Safety Assessment of Myristoyl Pentapeptide-4, Palmitoyl Pentapeptide-4, and Pentapeptide-4 as Used in Cosmetics

Status: Draft Final Report for Panel Review
Release Date: September 6, 2024
Panel Meeting Date: September 30 – October 1, 2024

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

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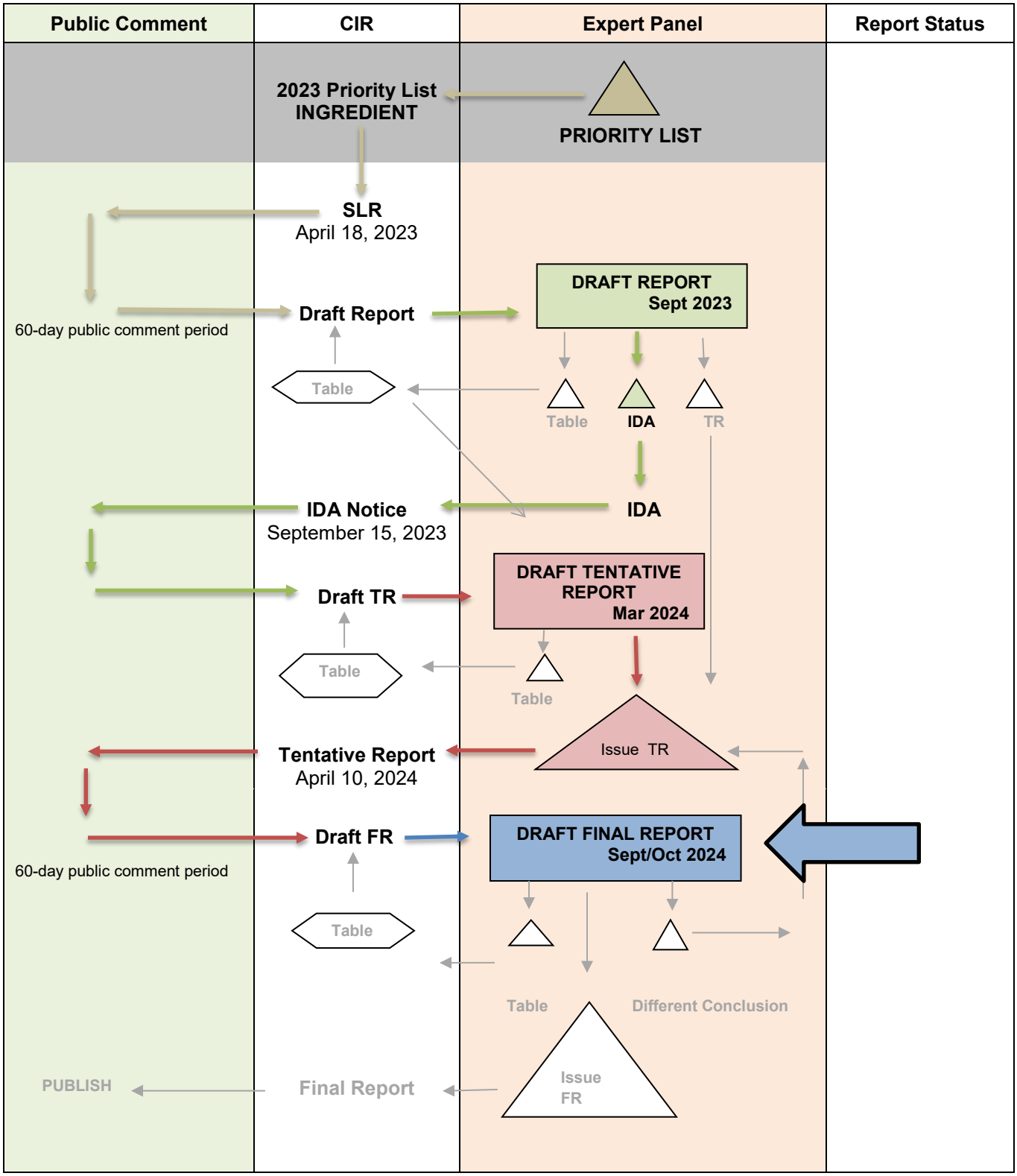
SAFETY ASSESSMENT FLOW CHART

INGREDIENT/FAMILY Pentapeptide Ingredients

MEETING September/October 2024



Cosmetic Ingredient Review

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Memorandum

To: Expert Panel for Cosmetic Ingredient Safety Members and Liaisons
From: Preethi S. Raj, M.Sc.
Senior Scientific Analyst/Writer, CIR
Date: September 6, 2024
Subject: Safety Assessment of Pentapeptides as Used in Cosmetics

Enclosed is the Draft Final Report of the Safety Assessment of Pentapeptides as Used in Cosmetics (identified as *report_Pentapeptides_092024* in the pdf). This is the third time the Panel is seeing a safety assessment of this ingredient. At the March 2024 meeting, the Panel considered negative human irritation and sensitization data and the limited dermal penetration of Palmitoyl Pentapeptide-4, further supported by log p values for all 3 ingredients. Consequently, the Panel issued a Tentative Report for public comment with the conclusion that Myristoyl Pentapeptide-4, Palmitoyl Pentapeptide-4, and Pentapeptide-4 (KTTKS and KTSKS sequences) are safe in cosmetics in the present practices of use and concentration described in the safety assessment.

Since the March 2024 meeting, no new data were received. Comments that were received from the Council for the Tentative Report have been addressed, and follow this memo (*PCPCcomments_Pentapeptides_092024*). A comments response checklist is also included (*response-PCPCcomments_Pentapeptides_092024*).

The following documents are included in the package for your review:

- a flow chart (*flow_Pentapeptides_092024*)
- ingredient history (*history_Pentapeptides_092024*)
- search strategy (*search_Pentapeptides_092024*)
- data profile (*dataprofile_Pentapeptides_092024*)
- transcripts (*transcripts_Pentapeptides_092024*)

The Panel should carefully review the revised exposure assessment section, Abstract, Discussion, and Conclusion and issue a Final Report.

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: May 6, 2024

SUBJECT: Tentative Report: Safety Assessment of Myristoyl Pentapeptide-4, Palmitoyl Pentapeptide-4 and Pentapeptide-4 as Used in Cosmetics (release date: April 18, 2023)

The Personal Care Products Council respectfully submits the following comments on the Tentative Report, Safety Assessment of Myristoyl Pentapeptide-4, Palmitoyl Pentapeptide-4 and Pentapeptide-4 as Used in Cosmetics.

Key Issues

Discussion – It would be helpful if the Discussion would acknowledge that all the information in the report is primarily on Palmitoyl Pentapetide-4, with a small amount of information on Pentapeptide-4 itself. The Discussion should then state why the Expert Panel considered it acceptable to read-across using data on Palmitoyl Pentapeptide-4 to support the safety of Myristoyl Pentapeptide-4. For example, the dermal penetration data on Palmitoyl Pentapeptide-4 showed limited dermal absorption. Log p values indicated that the dermal penetration of Myristoyl Pentapeptide-4 would be less than Palmitoyl Pentapeptide-4.

The way the Discussion is currently written suggests that the Expert Panel primarily considered human data to reach a conclusion concerning dermal irritation and sensitization. It should be made clear that the Expert Panel considered all the dermal irritation and sensitization data in a weight of evidence approach.

The Discussion states: “The Panel stated that although the HRIPTs were not performed at maximum use concentrations....” which implies the concentrations tested in the HRIPTs were lower than the maximum use concentration. The maximum use concentration reported was 0.0035%. The HRIPTs were at 0.018% and 0.01% (which resulted in dose of 5.54 $\mu g/cm^2$). Both are greater than the maximum use concentration. This needs to be rewritten. Somewhere in the report, it would also be helpful to calculate dermal doses ($\mu g/cm^2$) at the maximum use concentrations to compare to the dermal doses used in the HRIPT.

The Discussion states: “the negative human dermal irritation studies at less than the maximum use concentration....”. The maximum use concentration reported was 0.0035%. The human dermal irritation studies were completed on materials that contained 0.01% Palmitoyl Pentapeptide-4 and 0.018% Palmitoyl Pentapeptide-4, both greater, not less than the maximum use concentration. This needs to be rewritten.

Somewhere in the report, it should clearly state that in the Dictionary, Pentapeptide-4 and Myristoyl Pentapeptide-4 are associated with 1 sequence, KTTKS, and Palmitoyl Pentapeptide-4 is associated with two sequences, KTTKS and KTSKS. This is based on the CAS numbers and technical names included in the Dictionary monographs. This information could easily be added as a footnote to Table 1.

Additional Considerations

Cosmetic Use – It would be helpful to note that the high use concentration in leave-on products (0.0012% face and neck preparations) was also reported in eye lotions.

Non-Cosmetic Use – Please add “in” to the following: “has been tested [in] male albino Wistar rats”

Genotoxicity; Summary; Table 4 – The description of the Ames test of Pal-KTSKS needs to be revised. Only the preliminary cytotoxicity study used doses up to 5000 µg/plate. The test substance was considered cytotoxic at 5000 µg/plate, so the highest dose tested in the actual assays was 1600 µg/plate. As the observation of cytotoxicity can affect the interpretation of the study, the concentrations at which cytotoxicity was observed should be stated (especially in Table 4). Cytotoxicity was strain and metabolic activity dependent (generally more cytotoxic without metabolic activation), but there were doses for all conditions that were not cytotoxic. In Table 4, in the Procedure column, it would be helpful to state that they completed both a plate incorporation assay and a preincubation assay.

Dermal Irritation and Sensitization – In the description of the guinea pig maximization test it states: “Thirty guinea pigs (test animals: 10/sex; controls: 5/sex), received the test substance at an effective concentration of 0.0075%...” This sentence needs to be revised. A total of 30 guinea pigs were in the study. Only 20 of the guinea pigs received the test substance. The other 10 guinea pigs were controls.

	Pentapeptides – September 30 – October 1, 2024 – Preethi Raj	
	Comment Submitter: Personal Care Products Council **Date of Submission: May 6, 2024 (comments on Tentative Report)**	
#	**Comment**	**Response/Action**
1	Key Issues: Discussion – It would be helpful if the Discussion would acknowledge that all the information in the report is primarily on Palmitoyl Pentapeptide-4, with a small amount of information on Pentapeptide-4 itself. The Discussion should then state why the Expert Panel considered it acceptable to read-across using data on Palmitoyl Pentapeptide-4 to support the safety of Myristoyl Pentapeptide-4. For example, the dermal penetration data on Palmitoyl Pentapeptide-4 showed limited dermal absorption. Log p values indicated that the dermal penetration of Myristoyl Pentapeptide-4 would be less than Palmitoyl Pentapeptide-4. The way the Discussion is currently written suggests that the Expert Panel primarily considered human data to reach a conclusion concerning dermal irritation and sensitization. It should be made clear that the Expert Panel considered all the dermal irritation and sensitization data in a weight of evidence approach. The Discussion states: "The Panel stated that although the HRIPTs were not performed at maximum use concentrations...." which implies the concentrations tested in the HRIPTs were lower than the maximum use concentration. The maximum use concentration reported was 0.0035%. The HRIPTs were at 0.018% and 0.01% (which resulted in dose of 5.54 µg/cm2). Both are greater than the maximum use concentration. This needs to be rewritten. Somewhere in the report, it would also be helpful to calculate dermal doses (µg/cm2) at the maximum use concentrations to compare to the dermal doses used in the HRIPT. The Discussion states: "the negative human dermal irritation studies at less than the maximum use concentration....". The maximum use concentration reported was 0.0035%. The human dermal irritation studies were completed on materials that contained 0.01% Palmitoyl Pentapeptide-4 and 0.018% Palmitoyl Pentapeptide-4, both greater, not less than the maximum use concentration. This needs to be rewritten. Somewhere in the report, it should clearly state that in the Dictionary, Pentapeptide-4 and Myristoyl Pentapeptide-4 are associated with 1 sequence, KTTKS, and Palmitoyl Pentapeptide-4 is associated with two sequences, KTTKS and KTSKS. This is based on the CAS numbers and technical names included in the Dictionary monographs. This information could easily be added as a footnote to Table 1.	- Statement added that most of the data is on Palmitoyl Pentapeptide-4, and why it was acceptable to read-across - Added weight of evidence statement Here are the calculations for the 2nd HRIPT: Effective concentration x amount applied = amount applied to patch = 180 ppm x 160 mcg = 2.88 µg Patch size = 40 cm^2 Dose/area of skin = 2.88/40 = 0.072µg/cm^2 -have rewritten Discussion to state that the HRIPTs are at higher concentrations of use (compared to the reported max use concentration)
2	Cosmetic Use – It would be helpful to note that the high use concentration in leave-on products (0.0012% face and neck preparations) was also reported in eye lotions.	-added
3	Non-Cosmetic Use – Please add "in" to the following: "has been tested [in] male albino Wistar rats"	-added
4	Genotoxicity; Summary; Table 4 – The description of the Ames test of Pal-KTSKS needs to be revised. Only the preliminary cytotoxicity study used doses up to 5000 µg/plate. The test substance was considered cytotoxic at 5000 µg/plate, so the highest dose tested in the actual assays was 1600 µg/plate. As the observation of cytotoxicity can affect the interpretation of the study, the concentrations at which cytotoxicity was observed should be stated (especially in Table 4). Cytotoxicity was strain and metabolic activity dependent (generally more cytotoxic without metabolic activation), but there were doses for	-added these details/clarification

	all conditions that were not cytotoxic. In Table 4, in the Procedure column, it would be helpful to state that they completed both a plate incorporation assay and a preincubation assay.	
5	Dermal Irritation and Sensitization – In the description of the guinea pig maximization test it states: "Thirty guinea pigs (test animals: 10/sex; controls: 5/sex), received the test substance at an effective concentration of 0.0075%..." This sentence needs to be revised. A total of 30 guinea pigs were in the study. Only 20 of the guinea pigs received the test substance. The other 10 guinea pigs were controls.	-revised

CIR History of:

Myristoyl Pentapeptide-4, Palmitoyl Pentapeptide-4, and Pentapeptide-4

March 2013

The Panel initially reviewed a large family of ingredients called Palmitoyl Oligopeptides. Since, this grouping was quite broad, the Panel decided to table this report to regroup the ingredients. Palmitoyl Petnapeptide-4 was one of said ingredients.

Previously received (unpublished) data for Palmitoyl Pentapeptide-4 includes:

- January 2013: concentration of use info

- November 2012: data from industry:

- Palmitoyl Pentapeptide-4 tested at 0.01% (vehicle and other contents not specified):
  -acute dermal irritation and acute eye irritation in rabbits, acute oral toxicity in rats, 2-wk dermal irritation in guinea pigs, HET-CAM assay, acute dermal irritation in 10 subjects, HRIPT in 51 subjects
- GPMT (0.0075% in saline and 0.01% during induction; 0.0025% in saline during challenge)
- Ames test (0.5% Palmitoyl Pentapeptide, in ethanol and water)

July 2022; February 2023

-Concentration of use data submitted by Council**;** Updated frequency of use data received from the VCRP program

April 2023

-SLR posted on CIR website; comments on SLR received from Council

- A memo was received from the Council stating that in a study summarized in the report, Palmitoyl Pentapeptide-4 did not exceed the concentration of this ingredient in face and neck products reported to the PCPC concentration of use survey

May - July 2023

In response to the SLR, the following data were received:

- Summary Information on Palmitoyl Pentapeptide-4 (provides an overview of the individual data files listed below)
- EpiSKIN® test with MTT assay (formulation containing 0.12% Palmitoyl Pentapeptide-4)
- Human patch test (formulation containing 0.12% Palmitoyl Pentapeptide-4)
- HET-CAM assay (formulation containing 0.12% Palmitoyl Pentapeptide-4)
- In vitro ocular irritation: SkinEthic™ model (formulation containing 0.12% Palmitoyl Pentapeptide-4)
- HRIPT (formulation containing 0.12% Palmitoyl Pentapeptide-4)
- XenoScreen YES/YAS endocrine disruptor testing (formulation containing 0.12% Palmitoyl Pentapeptide-4)
- XenoScreen XL YES endocrine disruptor testing (formulation containing 0.12% Palmitoyl Pentapeptide-4)
- DPRA (81.6% Palmitoyl Pentapeptide-4)
- In vitro sensitization test using KeratinoSens™ cell line (81.6% Palmitoyl Pentapeptide-4)
- Ames test (81.6% Palmitoyl Pentapeptide-4)
- In vitro mammalian cell micronucleus test (81.6% Palmitoyl Pentapeptide-4)
- Phototoxicity test (Palmitoyl Pentapeptide-4, tested at 0.0015%)

September 2023

A Draft Report was presented to the Panel. After reviewing the available data, the Panel issued an IDA with the following data needs:

- Dermal irritation and sensitization data for the lysine-threonine-serine-lysine-serine (KTSKS) amino acid sequence
- Skin penetration and degradation data for Myristoyl Pentapeptide-4 (KTSKS sequence)
- Clarification of the concentration of use tested in the HRIPT study currently summarized in the report on Palmitoyl Pentapeptide-4 (Pal-lysine-threonine-threonine-lysine-serine; Pal-KTTKS) sequence

The following data were received:

- revised concentration of use data, clarifying that the previously reported max concentration of use was for an experimental product
- predicted log P values for all 3 ingredients, and both amino acid sequences
- Clarification of the concentration of use and other details in the HRIPT study on Palmitoyl Pentapeptide-4

March 2024

A Draft Tentative Report was presented to the Panel. The Panel considered the limited dermal penetration of these ingredients and the negative dermal irritation and sensitization in a weight of evidence approach, issuing a Tentative Report with a safe as used conclusion.

Comments on the Tentative Report were received from Council after the March meeting.

September 2024

A Draft Final Report is being presented for Panel review.

Pentapeptides Data Profile* – September 30 – October 1, 2024 – Preethi Raj

				Toxicokinetics			Acute Tox			Repeated Dose Tox			DART		Genotox		Carci		Dermal Irritation			Dermal Sensitization				Ocular Irritation		Clinical Studies	
	Reported Use	Method of Mfg	Impurities	log P/log K_{ow}	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
Myristoyl Pentapeptide-4	X																												
• Pal-KTTKS				X																									
• Pal-KTSKS				X																									
Palmitoyl Pentapeptide-4	X																												
• Pal -KTTKS		X	X	X	X	X		X		X					X					X	X		X	X		X	X		
• Pal-KTSKS		X	X	X											X				X		X	X		X	X	X			
Pentapeptide-4	X																												
• Pal-KTTKS				X	X	X																							
• Pal-KTSKS				X																									

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

[Pentapeptides]

Ingredient	CAS #	PubMed	FDA	HPVIS	NIOSH	NTIS	NTP	FEMA	EU	ECHA	ECETOC	SIDS	SCCS	AICIS	FAO	WHO	Web
Palmitoyl Pentapeptide-4	521091-64-5 214047-00-4	✓	NR	NR	NR	NR	NR	NR	✓*	NR	NR	NR	NR	NR	NR	NR	✓
Pentapeptide-4	NA	✓	NR	NR	NR	NR	NR	NR	✓*	NR	NR	NR	NR	NR	NR	NR	✓
Myristoyl Pentapeptide-4	NA	NR	NR	NR	NR	NR	NR	NR	✓*	NR	NR	NR	NR	NR	NR	NR	✓

NR- not reported; ✓ - data available; ✓*- data available, but not relevant

Search Strategy

PubMed

[*total # of hits / # hits that were useful*] – search last performed: 08/14/2024

((((((((((((((myristoyl pentapeptide-4) OR (Myristoyl Pentapeptide-3)) OR (Collasyn 514KS)) OR (Palmitoyl Pentapeptide-3)) OR (Palmitoyl Pentapeptide-4)) OR (521091-64-5)) OR (214047-00-4)) OR (1392416-25-9)) OR (149128-48-3)) OR (N2-(1-oxohexadecyl)-L-lysyl-L-threonyl-L-seryl-L-lysyl-L-serine)) OR (N2-(1-oxohexadecyl)-L-lysyl-L-threonyl-L-threonyl-L-lysyl-L-serine)) OR (Palmitoyl Pentapeptide-3)) OR (Lipopentapeptide 3)) OR (OriStar POPP)) OR (SpecPed SC-PP4)) OR (ApepPPP-5)) OR (BsPep-5)) OR (Matrixyl)
- 4,508/6 results

General Search

palmitoyl pentapeptide-4 cosmetic toxicity – 403,000/3

oligopeptide toxicity pentapeptide-4 – 264,000/4

LINKS

Search Engines

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

Pertinent Websites

- wINCI - https://incipedia.personalcarecouncil.org/winci/ingredient-custom-search/
- FDA databases http://www.ecfr.gov/cgi-bin/ECFR?page=browse
- FDA search databases: http://www.fda.gov/ForIndustry/FDABasicsforIndustry/ucm234631.htm;,
- Substances Added to Food (formerly, EAFUS): https://www.fda.gov/food/food-additives-petitions/substances-added-food-formerly-eafus
- GRAS listing: http://www.fda.gov/food/ingredientspackaginglabeling/gras/default.htm
- SCOGS database: http://www.fda.gov/food/ingredientspackaginglabeling/gras/scogs/ucm2006852.htm
- Indirect Food Additives: http://www.accessdata.fda.gov/scripts/fdcc/?set=IndirectAdditives
- Drug Approvals and Database: http://www.fda.gov/Drugs/InformationOnDrugs/default.htm
- FDA Orange Book: https://www.fda.gov/Drugs/InformationOnDrugs/ucm129662.htm
- (inactive ingredients approved for drugs: http://www.accessdata.fda.gov/scripts/cder/iig/
- HPVIS (EPA High-Production Volume Info Systems) - https://iaspub.epa.gov/oppthpv/public_search.html_page
- NIOSH (National Institute for Occupational Safety and Health) - http://www.cdc.gov/niosh/
- NTIS (National Technical Information Service) - http://www.ntis.gov/
  - technical reports search page: https://ntrl.ntgis.gov/NTRL/
- NTP (National Toxicology Program) - http://ntp.niehs.nih.gov/
- Office of Dietary Supplements https://ods.od.nih.gov/
- FEMA (Flavor & Extract Manufacturers Association) GRAS: https://www.femaflavor.org/fema-gras
- EU CosIng database: http://ec.europa.eu/growth/tools-databases/cosing/
- ECHA (European Chemicals Agency – REACH dossiers) – http://echa.europa.eu/information-on-chemicals;jsessionid=A978100B4E4CC39C78C93A851EB3E3C7.live1
- ECETOC (European Centre for Ecotoxicology and Toxicology of Chemicals) - http://www.ecetoc.org
- European Medicines Agency (EMA) - http://www.ema.europa.eu/ema/
- OECD SIDS (Organisation for Economic Co-operation and Development Screening Info Data Sets)- http://webnet.oecd.org/hpv/ui/Search.aspx
- SCCS (Scientific Committee for Consumer Safety) opinions: http://ec.europa.eu/health/scientific_committees/consumer_safety/opinions/index_en.htm
- AICIS (Australian Industrial Chemicals Introduction Scheme)- https://www.industrialchemicals.gov.au/
- International Programme on Chemical Safety http://www.inchem.org/
- FAO (Food and Agriculture Organization of the United Nations) - http://www.fao.org/food/food-safety-quality/scientific-advice/jecfa/jecfa-additives/en/
- WHO (World Health Organization) technical reports - http://www.who.int/biologicals/technical_report_series/en/
- www.google.com - a general Google search should be performed for additional background information, to identify references that are available, and for other general information

Botanical Websites, if applicable

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

Fragrance Websites, if applicable

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

SEPTEMBER 2023 PANEL MEETING – INITIAL REVIEW/DRAFT REPORT

Belsito Team – September 11, 2023

DR. BELSITO: Okay. Pentapeptides.

