# Safety Assessment of Acetyl Hexapeptide-8 Amide as Used in Cosmetics

Status: Draft Final Report for Panel Review
Release Date: February 16, 2021
Panel Date: March 11-12, 2021

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.; Daniel C. Liebler, Ph.D.; Lisa A. Peterson, Ph.D.; Ronald C. Shank, Ph.D.; Thomas J. Slaga, Ph.D.; and Paul W. Snyder, D.V.M., Ph.D. Previous Panel member involved in this assessment: James G. Marks, Jr., M.D. The Cosmetic Ingredient Review (CIR) Executive Director is Bart Heldreth, Ph.D. This report was prepared by Wilbur Johnson, Jr., M.S., Senior Scientific Analyst/Writer, CIR.



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Cosmetic Ingredient Review

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**Memorandum**

To: Expert Panel for Cosmetic Ingredient Safety Members and Liaisons

From: Wilbur Johnson, Jr.
Senior Scientific Analyst/Writer, CIR

Date: February 16, 2021

Subject: Safety Assessment of Acetyl Hexapeptide-8 Amide as Used in Cosmetics

Enclosed is the Draft Final Report of the Safety Assessment of Acetyl Hexapeptide-8 Amide as Used in Cosmetics (*acetyl032021rep*). At the December 2020 meeting of the Expert Panel for Cosmetic Ingredient Safety (Panel), the Panel issued a Revised Tentative Report with a conclusion stating that Acetyl Hexapeptide-8 Amide is safe in cosmetics at concentrations up to 0.005%, and that the available data are insufficient for evaluating safety at higher concentrations. It was agreed that a no-observed-adverse-effect-level (NOAEL) for type I and type III collagen synthesis would be needed in order to evaluate the safety of Acetyl Hexapeptide-8 Amide in cosmetic products at concentrations > 0.005%. These data have not been provided.

This report has been revised to address comments *(acetyl032021pcpc* - enclosed*)* on the revised tentative report that were received from the Council. Addtionally, the report has been revised to include 2021 FDA VCRP data (*acetyl032021FDA* - enclosed) that were received in January of this year. It should be noted that these data indicate that the reported use frequency of Acetyl Hexapeptide-8 Amide in cosmetics has decreased by more than 100 uses since this safety assessment was reviewed by the Panel in December of last year.

Also included in this package for your review are the report history (*acetyl032021hist*), flow chart (*acetyl032021flow*), literature search strategy (*acetyl032021strat*), ingredient data profile (*acetyl032021prof*), and minutes from prior Panel meetings (*acetyl032021min*).

The Panel should carefully consider the Abstract, Discussion, and Conclusion presented in this report. After reviewing these documents, the Panel should issue a Final Report with the conclusion that is stated in the first paragraph above.

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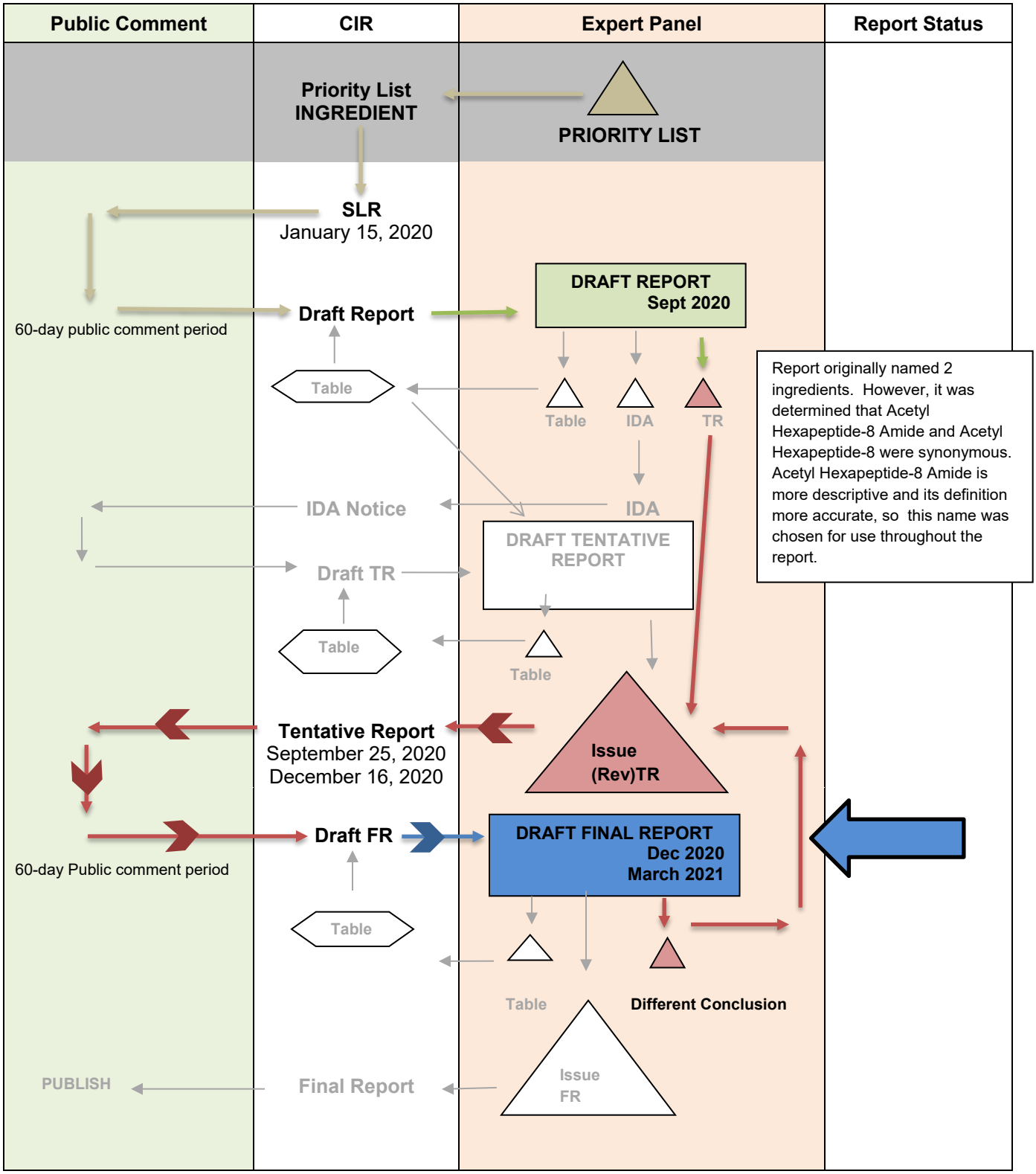
# SAFETY ASSESSMENT FLOW CHART

**INGREDIENT/FAMILY** Acetyl Hexapeptide-8 Amide

**MEETING** March 2021



CIR History of:

**Acetyl Hexapeptide-8 and Acetyl Hexapeptide-8 Amide**

A Scientific Literature Review (SLR) on Acetyl Hexapeptide-8 and Acetyl Hexapeptide-8 Amide was issued on January 15, 2020.

**Draft Report, Teams/Panel: September 14-15, 2020**

The draft report has been revised to include the Council's comments, and also includes use concentration data on Acetyl Hexapeptide-8 that were received from the Council. After reviewing the data included in this safety assessment, the Panel concluded that Acetyl Hexapeptide-8 Amide is safe in cosmetics in the present practices of use and concentration described in the safety assessment, and issued a Tentative Report.

Prior to the September 2020 Panel meeting, CIR was made aware that Acetyl Hexapeptide-8 is synonymous with Acetyl Hexapeptide-8 Amide. Accordingly, all of the data in the literature states Acetyl Hexapeptide-8 as the test material, but is fully applicable to the synonymous ingredient, Acetyl Hexapeptide-8 Amide. The name, Acetyl Hexapeptide-8 Amide, is more accurate, as the ingredient is used as the amidated peptide. Thus, the Amide name is used throughout the report. With this in mind, the Panel agreed that the subject of this safety assessment should be changed to Acetyl Hexapeptide-8 Amide.

The Panel noted the absence of systemic toxicity and genotoxicity data on Acetyl Hexapaptide-8 Amide. However, concern over the lack of these data was mitigated, after considering the peptide structure of this ingredient and associated low log Ko/w value of -7.68 (i.e. percutaneous absorption is unlikely), and the low maximum use concentration of 0.005% in leave-on cosmetic products. The Panel determined that these findings support the safe use of Acetyl Hexapeptide-8 Amide in cosmetic products.

Finally, the Panel discussed the issue of incidental inhalation exposure from the use of Acetyl Hexapeptide-8 Amide in face powders at concentrations up to 0.0001%. It was noted that conservative estimates of inhalation exposures to respirable particles during the use of loose powder cosmetic products are 400-fold to 1000-fold less than protective regulatory and guidance limits for inert airborne respirable particles in the workplace.

**Draft Final Report, Teams/Panel: December 7-8, 2020**

The draft report has been revised to include comments that were received from the Council.

The Panel concluded that Acetyl Hexapeptide-8 Amide is safe in cosmetics at concentrations up to 0.005%, and that the available data are insufficient for evaluating safety at higher concentrations. A revised tentative report with this conclusion was issued. The Panel agreed that a no-observed-adverse-effect-level (NOAEL) for type I and type III collagen synthesis would be needed in order to evaluate the safety of Acetyl Hexapeptide Amide in cosmetic products at concentrations > 0.005%.

The Panel stated their awareness of a consumer product purported to contain 10 to 30% Acetyl Hexapeptide; however, whether this product is a drug or cosmetic remains unknown. The Panel recognizes that Acetyl Hexapeptide-8 Amide is used in leave-on cosmetic products at concentrations up to 0.005%, based on vetted information sources, and that a drug effect (i.e., anti-wrinkle effect) on the dermis would not be likely at this low concentration. Nonetheless, the Panel acknowledges that the drug effect may be apparent at higher use concentrations.

The Panel noted the absence of systemic toxicity and detailed genotoxicity data on Acetyl Hexapaptide-8 Amide. Still, concern over the lack of these data was mitigated, after considering the peptide structure of this ingredient, the associated low partitioning coefficient of -6.3 (percutaneous absorption unlikely), and the low maximum use concentration of 0.005% in leave-on cosmetic products. On the subject of potential percutaneous absorption, the Panel also noted differing degrees of reported skin penetration by Acetyl Hexapeptide-8 Amide with in vitro models. The Panel felt that studies that utilized liquid chromatography with tandem mass spectrometry to measure the peptide were most dependable, and noted that these studies indicated minimal skin penetration.

**Draft Final Report, Teams/Panel: March 11-12, 2021**

The draft final report has been revised to include comments that were received from the Council.

| Acetyl Hexapeptide-8 Amide Data Profile* –March 11-12, 2021 – Wilbur Johnson, Jr. | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | Toxico-kinetics | | Acute Tox | | | Repeated Dose Tox | | | DART | | Genotox | | Carci | | Dermal Irritation | | | Dermal Sensitization | | | | Ocular Irritation | | Clinical Studies | |
| | Reported Use | GRAS | Method of Mfg | Constituents | Impurities | Dermal Penetration | ADME | Dermal | Oral | Inhalation | Dermal | Oral | Inhalation | Dermal | Oral | In Vitro | In Vivo | Dermal | Oral | In Vitro | Animal | Human | In Vitro | Animal | Human | Phototoxicity | In Vitro | Animal | Retrospective/ Multicenter | Case Reports |
| **Acetyl Hexapeptide-8 Amide** | **379** | | **X** | **X** | | **X** | | | **X** | | | | | | | **X** | | | | | **X** | | | | **X** | | | **X** | | |

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

**Acetyl Hexapeptide-8 and Amide – 10/28/2019;8/8/2020;10/20/2020;1/12/2021]**

| Ingredient | CAS # | InfoBase | SciFinder | PubMed | TOXNET | FDA | EU | ECHA | IUCLID | SIDS | HPVIS | NICNAS | NTIS | NTP | WHO | FAO | ECE-TOC | Web |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Acetyl Hexapeptide-8 | 616204-22-9 | | Yes | 4/4 | 2/0 | 0/0 | No | Process* | No | No | No | No | No | No | No | No | No | Yes |
| Acetyl Hexapeptide-8 Amide | | | Yes | 0/0 | 0/0 | 0/0 | No | No | No | No | No | No | No | No | No | No | No | Yes |

*ECHA – pre-registration process

**Search Strategy**

[*document search strategy used for SciFinder, PubMed, and Toxnet*]
[*identify total # of hits /# hits that were useful or examined for usefulness*]

**LINKS**

InfoBase (self-reminder that this info has been accessed; not a public website) - http://www.personalcarecouncil.org/science-safety/line-infobase
ScfFinder (usually a combined search for all ingredients in report; list # of this/# useful) - https://scifinder.cas.org/scifinder
PubMed (usually a combined search for all ingredients in report; list # of this/# useful) - http://www.ncbi.nlm.nih.gov/pubmed
Toxnet databases (usually a combined search for all ingredients in report; list # of this/# useful) – https://toxnet.nlm.nih.gov/ (includes Toxline; HSDB; ChemIDPlus; DAR; IRIS; CCRIS; CPDB; GENE-TOX)
FDA databases – http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/cfrsearch.cfm (CFR); then,
list of all databases: http://www.fda.gov/ForIndustry/FDABasicsforIndustry/ucm234631.htm; then,
http://www.accessdata.fda.gov/scripts/fcn/fcnnavigation.cfm?rpt=eafuslisting&displayall=true (EAFUS);
http://www.fda.gov/food/ingredientspackaginglabeling/gras/default.htm (GRAS);
http://www.fda.gov/food/ingredientspackaginglabeling/gras/scogs/ucm2006852.htm (SCOGS database);
http://www.accessdata.fda.gov/scripts/fdcc/?set=IndirectAdditives (indirect food additives list);
http://www.fda.gov/Drugs/InformationOnDrugs/default.htm (drug approvals and database);
http://www.fda.gov/downloads/AboutFDA/CentersOffices/CDER/UCM135688.pdf (OTC ingredient list);
http://www.accessdata.fda.gov/scripts/cder/iig/ (inactive ingredients approved for drugs)
EU (European Union); check CosIng (cosmetic ingredient database) for restrictions and SCCS (Scientific Committee for Consumer Safety) opinions - http://ec.europa.eu/growth/tools-databases/cosing/
ECHA (European Chemicals Agency – REACH dossiers) – http://echa.europa.eu/information-on-chemicals;jsessionid=A978100B4E4CC39C78C93A851EB3E3C7.live1
IUCLID (International Uniform Chemical Information Database) - https://iuclid6.echa.europa.eu/search
OECD SIDS documents (Organisation for Economic Co-operation and Development Screening Info Data Sets)- http://webnet.oecd.org/hpv/ui/Search.aspx
HPVIS (EPA High-Production Volume Info Systems) - https://ofmext.epa.gov/hpvis/HPVISlogon
NICNAS (Australian National Industrial Chemical Notification and Assessment Scheme)- https://www.nicnas.gov.au/
NTIS (National Technical Information Service) - http://www.ntis.gov/
NTP (National Toxicology Program ) - http://ntp.niehs.nih.gov/
WHO (World Health Organization) technical reports - http://www.who.int/biologicals/technical_report_series/en/
FAO (Food and Agriculture Organization of the United Nations) - http://www.fao.org/food/food-safety-quality/scientific-advice/jecfa/jecfa-additives/en/ (FAO);
FEMA (Flavor & Extract Manufacturers Association) - http://www.femaflavor.org/search/apachesolr_search/
Web – perform general search; may find technical data sheets, published reports, etc
ECETOC (European Center for Ecotoxicology and Toxicology Database) - http://www.ecetoc.org/

**SEPTEMBER 2020 PANEL MEETING – INITIAL REVIEW/DRAFT REPORT**

**Belsito Team – September 14, 2020**

**Acetyl Hexapeptide-8 and Acetyl Hexapeptide-8 Amide**

DR. BELSITO: Oh. Yeah. Sorry. Acetyl Hexapeptide. So this is the first time we're reviewing the safety assessment of acetyl hexapeptide-8 and hexapeptide-8 amide functioning as a skin conditioning agent, humectant, and also miscellaneous. We also have Wave-2 data on this. Wilbur, I'm assuming that will be incorporated into this?

MR. JOHNSON: Yes. Those comments will be addressed in text.

DR. BELSITO: Okay. We've got, for some reason my comments didn't save here. Okay. I'm sorry, but my review of this did not save on my computer. So Paul or Dan, do you wanna take over here?

DR. LIEBLER: Yeah. I can start with just a -- maybe I'll start with some of the chemistry stuff, Paul, and then I can kick it over to you for the tox?

DR. SNYDER: Sure.

DR. LIEBLER: Okay. So on page two of Wilbur's cover memo there's a question about adding some other ingredients that are either related or identical. It's in the third paragraph of the cover memo. And my comment on that was it's okay to add the other ingredients if they can be identified to be identical and have the same uses. Is there anything else you want to say about that, Wilbur, to further clarify it, or Monice, or anybody?

MR. JOHNSON: I have no further comments, Dr. Liebler.

DR. EISENMANN: This is Carol. Before you do -- Wilbur, before you do add it, please check with Joanne because I've had a little bit of a discussion with her and she's considering taking out the duplicates, the 24 names because those really are not recognized yet. So just before you do it see if for sure what she's gonna do because she was thinking about it. But I'm not sure -- she hasn't done it yet. I just looked in the dictionary. Okay?

DR. LIEBLER: So you're saying they might not be in the dictionary, Carol?

DR. EISENMANN: They are in the dictionary now, but Joanne is considering taking them out because they are duplicates.

DR. LIEBLER: Okay.

DR. EISENMANN: But she hasn't done it yet, so it means she has to contact all the suppliers that are associated with those names. So whether or not -- and change their names, which they might not be happy about. But I would, in the next week or so, check with her and see what her plans are for sure.

DR. LIEBLER: Okay.

DR. EISENMANN: I'm not sure she had (audio skip).

DR. LIEBLER: Thank you, Carol.

DR. BELSITO: My understanding that all four names refer to the same ingredient. Is that correct?

DR. EISENMANN: That's correct.

DR. LIEBLER: Yeah. All right. We'll let you guys all sort that out on your end. As long as it's the same ingredient it doesn't matter to us. So now to method of manufacture. The second paragraph, acetyl hexapeptide-8 amide. It says it's completely synthesized in the laboratory and has no excipient preservatives or anti-oxidants, which is all fine.

And then the next sentence is, acetyl hexapeptide-8 amide has also been derived for internal of the synaptic protein, synaptosomal nerve associated protein. And that implies that it's produced from an animal source. So we need to sort that out because if so, we'll need the animal products boilerplate.

So it may be that this is either synthesized or produced from a tissue source. But that needs to be clarified. In other words, is the cosmetic ingredient produced from either synthetic -- is either produced synthetically or from a tissue source.

