.38, and 0.19 mg/mL respectively, and no precipitate was observed at 0.094, 0.047, and 0.023 mg/mL.

The test item did not increase the pH and osmolality of GM measured using the current *US Pharmacopeia* as a guide (5). The pH ranged from 7.63 to 7.65 and the osmolality ranged from 473 to 496 mOsm/kg[1], compared to 7.64 and 495 mOsm/kg for the vehicle alone (Ethanol) diluted in GM at the same ratio as the test item.

On the basis of these results, Clo-IPE was formulated in ethanol for the *in vitro* micronucleus test.

3.4.3 Preparation of Test Item Formulations

On the day of treatment for Experiment 1, Clo-IPE at 75 mg/mL was serially diluted in ethanol by a factor of two to 38, 19, 9.4, 4.7, 2.3, 1.2, 0.59, and 0.29 mg/mL. For Experiment 2, Clo-IPE at 75 mg/mL was twice diluted by a factor of five to 15 and 3.0 mg/mL, and subsequently serially diluted by a factor of two to 1.5, 0.75, 0.38, 0.19, 0.094, 0.047, and 0.023 mg/mL. The two highest concentrations of 75 and 15 mg/mL were not treated. All formulations were vortexed immediately before diluting.

[1] An osmolality reading could not be obtained from the test item treated to GM at 0.023 mg/mL due to repeated instrument errors (sample pre-freeze), until ultimately, there was no remaining sample. These errors occur occasionally due the nature of the medium, but there was no impact to the integrity of the data as the osmolality for the highest treated concentration of 0.75 mg/mL was obtained.

3.4.4 Treatment with Test Item: Short-Term Treatment (4 Hours)

Duplicate wells were treated for each test item concentration and control. On the day of treatment, the test item was formulated at the concentrations indicated in section 3.4.3 and then diluted in GM and GM with S9 mix, as appropriate. Test item formulation or vehicle control (30 µL) were added per 3 mL of GM or GM with S9 mix. The positive controls were similarly diluted at 30 to 60 µL per 3 mL of GM or GM with S9 mix to achieve the appropriate final concentrations.

The medium from the overnight cultures was aspirated, and the cells were treated with 1 mL/well GM or GM with S9 mix containing test or control item, as appropriate. Additional cultures were included as 'pre-treatment' wells. Pre-treatment wells remained untreated and were harvested on the day of treatment as per section 3.4.6. These wells were used to determine replication increase (RI) and Relative Increase in Cell (Nuclear) Counts (RICC).

The cultures were incubated for 4 hours. Following incubation, the cell cultures were examined. For Experiment 1, samples of the treatment media were retained for pH and osmolality assessment. This was not possible for Experiment 2 because only the long-term treatment was performed. Because there was no evidence of a substantial increase in the number of mitotic cells compared with controls, indicating no treatment-induced mitotic arrest, the media was removed from all wells. Fresh GM (1 mL/well) was added and the cultures were incubated until 23 hours after the initiation of treatment, i.e. approximately 1.5 – 2.0 times the cell cycle length.

3.4.5 Treatment with the Test Item: Long-Term Treatment (23 Hours)

The treatment was similar to the short-term exposure with the exception that the cells were exposed to the test item and controls in the absence of S9 for 23 hours for Experiment 1 and 2, respectively (approximately 1.5 – 2.0 times the cell cycle length).

3.4.6 Pre-treatment Cell Harvest

On the day of cell treatment, the pre-treatment wells were harvested as per section 3.4.8. These cells were untreated and were used to determine RI and RICC. The cells were stored at 2-8°C for 2 days until the remaining treated samples were analyzed by flow cytometry.

3.4.7 Precipitation, pH and Osmolality of Treatment Medium

At the end of treatment, cell cultures were examined for precipitation and pH change, i.e. change in the colour of the treatment medium containing phenol red. The treatment medium of the two highest concentrations and the negative control

for the short-term exposure (Experiment 1) were sampled for pH and osmolality measurement, using the current *US Pharmacopeia* as a guide (5).

3.4.8 Staining, Lysis and Flow Cytometry

The following steps were performed using the Litron *In Vitro* MicroFlow® micronucleus analysis kit according to the manufacturer's instructions (6).

The treatment plates were removed from the incubator 23 hours after treatment and placed on ice for 20 min. The treatment media was aspirated and 300 µL per well of Nucleic Acid Dye A (ethidium monoazide, EMA) working solution was added. With the plate cover off, the cells (while remaining on ice) were exposed to visible light for 30 min to photoactivate EMA. This dye stains the nucleic acids of apoptotic and necrotic cells, while leaving viable cells unstained. The Nucleic Acid Dye A working solution was aspirated and the cells were washed with 1 mL/well cold 1x Buffer Solution. The cells were incubated in Complete Lysis Solution 1 (500 µL/well) for one hour protected from light. This solution contains Nucleic Acid Dye B (SYTOX green) which stains the nucleic acids of all cells (viable, apoptotic and necrotic) including the micronuclei. Following the 1-hour incubation, Complete Lysis Solution 2 containing Counting Beads was added (500 µL/well) and the cells were kept at room temperature for 30 min protected from light.

The stained samples were protected from light until analysis by flow cytometry. The samples were transferred to flow cytometry tubes and stored refrigerated for one (treated samples) to two (pre-treatment samples) days prior to analysis by flow cytometry. Samples were equilibrated to room temperature for approximately 30 min prior to analysis.

Samples were analyzed using a MACSQuant Analyzer 10 flow cytometer (Miltenyi Biotec) in accordance with standard operating procedures and the Litron *In Vitro* MicroFlow® micronucleus analysis kit manual (6). One negative and one positive control were used to set voltages, trigger and gates for the flow cytometer. All remaining samples were acquired with these settings. Each sample was mixed by the MACSQuant Analyzer 10 flow cytometer prior to acquisition, and 100 µL per sample was analyzed for the number of nuclei, beads, micronuclei and hypodiploid cells. The DNA from apoptotic and necrotic cells was excluded from the analysis due to double-staining by the Nucleic Acid Dyes A and B (EMA positive). The micronuclei, hypodiploid nuclei and normal chromatin of viable cells acquired by the flow cytometer were used to determine the proportion of micronuclei and hypodiploidy present in the samples, as well as the RI and cytotoxicity of treatment as per step 3.4.9. Micronuclei were scored in at least 2000 nuclei (at least 15,806 per replicate) for each non-cytotoxic test concentration and control.