MS. RAJ: Before you start, I just have some printouts for everyone. It's the data profile by sequence, because there are two sequences in the report.

DR. KLAASSEN: Right.

MS. RAJ: As well as the paper that you mentioned.

DR. BELSITO: I think everyone got the food paper, right?

DR. KLAASSEN: Mm-Hmm.

MS. RAJ: It's in there.

DR. BELSITO: Right. But everyone should have gotten it ahead of time because Bart sent it out. I mean, that was my major issue with this report, is that it sort of raised my antenna because it's used as anti-wrinkle, which can be used as a marketing claim. And the other study that was done in Asia just showed some improvementm in crow's lines.

But in the Fu study, I mean, the interesting thing is they didn't see any changes in trans-epidermal water loss and other endpoints where there was biological activity, except in the keratin profile. And so my question is, do we consider that kind of change that they noted to be biologic and therefore not a cosmetic or not?

DR. RETTIE: Is it up to us to decide whether it's biological or not? Isn't it -- doesn't it have to have something to do with the claims of the manufacturer?

DR. BELSITO: Well, no, the claims they're making are purely marketing. You know, it's being marketed as an anti-wrinkle. So their marketing claims keep it within the level of cosmetics and what dermatologists incorrectly called cosmeceuticals.

The dilemma I have is that we have the Fu paper and there are changes in the keratin profile. And then we have a manufacturer telling us that the Fu levels do not exceed the levels that we use in our product. So sometimes, Allan, we've had materials where they can cause -- the most common thing is a bleaching effect. And then we'll say, well, that would not be appropriate for a cosmetic.

But here we have a reference to this paper and then a reference below it, personal communication, to the PCPC, that the Fu study was using a material that does not exceed the levels that we're reporting here. So then it becomes up to us to say, okay, it didn't cause any changes in trans-epidermal water loss, blah, blah, blah. You know, there were some changes in the subtypes of keratin but we don't consider this a significant biological effect. Or we do, in which case we can't rule on the safety of that concentration.

DR. RETTIE: My reading was that it was a difference in percentages of the different subtypes of keratin.

DR. BELSITO: Right.

DR. RETTIE: And going back to you, is that -- I don't know how to evaluate that.

DR. BELSITO: I don't either. I'm a dermatologist, I'm not a keratin person. And you would think that if it changed -- if there was biological significance there would've been a change in trans-epidermal water loss, which wasn't seen in that report, right?

DR. RETTIE: So that's a more gross effect. And could we refer back to that transepidermal --

DR. BELSITO: What do you think?

DR. SNYDER: It's a biological effect, period.

DR. BELSITO: Yeah.

DR. SNYDER: It changed the profile.

DR. BELSITO: It's a biological --

DR. BELSITO: If the profile didn't have an apparent effect, other than a wrinkle --

DR. SNYDER: In toxicology, we talk about the fact that it has to alter the function or something. But we don't know. I mean, it clearly altered the function if it decreased wrinkles.

DR. RETTIE: Well, it didn't alter the function in terms of trans-epidermal water loss.

DR. SNYDER: That's only one function of it, though.

DR. BELSITO: Right. I mean, it's a conundrum.

DR. SNYDER: You weren't listening to Don this morning about the KeratinoSens.

MS. RAJ: Dr. Belsito, a very, I guess, simplified understanding of this paper, isn't it basically saying that the formulation that they tested, that included this peptide, was tolerable compared to, say, the tretinoin?

DR. BELSITO: Well, that was what they were saying. But also what they're reporting is a change in keratin profile.

DR. SNYDER: To me, that's a biological effect. That's a drug, right?

DR. BELSITO: Yeah. I mean, what they're showing is that, okay, there's -- you know, the retinoid increases erythema, increases dryness; this pentapeptide does not. The changes in baseline, the trans-epidermal water loss, we're seeing with the retinoid, but we're not seeing with the pentapeptide. But then there were changes in the keratin profile that were seen with the pentapeptide.

DR. RETTIE: So that's the only change.

DR. BELSITO: It's the only change reported in this paper.

DR. SNYDER: Don has a comment.

DR. BJERKE: Yeah, if I may. The way I kind of wrap my mind around this, is to look at whether it's a NOEL, no-observable-effect-level, or it's a no-observable-adverse-effect-level. Because there's no restriction on cosmetic ingredients that they can't have any biological activity. They all do. Look at salicylates, look at retinoids.

Almost everything that we do in parts of benefit to the appearance of the skin. And so, I think what we're talking about here is a -- we don't have an inert ingredient. We don't have something that we can say has no effects. But what we can say is it has no adverse effects. Because if you think about these, these peptides are present at, let's say, 5 to maybe 100, maybe max 200 part per million.

DR. BELSITO: Yeah, they're small.

DR. BJERKE: They don't get across the skin very well either, typically.

DR. BELSITO: I mean, yeah, if you look at Oil of Olay, Regenerist Micro-sculpting cream --

DR. BJERKE: Exactly.

DR. BELSITO: -- it has this pentapeptide in it.

DR. BJERKE: Right.

DR. BELSITO: It's the ingredients, da-da-da-da, and then in small print -- then the pentapeptide comes in at the end.

DR. BJERKE: Absolutely. I mean, based on this study, to try to ascribe something specifically to the pentapeptide as opposed to the overall formulation, I think it's somewhat difficult as well.

DR. BELSITO: I'm not arguing with you. I'm just pointing out that I saw this change and I didn't know how to deal with it, particularly because the next reference after was that the Fu study was what we have in our product possibly.

DR. BJERKE: Yeah, exactly. And then I think the way that we position this is it helps to improve the appearance of fine lines and wrinkles. If we were to say that this decreases wrinkles or removes wrinkles --

DR. BELSITO: No, I know. I know the marketing claim.

DR. BJERKE: Yeah. Yeah. And you pointed that out quite clearly, which is exactly how we approach these.

DR. BELSITO: I mean, I'm fine. I just want to point it out and have this discussion because it's the first time we've ever dealt with something where there's been any kind of effect that we've seen, that we haven't been able to simply say should not occur.

DR. BJERKE: Right. Right.

DR. BELSITO: Because as used, this change in keratin profile seems to be occurring, but there's no redness, there's no irritation, there's no increased trans-epidermal water loss. We have no other evidence of an adverse effect. And if everyone else is comfortable with that, I'll forget I ever mentioned it.

DR. RETTIE: I'm comfortable. I had a couple of comments.

DR. BELSITO: Paul?

DR. SNYDER: I'm fine.

DR. BELSITO: Okay. Go ahead, Allan, with your comments.

DR. RETTIE: I'm trying to find it. Well, one's just a note. I was curious about the reported log p(s) for these things. The Myristoyl Pentapeptide at a log p of (negative) -0.3?

DR. BELSITO: I had the same question.

DR. RETTIE: And then you jump up for the Palmitoyl to like 3.

DR. BELSITO: Yeah. Is this true for the others as well or just the Palmitoyl? Yeah.

DR. RETTIE: I mean, if you look at Palmitoyl versus Myristoyl, log p(s) is like -- the difference is one right, for the acids. So this just seemed -- I mean, they're calculated, so we just don't know. So I just let it go because it was estimated, but I was surprised by it. And the only other comment I had was under method of manufacture.

DR. BELSITO: Yeah, I have the same question.

DR. RETTIE: Yeah.

DR. BELSITO: In impurities, can the palmitoyl serve as a read across for the Myristoyl and the Pentapeptide-4?

DR. RETTIE: That was part of it. I was more specifically thinking about deleting the second paragraph under methods of manufacture. Seemed to me they were both provided by Sederma. And the first one came years ago and it was like a very condensed version. And the newer one, which is the first paragraph, is lots of information about peptide synthesis.

So, I thought you could probably strike the second one since they were both from the same manufacturer. And one was very generic, the other was quite specific.

DR. BELSITO: Okay. What page are you on, Allan, PDF what?

DR. SNYDER: Page 55.

DR. RETTIE: 55, is it?

DR. SNYDER: Yeah.

DR. BELSITO: So, you're saying?

DR. RETTIE: I'm saying -- Page 55, if I ever get there? There's a redundancy there is basically what I'm saying.

DR. BELSITO: So, the PCPC also raised, they are general to the production of peptide synthesis. It's unknown whether it's specific ingredients of cosmetics. But then one of the methods is exactly what you described.

DR. RETTIE: Yep. So the last paragraph in this section, on PDF Page 56.

DR. BELSITO: But first we should address the PCPC comment. Are we fine with dropping method --

DR. RETTIE: Yes, I think so.

DR. BELSITO: It says methods of manufacturing detailed are general to the production of peptide synthesis. Unknown whether they're specific to the ingredients that are used in cosmetics. But then the description that you are giving,

, is essentially the description for one of the cosmetics. So do we get rid of that whole paragraph?

DR. EISENMANN: I don't care. I just don't like that -- it's saying it's unknown.

DR. BELSITO: Right.

DR. EISENMANN: I mean, I don't care how you resolve it, but it's known, and it agrees with what you -- the general --

DR. BELSITO: Right. I mean, I agree. So what do we do with that paragraph? Get rid of it and then just discuss the synthesis for Palmitoyl Pentapeptide-4?

DR. KLAASSEN: That's what I would think.

DR. EISENMANN: That's fine.

DR. RETTIE: So, (inaudible) after -- general to the production of peptide synthesis?

DR. BELSITO: No, we're talking about getting rid of the entire paragraph because then it's described again under Palmitoyl. And then the other question is, can that method of manufacturing and impurities data for Palmitoyl Pentapeptide cover Pentapeptide-4 and Myristoyl, because we don't have manufacturing impurities for those two.

DR. RETTIE: But you would imagine it's not a long, big stretch to the synthesis of just Pentapeptide-4 or the Myristoylated version of it.

DR. BELSITO: I agree. I'm just pointing that out.

DR. RETTIE: Yep, I agree.

DR. BELSITO: So, we're fine with using the Palmitoyl to support the safety?

DR. RETTIE: Yes.

DR. SNYDER: Dan Liebler previously had raised the issue of clarification of the nomenclature of the composition. And it was his opinion they should be reviewed independently.

DR. RETTIE: The Palmitoyl versus the Myristoyl?

DR. SNYDER: Correct.

DR. BELSITO: Who did?

DR. SNYDER: Dan.

DR. KLAASSEN: Why?

DR. SNYDER: It was in our old notes.

DR. RETTIE: Was that because of potential biological effects being so different?

DR. SNYDER: Because this is essentially a Pentapeptide-4 report.

DR. RETTIE: Mm-Hmm.

DR. SNYDER: Yeah. So I don't know why -- what -- I don't know.

DR. BELSITO: Well, he's not here to answer it, so we have to rely on Allan.

DR. SNYDER: Well, no, I was trying to get Allan's take on that.

DR. BELSITO: Okay. You're trying to prompt Allan, huh?

DR. SNYDER: Well, yeah, a little bit.

DR. RETTIE: I didn't have awful lot of problems with it.

DR. SNYDER: Okay.

DR. RETTIE: I mean, for the Myristoylated and Palmitoylated compounds, those are going get cleaned. You're going to get Pentapeptide-4 from it.

DR. BELSITO: Okay.

DR. RETTIE: I think it's a decent group.

DR. BELSITO: You're sure, as opposed to Diglycerin?

DR. RETTIE: This one I'm pretty sure.

DR. BELSITO: Okay. Okay.

DR. SNYDER: All right. Okay.

DR. BELSITO: Okie doke.

DR. RETTIE: I had fun with this. My son is a peptide chemist. Got his input.

DR. SNYDER: Must be pretty cool for you. Yeah. That's pretty cool.

DR. BELSITO: Preethi, I had a question. That's the same question that Allan had about the numbers. Why minus three for Myristoyl and plus for the Palmitoyl?

MS. RAJ: So those two values come from a paper, if you see Reference Number 2, it's Abu Samah.

DR. BELSITO: Yeah.

MS. RAJ: So that's where I got them from. I could possibly -- I think there were PubChem pages, too, which had log p values if those would be more suitable to you. But I figured since they're comparing both of the ingredients in the same paper, that you could have confidence that they would be not just random, but.

DR. KLAASSEN: Maybe one was a typing error.

MS. RAJ: Um-hmm. Maybe.

DR. KLAASSEN: Those things do happen.

DR. BELSITO: Then in the introduction, you say the Palmitoyl Pentapeptide, and then the Pentapeptide has different amino acid groups. Is that true for the Myristoyl as well or just for the Palmitoyl?

MS. RAJ: Well I think the sequences are referring more to the Pentapeptide-4 molecule, which is the same for all three ingredients reviewed in this report, right? So there could potentially even be more sequences. But I think the reason why we added that language is because we have data, two sequences. And just to let you know, in the wINCI monograph for Palmitoyl Pentapeptide-4, both of the sequences for which we have data, on the report are listed.

DR. BELSITO: So then should we say other sequences are possible?

MS. RAJ: Yes, we could. I mean, if the chemists agreed.

DR. BELSITO: I mean, I'm just saying because as I read this now we're saying that --

DR. EISENMANN: They would name it different. Usually now they're naming each sequence separately. This was an early peptide and they kind of grandfathered in that there's two sequences.

DR. BELSITO: So there are only two sequences?

DR. EISENMANN: Right?

DR. BELSITO: But Myristoyl Pentapeptide could have one of these two sequences as well? And Pentapeptide-4 could as well?

MS. RAJ: I would think so.

DR. RETTIE: So is it six sequences we're dealing with or three is the question.

DR. BELSITO: We're dealing with two.

DR. EISENMANN: No, it's two sequences that might be attached. And it's very unlikely that Pentapeptide is used on its own because it wouldn't get into the skin is my understanding. It may be one of these things that they put a name in the dictionary to name something else.

DR. BELSITO: Right. Okay.

DR. EISENMANN: So, reference material.

DR. BELSITO: I'm just trying to clarify the two sequences that we're seeing. Could those also be for Myristoyl and for Pentapeptide-4? Or is it only the Palmitoyl that has those two sequences?

MS. RAJ: We did not find data with those sequences associated the other ingredients.

DR. BELSITO: But they could be?

MS. RAJ: I would think so, but I'll let the chemists speak on that. If they --

DR. BELSITO: Well, I mean the chemists aren't going to know what the cosmetic companies are doing.

DR. EISENMANN: I have to go back to the supplier and ask them again. And ask.

DR. BELSITO: Well, I mean, I just think we need clarification in the language, whether, you know -- because right now the way it's written, Myristoyl Pentapeptide and Palmitoyl have an additional saturated da-da-da-da. The amino acid sequence of the ingredients can vary.

So instead of just saying Palmitoyl, you say the amino acid sequence of the Pentapeptide portion of these ingredients can vary. The two variations are KTTKS and KTSKS, rather than linking them to Palmitoyl. Is that fair?

MS. RAJ: I just wonder though -- I mean, I'm just thinking out loud. Because these two sequences were on the Palmitoyl Pentapeptide monograph and not on the Pentapeptide-4 monograph, would that make any difference? I don't know.

DR. BELSITO: I mean, I'm just trying to be as accurate as possible. Because as I read this, do we say what the sequence is for Myristoyl Pentapeptide?

MS. RAJ: No.

DR. BELSITO: Fine.

DR. RETTIE: We don't associate it with either the KTTKS or the other one.

DR. BELSITO: Okay. So just leave it as it is. It's fine.

DR. RETTIE: But I'm wondering if some clarification is needed, that when you go back in time and you read all the information, when it first came up, there was a laundry list yay big and a lot of questions about nomenclature which I wasn't necessarily part of. But when I just read the Chemistry section here, Pentapeptide-4 is a synthetic peptide comprised of lysine, serine, and threonine linked in varied 5-amino sequences, two of which. So that doesn't seem to preclude other sequences to me.

DR. BELSITO: No, but what Carol's saying is those two have come under -- grandfathered as Pentapeptide-4.

DR. EISENMANN: I'll double check with Joanne, but I'm quite certain (inaudible) now.

DR. BELSITO: Pentapeptide-4 is that one or the other?

DR. RETTIE: Okay.

DR. BELSITO: But not any of the other combinations.

DR. RETTIE: That's doesn't come through in the way it's written to me at least, right now. Can we clarify that?

DR. BELSITO: So, what we can say is Pentapeptide-4, da-da-da-da. The two --

DR. SNYDER: Or varied 5-amino acid sequences (forming a pentapeptide) -- take out the two of which -- are lysine-threonine-threonine-lysine-serine. Just take out that two of which are. Just say what they are.

DR. BELSITO: The two referred to as Pentapeptide-4 are.

DR. SNYDER: Right.

DR. RETTIE: You could take out varied from 5-amino acid sequences. Just say which are linked, and 5-amino acid sequences, e.g.

MS. RAJ: Okay.

DR. BELSITO: Okay. So Pentapeptide-4 is a synthetic peptide comprised of -- what are you doing here, Allan?

DR. SNYDER: Comprised of either.

DR. BELSITO: Comprised of either.

DR. SNYDER: Or.

DR. RETTIE: Yeah.

DR. SNYDER: Just list the two, either or. The KTTKS and the KTSKS.

DR. EISENMANN: Is it either or, or is it a mix?

DR. BJERKE: I think we have to go back to the INCI dictionary, right? Because it's the same issue that we've been dealing with historically.

DR. EISENMANN: Yeah, I'm not sure.

DR. BJERKE: Yeah.

DR. SNYDER: All the rest of it is not important. We're defining what it is we're looking at.

DR. BELSITO: Is either serine-lysine-threonine -- okay. I got it.

DR. SNYDER: Yeah. Yep. Or, then the other.

DR. RETTIE: There might be more discussion with the other group tomorrow, particularly around biological activities of myristoylated and palmitoylated peptides, but I'm not sure that those are really relevant to what -- we'll see tomorrow. I'm expecting some --

DR. SNYDER: We've already reviewed myristic acid and palmitic acid.

DR. RETTIE: Very specifically when they're attached to proteins they have different biological functions.

DR. SNYDER: Okay. Fair enough.

DR. RETTIE: And in my opinion should not be treated together. But for peptides, these things are very specifically on a position that's not that relevant to their biological activity.

DR. SNYDER: Okay.

DR. RETTIE: It is my reading.

DR. SNYDER: Okay.

DR. BELSITO: Okay, Carol, are we going to get updates? Because right now, concentration of use is from 2013 from one of these.

MS. RAJ: No, that's just because data on Palmitoyl Pentapeptide-4 had previously been reviewed, but was not published. So that's why that data (inaudible).

DR. BELSITO: But we don't have 2022 or 2023 data for it.

MS. RAJ: We do. We do. It's in Table --

MS. FIUME: PDF Page 64.

MS. RAJ: Yes. Oh, you're saying it's --

MS. FIUME: So it's not recorded for the Pentapeptide-4. There was nothing (inaudible).

MS. RAJ: It's just for Palmitoyl Pentapeptide. And there's one concentration piece for (inaudible). That's actually the highest, if I'm not mistaken.

DR. EISENMANN: Yes, it is.

DR. BELSITO: So, the data in Table 3 is from 2023 -- the concentrations are from 2022 for all product categories?

MS. RAJ: Yes.

DR. BELSITO: Okay. Then I misunderstood. So, then in the cosmetics, you -- so this is PDF Page 56 under the Cosmetic, the third paragraph down. Historical concentration of use, where you say that Palmitoyl Pentapeptide-4 in 2013, I would get rid of that because it's misleading. We're looking at data for the current, we don't care about historical.

MS. RAJ: Okay.

DR. BELSITO: At least I don't care. Because that's where I thought you were giving me data from 2013.

MS. RAJ: But obviously we're still keeping the tox data?

DR. BELSITO: Yeah, the tox data is fine. But the concentration of use, I mean, I thought that it was old.

MS. RAJ: Okay.

DR. BELSITO: Under dermal penetration, Preethi, your second paragraph.

DR. SNYDER: It says Dermal Permeation, not Penetration.

DR. BELSITO: It's really more akin to dermal metabolism rather than penetration.

DR. SNYDER: You got Permeation instead of Penetration.

DR. BELSITO: Yeah. But the second paragraph is really more metabolism, no?