MS. FIUME: So would you want that as an IDA request?

DR. LIEBLER: Yes. So method of manufacture is unclear. I guess what it is, Monice and Wilbur, is just that I don't know if the cosmetic ingredient is solely produced synthetically and that this reference to the -- from the synaptic protein is another way that this ingredient could be made but that's not how a cosmetic grade ingredient is made.

It reads right now like the cosmetic ingredient could be from either synthetic or from this tissue source protein. So we need to know which is which. If they're both, you know, source of the cosmetic ingredient so be it, we just need to add the appropriate boilerplate and make sure we treat it in the discussion.

DR. EISENMANN: The definition of hexapeptide-8 says synthetic. So it's a synthetic material.

DR. LIEBLER: Okay. Then maybe we should just remove that sentence because it just creates confusion, the second sentence in that paragraph, Wilbur, acetyl hexapeptide-8 has also been derived from the in the terminal of the synaptic protein. I think you could delete that sentence then.

MR. JOHNSON: Okay. I'll do that.

DR. LIEBLER: Okay. Let's see. I had a couple of minor comments that -- I'm gonna kick it over to Paul, I think, for the tox.

DR. SNYDER: Sure. So we did have dermal penetration data in vitro. We had lots of data. And we had no dermal absorption in vivo data. We had limited tox data. There was only an acute tox test -- an acute tox oral study.

There was no short term, no sub-chronic, no chronic, no repro, no carcinogenicity, and one AIMS test which was negative. So I thought we would also be insufficient because I think we need a 28-day dermal because we have no absorption data -- in vivo absorption data or any toxicity data. So on a representative, whatever Dan would think would be a representative ingredient, hexapeptide, I think we should have a 28-day dermal because of those issues there. We did have, Don, HRIPT data at 0.05 percent. It was a relatively low number of patients. It was only 50, but it was negative.

DR. BELSITO: Mm-hmm.

DR. SNYDER: So I thought, whether you thought that was adequate or not.

DR. BELSITO: So can we just go back to your point, Paul, with the 28-day dermal. Dan, Paul was asking you which -- I guess there's really only one material, they're all the same.

DR. LIEBLER: Yeah. So I think that my expectation is a peptide will not be absorbed through the stratum corneum. But I can't point to a definitive piece of data. It's not quite the same as saying it's a large polymer but it's a six, you know, seven, probably about a 750 molecular weight molecule that's pretty polar. I don't think it's gonna go through the stratum corneum pretty much at all. I don't --

DR. KLAASSEN: I would second that. And in fact, you know, in the chemical properties it says it has a long-P of -6.3.

DR. SNYDER: Yeah.

DR. LIEBLER: The penetration --

DR. KLAASSEN: So the likelihood of that going through the skin is very, very unlikely.

DR. SNYDER: The penetration data does say they did the last study there it says, "Authors noted these results indicate that acetyl hexapeptide-8 is capable of permeating through the skin." So that's why I kind of defaulted to that because we had the in vitro data, but if you guys wanna make a statement about the lack of absorption based upon chemical characteristics I'm fine with that, then we don't need it.

DR. KLAASSEN: Personally, I don't think we need it.

DR. BELSITO: Dan?

DR. LIEBLER: I'm looking -- I'm just looking again really quick at the study Paul's referring to in PDF 13 because I have a note. This is one of the things I skipped over. So they said the concentration of acetyl hexapeptide-8 amide -- so this is the last paragraph -- hang on a second. It's the one, two, three, fourth paragraph on page 13 at the end of that paragraph. It's right before absorption, distribution, metabolism, and excretion.

And it describes the experiment. It says, "The concentration of acetyl hexapeptide-8 amide in the receptor fluid was quantified at two hours using high performance liquid chromatography. Total content of the peptide in the receptor reservoir was 30 percent of the amount that was deposited onto the membrane in the donor chamber. The authors noted that these results indicate that the peptide amide is capable of permeating through the skin."

Now, the problem is the detection system. I said this is probably not correct. If the analysis method is not mass-spec if it's just -- and they just say HPLC so I don't know what kind of detection. But if it's not mass-spec it's likely to be subject to interferences from similar peptides coming from the skin samples, not the applied peptide. So we would have to take a look at that paper, and I should have done that. I apologize for not doing it. Maybe I can pull that paper up.

DR. SNYDER: So Curt and Dan, what about the studies before that that talk about the relative permeation in relationship to water and oil emulsion, oil and water emulsion?

DR. KLAASSEN: Did they give a quantitative amount? They would -- is this the one that said it would be -- it was more under one kind of emulsion that another kind of an emulsion?

DR. SNYDER: Correct. Correct. Yeah.

DR. LIEBLER: Yeah. I frankly got lost in that description.

DR. SNYDER: So did I. That's why I deferred to the last one where it said it did permeate and that's why I said, okay then we need absorption data.

DR. LIEBLER: Yeah. So the paragraph above the one that I was talking about, it ends with no hexapeptide metabolite was detected in any layers. No peptide was detected in the dermis or buffer collected underneath the skin. So that leaves us with the water and oil, oil and water, water and oil. And those were tape-stripping experiments on porcine ear skin. I'm not sure if that system models, you know, human dermal absorption.

DR. KLAASSEN: Actually, pig skin is good.

DR. LIEBLER: Uh-huh.

DR. KLAASSEN: It's actually probably the best in comparison to human.

DR. LIEBLER: But tape-stripped?

DR. KLAASSEN: Well --

DR. BELSITO: The first --

DR. KLAASSEN: -- there's a way of, you know, determining --

DR. BELSITO: The first --

DR. KLAASSEN: -- how far it's gone down.

DR. LIEBLER: Yeah.

DR. BELSITO: The first paragraph is not tape-stripped in vitro.

DR. LIEBLER: Okay. Yeah. It did use LC-MS to --

DR. BELSITO: And then in the second one they tape-stripped it.

DR. LIEBLER: Yeah. Okay. And they did use LCMS, which is a bonafide analytical method for this in the first paragraph.

DR. KLAASSEN: Well, I guess it doesn't hurt to ask for the dermal study. I'd be very surprised if they have any toxicity from dermal application, but….

DR. BELSITO: We want a 28-day dermal at the highest concentration leave-on.

DR. LIEBLER: Yeah. It's only 0.05 percent so it's -- yeah.

DR. SNYDER: It's 0.005 percent, isn't it? Yeah. It's 0.005 percent in leave-on.

DR. LIEBLER: Monice, sorry. Can you send that to me again?

MS. FIUME: Sure.

DR. BELSITO: Yeah. So, and just while Dan is looking at that, yeah, Paul, and concern about the HRIPT usually want 100 subjects but it was done at 0.05 --

DR. SNYDER: Ten-fold higher than -- right.

DR. BELSITO: Right, than the use. So I'm okay with that.

DR. SNYDER: Okay. So I think it would be a, you know, I think we can either go insufficient data announcement or we can move forward with a safe as used depending upon the discussion regarding the absorption. And then if Dan gets clarification on the materials and methods because that's the only issues that I had.

DR. EISENMANN: One thing about that last dermal penetration study, what I wasn't clear about is if it says skin disks stratum corneum 2 centimeters squared. So was this a study just of looking at crossing the stratum corneum or was this more than just the stratum corneum? Because that's what I wasn't clear from the write-up.

DR. SNYDER: I just based on that last sentence.

DR. EISENMANN: Right.

DR. SNYDER: Is capable of permeating through the skin. So, again, we can have that verified with exactly what that meant.

DR. KLAASSEN: Yeah.

DR. SNYDER: Did it just penetrate the stratum corneum or did it actually go through?

DR. LIEBLER: Unfortunately, the paper Monice just sent me does not give enough information. Literally only says it was done by HPLC so it doesn't say what detection method was used. That's always a bad sign that they don't know what they're talking about because they don't write what's obviously important to people who know how to do this right. So --

DR. SNYDER: Exactly.

DR. LIEBLER: Yeah. I don't think we can have confidence in that report simply because it's not -- the experimental method is not adequately described.

DR. SNYDER: I think we can construct a very scientifically sound discussion point based upon the log P data, the physical characteristics of the ingredient that it would not likely be -- that it would not be absorbed and go from there. And with such a low concentration of use, even in a leave-on, a 0.05 percent, 0.005.

DR. BELSITO: Okay. So --

DR. LIEBLER: Yeah. That's a key point, Paul. I agree with the very low concentration of use.

DR. BELSITO: So then our discussion would simply revolve around the log Ko/w, the peptides not penetrating, and so we feel that the absence of adequate dermal penetration studies because we'll make some comment on the current studies, Dan? You felt the methodology was inappropriate?

DR. LIEBLER: Yeah. The last study, and then the second to last paragraph with the hairless guinea pigs, it didn't indicate penetration. It didn't indicate that it got through the skin. And then the emulsion studies, I'm not sure that the tape-stripped is relevant. And then the one above it --

DR. BELSITO: And the one above it you didn't like the description of methodology.

DR. LIEBLER: No. The one at the end was the one. The infinite dose was used. 250 mgs per centimeter squared. The problem is that they don't tell you how much actually went through with these different emulsions. I guess they did. 755 nanograms per centimeter squared, which is, I did calculate that. That's 0.0003 percent.

So in the one study with a model that Curt indicates should be a reasonable human skin model that -- this is the pig. The first paragraph under in vitro, they applied the, what they called the infinite dose 250 milligrams per centimeter squared, and they measured 755 nanograms per centimeter squared. And that's a penetration of 0.0003 percent. So if you combine that with the fact that we've got very low use concentrations in cosmetic products, this is really negligible significance at concentration of use.

DR. SNYDER: I'm surprised that's within detectable limits even.

DR. LIEBLER: Well, with the LCMS, yeah, they can easily do that.

DR. SNYDER: Oh, can they?

DR. LIEBLER: That's not hard to measure that level. But --

DR. BELSITO: So our discussion will center around the fact that the hairless guinea pigs it didn't go through skin, with the various emulsions the tape-strip is not relevant. And the other study -- the other part of that study where they looked at pig skin that was not tape-stripped they used an infinite dose of 250 milligrams and they measured very low penetration of 0.0003 percent --

DR. LIEBLER: Right. 0.0003 percent.

DR. BELSITO: Three zeros?

DR. LIEBLER: Three zeros and a three.

DR. BELSITO: Yeah. That's what I have.

DR. LIEBLER: Okay.

DR. BELSITO: And given the low concentration of use at 0.005 percent we weren't concerned.

DR. LIEBLER: Correct.

DR. BELSITO: Okay. Then the conclusion is safe as used?

DR. SNYDER: Yeah, again, I did mention there was only one genotox, one AIMES test. And so sometimes Tom likes to see a mammalian, but I didn't think with the -- I think that can also be negated in this absorption issue. It's just not gonna get absorbed.

DR. LIEBLER: I agree.

DR. KLAASSEN: I agree.

DR. LIEBLER: We can also point out that to the extent that any absorption does occur this ingredient is the substrate for numerous peptidases. And would be metabolized to amino acids that are already present in high concentration in all tissues.

DR. BELSITO: Okay.

DR. SNYDER: So I think -- did you get all that Don? So the first thing would be the clarification of the materials and method details regarding synthetic versus tissue sourced.

DR. BELSITO: Right.

DR. SNYDER: And then the absorption discussion, how we're gonna handle that.

DR. BELSITO: Right. So we're deleting the derivation from nerve tissue, nerve peptides. Yeah. And then basically everything else stays the same. But then in the discussion we talk about the lack of absorption through guinea pig skin, and that the pig skin studies, the tape-stripped isn't relevant to cosmetic use, and IN the other study the absorption was extraordinarily low, low use concentration mitigates our concern about lack of DART data. We have only an AIMS test with no mammalian genotoxicity, but again, the same argument, low penetration, low concentration of use. Correct?

DR. SNYDER: Perfect. Yep.

DR. BELSITO: Then our conclusion is safe as used.

DR. SNYDER: Correct.

DR. BELSITO: And then, Carol will work on determining what names this one ingredient goes under in the dictionary.

MS. FIUME: So can I ask, Wilbur, is this the report that we have a question of the cosmetic versus drug use? Did that need to be discussed with this? You're on mute.

MR. JOHNSON: One second, Monice.

MS. FIUME: Okay.

MR. JOHNSON: No. There was no need for discussion relating to any drug use, Monice.

MS. FIUME: Okay.

MR. JOHNSON: According to me --

MS. FIUME: Thank you.

MR. JOHNSON: You're welcome.

**Marks Team –September 14, 2020**

**Acetyl Hexapeptide-8 and Acetyl Hexapeptide-8 Amide**

DR. MARKS: Acetyl Hexapeptide-8. Oh, yeah. This one's a fun one. Would the real acetyl hexapeptide name appear? So this is a draft report. It's the first time we've seen these ingredients or ingredient, and we'll maybe have Bart clarify this since he's the chemist. So it's the first time reviewing the single ingredient, the acetyl hexapeptide-8, which is synonymous with the 8 amide, the 24 amide, and possibly also the acetyl hexapeptide-3, which is on page 10, and Wilbur says is the same also. So Bart, maybe to begin with, how many ingredients do we really have behind the doors? Is it one, two, three, four, five? And what are we going to title this?

DR. HELDRETH: Well, the title I'll let you work out, but basically these all work out to be the same thing. Most of these were named before the sequences were really fully understood, and that's why we got more than one name for essentially the same chemicals.

DR. MARKS: So are we going to call the one name that would be consistent -- do we want to call it acetyl hexapeptide-8?

DR. HELDRETH: Yeah. Probably acetyl hexapeptide-8 amide would be my recommendation.

DR. MARKS: Okay.

DR. HELDRETH: Because there is some confusion there with the acetyl hexapeptide-8 name. Basically, that's always used as the amide. So I would use the more descriptive name, the acetyl hexapeptide-8 amide.

DR. ANSELL: And that's our suggestion as well.

DR. MARKS: Oh, good, Jay. And how do you -- you say acetyl. I said acetyl. Is it the --

DR. PETERSON: Tomato, tomato.

DR. MARKS: Is that -- okay. Thank you, Lisa.

DR. ANSELL: Potato, potato.

DR. MARKS: I didn't want to pronounce the chemical name incorrectly. David, you'll find that every once in a while you need to ask your chemist colleague how do you pronounce it. So either one. Good. So I'm going to refer -- first time, I'm going to do the intro of this, although I'm seconding it. So I won't have to.

For my purposes, this is the first time reviewing a single ingredient, acetyl hexapeptide-8 amide. And it's synonymous with those other names. And does that include -- and that'll obviously be in the introduction to the paper, that first paragraph. And am I correct also, Bart, that acetyl hexapeptide-3 is another name for this? Did you see where I noted that on page 10?

DR. HELDRETH: Yeah. That's actually an old essentially discontinued name. It was one of the original names for it. So yeah. It's another synonym, but it's no longer considered a cosmetic ingredient name.

DR. MARKS: So would you leave out on page 10, or would you leave it in? Part of it is I'm reading in the beginning where they're all the same, and then I hit page 10. And I see that it says this is a synonym also.

DR. HELDRETH: Right. So when we first made this report, we were not aware that all of the other ones were synonyms. We thought it was just the acetyl hexapeptide-3 was a synonym. And then just as this was going to press, we found out from the nomenclature committee that indeed all of these ingredients were the same thing.

DR. MARKS: Okay.

DR. HELDRETH: So we'll flesh out the intro and make this a lot more clear in the next version that you see. We just kind of worked it in there very quickly so that it would be in the version that got to the Panel.

DR. MARKS: Great. Okay. Thanks, Bart, for clarifying that. Wilbur, are you back yet?

MR. JOHNSON: I'm here, Dr. Marks.

DR. MARKS: Good. Okay. So I won't ask Lisa, Ron, and Tom are the ingredients okay because there's only one ingredient. So we don't have a choice for this. Comments, needs, Lisa, Ron, Tom?

DR. SHANK: We have enough --

DR. SLAGA: There's a good bit of data. It's not an irritant. It's not a sensitizer and not genotoxic. So those parts okay.

DR. MARKS: It is absorbed.

DR. SLAGA: Yeah. It's absorbed.

DR. MARKS: And I wondered whether you needed repeat tox, DART, that sort of thing, Ron.

DR. SHANK: No, the maximum leave on concentration's very, very low. So the blood concentration from cosmetic use would be negligible.

DR. MARKS: Yeah. I wondered whether -- yeah. What I have is the use concentration at a leave on is 0.005 percent. Is that what you had?

DR. SHANK: Yes, it is.

DR. MARKS: So really, we don't need these -- even if it's absorbed, the concentration is so small that we would not expect it to be toxic.

DR. SHANK: That's right.

DR. MARKS: So we can second presumably a tentative report with a safe conclusion. Does that sound --

DR. SLAGA: That's what I would do.

DR. MARKS: Okay.

DR. PETERSON: I concur with that. My only comments are editorial, so I didn't have any needs.

DR. SHANK: Good.

DR. BERGFELD: I'd just like to make one comment. I found this to be an interesting ingredient because it produces edema, swelling of the skin, and used for wrinkles, which can obviously be temporary.

DR. MARKS: Okay. Any other comments anybody? So tomorrow I'll be seconding a motion presumably tentative report safe as used. Okay. Bart, thanks for clarifying the nomenclature. Did you hear that, Wilbur, where we're going to -- we're just going to use the 8 amide as the lead terminology for all these synonyms?

MR. JOHNSON: Yes. Okay. Thank you.

DR. MARKS: You're welcome.

**Full Panel –September 15, 2020**

**DR. BELSITO**: Okay, so, again this is the first time that we're looking at this report, and basically we're told that all the various names refer to the same cosmetic ingredient whether it's Acetyl Hexapeptide-8, Acetyl Hexapeptide-8 Amide, or then we were told about Acetyl Hexapeptide-24 and Acetyl Hexapeptide-24 Amide. And, it's not clear whether the 24 -- I gather from Carol -- will actually be added to the dictionary or not. But, be that as it may, it appears that we're really looking at one material here, not two as the title would suggest.

Our team looked at all of this and we thought that based upon the log K o/w, and that it was a peptide, it would not penetrate through the skin. We didn't feel the tape strips studies were relevant, nor were the infinite dose absorption studies. And, that although there was no mammalian genotox, because of our assumption that there would be lack of penetration and the very low measures at infinite dose penetration, we thought we could go with a safe as used for this ingredient.

**DR. MARKS**: Second.

**DR. BERGFELD:** Any further comment?

**DR. MARKS**: Yeah, I might also add, on Page 10, Acetyl Hexapeptide-3 was also mentioned as another synonym, so there may be actually five synonyms. And, Bart suggested yesterday that we refer to it -- use the amides. So, it's Acetyl Hexapeptide-8 Amide, being the term we use in the title. And then, Don, we agree with your team with your assessment of the safety of this ingredient. And we might also add Ron Shank brought up that the use concentration is 0.005 percent. So, that small concentration would also support the safety of the ingredient.