[redacted]

3.4.9 Calculations

The replication increase (RI) of the negative control cultures was determined to ensure that the treated cells underwent mitosis:

$$No.of\ Nuclei\ at\ harvest\ \div\ no.of\ Nuclei\ at\ pretreatment$$

The Relative Increase in Cell (Nuclear) Counts (RICC) was calculated using the nuclei from pre- and post-treatment cultures and controls as follows:

$$RICC\ =\ \frac{Increase\ in\ no.Nuclei\ in\ treated\ cultures\ (final-starting)}{Increase\ in\ no.Nuclei\ in\ control\ cultures\ (final-starting)}\times\ 100$$

The frequency of micronuclei (MN) was determined by:

$$\%\ MN\ =\ \frac{Micronuclei\ count}{Nuclei\ count}\times\ 100$$

The frequency of hypodiploid cells (HD) was determined by:

$$\%\ HD\ =\ \frac{Hypodiploid\ count}{Nuclei\ count}\times\ 100$$

In some cases, determination of the amount of dead/apoptotic cells in treated cultures versus negative controls can aid in the determination of cytotoxicity and prevent false positive results caused by apoptotic bodies. The amount of dead/apoptotic cells was calculated as fold EMA because EMA stains the nucleic acids of apoptotic and necrotic cells, while leaving viable cells unstained (7).

$$Fold\ EMA\ =\ \frac{Mean\ \%\ Parent\ EMA\ of\ treated\ cultures}{Mean\ \%\ Parent\ EMA\ of\ control\ cultures}$$

4. DATA AND REPORTING

4.1 Data Analysis

Data for individual wells was acquired separately using the flow cytometer and calculations were performed as per step 3.4.9. Nuclei count, micronuclei count, hypodiploid count, bead count, % Parent EMA positive, frequency of hypodiploidy and frequency of micronuclei are provided for each sample. Summary tables include the RI for the negative controls, as well as the average RICC and frequency of micronuclei and hypodiploidy per treatment and control.

No positive response was observed for the test item. This is indicated by a mean %MN ≥ 3-fold (4) (8) (9) and/or a mean %HD ≥ 7-fold (4) (9) the concurrent negative control that was greater than the upper tolerance interval (mean + 3 standard deviations (SD)) of the negative control historical data for at least one non-cytotoxic concentration. Therefore, no further statistical analysis was performed.

Statistical analysis was performed for the positive responses obtained by the positive controls. The %MN and %HD (as applicable) were compared to the concurrent negative control by calculating z', a statistic developed for the large sample sizes associated with flow cytometric *in vitro* micronucleus data that is based on the mean and variance (SD) of each sample/control. Using this statistic, false positive results are avoided, as a positive response must exhibit an appropriate separation between the mean + SD of the test sample (t) compared to the mean + SD of the negative control (c) (10).

$$z' = (|mean_t - mean_c| - 3(SD_t + SD_c)) \; / \; |mean_t - mean_c|$$

z' > 0.5 was considered significant, however %MN and %HD were not evaluated for z' under conditions of extreme cytotoxicity, i.e. RICC < 40% (3).

4.2 Assay Acceptance Criteria

The study was evaluated using the following assay acceptance criteria:

- the frequency of micronuclei in the concurrent negative control should be consistent with the normal characteristic range of the historical negative control data; and
- the replication increase of the negative control should be ≥ 1.8 to demonstrate that a substantial proportion of the cells treated with the vehicle control underwent cell division; and

- the positive controls should induce mean %MN ≥ 3-fold the concurrent negative controls that are consistent with the normal characteristic range of the historical positive control data; and

- the test item should be evaluated in all three experimental conditions (3-6 hours with and without S9, and 18-24 hours without S9) unless one yields positive results; and

- depending on the nature of the test item, the maximum test concentration should reach the limit of cytotoxicity or solubility, but will not exceed 10 mM, 2 mg/mL or 2 µL/mL, whichever is the lowest. If the maximum concentration is based on cytotoxicity, the highest concentration will aim to achieve a RICC of 45 ± 5% of the concurrent negative control; and

- micronuclei should be scored in at least 2000 nuclei per test item concentration and control (3).

4.3 Evaluation and Interpretation of Results

The test item was considered to be clearly negative if, in all experimental conditions examined:

- none of the non-cytotoxic test concentrations exhibited an increase in micronuclei (≥ 3-fold) or hypodiploidy (≥ 7-fold) compared with the concurrent negative control; and

- all results were within the normal characteristic distribution of the historical negative control data (tolerance interval).

A negative result indicated that, under the test conditions, the test item did not induce chromosome breaks and/or gain or loss in cultured CHO cells.

The test item was considered to be clearly positive if, in any of the experimental conditions examined:

- at least one of the non-cytotoxic test concentrations exhibited an increase in micronuclei (≥ 3-fold) or hypodiploidy (≥ 7-fold) compared with the concurrent negative control; and

- any results were greater than the normal characteristic distribution of the historical negative control data (tolerance interval); and

- the increase was concentration-related when evaluated with an appropriate trend test.

When all of these criteria were met, the test item was considered capable of inducing chromosome breaks and/or gain or loss in cultured CHO cells. An equivocal result was considered if the results did not meet the criteria for a clearly negative or positive response, even after additional experiments.

In the event that the controls fell slightly outside the normal (historical) range, the Study Director was allowed discretion in accepting the results of the experiment as valid as long as these data were not extreme outliers or due to technical issues and that there was evidence that the test system was 'under control'. Biological relevance of the results was considered first.

5. RESULTS

5.1 Experiment 1 (Main Study)

5.1.1 Test Item Exposure Conditions

CHO cells were treated with Clo-IPE up to the limit of solubility using three treatment conditions, 4 hours in the absence and presence of metabolic activation (S9) and 23 hours in the absence of S9. Clo-IPE was evaluated at 750, 380, 190, 94, 47, 23, 12, 5.9, and 2.9 µg/mL. Concurrent negative/vehicle and positive controls were included in each treatment condition to verify the test system (Table 1 and Appendix III).

Extreme, moderate, and slight precipitate was observed following Clo-IPE treatment at 750, 380, and 190 µg/mL in the absence of S9, while moderate and slight precipitate was observed at 750 and 380 µg/mL in the presence of S9, respectively (Table 1). No change in pH (indicated by a change in treatment media colour) was observed following treatment with Clo-IPE for all treatment conditions. In addition, for the short-term treatments, the pH for the two highest concentrations of 750 and 380 µg/mL was measured to be 7.46 and 7.44 in the absence of S9 and 7.41 and 7.42 in the presence of S9, respectively. These results did not differ from the concurrent negative controls where the pH was measured to be 7.44 and 7.43 in the absence and presence of S9, respectively (Table 1). For the short-term treatments, the osmolality at 750 and 380 µg/mL was measured to be 444 and 459 mOsmo/kg in the absence of S9 and 456 and 475 mOsm/kg in the presence of S9, respectively. These results did not differ from the concurrent negative controls where the osmolality was measure to be 447 and 466 mOsm/kg in the absence and presence of S9, respectively (Table 1).

