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

Status: Draft Report for Panel Review
Release Date: August 21, 2020
Panel Date: September 14-15, 2020

The Expert Panel for Cosmetic Ingredient Safety members are: Chair, Wilma F. Bergfeld, M.D., F.A.C.P.; Donald V. Belsito, M.D.; Curtis D. Klaassen, Ph.D.; Daniel C. Liebler, Ph.D.; James G. Marks, Jr., M.D.; Lisa A. Peterson, Ph.D.; Ronald C. Shank, Ph.D.; Thomas J. Slaga, Ph.D.; and Paul W. Snyder, D.V.M., Ph.D. The Cosmetic Ingredient Review (CIR) Executive Director is Bart Heldreth, Ph.D. This report was prepared by Wilbur Johnson, Jr., M.S., Senior Scientific Analyst, CIR.
Memorandum

To: Expert Panel for Cosmetic Ingredient Safety Members and Liaisons

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

Date: August 21, 2020

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

Enclosed is a Draft Report on the Safety Assessment of Acetyl Hexapeptide-8 and Acetyl Hexapeptide-8 Amide as Used in Cosmetics (acetyl092020rep). This is the first time the Panel is reviewing the safety assessment on these ingredients. Comments on the Scientific Literature Review (SLR) that was announced on January 15, 2020 were received from the Council, and the draft report has been revised to address these comments. The comments are enclosed (acetyl092020pcpc), along with the use concentration data (acetyl092020data) that were received from the Council.

Also included in this package for your review are the report history (acetyl092020hist), flow chart (acetyl092020flow), literature search strategy (acetyl092020strat), ingredient data profile (acetyl092020prof), and 2020 FDA VCRP data (acetyl092020FDA).

Of note, CIR was made aware, as these reports were going to press, that Acetyl Hexapeptide-8 is synonymous with Acetyl Hexapeptide-8 Amide. Accordingly, all of the data in the literature states Acetyl Hexapeptide-8 as the test material, but is fully applicable to the synonymous ingredient, Acetyl Hexapeptide-8 Amide. The name, Acetyl Hexapeptide-8 Amide, is more accurate, as the ingredient is used as the amidated peptide. Thus, the Amide name is used throughout the report. Furthermore, CIR was just made aware that not only are Acetyl Hexapeptide-8 and Acetyl Hexapeptide-8 Amide synonymous with each other, but they are also synonymous with Acetyl Hexapeptide-24 and Acetyl Hexapeptide-24 Amide. Thus, there appears to be 4 names for 1 ingredient. Unless the Panel objects, Acetyl Hexapeptide-24 and Acetyl Hexapeptide-24 Amide will be incorporated into the next iteration of the report.

After reviewing these documents, if the available data are deemed sufficient to make a determination of safety, the Panel should issue a Tentative Report with a safe as used, safe with qualifications, or unsafe conclusion, and Discussion items should be identified. If the data are not deemed sufficient, an Insufficient Data Announcement should be issued, and the data needs identified.
CIR History of:

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

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

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

The draft report has been revised to include the Council’s comments, and also includes use concentration data on Acetyl Hexapeptide-8 that were received from the Council.
# Acetyl Hexapeptide-8 and Acetyl Hexapeptide-8 Amide Data Profile*

**- September 14-15, 2020 – Wilbur Johnson, Jr.**

<table>
<thead>
<tr>
<th></th>
<th>Reported Use</th>
<th>GRAS</th>
<th>Method of Mfg</th>
<th>Composition</th>
<th>Impurities</th>
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<th>Acute Tox</th>
<th>Repeated Dose Tox</th>
<th>DART</th>
<th>Genotox</th>
<th>Carci</th>
<th>Dermal Irritation</th>
<th>Dermal Sensitization</th>
<th>Ocular Irritation</th>
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</tbody>
</table>

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

**Please note:** Acetyl Hexapeptide-8 is synonymous with Acetyl Hexapeptide-8 Amide. Accordingly, all of the data in the literature states Acetyl Hexapeptide-8 as the test material, but is fully applicable to the synonymous ingredient, Acetyl Hexapeptide-8 Amide. Therefore, if data were available in a category, it is indicated for both ingredients.
<table>
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<th>PubMed</th>
<th>TOXNET</th>
<th>FDA</th>
<th>EU</th>
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*ECHA – pre-registration process