**DR. BELSITO**: Yeah.

**DR. BERGFELD:** So we have had a motion and a second. We've had comments and clarifications. Any other statements to be made? Any other discussion points? Don, are you in agreement with the change of the title?

**DR. BELSITO**: Yeah, I'm fine. I pointed out that we had multiple names for one ingredient.

**DR. BELSITO**: Right, right. Okay. I'll call for the vote, though. All those against moving forward as a safe ingredient, please indicate by stating your name. I'll assume that all of you are for, so it's unanimous decision to move forward and approve this as a safe ingredient. Then, coming to the Benzophenones, this is going to be Dr. Marks.

**DECEMBER 2020 PANEL MEETING – DRAFT FINAL REPORT**

**Belsito Team –December 7, 2020**

**DR. BELSITO:** So this is the one that was supposed to go final. And we got this late-breaking report from the Women's Voices of the Earth indicating that this material is actually used as Botox in a jar, marketed as a cosmetic in concentrations up to 10 to 30 percent, not the 0.005 leave-on percentage that we were told.

I personally, when I read this last night, was not in a mood to go through the entire report and see whether it would be considered safe. We certainly don't have sensitization and irritation at that level. So I would say we should table this. Would that be the right move, Monice?

**MS. FIUME:** Actually, Don, I don't know which FDA rep is on the phone. Keith, is it you? Is there an FDA rep on this side? I was hoping FDA would be on the meeting because typically anti-wrinkle creams are not considered cosmetics. That is a drug use. So if it's used in an anti-wrinkle cream, that really wouldn't fall under the purview of the panel. It would be other types of creams maybe, but not an anti-wrinkle. That's a drug claim.

**DR. EISENMANN:** This is Carol. And I saw that higher concentrations on the Internet, and I had a discussion with CIR SSC and asked them about it. And what they said, is that it's so expensive that they've thought that that would be highly unlikely. And that they were all okay with if you concluded safe at the low concentration.

They think that the concentration may be of a mixture that contains this protein, but of course they don't know for sure. But I brought this up with them and they would be okay with the low concentration as a conclusion.

**DR. LIEBLER:** Well, that sounds like speculation, as valid as it may be. And the issue is whether or not this is a cosmetic product or a drug medicinal product. And so, we're looking for somebody from FDA to clarify that, Monice?

**MS. FIUME:** That would be preferable, because it's always been my understanding that anti-wrinkle is a drug use. And I see that Keith from FDA is on the participant list. Keith, can you hear us? Are you on this call?

**MR. WYATT:** Yes. I can. Can you hear me?

**MS. FIUME:** Now I can.

**MR. WYATT:** Yeah. I'm not going to confirm, but it's my understanding that anti-wrinkle it would be a drug use and a non-cosmetic. But I'm going to need to confirm that with Nakissa and Linda.

**MS. FIUME:** Okay. And then, Don, the other thing I wanted to say is, you know, this has been coming up a lot lately where we are getting information saying, on the Internet we see this concentration or use. And our sources are typically what we receive in the concentration of use survey and what we receive from the FDA VCRP. And that's why our conclusion is worded as, based on the information contained in this report. Those are our sources that we typically use for use frequency and concentration. So that is why we've always gone with that information.

**DR. BELSITO:** Okay.

**MR. WYATT:** Yeah. Does the 10 percent and 30 percent containers have claims of anti-wrinkle on the container?

**DR. BELSITO:** I don't know that.

**MS. FIUME:** I'm not familiar with the containers.

**MR. WYATT:** That might be a driver. If they're not claiming anti-wrinkle, then it might be something different. But technically, anti-wrinkle is a drug use.

**DR. LIEBLER:** Yeah. I've got a photo. From one of the links that Alexandra Scranton sent us, I've got a photo of the Argireline solution, ten percent. It says, "It targets the appearance of dynamic facial lines." "Targets the appearance of dynamic facial lines."

**MR. WYATT:** Then well, that's really not a claim of anti-wrinkle. It's a little more obscure than that. I'm going to need to confirm with my management.

**DR. LIEBLER:** Yeah. It's probably why they wrote it that way.

**MR. WYATT:** We'll get clarification and address this tomorrow during the group meeting. Does that sound reasonable?

**MS. FIUME:** Don?

**DR. BELSITO:** Yeah. I mean I think it does. I'm having issues. I'm not seeing my comments here for some reason. I have my thumb drive in a different computer. I don't think I have any other comments. Dan, can you help me out? Where would I be able to see comments?

**DR. LIEBLER:** So on the right-hand side there's a whole bunch of little symbols.

**DR. BELSITO:** Yeah?

**DR. LIEBLER:** And the one that looks like a Post-It Note like a comment bubble, click on that.

**DR. BELSITO:** Yeah. Okay, thank you.

**DR. LIEBLER:** Sure.

**DR. BELSITO:** I changed computers and -- yeah, I mean, I really didn't have any other comments on this until we got that information from Women's Voices of the Earth. Did anyone else have any comments on this report?

**DR. SNYDER:** I don't.

**DR. LIEBLER:** Yeah, I thought it looked good. I didn't have any edits.

**DR. BELSITO:** I had one edit, the introduction, but it was minor. Well, I think we really need clarification as how this is being marketed. I guess we can for now proceed as a final, with the notion that our understanding is that in cosmetics it's 0.005 percent, and an anti-wrinkle would be OTC drug because it has a biologic effect. And hopefully we can get further clarification from the FDA tomorrow and see what the other panel thinks about this. Is that fair?

**DR. LIEBLER:** Yeah. I agree.

**DR. BELSITO:** All right.

**MS. FIUME:** But Don, can then I bring that back to a point of procedure for the panel? Then as far as our information for concentration and frequency of use, should we just be relying on what we know that we have? Because I don't think -- it would be very difficult for the writers to go out and search the Internet and then try and prove the validity of what's in there.

**DR. SNYDER:** I agree, Monice. I think that we need to strengthen our use statement here under Use. I'm on PDF page 16. And to your point, I think we need to say a little bit more emphatically that the data used by the Expert Panel for Cosmetic Safety is based upon data that has been verified. Because again, it wouldn't be reasonable to go on the Internet and verify -- to

trust, but verify, these would go forever. And so, I think that we need to just emphatically say that the safety is based upon that. And then our conclusion, as you stated already, clearly states that it's based upon the uses reported in this report.

So I'm comfortable just going with this one final anyway. We do know that there's some new information. We can take it under discussion. We can find out whether there is an OTC use or not. It sounds like to me like it's not going to be clarified, even tomorrow, because of the wording on the label. And we can't verify the use because they don't have an ingredients list that includes the hexapeptide all by itself. Again, it's the trust but verify. We're kind of stuck.

So I don't think we should hold this report up. We can report it as it is and if we come back and find out that's used at a much higher concentration, then we can reopen, and we have procedures for that.

**MR. JOHNSON:** Good morning. This is Wilbur Johnson. I have a comment, please?

**DR. BELSITO:** Sure.

**MR. JOHNSON:** Yeah. Dr. Snyder, where would you want that statement to be included within the use section?

**DR. SNYDER:** Well, I think this time we should just have a discussion, Wilber. I think that we should have a little stronger sentence here saying that other reported uses that may occur on the Internet or something have not been verified or something. That's something we can discuss.

I'm just kind of thinking here on my feet at getting us new information. And to Monice's point that, yes, you could probably survey the Internet and you're going to probably find just about anything on there. But it's not verifiable data. I mean, this is all science driven. We have verifiable data. You know what I mean? And that's the only thing we can base it on. Otherwise, it's all speculation and who knows?

I'm just saying if there's a way we can beef this up a little bit. Because, again, it's an informative thing that if there is other -- there be may be other information out there that -- we know there's other information now for this report, but we can't verify it and it probably can't be verified. I was just wondering if we could beef this up. I was just reading this as Don was going through his iteration about the new data.

**DR. BELSITO:** Well, but I mean we do have data. At least these websites that suggest that it is being used at a higher concentration in these creams. I don't know that we can ignore that, Paul. We have to address it in some fashion.

It would be nice, it would be easy, if the FDA were to say well, these products are clearly drug and not cosmetic. So I would prefer to hear what they have to say. I don't think we can totally discount this. There's a lot of "cosmeceuticals" out there that have not been reviewed by FDA, have not been filed as OTC drugs. And, I mean, I just don't think we can ignore this information.

**DR. SNYDER:** Well, no, I wasn't suggesting that we ignore it. I just think that we haven't verified it. Otherwise, I think where we're going to end up is we're going to end up every report is going to be insufficient. Because there's going to be these spurious things that we now know about. And like you said, who's going to do sensitization tests at 30 percent? When the maximum concentration that we know is probably used is 0.05 percent. That's a huge difference. It's not like we're talking 0.05 percent, 0.1 percent.

And so, my concern is that these things are all going to be in limbo, we're going to have to base it on some data. And then why isn't the cosmetic registration program capturing these Internet ones? It's voluntary but if -- I mean, do they need to develop some other mechanism where they say, hey, we see this, it's being reviewed. Can you verify that this is in fact a cosmetic use and not an OTC use and et cetera, et cetera?

**DR. LIEBLER:** Is Sephora part of the trade group? That's who makes this stuff, or who sells it. I don't know if Sephora is a cosmetic producer or just a marketer/retailer.

**MS. FIUME:** I don't know the member list. Carol, do you have any idea?

**DR. EISENMANN:** No. I don't.

**DR. LIEBLER:** I'm just looking at their Google search. Sephora is a French multinational retailer of personal care and beauty products.

**DR. BELSITO:** Yeah, they're --

**DR. LIEBLER:** So this is retail.

**DR. BELSITO:** They're a retail company.

**DR. EISENMANN:** Yeah. I know they don't respond to the concentration of use surveys.

**DR. LIEBLER:** Yeah.

**DR. SADRIEH:** Hi. This is Nakissa Sadrieh. Can you hear me?

**DR. BELSITO:** Yes.

**DR. LIEBLER:** Yes.

**DR. SADRIEH:** Yes. I was in the other team, I was asked to call in in case you had a question from FDA. Is there anything that you needed to ask about this ingredient that I could answer?

**DR. BELSITO:** Well, the question, Nakissa, was how this would be considered by the FDA in terms of Botox in a jar, anti-wrinkle claims. Have you looked at these products? What is your opinion? Are they OTC drugs? Are they, in fact, cosmetics?

**DR. SADRIEH:** So the product and the ingredient, obviously, are looked at differently. We regulate the product. So that means that we would look at the claims on a product to determine if it's misbranded or rated -- excuse me. And the claims are what make it possibly a drug, an unapproved drug. Anti-wrinkle basically is a little bit one of these claims that could potentially be a drug claim. But we look at the entirety of the information when we look at the claims data.

So it's not just what's on the package. We look on the Internet, when it's associated with the sale of a product, what other things are stated on the website of the company, testimonials. Those are all considered part of the labeling of the product. So advertisement is not. Advertisement in a magazine and all that, that would be FTC. But anything that's associated with the sale of the product, which means it was on the website, would technically be considered labeling.

So if, for example, in addition to anti-wrinkle they make claims about collagen growth or effects on structure function, then those would make a product potentially an unapproved drug. And we wouldn't say that it's an OTC drug because that's already making -- we just say drug; and then it's for CDER to determine whether they consider it an OTC or not. Because it depends on whether a submission had been received prior to that, whether there was an NDA for the ingredient. And I don't know that this ingredient that there is one. So the sort of drug aspect of it becomes a further complication.

I know that for other ingredients, such as other prostaglandin analogs that were used in eyelash products, we considered them as drugs because they were in drug products. And so when they were putting them in cosmetic products, at similar levels, then that would clearly be a different issue. So that's why I think that it's a little complicated to kind of have a blanket statement as to what our policy is about these things, because we have to look at the entirety of the information.

And so, on a product it's possible that there are some products, obviously, that have higher levels. I don't know where the information comes from where they're saying that it can be up to 30 percent. If they have survey data, or if the company is stating on their website that that's the case, then clearly one would have to kind of agree with that. But then I think that we would then have to look at CIR's process for determining how they establish what the highest levels of an ingredient are. Because if you're saying that it's orders of magnitude lower than -- and you're not capturing something that a company's stating, then that's a separate issue.

So I guess what I'm trying to say is that, yes, anti-wrinkle could be a drug claim, effects on structure and function claims, such as affecting collagen. So if you have data saying that there are effects on, for example, collagen sort of synthesis or other types of things that might affect structure function, then those clearly would take a different meaning at such a high level. And potentially it could make the ingredients, when added to a cosmetic, more like a drug, especially if you look at the other ingredients in a product which might increase the dermal absorption of it.

So unfortunately, I can't really give you an easy answer that, yes, at this level it would be a drug. It would depend on a number of factors because, again, we would be looking at the totality of the information. The claims, however, the safety data maybe can be looked at differently given the higher concentration. So I don't know whether that was helpful.

**DR. SNYDER:** Yes, thank you. That was helpful. So Don, have we reviewed prostaglandin?

**DR. BELSITO:** Not that I'm aware of.

**DR. SNYDER:** Because I thought that was an interesting analogy, saying that the cosmetic -- we could approve prostaglandins for use in cosmetics, and say that we're not purview to the use of prostaglandins for eyelash growth promotion or whatever that is that they're used for. So then that would be -- is that an approach we could take here? To say that -- we could specifically say in the use that we're aware of higher uses and, based upon their advertisements, they're purported to be a biological effect for modulation of collagen. And we don't have data to support that, or that would be not in the purview of the panel.

Because, I mean, this whole report is totally almost exclusively based on low concentration of use, 0.05 percent maximum leave on, and very little penetration potential. So that kind of goes out the window if we're talking about 30 percent now.

**DR. BELSITO:** No. I mean I agree. And also, we have under Other Relevant Studies effect on skin histology, they would treat it with 10 percent oil and water. I didn't even really look at this before. I thought it wasn't relevant.

**DR. LIEBLER:** So our conclusion says it's safe in cosmetics in the present practices of use and concentration described in the safety assessment. I mean, it doesn't sound like we're going to get a resolution of the question, of is this a cosmetic use or not,

any time soon. And our report describes the use of this ingredient in cosmetic products at the described concentration which are far lower than are described in these internet marketed products.

**DR. SNYDER:** At reference eight actually is a synthetic hexapeptide with anti-wrinkle activity.

**DR. BELSITO:** Yeah. So actually, one way of getting around this is if you look on PDF page 18, at 10 percent this clearly has a drug effect. If you look at the effect on skin histology under Other Relevant Studies.

**DR. SNYDER:** Right. Decrease the wrinkle depth by 20 percent.

**DR. BELSITO:** Yeah. And increase collagen. So I think in our discussion we can clearly point out that, at higher levels there appears to be a biological effect on skin which would not be considered a cosmetic. In which case, Nakissa, I think the FDA should begin looking at these products seeing how they're being marketed. Because clearly, at least, there's one study to suggest that a ten percent concentration of acetyl hexapeptide-8 would have a biologic effect on skin.

**DR. SADRIEH:** Yeah. The problem is that we don't get the -- first of all products are not, obviously, the registration is not mandatory. And then the concentrations of use are never indicated to us. So the only way we would know, is that we would have to do a survey of our own and actually measure the concentrations. Because even if it's reported to be a certain amount on the label, one can't be sure that it's correct.

But yes, what you're highlighting is it's an issue that we face regularly. And I guess my question is, is there a way to determine a concentration at which the biological effect would not be considered a drug effect? You know, in terms of drug effect meaning that it doesn't have an effect on the structure and the function of the body?

Because we all know that any ingredient can have an effect at the right concentration, if it's somehow made available to the target on which it's supposed to have an effect. And so, I guess this has been sort of a universal question for me; is how do we determine the maximum concentration that would -- for any ingredient really -- that would allow it to be safely used in a cosmetic without sort of stepping over the types of effects that might render it potentially a drug because of effects on structure and function? And I don't know whether that's a kind of risk assessment that has to be done for every single ingredient in order to kind of come up with the maximum concentration of use.

**DR. SNYDER:** Well, it's not really necessary. We just need to no effect level for this effect on the dermis. And we don't have it, Don, so we're almost obligated to go -- because there is a biological effect, now we're almost obligated to go insufficient for no effect level on the skin. Because we only have it at ten percent and we don't know what it does at lower concentrations. We don't know what the NOEL, what the no observed effect level is for that effect on the skin.

**DR. BELSITO:** Well, at point 0.005 it's probably unlikely to have an effect, but you do have a point there, Paul.

**DR. SNYDER:** I personally believe -- my scientific opinion would be that there is not going to be an effect, at that low a concentration, with that level of penetration. However, it is a biological effect, but we don't have the NOAEL for that effect.

**DR. SADRIEH:** Which means you probably can't even do a risk assessment because you don't have point of departure, right?

**DR. LIEBLER:** This would probably be very difficult to do. Just reading the description of the study at the end of PDF 18, on to the top of PDF 19. It describes the endpoint is essentially morphologic analysis of the collagen fibers. And it says when compared to the age model group, the skin of mice treated with the ingredient was thicker, and greater number of collagen fibers, and the fibers were dense and compact. But then it says the difference between the age model group and placebo control group was insignificant.

**DR. BELSITO:** Right. So there was an effect, but statistically it was not a significant effect.

**DR. LIEBLER:** Right.

**MS. FIUME:** And then on PDF page 20 under Clinical Studies there's also two human studies at ten percent where they're measuring, I guess, wrinkle depth but also looking at irritation with the ten percent product.

**DR. LIEBLER:** Um hmm.

**MS. FIUME:** Just as maybe a bit more information. But I guess this is taking me back to my original question, and as Paul had stated. If your discussion covers the fact that anti-wrinkle is a drug effect, and that's not expected in a cosmetic, and if the conclusion states that these are safe based on the information as presented in the report, does trying to decide where the difference comes in between a cosmetic effect and a drug effect actually go beyond the scope of the document? If we're saying safe in the present practices of use and concentration, described in the safety assessment, and the discussion states that the intended effect cannot be a drug effect, does that provide you with a conclusion that is typical with our procedures?

**DR. KLAASSEN:** I think it is. I think exactly what you're saying, Monice, is appropriate.

**DR. SADRIEH:** I guess my question would be that if the present practices of use indicate that there are levels that are much higher then, does that not constitute also present practices of use? That's the only kind of concern that I have if it is used at that level.

And then the other thing is, if you're putting a limit on the level that is lower than what the level is in, potentially, some products, then from the FDA's perspective then, we don't have the authority to do anything because we don't have an opinion about that. We haven't done an evaluation of it. And how do we enforce that because this is really not -- our conclusion's not enforceable?

So I would sort of like to argue that we want to be careful about the conclusions. Because if we can't cover all products, or at least to the extent that we know the level at which it's used and whether that the safety data actually covers it, then making a conclusion that is not helpful to either consumers or manufacturers then creates a burden on how one enforces that. But that's just my opinion right now.

