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

Status: Draft Final Report for Panel Review
Release Date: November 13, 2020
Panel Date: December 7-8, 2020

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, 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, CIR

Date: November 13, 2020

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

Enclosed is a draft final report of the Safety Assessment of Acetyl Hexapeptide-8 Amide as Used in Cosmetics (*acetyl122020rep*). The safety of Acetyl Hexapeptide-8 Amide (synonymous with Acetyl Hexapeptide-8 (sans "Amide")), as used in cosmetics, is reviewed in this safety assessment. It should be noted that while Acetyl Hexapeptide-8 Amide is synonymous with the in-use name, Acetyl Hexapeptide-8, with both names being found in the *International Cosmetic Ingredient Dictionary and Handbook*, 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).

A Tentative Report with the following conclusion was issued at the September 2020 Panel meeting: Acetyl Hexapeptide-8 Amide is safe in cosmetics in the present practices of use and concentration described in this safety assessment. Comments on the tentative report were received from the Council, and the draft final report has been revised to address these comments (acetyl*122020pcpc*). The comments are enclosed.

Also included in this package for your review are the report history (*acetyl122020hist*), flow chart (*acetyl122020flow*), literature search strategy (*acetyl122020strat*), ingredient data profile (*acetyl122020prof*), minutes from the September 2020 Panel meeting (*acetyl122020min*), and 2020 FDA VCRP data (*acetyl122020FDA*).

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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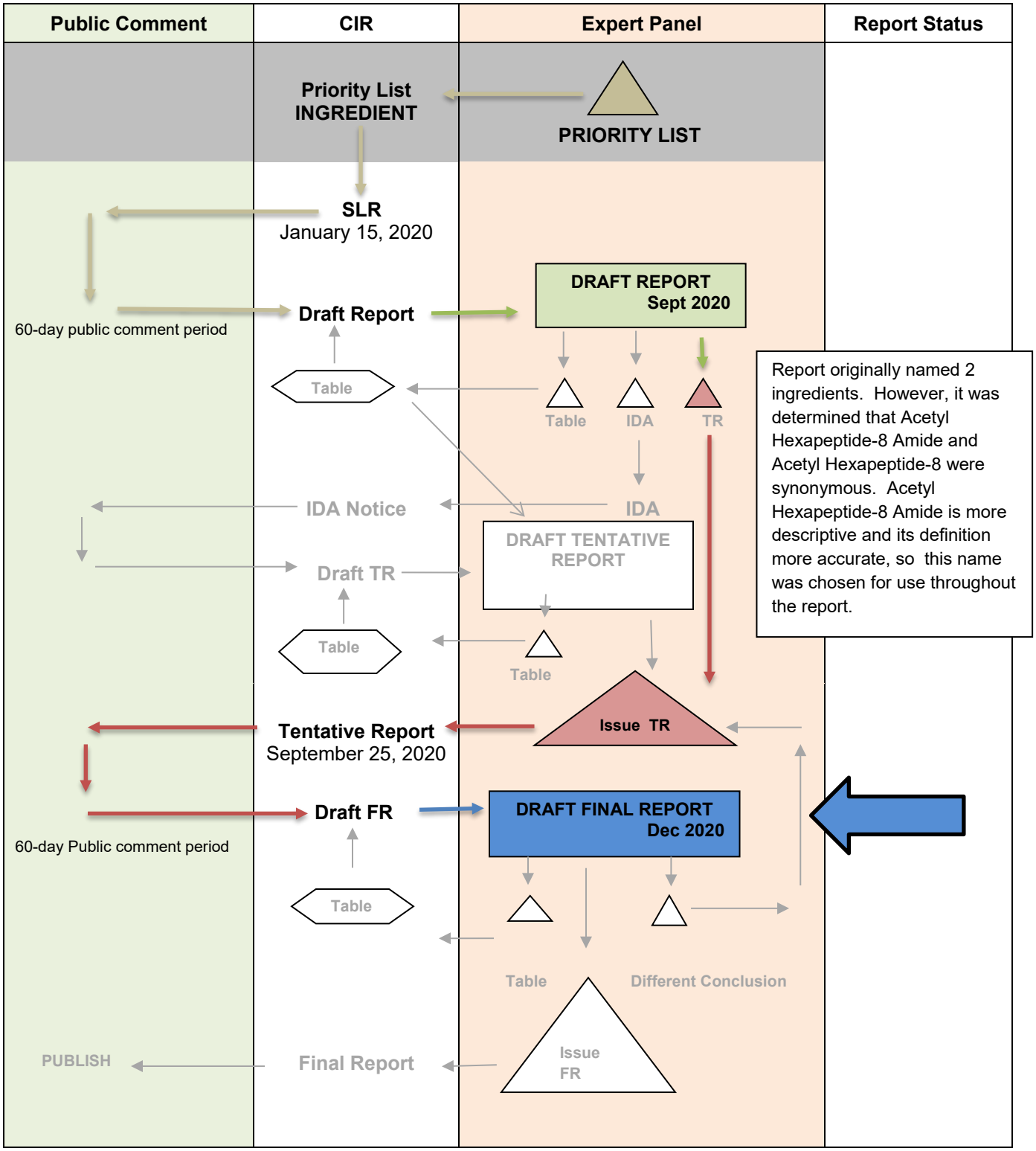
SAFETY ASSESSMENT FLOW CHART