RICC, which is based on the number of nuclei present in the treated samples versus the negative control samples, is used as a measure of cytotoxicity. Dose-dependent cytotoxicity was observed in all treatment conditions following treatment with Clo-IPE (Table 1). RICC values ranged from 96 to -32% (4 hours in the absence of S9), 101 to -41% (4 hours in the presence of S9), and 88 to -31% (23 hours in the absence of S9). When the maximum test item concentration is based on cytotoxicity, the highest concentration for evaluation should aim to achieve an RICC of 45 ± 5% (3). Therefore, the highest non-cytotoxic concentration was 12, 190, and 5.9 µg/mL for all treatment conditions, with RICC values of 75, 90 and 61% for 4 hours in the absence and presence of S9, and 23 hours in the absence of S9, respectively. Although the RICC values were greater than the target of 45 ± 5%, the next highest concentrations of Clo-IPE yielded cytotoxic (<40%) RICC values of 34, -41 and 17% for 4 hours in the absence and presence of S9, and 23

hours in the absence of S9, respectively, demonstrating a steep dose response for each treatment condition. Because only two doses were available for the evaluation of micronuclei for the long-term treatment, this condition was repeated in Experiment 2 with a modified dose design. Because results were available for at least three Clo-IPE concentrations over a range of cytotoxicity in the short-term treatment conditions, the test item was evaluated in accordance with OECD guidelines up to the limit of cytotoxicity (3).

5.1.2 Test Item Micronuclei and Hypodiploidy Results

For the 4-hour treatment in the absence of S9, no dose-dependent increase in mean %MN ≥ 3-fold (0.75 to 0.81-fold) or mean %HD ≥ 7-fold (0.74 to 0.95-fold) the concurrent vehicle control was observed for any non-cytotoxic concentration of Clo-IPE (Table 1). The mean %MN values (ranging from 0.50 to 0.54) and mean %HD values (ranging from 0.28 to 0.36) were below the upper tolerance interval of the negative control historical data (1.02 for %MN and 0.52 for %HD) (Appendix IV). Therefore, the results were consistent with a negative response.

For the 4-hour treatment in the presence of S9, no dose-dependent increase in mean %MN ≥ 3-fold (0.76 to 0.97-fold) or mean %HD ≥ 7-fold (0.81 to 1.15-fold) the concurrent vehicle control was observed for any non-cytotoxic concentration of Clo-IPE (Table 1). The mean %MN values (ranging from 0.49 to 0.62) and mean %HD values (ranging from 0.26 to 0.37) were below the upper tolerance interval of the negative control historical data (1.18 for %MN and 0.48 for %HD) (Appendix IV). Therefore, the results were consistent with a negative response.

For the 23-hour treatment in the absence of S9, no dose-dependent increase in mean %MN ≥ 3-fold 0.40 to 0.73-fold) or mean %HD ≥ 7-fold (0.65 to 1.01-fold) the concurrent vehicle control was observed for any non-cytotoxic concentration of Clo-IPE (Table 1). The mean %MN values (ranging from 0.49 to 0.89) and mean %HD values (ranging from 0.35 to 0.55) were below the upper tolerance interval of the negative control historical data (1.86 for %MN and 0.73 for %HD) (Appendix IV). The results were consistent with a negative response but this condition was repeated in Experiment 2 as three doses were not available for evaluation in accordance with OECD guidelines (3).

In the absence of any indication of a positive response, no statistical analysis was performed for increase in %MN or %HD following Clo-IPE treatment for any treatment condition.

5.1.3 Negative and Positive Controls

The RI of the negative/vehicle controls in all treatment conditions ranged from 3.45 to 4.26, meeting the acceptance criteria (≥ 1.8) and demonstrating that a substantial proportion of negative control cells underwent cell division (Table 1). The %MN

and %HD values for the vehicle controls in all treatment conditions fell within the tolerance interval of the historical negative control data (Appendix IV) meeting acceptance criteria and indicating that the vehicle control was appropriate for testing.

At least one concentration of the positive controls for each condition met the acceptance criteria of producing increases in %MN that were ≥ 3-fold when compared with the concurrent vehicle controls and that fell within the normal characteristic range of the historical positive control data (Table 1 and Appendix III). Consistent with its mode of action (aneugen), COL induced increases in %HD that were ≥ 7-fold when compared with the concurrent vehicle control and that fell within the normal characteristic range of the historical positive control data (Table 1 and Appendix III). The positive controls produced statistically significant results ($z' > 0.5$) with the exception of %MN for CP at 4.0 µg/mL which demonstrated slightly higher treatment-induced variability (Appendix III). Because the other concentration of CP (2.0 µg/mL) met the study acceptance criteria for positive controls and the increase was statistically significant, the lack of statistical significance for CP at 4.0 µg/mL had no adverse impact on the outcome of the study or the interpretation of results.

The negative and positive control results met all acceptance criteria for a valid assay.

5.2 Experiment 2 (Main Study – 23 Hour Treatment)

5.2.1 Test Item Exposure Conditions

CHO cells were treated with Clo-IPE up to the limit of cytotoxicity for 23 hours in the absence of S9. Clo-IPE was tested at 30, 15, 7.5, 3.8, 1.9, 0.94, 0.47, and 0.23 µg/mL. Concurrent negative/vehicle and positive controls were included to verify the test system (Table 2 and Appendix III).

No precipitate was observed following treatment with Clo-IPE (Table 2). No change in pH (indicated by a change in treatment media colour) was observed following treatment with Clo-IPE for all treatment conditions. As per study plan, the measurement of the pH and osmolality of the treatment medium was not required for the long-term treatment.

Dose-dependent cytotoxicity was observed in all treatment conditions following treatment with Clo-IPE (Table 2). RICC values ranged from 102 to -25%. When the maximum test item concentration is based on cytotoxicity, the highest concentration for evaluation should aim to achieve an RICC of 45 ± 5% (3). Therefore, the highest non-cytotoxic concentration was 7.5 µg/mL with an RICC value of 59%. Although the RICC values was slightly greater than the target of 45 ± 5%, the next highest concentration of Clo-IPE yielded a cytotoxic (<40%) RICC

value of 13%, demonstrating a steep dose response. Because results were available for at least three Clo-IPE concentrations over a range of cytotoxicity in the long-term treatment condition, the test item was evaluated in accordance with OECD guidelines up to the limit of cytotoxicity (3).

5.2.2 Test Item Micronuclei and Hypodiploidy Results

For the 23-hour treatment in the absence of S9, no dose-dependent increase in mean %MN ≥ 3-fold 0.99 to 1.43-fold) or mean %HD ≥ 7-fold (1.03 to 1.24-fold) the concurrent vehicle control was observed for any non-cytotoxic concentration of Clo-IPE (Table 2). The mean %MN values (ranging from 0.90 to 1.35) and mean %HD values (ranging from 0.40 to 0.48) were below the upper tolerance interval of the negative control historical data (1.86 for %MN and 0.73 for %HD) (Appendix IV). Therefore, the results were consistent with a negative response.

In the absence of any indication of a positive response, no statistical analysis was performed for increase in %MN or %HD following Clo-IPE treatment.

5.2.3 Negative and Positive Controls

The RI of the negative/vehicle controls was 4.67, meeting the acceptance criteria (≥ 1.8) and demonstrating that a substantial proportion of negative control cells underwent cell division (Table 2). The %MN and %HD values for the vehicle controls fell within the tolerance interval of the historical negative control data (Appendix IV) meeting acceptance criteria and indicating that the vehicle control was appropriate for testing.