**MR. JOHNSON:** I have just one question. Doesn't present practices of use only relate to the VCRP data?

**MS. FIUME:** And the survey data.

**MR. JOHNSON:** And the study --

**MS. FIUME:** And that's why we have the first paragraph under Cosmetic Use. And that's why the conclusions are written that way, to say as given in the safety assessment. And then for the panel, there's also a question in the comments asking is it really an in vivo effect or is it only seen in vitro?

**DR. BELSITO:** We don't know the --

**MS. FIUME:** Because we don't know the mechanisms of action in vivo, perhaps it's only hydration. I guess for as you -- and the panel has a purview to decide whatever they want on this, but I'd like to stay within our procedures as we look at the issues in the report.

**DR. BELSITO:** But hydration would be a cosmetic effect. I mean, I think I'm just betwixt and between here. I don't think we can ignore the fact that this information was brought to our attention. Is it something that we could raise or discuss some more in the discussion, that we're aware of -- I mean, we can't say that we're not aware that these products are being marketed. We're not aware of the actual concentrations because they weren't reported to us. But yeah, where do we put this information? I don't think we should just ignore it.

**MS. FIUME:** Again, it's hard to decide, based on what Nakissa said, not knowing whether or not it's truly a drug effect. But technically, if it was a drug effect, it would go under the Non-cosmetic Use section and be listed there.

**DR. SNYDER:** Yeah. Again, I want to reiterate, that discussion, I think, is two different points here. The first point is, is whether the anti-wrinkle is under the purview of the cosmetics. I think our bigger conundrum for us, as the Expert Panel for Safety of Cosmetic Ingredients, is that we now recognize there is a potential biological effect at ten percent, we don't know what the no observed effect level is. And so, even in cosmetic use, at cosmetic concentration, we don't know where there's no effect for that biological effect. That's more of my conundrum right now.

**DR. BELSITO:** But we -- do they --

**DR. KLAASSEN:** But Paul, Paul --

**DR. SNYDER:** Yeah.

**DR. BELSITO:** Go ahead, Curt.

**DR. KLAASSEN:** Doesn't it say that this wasn't even a statistically significant effect at ten percent?

**DR. BELSITO:** It did.

**DR. KLAASSEN:** Okay. So therefore --

**DR. SNYDER:** Well, but you have to go to page -- there's another Reference 20 under the Other Clinical Reports. It said at the bottom line of the first paragraph, it said the ten percent Acetyl Hexapeptide --

**DR. BELSITO:** What page, Paul? What PDF page?

**DR. SNYDER:** Page 20.

**DR. BELSITO:** Okay.

**DR. SNYDER:** Other Clinical Reports, the last sentence of that first paragraph says, ten percent of acetyl hexapeptide-8 amide decreased the depth of skin wrinkles by 20 percent by day 15 and 30 percent by day 30.

**DR. BELSITO:** Well, we don't know -- I mean, that was the chat message before. We don't know the mechanism of that; it could simply be hydration.

**DR. SNYDER:** Yeah. But again, we don't know.

**DR. LIEBLER:** Well, of course, I (audio skip) in the contralateral side without peptide should have had the same thing. And it apparently didn't from the description here. The oil and water emulsion containing the peptide decreased the depth of skin wrinkles. So they evidently did have a control, a vehicle control.

**DR. BELSITO:** Yeah. So the vehicle by ten percent and the "Acetyl Hexapeptide-8 Amide" by 20 percent day 15, 30 percent by day 30.

The next one talks about transepidermal water loss. So it could all be just hydration at that level. Because it says, when compared to the placebo group, statistically significant decrease in transepidermal water loss was observed. But then at 60 days the decrease was not statistically significant. Makes no sense. But clearly, people have been playing with this ten percent in creams, based upon this other data that we're getting. And then now I'm conflicted here.

**MS. FIUME:** Do the current concentrations of use that are reported, and the penetration data in the report, give you any comfort level as to for what's reported for the cosmetic uses based on the survey?

**DR. BELSITO:** The point of -- no, obviously, Monice, because we're going safe as used. I've got to go back -- again, I saw this email at ten o'clock last night and didn't really have a chance to completely review this report. In fact, we don't really have ADME do we? We just assumed, looking at the molecule, that it wouldn't penetrate. There's no absorption distribution metabolism here.

**MS. FIUME:** No. There's dermal penetration data on PDF page 17, but correct, no ADME.

**DR. BELSITO:** Where's the penetration data?

**DR. SNYDER:** PDF page 18, Don, it goes 17 to 18.

**DR. BELSITO:** I see. Skin penetration -- what was the level that they used here?

**DR. SNYDER:** They used 0.05 percent. If you go to the top of page 18, it's the top paragraph there. They used 0.05 percent. It said there was a total content of peptide, and receptor reservoir was 30 percent of the amount that was deposited.

**DR. BELSITO:** Yeah. So it would be -- it permeated more rapidly and significantly higher from a multiple water and oil -- water and oil and water, and oil and water emulsions.

**DR. LIEBLER:** Yeah. This is the study I flagged last time we talked about this report. Because they just described the quantification using what they call high-performance liquid chromatography. And that's a really inadequate description of how you would measure a peptide, in a solution that contains probably other peptides leaching out from the skin preparation that was studied.

And I actually looked at the paper, and they provided no further description. So the way that you detect the peptide, that when you do HPLC or high-performance liquid chromatography, it's all a difference. And I had no assurance that this assay wasn't subject to lots of interference from other things.

I actually found it inconsistent with the studies (inaudible), which failed to detect the peptide penetrating. And then, the one in the first paragraph on PDF 17, under Dermal Penetration, used a much more reliable method, LC-MS. So they are able to very specifically detect that peptide.

**DR. BELSITO:** What page are you on there, Dan?

**DR. LIEBLER:** I'm at the top of PDF 17, under Toxicokinetic Studies, Dermal Penetration In Vitro. That first paragraph. It looked like there was a statistically significant difference but, only from the multiple water and oil water emulsion, and oil and water emulsion. But the amount of material that apparently penetrated is very small. So this is consistent with what we would expect for a peptide applied topically. There's going to be some effect of the other components of the solution, whether it's oils or detergents. And in this study they used both.

So anyway, I think this is consistent with our expectation that a peptide would have relatively little penetration of the skin. And that last paragraph, that seemed to suggest there was a lot, I don't trust the assay. And the description provided doesn't provide significant description to allow us to trust the data.

**MR. JOHNSON:** I just have a --

**DR. LIEBLER:** Go ahead, Wilbur.

**MR. JOHNSON:** Yeah. I have a comment. Thank you. Looking at the first paragraph of the discussion, relating to skin penetration, do you consider that information to still be valid?

**DR. LIEBLER:** First paragraph on PDF 17?

**DR. BELSITO:** No.

**MR. JOHNSON:** No. The second section.

**DR. LIEBLER:** Sorry. Say that again, Wilbur?

**DR. BELSITO:** Discussion --

**MR. JOHNSON:** PDF page 21, the first paragraph of the discussion.

**DR. LIEBLER:** I'm going to it now.

**MR. JOHNSON:** Okay.

**DR. LIEBLER:** That paragraph's still fine, Wilbur.

**MR. JOHNSON:** Okay. Thank you. And it doesn't need to be amended in any way, Dr. Liebler?

**DR. LIEBLER:** No.

**MR. JOHNSON:** Okay. Thank you.

**DR. SADRIEH:** This is Nakissa. I just have a question. The study that looked at the various skin models, the pig, the cadaver, and the cosmetic surgery skin, they all used the same HPLC method? I guess I'm asking the question because, you know, the 30 percent that was absorbed in the cosmetic surgery skin is clearly more than the others, but I'm just saying that, you know, is the same detection method used to quantitate the amount of absorption?

**DR. LIEBLER:** No. Nakissa, no. It appears this is a separate study with a different method. And this is the one that I described as being inadequately described. But if it was just HPLC, it just doesn't tell you what the detection method is. HPLC is simply the separation of the peptides going into some type of detector.

The paragraphs above that refer to mass spectrometry as the detection method, which is really the state of the art. Whereas that last study it's not clear what they were doing. And it could have been UV-Vis detection, in which case it would have been completely nonspecific. But it simply doesn't say.

**DR. SADRIEH:** Thank you.

**DR. LIEBLER:** But that's the one that suggested that a lot of the peptide got through. And I don't think that conclusion is reliable.

**DR. SADRIEH:** Thanks.

**DR. LIEBLER:** Sure. So, I'm kind of where Paul is here. I think the problem is we've got this report -- we've got these reports that we can't ignore of a biological effect in humans, and we have a very high use concentration relative to everything else we have, relative to what's reported. But we know that there are products that may be considered cosmetics at the end of the day. And we have no -- we don't have a NOEL. And we're stuck with that inconvenient fact.

**DR. SADRIEH:** And so, I think that the -- and the Wang paper, that's the one that looked at the type I and the type III collagen. And that's the one that found a statistically significant effect, right?

**DR. BELSITO:** At the end of the day it was not significant I think, right?

**DR. SADRIEH:** I think that the table kind of indicates that it might be, because they put an asterisk. But I'm not sure, maybe it's not.

**DR. LIEBLER:** I'm just reading from the language in the report here, the bottom of PDF 19. It says, when compared to the age model group, the skin of mice treated with the peptide was thicker, and greater number of collagen fibers, fibers more dense and compact. The difference between the age model group and placebo was insignificant. So they describe what was observed, but it was insignificant, probably because it's really hard to measure that well enough to establish differences.

**DR. BELSITO:** But then they go on, Dan, and they say, additionally when compared to the age model group, type I collagen fibers increased, and type III collagen decreased, in the Acetyl Hexapeptide-8 Amide treatment group.

**DR. LIEBLER:** That's correct.

**DR. BELSITO:** And those changes were significant.

**DR. LIEBLER:** Yeah. They apparently are given the p-values.

**DR. SADRIEH:** That's how I read the table.

**DR. LIEBLER:** So you get sort of mix of significant and insignificant findings.

**DR. SNYDER:** The age model group is not the treatment group. It's the model they used, and then the model got placebo or the model got the treatment. So that's not saying that it's insignificant with the treatment group -- the test article. That's the way I read that.

**MR. JOHNSON:** I have a question. Is the anti-wrinkle effect considered a toxic effect?

**DR. SNYDER:** No.

**DR. BELSITO:** No. It would be a beneficial effect, but if it's biologic, it's not cosmetic. If it's hydration that makes the appearance of wrinkles less, then that would be a cosmetic effect, right? It would not be a drug effect, rather, it would be in line with cosmetics that hydrate.

**DR. SADRIEH:** Right. So the claims make it a cosmetic. But the effect on the structure and function, that would be a drug manifestation, basically, drug effects.

**DR. BELSITO:** Okay. Well then, could we use this paragraph to say that data indicate that at ten percent there may be a drug effect to this and that -- in the discussion?

**DR. LIEBLER:** I think we could do that.

**DR. BELSITO:** And then really strengthen the discussion to say we're looking at this as reported in cosmetics, where the maximum concentration is 0.005 percent.

**DR. LIEBLER:** Right.

**DR. BELSITO:** And that data would seem to indicate that in higher concentrations, that it may actually have a drug effect by enhancing type III collagen.

**DR. LIEBLER:** So Wilber, this would be a new paragraph, a new second paragraph for the discussion.

**MR. JOHNSON:** Okay.

**DR. SNYDER:** And I think that discussion needs to be beefed up, Dan, by you regarding the HPLC methodology versus the mass spec methodology, because we do have that one part where it says 30 percent penetration. And you're thinking that's way, way, way overstated.

**DR. LIEBLER:** I think it's likely -- yeah.

**DR. KLAASSEN:** I agree.

**DR. LIEBLER:** I'll write language into this, Wilbur. I'll add that to the end of the first paragraph.

**MR. JOHNSON:** Okay.

**DR. BELSITO:** And are we going to be able to get this back by tomorrow so that we can make it final, or are we going to table this and look at it again?

**DR. LIEBLER:** I think we can bring this up, bring this to the full panel meeting tomorrow. I'm going to write this right now.

**DR. BELSITO:** Okay. So David's reporting in full session, so it will be fun. Okay. So Dan, you'll work with Wilbur to beef up the discussion?

**DR. LIEBLER:** Yeah.

**DR. BELSITO:** Okay. Wilber, you're okay with that?

**MR. JOHNSON:** Yes. I am, thank you.

**DR. BELSITO:** Okay.

**DR. LIEBLER:** So Wilber, you take the lead on the skin ten percent, I'll take the lead on the analytical stuff on skin penetration, okay?

**MR. JOHNSON:** Okay. Sure.

**DR. BELSITO:** Okay. So Wilbur's going to add the ten percent in the apparent biological effect and, Dan, you're going to do the penetration?

**DR. LIEBLER:** Yes.

**DR. BELSITO:** Anything else on this? Okay. Hearing nothing, I think we've beaten this horse dead. He has no more collagen left. Okay. So we're moving on --

**DR. SADRIEH:** I will leave now and go to the other team if that's all right then?

**DR. BELSITO:** Yeah. And thanks for your support, Nakissa.

**DR. LIEBLER:** Sure. Thanks, Nakissa.

**DR. SADRIEH:** Thank you very much. Okay. Bye bye.

**DR. BELSITO:** Bye bye. Let me save my comments here.

**Cohen Team – December 7, 2020**

**DR. COHEN:** And this is Wilbur's. And this is a draft final report passing through from draft report. So the Panel last reviewed this in September, and we had a reported max use of 0.005 percent. We had clarity on the multiple nomenclatures used, and the amide was settled upon.

And then we received a late-breaking correspondence from Women's Voices for the Earth, indicating real use concentrations that were much higher than the max use we had listed. This appearing in anti-aging cosmetics labeled with argireline, which is Acetyl Hexapeptide-8. Some of these examples given showed Acetyl Hexapeptide-8 listed as the second or third ingredient, and in our own report describes the use of it a ten percent cream. So I could use some help from the group on how we proceed from here.

I guess, Bart, if I can ask you to comment on how to handle this information and what might the next steps be.

**DR. HELDRETH:** Yeah, this is a comment that we run into when we're looking at concentrations of use in ingredients in cosmetic products. Typically, we consult with FDA's Voluntary Cosmetic Registration Program, and as well as concentrations of use that we get directly from the Council for their survey.

One of the issues we have with looking at concentrations of use that you get by looking at, you know, different online retailers, or even going out in the market, is we don't know just by looking at what we've received is this a cosmetic or is this an over-the-counter drug? And, if an FDA official is on the line and able to speak to this matter, I'm sure they'll have more expertise than I. But my understanding is, if these products are sold saying that they will actually reduce wrinkles, then these products would fall outside the scope of this Panel as being drugs instead of cosmetics.

So that's why these numbers differ from what we have in our report, because we are not confident that we can say that these are cosmetic products versus drugs.

**DR. SLAGA :** Drug.

**DR. COHEN:** And any comments from the group or from -- is FDA on? Can they comment on that?

**DR. SADRIEH:** Yes. Hi. This is Nakissa Sadrieh. I'm the FDA representative. So, yes, I think I participated in this discussion in the other team as well.

Just to clarify, it's a claim that makes the product a cosmetic -- or makes it a cosmetic or a drug. And anti-aging technically could be considered a drug claim because it is assumed that it affects the structure and the function of the body. However, the FDA doesn't just regulate based on a single claim. We look at the labeling in its totality. And what constitutes labeling for product is what's on the label of the package, but also what's on the company's website. The information that's provided on the website, testimonials, and other things about how the products works and what the manufacturer believes the kind of effects of the product is going to be.

Again, what's in a magazine, that is not considered labeling, that's advertisements. So, what is associated with the sale of the product -- meaning webpage -- and other types of promotional material that is in close contact with the sale of product, that would be considered labeling. So the FDA regulates products based on claims. And so, if the product makes purely cosmetic claims, then it's a cosmetic. But, if there are drug claims, it becomes a drug.

The ingredients -- there isn't an ingredient that just makes something a drug versus a cosmetic, although there have been situations where just the presence of an ingredient was assumed to possibly give the product drug characteristics. I used the example of prostaglandin analogues in the other team, where those ingredients in eyelash enhancers potentially could make the product considered to be a drug.

However, I guess, in this case, we, the Voluntary Cosmetic Registration Program, VCRP, does not collect data on the amounts that are in products. And, clearly, the VCRP's not even comprehensive of all that's all on the market. So it's possible that there are products out that are not listed in VCRP. And also we don't know what the levels are. The question becomes whether data exists at the levels that are supposed to be in a product that lead to potentially drug-like effects. And drug-like effects means that it has an effect on the structure and the function of the body, and it has some sort of efficacy.

So if, for example, something causes, you know, collagen growth, that would be considered an effect on the structure and the function of the body. And then that would make an ingredient possibly a drug ingredient, depending on, obviously, the claims that the product is making.

So it's not -- you know, I'm not going to be able to give you an answer that, you know, at this concentration, this a drug, at this concentration, it's a cosmetic. It depends on a number of factors that have to be evaluated. The FDA regulates the product, but not the actual ingredient. The only ingredients that are evaluated pre-market are color additives. All other ingredients are not pre-market evaluated, and so they are allowed to be used in cosmetics unless they are proven by the FDA to be unsafe. So the

onus is on the FDA to prove harm in order to try to remove an ingredient and not to show safety -- or lack of safety. You know what I mean? And the company is not supposed to provide that information to FDA except for a color additive.

So I tried to give a quick kind of summary of FDA's sort of, you know, jurisdiction, and really how it regulates a product versus an ingredient and how you show safety or demonstrate harm.

**DR. COHEN:** So thank you.

**DR. SADRIEH:** But the bottom line is it's utterly impossible for me to say whether this ingredient is a drug or not, because it depends on how the product is marketed.

**DR. BERGFELD:** It states it's a skin conditioner, and by study showed that it decreased wrinkles. But we know, as a clinical investigator with retinoid products topically that went to the FDA, that the controlled placebo was a conditioner of skin, and it decreased wrinkles about 16 percent. That was a placebo. The active was in the area of about a 40 percent decrease. So I think that skin conditioning, on a whole, can reduce fine wrinkles, so I think this is a cosmetic use.

**MR. GREMILLION:** Could I ask --

**DR. COHEN:** So I have -- no, go ahead.

**MR. GREMILLION:** Thank you. Is there a database, analogous to the VCRP, for over-the-counter drug products that are products that FDA's made a determination that they are drugs? You know, maybe that would be one way to address that ambiguity that Bart highlighted.

**DR. SADRIEH:** I mean, even if -- first of all, I don't know of a particular database like that. I mean, there's a -- you know, you can search based on the active ingredients on the FDA's website under drug info. But the fact that something is a drug ingredient doesn't mean that the product -- the ingredient cannot be used in a cosmetic.