INGREDIENT/FAMILY Acetyl Hexapeptide-8 Amide

MEETING December 2020



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.

Acetyl Hexapeptide-8 and Acetyl Hexapeptide-8 Data Profile* – December 7-8, 2020 – 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]

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 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
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.

Acetyl Hexapeptide-8 and Acetyl Hexapeptide-8 Amide

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.

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

Status: Draft Final Report for Panel Review
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The Expert Panel for Cosmetic Ingredient Safety members are: Chair, Wilma F. Bergfeld, M.D., F.A.C.P.; Donald V. Belsito, M.D.; David E. Cohen, M.D.; Curtis D. Klaassen, Ph.D.; 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, 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 cosmetics in the present practices of use and concentration described in this safety assessment.

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 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, 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.[4]

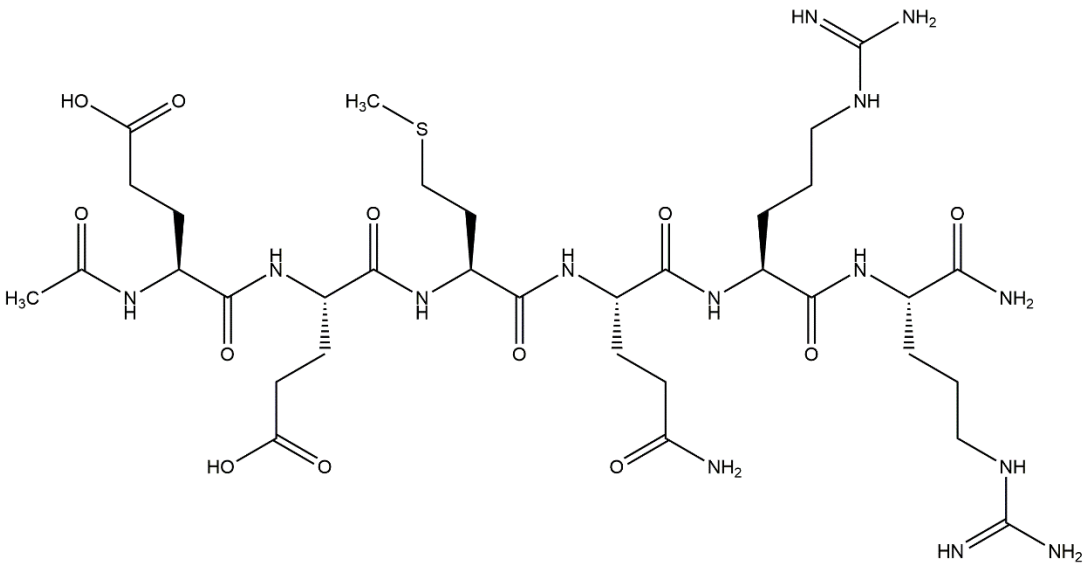

Figure 1. Acetyl Hexapeptide-8 Amide.

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.[11] 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.[12]

According to 2020 VCRP data, Acetyl Hexapeptide-8 is reported to be used in 452 cosmetic products (422 leave-on and 30 rinse-off), and an additional 33 uses are reported with the name Acetyl Hexapeptide-3 (32 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).

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.[13-15]

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.[16]

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.[17] (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.[18] 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.[19] 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 Hexpeptide-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.[20] 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.[17] 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 2020 VCRP data, Acetyl Hexapeptide-8 is reported to be used in 452 cosmetic products (422 leave-on and 30 rinse-off); an additional 33 uses (32 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 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 Hexpeptide-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 twice daily for 6 wk. When compared to the normal control group, the following changes were observed in the aged model group: thinner skin, significant reduction in amount of collagen fibers in the dermis, and fibers were bound more loosely.