MS. RAJ: I think that's how they described it in the paper, but --

DR. BELSITO: Well, it says the dermal stability was evaluated in vitro.

DR. SNYDER: But it's okay if you look at -- if we would of have the normal category ADME instead of dermal permeation. So I'd change that dermal permeation to ADME absorption, distribution, metabolism.

MS. FIUME: Actually, we do break out the dermal penetration versus ADME.

DR. SNYDER: Okay.

MS. FIUME: So, it's typically broken out.

DR. BELSITO: But the second paragraph is not really penetration, it's more metabolism. Right?

DR. RETTIE: Which page are we on?

DR. SNYDER: Page 57.

DR. BELSITO: Page 57, the second paragraph. At predetermined times, the amount of Palmitoyl Pentapeptide-4 and Pentapeptide-4 present incubated mixtures was sampled and analyzed. Pentapeptide-4 was almost fully degraded in the dermal skin extract and whole skin homogenate. I mean, it's really metabolism.

DR. KLAASSEN: Yeah, just give it a new heading.

MS. FIUME: So you would want that as ADME?

DR. BELSITO: Yeah.

DR. KLAASSEN: Yeah. That's fine.

MS. RAJ: Just the second paragraph?

DR. KLAASSEN: Correct?

DR. BELSITO: Yeah. The first paragraph is penetration, not permeation. And the second paragraph is ADME.

DR. RETTIE: This supports all the expectations that (inaudible) will slightly impede metabolism, which is what you'd expect.

DR. BELSITO: So, um, we have no DART data here, so I just have a question. Assuming in concentrations maximum 0.05 percent limited percutaneous absorption in vitro; are we okay with the lack of systemic data?

DR. SNYDER: I think so. There was an endocrine study that was negative at 0.12 percent. So there's no -- I think so.

DR. BELSITO: So that would go in the Discussion?

DR. SNYDER: Yeah.

DR. BELSITO: Okay. So low maximum use concentration, limited percutaneous absorption data, in vitro, the absence of endocrine activity at certain concentration -- 0.1 percent obviated the Panel's need for DART data.

MS. RAJ: And I guess the genotox takes care of carci?

DR. BELSITO: Yeah. The dermal, as Don reviewed this morning, two out three in vitro means non-sensitizing. This is PDF Page 59, the second paragraph. So we have in vitro data supporting non-sensitization as well.

DR. RETTIE: I wondered about the estrogenic activity that was reported. The concentrations of some kind of context in the YAS agonist assay. Is there any concern about that?

DR. SNYDER: I didn't pick up on that.

DR. RETTIE: So it's at the bottom of Page 60, last paragraph?

DR. SNYDER: Yeah, that's nothing I don't think.

DR. RETTIE: So, that's 6.9 micromolar. Is that what it's talking about?

DR. SNYDER: Yeah.

DR. RETTIE: So that's pretty bad. Yeah. Okay.

DR. SNYDER: You have more, Don?

DR. BELSITO: I'm just trying to look here. Yeah. Under phototoxicity studies. So, basically, they were just doing a UV absorbance and it didn't absorb. So, the test article was predicted to be non-photo toxic and also non-photo allergenic, both endpoints.

Henry's law, less than a thousand, you're fine. And then, I just had a question on the HET CAM study for ocular toxicity. It says published in the *Journal Official Republique Francaise*. Do we know what protocol that was? Because there's no OECD test guidelines for HET CAM. There's an ECVAM protocol, was that the one that was used?

MS. RAJ: I believe so. I mean, whatever was provided in the data is what we put.

DR. BELSITO: Yeah. Rather than putting as published in whatever journal, if they say what protocol they used, I would get rid of the journal and just put the protocol.

MS. RAJ: Just in case the actual protocol numbers isn't found, what would you want then, Dr. Belsito?

DR. BELSITO: Then I think you just reference --

DR. EISENMANN: I'm pretty sure that's France's, like, federal register. That's not a journal. That's like a regulatory.

DR. BELSITO: Okay. So they're probably using an ECVAM criteria?

DR. EISENMANN: I don't know what they were using. But that's like the regular requirement of --

DR. BELSITO: If you could just see if they state what protocol they used for the HET CAM, because there are different protocols. I think the Japanese protocol differs from the European.

DR. KLAASSEN: I'm thinking this is a French CAM.

DR. BELSITO: I think we're getting to a safe as used, but then I just want to point out that in -- that's why I'm getting to the ocular irritation studies -- that one of them had a test article was classified as moderately irritating and this is max uses in an eye area formulation. So, and our safe is used, do we do formulated to be non-irritating?

MS. FIUME: You can. I mean, a lot of times -- the panel has gone both ways on it.

DR. BELSITO: It doesn't hurt.

MS. FIUME: So, yeah.

DR. BELSITO: Because once I got past the issue of biological effect, I thought we could go safe as used when formulated not to be irritating, based upon that ocular irritation. Which I think is probably not real, but there's certainly -- well we don't know

in the Fu study whether it was 0.05 percent. But there was no erythema, no dryness, so there was no evidence of irritation in that study. But we don't know the concentration. So is everyone okay with that conclusion?

DR. SNYDER: Yes.

DR. BELSITO: Okay. So the discussion, Preethi, would be we noted some changes in the keratin profile, but they do not appear to be adverse effects based upon lack of erythema, dryness, trans-epidermal water loss. That the molecule -- low concentration, not likely to be absorbed, lack of endocrine disruption obviated the needs for DART. Anything else in the discussion?

DR. RETTIE: I don't think not observed is appropriate, not for the palmitoylated one anyway.

DR. BELSITO: I'm sorry. Not observed what?

DR. RETTIE: I think you included not absorbed in the list of things that would mitigate concerns. But the palmitoylated peptide is going to be absorbed.

DR. EISENMANN: Into the skin, but not beyond the --

DR. RETTIE: Not beyond it? Is that what it said? Little to no permeation. You're correct. All good.

MS. FIUME: Can I ask for clarification for the Discussion just so it doesn't come back in the future? Does anything need to be discussed regarding the different sequences being that these have been broken up?

DR. BELSITO: No. I wouldn't go that far. Can we take a five minute bio break? Back at 3:15. And then we got to start boogying.

DR. SNYDER: Re-reviews, we're in good shape. We'll be fine.

DR. BELSITO: Yeah. That's what I hear. I don't know we're going to argue about read across.

[BREAK]

Cohen Team – September 11, 2023

DR. COHEN: Myristoyl Pentapeptide-4, Palmitoyl Pentapeptide-4, and Pentapeptide-4. This is a draft report of the safety assessment of these. This is the first time we're seeing these three ingredients, which comprised of five amino acid sequence of a lysine-serine and threonine. And these are sub-fragments of Type 1 collagen propeptide.

The Panel previously reviewed the safety of individual amino acids comprising of these ingredients as well as myristic acid and palmitic acid. And in 2013, the alpha amino acids were considered safe.

In 2019, the panel issued a final report on myristic and palmitic acid with the conclusions they were safe. Apropos to our last conversation, the hydrophilic and charged nature of Pentapeptide-4 makes it difficult for it to pass through an intact stratum corneum. These function as skin conditioning agents.

We have method of manufacturing and impurity. We have a frequency of use of 239 formulations, of which 223 are leave on with a maximum concentration of Palmitoyl Pentapeptide of 0.0035 percent. And Myristoyl Pentapeptide has four uses at 0.05 percent in an eye makeup preparation. So, we have these reported near the eye. It's reported to be in a face powder, which could possibly be inhaled. We have HRIPT in excess of the max use.

We received an article from Don which was referring to a cosmetic product that had retinyl propionate in it as well. So I know it's a little hard to pull out any adverse issues from the chemicals at hand, so I'll open it up for discussion.

DR. HELDRETH: I have one additional thing that Preethi wanted me to hand along. Was that we were looking at this report carefully and the ingredients therein, and realizing that there's actually two different amino acid sequences represented by the same ingredient name. So, Preethi prepared a data table, broken out by the two different amino acid sequences, with the thought that maybe you wouldn't think that read across would be appropriate between the two.

DR. ROSS: Yeah, I mean, I struggled with that. And I haven't seen this new table, but because they are different peptide structures I didn't think you could read across. And you know we only have the penetration and degradation and masking from one of those forms, and that's the KTTKS. The KTSKS -- this is going to get real confusing -- we don't have that. And so, I thought that probably should be done. The Palmitoyl KTSKS and masking.

Due to the lack of penetration stability, which David referred to, in the skin, I wasn't too concerned with systemic tox but there was no dermal effects on the, again, the KTSKS. So, I thought that needed to be done.

DR. COHEN: Can you repeat so I have that again?

DR. ROSS: Yeah. I mean, if we can get some sort of dermal toxicity on the KTSKS --

DR. COHEN: KTSKS.

DR. ROSS: But, I mean, the first thing, I think, would be -- the first point of departure here would be do we see penetration and degradation with the KTSKS. The Palmitoyl KTSKS? Because as, I think, Preethi has split this out here -- yeah. So, you see here, David, there's very little data with the KTSKS.

DR. COHEN: Right. And so, can you read-across with different peptide sequences?

DR. ROSS: I don't think so. There might be some discussion of that tomorrow, but I wouldn't have thought so, no. I'm sure Susan has an opinion on that.

DR. COHEN: Susan? Susan, what are your thoughts on read-across?

DR. TILTON: Yeah, I mean, I had the same questions, actually, about whether or not we could read across for the different peptides. I do think, especially, since we're at the draft report stage that we could request data on absorption or penetration and degradation dermally.

DR. COHEN: For the same one David mentioned, the KTSKS?

DR. TILTON: Yes. KTTKS?

DR. COHEN: Wait, K --

DR. ROSS: KTSKS.

DR. COHEN: KTS- -- well, let me see.

DR. TILTON: Oh.

DR. COHEN: We don't have acute tox on the KTSKS. We don't have that. Okay. I think that makes sense because we don't know what the biological signal will be by switching the two peptides around, right?

DR. ROSS: We don't --

DR. HELDRETH: I'll say in a previous safety assessment by CIR -- or by the Expert Panel I should say -- this had to be at least five or six years ago, the Panel did a report on so-called oligopeptides. And we ran into very similar situation where one ingredient name resulted in two different amino acid sequences that could fit under that name.

So, the Panel drafted the Discussion and their Conclusion at those sequences. So, it was something like oligopeptide X was safe as used when the amino acid sequences KTSKS or something like that.

And so, in the same report, you're looking at one ingredient, per se, but you're really looking at two different chemicals.

DR. SLAGA: Right.

DR. COHEN: I think we're all in agreement on that. So, what other data needs might we have? I just don't want to leave it hanging with just one and not have everything now.

DR. ROSS: What have you got there, David? What's your list so far?

DR. COHEN: Dermal tox on KTSKS. We have -- oh, I got to look back on this one. We have human sensitization on both, and I think they're at good concentrations.

DR. ROSS: The human HRIPT -- was the concentration specified for KTTKS? I have in my notes that it wasn't specified.

DR. HELDRETH: Yeah. I don't think it was specified. If you see on the list there you'll see sometimes there's an X next to, say like Palmitoyl Pentapeptide-4, you see there's an X under reported use. Having an X there in that category means that we have data, but it didn't specify if it's -- what amino acid sequence.

DR. ROSS: Could we try and get that concentration or, I mean, it would be impossible to get?

DR. HELDRETH: We could ask for it.

DR. ROSS: What's -- yeah.

DR. COHEN: Why would it be impossible?

DR. ROSS: I mean, it must be there somewhere.

DR. COHEN: Yeah. K- -- well.

DR. ROSS: The Palmitoyl KTTKS.

DR. COHEN: Well, we have KTTKS at 0.01 percent.

DR. ROSS: What page are you on?

DR. COHEN: I'm on 66. This is for irritation. And for sensitization for KTTKS, on the HRIPT, we don't have concentration so we need that.

DR. ROSS: Yeah. That's right.

DR. COHEN: Concentration --

DR. ROSS: Wasn't specified.

DR. COHEN: -- of the HRI- -- I mean, they just need to pull a report for that one. It's referenced. Now, we're going to read across for Pentapeptide-4 and Myristoyl Pentapeptide-4.

DR. ROSS: I don't know. Are we?

DR. COHEN: That's why I'm asking.

DR. ROSS: I mean, there are very few uses with the Myristoyl, but it's mainly ocular. But yeah, I mean, those groups are going to govern penetration. In a different way, they're going to get to different spots. I mean, you could make an argument for reading across, but I think being conservative, maybe we shouldn't read across them.

DR. COHEN: So, then we need the full dossier for the Myristoyl Pentapeptide-4.

DR. ROSS: You would. I think you'd need to start out with those skin penetration studies, because there's not much point in doing a full dossier of toxicity if it doesn't get in, right?

DR. COHEN: So, we need dermal tox for not only KTSKS but for the --

DR. ROSS: First and foremost, you need the skin penetration and degradation study. And I think that should be number one for the Palmitoyl as well.

DR. SLAGA: That was the only need that I found.

DR. COHEN: Which one?

DR. SLAGA: Was the penetration and --

DR. ROSS: Yeah.

DR. COHEN: For the Myristoyl?

DR. SLAGA: Yeah.

DR. COHEN: And you mentioned one other thing right after that. I didn't catch it. Skin penetration for the Myristoyl and --

DR. ROSS: And degradation.

DR. COHEN: And degradation. Yeah, penetration and degradation for Myristoyl.

DR. ROSS: Now the Myristoyl is used mainly ocular, four uses. So, we've got no ocular data with the Myristoyl.

DR. COHEN: Ocular tox?

DR. ROSS: Well, yeah, some sort of molecular test. A HET-CAM or something like that. Because the maximum concentration of that one, I think, is a lot higher than the Palmitoyl.

DR. COHEN: Hold on, that's -- the Palmitoyl goes by the eye, right, at point 0.05 percent right?

DR. ROSS: Myristoyl right?

DR. COHEN: I have Palmitoyl Pentapeptide-4 is used to up to 0.05 percent in eye makeup preparations.

DR. ROSS: Yeah, I question whether that was correct or not.

DR. COHEN: Because in another place it says Myristoyl Pentapeptide-4, right?

DR. ROSS: Exactly. So, I think the max use that I got from --

DR. COHEN: We've got to go to the table and make sure did I pull this out correctly.

DR. ROSS: Yeah, let's look at the table. The max use on Palmitoyl is 0.0035 percent.

DR. COHEN: It's 0.0035 percent for which?

DR. ROSS: For the Palmitoyl.

DR. COHEN: Where did this come from?

DR. ROSS: And the max use on the Myristoyl is 0.05 percent, it's a lot higher. So even if you were doing read across, you don't have a high enough concentration so you're going to need it in those ocular tests of some sort. But yeah, I had a comment in here that maybe that was a misstatement.

DR. COHEN: Oh, you saw the same thing?

DR. ROSS: Yeah. I can find the sticky note.

DR. COHEN: I'm just putting one more thing down before I hit that.

DR. ROSS: Yeah. That was on Page 56 of the PDF. And it says that Palmitoyl Pentapeptide-4 is used at up to 0.05 percent in eye makeup. And so, I asked the question should that be Myristoyl?

DR. COHEN: Yeah, because in Table 3 there's no max use at that concentration listed.

DR. ROSS: Correct. Yeah. So, I think it just --

DR. COHEN: It's a typo.

DR. ROSS: It's a typo, yeah.

DR. HELDRETH: Where is that, again?

DR. ROSS: I just had it there. So, I think I said --

DR. COHEN: I'll find it --

DR. ROSS: -- 56 of the PDF that I have.

DR. COHEN: It's in Cosmetic Use. Use Cosmetic. One, two, three, fourth paragraph, second part of the first line.

DR. ROSS: The paragraph starts, "Some of the ingredients are reported to be used in products that are applied near the eye."

DR. COHEN: And it says Palmitoyl Pentapeptide-4 is used up to 0.05 percent in eye makeup preparations. We don't think is correct, but just need to double check that. Because either the sentence is wrong, or the table is wrong.

DR. ROSS: Yeah. So, we've got penetration and degradation on the different Palmitoyl.

DR. COHEN: Wait, not Myristoyl?

DR. ROSS: And Myristoyl.

DR. COHEN: Palmitoyl and Myristoyl?

DR. ROSS: Yeah.

DR. COHEN: So, the KTSKS?

DR. ROSS: Yeah.

DR. COHEN: All right, is it --

DR. ROSS: KTSKS.

DR. COHEN: Pal-KTSKS.

DR. ROSS: Yeah.

DR. COHEN: Concentration of the HRIPT at KTTKS. Ocular data tox.

DR. ROSS: Yeah, some sort of molecular test for the Myristoyl.

DR. COHEN: You mean molecular or in vitro test?

DR. ROSS: Anything. I'll take anything that shows some sort of safety at 0.05 percent on ocular cells.

DR. COHEN: We can't read across. Okay.

DR. ROSS: But, you know, if for example, Myristoyl doesn't get in, or the other form of Palmitoyl doesn't get in, do we need any of the other tests if these things don't get in and they're rapidly degraded.

DR. COHEN: No.

DR. BERGFELD: Eye is different than skin.

DR. COHEN: No, no.

DR. ROSS: Even with skin, yeah.

DR. COHEN: No, no, he's saying if the Palmitoyl and the -- if the Myristoyl doesn't get in, do we need the whole repertoire of the whole HRIPT and everything else if the sequence is the same? Right? The peptide sequence is going to be the same.

DR. ROSS: The peptide sequence would be the same, yeah.

DR. TILTON: Yeah, I think we could use sort of an if/then situation.

DR. ROSS: Yeah.

DR. COHEN: And by the way, does anyone remember the Myristoyl Pentapeptide uses which sequence? Is it KTTKS? Do we know, do we remember?

DR. HELDRETH: So, the reason that it's listed this way without either sequence written, is because we don't -- the study that has the X there didn't determine which amino acid sequence.

DR. ROSS: Oh, really?

DR. HELDRETH: So, you think about it as Pentapeptide-4 without any fatty acid attached to it. So, it could be either sequence, but many times the research papers don't tell you. I think, a lot of times the authors didn't even know that they were looking at potentially two different sequences.

DR. COHEN: So, if we have the full dossier of both sequence -- but we don't even know if those two sequences are the ones used in the Myristoyl, do we?

DR. HELDRETH: Yes, we do. So, think about there's Pentapeptide-4, it's got the two amino acid sequences. So, then Palmitoyl Pentapeptide can be a palmitoylated version of either one, as can Myristoyl be a myristoylated version of either sequence there.

DR. COHEN: Yeah.

DR. HELDRETH: But we found data on the two different amino acid sequences for Palmitoyl Pentapeptide-4, but not for the Myristoyl and not for the unesterified Pentapeptide-4.

DR. COHEN: So, the anchor to read across is going to be on the Palmitoyl Pentapeptide because we know the two sequences there. Right? So, we can assume it's one or the other based on the other two, but we need all the data on both of them. Does that make sense or no?

DR. ROSS: But if it doesn't get in and it's degraded in the skin, and you're looking at many skin effects, specifically, rather than systemic effects.

DR. COHEN: So, we have irritation and sensitization. We don't have any carci data on it. But we don't know if there's any other biologic signals from this in the skin.

DR. ROSS: Yeah. I think that was part of sort of what Don was getting at with his paper. Are these things having other effects on the skin, specifically?

DR. COHEN: They are very complicated with the other ingredients though, because it had a retinoid in it.

DR. ROSS: Yeah, but the cellular effects in skin, though, I think the last time this was discussed there was a lot of discussion about angiogenesis. I didn't see that cropping up at all in this document, which is fine.

DR. COHEN: So, Tom, do we need carcinogenicity data or other -- we have in vitro genotox, right?

DR. SLAGA: Yeah. We have sufficient genotox and it's not an irritant, so I don't think we need any carcinogenicity.

DR. COHEN: Okay.

DR. ROSS: I don't think so either.

DR. COHEN: Okay.

DR. ROSS: I agree with Tom there. One thing I would say, one of these things was estrogenic in the saccharomyces study. We have no DART data.

But if you actually go back into that saccharomyces yeast study, I went back into it actually. The max concentration we have of the Palmitoyl Pentapeptide is 0.0035 percent, okay. And when I did the cross calculation on molarity that comes out to 4.3 times 10 to the minus 5. And the reason I'm saying that, is that if you go to the actual data produced from industry -- I think that's PDF Page 231 -- there was no agonist activity at that concentration. So, I think we're okay there.

So even though it had some estrogen antagonist activity --

DR. COHEN: 231?

DR. ROSS: Yeah. It's a long way down, I know.

DR. COHEN: That's a lot of thumb action on the little pad here.

DR. ROSS: You'll see a graph. You've actually got to go into the real data.

DR. COHEN: Inactive.

DR. ROSS: So, I think we're okay there.

DR. COHEN: Was the concentration appropriate, you said?

DR. ROSS: Yeah.

DR. BERGFELD: Concentration is no effect.

DR. ROSS: Yeah.

DR. COHEN: Yeah. No. I just want to make sure we weren't dealing with an order or two orders of magnitude lower a concentration that we're dealing with here, because then it wouldn't be fungible to this discussion, right? Okay.

DR. ROSS: So, I think we're okay.

DR. COHEN: All right, that'll be good discussion for tomorrow.

DR. BERGFELD: Summarize what you're going to ask for, please.

DR. COHEN: Okay, so we do not feel we can read across with the different peptide sequences. We'd like dermal tox on KTSKS, skin penetration and degradation from Myristoyl and palmitic KTSKS. Ocular tox or in vitro ocular data on Myristoyl pentapeptide-4, and the concentration of the HRIPT for KTTKS.

DR. ROSS: Can you lead with the degradation and penetration of the skin? I believe that's the first.

DR. COHEN: Should we lead with not read-across, though?

DR. ROSS: Oh, yeah. You could lead with not read-across, but then ask for the penetration and degradation in the skin. I think that's the first issue.

DR. COHEN: Will do.