I mean, you know, we know that salicylic acid is used in cosmetics. We know that hydroquinone is used in cosmetics. We know that a lot of sunscreen ingredients are used in cosmetics, but they are not labeled to be used for the same purposes. So the sunscreen is not labeled to be used as a sunscreen. Salicylic acid is not used to treat ache on the product.

So just having an ingredient -- I mean, you know, as a toxicologist, I guess, you know, there is no ingredient that's not going to have an effect. At a concentration in a given formulation, everything is potentially going to have an effect. But the question is really, you know, is that an effect that's going to be harmful? And, you know, what level of risk one is willing to accept?

So I don't think that looking at a list of ingredient, that potentially might be a drug product, would help determine whether something is or isn't a drug. There are lots of ingredients in drugs that are safe when used in cosmetics.

**MR. GREMILLION:** Yeah. What I was thinking was looking at the products and seeing -- for example, this iQ Natural that's sold at Walmart, I think it's 30 percent concentration. Seeing if that product was in the list of drug products, then we would know that it's not reported to the VCRP because the manufacturer's considering itself a drug product, if that makes sense.

**DR. SADRIEH:** I mean, it still depends on what the claims are.

**MR. GREMILLION:** But it sounds like that is not like --

**DR. SADRIEH:** If the product --

**MR. GREMILLION:** Yeah.

**DR. SADRIEH:** -- just says that it's 50 percent something without making any claims. Because a lot of people that sell products that just say, you know -- I don't know -- five percent retinoic acid, they don't say anything else. I mean, there's no claim. So the question is it's not even a cosmetic. It's not a -- you know, it's just -- what is it?

**DR. COHEN:** So I couldn't find that 30 percent one that -- it seemed like a dead link. But for the 20 percent product, on the site, there's a claim, "It eliminates fine lines and wrinkles." And the ten percent one, they used the term "controlled" studies. So, I mean, I think those are getting close to something that's not a cosmetic.

But, I guess, the issue that is just more obvious here is that the max use concentration, and the concentrations we're talking about in this safety assessment, is at 0.005 max use, and these other products are coming in multiple orders of magnitude above it. So I think it's at least a discussion point that we need to discuss. And, again, I think, just administratively, in our clinical studies, in a portion of our report, we talk about ten percent. So (audio interference) we move on, and then I'll ask our group if there's other comments as well.

**DR. BERGFELD:** So David where are we now? Are we in the discussion area of what we're going to discuss?

**DR. COHEN:** I think this goes in the discussion.

**DR. BERGFELD:** Yeah.

**DR. COHEN:** I think it goes in the discussion. The issue is there's such a large difference between our safety assessment, in what we looked at, and what's out there in products. I mean, again, this is me not sometimes understanding the mechanics of this. But this looked like a large difference that we need to at least address in the discussion and make very clear.

**DR. HELDRETH:** Yes. If I may interject here, you know, we often come across these sorts of information sources, like Wikipedia or online retailers listing that they have such ingredients in certain products. And there's just no clarification possible to know is this a drug product, is this a cosmetic, is this some other sort of classification?

And so, instead of following those sources of information, historically CIR has depended on the Council's concentration of use survey of the manufactures who make these products. And that's the concentrations that we provide in our table. And so then that typically is what the Panel will base their assessment on is those max concentration of use.

But when we get reports like this one from Women's Voices for the Earth, where we have no way to vet what type of product this is, it usually falls outside of the scope of the Panel, since we really don't know what to do with this information. Alex, did you want to add to that?

**MS. KOWCZ:** Yeah, Bart. I just wanted to add to your point because I have worked with this material in my past life. And it is an extremely expensive material. I would probably ask Carol if we can go back and find out, you know, where this ten percent is coming from. Because it maybe -- I think, it's a zero number, because to me, it sounds like it would be ten percent of the raw material itself, or maybe ten parts per million. But there's no way it is using ten percent of this material in a product.

So we can try to find out a little bit more information, Bart. That's just my -- as I'm sitting here listening to everything, I just don't think that this could possibly be used at that high of a level. But we can find out for the next meeting, if that would help at all. And you're right, we go only by what our concentration of use surveys told us, and it is definitely 0.005 percent that we have found the highest usage of. Thank you.

**DR. COHEN:** So that would be a great question for discussion.

**MR. GREMILLION:** The Walmart -- sorry. If I could point out the product that sold at Walmart which -- and Alex, I'd be curious whether this -- iQ Natural is the company -- if they're a member of the Council. But it says 30 percent Lipotec Argireline.

**MS. KOWCZ:** They are not. They are not. Yes, Argireline is the hexapeptide. That's the actually raw material. So that's also -- it's produced by Lipotec, a Spanish Company. So we're pretty familiar with the material itself. And I know it has two different names, but it's really the acetyl hexapeptide.

So that is the material, Thomas. You're absolutely right. But I find it very hard to believe that it would be there at such an extremely high level. So we'll try to find out.

**DR. BERGFELD:** (inaudible) FDA.

**MR. GREMILLION:** Yeah, I guess -- I mean, that --

**MS. KOWCZ:** And I think as Nakissa said before, from the FDA, it really has a lot to do with the claim. It's just we'll try to find out as much as we can, but they are not a member of the Council, so it would be pretty difficult for us, Thomas.

**DR. BERGFELD:** I think that it would be appropriate to send a letter or a note to the FDA, regarding this question and toss it over to them. I don't think this belongs in our purview.

**MS. KOWCZ:** Thanks, Wilma.

**DR. COHEN:** So, our group, Lisa, Ron, Tom, comments, or thoughts?

**DR. SHANK:** Well, what should we do? Table this until we get clarification about higher use concentrations?

**DR. HELDRETH:** I don't think we'll ever get (audio interference).

**DR. SHANK:** The report as is sounds good to me.

**DR. HELDRETH:** -- on all products in the market.

**DR. SLAGA :** (inaudible).

**DR. BERGFELD:** Can't hear you, Tom.

**DR. COHEN:** I don't know if that's Tom talking to us or somebody else.

**DR. HELDRETH:** I think he was talking to someone else at the moment.

**DR. COHEN:** Ah.

**DR. BERGFELD:** Tom is muted.

**DR. HELDRETH:** I don't think we'll ever get --

**DR. COHEN:** Thomas, any comments on what we've heard? Lisa?

**DR. PETERSON:** Well, it sounds very complicated, and I guess I like the idea of, you know, if this an FDA issue of a product on the market, which, you know, they're not following -- yeah, I'm not a hundred percent sure. It doesn't, from my listening of the conversation, it doesn't necessarily seem to fit within our purview, but perhaps another agency's purview. That's sort of how I'm reading it.

But I also -- to Ron's point, I mean, there might be some value in just tabling this until we can get some better clarification of the issue.

**DR. BERGFELD:** Excuse me. Are you wanting clarification of the actual concentrations used, questioning the FDA report of 2015? Or are you just asking -- what?

**DR. PETERSON:** I think I'm just --

**DR. BERGFELD:** Because I think we have enough information here on what we have. And I think that the higher concentrations are not our issue unless --

**DR. PETERSON:** Okay. Yeah, I will take you at that, that if it's our issue, then I think we can move forward.

**DR. BERGFELD:** Right.

**DR. PETERSON:** And I'm going to follow your lead, because that's what my level of experience. But it makes sense to me, that if it's not part of our purview, it doesn't -- you know, we can only act on the information given to us by --

**DR. BERGFELD:** Well, maybe --

**DR. COHEN:** All right. Well, but the question is --

**DR. PETERSON:** -- made by the -- you know, do you do normally --

**DR. COHEN:** Go ahead.

**DR. BERGFELD:** Lisa?

**DR. PETERSON:** Well, I think if it's not part of our purview, then we can go forward. And, to me, that's the judgment of others, not my judgment, because I'm just too new to the game. And it makes sense to me to say that it's not part of our purview. But the issue should be dealt with, so I think it should be forwarded to the appropriate organization.

**DR. COHEN:** I suppose, my only lingering issue is, do we have reasonable certainty that these are not cosmetic products that do fall under our purview? I agree that the report, as is, is very sufficient. But these other products sold over the counter may in fact be drugs, but it's not clear. And I don't know if what we've heard just now has clarified that. I'm okay with it being in the discussion and moving forward.

**DR. BERGFELD:** Well, I think what I've --

**DR. COHEN:** Go ahead, Wilma.

**DR. BERGFELD:** I think what you've heard from the FDA is that this group hasn't volunteered to tell what they are. We don't know who they are. And so, they're out there practicing and they're putting forth a drug and selling it as a cosmetic with no claim. So we can't get into that. We're not a policing group.

**DR. COHEN:** Okay.

**DR. BERGFELD:** That's the FDA.

**DR. SHANK:** I agree.

**DR. COHEN:** Could I --

**DR. SHANK:** Can we change the conclusion a little bit? The conclusion we usually say, safe as used under present practices of use and concentrations. Maybe in this case, we should specify a limit --

**DR. BERGFELD:** Within this document.

**DR. SLAGA :** -- a maximum limit on the concentration, which is covered by the report.

**MR. GREMILLION:** I was going to make a similar point just to -- if the wording is going to be safe as used, I think the report should make clear, you know, to recognize these other products are out there, and they're excluded from that conclusion.

**DR. BERGFELD:** Well, we don't want to go there, Tom. We do not want to go there.

**MR. GREMILLION:** I think otherwise saying, safe as used is --

**DR. BERGFELD:** You can put the concentrations in the discussion. Those that --

**MR. GREMILLION:** Yeah, well, I mean, these aren't products --

**DR. BERGFELD:** In this doc- --

**MR. GREMILLION:** -- you know, being sold by a small vendors, these are large retailers. And I think just reading, "safe as used," would lead a lot of people to think, well, it's safe as used on the market where sold by large stores like these that are selling these. So I don't know how you would go about just kind of acknowledging that some of these things are out there, but I think otherwise, "safe as used," it could be misleading.

**DR. HELDRETH:** That's why our conclusion is safe as used in the present practices of concentration and use, as described in this discussion.

**DR. BERGFELD:** Well, if you put it in your discussion --

**MS. KOWCZ:** This is Alex.

**DR. COHEN:** Go ahead. Our skin sensitization data's at 0.05 percent. So, in the report, there's a discussion of ten percent. So, when we are describing safe as used in present practice, what message are we sending?

**DR. HELDRETH:** So typically, when we mean "present practices of use and concentration as described in this report," we looked at the worst-case scenarios that are in our concentration of use table. Because those are the maximum use concentrations that were reported to us, by manufactures, as the highest concentrations they put in their cosmetic products.

So our conclusion, when we say, "safe as used under present practices of use and concentration described in this safety assessment," it means no higher than those concentrations in those specific product categories.

**DR. COHEN:** Okay.

**DR. HELDRETH:** So, if we looked at PDF Page 23, under concentration of use, the leave-on max concentration, it ranges from 0.00005 to 0.005. So a conclusion of safety in this report would only cover up to that 0.005. If people are really putting products on the market and putting ten percent in their product, this conclusion does not apply to those products.

**DR. COHEN:** Yeah. I can agree. I agree with that. I'd like to see the discussion cover this issue though.

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

**DR. COHEN:** Is that reasonable?

**DR. HELDRETH:** Yeah, we can say that. You know, we received information about other products that are on the market, but it's unclear what the characterization is for these products. And, if this concentration is really being used, then this conclusion doesn't apply to those products, because we just don't have enough information about those products.

**DR. COHEN:** So again, administratively, if this is a draft final report, and tomorrow we approve it as safe as used in present practice and concentration, how do we get sort of another crack at looking at the discussion?

**DR. HELDRETH:** This would technically be our last crack at contributing to the discussion, and hammering out what you want to see in that discussion. So, if you have specific language you want Wilbur to include in that discussion, certainly now would be the time to initially present it, and tomorrow make sure that everything is given to him.

**DR. COHEN:** Well, I think I would articulate it as that we received information about --

**DR. BERGFELD:** Well, there is another option. It can go out.

**DR. COHEN:** Yeah, Wilma. Were you going to say something, Wilma? You said it can go out?

**DR. BERGFELD:** I forgot what I was going to say. It was important though.

**DR. COHEN:** Yes, you said it could go --

**DR. BERGFELD:** Oh, yes. I'm sorry. Thank you. It still can go out as a final after you draft the discussion. Because if you change it on the next round -- when you go to finalize the final, if you change it again, it has to go out again for another 60 days. If it's deemed editorial, it doesn't. So there is another option.

**DR. HELDRETH:** Yes, that's correct. If you feel like the changes to the discussion are so substantive, that you would like to see it go out for review, we can do that and issue another draft report.

**DR. COHEN:** I think it's sufficiently vague right now, and that maybe we would want to get some further input on this one. I'm happy to take advice from more experienced members on this. This has to go into the discussion somehow, and it's rather perfunctory to the problem.

**DR. BERGFELD:** Well, we'll offer you some advice --

**DR. COHEN:** Wilma?

**DR. BERGFELD:** If you insist on putting it in, you could put it in.

**DR. COHEN:** Wilma?

**DR. BERGFELD:** If you insist on putting it in, you can put it in as a couple of sentences just to show that you are aware of these products. But you deem the fact that this is not something that we were able to review. I think you had some better sentence -- previous discussion here. And add it to the already two paragraphs that are in that discussion, which describe the concentrations --

**DR. COHEN:** Yeah.

**DR. BERGFELD:** -- which can be added, and also inhalation. But I think we can move forward with this.

**DR. COHEN:** Okay. Okay. I think I'm comfortable with that.

**DR. BERGFELD:** No, we're not a policing agency.

**DR. COHEN:** No. No. I appreciate that. I really do. Lisa, Ron, Tom, are you okay with us proceeding, and a line or two will go in regarding that discussion of the information we got?

**DR. SHANK:** Yes.

**DR. SADRIEH:** I just have a question. This is Nakissa again.

**DR. BERGFELD:** Yep.

**DR. SADRIEH:** So, I mean, when you say you're not a, you know, policing agency, are you saying that at levels higher than the levels that you're saying are safe, that it's not safe?

**DR. SHANK:** No.

**DR. SADRIEH:** Because I mean, I think, you know, we need to be clear on if there's a level at which it's not considered safe, or that there is data to show safety.

**DR. HELDRETH:** When the Panel issues a safety assessment conclusion that says, "safe as used in the present practices of use and concentration as described in the safety assessment," they're not saying other uses or concentrations are unsafe. The conclusion just simply doesn't apply to those other uses. And that's, particularly, I think, appropriate in this case since we don't have clarity on those other products that have been submitted to us.

**DR. COHEN:** Yeah.

**DR. SADRIEH:** Thank you.

**DR. COHEN:** Okay.

**MR. JOHNSON:** Excuse me, Dr. Cohen. This is Wilbur Johnson.

**DR. BERGFELD:** They represent a sneaky group coming in with --

**MR. JOHNSON:** Yeah. I'd like to know, specifically, the language that should be included in the discussion.

**DR. COHEN:** We might need to edit this a little bit, but information was provided to the Panel regarding the use of over-the counter-products that contained higher concentrations of Acetyl Hexapeptide-8 Amide (audio skip) than is listed in the table of frequency in concentration of use. And, Wilma, help me if I'm hitting any live rails here. It is unclear whether these are over-the-counter drugs.

**DR. BERGFELD:** I think that you have to be more generic than that.

**DR. COHEN:** Okay. Can you recommend something?

**DR. BERGFELD:** I think it has to be, the Panel's been made aware that there may be formulations for public consumption that are at higher levels; however, details are unknown regarding these products. And this report does not reflect information on these products. Something like that.

**DR. COHEN:** Yeah, that's what I was going to say, and this report doesn't reflect that.

**DR. BERGFELD:** This has to be very simple, that you're aware, but you didn't act.

**DR. COHEN:** Yes.

**DR. SHANK:** Mm-hmm.

**MR. JOHNSON:** Well, Dr. Bergfeld, is there any need to have a reference for the higher concentrations that have been identified? For example, this product that, you know, supposedly contains 10 percent to a 30 percent Acetyl Hexapeptide-8?

**DR. BERGFELD:** Do we have a reference? We have a ten percent patch test.

**DR. COHEN:** No. We just have the letter.

**DR. BERGFELD:** We have Tom who went to Walmart.

**DR. COHEN:** No, we have the letter with the with the hotlinks.

**DR. HELDRETH:** I think what Wilbur is trying to get at here is, if we put something in the discussion that's not in the body of the report earlier on, do we need to have a citation next to it? Typically, we do not put citations in our Discussion section.

**DR. BERGFELD:** Right.

**DR. HELDRETH:** So, at least my opinion -- but it's the Panel's call -- is that it doesn't need a reference.

**DR. BERGFELD:** No, but we have now is hearsay. I just think we've been made aware of it.

**MR. JOHNSON:** Okay. Okay. Thank you. That works.

**DR. BERGFELD:** But they're may be other public -- there may be other products at higher concentrations available to the public; however, information on these are not available.

**MR. JOHNSON:** Okay. Thank you.

**DR. SHANK:** Maybe that should be put in the Use section of the report and not in the discussion.

**DR. BERGFELD:** That could be. Good idea.

**MR. JOHNSON:** Okay. But without a reference?

**DR. BERGFELD:** No, without a reference.

**DR. SHANK:** Well, if you can find a reference.

**DR. BERGFELD:** We don't have a reference.

**DR. SHANK:** Yes, but --

**DR. BERGFELD:** Go to Walmart.

**DR. SHANK:** But maybe put it in the Use section of the report, not in the discussion.

**DR. BERGFELD:** I like that.

**DR. HELDRETH:** Yeah. If you put it in the Use section instead of the discussion, we could cite this letter.

**DR. SHANK:** Okay.

**DR. COHEN:** You know there is a line in the Non-Cosmetic section, in the absence of any published information, that Acetyl Hexapeptide Amide is an approved drug. It should be noted that studies relating to the potential drug use of this peptide are available. But it's not approved as a drug in the U.S. So the question is does it go into use under the Non-Cosmetic part or the Cosmetic part?

**DR. BERGFELD:** Well, it doesn't make a claim, so you don't know, do you? It's probably in skin-aging area product line.

**DR. COHEN:** I don't want to be the one adjudicating those claims right now.

**MR. GREMILLION:** So the products sold at Walmart says it smooths wrinkles, hydrates, and plumps skin. I don't know if that adds anything to the discussion.

**DR. BERGFELD:** Mm-hmm.

**DR. HELDRETH:** So smoothing wrinkles would indicate a change in structure.

**DR. BERGFELD:** All moisturizers do that.

**DR. COHEN:** So perhaps we keep it in the discussion.

**DR. BERGFELD:** So it would go under Cosmetic.

**DR. COHEN:** You want it under Cosmetic, under Use?

**DR. BERGFELD:** Yeah, I think it goes under Cosmetic. I don't think we should make a big deal of it, because it's really out our purview.