All concentrations of the positive controls met the acceptance criteria of producing increases in %MN that were ≥ 3-fold when compared with the concurrent vehicle controls and that fell within the normal characteristic range of the historical positive control data (Table 2 and Appendix III). Consistent with its mode of action (aneugen), COL induced increases in %HD that were ≥ 7-fold when compared with the concurrent vehicle control and that fell within the normal characteristic range of the historical positive control data (Table 2 and Appendix III). The positive controls produced statistically significant results ($z' > 0.5$) (Appendix III).

The negative and positive control results met all acceptance criteria for a valid assay.

6. DISCUSSION

6.1 Assay Acceptance Criteria

The RI of the negative controls met the acceptance criteria of being ≥ 1.8 demonstrating that a substantial proportion of the negative control cells underwent cell division. The frequencies of MN and HD for the concurrent negative controls were within the historical negative control data. At least one concentration of the positive controls for each treatment condition produced an increase in %MN that was ≥ 3-fold when compared with the concurrent negative controls and that fell within the normal characteristic range of the historical positive control data. Consistent with its mode of action (aneugen), COL induced increases in %HD that were ≥ 7-fold when compared with the concurrent negative control and that fell within the normal characteristic range of the historical positive control data.

The test item, Clo-IPE, was evaluated up to the limit of cytotoxicity using three treatment conditions, 4 hours in the absence and presence of S9 (Experiment 1) and 23 hours in the absence of S9 (Experiment 2). At least three analyzable concentrations of the test item were available for each treatment condition, and micronuclei were scored in at least 2000 nuclei per non-cytotoxic test item concentration and control (≥ 15,806 per replicate). Therefore, all assay acceptance criteria were met and Clo-IPE was tested with a sufficient number of concentrations up to the limit of cytotoxicity in accordance with OECD guidelines (3).

6.2 Test Item Genotoxicity

In all experimental conditions, no non-cytotoxic concentration of the test item exhibited an increase in micronuclei (≥ 3-fold) or hypodiploidy (≥ 7-fold) compared with the concurrent negative control, and all results were within the range of the laboratory historical negative control data. Therefore, Clo-IPE was considered to be negative (not genotoxic), i.e. the test item did not induce chromosome breaks and/or gain or loss in cultured CHO cells.

7. CONCLUSION

The test item, Clo-IPE, did not induce micronuclei (via chromosome breaks or loss of whole chromosome(s)) or hypodiploidy (chromosome loss, sub-2n nuclei) in cultured CHO cells in the absence (4 and 23hours treatment) and presence (4 hours treatment) of metabolic activation when tested up to the limit of cytotoxicity, under the conditions of the test.

8. ARCHIVE

The original copy of the study plan, all raw data that were generated at Nucro-Technics, and a copy of the final report will be stored in Nucro-Technics' archives for 5 years. After 5 years, the Sponsor will be notified. As per the Sponsor's instructions (to be submitted in writing), records will be disposed of, retained for an additional fee or returned to the Sponsor.

Health Canada recommends that Sponsors store all documentation, study plan, raw data and final report of each study in support of an approved drug product for a minimum of 10 years (from the market notification date) and a longer period when required by the Food and Drug Regulations (11).

9. REFERENCES

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4) Bryce, S.M., Avlasevich, S.L., Bemis, J.C. and Dertinger, S.D. Miniaturized flow cytometry-based CHO-K1 micronucleus assay discriminates aneugenic and clastogenic modes of action. Environmental and Molecular Mutagenesis 52:280-286, 2011.

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10) Wojciechowski, J.P., Gleason, C.R., Roberts, D.J. and Custer, L.L. Novel statistical approach for evaluating flow cytometric *in vitro* micronucleus data. Environmental and Molecular Mutagenesis 57:623-629, 2016.

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Table 1: Results Summary - Experiment 1

Treatment	Dose (µg/mL)	Ppt	pH	Osm	Mean Nuclei	RI	Increase in Nuclei	RICC	% HD			% MN		
									Mean	z'	Fold Increase	Mean	z'	Fold Increase
Pre-treatment	-	-	-	-	7869	-	-	-	-	-	-	-	-	-
4 hours - S9														
Ethanol	0	NP	7.44	447	32211	4.09	24342	100	0.39	-	1.00	0.67	-	1.00
Clo-IPE	2.9	NP	-	-	31266	-	23397	96	0.36	-	0.95	0.54	-	0.81
	5.9	NP	-	-	28952	-	21083	87	0.28	-	0.74	0.50	-	0.75
	12	NP	-	-	26056	-	18187	75	0.31	-	0.79	0.54	-	0.81
	23	NP	-	-	16162	-	8293	34	1.32	-	3.42	1.43	-	2.15
	47	NP	-	-	18	-	-7851	-32	2.17	-	5.65	32.78	-	49.23
	94	NP	-	-	4	-	-7865	-32	0.00	-	0.00	10.00	-	15.02
	190[a]	SP	-	-	-	-	-	-	-	-	-	-	-	-
	380[a]	MP	7.44	459	-	-	-	-	-	-	-	-	-	-
	750[a]	EP	7.46	444	-	-	-	-	-	-	-	-	-	-
MMC	0.20	NP	-	-	24562	-	16693	69	0.56	-	1.46	3.32	0.92	4.99
	0.25	NP	-	-	23011	-	15142	62	0.76	-	1.99	4.74	0.65	7.12

Ethanol: Ethanol (100%)
MMC: Mitomycin C
Ppt: Precipitate (NP: None, SP: Slight, MP: Moderate, EP: Extreme)
Osm: Osmolality (mOsm/kg)
RI: Replication Increase = No. Nuclei at Harvest / No. Nuclei at Pre-treatment
Increase in Nuclei = Nuclei at Harvest - Nuclei at Pre-treatment
RICC: Relative Increase in Cell (Nuclear) Counts = Increase in Nuclei (treatment) / Increase in Nuclei (control) × 100
RICC < 40% is cytotoxic; %MN and %HD not biologically relevant in this case (greyed out)
HD: Hypodiploid
MN: Micronuclei
%HD = Hypodiploid count / Nuclei count × 100
%MN = Micronuclei count / Nuclei count × 100
%HD Fold Increase = Mean %HD of treatment / Mean %HD of negative control
%MN Fold Increase = Mean %MN of treatment / Mean %MN of negative control
SD: Standard deviation
$z' = (|mean_t\text{-}mean_c| - 3(SD_t + SD_c)) / |mean_t\text{-}mean_c|$
z' > 0.5 is signficant (only calculated for non-cytotoxic doses with %HD Fold Increase ≥ 7 or %MN Fold Increase ≥ 3 and %HD or %MN > tolerance interval of negative control historical data)
No change in media colour was observed for any treatment indicating no pH change
a: Samples not assessed by flow cytometry due to overt cytotoxicity
"-": Not assessed

Table 1: Results Summary - Experiment 1 (continued)