DR. ROSS: And then if we need additional tox after that -- I think Susan commented earlier we could do this as an if/then kind of thing.

DR. COHEN: So, the dermal tox for KTSKS is part of the if/then statement?

DR. ROSS: Yeah, if it gets in. Yeah.

DR. COHEN: Got it. Are we satisfied with that insufficiency list? Yeah.

DR. BERGFELD: You felt that human studies were fine? I forgot if they had them.

DR. COHEN: The human studies, we're okay. We just don't have a concentration on the HRIPT.

DR. BERGFELD: Okay.

DR. COHEN: I mean, I think that's very doable to get that. I mean, it's a cited report. It probably was just left off when it came in. Okay.

DR. BERGFELD: Going out as an IDA?

DR. COHEN: Yes. That was a complicated one.

DR. ROSS: Yeah.

Full Panel – September 12, 2023

DR. BELSITO: Yep. Okay, so this is a draft report on the safety assessment of the Myristoyl Pentapeptide-4, Palmitoyl Pentapeptide-4, and Pentapeptide-4 as used in cosmetics. It's the first time that we're looking at this material.

I had a little concern about whether there was biological activity. Because in the Fu study, although there was no increase in trans-epidermal water loss, there was no erythema, there was no dryness. There was a slight change in the keratin profile. We discussed this somewhat extensively in our group, and we felt that these effects were not adverse effects and didn't appear to alter biological function. And so, we were fine with going ahead with a safe as use conclusion for these.

DR. SNYDER: No.

DR. BELSITO: Oh wait a minute. I'm sorry.

DR. SNYDER: With the caveat, non-irritating due to --

DR. BELSITO: Right. Non-irritating because of some irritation studies around the eye. Correct.

DR. BERGFELD: You're putting that in the Conclusion or the Discussion?

DR. BELSITO: When formulated to be non-irritating.

DR. BERGFELD: Okay. Dr. Cohen?

DR. COHEN: So we contemplated this quite a bit as well. We felt, primarily, that because the peptide sequences were different that we couldn't read across. I think if we read-across we might have come to similar conclusions, but we didn't have a comfort level that we could say the biologic activity was going to be the same if you switch the sequence of the amino acids.

So, we proposed an IDA asking for HRIPT for the KTSKS, skin penetration and degradation for Myristoyl and Palmitoyl KTSKS, and if it gets in, dermal tox, and some ocular tox or in vivo data on Myristoyl Pentapeptide. And the concentration of the HRIPT in the KTTKS report because it was listed as blank.

DR. BERGFELD: Any other comments? We have a motion and we have a counter opinion.

DR. RETTIE: Could I just make a comment on the read across. Sure they're different pentapeptides, but the difference is switching a serine at a three position to a threonine at the three position, which is the most conservative change you can make. And so, on the basis of that, at least, I felt more comfort that we could read across. I think that was maybe our basis for coming to a different conclusion on the read across than you.

DR. COHEN: We certainly didn't have a level of confidence, perhaps, based on -- I certainly couldn't say that that substitution was going to be biologically similar. You've made a comment about the conservativeness of that substitution, but you want to comment on it, David?

DR. RETTIE: I mean there's just both alcohols, serine and threonine. Very similar physical chemical properties?

DR. ROSS: No, it is -- I mean, it's a reasonable discussion to have because it is a relatively minor change. But since this was the first time we were seeing this, we wanted to ask for, and we felt it would be valuable for a second or third time we see it, to look at the other peptide and look at penetration -- from Palmitoyl -- look at penetration and degradation in the skin to have similar data with that.

It's a judgment call whether they're going to be suitable for read across or not. We often get into this discussion, is it a read across or is it not a read across? And we came down on the side that it wasn't in this case, and you came down on the other side of that it sounds like. So, I guess we have to come to some resolution of that.

DR. BERGFELD: You have proposed --

DR. BELSITO: This is not in my area of expertise, but if we think that the substitution is minor and would not result in any significant changes -- again, I'm very much aware that we should not be asking for data that the next go around if we don't get it, we go, oh yeah, okay, we can read across. So, if you really believe that we can't do that, that's one thing. If it's like, oh, it's the first time and it would be nice to have it, but I'm going to say it's okay if I don't get it, then I don't think that's reasonable.

DR. ROSS: I think I said that if it was the first time, the second time or the third time, it would be valuable to have. So, it's not just asking for it because it's the first time. I think we were in the position that there wasn't a read across here. And, I guess, as I pointed out, you've come down on the other side of that.

DR. BELSITO: So, you felt -- because you said it was a minor substitution. But you still felt that it could not be read across?

DR. ROSS: Yeah, it's peptides. I'm not sure what the activity of different peptides are going to be.

DR. COHEN: Yeah, we just didn't have comfort.

DR. RETTIE: That's a very conservative approach and it's very difficult to argue with. Like I said, I was moved by the similarity in the two amino acid structures. Any time I made a serine to threonine mutation in any of my CYP enzymes it didn't make any difference, but that's a whole different kettle of fish. I mean I can acquiesce to your cautious approach since it is the first time we're looking at this.

DR. BELSITO: Okay, fine. The only other question that I then have is, since we're saying formulated to be non-irritating, why do you want additional ocular data on Myristoyl Pentapeptide?

DR. COHEN: Don, I think that would cover that, right? We weren't creating our IDA based on your conclusion.

DR. ROSS: Yeah, we didn't have the non-irritating in our conclusion.

DR. COHEN: We didn't have a conclusion.

DR. ROSS: Yeah, I mean the Myristoyl, there was four uses and I think they were all essentially ocular.

DR. BELSITO: Right. It's .05.

DR. ROSS: And so, that's why we went with some molecular tests for ocular irritation.

DR. BELSITO: Well, we have ocular irritation, it's just on Palmitoyl.

DR. ROSS: Yeah, exactly.

DR. BERGFELD: So I gather, Don, you're rescinding your motion?

DR. BELSITO: I believe it was David's motion, no?

DR. BERGFELD: Yours.

DR. COHEN: No, it's yours.

DR. BELSITO: Oh, my motion. Yeah, fine. So, insufficient for -- David repeat your needs.

DR. COHEN: We wanted an HRIPT at 0.12 percent for KTSKS.

DR. SNYDER: HRIPT or just sensitization data?

DR. COHEN: Thank you. Irritation and sensitization. Thank you. That's right, especially after the last lecture we had. irritation and sensitization for KTSKS. Skin penetration and degradation for Myristoyl and Palmitoyl, KTSKS, and if it gets in, dermal tox. Concentration of the HRIPT report for KTTKS.

DR. BELSITO: Since we're saying formulated to be nonirritating, do you still need the irritation data?

DR. COHEN: Well it was sensitization as well.

DR. BELSITO: Well, but they could do sensitization in vitro. It'd be a lot cheaper.

DR. COHEN: No, but the report's done. The report is in there, it just didn't list the concentration. So, if the old report can be just pulled up and we see what the concentration is, we don't need to go invent new data.

DR. BELSITO: Okay, so what you're asking for is that they clarify the data that's already existing?

DR. COHEN: Exactly, exactly. We weren't asking for new data.

DR. BELSITO: Because it sounded like you were asking for new data.

DR. ROSS: It did sound a bit like that, actually, I agree with you. But, I think, clarification was what we were after. And it's on PDF Page -- for the writers -- it's on PDF Page 67. And it's the second entry in the Human Table.

DR. COHEN: It's just blank.

DR. ROSS: Just says not specified. Test concentration.

DR. BELSITO: Okay, fine.

DR. BERGFELD: All right, so we have a second?

DR. BELSITO: Yes.

DR. BERGFELD: And we understand what we need?

DR. BELSITO: Yep.

DR. BERGFELD: All right.

DR. BELSITO: I do.

DR. BERGFELD: It's all been clarified, I'll call for the vote. All those in favor of an IDA? Thank you. I saw all the raised hands. Thank you. Unanimous. Moving on to our next ingredient, Dr. Cohen, Charcoal ingredients.

MARCH 2024 PANEL MEETING – SECOND REVIEW/DRAFT AMENDED REPORT

Belsito Team – March 28, 2024

DR. BELSITO: Okay. So, then, pentapeptides. Again, we had a Wave 2 from PCPC. They were busy. In this case, I agreed with all their comments. Allan? Curt? Paul?

DR. RETTIE: Yeah, they're all fine for me.

DR. SNYDER: Same for me.

DR. KLAASSEN: Yes. Yes. Yes.

DR. BELSITO: Okay. Basically, we're looking at this draft tentative report on these pentapeptides as used in cosmetics. It's the second time we're looking at the safety assessment of three ingredients. In the last review, data for the two amino acid sequences of Pentapeptide-4. That included namely lysine-threonine-threonine-lysine-serine, the so-called KTTKS, and lysine-threonine-serine-lysine-serine, the KTSKS. It's been, throughout the report, indicated that way.

When we last looked at this in September of 2023, we issued an insufficient data announcement. What we wanted was irritation and sensitization for the KTSKS amino acid sequence, skin penetration and degradation data for Myristoyl

Pentapeptide-4, the KTSKS sequence, and clarification of the concentration of use tested in the HRIPT study summarized in the report on Palmitoyl Pentapeptide, KTTKS sequence.

We received data from the IDA with previously reported maximum use concentration of 0.05 percent for Myristoyl Pentapeptide-4 in an eye makeup. It was verified to be an experimental product that was never developed. That was not a concentration of concern, i.e., at 0.05 percent. The corrected maximum concentration for Palmitoyl Pentapeptide-4 is 0.0035 percent in a hair conditioner. The highest reported leave-on is 0.012 percent Palmitoyl Pentapeptide-4 in face and neck preparations.

Then we also got additional data that, if you can read there, I won't read it all. I just made a note that, in terms of the additional data, if we're okay with the log P data for absorptions and no longer have a need for degradation data for Palmitoyl KTSKS, then I thought these were all safe as used. It depends upon whether you're okay with the log P data. If we have irritation at use concentration on epiderm and at 58 percent use concentration, 0.018 percent in an HRIPT, do we have sensitization and irritation covered? I don't know whether you were all okay with the log P data.

DR. SNYDER: I defer to Curt on that one.

DR. BELSITO: Pardon? You said you'd defer to Curt, Paul?

DR. SNYDER: Yeah, on the log P data interpretation.

DR. KLAASSEN: What is the log P? I don't even see it.

DR. RETTIE: They corrected the log Ps in the table, providing calculated log Ps. The numbers didn't make much sense from (audio skip).

DR. KLAASSEN: 1.85 and 2.7? These are --

DR. RETTIE: Yep. Myristoylated was 1.8. Palmitoylated was 2.5 to 2.7.

DR. KLAASSEN: Right.

DR. RETTIE: The naked pentapeptide was minus four. I feel, though, that one made sense.

DR. KLAASSEN: Okay.

DR. BELSITO: You think that they all made sense, Allan? Is that what you said?

DR. RETTIE: Much more sense than the previous iteration where the numbers jumped all over the place.

DR. BELSITO: Okay.

DR. RETTIE: They've given a consistent way of calculating it, which is by a calculated method. I thought they had cleared that up. What do you think, Curt?

DR. KLAASSEN: Okay, but, Don, you wanted to use these PKAs -- what was your question, Don, about the PKAs?

DR. BELSITO: Can we use those data to assume absorption and, therefore, no longer need degradation data for the Palmitoyl KTSKS since it would not be significantly absorbed? The Palmitoyl KTKS is 2.72. Is that correct?

DR. KLAASSEN: Yeah, that's a Palmitoyl Pentapeptide-4 is 2.7, which means that's very lipid soluble.

DR. RETTIE: Yep.

DR. KLAASSEN: I guess I would not want to say that that would not be absorbed.

DR. RETTIE: Yeah, I agree. I think it would be absorbed.

DR. RETTIE: Right. Whereas, now, the Pentapeptide-4, that's a minus 4.12. Now, I would agree that that's not likely to be absorbed because that's quite water soluble. You agree with me, Allan?

DR. RETTIE: Yes, just based on log P, I agree with you there.

DR. KLAASSEN: Yeah.

DR. RETTIE: Then there's the question -- then there's the question of the extensive hydrolysis in skins --

DR. KLAASSEN: Yeah.

DR. RETTIE: -- which we need to consider on top of the log P, I think, with regard to what would be absorbed.

DR. KLAASSEN: Yes. I think we need to be a little cautious here.

DR. RETTIE: Yeah, I think there'd be little or no systemic penetration of the intact peptides, but there would be some.

DR. BELSITO: Then, given the level of use --

DR. RETTIE: They're used at very low concentrations. Right?

DR. BELSITO: Right.

DR. KLAASSEN: Yeah, 0.0012 percent.

DR. BELSITO: I don't know why we were concerned about the degradation products. What was the basis for that ask that we wanted skin penetration and degradation data for Myristoyl Pentapeptide-4, the KTSKS sequence?

DR. RETTIE: If I remember correctly, this was coming from the other team who didn't feel confident that you could, quote, read-across from the KT -- the other one, the KTTKS sequence, to the KTSKS sequence. We went back and forth on that. I thought it was very, very much a conservative change. In the end, we acquiesced for their need to consider these separately. I think that's where the ask came from.

DR. BELSITO: Okay. Monice, can you help us out?

MS. FIUME: I'm trying to look through the minutes right now to see where, exactly, it came from. Dr. Rettie, you were correct. It was the Cohen team that had the concern. I'm just trying to see where the degradation aspect came in.

Dr. Cohen said, "We do not feel we can read across with the different peptide sequences. We'd like dermal tox on KTSKS, skin penetration and degradation on Myristoyl and Palmitic KTSKS." Dr. Ross said, "Can you lead with the degradation and penetration of the skin? I believe that is first." That was in the full panel meeting. They were not happy with any type of read-across.

DR. RETTIE: I think this will be a good test case for the read-across group to consider as we look at historical data that we looked at over the years. Yeah, this was an unusual one, I thought.

DR. KLAASSEN: I guess my question is, even with the hydrolysis, most likely, I mean, you're going to get amino acids. What are we concerned about absorption of a little bit of amino acids?

DR. BELSITO: I don't know.

DR. SNYDER: Yeah, I would agree with you, Curt.

DR. KLAASSEN: And at extremely low concentrations that it's used at, I just don't have a problem at all with this.

DR. BELSITO: Okay. We're saying that we don't feel we need this information?

DR. KLAASSEN: I don't think so.

DR. RETTIE: Yeah, I'm skeptical too.

DR. SNYDER: Well, I think we frame it from the standpoint that there's -- the log P suggests little to no penetration, a low concentration of use, and the dermal degradation hydrolysis to amino acids, therefore we're not concerned about dermal exposure, right? That's kind of the summary of it?

DR. KLAASSEN: Yes.

DR. RETTIE: Yeah, except for the first part. I don't agree with you about the log Ps because the log Ps of the Palmitoylated and Myristoylated pentapeptides are adequate for (audio skip).

DR. BELSITO: The degradation would be to amino acids --

DR. RETTIE: Mm-hmm. Yes.

DR. BELSITO: -- myristic acid, I presume -- myristic alcohol.

DR. RETTIE: Yeah.

DR. BELSITO: And the concentrations are low, and so no concern.

DR. KLAASSEN: I would say extremely low.

DR. BELSITO: Okay.

MS. FIUME: Okay. They also mentioned masking -- the penetration, degradation, and masking. I'm not sure what is meant by that. Allan, do you know what Dr. Ross's concern would be with that?

DR. RETTIE: With masking? Could you repeat that, Monice?

MS. FIUME: Yeah. It says, "But because they are different peptide structures, I didn't think we could read across. As you know, we only have the penetration and degradation and masking from one of those forms."

DR. BELSITO: I think that was probably a typo.

MS. FIUME: It repeated several times. They may have -- the transcribers may have had it incorrect several times. I don't know what else -- I don't know what word would have been meant.

DR. BELSITO: Well, our comment back is going to be we don't feel this data was necessary. We just let it go in the first slide because it was insufficient. We feel now that degradation would be to amino acids and myristic alcohol in concentrations extremely low and are not of concern. Is that a fair summary?

DR. KLAASSEN: Yes.

DR. RETTIE: Yeah, I think so.

DR. SNYDER: And the low concentrations of use.

DR. BELSITO: Right. The concentrations are extremely low given the low concentration of use.

DR. RETTIE: And the degradation of the two acids, myristic acid and palmitic acid.

DR. BELSITO: I missed what you said, Allan, something about --

DR. RETTIE: I just -- it would be the acids that would be released. It's the myristic and palmitic acids, not the alcohols.

DR. BELSITO: Okay. Okay. Thank you.

DR. RETTIE: Small point.

DR. BELSITO: Okay. Degradation would be to amino acids in myristic acid, and concentrations are extremely low given low use concentration, so not a concern.

DR. RETTIE: That would be a good summary, I think, from our end.

DR. BELSITO: Okay.

DR. RETTIE: I just had a question. Maybe Monice knows here. There's two peptide sequences here, the KKTS and then KTSKS. I was trying to find it in the literature where the KTSKS one pops up. The KKTS one is part of the signal peptide naturally occurring, and of course, SER (phonetic) has been done around this. But I couldn't really see anything about the relative potencies of the other one versus the one that's more commonly used. Do you know if you found anything like that, Monice?

MS. FIUME: I don't. Are you asking why they're both mentioned? Or -- I'm sorry, Allan.

DR. RETTIE: Why they both -- yeah, I don't where the serine variant came from and I couldn't find anything in the literature about it.

MS. FIUME: I believe it was from unpublished --

DR. RETTIE: It doesn't seem to be a nat- --

MS. FIUME: I believe it was from unpublished data if I remember correctly. Let me see if I can find that information.

DR. RETTIE: It's just that the major use is for the other one by a huge amount. I think there's only one use for this serine variant.

MS. FIUME: Did we say it was at the September -- yeah, September meeting.

DR. BELSITO: September meeting.

DR. RETTIE: Yeah, we don't need to get hung up on this. I was just curious if you knew anything off the top of your head, Monice.

MS. FIUME: I know it was from the unpublished data. I was just trying to look for some of the details.

DR. RETTIE: Yeah, maybe you could just send me a link to that later so I can follow up.

MS. FIUME: Okay. I think, yeah --

DR. RETTIE: Thank you.

MS. FIUME: -- it's in the memo. We said, "Of note, data for two amino acid sequences have been included." Then I will send you the data file that talks about it, about the two sequences.

DR. RETTIE: Fine. That would be great.

MS. FIUME: Okay.

DR. BELSITO: Given what we've been through and the sensitization, irritation, HRIPT data we have, are we okay with going as safe as used for these three ingredients?

DR. SNYDER: I was.

DR. BELSITO: Allan? Curt?

DR. RETTIE: I was too, other than this note that I have here that one's an endocrine agonist at 0.12 percent. Did you look at that, Paul?

DR. SNYDER: Previously.

DR. RETTIE: You weren't concerned about it.

DR. SNYDER: No.

DR. RETTIE: Because of the concentration.

DR. SNYDER: Correct.

DR. RETTIE: Okay.

DR. BELSITO: And that would be in the discussion. What was the concentration for the endocrine agonist?

DR. RETTIE: I think it's 0.12 percent is what I have noted here. That's for the Pal-KTSKS.

DR. BELSITO: Endocrine agonist for the Pal-KTKS?

DR. RETTIE: Pal-KTSKS is what I have noted.

DR. BELSITO: No concern because of use concentration. Anything else that needs to go in the discussion?

MS. FIUME: Don, I think I may have missed it. The original request for dermal irritation and sensitization is no longer needed for what reason?

DR. BELSITO: We have irritation at use concentration on epiderm and at 0.18 percent in an HRIPT with -- and equal to six -- 106, but that's not quite the use concentration. Then we also have two negative in vitro for sensitization, so two negative in vitro, an HRIPT, while not at as high as use concentration, was negative, and negative irritation on epiderm at 15 -- at higher than use concentration.

MS. FIUME: Thank you.

DR. SNYDER: Don, on page 72, under Endocrine Activity, I don't see a positive agonist effect there, Allan. That' probably why I didn't --

DR. RETTIE: Hm.

DR. SNYDER: I mean, it actually says no estrogenic antagonist activities were observed. "No inhibition of cellular growth or estrogenic agonist activity was observed at any concentration tested." So I don't know where that came from. Because I have my notes now and I went back and looked, and it was negative.

DR. BELSITO: What page, Curt -- I mean, Paul?

DR. SNYDER: Page 72.

DR. BELSITO: Oh, yeah.

DR. RETTIE: Yeah, yeah. You're right.

DR. SNYDER: Yeah. It's just -- there's no data, Don.

DR. RETTIE: Yeah. Yes, yes.

DR. SNYDER: Yeah, there's no data, yeah.

DR. RETTIE: Yeah. Yep. Let me put a big X through that thing.

DR. BELSITO: Okay.

DR. RETTIE: Sorry to mislead you.

DR. SNYDER: That's all right. That's all right. That's why I keep good notes.

DR. RETTIE: Yeah, you came up when I -- Dr. Snyder, there was an endocrine study that --

DR. BELSITO: The inhalation is fine. That part of the discussion is fine. The keratin effects are fine. Okay. Yeah, I think the discussion is pretty well written. Then we put in the sensitization and irritation is covered -- "Noted absence of dermal irritation, sensitization, and other available data." I mean, I think the discussion is fine. I don't know if anything else needs to be added to it. Paul? Curt? Allan? Do you have any additions to --

DR. SNYDER: No, I do not, Don.

DR. BELSITO: Okay.

DR. KLAASSEN: No, that -- no.

DR. RETTIE: That looks good.

DR. BELSITO: Okay, so the discussion as is and the conclusion as safe as used. Okay. Anything else on the pentapeptides?

Cohen Team – March 28, 2024

DR. COHEN: So, myristoyl pentapeptide-4, palmitoyl pentapeptide-4, and pentapeptide-4. This is a draft tentative report and it's the second time we're seeing this of three (inaudible) ingredients. At the last reviewed data for two amino acid sequences pentapeptide-4 have been included namely the lysine-threonine-threonine-lysine-serine or KTTKS and the lysine-threonine-serine-lysine-serine KTSKS.