**DR. PETERSON:** I think that's a --

**DR. COHEN:** Okay. And we'll send a letter to FDA on it?

**DR. SHANK:** Yes.

**DR. BERGFELD:** I think we can just send a note over to FDA, saying that we've been made aware of this, and that we've shifted the responsibility for follow up to them.

**DR. COHEN:** Okay.

**DR. HELDRETH:** Dr. Bergfeld, alternatively, since we're really not clear if this falls in the cosmetic or non-cosmetic, we could just add a sentence in the introduction saying that we received reports on this, but we don't have enough information to proceed.

**DR. BERGFELD:** Yeah. Where --

**DR. HELDRETH:** And that we don't have to classify it.

**DR. SHANK:** That's okay.

**DR. COHEN:** What do you mean, we don't have enough information to proceed? Proceed on what?

**DR. BERGFELD:** Okay.

**DR. HELDRETH:** We don't know enough about these products to include them in our safety assessment.

**DR. COHEN:** Are we saying that specifically?

**DR. HELDRETH:** Well, that's up to you. I mean, we had been talking for quite a while right now about whether or not these are cosmetic products, or whether they're drugs or, and I don't think --

**DR. COHEN:** We do know. Right.

**DR. HELDRETH:** -- we don't know.

**DR. COHEN:** We don't have enough information to proceed with their safety assessment, I suppose.

**DR. HELDRETH:** That's right. We don't have enough information to know whether or not to include them.

**DR. COHEN:** Okay. I'm very comfortable with that. All right. Any other comments?

**DR. PETERSON:** I think it's a good place for it.

DR. COHEN: Okay. Tom, Ron, any further remarks? Okay. Let's move on.

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**DR. COHEN**: Yes, so Acetyl Hexaeptide-8 Amide, this was reviewed by the Panel in September. We had some clarity on nomenclature settling on the amide. We also reviewed some late breaking correspondence regarding concentration of use in certain products. After substantial conversation about this, we concluded with a safe in present practice of use and concentration.

**DR. BERGFELD:** Is that a motion?

**DR. COHEN**: Yes.

**DR. BERGFELD:** And, is there a second?

**DR. SHANK:** Second.

**DR. BERGFELD:** Doctor -- Ron is seconding?

**DR. SHANK:** Yes.

**DR. BERGFELD:** Any further discussion, Dr. Belsito's team?

**DR. BELSITO**: Yeah, so we felt that we really could not ignore the data that was sent by Women's Voices for the Earth. We spoke with the FDA representative, and it really wasn't clear about where these products, where this ingredient was used from 10 to 30 percent, fell in terms of whether they were cosmetic products or not.

We have that information; we can't ignore it. Because if, in fact, it's being marketed as a cosmetic, and despite our report that the present practices of use are .008, we do have information that there are products out there at 10 to 30 percent, there're categories that we do not know.

So, we felt that we could go with a sufficient up to .008 percent in cosmetic products. But that we would need additional data looking at the biological effects of products containing from 10 to 20 percent, particularly on collagen synthesis. And Dan and

Wilbur drafted a little bit of information on that. So I'll let them do that because again I'm being bothered by clinic. Dan and Wilbur, do you want to --

**DR. COHEN**: Don, could we --

**MR. JOHNSON:** If you don't mind, please, I'll read the discussion.

**DR. BELSITO**: Yes, please.

**MR. JOHNSON:** Okay, the available in vitro and in vivo data indicate that Acetyl Hexaeptide-8 Amide may have drug activity, i.e. anti-wrinkle effect, by exerting an effect on type III collagen in the dermis at a concentration of 10 percent. However, the Panel recognizes that Acetyl Hexaeptide-8 Amide is being used in leave-on cosmetic products at concentrations up to 0.005 percent, and has the understanding that this drug effect on the dermis would not be likely at this low concentration. However, the Panel acknowledges that the drug effect may be apparent at higher use concentrations.

The Panel noted different degrees of reported skin penetration by Acetyl Hexaeptide-8 Amide with in vitro models. The Panel felt that studies that employed LCMS/MS, to measure the peptide, were most reliable, and noted that these indicated minimal skin penetration.

**DR. COHEN**: Don, we labored over the very same issues. And the question was that the VCRP had the max use concentration of 0.005, and our safety assessment was going to be based on the available data that we had. And it was unclear to us how these drugs were being marketed -- how these products were being marketed, perhaps even as drugs. And we came to a conclusion that we would send a note to FDA for their review of this issue.

**DR. BELSITO**: But in the meantime, David, that doesn't tell us whether they're being marketed as a drug or as a cosmetic product. And, we do have -- I mean, of course we -- this information in our report, but we also have confirmation from outside sources that would suggest that the levels that are being used are considerably higher than what we're looking at.

And we were not able to get clarification from the FDA yesterday as to whether these were being marketed, what was their claims for anti-wrinkle or biological effects. There was also some question as to whether this functioned merely as a hydrating agent and made the skin appear less wrinkled, in which case that would be cosmetic if it wasn't having a biological effect.

So, we just thought that we certainly could go sufficient at .005 percent. But at this point insufficient for concentrations that are higher, until we get a dose response effect, on this material, on collagen synthesis.

**DR. BERGFELD:** So, Don, what you're actually asking is possibly David consider rescinding his motion?

**DR. BELSITO**: Well, what I'm saying is that it's a split conclusion. It's safe --

**DR. BERGFELD:** Right, another conclusion.

**DR. BELSITO**: -- up to .005 percent. Right. But above that the data is, you know, incomplete and --

**DR. BERGFELD:** Right.

**DR. BELSITO**: -- insufficient data to determine the safety of this material above that concentration.

**DR. BERGFELD:** Dr. Cohen, can you respond?

**DR. COHEN**: Don, I like that. Yeah, I like that. We have a question from Thomas -- or a comment.

**DR. GREMILLION**: You know, actually, I think my comment may be superfluous in light of how this seems to be going, so I'll let you proceed.

**DR. BERGFELD:** Okay.

**DR. BELSITO**: Dan, did you have a comment?

**DR. LIEBLER**: No, I don't have any further comment. I think you captured our thinking in your comments, Don.

**DR. COHEN**: Lisa, Tom, Ron?

**DR. BERGFELD:** Wilbur has a comment.

**DR. COHEN**: Oh, I can't see it.

**DR. BERGFELD:** Okay, Wilbur has a comment.

**MR. JOHNSON:** Yes, Dr. Bergfeld, I would just like for the conclusion to be, you know, read as it should be stated in the report.

**DR. BERGFELD:** Well, first of all, Wilbur, we need to rescind the first motion. So, let us act on that first.

**MR. JOHNSON:** Sure, Thank you.

**DR. BERGFELD:** So, Dr. Cohen, you rescind?

**DR. COHEN**: Yes, I'll rescind my last motion and put in the new motion for safe -- sufficient in concentrations of 0.005 or less and insufficient for concentrations greater than that.

**DR. BERGFELD:** And that's the content of your conclusion, Dr. Belsito?

**DR. BELSITO**: Yeah, that will cover it.

**DR. BERGFELD:** That will cover it.

**DR. BELSITO**: And then in the discussion the insufficiency would be at what level this material has an effect on collagen types I and III.

**DR. BERGFELD:** So that would be a request in the discussion area, information needed? Yeah.

**DR. BELSITO**: Yeah, and it would be the insufficiency request.

**DR. BERGFELD:** So we have a motion that I'm going to assume has been rescinded, and one has been proposed. And do we have a second for the second proposal of the conclusion? David, are you seconding it then? Yes.

**DR. COHEN**: Well -- yes.

**DR. BERGFELD:** Yeah. And, is there any other discussion that we need to comment on, in the text or in the discussion?

**DR. HELDRETH**: I have one comment, Dr. Bergfeld.

**DR. BERGFELD:** Okay.

**DR. HELDRETH**: So, for the use section of our reports, typically we're referencing the Council's survey for concentrations of use. But it seems like in this case we have an alternative source for these higher concentrations of use. What would the Panel like Wilbur to reference for those higher concentrations of use in the use section?

**DR. BELSITO**: So, Bart, I think what I would suggest, at least personally, is that we have the current concentration of use in the tables as we have. And then add a paragraph that recently the Panel became aware that there were products being marketed that contained up to 10 to 30 percent. It was unclear as to the status of these, whether they were considered over the counter drugs or whether they were cosmetics. It was unclear whether they were acting by hydrating the skin or by actually having a biological function by effecting collagen synthesis. And, at this point, we felt that the data was not sufficient to determine whether it was a cosmetic or a pharmaceutical effect, and hence our split conclusion.

**DR. HELDRETH**: Okay. And then --

**DR. SNYDER**: We actually have two references. Reference number eight and reference number 20 are two different anti-wrinkle creams. Which clearly gives the concentration and show the biological effect on collagen. So, I think that suffices where our data came from to raise our concerns. So I think the discussion can capture those two references, irrespective of any data from the reporting program.

**DR. BERGFELD:** Paul, we usually don't put references in the discussion, so where else might you put that?

**DR. BELSITO**: Well, Wilma, these --

**DR. BERGFELD:** Yeah, I understand.

**DR. BELSITO**: -- were actually in the original report, and just, I think we all ignored them because we weren't looking at concentrations that high.

**DR. BERGFELD:** Okay. So we do have them within the text of the document?

**DR. BELSITO**: Yes.

**DR. BERGFELD:** Okay. So, any other points of --

**DR. HELDRETH**: Yeah, I just have one more point to add. So, if this becomes a consensus of the Panel to vote this, this would be a new restriction to the conclusion, and so this report would not come out of this meeting as a final, it would have to go out for public comment again. So it'll get revised and be a new tentative report and come back to the Panel again in a future meeting.

**DR. BERGFELD:** I think that's --

**DR. BELSITO**: That would be fine.

**DR. COHEN**: Yeah.

**DR. BERGFELD:** All right, any other -- if there's no other discussion then we'll call for the vote of a split decision. All those -- Wilbur?

**DR. PETERSON**: There are a couple of people with their hands waved.

**DR. BERGFELD:** I don't see them. Who are they?

**MR. JOHNSON:** Yes, Dr. Bergfeld, I just have a comment.

**DR. BERGFELD:** Go ahead.

**MR. JOHNSON:** Should there be any mention of data needed in order to complete the safety assessment, in that part of this conclusion relates to insufficient data to determine safety above 0.005 percent?

**DR. BELSITO**: Yes, I said that Wilbur. It would be a no adverse effect level in terms of collagen -- type III and type I collagen. So, at what level does this material have absolutely no effect on collagen synthesis?

**MR. JOHNSON:** You said type II and type III, Dr. Belsito?

**DR. BELSITO**: Type I and III is what was in the report.

**DR. BERGFELD:** Is there another question out there?

**MR. JOHNSON:** I and III, thank you.

**DR. BERGFELD:** Is there another comment by anyone? I only see Wilbur's hand up.

**MR. JOHNSON:** No, I took it down, Dr. Bergfeld.

**DR. BERGFELD:** Okay.

**DR. HELDRETH**: I see Dr. Sadrieh, from FDA, and Dr. -- yeah, just Dr. Sadrieh.

**DR. BERGFELD:** Okay.

**DR. SADRIEH**: Yes, hi, this is Nakissa Sadrieh from FDA. I just had a question. There was mention of a letter being sent to FDA. Is that still the plan? And I just wanted to know what the request will be from FDA in terms of providing information to help you finalize your report.

**DR. BERGFELD:** Dr. Cohen?

**DR. COHEN**: I think in light of the final vote we can hold off on that until we get further information. And then we can really look at that if we need to.

**DR. SADRIEH**: Okay, thank you very much for that clarification.

**DR. BERGFELD:** Thank you.

**DR. SADRIEH**: I also just wanted to mention that you said that you were looking at the levels in VCRP? VCRP doesn't have any levels listed in it. So, you know, usually you can get the concentrations of use from VCRP.

**DR. COHEN**: It's the max --

**DR. SADRIEH**: Yup.

**DR. COHEN**: Thank you for clarifying.

**DR. SADRIEH**: Thank you.

**DR. COHEN**: That's me getting use to the process.

**DR. SADRIEH**: So that's all right, yes. Thank you.

**DR. BERGFELD:** Don, do you have a question, Don? Your hand is up. No?

**DR. BELSITO**: No, if my hand is up I accidentally hit the hand button.

**DR. BERGFELD:** Okay. Anyone else wanting to make a comment that I can't see?

**DR. LIEBLER**: Unhand that button, Don.

**DR. BERGFELD:** I'm going to call the question then. All those in favor of a split conclusion on this ingredient -- please indicate your opposition first. Hearing none, it's unanimous, it passes.

# Safety Assessment of Acetyl Hexapeptide-8 Amide as Used in Cosmetics

Status: Draft Final Report for Panel Review
Release Date: February 16, 2021
Panel Date: March 11-12, 2021

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.; Daniel C. Liebler, Ph.D.; Lisa A. Peterson, Ph.D.; Ronald C. Shank, Ph.D.; Thomas J. Slaga, Ph.D.; and Paul W. Snyder, D.V.M., Ph.D. Previous Panel member involved in this assessment: James G. Marks, Jr., M.D. The Cosmetic Ingredient Review (CIR) Executive Director is Bart Heldreth, Ph.D. This report was prepared by Wilbur Johnson, Jr., M.S., Senior Scientific Analyst/Writer, CIR.



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**ABSTRACT**: The Expert Panel for Cosmetic Ingredient Safety (Panel) reviewed the safety of Acetyl Hexapeptide-8 Amide (synonymous with Acetyl Hexapeptide-8 (sans "Amide")) in cosmetic products; this ingredient is reported to function as a skin conditioning agent - miscellaneous in cosmetics. The Panel reviewed data relevant to the safety of this ingredient in cosmetic formulations, and concluded that Acetyl Hexapeptide-8 Amide is safe in the present practices of use in cosmetics at concentrations up to 0.005%. The Panel further concluded that the available data are insufficient to make a determination that Acetyl Hexapeptide-8 Amide is safe in cosmetic formulations at concentrations greater than 0.005%.

## INTRODUCTION

The safety of Acetyl Hexapeptide-8 Amide (synonymous with Acetyl Hexapeptide-8 (sans "Amide")), as used in cosmetics, is reviewed in this safety assessment. According to the web-based *International Cosmetic Ingredient Dictionary and Handbook* (wINCI; *Dictionary*), Acetyl Hexapeptide-8 Amide functions as a skin conditioning agent - miscellaneous.[1] While Acetyl Hexapeptide-8 Amide is synonymous with in-use name, Acetyl Hexapeptide-8, and both are included in the *Dictionary*, the following synonyms have been retired or deleted from the *Dictionary*: Acetyl Hexapeptide-3, Acetyl Hexapeptide-24, and Acetyl Hexapeptide-24 Amide. Since the name, "Acetyl Hexapeptide-8 Amide," is more descriptive and its definition more accurate, this name was chosen for use throughout the report (i.e., instead of Acetyl Hexapeptide-8).

In 2018, the Expert Panel for Cosmetic Ingredient Safety (Panel) published a safety assessment of tripeptide-1, hexapeptide-12, their metal salts and fatty acyl derivatives, and palmitoyl tetrapeptide-7 as used in cosmetics.[2] The Panel concluded that these ingredients are safe in cosmetics in the present practices of use and concentration, as described in that safety assessment. (This report is available on the Cosmetic Ingredient Review (CIR) website. https://www.cir-safety.org/ingredients) Though the peptide sequences in those ingredients that have been reviewed differ from the peptide sequence in Acetyl Hexapeptide-8 Amide, it is important to note that the Panel has evaluated the safety of ingredients in which a distinct peptide sequence is part of the chemical structure.

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 exhaustive search of the world's literature. A list of the typical search engines and websites that are used and the sources that are typically explored, as well as the endpoints that the Panel typically evaluates, is provided on the CIR website (https://www.cir-safety.org/supplementaldoc/preliminary-search-engines-and-websites; https://www.cir-safety.org/supplementaldoc/cir-report-format-outline). Unpublished data may be provided by the cosmetics industry, as well as by other interested parties.

## CHEMISTRY

### Definition and Structure

Acetyl Hexapeptide-8 Amide (CAS No. 616204-22-9, synthetic peptide also known as Acetyl Hexapeptide-8, Acetyl Hexapeptide-3, Acetyl Hexapeptide-24, and Acetyl Hexapeptide-24 Amide) is defined as the product obtained by the acetylation of hexapeptide-8, in which the C-terminus is an amide.[3] The sequence for this acetylated and amidated peptide is Ac-Glu-Glu-Met-Gln-Arg-Arg-$NH_2$ (acetyl group-glutamic acid-glutamic acid-methionine-glutamine-arginine-arginine-amino group).[4]

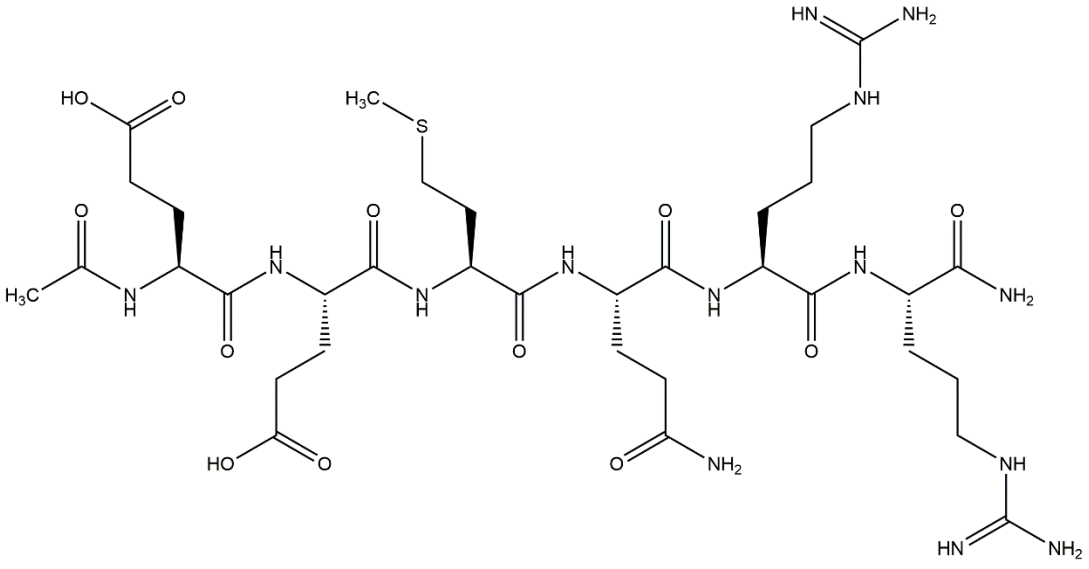

**Figure 1**. Acetyl Hexapeptide-8 Amide.

### Chemical Properties

Acetyl Hexapeptide-8 Amide is a white powder with a molecular weight of 889.1 Da.[4,5] It is soluble in water, and has a log P of -6.3.[6,7]

### Method of Manufacture

One method of manufacture of Acetyl Hexapeptide-8 Amide is via solid-phase peptide synthesis in which the 9-fluorenylmethoxycarbonyl group (Fmoc group) is used as a temporary protecting group for the *N*-terminus.[8,9] This ingredient has been also been synthesized by solid phase on a *p*-methylbenzhydrilamine resin; this allows the cleavage of the peptide amide in acid conditions with the concomitant deprotection of the side chains protection. The resulting peptidyl resin was treated at room temperature with a mixture of trifluoroacetic acid/thioanisol/water (95/2.5/2.5, v/v/v, 7 ml/g resin) for 2 h. The crude peptides were precipitated by filtration into cold diethyl ether and vacuum-dried. The crude product was dissolved in 10% acetic acid for de-*tert*-butylation at 60 ºC and then purified.