Treatment	Dose (µg/mL)	Ppt	pH	Osm	Mean Nuclei	RI	Increase in Nuclei	RICC	% HD			% MN		
									Mean	z'	Fold Increase	Mean	z'	Fold Increase
4 hours + S9														
Ethanol	0	NP	7.43	466	27182	3.45	19313	100	0.32	-	1.00	0.65	-	1.00
Clo-IPE	2.9	NP	-	-	27356	-	19487	101	0.29	-	0.90	0.61	-	0.95
	5.9	NP	-	-	26845	-	18976	98	0.30	-	0.91	0.50	-	0.77
	12	NP	-	-	27144	-	19275	100	0.26	-	0.81	0.52	-	0.81
	23	NP	-	-	26762	-	18893	98	0.30	-	0.93	0.49	-	0.76
	47	NP	-	-	26068	-	18199	94	0.27	-	0.84	0.55	-	0.86
	94	NP	-	-	25441	-	17572	91	0.37	-	1.15	0.62	-	0.97
	190	SP	-	-	25216	-	17347	90	0.32	-	0.97	0.50	-	0.78
	380	SP	7.42	475	2	-	-7867	-41	0.00	-	0.00	75.00	-	116.13
	750	MP	7.41	456	0	-	-7869	-41	0.00	-	0.00	0.00	-	0.00
CP	2.0	NP	-	-	18535	-	10666	55	0.55	-	1.69	2.81	0.57	4.35
	4.0	NP	-	-	16237	-	8368	43	1.12	-	3.47	5.72	0.42	8.86

Ethanol: Ethanol (100%)
CP: Cyclophosphamide
Ppt: Precipitate (NP: None, SP: Slight, MP: Moderate, EP: Extreme)
Osm: Osmolality (mOsm/kg)
RI: Replication Increase = No. Nuclei at Harvest / No. Nuclei at Pre-treatment
Increase in Nuclei = Nuclei at Harvest - Nuclei at Pre-treatment
RICC: Relative Increase in Cell (Nuclear) Counts = Increase in Nuclei (treatment) / Increase in Nuclei (control) × 100
RICC < 40% is cytotoxic; %MN and %HD not biologically relevant in this case (greyed out)
HD: Hypodiploid
MN: Micronuclei
%HD = Hypodiploid count / Nuclei count × 100
%MN = Micronuclei count / Nuclei count × 100
%HD Fold Increase = Mean %HD of treatment / Mean %HD of negative control
%MN Fold Increase = Mean %MN of treatment / Mean %MN of negative control
SD: Standard deviation
$z' = (|mean_t\text{-}mean_c| - 3(SD_t + SD_c)) / |mean_t\text{-}mean_c|$
z' > 0.5 is signficant (only calculated for non-cytotoxic doses with %HD Fold Increase ≥ 7 or %MN Fold Increase ≥ 3 and %HD or %MN > tolerance interval of negative control historical data)
No change in media colour was observed for any treatment indicating no pH change
"-": Not assessed

Table 1: Results Summary - Experiment 1 (continued)

Treatment	Dose (µg/mL)	Ppt	pH	Osm	Mean Nuclei	RI	Increase in Nuclei	RICC	% HD			% MN		
									Mean	z'	Fold Increase	Mean	z'	Fold Increase
23 hours - S9														
Ethanol	0	NP	-	-	33525	4.26	25656	100	0.54	-	1.00	1.22	-	1.00
Clo-IPE	2.9	NP	-	-	30377	-	22508	88	0.55	-	1.01	0.89	-	0.73
	5.9	NP	-	-	23584	-	15715	61	0.35	-	0.65	0.49	-	0.40
	12	NP	-	-	12131	-	4262	17	0.33	-	0.62	0.50	-	0.41
	23	NP	-	-	6377	-	-1492	-6	0.27	-	0.50	0.43	-	0.35
	47	NP	-	-	0	-	-7869	-31	0.00	-	0.00	0.00	-	0.00
	94	NP	-	-	1	-	-7868	-31	0.00	-	0.00	0.00	-	0.00
	190[a]	SP	-	-	-	-	-	-	-	-	-	-	-	-
	380[a]	MP	-	-	-	-	-	-	-	-	-	-	-	-
	750[a]	EP	-	-	-	-	-	-	-	-	-	-	-	-
COL	0.15	NP	-	-	24113	-	16244	63	4.65	0.72	8.64	2.97	-	2.43
	0.18	NP	-	-	20792	-	12923	50	8.06	0.94	14.97	5.93	0.73	4.85

Ethanol: Ethanol (100%)
COL: Colchicine
Ppt: Precipitate (NP: None, SP: Slight, MP: Moderate, EP: Extreme)
Osm: Osmolality (mOsm/kg)
RI: Replication Increase = No. Nuclei at Harvest / No. Nuclei at Pre-treatment
Increase in Nuclei = Nuclei at Harvest - Nuclei at Pre-treatment
RICC: Relative Increase in Cell (Nuclear) Counts = Increase in Nuclei (treatment) / Increase in Nuclei (control) × 100
RICC < 40% is cytotoxic; %MN and %HD not biologically relevant in this case (greyed out)
HD: Hypodiploid
MN: Micronuclei
%HD = Hypodiploid count / Nuclei count × 100
%MN = Micronuclei count / Nuclei count × 100
%HD Fold Increase = Mean %HD of treatment / Mean %HD of negative control
%MN Fold Increase = Mean %MN of treatment / Mean %MN of negative control
SD: Standard deviation
$z' = (|mean_t-mean_c| - 3(SD_t + SD_c)) / |mean_t-mean_c|$
z' > 0.5 is signficant (only calculated for non-cytotoxic doses with %HD Fold Increase ≥ 7 or %MN Fold Increase ≥ 3 and %HD or %MN > tolerance interval of negative control historical data)
No change in media colour was observed for any treatment indicating no pH change
a: Samples not assessed by flow cytometry due to overt cytotoxicity
"-": Not assessed

Table 2: Results Summary - Experiment 2

Treatment	Dose (µg/mL)	Ppt	Mean Nuclei	RI	Increase in Nuclei	RICC	% HD			% MN		
							Mean	z'	Fold Increase	Mean	z'	Fold Increase
Pre-treatment	-	-	6231	-	-	-	-	-	-	-	-	-
					23 hours - S9							
Ethanol	0	NP	29109	4.67	22878	100	0.39	-	1.00	0.91	-	1.00
Clo-IPE	0.23	NP	29107	-	22876	100	0.45	-	1.15	0.90	-	0.99
	0.47	NP	29623	-	23392	102	0.45	-	1.16	0.92	-	1.02
	0.94	NP	28599	-	22368	98	0.42	-	1.07	0.92	-	1.01
	1.9	NP	27748	-	21517	94	0.48	-	1.24	1.30	-	1.43
	3.8	NP	26098	-	19867	87	0.47	-	1.21	1.35	-	1.48
	7.5	NP	19764	-	13533	59	0.40	-	1.03	0.95	-	1.04
	15	NP	9241	-	3010	13	0.27	-	0.70	0.45	-	0.50
	30	NP	458	-	-5773	-25	0.22	-	0.58	0.41	-	0.45
COL	0.15	NP	22269	-	16038	70	10.15	0.90	26.11	7.03	0.69	7.74
	0.18	NP	18551	-	12320	54	15.30	0.91	39.36	14.12	0.78	15.54