So, at the September meeting when we had the draft report we issued an IDA with the following needs, dermal irritation and sensitization data for the KTSKS amino acid sequence. Skin penetration and degradation data for KTSKS sequence and clarification of the concentration of use tested in the HRIPT study for the KTTKS sequence.

We got some information about max use and that while we had 0.05 percent for the myristoyl pentapeptide-4, this product was never developed and so we're told that the max concentration now is 0.0035 percent for palmitoyl pentapeptide-4 in hair conditioners and the highest reported leave on concentration of 0.012 for palmitoyl pentapeptide-4 in face and neck preparation.

DR. EISENMANN: Actually, it's 0.0012 there's an -- okay.

DR. COHEN: I was going to say the memo says 0.12 but the report says 0.0012 percent. I had that, and Carol, I saw that in your Wave 2. So, the additional second wave comments are noted. I'd open it up for questions. Do you feel that we've gotten our data needs met? David, you could kick off.

DR. ROSS: So, trying to do this quickly. My laundry list is nowhere near as long as it was on the previous one. But first of all, we had an incorrect request in there. We actually asked for penetration and degradation for the palmitoyl pentapeptide. The request came back for the myristoyl pentapeptide and not surprisingly we didn't get it because, I think, we're interested in that. But we did get log Ps/partition coefficients of all these peptides which I'll get to in a minute. And we got the clarification of the HRIPT concentration. But the issue last time, I think, if I remember was a lack of read across.

DR. COHEN: Right.

DR. ROSS: We spent a long time discussing that with the different peptides and that was one of the reasons why we went with the IDA. I still think that NOAEL read across is appropriate. I'll give you the reasons for that in second, but I think we may be able to clear both of the palmitoyl peptides and I'll give you the reasons for that as well.

On the read across this all revolves around a serine to threonine change in the peptides. There's good examples in the literature where that change in proteins causes big differences in proteolytic activity and also in phosphorylation and these things on skin are matrikines and so I think it's relevant that we don't read across. I think that's a reasonable conclusion. But when we get to the log Ps that were provided, it shows that the palmitoyl KTSKS is likely -- is less likely to get across the skin than the palmitoyl KTTKS where we actually have the skin penetration data.

So indirectly, even though we didn't ask for specifically the correct thing, we got some log Ps which allow us to get to the point that I think we can clear both peptides because that KTSKS is less likely to get in than the KTTKS.

So, both sequences, in my opinion, are okay and both sequences are okay in dermal and ocular irritation at max concentrations. So, I think both the palmitoyl sequences are fine. Insufficient on the myristoyl and on the pentapeptide-4 because we have no concentration data. We have no concentration of use data. So how can you make any conclusions when you don't know what the concentration is. So, I think at least I'm insufficient on those two minimal uses, but the major uses on the palmitoyl I think, at this point, I could go safe as used.

DR. TILTON: I agree, David. I also felt comfortable with the log P data allowing us to make that comparison and feel more confident for both sequences. So, I also have safe as used when formulated to be non-sensitizing based on data for both sequences of the palmitoyl pentapeptide-4.

DR. COHEN: Hold on a second. So, what is our remaining IDA for the myristoyl and the pentapeptide-4?

DR. ROSS: We have very little data. I've got a data table in here. I don't think there's much in it. We certainly have no concentrations of use at the present time for these things.

DR. TILTON: Yeah. I would say that would be the primary thing is we don't have concentrations of use.

DR. COHEN: We didn't ask for that in the original IDA, though, right?

DR. ROSS: No.

DR. COHEN: We asked for the concentration of use in the HRIPT, but we didn't ask for --

DR. TILTON: There were previously concentrations of use.

DR. COHEN: And they disappeared?

DR. ROSS: Well, that was the one that was the high concentration in the eye.

DR. COHEN: Yeah, and then it went away, right?

DR. ROSS: Then it went away. So, I mean, we got four uses on the myristoyls and we got one use on the pentapeptide-4. We got 239 uses on the palmitoyl. Far and away the minor use categories of these dossiers, right?

DR. BERGFELD: Does the myristoyl make any difference, like the serine?

DR. ROSS: The myristoyl, you know, that's the transport mechanism really and so once it's cleaved off in the skin the peptide's released to do its thing, so it controls a little bit of where it's going to get there. If you look at the log Ps, we also got those for the myristoyls and I seem to recall they're a little less. Yeah. You don't get as much penetration with a myristoyls as you do with the palmitoyls, but we've got no other data on the myristoyls. And as Susan says, the major thing is we don't know what they're being used at. What concentrations.

DR. TILTON: Generally, if we say safe as used and there is no reported concentrations used, I mean, but generally that means that's covered correctly. I mean, the way it's presented in the report, there are no concentrations --

DR. COHEN: Well, the problem --

DR. TILTON: -- we're not approving.

DR. BERGFELD: Four uses.

DR. COHEN: -- if you accept a read across then you might use the concentrations from the other one but we've already said we can't read across so you can't go ahead with the other concentrations, right?

DR. SLAGA: Right.

DR. COHEN: Unless you say we can read across with regard to the concentration from the palmitoyl as you're saying the myristoyl is just a carrier to a certain point and then if it's got less of a chance of getting in why can't you read across in that regard?

DR. ROSS: I think you could hear an argument tomorrow that there may be a read across for myristoyl and palmitoyl, but I think I'd be comfortable going out and asking for the concentrations of those.

DR. COHEN: Are we talking about for the myristoyl and pentapeptide-4?

DR. ROSS: Yeah, for the myristoyl. We just don't know what it's being used in. I'm not sure about the read across yet on -- you know, if these sequences are released in the skin, you could argue that it's going to be the same whether it's one pentapeptide or another, whether it's myristoyl as the carrier or palmitoyl as the carrier. And so, yeah, an argument can be made for read across, but we know we've got absolutely no data for the myristoyl.

DR. BERGFELD: Bart, have we ever covered myristoyl before?

DR. HELDRETH: I mean, we've certainly covered that chain length on attached to other chemical entities but I'm not sure that --

DR. COHEN: You mean, that pentapeptide -- that pentapeptide we've looked at?

DR. HELDRETH: Not necessarily this sequence, no.

DR. ROSS: Myristic acid.

DR. COHEN: I'm presenting this one tomorrow, guys, so.

DR. ROSS: Yeah. Well, I mean, I would go with safe as used for the palmitoyls and we'd like some discussion around the myristoyl and if we need -- we certainly need concentrations on that, but do we need anything else?

DR. COHEN: Well, so, have the exercise go is that's just getting the pentapeptide to its target and you have data on the penetration of that pentapeptide already. So, what's your worst-case scenario, David? Give me data that would give you pause to clear it.

DR. ROSS: Well, I think my problem is that I don't know what concentration is being used there.

DR. COHEN: Well, if we accept some form of read across, wouldn't the max use concentration be 0.0035?

DR. ROSS: If you were doing a read across to the palmitoyls it would be. Correct.

DR. COHEN: Right. Okay, so if myristoyl if you were told that the max concentration was 0.0035, would you clear myristoyl?

DR. ROSS: Good question, and I would probably -- down that road, yes. I have notes in here where I made that argument to myself when I was prepping this and I came up with the fact that, yeah, I would probably clear the KTTKS. Would I clear the KTSKS? Probably. I know it's not getting in, it's not going to be a systemic toxicity concern, and we know the palmitoyl KTSKS is okay for skin sensitization and ocular, so yeah.

DR. COHEN: Did I get it right? The KTSKS is less likely to cross than the KTTKS? Did I miss that or get it right?

DR. TILTON: And I would support that. I just didn't know if we needed to have reported use concentrations.

DR. COHEN: Well, this happens a lot that we don't get use concentrations when there's a number of products there and so if we don't have max use but this is falling into the same report -- I think I'm getting it right -- that it would be predicated on the max use on the one that's listed as 0.0035 as listed in this report and that's it, right? So, if we don't have max use of 0.1, right, and we don't have that. That's not in here. So why wouldn't we clear the other ones if you felt that the sequences would have the same behavior.

DR. HELDRETH: Yeah. Historically if the Panel felt that there was enough relation between one or more ingredients in there and some of them had no uses, we would often have a caveat on our conclusion for those ingredients without reported use or concentration, the expectation of the Panel is that the use in the same use categories and concentrations as other ingredients in the report.

But if that's not the case here that the consensus of comfort of using that caveat here then I think the next step would actually be issuing a second IDA here for those concentrations of use since it wasn't asked for before. I don't think it would be appropriate to go forward and get an insufficient data conclusion since it's a new request. So, I think those are kind of the two pathways that you have to pick from.

DR. COHEN: It's new because the old was pulled, right?

DR. HELDRETH: Right.

DR. COHEN: So, it's a little different than when we just forgot to ask, or we thought of it afterwards. But I guess the real question is when we present this tomorrow, we have lots of Table 3's that have NRs in the concentration of use, you know, when we have 13 things listed. And we don't even have in the discussion that statement, Bart, it's just sort of presumed that the max use is the max use for the group. I mean, max concentration is for the group. So are we creating more work than is needed if it's 0.0035 are we okay?

DR. TILTON: I'm okay.

DR. COHEN: Across the group, right?

DR. TILTON: Yes.

DR. HELDRETH: I think Carol has her hand up.

DR. COHEN: I'm sorry, who does?

DR. HELDRETH: Carol.

DR. EISENMANN: I was just going to mention that as far as we are aware, the myristoyl is only being sold as one sequence, KTTKS, and not the other sequence. So, you asked for data on the other sequence of the myristoyl, you're not going to get it because as far as we know it doesn't exist.

DR. ROSS: And Carol, KTTKS is the sequence where we have the most data?

DR. EISENMANN: Correct.

DR. ROSS: Yeah.

DR. COHEN: But David, you said KTSKS is less likely to get across than the KTTKS, right?

DR. ROSS: Based on partition coefficient, correct.

DR. COHEN: Yeah.

DR. EISENMANN: And if I ask for more concentration of use data, I'm unlikely to get it.

DR. ROSS: Yeah.

DR. COHEN: Yeah.

DR. BERGFELD: I think you could handle this in the discussion and clear it.

DR. COHEN: I'm kind of inclined to clear the group based on the concentration. And, Susan, I just wanted to come back around for clarification. Did you make a comment when formulated to be non-sensitizing?

DR. BERGFELD: Yes, she did.

DR. TILTON: I did. That is what I had written down. But I guess that was due to maybe some of the ocular irritation.

DR. ROSS: I didn't have that (audio skip) .

DR. TILTON: I'm sorry.

DR. ROSS: I didn't have the non-sensitizing. Did anyone else catch that?

DR. COHEN: No, no. I didn't have non-sensitizing. This would be a non-irritating but let's go just review that again.

DR. TILTON: You are correct. I'm not seeing in my notes cause for concern for sensitization for either sequence.

DR. ROSS: Yeah. I cleared them on both dermal and --

DR. TILTON: Yeah.

DR. ROSS: -- both sequences.

DR. TILTON: So, we can remove that.

DR. COHEN: So, are we going to go out as safe as used?

DR. ROSS: Yeah.

DR. COHEN: So, I'm going to go out with the safe as used and then with the discussions about the partition coefficients, the concentration of use we can do during the discussion if we get a second.

DR. ROSS: Yeah.

DR. COHEN: And if we don't get a second, we'll just flow with the conversation, but I think this is going to be a simple presentation, a simple motion, and may have further discussions later, right?

DR. ROSS: I think they'll agree with you because they were there last time on this one. So.

DR. COHEN: I know that. I know that, but we did it for good reason and I think that us prevailing on that was very positive.

DR. ROSS: I think the precedent of not reading across the different peptide sequences was a good one to make. So, I think that was a good discussion, yes.

DR. COHEN: Well, that's kudos to you guys on this panel.

DR. BERGFELD: Can I ask you a question? Do you need to mention in the discussion that there are no DART and no carcinogenicity?

DR. ROSS: I think they were in the dossier that we didn't have that. The discussion, should we mention it now?

DR. BERGFELD: Yeah. And the reason that we were not concerned about that was penetration probably.

DR. TILTON: But there's also negative genotox.

DR. BERGFELD: Negative genotox, yeah.

DR. ROSS: Yeah. Good point.

DR. TILTON: And there was also no reported estrogenic, antagonistic, or androgenic agonistic -- agonist or antagonist activity, no inhibition of cell growth or estrogenic agonist activity so I wasn't concerned about DART.

DR. BERGFELD: But we could put that in the discussion.

DR. ROSS: Yeah.

DR. COHEN: Okay. Okay, so is that noted already, or do I need to bring that up tomorrow?

DR. BERGFELD: You have to put that in because it's not there.

DR. COHEN: So negative genotox and negative endocrine.

DR. BERGFELD: And estrogen.

DR. ROSS: I think there was some estrogenic activity in the YES assay, but the concentrations were much greater than our range of use. I think I commented on that last time in the transcript. Then the concentration calculation on the original data and so it wasn't relevant to our range of use. I think we're good basically on that.

Full Panel – March 29, 2024

DR. COHEN: Thank you. Myristoyl Pentapeptide-4, Palmitoyl Pentapeptide-4 and Pentapeptide-4, this is a Draft Tentative Report of the safety of these items. And it's the second time we're seeing these three ingredients. As noted during the last review, data for two amino acid sequences of Pentapeptide-4 have been included, namely lysine-threonine-threonine-lysine-serine, or KTTKS. And lysine-threonine-serine-lysine-serine, KTSKS.

At the September meeting, we issued an Insufficient Data Announcement with the needs including dermal irritation and sensitization of KTSKS, skin penetration and degradation data for KTSKS, and clarification of the concentration of use for an HRIPT study.

We should note that in the previous report the maximum concentration of 0.05 percent for Myristoyl Pentapeptide-4 in other eye makeup products was never developed. So consequently, the corrected maximum concentration for use in these products is 0.0035 percent Palmitoyl Pentapeptide-4 in a hair conditioner. And for leave-on it's 0.0012. We had some additional data provided, and deliberated that, and our motion is safe as used.

DR. BELSITO: Second.

DR. BERGFELD: Any further discussion or edits that are important to mention?

DR. BELSITO: Yeah, the Council comments are all of which we expected.

DR. COHEN: Yup.

DR. BERGFELD: Okay, any other comments, David?

DR. COHEN: No, I think we were going to discuss the negative genotox and the minimum endocrine issues in the Discussion. And we reviewed the logP values yesterday, but, no, there's nothing really major.

DR. BERGFELD: All right, I will call the question then, all those against? Abstaining? Unanimously approved. Moving on to our next ingredient, which is the Copper Gluconate, Dr. Belsito.

Safety Assessment of Myristoyl Pentapeptide-4, Palmitoyl Pentapeptide-4, and Pentapeptide-4 as Used in Cosmetics

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ABBREVIATIONS

ARE	antioxidant/electrophile response element
CAS	Chemical Abstracts Service
CIR	Cosmetic Ingredient Review
Council	Personal Care Products Council
CPSC	Consumer Product Safety Commission
Dictionary	web-based *International Cosmetic Ingredient Dictionary and Handbook* (wINCI)
DHT	5α-dihydrotestosterone
DMSO	dimethyl sulfoxide
DPBS	Dulbecco's phosphate buffer solution
DPRA	direct peptide reactivity assay
E2	17-β estradiol
EC_{10}	10% effect concentration
ECVAM DB-ALM	European Centre for Validation of Alternative Methods Database on Alternative Methods
FCA	Freund's complete adjuvant
Fmoc	fluorenylmethoxycarbonyl
Fmoc-Lys(Boc)-OH	N_α-fluorenylmethoxycarbonyl-N_ε-(*t*-butoxycarbonyl)-lysine
Fmoc-Ser(tBu)-OH	N_α-fluorenylmethoxycarbonyl-*O*-(*t*-butyl)-L-serine
Fmoc-Thr(tBu)-OH	N_α-fluorenylmethoxycarbonyl-*O*-(*t*-butyl)-L-threonine
FDA	Food and Drug Administration
GLP	good laboratory practices
HEPES	4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid
hERα	human estrogen receptor α
hAR	human androgen receptor
HET-CAM	hen's egg-chorioallantoic membrane
HRIPT	human repeated insult patch test
I_{max}	maximal response
ICH Q3C	International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use Guideline for Residual Solvents
KTTKS	lysine-threonine-threonine-lysine-serine; Pentapeptide-4
KTSKS	lysine-threonine-serine-lysine-serine; Pentapeptide-4
LC-MS/MS	liquid chromatography with tandem mass spectrophotometry
LoD	limit of detection
LOQ	limit of quantification
LPPS	liquid-phase peptide synthesis
MTT	3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide
NR	none reported
OD	optical density
OECD	Organisation for Economic Cooperation and Development
Pal-KTTKS	Palmitoyl Pentapeptide-4
Panel	Expert Panel for Cosmetic Ingredient Safety
PBS	phosphate-buffered solution
PCI	primary cutaneous irritation
SDS	sodium dodecyl sulfate
SLS	sodium lauryl sulfate
SPPS	solid-phase peptide synthesis
TG	test guideline
US	United States
UVA/UVB	ultraviolet light A/ultraviolet light B
VCRP	Voluntary Cosmetic Registration Program
YAS	Yeast Androgen Screen
YES	Yeast Estrogen Screen

ABSTRACT

The Expert Panel for Cosmetic Ingredient Safety (Panel) assessed the safety of Myristoyl Pentapeptide-4, Palmitoyl Pentapeptide-4, and Pentapeptide-4 (KTTKS and KTSKS sequences), which are reported to function as skin-conditioning agents in cosmetic products. The Panel reviewed the available data to determine the safety of these ingredients and concluded these ingredients are safe in cosmetics in the present practices of use and concentration described in this safety assessment.

INTRODUCTION

This assessment reviews the safety of Myristoyl Pentapeptide-4, Palmitoyl Pentapeptide-4, and Pentapeptide-4 as used in cosmetic formulations. According to the web-based *International Cosmetic Ingredient Dictionary and Handbook* (*Dictionary*), these ingredients are reported to function in cosmetics as skin-conditioning agents (Table 1).[1]

The 3 ingredients included in this safety assessment are synthetic peptides which comprise a 5-amino-acid-sequence (pentapeptide) containing lysine, serine, and threonine. One such sequence is lysine-threonine-threonine-lysine-serine, also represented as Lys-Thr-Thr-Lys-Ser, or, KTTKS.[2] Myristoyl Pentapeptide-4 and Palmitoyl Pentapeptide-4 have an additional saturated fatty acid group attached to the peptide structure, namely myristic acid and palmitic acid, respectively. The amino acid sequence of the pentapeptide portion of these ingredients can vary; thus, data for two variations of Pentapeptide-4, namely, KTTKS and KTSKS (Lys-Thr-Ser-Lys-Ser), are included in this report.

The Panel has also previously reviewed the safety of the individual amino acids comprising these ingredients, as well as myristic acid and palmitic acid. In 2013, the Panel published a final report with the conclusion that α-amino acids are safe in the present practices of use and concentration in cosmetics as described in the safety assessment.[3] The safety of myristic acid and palmitic acid has been evaluated in several reviews[4-7] which can be accessed on the Cosmetic Ingredient Review (CIR) website (https://cir-reports.cir-safety.org). Ultimately, in 2019, the Panel issued a final report on the safety of myristic acid and palmitic acid (as part of the safety assessment of fatty acids and fatty acid salts) with the conclusion that the ingredients are safe in cosmetics in the present practices of use and concentration described in the safety assessment when formulated to be non-irritating and non-sensitizing, which may be determined based on a quantitative risk assessment.[7]

This safety assessment includes relevant published and unpublished data that are available for each endpoint that is evaluated. Published data are identified by conducting an extensive search of the world's literature; a search was last conducted January 2024. A listing of the search engines and websites that are used and the sources that are typically explored, as well as the endpoints that the Panel typically evaluates, is provided on the CIR website (https://www.cir-safety.org/supplementaldoc/preliminary-search-engines-and-websites; https://www.cir-safety.org/supplementaldoc/cir-report-format-outline). Unpublished data are provided by the cosmetics industry, as well as by other interested parties.

CHEMISTRY

Definition and Structure

Pentapeptide-4 (CAS No. 149128-48-3) is a synthetic peptide comprised of the amino acids, lysine, serine, and threonine (forming a pentapeptide), in either the lysine-threonine-threonine-lysine-serine (also represented as Lys-Thr-Thr-Lys-Ser; i.e, KTTKS) or, lysine-threonine-serine-lysine-serine (also represented as Lys-Thr-Ser-Lys-Ser, i.e. KTSKS) sequence (Figure 1).[1,2] Myristoyl Pentapeptide-4 (CAS No. 1392416-25-9) and Palmitoyl Pentapeptide-4 (CAS No. 521091-64-5; 214047-00-4) each have a myristic acid or palmitic acid group, respectively, attached to the *N*-capped end of this sequence. The definitions and structures of the ingredients included in this review are provided in Table 1.