**Search Strategy**

[document search strategy used for SciFinder, PubMed, and Toxnet]

[identify total # of hits /# hits that were useful or examined for usefulness]
**LINKS**

InfoBase (self-reminder that this info has been accessed; not a public website) - [http://www.personalcarecouncil.org/science-safety/line-infobase](http://www.personalcarecouncil.org/science-safety/line-infobase)

SciFinder (usually a combined search for all ingredients in report; list # of this/# useful) - [https://scifinder.cas.org/scifinder](https://scifinder.cas.org/scifinder)

PubMed (usually a combined search for all ingredients in report; list # of this/# useful) - [https://www.ncbi.nlm.nih.gov/pubmed](https://www.ncbi.nlm.nih.gov/pubmed)

Toxnet databases (usually a combined search for all ingredients in report; list # of this/# useful) – [https://toxnet.nlm.nih.gov/](https://toxnet.nlm.nih.gov/) (includes Toxline; HSDB; ChemIDPlus; DAR; IRIS; CCRIS; CPDB; GENE-TOX)


EU (European Union); check CosIng (cosmetic ingredient database) for restrictions and SCCS (Scientific Committee for Consumer Safety) opinions - [http://ec.europa.eu/growth/tools-databases/cosing/](http://ec.europa.eu/growth/tools-databases/cosing/)


HPVIS (EPA High-Production Volume Info Systems) - [https://ofmext.epa.gov/hpvis/HPVISLogon](https://ofmext.epa.gov/hpvis/HPVISLogon)

NICNAS (Australian National Industrial Chemical Notification and Assessment Scheme) - [https://www.nicnas.gov.au/](https://www.nicnas.gov.au/)

NTIS (National Technical Information Service) - [http://www.ntis.gov/](http://www.ntis.gov/)

NTP (National Toxicology Program) - [http://ntp.niehs.nih.gov/](http://ntp.niehs.nih.gov/)


FEMA (Flavor & Extract Manufacturers Association) - [http://www.femaflavor.org/search/apachesolr_search/](http://www.femaflavor.org/search/apachesolr_search/)

Web – perform general search; may find technical data sheets, published reports, etc

ECETOC (European Center for Ecotoxicology and Toxicology Database) - [http://www.ecetoc.org/](http://www.ecetoc.org/)

**Botanical Websites, if applicable**

Dr. Duke’s - [https://phytochem.nal.usda.gov/phytochem/search](https://phytochem.nal.usda.gov/phytochem/search)


GRIN (U.S. National Plant Germplasm System) - [https://npgsweb.ars-grin.gov/gringlobal/taxon/taxonymsimple.aspx](https://npgsweb.ars-grin.gov/gringlobal/taxon/taxonymsimple.aspx)


**Fragrance Websites, if applicable**

RIFM (the Research Institute for Fragrance Materials) should be contacted

Qualifiers
Absorption
Acute
Allergy
Allergic
Allergenic
Cancer
Carcinogen
Chronic
Development
Developmental
Excretion
Genotoxic
Irritation
Metabolism
Mutagen
Mutagenic
Penetration
Percutaneous
Pharmacokinetic
Repeated dose
Reproduction
Reproductive
Sensitization
Skin
Subchronic
Teratogen
Teratogenic
Toxic
Toxicity
Toxicokinetic
Toxicology
Tumor
Safety Assessment of Acetyl Hexapeptide-8 and Acetyl Hexapeptide-8 Amide as Used in Cosmetics

Status: Draft Report for Panel Review
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INTRODUCTION

The safety of Acetyl Hexapeptide-8 and Acetyl Hexapeptide-8 Amide, as used in cosmetics, is reviewed in this safety assessment. According to the web-based International Cosmetic Ingredient Dictionary and Handbook (wINCI; Dictionary), Acetyl Hexapeptide-8 functions as a skin-conditioning agent-humectant and Acetyl Hexapeptide-8 Amide functions as a skin-conditioning agent-miscellaneous. It seems that Acetyl Hexapeptide-8 and Acetyl Hexapeptide-8 Amide were, until recently, each labeled as Acetyl Hexapeptide-8 (i.e. the “Amide” name was not in use, though the chemical was). Accordingly, all of the data in the literature states Acetyl Hexapeptide-8 as the test material, when the ingredient under investigation therein was actually amidated, i.e. Acetyl Hexapeptide-8 Amide.