Ethanol: Ethanol (100%)
COL: Colchicine
Ppt: Precipitate (NP: None, SP: Slight, MP: Moderate, EP: Extreme)
RI: Replication Increase = No. Nuclei at Harvest / No. Nuclei at Pre-treatment
Increase in Nuclei = Nuclei at Harvest - Nuclei at Pre-treatment
RICC: Relative Increase in Cell (Nuclear) Counts = Increase in Nuclei (treatment) / Increase in Nuclei (control) × 100
RICC < 40% is cytotoxic; %MN and %HD not biologically relevant in this case (greyed out)
HD: Hypodiploid
MN: Micronuclei
%HD = Hypodiploid count / Nuclei count × 100
%MN = Micronuclei count / Nuclei count × 100
%HD Fold Increase = Mean %HD of treatment / Mean %HD of negative control
%MN Fold Increase = Mean %MN of treatment / Mean %MN of negative control
SD: Standard deviation
$z' = (|mean_t\text{-}mean_c| - 3(SD_t + SD_c)) / |mean_t\text{-}mean_c|$
z' > 0.5 is signficant (only calculated for non-cytotoxic doses with %HD Fold Increase ≥ 7 or %MN Fold Increase ≥ 3 and %HD or %MN > tolerance interval of negative control historical data)
No change in media colour was observed for any treatment indicating no pH change
"-": Not assessed

STUDY-SPECIFIC SUPPLEMENT

STUDY-SPECIFIC SUPPLEMENT NO. 1

STUDY PLAN NO. LAS/403393

The following study-specific details are being added to Study Plan No. LAS/403393:

Experiment: 1

Solvent/Vehicle: Ethanol (100%)

Concentration Interval 2-fold

Dose volume: 10 µL/mL

Test Item Dose Design: Designed to reach the limit of solubility in the test system

Test Item Concentrations:

Target Formulation Concentrations (mg/mL) All treatment conditions ± S9	Target Final Concentrations (mg/mL) All treatment conditions ± S9
75[1]	0.75
38	0.38
19	0.19
9.4	0.094
4.7	0.047
2.3	0.023
1.2	0.012
0.59	0.0059
0.29	0.0029

Osmolality and pH: Osmolality and pH measurements will be performed for 4 hours with and without S9 (short-term treatment) on the two highest test item concentrations and the vehicle control.

---

[1] The stock solution was prepared by the Sponsor in ethanol at 10% w/w (approximately 75 mg/mL; as stated on the Certificate of Analysis).

STUDY-SPECIFIC SUPPLEMENT

STUDY-SPECIFIC SUPPLEMENT NO. 1

STUDY PLAN NO. LAS/403393

Approved by:

[redacted] _____ February 12, 2024

Study Director
Nucro-Technics

Date

Reviewed by:

[redacted] _____ Feb 12, 2024

Quality Assurance Associate
Nucro-Technics

Date

STUDY-SPECIFIC SUPPLEMENT

STUDY-SPECIFIC SUPPLEMENT NO. 2

STUDY PLAN NO. LAS/403393

The following study-specific details are being added to Study Plan No. LAS/403393:

Experiment: 2

Solvent/Vehicle: Ethanol (100%)

Concentration Interval 2-fold

Dose volume: 10 µL/mL

Test Item Dose Design: Designed to reach the limit of cytotoxicity in the test system with at least three analyzable non-cytotoxic concentrations, which was not achieved in Experiment 1.

Test Item Concentrations:

Target Formulation Concentrations (mg/mL) 18 to 24 hours -S9	Target Final Concentrations (µg/mL) 18 to 24 hours -S9
75[1]	Not treated
15	Not treated
3.0	30
1.5	15
0.75	7.5
0.38	3.8
0.19	1.9
0.094	0.94
0.047	0.47
0.023	0.23

Osmolality and pH: Osmolality and pH measurements will not be performed. Treatment medium will be examined for changes in pH (colour change of treatment medium containing phenol red).

---

[1] The stock solution was prepared by the Sponsor in ethanol at 10% w/w (approximately 75 mg/mL; as stated on the Certificate of Analysis).

STUDY-SPECIFIC SUPPLEMENT

STUDY-SPECIFIC SUPPLEMENT NO. 2

STUDY PLAN NO. LAS/403393

Approved by:

[redacted] _____ 03-01-24

Study Director
Nucro-Technics

Date

Reviewed by:

[redacted] _____ Mar 04, 2024

Quality Assurance Associate
Nucro-Technics

Date

Appendix III: Individual Results - Experiment 1

Treatment	Dose (µg/mL)	Nuclei count	Bead count	HD count	MN count	EMA (%-#)	Mean EMA	Fold EMA	%MN	%HD
Pre-treatment	-	8583	2102	23	52	0.07	0.08	-	0.61	0.27
		7496	1870	22	37	0.04			0.49	0.29
		7766	2031	20	45	0.10			0.58	0.26
		7630	2533	23	53	0.12			0.69	0.30
				4 hours - S9						
Ethanol	0	32349	3661	120	228	0.05	0.06	1.00	0.70	0.37
		32072	3669	128	201	0.07			0.63	0.40
Clo-IPE	2.9	31173	3548	113	170	0.05	0.06	0.92	0.55	0.36
		31358	3653	115	166	0.06			0.53	0.37
	5.9	28888	3558	83	147	0.04	0.04	0.58	0.51	0.29
		29016	3552	81	142	0.03			0.49	0.28
	12	26626	3573	75	133	0.04	0.05	0.75	0.50	0.28
		25485	3455	84	149	0.05			0.58	0.33
	23	15597	3368	215	233	0.11	0.12	2.00	1.49	1.38
		16727	3596	210	230	0.13			1.38	1.26
	47	13	2841	0	4	73.02	65.26	1087.67	30.77	0.00
		23	2770	1	8	57.50			34.78	4.35
	94	5	2805	0	1	57.14	70.24	1170.58	20.00	0.00
		2	3103	0	0	83.33			0.00	0.00
	190[a]	-	-	-	-	-	-	-	-	-
		-	-	-	-	-			-	-
	380[a]	-	-	-	-	-	-	-	-	-
		-	-	-	-	-			-	-
	750[a]	-	-	-	-	-	-	-	-	-
		-	-	-	-	-			-	-
MMC	0.20	24498	3656	144	810	0.21	0.22	3.58	3.31	0.59
		24626	4078	133	821	0.22			3.33	0.54
	0.25	22976	3679	162	1021	0.22	0.24	4.00	4.44	0.71
		23046	3690	190	1160	0.26			5.03	0.82

Ethanol: Ethanol (100%)
MMC: Mitomycin C
MN: Micronuclei
HD: Hypodiploid
EMA: Ethidium monoazide
%-#: % Parent (Flow Cytometry Parameter)
%MN = Micronuclei count / Nuclei count × 100
%HD = Hypodiploid count / Nuclei count × 100
Fold EMA = Mean % Parent EMA (test) / Mean % Parent EMA (control) (Not determined when Mean EMA (control) = 0.00)
Fold EMA > 10 is indicative of cytotoxicity
a: Samples not assessed by flow cytometry due to overt cytotoxicity
"-": Not assessed/determined