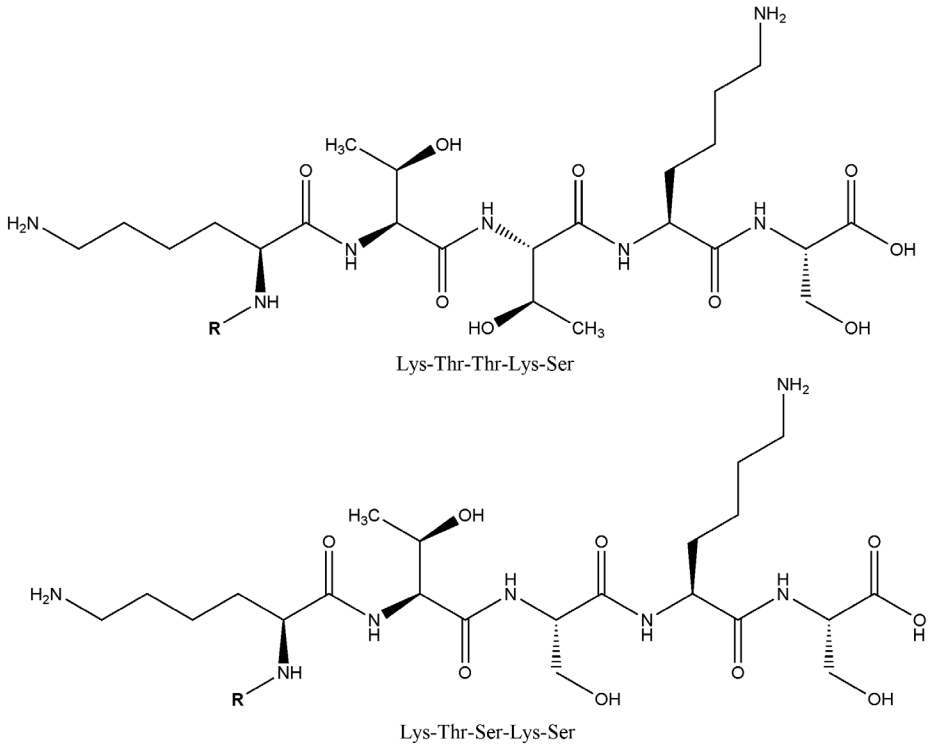


Figure 1. Pentapeptide-4 (when R is hydrogen) and *N*-capped derivatives (when R is the residue of myristic or palmitic acid)

Pentapeptide-4 is a subfragment of type I collagen propeptide, and is regarded as a signal peptide and a matrikine, which possesses the ability to enhance dermal remodeling by triggering cellular processes, such as inhibiting collagenase activity and increasing extracellular matrix production.[2,8-11] The hydrophilic and charged nature of Pentapeptide-4 makes it difficult for it to pass through the intact stratum corneum.[12] However, through the attachment of a fatty acid, such as palmitic acid, which has a 16-carbon chain, the peptide is rendered more lipophilic and is more easily able to penetrate into the skin.[13]

Chemical Properties

Myristoyl Pentapeptide-4, Palmitoyl Pentapeptide-4, and Pentapeptide-4 have molecular weights of 774 g/mol,[14] 802.1 g/mol,[15,16] and 563.6 g/mol,[17] respectively. Additionally, these ingredients have the following predicted log p values for the KTTKS and KTSKS sequences, respectively: Myristoyl Pentapeptide-4 (1.85; 1.6), Palmitoyl Pentapeptide-4 (2.72; 2.52) , and Pentapeptide-4 (-4.12; -4.39).[18] Chemical properties for ingredients in this report are further outlined in Table 2.

Method of Manufacture

Palmitoyl Pentapeptide-4

Two samples of Palmitoyl Pentapeptide-4 (Pal-KTTKS and Pal-KTSKS) are described by a supplier as being obtained via solid phase synthesis at room temperature using Fmoc-amino acid derivatives.[19] An N_α-fluorenylmethoxycarbonyl-N_ε-(*t*-butoxycarbonyl)-lysine (Fmoc-Lys(Boc)-OH) complex is first activated with a coupling agent and reacted on serine-protected resin. Deprotection of the Fmoc residue with a base produces a dipeptide on the resin. For the Pal-KTTKS sequence, both activation and coupling are achieved using the N_α-fluorenylmethoxycarbonyl-*O*-(*t*-butyl)-L-threonine (Fmoc-Thr(tBu)-OH) complex, and deprotection is achieved with the Fmoc-Lys(Boc)-OH group. For the Pal-KTSKS sequence, the N_α-fluorenyl-methoxycarbonyl-*O*-(*t*-butyl)-L-serine (Fmoc-Ser(tBu)-OH), Fmoc-Thr(tBu)-OH, and Fmoc-Lys(Boc)-OH groups are utilized for activation, coupling, and deprotection, respectively. After the last Fmoc-deprotection step, palmitic acid is reacted in the same manner in each process and the resulting products are fully deprotected and purified to yield the final amino acid sequences (Pal-Lys-Thr-Thr-Lys-Ser-OH and Pal-Lys-Thr-Ser-Lys-Ser-OH).

Impurities

Palmitoyl Pentapeptide-4

The impurities found in a sample of Palmitoyl Pentapeptide-4 (Pal-KTTKS), as described by a supplier, were: acetate (< 10%), palmitic acid (< 5%), water (< 5%), and residual solvents (in accordance with the International Council for Harmonisation Of Technical Requirements for Pharmaceuticals for Human Use Guideline for Residual Solvents (ICH Q3C)).[15] Two distinct samples of Palmitoyl Pentapeptide-4, each comprising the Pal-KTTKS or Pal-KTSKS sequence, were described by a supplier as having ≥ 90% purity at 210 nm.[19] The supplier described the impurities in the first sample of Palmitoyl Pentapeptide-4 (Pal-KTTKS) as stereoisomers of Pal-KTTKS-OH, myristine-lysine-threonine-threonine-lysine-serine-OH, and stearyl-lysine-threonine-threonine-lysine-serine-OH. The impurities in the second Palmitoyl Pentapeptide-4

sample (Pal-KTSKS) were described by the supplier as stereoisomers of Pal-KTSKS-OH, myristyl-lysine-threonine-serine-lysine-serine-OH, and stearyl-lysine-threonine-serine-lysine-serine-OH.

USE

Cosmetic

The safety of the cosmetic ingredients addressed in this assessment is evaluated based on data received from the US Food and Drug Administration (FDA) and the cosmetics industry on the expected use of these ingredients in cosmetics and does not cover their use in airbrush delivery systems. Data included herein were obtained from the FDA's Voluntary Cosmetic Registration Program (VCRP) database in 2023 (frequency of use) and in response to a survey conducted by the Personal Care Products Council (Council) (maximum use concentrations). The data were provided by cosmetic product categories, based at that time on 21CFR Part 720. For most cosmetic product categories, 21CFR Part 720 does not indicate type of application and, therefore, airbrush application is not considered. Airbrush delivery systems are within the purview of the US Consumer Product Safety Commission (CPSC), while ingredients, as used in airbrush delivery systems, are within the jurisdiction of the FDA. Airbrush delivery system use for cosmetic application has not been evaluated by the CPSC, nor has the use of cosmetic ingredients in airbrush technology been evaluated by the FDA. Moreover, no consumer habits and practices data or particle size data are publicly available to evaluate the exposure associated with this use type, thereby preempting the ability to evaluate risk or safety.

According to 2023 VCRP survey data, Palmitoyl Pentapeptide-4 has the greatest reported frequency of use; it is reported to be used in 239 formulations, 223 of which are leave-on products (Table 3).[20] Myristoyl Pentapeptide-4 is reported to have 4 uses, while Pentapeptide-4 has 1 reported use. The results of the concentration of use survey conducted by the Council in 2022, and revised in 2023, indicate Palmitoyl Pentapeptide-4 has the highest maximum reported concentration of use, at up to 0.0035% in hair conditioners.[21] The highest leave-on maximum concentration of use reported is 0.0012% Palmitoyl Pentapeptide-4 in face and neck preparations and in eye lotions. Concentration of use data were not reported for the other 2 ingredients.

Some of these ingredients are reported to be used in products that are applied near the eye; Palmitoyl Pentapeptide-4 is used at up to 0.0012% in eye lotions. Palmitoyl Pentapeptide-4 is reported to be used in a face powder (concentration not provided) and could possibly be inhaled. In practice, as stated in the Panel's respiratory exposure resource document (https://www.cir-safety.org/cir-findings), most droplets/particles incidentally inhaled from cosmetics would be deposited in the nasopharyngeal and tracheobronchial regions and would not be respirable (i.e., they would not enter the lungs) to any appreciable amount. 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.

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

The Pentapeptide-4 ingredients named in the report are not restricted from use in any way under the rules governing cosmetic products in the European Union.[22]

Non-Cosmetic

Palmitoyl Pentapeptide-4 (Pal-KTTKS) has been tested in male albino Wistar rats for its wound-healing effects.[23] Palmitoyl Pentapeptide-4 applied in a patch (0.1 and 1 mg) and cream (1 mg) form had a larger impact on wound healing in animals, compared to negative controls (untreated) and positive controls (ready-to-wear dressing; $p < 0.05$).

TOXICOKINETIC STUDIES

Dermal Penetration

In Vitro

Palmitoyl Pentapeptide-4; Pentapeptide-4

The permeability of Palmitoyl Pentapeptide-4 (Pal-KTTKS) and Pentapeptide-4 (KTTKS) was evaluated in an in vitro study using 3 replicate skin samples of CrlOri: SKH1-hr strain hairless mice.[24] Intact hairless mouse skin was mounted on Franz diffusion cells with the epidermal side facing the donor compartment. In the receptor compartment, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) buffer was mixed with 15% ethanol containing phenylmethane-sulfonylfluoride and 1,10-phenanthroline at final concentrations of 5 and 1 mM, respectively, as proteolytic enzyme inhibitors. The donor compartment was loaded with a 1 ml of Palmitoyl Pentapeptide-4 or Pentapeptide-4 (100 µg/ml in 15% ethanol) solution. After 24-h incubation, the skin was removed from the diffusion cell and the remaining donor solution on the skin surface was washed 4 times with 1 ml of distilled water. Upon drying, separation, and mincing of the skin layers (stratum corneum, epidermis, and dermis), the amount of Palmitoyl Pentapeptide-4 or Pentapeptide-4 distributed in each skin layer was extracted using 1 ml of methanol for 24 h with continuous shaking. The extracted samples were centrifuged and

the supernatants were analyzed using liquid chromatography with tandem mass spectrometry (LC-MS/MS). No detectable level of Pentapeptide-4 was observed in the receptor solution over an observation period of 48 h. A trace amount of Palmitoyl Pentapeptide-4 was detected in the receptor solution after 24 h by LC-MS/MS; however, it was below the limit of quantification (LOQ; < 0.5 µg/ml). No amount of Pentapeptide-4 was detected in any of the skin layers over a period of 24 h. Palmitoyl Pentapeptide-4 was observed in every skin layer: 4.2 ± 0.7 µg/cm^2 in the stratum corneum, 2.8 ± 0.5 µg/cm^2 in the epidermis, and 0.3 ± 0.1 µg/cm^2 in the dermis. Overall, 14.6% of the applied Palmitoyl Pentapeptide-4 was retained in the skin: 8.3% in the stratum corneum, 5.6% in the epidermis, and 0.6% in the dermis. Therefore, the researchers concluded that neither Palmitoyl Pentapeptide-4 nor Pentapeptide-4 could permeate through full-thickness hairless mouse skin over the time period used in these experiments.

Absorption, Distribution, Metabolism, and Excretion (ADME)

In Vitro

Palmitoyl Pentapeptide-4; Pentapeptide-4

The dermal stability of Palmitoyl-Pentapeptide-4 (Pal-KTTKS) and Pentapeptide-4 (KTTKS) was evaluated in vitro in epidermal and dermal skin extracts and whole skin homogenate prepared from hairless mouse skin.[24] Pentapeptide-4 (200 µl) or Palmitoyl Pentapeptide-4 (40 µg/ml in 10 mM HEPES buffer, pH 7.4, as peptide concentration) was incubated with 200 µl of the epidermal skin extract, dermal skin extract, or whole skin homogenates at 37 °C for 120 min. At predetermined times, the amount of Palmitoyl Pentapeptide-4 and Pentapeptide-4 present in the incubated mixtures was sampled and analyzed by LC-MS/MS. Pentapeptide-4 was almost fully degraded in the dermal skin extract and whole skin homogenate, with 3.2% remaining in the dermal skin extract at 30 min and 1.5% remaining in the whole skin homogenate at 60 min. The degradation of Pentapeptide-4 in the epidermal skin extract was slower than that seen in the dermal skin extract and whole skin homogenate, which was potentially attributed to lower amounts of proteolytic enzymes. Palmitoyl Pentapeptide-4 was more stable in the skin extracts over time, compared to Pentapeptide-4. The concentration of Palmitoyl Pentapeptide-4 detected in the epidermal skin extract after 120 min was similar to the initial concentration. After 60 min, 11.2% Palmitoyl Pentapeptide-4 remained in the whole skin homogenate, and, after 120 min, 9.7% Palmitoyl Pentapeptide-4 remained in the dermal extract.

TOXICOLOGICAL STUDIES

Acute Toxicity Studies

Oral

Palmitoyl Pentapeptide-4

The acute oral toxicity of Palmitoyl Pentapeptide-4 (Pal-KTTKS), tested at 0.01% (vehicle not specified), was evaluated in Sprague-Dawley rats (5/sex), in accordance with Organisation for Economic Co-operation and Development (OECD) test guideline (TG) 401.[15,25] A single dose of the test substance (20 ml/kg) was administered via gavage. Mortality, clinical abnormalities, and body weight gain were monitored for a period of up to 14 d; all animals were killed at the end of the study. No deaths occurred during the study and no apparent changes or abnormalities were observed in general behavior, body weight gain, or upon necropsy.

Short-Term Toxicity Studies

Dermal

Palmitoyl Pentapeptide-4

Groups of guinea pigs (5/sex; strain not specified) were treated with 0.01% Palmitoyl Pentapeptide (0.05 ml; vehicle not specified; Pal-KTTKS) in a 2-wk dermal irritation study.[15,26] No deaths or clinical signs related to treatment were noted during the study; internal organs were not examined. No further details were provided.

Subchronic, and Chronic Toxicity Studies

No subchronic or chronic toxicity studies were found in the published literature, and unpublished data were not submitted.

DEVELOPMENTAL AND REPRODUCTIVE TOXICITY STUDIES

No developmental and reproductive toxicity studies were found in the published literature, and unpublished data were not submitted.

GENOTOXICITY STUDIES

Details of the in vitro genotoxicity studies summarized below are provided in Table 4.

A solution of 0.5% Palmitoyl Pentapeptide-4 (Pal-KTTKS) in distilled water and ethanol (75/25), tested at 2% in distilled water, was not mutagenic in an Ames test at concentrations up to 5000 µg/plate using *Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537 and *Escherichia coli* WP2*uvr*A.[15,27] In another Ames test, performed in accordance with OECD TG 471, Palmitoyl Pentapeptide-4 (81.6% pure, Pal-KTSKS) in dimethyl sulfoxide (DMSO) was not mutagenic when tested at concentrations up to 1600 µg/plate using *S. typhimurium* strains TA98, TA100, TA102, TA1535, and TA1537,

with or without metabolic activation.[19,28] Signs of cytotoxic activity were strain- and metabolic activity dependent and there were doses in all test conditions that were not cytotoxic. The genotoxic potential of Palmitoyl Pentapeptide-4 (> 96% pure, Pal-KTSKS) in water was evaluated in an in vitro mammalian cell micronucleus test in accordance with OECD TG 487 using cultured human lymphocytes.[19,29] Cells were treated with 250, 500, or 1000 µg/ml of the test article in the presence of metabolic activation for 4-h, followed by a 24-h recovery period; cells were also treated with 375, 500, or 750 µg/ml of the test article in the absence of metabolic activation for 4 h, followed by a 24-h recovery period (short treatment). In an additional assay, cells were treated with concentrations of 250, 320, or 400 µg/ml Palmitoyl Pentapeptide-4 for 24 h without a recovery period (continuous treatment). Neither statistically nor biologically significant increases in the number of micronucleated cells were observed with either treatment period; the test article was deemed not genotoxic.

CARCINOGENICITY STUDIES

No carcinogenicity studies were found in the published literature, and unpublished data were not submitted.

OTHER RELEVANT STUDIES

Endocrine Activity

Palmitoyl Pentapeptide-4

The estrogenic and androgenic activity of a formulation containing 0.12% Palmitoyl Pentapeptide-4 (other contents not specified; Pal-KTSKS) was evaluated in transformed yeast cells using the XenoScreen Yeast Estrogen Screen (YES) and Yeast Androgen Screen (YAS) assays.[19,30] *Saccharomyces cerevisiae* cells were genetically transformed with human estrogen receptor α (hERα) and human androgen receptors (hAR) and, additionally, had an expression plasmid carrying the reporter gene lacZ inserted. Binding of the test article with hERα or hAR receptors resulted in the interaction of these receptors with the corresponding response elements on the expression plasmid, in turn affecting β-galactosidase gene expression. Thus, the amount of secreted β-galactosidase, which was correlated with colorimetric quantification of the conversion of the yellow substrate, chlorophenol red-β-D-galactopyranoside, into a red product at 570 nm (corrected for unspecific absorption and light scattering at 690 nm), indicated the estrogenic or androgenic activity of the test article. The difference between these optical density (OD) absorbance values (OD_{690} - OD_{570}) was used to calculate growth factor values and induction ratios. Eight serial dilutions of the test article (half-log steps) in DMSO, resulting in final concentrations of 3.16×10^{-6} - 1×10^{-2} M, were added to yeast cells in the agonist assays. For the agonist YES assay, 17-β estradiol (E2) was used as the positive control at 7 final concentrations between 1×10^{-11} – 1×10^{-8} M; 5α-dihydrotestosterone (DHT) was used as the positive control for the agonist YAS assay at 7 final concentrations between 1×10^{-9} – 1×10^{-6} M, using half-log dilution steps. DMSO (1%) was used as the solvent control. The inhibitory activity of the test article dilutions were evaluated in the presence of E2 (1.3×10^{-9} M) in an antagonistic YES assay and in the presence of DHT (3×10^{-8} M) in an antagonistic YAS assay. Serial dilutions of 4-hydroxytamoxifen and flutamide were used as antagonist positive controls. The test article showed estrogenic activity with an EC_{10} value of 6.9×10^{-3}; cellular toxicity (growth factors ≤ 0.5) was observed at the two highest test concentrations (1×10^{-2} and 3.16×10^{-3} M) in the YAS assay. No estrogenic antagonist, androgenic agonist, or androgenic antagonist activities were observed.

Similarly, the estrogen agonist effects of a formulation containing 0.12% Palmitoyl Pentapeptide-4 (other contents not specified; Pal-KTSKS), were assessed in a XenoScreen XL YES assay.[31] Lyticase and a detergent were used to facilitate the secretion of the intracellularly synthesized β-galactosidase. Test article samples were serially diluted in 8 steps (half-log steps) in water with 1% DMSO, with concentrations ranging from 5.21×10^{-5} – 6.7×10^{-3} M. E2 was used as the positive control in 8 final concentrations between 2.1×10^{-12} – 6.7×10^{-9} M, using half-log dilution steps; 1% DMSO served as the solvent control. The limit of detection (LoD) for estrogenic activity was 1.49×10^{-11} M E2. No inhibition of cellular growth or estrogenic agonist activity was observed at any concentration tested.

DERMAL IRRITATION AND SENSITIZATION STUDIES

Details on the dermal irritation and sensitization data summarized below can be found in Table 5.

A formulation containing 0.12% Palmitoyl Pentapeptide-4 (tested as supplied; Pal-KTSKS) did not cause irritation when applied to a reconstructed human epidermis model (EpiSkin®) in a cutaneous primary irritation test performed in accordance with OECD TG 439.[19,32] Palmitoyl Pentapeptide-4, tested at 0.01% (vehicle not specified; Pal-KTTKS), was not irritating in an acute dermal irritation test performed in accordance with OECD TG 404 using New Zealand white rabbits nor in a 2-wk dermal irritation study performed in accordance with OECD TG 404 using guinea pigs.[15,26,33] A trade name mixture containing 0.01% Palmitoyl Pentapeptide-4 (applied neat; Pal-KTTKS) was tested for acute skin irritation using 10 subjects.[15,34] Very slight erythema was observed in 1 of the subjects and the primary cutaneous irritation (PCI) score was determined to be 0.10. The test substance was considered to be well-tolerated. A formulation containing 0.12% Palmitoyl Pentapeptide-4 (tested at 15% in distilled water; Pal-KTSKS) was not irritating when applied for 48 h, under semi-occlusive conditions in a patch test using 11 subjects.[19,35]

Palmitoyl Pentapeptide-4 (81.6% pure, Pal-KTSKS) was predicted to be non-sensitizing when tested at 5 mM (5 µl) and 25 mM (250 µl) in water in a direct peptide reactivity assay (DPRA) performed in accordance with OECD TG 442C.[19,36]

Palmitoyl Pentapeptide-4 (81.6% pure; Pal-KTSKS) was tested at up to 200 µM (0.05 ml) in DMSO using the KeratinoSens™ cell line in an antioxidant/electrophile response element (ARE)-Nrf2 luciferase assay, performed in accordance with OECD TG 442D.[19,37] The test article yielded a maximal response value (I_{max}) of 1.35 compared to an I_{max} of 5.12 for the positive control, cinnamaldehyde; the test article was predicted to be non-sensitizing. A guinea pig maximization test was performed in accordance with OECD TG 406, to evaluate the sensitization potential of Palmitoyl Pentapeptide-4 (0.01%; Pal-KTTKS).[15,38] Twenty guinea pigs (10/sex) received the test substance at an effective concentration of 0.0075% (w/w; in saline) followed by an undiluted epicutaneous application during induction, and a dermal application of the test substance at an effective concentration of 0.0025%, in saline, during challenge. Ten control animals were used (5/sex). No skin reactions were observed during evaluation of the test sites 24 and 48 h after patch removal; the test substance was deemed non-sensitizing. A formulation containing 0.12% Palmitoyl Pentapeptide-4 (tested at 15% in distilled water; resulting in 0.072 µg/cm^2 applied Palmitoyl Pentapeptide-4; Pal-KTSKS) was not irritating or sensitizing when applied under semi-occlusive conditions in a human repeated insult patch test (HRIPT) using 106 subjects.[19,39] The undiluted application of a trade name mixture containing 0.01% Palmitoyl Pentapeptide-4 (Pal-KTTKS) to a 3.61 cm^2 area, resulting in 5.54 µg/cm^2 applied Palmitoyl Pentapeptide-4, did not cause irritation or sensitization in an occlusive HRIPT using 51 subjects.[15,40,41]

Phototoxicity Studies

Palmitoyl Pentapeptide-4

The potential for a sample of Palmitoyl Pentapeptide-4 (tested at 0.0015%; Pal-KTSKS), in water, to absorb ultraviolet light A (UVA) and ultraviolet light B (UVB) was evaluated, in accordance with OECD TG 101.[19,42] The diluted article (1 ml) was placed in a calibrated spectrophotometer in order to read UVA/UVB absorption. No absorbance peak was observed between 290 and 400 nm, which was suggestive of a molar extinction coefficient (ε; a measure of how strongly a chemical species or substance absorbs light at a particular wavelength; is an intrinsic property of chemical species that is dependent on structure) < 1000 M^{-1} cm^{-1}. The test article was predicted to be non-phototoxic.