According to a manufacturer of Acetyl Hexapeptide-8 Amide, this ingredient is completely synthesized in the laboratory and no excipients, preservatives, or antioxidants are used during the manufacturing process.[4] Another source indicates that Acetyl Hexapeptide-8 Amide is synthesized in accordance with good manufacturing practice (GMP) guidelines, and involves a final freeze-drying step.[6] These freeze-dried products are commonly obtained as a polymorphous crystalline powder.

### Composition/Impurities

According to a manufacturer of Acetyl Hexapeptide-8 Amide, no excipients, preservatives, or antioxidants are present.[4] Furthermore, according to this manufacturer's product specification, Acetyl Hexapeptide-8 Amide is > 95% pure and contains < 5% water. Another manufacturer has stated that the peptide purity of Acetyl Hexapeptide-8 Amide is > 80%, and that the results of an amino acid analysis indicate the presence of glutamic acid (2.7 to 3.3%), methionine (0.6 to 1%), and arginine (1.8 to 2.2%).[6]

Furthermore, Acetyl Hexapeptide-8 Amide is supplied either as a powder or provided as a tradename mixture that is an aqueous solution containing 0.5 g/l of the powder (i.e., 0.05% aqueous solution; pure active peptide in solution estimated at ~0.56 mM).[5,6] A 0.05% aqueous tradename mixture also contains 0.3% phenonip, which is a broad spectrum preservative with the following composition: phenoxyethanol, methylparaben, ethylparaben, propylparaben, butylparaben, and isobutylparaben.[6] According to another source, a tradename mixture contains Acetyl Hexapeptide-8 Amide (0.5 g/l), phenonip (0.5%), and water (99.45%).[10]

## USE

### Cosmetic

The safety of the cosmetic ingredient addressed in this safety assessment is evaluated based, in part, on data received from the United States (US) Food and Drug Administration (FDA) and the cosmetics industry on the expected use of this ingredient in cosmetics. Use frequencies of individual ingredients in cosmetics are collected from manufacturers and reported by cosmetic product category in FDA's Voluntary Cosmetic Registration Program (VCRP) database. Use data are submitted by the cosmetics industry in response to surveys, conducted by the Personal Care Products Council (Council), of maximum reported use concentrations by product category.

According to 2021 VCRP data, Acetyl Hexapeptide-8 is reported to be used in 333 cosmetic products (311 leave-on and 22 rinse-off), and an additional 21 uses are reported with the name Acetyl Hexapeptide-3 (20 leave-on and 1 rinse-off; Table 1).[11] According to the *Dictionary*,[1] Acetyl Hexapeptide-3 is listed as a technical name for Acetyl Hexapeptide-8; therefore data for both of these ingredients are captured in the table. The results of a concentration of use survey conducted by the Council in 2019 indicate that Acetyl Hexapeptide-8 is used at concentrations up to 0.005% (in eye lotions and face and neck products; not spray), which is the highest reported maximum use concentration for leave-on formulations.[12] In rinse-off products, Acetyl Hexapeptide-8 is reported to be used at concentrations up to 0.000005% (skin cleansing products). The Panel is aware of products with higher use concentrations, but whether these products are used as cosmetics is not clear.[8,13]

Cosmetic products containing Acetyl Hexapeptide-8 may be applied to the skin or near the eyes at concentrations up to 0.005% (stated above). Acetyl Hexapeptide-8 also could be incidentally ingested during product use (e.g., use in lipsticks at concentrations up to 0.00025%). Products containing Acetyl Hexapeptide-8 may be applied as frequently as several times per day and may come in contact with the skin for variable periods following application. Daily or occasional use may extend over many years.

Acetyl Hexapeptide-8 is reported to be used in face powders at concentrations up to 0.0001%. Conservative estimates of inhalation exposures to respirable particles during the use of loose powder cosmetic products are 400-fold to 1000-fold less than protective regulatory and guidance limits for inert airborne respirable particles in the workplace.[14-16]

Acetyl Hexapeptide-8 is not included on the European Union's list of substances that are restricted or list of substances that are prohibited in cosmetic products.[17]

**Non-Cosmetic**

In the absence of any published information indicating that Acetyl Hexapeptide-8 Amide is an approved drug, it should be noted that studies relating to the potential drug use of this peptide are available. Even though Acetyl Hexapeptide-8 Amide is not currently approved for drug use in the US, a National Institutes of Health (NIH) study purporting the safety of a topical drug use (treatment of blepharospasm) has been published.[18] (These studies are included in the Clinical Studies section of this safety assessment.)

## TOXICOKINETIC STUDIES

### Dermal Penetration

#### In Vitro

The influence of different vehicles (multiple water-in-oil-in-water, oil-in-water, and water-in-oil emulsions) on the skin (porcine) penetration of Acetyl Hexapeptide-8 Amide was studied using Franz diffusion cells.[19] The composition of the multiple water-in-oil-in-water emulsion was described as follows: isopropyl myristate (20%), distilled water (75.99%), octyldodecanol and octyldodecyl xyloside and PEG-30 dipolyhydroxystearate (1.5%), and sucrose stearate (2.5%). Five parallel experiments for each formulation were performed. Porcine skin was cut with a dermatome set at 700 µm. Cut skin pieces were clamped between the donor and receptor chambers of the diffusion cells. The permeation area of the diffusion cell was 0.95 $cm^2$. The acceptor compartment was filled with 2 ml of 0.1% formic acid. An infinite dose (250 $mg/cm^2$) of Acetyl Hexapeptide-8 Amide (in emulsion) was applied onto the skin in the donor chamber. Samples (5 µl) for the analysis of permeated Acetyl Hexapeptide-8 Amide were taken after 2, 4, 6, and 8 h, and permeation was quantified using liquid chromatography with tandem mass spectrometry (LC-MS/MS). Acetyl Hexapeptide-8 Amide permeated more rapidly and to a statistically significantly higher extent from the multiple water-in-oil-in-water and the oil-in-water emulsions, while skin permeation of Acetyl Hexapeptide-8 Amide from the water-in-oil emulsion was undetectable. After 8 h, skin permeation was ranked in the order of multiple water-in-oil-in-water emulsion > oil-in-water emulsion > water-in-oil emulsion. A statistically significant difference ($p < 0.01$) between the cumulative permeated amount of Acetyl Hexapeptide-8 Amide after 8 h from the multiple water-in-oil-in-water emulsion (755 ± 149 $ng/cm^2$) and the oil-in-water emulsion (456 ± 120 $ng/cm^2$) was found.

In the same study, tape-stripping experiments using full-thickness porcine ear skin were also performed. The same emulsions were used, and 4 experiments for each formulation were performed. An Acetyl Hexapeptide-8 Amide emulsion (5 $mg/cm^2$) was applied and distributed with a saturated gloved finger, and the tape-stripping procedure was initiated after an exposure time of 1 h. After a residence time of 1 h, 46.7 ± 6.2 $ng/cm^2$ of applied Acetyl Hexapeptide-8 Amide penetrated into the stratum corneum from the multiple emulsion. The amounts that entered the stratum corneum from the oil-in-water and the water-in-oil emulsions were 24.7 ± 4.9 $ng/cm^2$ and 9.5 ± 2.1 $ng/cm^2$, respectively. Therefore, the multiple water-in-oil-in-water emulsion led to 4.91 ± 0.66-fold and 1.89 ± 0.25-fold higher skin deposition of Acetyl Hexapeptide-8 Amide than the water-in-oil and oil-in-water emulsion, respectively. The oil-in-water emulsion showed 2.61 ± 0.52-fold increased skin penetration of Acetyl Hexapeptide-8 Amide when compared to the water-in-oil emulsion. According to the results of these experiments, the penetration of Acetyl Hexapeptide-8 Amide from the different emulsions was in the order of multiple water-in-oil-in-water emulsion > oil-in-water emulsion > water-in-oil emulsion.

The skin penetration of Acetyl Hexapeptide-8 Amide was evaluated using hairless guinea pig skin and human cadaver skin assembled in in vitro diffusion cells.[20] The composition of the receptor fluid was: anhydrous calcium chloride (140 mg/ml), dextrose (1000 mg/ml), 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES, 5960 mg/l), magnesium sulfate heptahydrate (200 mg/ml), potassium chloride (400 mg/ml), monobasic potassium dihydrogen phosphate (60 mg/ml), sodium bicarbonate (350 mg/ml), sodium chloride (7000 mg/ml), sodium phosphate dibasic (50 mg/ml), and gentamicin sulfate (50 mg/ml). An oil-in-water emulsion containing 10% Acetyl Hexapeptide-8 Amide (dose = 2 $mg/cm^2$) was applied to the skin for 24 h. Skin disks were tape stripped to determine the amount of peptide in the stratum corneum. Skin penetration was measured in skin layers using hydrophilic interaction LC-MS/MS and electrospray ionization. Stable isotopically-labeled hexapeptides were used as internal standards for the quantitation of native hexapeptides to correct for matrix effects that are associated with electrospray ionization. Study results indicated that the majority of Acetyl Hexapeptide-8 Amide was washed from the surface of both skin types (guinea pig and human). For the Acetyl Hexapeptide-8 Amide that actually penetrated the skin, it remained mostly in the stratum corneum of hairless guinea pig skin (0.54% of applied dose) and human skin (0.22% of applied dose). Peptide levels were found to decrease as each layer was removed by tape stripping. The total amount of Acetyl Hexapeptide-8 Amide that was found in the epidermis was similar (at 0.01%) when hairless guinea pig skin and human skin were compared. Also, for both skin types, no peptide was detected in the dermis or buffer collected underneath the skin. No hexapeptide metabolite was detected in any layers of hairless guinea pig or human skin, or buffer collected underneath the skin.

In another study, the skin penetration of 0.05% aqueous Acetyl Hexapeptide-8 Amide was studied using human skin that had been obtained from different donors who had undergone cosmetic surgery.[8] All fat was removed from fresh frozen pieces of skin. The epidermis was teased away from underlying dermis, and the stratum corneum (~ 2 $cm^2$ skin disks) was used in skin penetration experiments. The experiments were performed using a glass cell with an upper chamber (donor

chamber) and a lower chamber (receptor chamber). The average diffusion area was 1.3 $cm^2$, and the receptor chamber volume was 4 ml. Skin disks (stratum corneum, ~ 2 $cm^2$) were mounted between the 2 chambers. Isotonic phosphate buffer (pH = 7.4) with 0.01% sodium azide as preservative, was used as the receptor fluid. Samples (0.5 ml) of 0.05% aqueous Acetyl Hexapeptide-8 Amide were poured into the donor chamber and 100 µl aliquots of receptor fluid were periodically withdrawn for analysis. The concentration of the Acetyl Hexapeptide-8 Amide in the receptor fluid was quantified at 2 h using high-performance liquid chromatography. The total content of peptide in the receptor reservoir was 30% of the amount that was deposited onto the membrane in the donor chamber. The authors noted that these results indicate that the Acetyl Hexapeptide-8 Amide is capable of permeating through the skin.

### Absorption, Distribution, Metabolism, and Excretion (ADME)

Data on the absorption (in vivo), distribution, metabolism, and excretion of Acetyl Hexapeptide-8 Amide were neither found in the published literature, nor were these data submitted.

## TOXICOLOGICAL STUDIES

### Acute Toxicity Studies

**Oral**

The acute oral toxicity of Acetyl Hexapeptide-8 Amide was evaluated using rats (number and strain not stated).[6] It was concluded that the test substance was non-toxic when administered orally ($LD_{50}$ > 2500 mg/kg).

### Short-Term, Subchronic, and Chronic Toxicity Studies

Short-term, subchronic, and chronic toxicity studies of Acetyl Hexapeptide-8 Amide were neither found in the published literature, nor were these data submitted.

## DEVELOPMENTAL AND REPRODUCTIVE TOXICITY STUDIES

Developmental and reproductive toxicity studies of Acetyl Hexapeptide-8 Amide were neither found in the published literature, nor were these data submitted.

## GENOTOXICITY STUDIES

### In Vitro

Acetyl Hexapeptide-8 Amide was evaluated for genotoxicity potential in the Ames test, using the following *Salmonella typhimurium* strains: TA97, TA98, TA100, TA102, and TA1537.[5,6] The primary reference for these data was unavailable. Over the range of concentrations tested, 0.05 to 5 mg/plate, the test substance was non-genotoxic.

## CARCINOGENICITY STUDIES

Carcinogenicity studies of Acetyl Hexapeptide-8 Amide were neither found in the published literature, nor were these data submitted.

## OTHER RELEVANT STUDIES

### Effect on Skin Histology

The effect of Acetyl Hexapeptide-8 Amide on skin histology was studied using groups of 10 Kunming mice, described as follows: normal control group, aged model group, placebo control group, and Acetyl Hexapeptide-8 Amide treatment group (aged mice only).[13] Aged models of the mice were established, and the histological changes before and after treatment were compared. Each vial of the test substance contained 10% Acetyl Hexapeptide-8 Amide in an oil-in-water emulsion without preservatives. The placebo control solution was a non-active oil-in-water emulsion without Acetyl Hexapeptide-8 Amide. Initially, 3 groups were injected s.c. with 0.1 ml/10 g of 10% D-galactose in skin of the nape and back daily for 6 weeks to establish the subacute aged models. The aging model induced by D-galactose was a common laboratory tool that was used to simulate senescence. Mice of the normal control group were injected s.c. with 0.1 ml/10 g saline (same areas). At the same time, the Acetyl Hexapeptide-8 Amide emulsion was applied to a shaved 2 x 2 cm site on the back of each test animal twice per day. The placebo solution was applied (shaved 2 x 2 cm site) to animals of the placebo control group. After 6 weeks, skin tissues (~ 1 x 1 cm) from the application sites of placebo control and test mice were removed. Skin tissues (~ 1 x 1 cm, from same site) were also removed from mice of the other 2 groups. Paraffin sections from all tissues were made and stained with hematoxylin-eosin (HE) stain and picrosirius-polarization (PSP) stain for microscopic examination.

After 6 weeks, all of the animals were alive and did not exhibit any side effects. The following changes were reported after 6 weeks for the aged model group, as compared to the normal control group: thinner skin, significant reduction in amount of collagen fibers in in the dermis, and fibers were bound more loosely. When compared to the aged model group, the skin of mice treated with Acetyl Hexapeptide-8 Amide was thicker with a greater number of collagen fibers, and the

fibers were dense and compact. The difference between the aged model group and the placebo control group was insignificant. Additionally, when compared to the aged model group, type I collagen fibers increased ($p < 0.01$) and type III collagen fibers decreased ($p < 0.05$) in the Acetyl Hexapeptide-8 Amide treatment group.

### Cytotoxicity

The cytotoxicity of Acetyl Hexapeptide-8 Amide was evaluated in an in vitro proliferation assay using the formazan-based antiproliferation assay (EZ4U assay).[5] Human embryonic kidney (HEK)-293 and neuroblastoma (IMR-32) cell lines, as well as human epidermal fibroblasts, were incubated for 48 h with test substance concentrations ranging from 0.01 µM to 100 µM. Doxorubicin, a commonly used drug in cancer chemotherapy, served as the reference compound. Significant antiproliferative activity of Acetyl Hexapeptide-8 Amide was observed at concentrations above 10 µM. Calculated half-maximal inhibitory concentration ($IC_{50}$) values for Acetyl Hexapeptide-8 Amide were 34.862 µM (in HEK-293 cells) and 64.458 (in IMR-32 cells). In human epidermal fibroblasts, a dose-dependent antiproliferative effect was observed; 67% inhibition was observed at 100 µM Acetyl Hexapeptide-8 Amide (highest test concentration). The data showed very strong antiproliferative effect of doxorubicin against the IMR-32 cell line ($IC_{50}$ = 0.0051 µM) and the HEK-293 cell line ($IC_{50}$ = 0.455 µM). The authors noted that the $IC_{50}$ value of Acetyl Hexapeptide-8 Amide (34.862 µM) was approximately 75-fold higher than the $IC_{50}$ of doxorubicin against the HEK-293 cell line, and more than 10,000-fold higher against the IMR-32 cell line. The authors also noted that the significant effect of Acetyl Hexapeptide-8 Amide in human epidermal fibroblasts was observed at 100 µM, whereas the significant effect of doxorubicin (at 5.628 µM) was at an 18-fold lower concentration. An $IC_{50}$ value for Acetyl Hexapeptide-8 Amide in human epidermal fibroblasts was not reported, but it was noted that the test substance exhibited a 67% antiproliferative effect after 48 h of incubation at a concentration of 100 µM. Finally, the authors stated that, given the cytotoxic activity of Acetyl Hexapeptide-8 Amide against human epidermal fibroblasts, the use of Acetyl Hexapeptide-8 Amide at very high doses or for a very long period of time must be considered potentially dangerous for patients.

### Inhibition of Catecholamine Release

The inhibitory activity of Acetyl Hexapeptide-8 Amide (tested at 100 µM) on calcium-evoked neurotransmitter release from digitonin-permeabilized chromaffin cells was studied.[8] Detergent-permeabilized chromaffin cells release both noradrenaline and adrenaline in response to an increase in intracellular calcium. Acetyl Hexapeptide-8 Amide (100 µM), caused 30% inhibition of the total catecholamine exocytosis. Botulinum neurotoxin A (BoNT A) (20 nM) caused up to 60% inhibition of catecholamine release. A 26-mer peptide (1 µM) derived from the C-terminal end of SNAP-25 (ESUP-E) caused up to 55% inhibition of catecholamine release. Dose response curves indicated an $IC_{50}$ of 110 µM for the test substance, which was 5000 x higher than the characteristic of BoNT A, and 400 x higher than that of ESUP-E.