Appendix III: Individual Results - Experiment 1 (continued)

Treatment	Dose (µg/mL)	Nuclei count	Bead count	HD count	MN count	EMA (%-#)	Mean EMA	Fold EMA	%MN	%HD
				4 hours + S9						
Ethanol	0	27332	4171	78	168	0.12	0.11	1.00	0.61	0.29
		27032	4001	98	183	0.10			0.68	0.36
Clo-IPE	2.9	25274	4124	56	146	0.10	0.10	0.86	0.58	0.22
		29438	4095	107	191	0.09			0.65	0.36
	5.9	26225	4012	59	113	0.09	0.08	0.68	0.43	0.22
		27465	4059	101	156	0.06			0.57	0.37
	12	26372	4034	59	127	0.09	0.08	0.73	0.48	0.22
		27916	4040	84	157	0.07			0.56	0.30
	23	26370	4245	79	118	0.09	0.08	0.68	0.45	0.30
		27153	4055	83	146	0.06			0.54	0.31
	47	25798	4175	67	136	0.07	0.10	0.91	0.53	0.26
		26338	4067	75	153	0.13			0.58	0.28
	94	26024	3262	99	173	0.08	0.10	0.91	0.66	0.38
		24857	3323	90	145	0.12			0.58	0.36
	190	25502	3230	68	136	0.10	0.11	0.95	0.53	0.27
		24929	3335	91	117	0.11			0.47	0.37
	380	2	2909	0	0	91.67	87.27	793.32	0.00	0.00
		2	3221	0	3	82.86			150.00	0.00
	750	0	2931	0	0	100.00	96.16	874.14	0.00	0.00
		0	3025	0	0	92.31			0.00	0.00
CP	2.0	17937	3122	104	537	0.19	0.20	1.82	2.99	0.58
		19133	3145	98	501	0.21			2.62	0.51
	4.0	16667	3365	185	843	0.28	0.32	2.86	5.06	1.11
		15806	2732	180	1009	0.35			6.38	1.14

Ethanol: Ethanol (100%)
CP: Cyclophosphamide
MN: Micronuclei
HD: Hypodiploid
EMA: Ethidium monoazide
%-#: % Parent (Flow Cytometry Parameter)
%MN = Micronuclei count / Nuclei count × 100
%HD = Hypodiploid count / Nuclei count × 100
Fold EMA = Mean % Parent EMA (test) / Mean % Parent EMA (control) (Not determined when Mean EMA (control) = 0.00)
Fold EMA > 10 is indicative of cytotoxicity
"-": Not assessed/determined

Appendix III: Individual Results - Experiment 1 (continued)

Treatment	Dose (µg/mL)	Nuclei count	Bead count	HD count	MN count	EMA (%-#)	Mean EMA	Fold EMA	%MN	%HD
				23 hours - S9						
Ethanol	0	33637	4180	185	383	0.10	0.10	1.00	1.14	0.55
		33413	4109	176	436	0.10			1.30	0.53
Clo-IPE	2.9	30689	4176	161	216	0.06	0.07	0.65	0.70	0.52
		30064	4037	170	326	0.07			1.08	0.57
	5.9	23694	3979	81	95	0.06	0.06	0.55	0.40	0.34
		23473	4152	85	137	0.05			0.58	0.36
	12	12721	3817	41	61	0.04	0.05	0.45	0.48	0.32
		11540	3786	40	61	0.05			0.53	0.35
	23	6376	3506	20	19	0.14	0.15	1.45	0.30	0.31
		6378	3594	14	36	0.15			0.56	0.22
	47	0	2942	0	1	75.00	87.50	875.00	0.00	0.00
		0	2947	0	0	100.00			0.00	0.00
	94	1	3151	0	0	50.00	58.34	583.35	0.00	0.00
		1	2982	0	0	66.67			0.00	0.00
	190[a]	-	-	-	-	-	-	-	-	-
		-	-	-	-	-			-	-
	380[a]	-	-	-	-	-	-	-	-	-
		-	-	-	-	-			-	-
	750[a]	-	-	-	-	-	-	-	-	-
		-	-	-	-	-			-	-
COL	0.15	23347	3895	1026	659	0.15	0.18	1.75	2.82	4.39
		24878	3985	1222	778	0.20			3.13	4.91
	0.18	20975	4042	1710	1289	0.20	0.24	2.40	6.15	8.15
		20608	3981	1641	1176	0.28			5.71	7.96

Ethanol: Ethanol (100%)
COL: Colchicine
MN: Micronuclei
HD: Hypodiploid
EMA: Ethidium monoazide
%-#: % Parent (Flow Cytometry Parameter)
%MN = Micronuclei count / Nuclei count × 100
%HD = Hypodiploid count / Nuclei count × 100
Fold EMA = Mean % Parent EMA (test) / Mean % Parent EMA (control) (Not determined when Mean EMA (control) = 0.00)
Fold EMA > 10 is indicative of cytotoxicity
a: Samples not assessed by flow cytometry due to overt cytotoxicity
"-": Not assessed/determined

Appendix III: Individual Results - Experiment 2

Treatment	Dose (µg/mL)	Nuclei count	Bead count	HD count	MN count	EMA (%-#)	Mean EMA	Fold EMA	%MN	%HD
Pre-treatment	-	5620	2246	7	26	0.09	0.08	-	0.46	0.12
		6942	2656	21	37	0.10			0.53	0.30
		5739	2596	16	23	0.02			0.40	0.28
		6622	2924	17	24	0.10			0.36	0.26
				23 hours - S9						
Ethanol	0	29480	4707	103	272	0.08	0.08	1.00	0.92	0.35
		28737	4439	123	257	0.08			0.89	0.43
Clo-IPE	0.23	29801	4581	128	291	0.05	0.07	0.88	0.98	0.43
		28413	4616	131	232	0.09			0.82	0.46
	0.47	29928	4507	138	310	0.06	0.06	0.75	1.04	0.46
		29317	4539	130	238	0.06			0.81	0.44
	0.94	28324	4544	121	311	0.06	0.06	0.75	1.10	0.43
		28874	4539	117	215	0.06			0.74	0.41
	1.9	27514	4569	157	467	0.12	0.09	1.06	1.70	0.57
		27981	4694	111	251	0.05			0.90	0.40
	3.8	26737	4661	149	405	0.13	0.12	1.44	1.51	0.56
		25459	4554	97	300	0.10			1.18	0.38
	7.5	19729	4485	83	142	0.07	0.09	1.06	0.72	0.42
		19799	4532	76	232	0.10			1.17	0.38
	15	10012	4454	19	41	0.06	0.12	1.44	0.41	0.19
		8470	4135	30	42	0.17			0.50	0.35
	30	529	3543	1	3	3.96	4.54	56.75	0.57	0.19
		386	3442	1	1	5.12			0.26	0.26
COL	0.15	22648	4401	2257	1495	0.21	0.28	3.44	6.60	9.97
		21890	4586	2262	1632	0.34			7.46	10.33
	0.18	18349	4679	2860	2470	0.53	0.56	7.00	13.46	15.59
		18753	4974	2815	2771	0.59			14.78	15.01