OCULAR IRRITATION STUDIES

Details on the ocular irritation studies summarized below can be found in Table 6.

A formulation containing 0.12% Palmitoyl Pentapeptide-4 (300 µl dose; Pal-KTSKS) was tested in an in vitro hens egg-chorioallantoic membrane (HET-CAM) assay, performed in agreement with French Good Laboratory Practices (GLP) and the European Directive 2004/10/EC.[19,43] The mean score calculated for hyperemia, hemorrhage, and coagulation, opacity, and/or thrombosis was 4.25; the test article was classified as slightly irritating. In another HET-CAM assay, a trade name mixture containing 0.01% Palmitoyl Pentapeptide-4 (Pal-KTTKS), which was tested as supplied, produced a mean irritation index of 6.0; the mean irritation index of the positive control, sodium dodecyl sulfate, was 12.0.[15,34] The test article was classified as moderately irritating. The ocular irritation potential of a formulation containing 0.12% Palmitoyl Pentapeptide-4 (tested at 30% in glycerin and water; Pal-KTSKS) was tested in a SkinEthic™ human corneal epithelial model, in accordance with OECD TG 492.[19,44] Mean cell viability when tested with the test article was 104.3%; the test article was considered not irritating. Palmitoyl Pentapeptide-4 tested at 0.01% (vehicle not specified; Pal-KTTKS) was assessed for ocular irritation in 3 male New Zealand white rabbits, in accordance with OECD TG 405.[15,45] A single dose of 0.1 ml was instilled into the conjunctival sac of the left eye, and the eye was not rinsed. All mean values for chemosis, redness of the conjunctiva, iris lesions, and corneal opacity were 0 at each tested time interval. The test substance was deemed non-irritating to rabbit eyes under the conditions of this study.

CLINICAL STUDIES

Use Studies

Palmitoyl Pentapeptide-4 has been tested in several clinical studies for its use as an anti-wrinkle agent. A moisturizer containing 3 ppm Palmitoyl Pentapeptide-4 was well tolerated in a 12-wk, double-blind, placebo-controlled, split face, left-right randomized clinical study performed in 93 female subjects.[46] In an 8-wk, randomized parallel-group study conducted in 196 women, a cosmetic product regimen containing niacinamide, Palmitoyl Pentapeptide-4, palmitoyl-lysine-threonine, retinyl propionate, and carnosine in a moisturizing base was well tolerated compared to a moisturizer containing 0.02% tretinoin;[47] although the concentration of Palmitoyl Pentapeptide-4 in the moisturizing base is not provided, it was reported to not exceed the maximum reported concentration of use of this ingredient in non-spray face and neck products that was reported to the Council in response to the use survey (i.e., 0.0012%).[48] Palmitoyl Pentapeptide-4 was also well tolerated in another 8-wk, double-blind randomized trial evaluating the effectiveness of 3 cream formulations containing either acetylhexapeptide-3, Pentapeptide-4, or placebo (concentrations not provided).[49]

SUMMARY

This assessment reviews the safety of Myristoyl Pentapeptide-4, Palmitoyl Pentapeptide-4, and Pentapeptide-4 as used in cosmetic formulations. These 3 synthetic peptides are comprised of a varied 5-amino-acid-sequence containing lysine, threonine, and serine; this report reviews the safety of two sequences, namely Pal-KTTKS and Pal-KTSKS. According to the *Dictionary*, these ingredients are reported to function in cosmetics as skin-conditioning agents. As reported in 2023 VCRP data, Palmitoyl Pentapeptide-4 is used in 239 formulations. Palmitoyl Pentapeptide-4 had the highest maximum

concentration of use reported in response to a 2022 concentration of use survey; it is used at up to 0.0035% in hair conditioners.

The permeability of Palmitoyl Pentapeptide-4 (Pal-KTTKS) and Pentapeptide-4 (KTTKS) was evaluated in an in vitro study using hairless mice skin. Either 1 ml of Palmitoyl Pentapeptide-4 or Pentapeptide-4 was incubated with skin samples for 24 h; the amount of each substance distributed in each skin layer was extracted using methanol and analyzed using LC-MS/MS. Pentapeptide-4 was not detected in the receptor solution after an observation period of 48 h; a trace amount of Palmitoyl Pentapeptide-4 was detected after 24 h, but it was below the LOQ at < 0.5 µg/ml. No amount of Pentapeptide-4 was detected in any of the skin layers over a period of 24 h. Palmitoyl Pentapeptide-4 was observed in every skin layer at: 4.2 ± 0.7 µg/cm^2 in the stratum corneum, 2.8 ± 0.5 µg/cm^2 in the epidermis, and 0.3 ± 0.1 µg/cm^2 in the dermis. Overall, 14.6% of the applied Palmitoyl Pentapeptide-4 was retained in the skin: 8.3% in the stratum corneum, 5.6% in the epidermis, and 0.6% in the dermis. The researchers concluded that Palmitoyl Pentapeptide-4 and Pentapeptide-4 did not permeate through full-thickness mouse skin.

The in vitro dermal stability of Palmitoyl Pentapeptide-4 and Pentapeptide-4 was evaluated in several mouse skin extracts. Either 200 µl Pentapeptide-4 or 40 µg/ml Palmitoyl Pentapeptide-4 (in 10 mM HEPES buffer) was incubated with 200 µl of the epidermal skin extract, dermal skin extract, or whole skin homogenates at 37 °C for 120 min. The amounts of each substance present in the incubated mixtures were sampled and analyzed by LC-MS/MS. Pentapeptide-4 was almost fully degraded in the dermal skin extract and whole skin homogenate, with 3.2% remaining in the dermal skin extract at 30 min and 1.5% remaining in the whole skin homogenate at 60 min. Pentapeptide-4 degradation was slower in the epidermal skin extract which was attributed to lower amounts of proteolytic enzymes. Palmitoyl Pentapeptide-4 was more stable in the skin extracts over time; the amount detected in the epidermal skin extract after 120 min was similar to the initial concentration. After 60 min, 11.2% Palmitoyl Pentapeptide-4 remained in the whole skin homogenate and after 120 min, 9.7% Palmitoyl Pentapeptide-4 remained in the dermal extract.

In an acute oral toxicity study, performed in accordance with OECD TG 401, groups of Sprague-Dawley rats (5/sex) received a single dose of Palmitoyl Pentapeptide-4 (20 ml/kg; Pal-KTTKS), tested at 0.01%, via gavage. No deaths occurred during the study and no abnormalities were observed in the general behavior, body weight gain, or upon necropsy. No deaths or clinical signs related to treatment were noted in groups of guinea pigs (5/sex) treated with 0.01% Palmitoyl Pentapeptide (0.05 ml) in a 2-wk dermal irritation study.

A solution of 0.5% Palmitoyl Pentapeptide-4 (Pal-KTTKS) in distilled water and ethanol (75/25), tested at 2% in distilled water, was not mutagenic at up to 5000 µg/plate, with or without metabolic activation using *S. typhimurium* strains TA98, TA100, TA1535, TA1537 and *E. coli* WP2*uvr*A. Palmitoyl Pentapeptide-4 (Pal-KTSKS) in DMSO was not mutagenic to *S. typhimurium* strains TA98, TA100, TA1535, and TA1537, with or without metabolic activation in another Ames test performed in accordance with OECD TG 471. Signs of cytotoxic activity were strain- and metabolic activity dependent and an absence of cytotoxicity was observed under all test conditions. In an in vitro mammalian cell micronucleus test, performed in accordance with OECD TG 487, cultured human lymphocytes were treated for 4 h with up to 1000 µg/ml Palmitoyl Pentapeptide-4 (Pal-KTSKS) in the presence of metabolic activation (24-h recovery), and for 4 h with up to 750 µg/ml Palmitoyl Pentapeptide-4 in the absence of metabolic activation (24-h recovery). Additionally, cells were treated continuously for 24 h (without a recovery period), in the absence of metabolic activation, with up to 400 µg/ml Palmitoyl Pentapeptide-4. Neither statistically nor biologically significant increases in the number of micronucleated cells were observed with the short-term or continuous treatments; the test article was deemed non-genotoxic.

When tested in XenoScreen YES and YAS agonist and antagonist assays, a formulation containing 0.12% Palmitoyl Pentapeptide-4 (Pal-KTSKS) showed estrogenic activity with an EC_{10} value of 6.9×10^{-3}; cellular toxicity (growth factors ≤ 0.5) was observed at the two highest test (1×10^{-2} and 3.16×10^{-3} M) in the YAS assay. No estrogenic antagonist, androgenic agonist, or androgenic antagonist activities were observed. The same test article did not exhibit inhibition of cellular growth or estrogen agonist activity at any concentration tested in another Xenoscreen XL YES assay; the LoD for estrogenic activity was 1.49×10^{-11} M E2.

A formulation containing 0.12% Palmitoyl Pentapeptide-4 in glycerin and water (tested as supplied; Pal-KTSKS) was not irritating to an EpiSkin® model in a cutaneous primary irritation test performed in accordance with OECD TG 439. Palmitoyl Pentapeptide-4, tested at 0.01% (Pal-KTTKS), was not irritating to rabbit skin in an acute dermal irritation study, nor was it irritating to guinea pig skin in a 2-wk dermal irritation study. In a clinical acute irritation study using 10 subjects, a trade name mixture containing Palmitoyl Pentapeptide-4 (0.01%) was well tolerated; very slight erythema was seen in 1 of the subjects, and the PCI was 0.10. A formulation containing 0.12% Palmitoyl Pentapeptide-4 (in distilled water; Pal-KTSKS) was not irritating in a human patch test using 11 subjects.

Palmitoyl Pentapeptide-4 (81.6% pure; Pal-KTSKS) was predicted to be non-sensitizing when tested in a DPRA (OECD TG 442C) and a ARE-Nrf2 luciferase assay (OECD 442D). In a guinea pig maximization test, Palmitoyl Pentapeptide-4 (0.01%; Pal-KTTKS) was not sensitizing when injected at effective test concentrations of 0.0075% in saline during intradermal induction, applied at 0.01% during epicutaneous induction, and applied at 0.0025% in saline during challenge. A formulation containing 0.12% Palmitoyl Pentapeptide-4 (tested at 15% in distilled water; Pal-KTSKS) was not irritating or sensitizing when tested under semi-occlusive conditions in an HRIPT using 106 subjects. No irritation or

sensitization was observed in an occlusive HRIPT in which 51 subjects were treated with a trade name mixture containing 0.01% Palmitoyl Pentapeptide-4 (Pal-KTTKS).

The potential for a sample of Palmitoyl Pentapeptide-4 (tested at 0.0015%; Pal-KTSKS) to cause phototoxicity was evaluated in an UVA/UVB spectrum test performed in accordance with OECD TG 101. No absorbance peak was observed between 290 and 400 nm, which was suggestive of a molar extinction coefficient < 1000 M^{-1} cm^{-1}; the test article was predicted to be non-phototoxic.

A formulation containing 0.12% Palmitoyl Pentapeptide-4 (300 µl dose; Pal-KTSKS) yielded a mean irritation score of 4.25 when tested in a HET-CAM assay and was classified as slightly irritating. Similarly, the ocular irritation potential of a trade name mixture containing 0.01% Palmitoyl Pentapeptide-4 (tested as supplied) was evaluated in another HET-CAM assay. The mean irritation index for the test substance, when tested as supplied, was 6.0, compared to a score of 12.0 for the positive control, sodium dodecyl sulfate. Thus, the test substance was classified as a moderate ocular irritant. Mean cell viability of a SkinEthic™ human corneal epithelial model when tested with a formulation containing 0.12% Palmitoyl Pentapeptide-4 (in glycerin and water; Pal-KTSKS) was 104.3%; the test article was considered non-irritating. In an acute ocular irritation study, a single, 0.1 ml dose of Palmitoyl Pentapeptide-4 tested at 0.01% (vehicle not specified; Pal-KTTKS) was not irritating to New Zealand white rabbit eyes.

Clinically, a moisturizer containing 3 ppm Palmitoyl Pentapeptide-4 was well-tolerated in a 12-wk, double blind placebo-controlled, split face, left-right randomized clinical study performed in 93 female subjects. Palmitoyl Pentapeptide-4 has also been shown to be well tolerated in other randomized trials where it was tested in cosmetic formulations (concentration did not exceed the maximum reported concentration of use in face and neck products).

DISCUSSION

This assessment reviews the safety of Myristoyl Pentapeptide-4, Palmitoyl Pentapeptide-4, and Pentapeptide-4 as used in cosmetic formulations. The Panel concluded these ingredients are safe in cosmetics in the present practices of use and concentration described in this safety assessment.

The amino acid sequence of the pentapeptide portion of these ingredients can vary; one sequence is lysine-threonine-threonine-lysine-serine (i.e., Lys-Thr-Thr-Lys-Ser, or, KTTKS), and the other is Lys-Thr-Ser-Lys-Ser (or KTSKS). According to the *Dictionary*, Palmitoyl Pentapeptide-4 is associated with the Pal-KTTKS and Pal-KTSKS sequences; Pentapeptide-4 and Myristoyl Pentapeptide-4 are only associated with the KTTKS amino acid sequence. Dermal penetration data on Palmitoyl Pentapeptide-4 demonstrated limited dermal absorption, and log p values received for all 3 ingredients indicated that the dermal penetration of Myristoyl Pentapeptide-4 would be less than Palmitoyl Pentapeptide-4. Thus, the Panel found the data sufficient to conclude on the safety of Palmitoyl Pentapeptide-4, Myristoyl Pentapeptide-4, and Pentapeptide-4, when these ingredients are comprised of one of either the KTTKS or KTSKS sequences.

The Panel considered negative HRIPT data for test concentrations that were higher than the maximum reported use concentrations in a weight of evidence approach. These results mitigated any concerns regarding sensitization. Additionally, the Panel noted a negative in vitro dermal irritation study which supported these results. The Panel noted the lack of developmental and reproductive toxicity and carcinogenicity data; however, the low reported maximum concentration of use for these ingredients, the limited percutaneous absorption evidenced in vitro, the negative genotoxicity studies, and negative results obtained in response to a formulation containing 0.12% Palmitoyl Pentapeptide-4 (Pal-KTSKS; test concentrations: 5.21×10^{-5} – 6.7×10^{-3} M) in an estrogen agonist assay mitigated the need for such data. The Panel also noted some changes in the keratin profile of subjects treated with a facial cream containing Palmitoyl Pentapeptide-4, suggesting a potential biologic effect; however, these were not considered adverse effects based upon the lack of erythema, dryness, and transepidermal water loss. The Panel also considered the available method of manufacturing and impurities data for Palmitoyl Pentapeptide-4. Furthermore, the Panel acknowledged its previous safety review of the individual amino acids comprising these ingredients, as well as the safety assessments of myristic and palmitic acid.

The Panel also discussed the issue of incidental inhalation exposure that could result from the use of formulations containing these ingredients; for example, Palmitoyl Pentapeptide-4 is reported to be used in a face powder (concentration not provided) and could be possibly inhaled. Inhalation toxicity data were not available. Coupled with the small actual exposure in the breathing zone and the low concentrations at which these ingredients are used (or expected to be used) in potentially inhaled products, the available information indicates that incidental inhalation would not be a significant route of exposure that might lead to local respiratory or systemic effects. A detailed discussion and summary of the Panel's approach to evaluating incidental inhalation exposures to ingredients in cosmetic products is available at https://www.cir-safety.org/cir-findings.

The Panel's respiratory exposure resource document (see link above) notes that airbrush technology presents a potential safety concern, and that no data are available for consumer habits and practices thereof. As a result of deficiencies in these critical data needs, the safety of cosmetic ingredients applied by airbrush delivery systems cannot be assessed by the Panel. Therefore, the Panel has found the data insufficient to support the safe use of cosmetic ingredients applied via an airbrush delivery system.

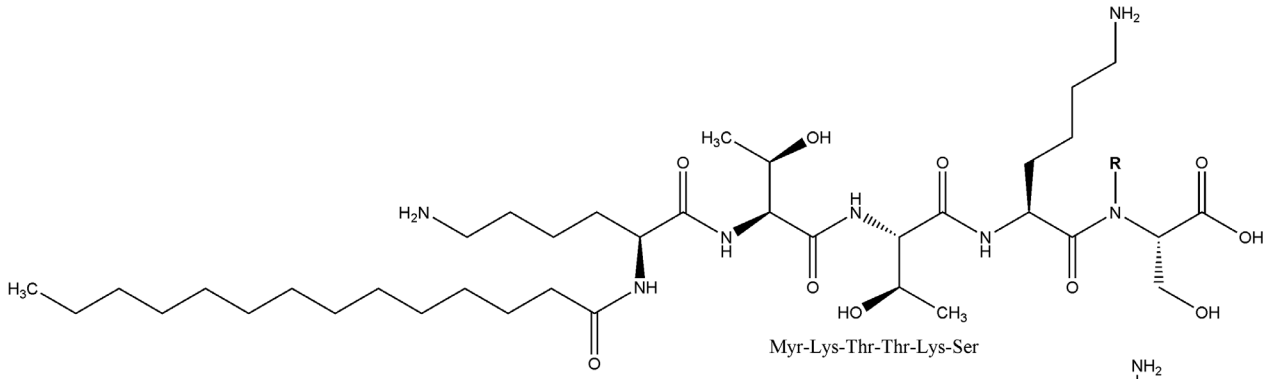
CONCLUSION

The Expert Panel for Cosmetic Ingredient Safety concluded that Myristoyl Pentapeptide-4, Palmitoyl Pentapeptide-4, and Pentapeptide-4 (KTTKS and KTSKS sequences) are safe in cosmetics in the present practices of use and concentration described in this safety assessment.

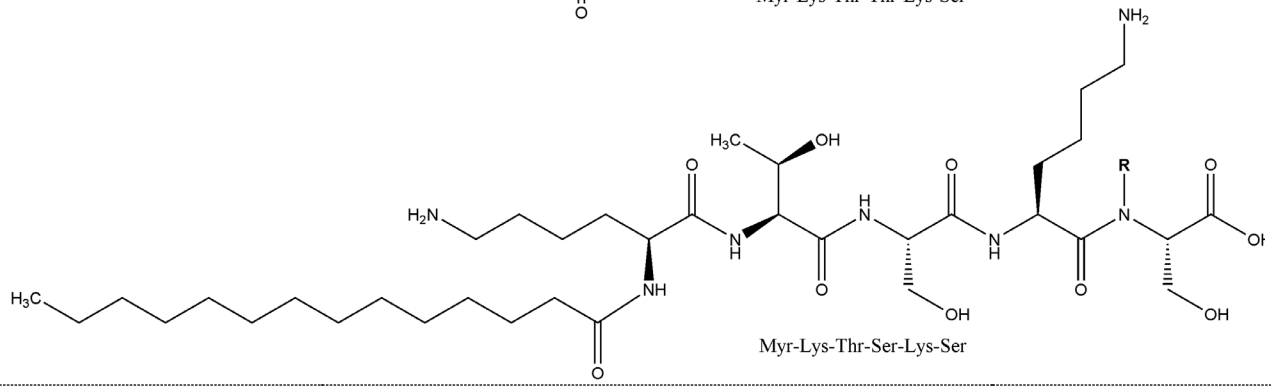
TABLES

Table 1. Definitions, structures, and functions of the ingredients in this assessment[1],CIR Staff

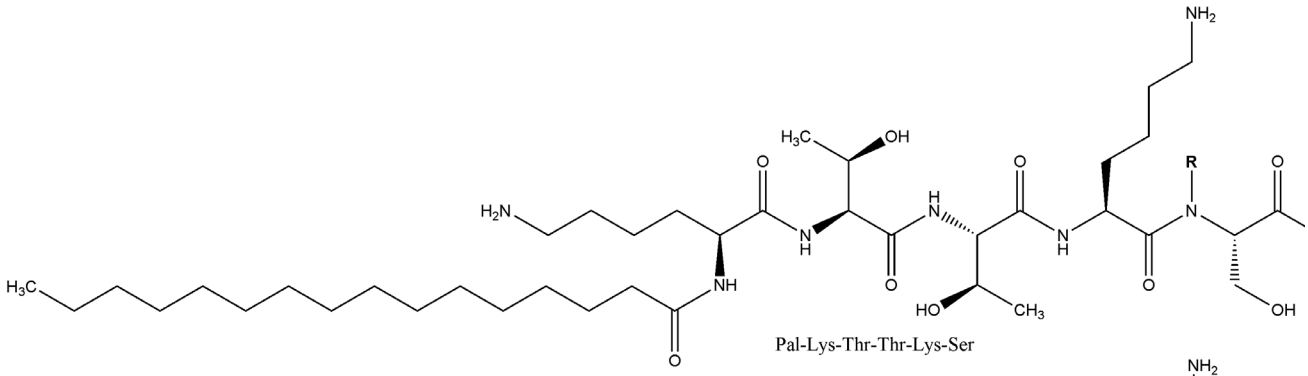
Ingredient	Definition	Function
Myristoyl Pentapeptide-4 1392416-25-9	Myristoyl Pentapeptide-4 is the reaction product of myristic acid and Pentapeptide-4.	Skin-conditioning agent - miscellaneous
Palmitoyl Pentapeptide-4 521091-64-5 214047-00-4	Palmitoyl Pentapeptide-4 is the reaction product of palmitic acid and Pentapeptide-4.	Skin-conditioning agent - miscellaneous