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

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

CHEMISTRY

Definition and Structure

Acetyl Hexapeptide-8 (also known as acetyl hexapeptide-3) is defined as the product obtained by the acetylation of hexapeptide-8. The sequence for this acetylated peptide is Ac-Glu-Glu-Met-Gln-Arg-Arg. Acetyl Hexapeptide-8 Amide (CAS No. 616204-22-9) is defined as the product obtained by the acetylation of hexapeptide-8 in which the C-terminus is an amide. The sequence for this acetylated and amidated peptide is Ac-Glu-Glu-Met-Gln-Arg-Arg-NH2.

Figure 1. Acetyl Hexapeptide-8 (as described)
However, when Acetyl Hexapeptide-8 is used in cosmetic formulations, it is typically amidated. In other words, regardless of whether the name Acetyl Hexapeptide-8 or the name Acetyl Hexapeptide-8 Amide is used, the structure in Figure 2 is the chemical used (i.e. Acetyl Hexapeptide-8 Amide). Furthermore, Acetyl Hexapeptide-24 is apparently identical to Acetyl Hexapeptide-8, as is Acetyl Hexapeptide-24 Amide. In summation, Acetyl Hexapeptide-8 = Acetyl Hexapeptide-8 Amide = Acetyl Hexapeptide-24 = Acetyl Hexapeptide-24 Amide. The toxicity data throughout this report is assigned to Acetyl Hexapeptide-8 Amide, but is directly applicable to these 3 synonyms.

**Chemicals Properties**

Acetyl Hexapeptide-8 has a molecular weight of 889.98 Da and an estimated octanol/water partitioning coefficient (log $K_{ow}$) of -7.68. However, as mentioned above, Acetyl Hexapeptide-8 is supplied as the amide, i.e. Acetyl Hexapeptide-8 Amide. Acetyl Hexapeptide-8 Amide is a white powder with a molecular weight of 889.0 Da. It is soluble in water and has a log P of -6.3.

**Method of Manufacture**

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

According to a manufacturer of Acetyl Hexapeptide-8 Amide, this ingredient is completely synthesized in the laboratory and no excipients, preservatives, or antioxidants are used during the manufacturing process. Acetyl Hexapeptide-8 Amide has also been derived from the $N$-terminal of the synaptic protein, synaptosomal nerve-associated protein 25 (SNAP-25, 12-17 amino acids) with the following amino acid sequence: Ac-Glu-Glu-Met-Gln-Arg-Arg-NH$_2$. Another source indicates that Acetyl Hexapeptide-8 Amide is synthesized in accordance with good manufacturing practice (GMP) guidelines, and involves a final freeze-drying step. These freeze-dried products are commonly obtained as a polymorphous crystalline powder.

**Composition/Impurities**

According to a manufacturer of Acetyl Hexapeptide-8 Amide, no excipients, preservatives, or antioxidants are present. Furthermore, according to this manufacturer’s product specification, Acetyl Hexapeptide-8 Amide is > 98% pure and contains < 5% water. Another manufacturer has stated that the peptide purity of Acetyl Hexapeptide-8 Amide is > 80%, and that the results of an amino acid analysis indicate the presence of glutamic acid (2.7 to 3.3%), methionine (0.6 to 1%), and arginine (1.8 to 2.2%).