Ethanol: Ethanol (100%)
COL: Colchicine
MN: Micronuclei
HD: Hypodiploid
EMA: Ethidium monoazide
%-#: % Parent (Flow Cytometry Parameter)
%MN = Micronuclei count / Nuclei count × 100
%HD = Hypodiploid count / Nuclei count × 100
Fold EMA = Mean % Parent EMA (test) / Mean % Parent EMA (control) (Not determined when Mean EMA (control) = 0.00)
Fold EMA > 10 is indicative of cytotoxicity
"-": Not assessed/determined

Appendix IV: Historical Control Data

	3-6 Hours -S9	3-6 Hours +S9	18-24 Hours -S9
		Negative Controls - % MN	
Mean	0.50	0.61	0.70
SD	0.17	0.19	0.39
Min	0.22	0.29	0.24
Max	1.31	1.18	2.54
LTI	0.00	0.03	0.00
UTI	1.02	1.18	1.86
N	133	137	144
		Positive Controls - % MN	
	MMC	CP	COL
Mean	5.42	4.39	7.58
SD	2.82	2.05	3.88
Min	1.04	1.51	2.32
Max	13.95	10.67	19.05
LTI	0.00	0.00	0.00
UTI	13.88	10.52	19.24
N	164	172	129
		Negative Controls - % HD	
Mean	0.22	0.23	0.29
SD	0.10	0.08	0.15
Min	0.07	0.05	0.07
Max	0.76	0.52	0.81
LTI	0.00	0.00	0.00
UTI	0.52	0.48	0.73
N	131	133	142
		Positive Controls - % HD	
	MMC	CP	COL
Mean	-	-	8.08
SD	-	-	2.88
Min	-	-	0.67
Max	-	-	15.94
LTI	-	-	0.00
UTI	-	-	16.73
N	-	-	143

Historical data collected from August 2018 to November 2023

MN: Micronuclei

HD: Hypodiploid

SD: Standard deviation

LTI: Lower tolerance interval = Mean - (3 x SD) or 0, whichever is greater

UTI: Upper tolerance interval = Mean + (3 x SD)

N: Number of samples

Min: Minimum value

Max: Maximum value

MMC: Mitomycin C

CP: Cyclophosphamide

COL: Colchicine

"-": Not applicable

Submission To: CIR	Submitted By: COMPANY via EAS Consulting Group
April 29, 2024	Roadmap for **Ethyl Tafluprostamide** / **DDDE**

Delivered via email

From: John Bailey, PhD
EAS Consulting Group

To: Bart Heldreth, PhD
Executive Director
Cosmetic Ingredient Review
Washington, DC

April 29, 2024

Re: Roadmap for Ethyl Tafluprostamide/DDDE and request for extension to respond to the IDA

Dear Bart,

As you know, at its December 2023 meeting, the CIR issued an IDA for Ethyl Tafluprostamide/DDDE.

On behalf of my client, I am pleased to provide to the CIR a roadmap that sets out my client's plan for obtaining the information specified in the IDA. The roadmap includes testing to obtain confirmatory, bridging data with read-across analogs and mechanistic information on downstream targets of DDDE. The roadmap also includes an assessment of the potential effect of geminal flourines, an issue that was raised in the discussion at the CIR meeting but was not included in the IDA.

The roadmap provided below includes my client's good faith estimates of completion dates based on information ToxMinds has received from the CROs with whom they are working to conduct the necessary testing. Based on the timelines provided by the CROs, my client anticipates completion of the testing by the end of September 2024 and submission of a Supplemental Report to the CIR in October – November 2024. Therefore, we are requesting that the CIR move consideration of prostaglandin analogs to its **December 2024** meeting.

Action/Test	**Responsible Party**	**Completion Date** ***Status – Estimated Completion Date***
Review and develop a scientifically robust strategy to address the insufficient data announcement (IDA) from CIR for: • acute toxicity data • repeated dose toxicity data • developmental and reproductive toxicity data • in vivo genotoxicity data Additionally, • confirmatory data (e.g., receptor interaction studies and downstream profiles of adverse effects) to support read across • mechanistic information on possible targets	ToxMinds	**Completed**
Selection of experienced and accredited CROs to conduct testing to respond to the IDA	ToxMinds	**Completed**

Submission To: CIR	Submitted By: COMPANY via EAS Consulting Group
April 29, 2024	Roadmap for **Ethyl Tafluprostamide** / **DDDE**

Action/Test	Responsible Party	Completion Date *Status – Estimated Completion Date*
Obtain confirmatory data (e.g. receptor interaction studies and downstream profiles of adverse effects) to support read-across		
In vitro testing to determine the potency of Ethyl Tafluprostamide/DDDE, Tafluprost and Bimatoprost to bind the prostanoid (PGF2alpha) receptor	Eurofins	**CRO selected**; *sample preparation ongoing; testing time – 4-8 weeks*
Evaluate feasibility of conducting *in vitro* testing to determine the potency of Ethyl Tafluprostamide / DDDE, Tafluprost, and Bimatoprost to bind prostamide receptors	ToxMinds	**Completed**; no assays with isolated prostamide receptors are available, testing is not feasible
Acute toxicity – *In vitro* neutral red uptake (NRU) assay (OECD GD 129) with Ethyl Tafluprostamide/DDDE, Tafluprost, Bimatoprost	Charles River	**CRO selected**; *sample preparation ongoing; testing time – 7-8 weeks*
Development toxicity - ReproTracker assay with Ethyl Tafluprostamide/DDDE, Tafluprost, Bimatoprost	Toxys	**CRO selected**; *sample preparation ongoing; testing time – 12 weeks*
Toxprofiler assay with Ethyl Tafluprostamide/DDDE, Tafluprost, Bimatoprost	Toxys	**CRO selected**; *sample preparation ongoing; testing time – 4 weeks*
Obtain mechanistic information on possible targets		
In silico endocrine receptor/activation predictions for Ethyl Tafluprostamide/DDDE, Tafluprost, and Bimatoprost	ToxMinds	**Completed**
Literature research of endocrine receptor activation by analogues, Tafluprost and Bimatoprost	ToxMinds	**Completed**
Geminal Flourines		
Analysis of differences in metabolism due to geminal flourines in the suitability of using Tafluprost or Bimatoprost in read-across analyses of Ethyl Tafluprostamide/DDDE	ToxMinds	Scheduled for May 2024; to be completed June 2024
Response to the IDA		
Prepare report with the newly generated bridging data on the target (Ethyl Tafluprostamide/DDDE) and analogues (Tafluprost and Bimatoprost) to support the read-across hypothesis and justify filling the data gaps for systemic toxicity endpoints by using read across.	ToxMinds	Scheduled for September – October 2024
Submit Supplemental Report #2 to the CIR	John Bailey	October - November 2024