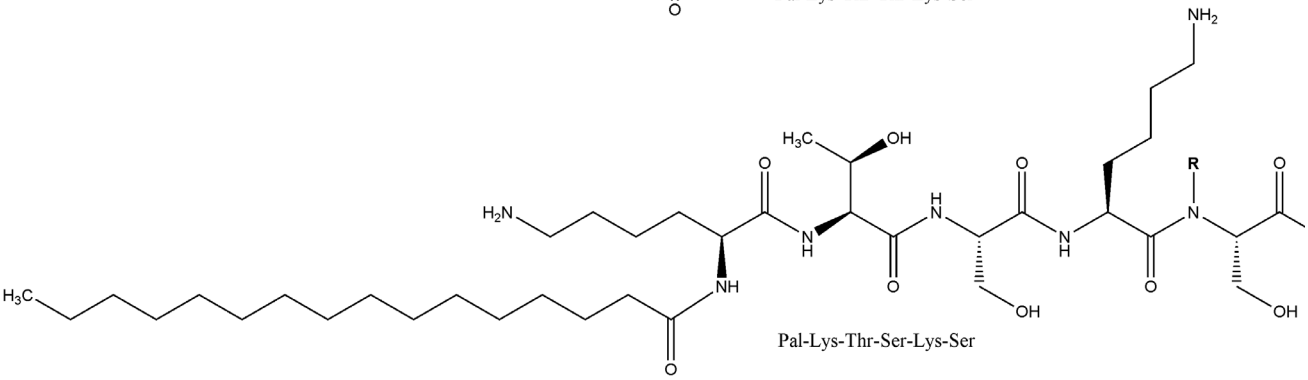
Myr-Lys-Thr-Thr-Lys-Ser



Myr-Lys-Thr-Ser-Lys-Ser



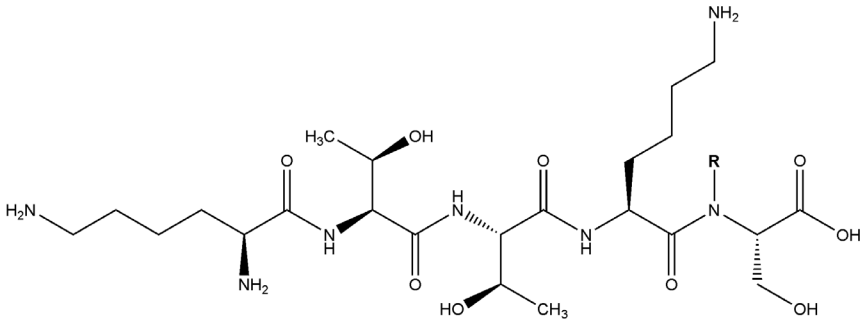
Pal-Lys-Thr-Thr-Lys-Ser



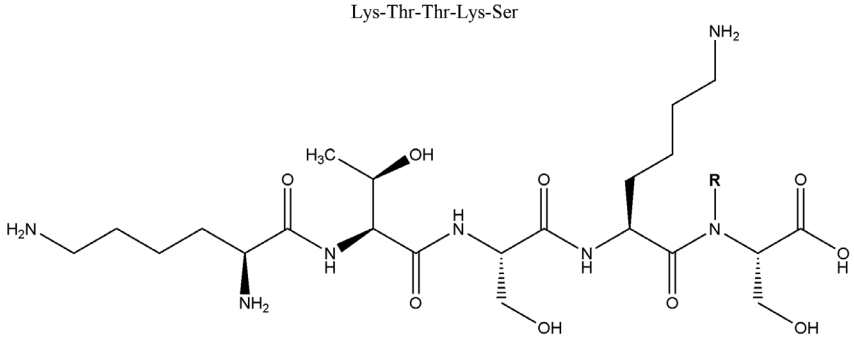
Pal-Lys-Thr-Ser-Lys-Ser

Table 1. Definitions, structures, and functions of the ingredients in this assessment[1],CIR Staff

Ingredient	Definition	Function
Pentapeptide-4 149128-48-3	Pentapeptide-4 is a synthetic peptide containing lysine, serine, and threonine.	Skin-conditioning agent - miscellaneous



Lys-Thr-Thr-Lys-Ser



Lys-Thr-Ser-Lys-Ser

Table 2. Chemical properties

Property	Value	Reference
	Myristoyl Pentapeptide-4	
Molecular Weight (g/mol)	774 (Myr-Lys-Thr-Thr-Lys-Ser) 759.99 (Myr-Lys-Thr-Ser-Lys-Ser)	14
Topological Polar Surface Area (Å^2)	296 (estimated; Myr-Lys-Thr-Thr-Lys-Ser)	14
log p	1.85 (estimated; Myr-Lys-Thr-Thr-Lys-Ser) 1.6 (estimated; Myr-Lys-Thr-Ser-Lys-Ser)	18
	Palmitoyl Pentapeptide-4	
Physical Form	Powder	15
Color	White	15
Molecular Weight (g/mol)	802.1 (Pal-Lys-Thr-Thr-Lys-Ser) 788.04 (Pal-Lys-Thr-Ser-Lys-Ser)	15,16
Topological Surface Area (Å^2)	296 (estimated; Pal-Lys-Thr-Thr-Lys-Ser)	16
log p	2.72 (estimated; Pal-Lys-Thr-Thr-Lys-Ser) 2.52 (estimated; Pal-Lys-Thr-Ser-Lys-Ser)	18
	Pentapeptide-4	
Molecular Weight (g/mol)	563.65 (Lys-Thr-Thr-Lys-Ser) 549.63 (Lys-Thr-Ser-Lys-Ser)	17
Topological Polar Surface Area (Å^2)	292 (estimated; Lys-Thr-Thr-Lys-Ser)	17
log p	-4.12 (estimated; Lys-Thr-Thr-Lys-Ser) -4.39 (estimated; Lys-Thr-Ser-Lys-Ser)	18

Table 3. Frequency (2023)[20] and concentration (2022)[21] of use according to likely duration and exposure by product category

	Myristoyl Pentapeptide-4		Palmitoyl Pentapeptide-4		Pentapeptide-4	
	# of Uses	Max Conc of Use (%)	# of Uses	Max Conc of Use (%)	# of Uses	Max Conc of Use (%)
Totals*	**4**	**NR**	**239**	**0.000005-0.0035**	**1**	**NR**
summarized by likely duration and exposure**						
Duration of Use						
Leave-On	*4*	*NR*	*223*	*0.00036 – 0.0012*	*1*	*NR*
Rinse-Off	*NR*	*NR*	*16*	*0.000005 – 0.0035*	*NR*	*NR*
Diluted for (Bath) Use	*NR*	*NR*	*NR*	*NR*	*NR*	*NR*
Exposure Type						
Eye Area	4	NR	31	0.0012	NR	NR
Incidental Ingestion	NR	NR	NR	NR	NR	NR
Incidental Inhalation-Spray	NR	NR	117[a]; 64[b]	NR	1[a]	NR
Incidental Inhalation-Powder	NR	NR	1; 64[b]	0.00036 – 0.0012[c]	NR	NR
Dermal Contact	4	NR	236	0.000005 – 0.0012	1	NR
Deodorant (underarm)	NR	NR	NR	NR	NR	NR
Hair - Non-Coloring	NR	NR	3	0.00035 – 0.0035	NR	NR
Hair-Coloring	NR	NR	NR	NR	NR	NR
Nail	NR	NR	NR	NR	NR	NR
Mucous Membrane	NR	NR	2	0.000005	NR	NR
Baby Products	NR	NR	NR	NR	NR	NR
as reported by product category						
Eye Makeup Preparations						
Eye Lotion			21	0.0012		
Other Eye Makeup Preparations	4	NR	10	NR		
Hair Preparations (non-coloring)						
Hair Conditioner			1	0.0035		
Rinses (non-coloring)			1	NR		
Shampoos (non-coloring)			1	0.00035		
Makeup Preparations						
Face Powders			1	NR		
Foundations			4	NR		
Personal Cleanliness Products						
Bath Soaps and Detergents			1	0.000005		
Other Personal Cleanliness Products			1	NR		
Skin Care Preparations						
Cleansing			10	0.000005		
Face and Neck (exc shave)			59	0.0012 (not spray)		
Body and Hand (exc shave)			5	0.00036 (not spray)		
Moisturizing			101	0.00059 (not spray)	1	NR
Night			8	NR		
Paste Masks (mud packs)			1	NR		
Skin Fresheners			8	NR		
Other Skin Care Preparations			6	NR		

NR – not reported

*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.

**likely duration and exposure are derived based on product category (see Use Categorization https://www.cir-safety.org/cir-findings)

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

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

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

Table 4. Genotoxicity studies

Test Article	Vehicle	Concentration/Dose	Test System	Procedure	Results	Reference
IN VITRO						
0.5% Palmitoyl Pentapeptide-4 in distilled water/ethanol (75/25) Pal-KTTKS	distilled water	tested at 2% 312.5, 625, 1250, 2500, and 5000 µg/plate, with or without metabolic activation	*S. typhimurium* TA98, TA100, TA1535, TA1537, and *E. coli* WP2*uvr*A	Ames test. For positive controls, sodium azide, 9-aminoacridine, 2-nitrofluorene, and 4-nitroquinoline were tested in the absence of metabolic activation, while 2-anthramine was tested in the presence of metabolic activation. Revertant colonies were scored after 48 to 72 h of incubation at 37 °C.	Not mutagenic. Results for the vehicle and positive controls were as expected.	15,27
Palmitoyl Pentapeptide-4, 81.6% pure Pal-KTSKS	DMSO	1.6, 5, 16, 50, 160, 500, and 1600 µg/plate, with or without metabolic activation	*S. typhimurium* TA98, TA100, TA102, TA1535, and TA1537	Ames test. OECD TG471. In the absence of metabolic activation, sodium azide and mitomycin were tested in water, and 2-nitroflourene and 9-aminoacridine were tested in DMSO, for positive controls. In the presence of metabolic activation, 2-aminoanthracine was tested in DMSO as a positive control. Both a plate incorporation and a preincubation assay were performed.	Not mutagenic. Cytotoxic effects were observed in the plate incorporation assay under the following test conditions: Without metabolic activation: - until 50 µg/plate for TA98 - until 160 µg/plate for TA100, TA102, and TA1535 - until 500 µg/plate for TA1537 [confirmed in the preincubation assay until 50 µg/plate for TA100 and TA102, until 160 µg/plate for TA98 and TA1535, and until 500 µg/plate for TA1537]. With metabolic activation: - until160 µg/plate for TA100 - until 500 µg/plate for TA98, TA102, TA1535, and TA1537 [confirmed in the preincubation assay until 160 µg/plate for TA102, until 500 µg/plate for TA98, TA100, TA1535, and TA1537] Controls produced expected results.	19,28
Palmitoyl Pentapeptide-4, > 96% pure Pal-KTSKS	sterile water	as supplied <u>with metabolic activation:</u> 4-h treatment, 24-h recovery: 250, 500, or 1000 µg/ml <u>without metabolic activation</u>: 4-h treatment, 24-h recovery: 375, 500, or 750 µg/ml 24-h, continuous treatment: 250, 320, or 400 µg/ml	Cultured human peripheral blood lymphocytes	Micronucleus test. OECD TG 487. Cells were treated for 4 h, with a 24-h recovery period, with and without metabolic activation (short treatment). In an additional assay, cells were treated for 24 h without a recovery period (continuous treatment). Cells treated were treated for 4 h followed by a 24-h recovery period, with cyclophosphamide in the presence of metabolic activation and with mitomycin in the absence of metabolic activation. Mitomycin and griseofulvin were used as positive controls in the 24-h, continuous assay.	Not genotoxic. Neither statistically or biologically significant increases in the number of micronucleated cells were observed with the short-term or continuous treatments.	19,29

DMSO – dimethyl sulfoxide; OECD – Organisation for Economic Cooperation and Development; TG – test guideline

Table 5. Dermal irritation and sensitization studies

Test Article	Vehicle	Test Concentration/Dose	Test Population/ System	Procedure	Results	Reference
IRRITATION						
IN VITRO						
Formulation containing 0.12% Palmitoyl Pentapeptide-4, glycerin, and water Pal-KTSKS	tested as supplied	10 µl; 100% (effective test concentration: 0.12% Palmitoyl Pentapeptide-4)	EpiSkin® reconstructed human epidermis model	Cutaneous primary irritation test. OECD TG 439. The test article, positive control (10 µl SDS), and negative control (10 µl PBS) were in contact with the epidermis model for 15 min, followed by a 42-h incubation period. Cell viability was evaluated via an MTT assay.	Predicted to be not irritating. The test article, as supplied did not stain the cells or interact with MTT.	19,32
ANIMAL						
Palmitoyl Pentapeptide-4 Pal-KTTKS	not specified	0.01%; 0.5 ml	3 male New Zealand white rabbits	Acute dermal irritation study. OECD TG 404. Semi-occlusive application of the test substance was made to shaved skin for 4 h. Skin reactions were observed 1, 24, 48, and 72 h after patch removal. Mean values for erythema and edema were calculated for each animal.	Not irritating. Very slight erythema was observed in 1 animal, only on day 1. All erythema and edema mean scores over 24, 48, and 72 h were 0.	15,33
Palmitoyl Pentapeptide-4 Pal-KTTKS	not specified	0.01%; 0.05 ml	Guinea pigs (5/sex; strain not specified)	2-wk dermal irritation study. Open application to a shaved, 2 cm^2 area of the left flank daily for 14 d; the site was not rinsed. Purified water applied to the right flank served as the control. Skin reactions were evaluated before and approximately 24 h after each application; these values were used to calculate daily irritation and weekly mean irritation indices.	Non-irritating. Very slight erythema was noted in 1 animal on days 12 and 13. According to the researchers, these reaction were not attributed to an irritant effect of the test substance because they were very slight and only occurred in 1 animal.	15,26
HUMAN						
Trade name mixture containing 0.01% Palmitoyl Pentapeptide-4 Pal-KTTKS	tested as supplied	0.02 ml (effective test concentration: 0.01% Palmitoyl Pentapeptide-4)	10 subjects	Acute skin irritation study. A single occlusive, neat application of the test substance was made to a 50 mm^2 area of the back for 48 h using Finn chambers. Untreated sites covered with an occlusive patch served as negative controls. Skin reactions were scored 30 min after patch removal.	Well-tolerated. Very slight erythema (hardly visible) in 1 of the subjects. PCI = 0.10.	15,34
Formulation containing 0.12% Palmitoyl Pentapeptide-4 Pal-KTSKS	distilled water	160 µl; 15% (effective test concentration: 0.018% Palmitoyl Pentapeptide-4)	11 subjects; phototype II - IV	Patch test; semi-occlusive application to 400 mm^2 for 48 h; test sites were scored before patching and 15 – 30 min after patch removal	Not irritating. No reactions were observed in either the test or control subjects.	19,35
SENSITIZATION						
IN CHEMICO/ IN VITRO						
Palmitoyl Pentapeptide-4, 81.6% pure Pal-KTSKS	water	5 (50 µl) and 25 mM (250 µl)	cysteine and leucine	DPRA; OECD TG 442C and ECVAM DB-ALM Protocol No 154; 24-h incubation period; each concentration was tested 3 times; mean percent depletion of cysteine and lysine was evaluated; positive control: cinnamaldehyde in acetonitrile; negative control: peptide in buffer	Prediction of non-sensitizing. Mean percent depletion of cysteine and lysine was 4.58%, reflecting no or minimal reactivity.	19,36
Palmitoyl Pentapeptide-4, 81.6% pure Pal-KTSKS	DMSO	0.98 – 2000 µM; 0.05 ml	KeratinoSens™ cell line	ARE-Nrf2 Luciferase test method; OECD TG 442D and ECVAM DB-ALM protocol 155; performed 2 times; positive control: cinnamaldehyde; negative controls: 1% DMSO in treatment medium	Prediction of non-sensitizing. I_{max} of 1.35, compared to 5.12 for positive control.	19,37

Table 5. Dermal irritation and sensitization studies

Test Article	Vehicle	Test Concentration/Dose	Test Population/ System	Procedure	Results	Reference
			ANIMAL			
Palmitoyl Pentapeptide-4, 0.01% Pal-KTTKS	saline	Induction: 75% (effective concentration 0.0075%); topical induction: applied neat (effective concentration 0.01%) Challenge: 25% (effective concentration: 0.0025%)	Guinea pigs (strain not specified) test animals: 10/sex controls: 5/sex	OECD TG 406. Guinea pig maximization test. Saline solution and mercaptobenzothiazole in corn oil served as negative and positive controls, respectively. On day 1, the test substance was mixed with FCA and injected intradermally in the back. After pretreatment of the test site with 10% SLS (pet) on day 7, the test substance was applied on day 8 under occlusion to the same region for 48 h. After a non-treatment period of 12 d, both test and control animals received an occlusive challenge application of the test substance to the right flank, as well as an occlusive application of the vehicle control to the left flank, both for 24 h. Skin reactions were evaluated 24 and 48 h after patch removal.	Not sensitizing. Controls yielded expected results.	15,38
			HUMAN			
Formulation containing 0.12% Palmitoyl Pentapeptide-4 Pal-KTSKS	distilled water	160 µl; 15% (effective concentration: 0.018% Palmitoyl Pentapeptide-4) This results in 0.072 µg/cm^2 of Palmitoyl Pentapeptide-4	106 subjects; phototype II - III	HRIPT; semi-occlusive conditions (400 mm^2); induction: 9 applications (48 – 72 h) were made to the upper back over a 3-wk period. Concurrent applications of distilled water under the same conditions served as control sites. Challenge: after a non-treatment period of 2 wk, a 48-h application was made to an induction site and an untreated site. Treated sites were scored before patching, 15 – 30 min after patch removal, and, additionally, 48 h after patch removal during the challenge phase	Not irritating or sensitizing. No reactions were induced during the induction or challenge phases.	19,39
Trade name mixture containing 0.01% Palmitoyl Pentapeptide-4 Pal-KTTKS	NA	applied undiluted: 55.4 mg/cm of the mixture This results in 5.54 µg/cm^2 of Palmitoyl Pentapeptide-4	51 subjects	HRIPT; The remaining ingredients in the mixture included: glycerin (qsp 100), water (25%), butylene glycol (20%), carbomer (1%), polysorbate 20 (0.5%), sodium lactate (max 1%); 2 g of the test material applied to a 3.61 cm^2 area under occlusive conditions.	Non-irritating and non-sensitizing	15,40,41

ARE – antioxidant/electrophile response element; DPRA – direct peptide reactivity assay; ECVAM DB-ALM - European Centre for Validation of Alternative Methods Database on Alternative Methods; FCA – Freund's Complete Adjuvant; HRIPT – human repeated insult patch test; MTT - 3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide; NA – not applicable; OECD – Organisation for Economic Cooperation and Development; PBS – phosphate-buffered solution; PCI – primary cutaneous irritation; SDS – sodium dodecyl sulfate; SLS – sodium lauryl sulfate; TG – test guideline

Table 6. Ocular irritation studies

Test Article	Vehicle	Test Concentration/Dose	Test Population	Procedure	Results	Reference
IN VITRO						
Formulation containing 0.12% Palmitoyl Pentapeptide-4 Pal-KTSKS	water	300 µl; 10% (effective test concentration: 0.012% Palmitoyl Pentapeptide-4)	4 eggs (test article); 2 eggs (reference controls)	In vitro HET-CAM assay; performed in agreement with French GLP, the European Directive 2004/10/EC, and the August 2004 decree from the *Journal Officiel Republique Francaise*; positive control: 0.4 and 3.2% lauryl sulfobetaine in saline solution; negative control: 0.05% lauryl sulfobetaine in saline solution	Classified as slightly irritating. The mean score calculated for hyperemia, hemorrhage, and coagulation, opacity, and/or thrombosis was 4.25.	19,43
Trade name mixture containing 0.01% Palmitoyl Pentapeptide-4 Pal-KTTKS	tested as supplied	dose not specified (effective test concentration: 0.01% Palmitoyl Pentapeptide-4)	HET-CAM	In vitro HET-CAM assay; 1996 HET CAM protocol published in the *Journal Officiel Republique Francaise*; positive control: SDS (0.05% (w/v))	Classified as moderately irritating. The mean irritation index for the SDS was 12, while the mean irritation index for the test substance was 6	15,34
Formulation containing 0.12% Palmitoyl Pentapeptide-4; glycerin, and water Pal-KTSKS	water	30 µl; 30% (effective test concentration: 0.036% Palmitoyl Pentapeptide-4)	human immortalized comeal epithelial cells	SkinEthic™ human corneal epithelial model. OECD TG 492, in agreement with French GLP, European Directive 2004/10/CE, and 2004 decree published in the *Journal Officiel Republique Francaise*. 2 epithelia were used as replicates; 30 min incubation period; positive control: methyl acetate; negative control: DPBS; cell viability evaluated via MTT assay	Not irritating. Mean cell viability for the test article was 104.3%. Positive controls yielded expected results.	19,44
ANIMAL						
Palmitoyl Pentapeptide-4 Pal-KTTKS	not specified	0.01%; 0.1 ml	3 male New Zealand white rabbits	OECD TG 405. A single dose was instilled into the conjunctival sac of the left eye. Treated eyes were not rinsed; right eyes served as control Ocular reactions were evaluated 1, 24, 48 and 72 h. Mean values for chemosis, redness of the conjunctiva, iris lesions, and corneal opacity were calculated for each animal	Classified as non-irritant. All mean values were 0 at each time interval.	15,45

DPBS – Dulbecco's phosphate buffer solution; GLP – good laboratory practices; HET-CAM – hen's egg-chorioallantoic membrane test; MTT – 3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide; OECD – Organisation for Economic Cooperation and Development; SDS – sodium dodecyl sulfate; TG – test guideline

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