### Effect on *N*-Ethylmaleimide-Sensitive Factor Attachment Protein Receptor (SNARE) Complex Formation

An experiment was performed to determine if 10% Acetyl Hexapeptide-8 Amide prevents or destabilizes formation of the SNARE complex in vitro.[8] Recombinant synaptic proteins vesicle-associated membrane protein (VAMP), syntaxin, and in vitro transcribed and translated [$^{35}$S]SNAP-25 were used. Incubation of the 3 synaptic proteins led to the formation of protein complex of 75 kDa that was resistant to the chaotropic detergent sodium dodecyl sulfate (SDS), but sensitive to heat. These are 2 well-known properties of the SNARE complex. When the proteins were incubated with Acetyl Hexapeptide-8 Amide (at 1 mM and 2 mM), formation of the SNARE complex was prevented in a dose-dependent manner. At 2 mM, the 75 kDa band was undetectable, suggesting complete abrogation of complex formation by the small peptide. The authors noted that these results indicate that Acetyl Hexapeptide-8 Amide can prevent the assembly of the protein complex that drives calcium-dependent exocytosis in secretory cells, implying that this peptide may modulate neurotransmitter release from these cells.

## DERMAL IRRITATION AND SENSITIZATION STUDIES

### Irritation

#### Animal

The skin irritation potential of a tradename mixture containing 0.05% aqueous Acetyl Hexapeptide-8 Amide was evaluated using albino male rabbits (number not stated).[6] The test protocol was not provided. There were no signs or erythema or edema at 7 d after removal of the test substance.

### Sensitization

#### Human

The skin sensitization potential of a tradename mixture containing 0.05% aqueous Acetyl Hexapeptide-8 Amide was evaluated in a human repeated insult patch test (HRIPT) involving 50 subjects.[6] The test substance did not cause skin sensitization in any of the subjects tested. Details relating to the test protocol and study results were not included.

## OCULAR IRRITATION STUDIES

### In Vitro

The ocular irritation potential of a solution of Acetyl Hexapeptide-8 Amide (concentrations not stated) was evaluated using the neutral red uptake test.[6] Details relating to the test protocol were not included. It was concluded that the test substance is potentially not irritating to the eyes.

## CLINICAL STUDIES

### Other Clinical Reports

Ten healthy women applied an oil-in-water emulsion containing 10% Acetyl Hexapeptide-8 Amide twice per day for 30 d.[8] The emulsion without Acetyl Hexapeptide-8 Amide was applied to the contralateral side. Skin topography analysis was performed by obtaining silicon imprints from the lateral preorbital region of each subject. Silicon imprints, obtained after 0, 15, and 30 d, were analyzed by confocal laser scanning microscopy to assess the evolution of the skin surface before and after treatment. Topical application of 10% Acetyl Hexapeptide-8 Amide (in oil-in-water emulsion) resulted in significant attenuation of the depth and roughness of the wrinkles. The oil-in-water emulsion did not cause significant changes in skin topography. Quantitative analysis and normalization of the silicon replicas showed that the oil-in-water emulsion reduced by 10% the depth of the skin wrinkles (not identified as a statistically significant finding). The oil-in-water emulsion containing 10% Acetyl Hexapeptide-8 Amide decreased the depth of skin wrinkles by 20% by day 15 and by 30% by day 30.

The effect of Acetyl Hexapeptide-8 Amide on the skin was evaluated using 8 subjects.[21] Skin properties were studied using skin microtopography and transepidermal water loss. Four subjects were each given a 50 g vessel containing an Acetyl Hexapeptide-8 Amide (10% w/w) cream. The other 4 subjects were each given a 50 g vessel containing a placebo cream that did not contain Acetyl Hexapeptide-8 Amide. The subjects were instructed to apply the cream twice daily for 2 months (60 d). Skin surface evaluation and measurement of transepidermal water loss were performed before treatment, day 0, and then on days 20, 40, and 60. Self-evaluation was performed after the 2-month treatment (day 60). Side effects were also evaluated by the volunteers. To evaluate the tolerability and potential irritant power of the Acetyl Hexapeptide-8 Amide (10% w/w) cream, the subjects were asked to answer whether they experienced the following effects on the skin: warmth, dryness, stinging, redness, desquamation, dryness, itching, or ocular irritation. These variables were scored on a scale of 1 (slight) to 4 (great). Also, when compared to the placebo group, a statistically significant decrease in transepidermal water loss was observed after 20 d ($p = 0.025$) and 40 d ($p = 0.028$) of application of the Acetyl Hexapeptide-8 Amide (10% w/w) cream. At 60 d, the decrease in transepidermal water loss was not statistically significant. None of the following effects was reported after application of the Acetyl Hexapeptide-8 Amide (10% w/w) cream: warmth, dryness, stinging, redness, desquamation, dryness, itching, or ocular irritation.

### Eyelid Irritation

A double-blind, placebo-controlled randomized trial on topically applied Acetyl Hexapeptide-8 Amide was conducted using 24 blepharospasm patients who were receiving botulinum neurotoxin therapy (orbicularis oculi muscle injections) at regular 3-mo intervals.[18] On the day after injection of botulinum neurotoxin, 12 patients applied an emulsion containing 0.005% Acetyl Hexapeptide-8 Amide twice daily to the eyelids. Topical application (repeated daily for ~ 7 mo) was standardized and targeted the eyelids only, independent of involvement of the orbital orbicularis oculi or surrounding muscles. A placebo (emulsion without Acetyl Hexapeptide-8 Amide) was applied topically to another 12 blepharospasm patients according to the same procedure. No severe adverse events were observed during the study. Four subjects (2 test and 2 placebo) experienced minor, self-limiting eyelid irritation. The irritation reactions observed did not necessitate any modifications of the test procedure.

## SUMMARY

The safety of Acetyl Hexapeptide-8 Amide (and thus, the synonym, Acetyl Hexapeptide-8), as used in cosmetics, is reviewed in this safety assessment. According to the *Dictionary*, Acetyl Hexapeptide-8 Amide is reported to function as a skin conditioning agent - miscellaneous.

According to 2021 VCRP data, Acetyl Hexapeptide-8 is reported to be used in 333 cosmetic products 311 leave-on and 22 rinse-off); an additional 21 uses (20 leave-on and 1 rinse-off) are reported under the name acetyl hexapeptide-3. The results of a concentration of use survey conducted by the Council in 2019 indicate that Acetyl Hexapeptide-8 is being used at concentrations up to 0.005% (in eye lotions and face and neck products; not spray), which is the highest reported maximum use concentration for leave-on formulations. In rinse-off products, Acetyl Hexapeptide-8 is reported to be used at concentrations up to 0.000005%. The Panel is aware of products with higher use concentrations, but whether these products are used as cosmetics is not clear.

The in vitro skin penetration of Acetyl Hexapeptide-8 Amide has been demonstrated using porcine skin. Differences in the skin penetration of Acetyl Hexapeptide-8 Amide through porcine skin were observed when various vehicles for the test

substance were used. For example, statistically significant difference ($p < 0.01$) between the cumulative permeated amount of Acetyl Hexapeptide-8 Amide after 8 h from the multiple water-in-oil-in-water emulsion (755 ± 149 ng/cm$^2$) and the oil-in-water emulsion (456 ± 120 ng/cm$^2$) was found. Overall, the penetration of Acetyl Hexapeptide-8 Amide from the different emulsions was in the order of multiple water-in-oil-in-water emulsion > oil-in-water emulsion > water-in-oil emulsion.

In another study, the skin penetration of Acetyl Hexapeptide-8 Amide was evaluated using hairless guinea pig skin and human cadaver skin in vitro. For the Acetyl Hexapeptide-8 Amide that actually penetrated the skin, it remained mostly in the stratum corneum of hairless guinea pig skin (0.54% of applied dose) and human skin (0.22% of applied dose). Peptide levels were found to decrease as each layer was removed by tape stripping. The total amount of Acetyl Hexapeptide-8 Amide that was found in the epidermis was similar (at 0.01%) when hairless guinea pig skin and human skin were compared. Also, for both skin types, no peptide was detected in the dermis or buffer collected underneath the skin. No hexapeptide metabolite was detected in any layers of hairless guinea pig or human skin, or buffer collected underneath the skin.

The skin penetration of 0.05% aqueous Acetyl Hexapeptide-8 Amide was studied using human skin (stratum corneum) that had been obtained from different donors who had undergone cosmetic surgery. The total content of peptide in the receptor reservoir of the diffusion cell was 30% of the amount that was deposited onto the membrane in the donor chamber. These results indicate that Acetyl Hexapeptide-8 Amide is capable of permeating through the skin.

In an acute oral toxicity study, Acetyl Hexapeptide-8 Amide was evaluated using rats (number and strain not stated). The test substance was non-toxic when administered orally ($LD_{50}$ > 2500 mg/kg).

Acetyl Hexapeptide-8 Amide was evaluated for genotoxicity potential in the Ames test, using the following *S. typhimurium* strains: TA97, TA98, TA100, TA102, and TA1537. Over the range of concentrations tested, 0.05 to 5 mg/plate, the test substance was non-genotoxic.

The effect of Acetyl Hexapeptide-8 Amide (10% in oil-in-water emulsion without preservatives) on skin histology was studied using groups of 10 Kunming mice, one of which was an aged model group. The test substance was applied (aged mice only) twice daily for 6 wk. When compared to the aged model group, the skin of mice treated with Acetyl Hexapeptide-8 Amide was thicker, with a greater number of collagen fibers, and the fibers were dense and compact.

The cytotoxicity of Acetyl Hexapeptide-8 Amide was evaluated in an in vitro proliferation assay using the formazan-based antiproliferation assay (EZ4U assay). Human embryonic kidney (HEK)-293 and neuroblastoma (IMR-32) cell lines, as well as human epidermal fibroblasts, were incubated for 48 h with test substance concentrations ranging from 0.01 μM to 100 μM. Significant antiproliferative activity was observed at concentrations above 10 μM. Particularly, the significant effect of Acetyl Hexapeptide-8 Amide in human epidermal fibroblasts was observed at 100 μM.

The inhibitory activity of Acetyl Hexapeptide-8 Amide (tested at 100 μM) on calcium-evoked neurotransmitter release from digitonin-permeabilized chromaffin cells was studied. Acetyl Hexapeptide-8 Amide (100 μM), caused 30% inhibition of the total catecholamine exocytosis.

An experiment was performed to determine if Acetyl Hexapeptide-8 Amide prevents or destabilizes formation of the SNARE complex in vitro. Recombinant synaptic proteins VAMP, syntaxin, and in vitro transcribed and translated [$^{35}$S]SNAP-25 were used. Incubation of the 3 synaptic proteins led to the formation of protein complex of 75 kDa that was resistant to the chaotropic detergent SDS, but sensitive to heat. When the proteins were incubated with Acetyl Hexapeptide-8 Amide (at 1 mM and 2 mM), formation of the SNARE complex was prevented in a dose-dependent manner. These results indicate that Acetyl Hexapeptide-8 Amide can prevent the assembly of the protein complex that drives calcium-dependent exocytosis in secretory cells, implying that this peptide may modulate neurotransmitter release from these cells.

The skin irritation potential of a tradename mixture containing 0.05% aqueous Acetyl Hexapeptide-8 Amide was evaluated using albino male rabbits (number not stated). There were no signs or erythema or edema at 7 d after removal of the test substance. A tradename mixture containing Acetyl Hexapeptide-8 Amide (0.05% aqueous) was evaluated for skin sensitization potential in an HRIPT involving 50 subjects. The test substance did not cause skin sensitization in any of the subjects tested.

The ocular irritation potential of a solution of Acetyl Hexapeptide-8 Amide (concentrations not stated) was evaluated using the neutral red uptake test. Results indicated that the test substance is potentially not irritating to the eyes.

## DISCUSSION

The Panel noted the absence of systemic toxicity and detailed genotoxicity data on Acetyl Hexapeptide-8 Amide. However, concern over the lack of these data was mitigated after considering the peptide structure of this ingredient, the associated low log P value of -6.3 (percutaneous absorption unlikely), and the low maximum use concentration of 0.005% in leave-on cosmetic products that was reported in the Council survey. On the subject of potential percutaneous absorption, the Panel also noted differing degrees of reported skin penetration by Acetyl Hexapeptide-8 Amide with in vitro models. The Panel felt that studies that employed LC-MS/MS to measure the peptide were most reliable, and noted that these indicated minimal skin penetration.

The Panel recognized reports of products containing higher use concentrations of Acetyl Hexapeptide-8 than what was reported in the Council survey, and acknowledged that whether these products are drugs or cosmetics remains unknown. In dermal studies, 10% Acetyl Hexapeptide-8 Amide may have drug activity (i.e., an anti-wrinkle), as indicated by an effect on type I and type III collagen in the dermis. However, the Panel noted that whether the mechanism of action of this product is via hydration of the skin or a biological effect on collagen synthesis is unclear. The Panel was of the understanding that Acetyl Hexapeptide-8 Amide would not be likely to produce a drug (anti-wrinkle) effect when used at a low concentration (i.e., 0.005%) in leave-on cosmetic products. However, the Panel acknowledges that the drug effect may be apparent at higher use concentrations, but the threshold is not known. Additionally, use concentrations > 0.005% are unsupported by the available safety test data. Therefore, the Panel determined the available data were insufficient to determine safety at concentrations > 0.005%, and the following data are needed:

- an NOAEL for type I and type III collagen synthesis.

The Panel also discussed the issue of incidental inhalation exposure from the use of Acetyl Hexapeptide-8 in face powders at concentrations up to 0.0001%. It was noted that conservative estimates of inhalation exposures to respirable particles during the use of loose powder cosmetic products are 400-fold to 1000-fold less than protective regulatory and guidance limits for inert airborne respirable particles in the workplace.

## CONCLUSION

The Expert Panel for Cosmetic Ingredient Safety (Panel) concluded that Acetyl Hexapeptide-8 Amide is safe in cosmetics in the present practices of use and concentration described in this safety assessment when the concentration does not exceed 0.005%. The Panel further concluded that the available data are insufficient to make a determination that Acetyl Hexapeptide-8 Amide is safe in cosmetic formulations at concentrations > 0.005%.

# TABLE

**Table 1.** Frequency (2021) and concentration of use (2019) of Acetyl Hexapeptide-8 Amide according to duration and type of exposure.[11,12]

| | # of Uses Reported as Acetyl Hexapeptide-8 | # of Uses Reported as acetyl hexapeptide-3 | Conc. (%) |
|---|---|---|---|
| **Totals*** | **333** | **21** | **0.000005-0.005** |
| ***Duration of Use*** | | | |
| *Leave-On* | *311* | *20* | *0.00005-0.005* |
| *Rinse off* | *22* | *1* | *0.000005* |
| *Diluted for (bath) Use* | *NR* | *NR* | *NR* |
| ***Exposure Type*** | | | |
| Eye Area | 35 | 7 | 0.00005-0.005 |
| Incidental Ingestion | 2 | NR | 0.00025 |
| Incidental Inhalation- Sprays | 127[a]; 102[b] | 2[a]; 6[b] | NR |
| Incidental Inhalation- Powders | 102[b]; | 6[b] | 0.0001; 0.00026-0.005[c] |
| Dermal Contact | 329 | 21 | 0.000005-0.005 |
| Deodorant (underarm) | NR | NR | NR |
| Hair - Non-Coloring | 2 | NR | NR |
| Hair-Coloring | NR | NR | NR |
| Nail | NR | NR | NR |
| Mucous Membrane | 10 | NR | 0.00025 |
| Baby Products | NR | NR | NR |

*Because each ingredient may be used in cosmetics with multiple exposure types, the sum of all exposure types may not equal the sum of total uses.

NR = Not Reported

[a]It is possible that these products may be sprays, but it is not specified whether the reported uses are sprays

[b] Not specified these products are sprays or powders, but it is possible the use can be as a spray or powder, therefore the information is captured in both categories

[c]It is possible that these products may be powders, but it is not specified whether the reported uses are powders

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**2021 VCRP Data**

**Acetyl Hexapeptide-8**

| | | |
|---|---|---|
| Eye Lotion | 03D | 17 |
| Other Eye Makeup Preparations | 03G | 18 |
| Shampoos (non-coloring) | 05F | 1 |
| Other Hair Preparations | 05I | 1 |
| Foundations | 07C | 8 |
| Lipstick | 07E | 2 |
| Makeup Fixatives | 07H | 1 |
| Other Makeup Preparations | 07I | 2 |
| Bath Soaps and Detergents | 10A | 4 |
| Other Personal Cleanliness Products | 10E | 4 |
| Aftershave Lotion | 11A | 1 |
| Cleansing | 12A | 1 |
| Face and Neck (exc shave) | 12C | 85 |
| Body and Hand (exc shave) | 12D | 17 |
| Moisturizing | 12F | 117 |
| Night | 12G | 6 |
| Paste Masks (mud packs) | 12H | 12 |
| Skin Fresheners | 12I | 3 |
| Other Skin Care Preps | 12J | 32 |
| Suntan Gels, Creams, and Liquids | 13A | 1 |
| **Total** | | **333** |

**Acetyl Hexapeptide-3**

| | | |
|---|---|---|
| Eye Lotion | 03D | 5 |
| Other Eye Makeup Preparations | 03G | 2 |
| Face and Neck (exc shave) | 12C | 6 |
| Moisturizing | 12F | 1 |
| Night | 12G | 1 |
| Paste Masks (mud packs) | 12H | 1 |
| Other Skin Care Preps | 12J | 5 |
| **Total** | | **21** |

Personal Care Products Council
Committed to Safety, Quality & Innovation

**Memorandum**

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

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

**DATE:** January 11, 2021

**SUBJECT:** Revised Tentative Report: Safety Assessment of Acetyl Hexapeptide-8 Amide as Used in Cosmetics (release date: December 16, 2020)

The Personal Care Products Council respectfully submits the following comments on the revised tentative report, Safety Assessment of Acetyl Hexapeptide-8 Amide as Used in Cosmetics.

Key Issue
Definition and Structure – This section should clearly state that Hexapeptide-8 is a synthetic peptide. This would be consistent with the COSING definition of Acetyl Hexapeptide-8 and the Dictionary definition of Hexapeptide-8. The Dictionary considers Hexapeptide-8 to be a reference material with the following definition "a synthetic peptide containing arginine, glutamic acid, glutamine and methionine." It was defined so that the name Hexapetide-8 could be used to name other ingredients.

Additional Considerations
Definition and Structure – Please define the abbreviations used for the amino acids that make up this peptide.

Method of Manufacture – Please revise: "ingredient has been also been synthesized"

Effect on Skin Histology – In the description of reference 13, it is not clear if there was a group of non-aged mice treated with Acetyl Hexapeptide-8 Amide. It should be made clear if all mice treated with Acetyl Hexapeptide-8 Amide were "aged" mice.

Summary – Please state the FDA product category for the highest rinse-off concentration.

In the Summary, the description of reference 13 appears to give the results of the aging treatment. The results of treatment with Acetyl Hexapeptide-8 Amide should also be stated.