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 Hexapaptide-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.

The Panel 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.

TABLE

Table 1. Frequency (2020) 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*	**452**	**33**	**0.000005-0.005**
Duration of Use			
Leave-On	*422*	*32*	*0.00005-0.005*
Rinse off	*30*	*1*	*0.000005*
Diluted for (bath) Use	*NR*	*NR*	*NR*
Exposure Type			
Eye Area	61	1	0.00005-0.005
Incidental Ingestion	4	NR	0.00025
Incidental Inhalation- Sprays	154[a]; 134[b]	5[a]; 16[b]	NR
Incidental Inhalation- Powders	1; 134[b];	16[b]	0.0001; 0.00026-0.005[c]
Dermal Contact	447	33	0.000005-0.005
Deodorant (underarm)	NR	NR	NR
Hair - Non-Coloring	1	NR	NR
Hair-Coloring	NR	NR	NR
Nail	NR	NR	NR
Mucous Membrane	13	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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2020 FDA VCRP DATA

ACETYL HEXAPEPTIDE-8	Eye Lotion	03D	33
ACETYL HEXAPEPTIDE-8	Other Eye Makeup Preparations	03G	28
ACETYL HEXAPEPTIDE-8	Shampoos (non-coloring)	05F	1
ACETYL HEXAPEPTIDE-8	Face Powders	07B	1
ACETYL HEXAPEPTIDE-8	Foundations	07C	13
ACETYL HEXAPEPTIDE-8	Lipstick	07E	4
ACETYL HEXAPEPTIDE-8	Makeup Bases	07F	3
ACETYL HEXAPEPTIDE-8	Rouges	07G	1
ACETYL HEXAPEPTIDE-8	Makeup Fixatives	07H	1
ACETYL HEXAPEPTIDE-8	Other Makeup Preparations	07I	4
ACETYL HEXAPEPTIDE-8	Bath Soaps and Detergents	10A	4
ACETYL HEXAPEPTIDE-8	Other Personal Cleanliness Products	10E	5
ACETYL HEXAPEPTIDE-8	Aftershave Lotion	11A	2
ACETYL HEXAPEPTIDE-8	Cleansing	12A	1
ACETYL HEXAPEPTIDE-8	Face and Neck (exc shave)	12C	124
ACETYL HEXAPEPTIDE-8	Body and Hand (exc shave)	12D	10
ACETYL HEXAPEPTIDE-8	Moisturizing	12F	133
ACETYL HEXAPEPTIDE-8	Night	12G	18
ACETYL HEXAPEPTIDE-8	Paste Masks (mud packs)	12H	19
ACETYL HEXAPEPTIDE-8	Skin Fresheners	12I	2
ACETYL HEXAPEPTIDE-8	Other Skin Care Preps	12J	44
ACETYL HEXAPEPTIDE-8	Suntan Gels, Creams, and Liquids	13A	1
			452

ACETYL HEXAPEPTIDE-3	Eye Lotion	03D	1
ACETYL HEXAPEPTIDE-3	Foundations	07C	3
ACETYL HEXAPEPTIDE-3	Face and Neck (exc shave)	12C	14
ACETYL HEXAPEPTIDE-3	Body and Hand (exc shave)	12D	2
ACETYL HEXAPEPTIDE-3	Moisturizing	12F	4
ACETYL HEXAPEPTIDE-3	Night	12G	1
ACETYL HEXAPEPTIDE-3	Paste Masks (mud packs)	12H	1
ACETYL HEXAPEPTIDE-3	Other Skin Care Preps	12J	7
			33

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: September 4, 2020

SUBJECT: Draft Report: Safety Assessment of Acetyl Hexapeptide-8 and Acetyl Hexapeptide-8 Amide as Used in Cosmetics (draft prepared for the September 14-15, 2020 meeting of the Expert Panel for Cosmetic Ingredient Safety)

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

Dermal Penetration – It is not clear what is meant by n=5 in the following sentence: "Five parallel experiments for each formulation (n = 5) were performed." Because the sentence states that there were 5 experiments, either "(n=5)" should be deleted or changed to "(n=3)" to indicate the number of formulations tested.

Cytotoxicity – It is not clear if the following information in this section is just about the control, Doxorubicin, or if some of these results were for Acetyl Hexapeptide-8 Amide. "Doxorubicin, a commonly used drug in cancer chemotherapy, served as the reference compound. Significant antiproliferative activity was observed at concentrations above 10 µM. Calculated half-maximal inhibitory concentration (IC_{50}) values 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 (highest test concentration). "