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

**USE**

**Cosmetic**

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

Acetyl Hexapeptide-3 is listed as a technical name for Acetyl Hexapeptide-8, and uses are listed in the VCRP for both names. Therefore, that information is captured as such in the use table. According to 2020 VCRP data, Acetyl Hexapeptide-8 is reported to be used in 452 cosmetic products (422 leave-on and 30 rinse-off) as Acetyl Hexapeptide-8, and an additional 33 uses are reported with the name acetyl hexapeptide-3 (32 leave-on and 1 rinse-off; Table 1). The results of a concentration of use survey conducted by the Council in 2019 indicate that Acetyl Hexapeptide-8 is used at concentrations up to 0.005% (in eye lotions and face and neck products; not spray), which is the highest reported maximum use concentration for leave-on formulations. In rinse-off products, Acetyl Hexapeptide-8 is reported to be used at concentrations up to 0.000005% (skin cleansing products). According to VCRP and Council survey data, Acetyl Hexapeptide-8 Amide is not reported to be used in cosmetic products.

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

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

Acetyl Hexapeptide-8 and Acetyl Hexapeptide-8 Amide are not included on the European Union’s list of substances that are restricted or list of substances that are prohibited in cosmetic products.

**Non-Cosmetic**

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

**TOXICOKINETIC STUDIES**

**Dermal Penetration**

**In Vitro**

The influence of different vehicles (multiple water-in-oil-in-water, oil-in-water, and water-in-oil emulsions) on the skin (porcine) penetration of Acetyl Hexapeptide-8 Amide was studied using Franz diffusion cells. The composition of the multiple water-in-oil-in-water emulsion was described as follows: isopropyl myristate (20%), distilled water (75.99%), octyldodecanol and octyldodecyl xyloside and PEG-30 dipolyhydroxystearate (1.5%), and sucrose stearate (2.5%). Five parallel experiments for each formulation (n = 5) were performed. Porcine skin was cut with a dermatome set at 700 µm. Cut skin pieces were clamped between the donor and receptor chambers of the diffusion cells. The permeation area of the diffusion cell was 0.95 cm². The acceptor compartment was filled with 2 ml of 0.1% formic acid. An infinite dose (250 mg/cm²) of Acetyl Hexapeptide-8 Amide (in emulsion) was applied onto the skin in the donor chamber. Samples (5 µl) for the analysis of permeated Acetyl Hexapeptide-8 Amide were taken after 2, 4, 6, and 8 h, and permeation was quantified using liquid chromatography with tandem mass spectrometry (LC-MS/MS). Acetyl Hexapeptide-8 Amide permeated more rapidly and to a statistically significantly higher extent from the multiple water-in-oil-in-water and the oil-in-water emulsions, while skin permeation of Acetyl Hexapeptide-8 Amide from the water-in-oil emulsion was undetectable. After 8 h, skin permeation was ranked in the order of multiple water-in-oil-in-water emulsion > oil-in-water emulsion > water-in-oil emulsion. A statistically significant difference (p < 0.01) between the cumulative permeated amount of Acetyl
Hexapeptide-8 Amide after 8 h from the multiple water-in-oil-in-water emulsion (755 ± 149 ng/cm²) and the oil-in-water emulsion (456 ± 120 ng/cm²) was found.

In the same study, tape-stripping experiments using full-thickness porcine ear skin were also performed. The same emulsions were used, and 4 experiments for each formulation were performed. An Acetyl Hexapeptide-8 Amide emulsion (5 mg/cm²) was applied and distributed with a saturated gloved finger, and the tape-stripping procedure was initiated after an exposure time of 1 h. After a residence time of 1 h, 46.7 ± 6.2 ng/cm² of applied Acetyl Hexapeptide-8 Amide penetrated into the stratum corneum from the multiple emulsion. The amounts that entered the stratum corneum from the oil-in-water and the water-in-oil emulsions were 24.7 ± 4.9 ng/cm² and 9.5 ± 2.1 ng/cm², respectively. Therefore, the multiple water-in-oil-in-water emulsion led to 4.91 ± 0.66-fold and 1.89 ± 0.25-fold higher skin deposition of Acetyl Hexapeptide-8 Amide than the water-in-oil and oil-in-water emulsion, respectively. The oil-in-water emulsion showed 2.61 ± 0.52-fold increased skin penetration of Acetyl Hexapeptide-8 Amide when compared to the water-in-oil emulsion. According to the results of these experiments, the penetration of Acetyl Hexapeptide-8 Amide from the different emulsions was in the order of multiple water-in-oil-in-water emulsion > oil-in-water emulsion > water-in-oil emulsion.

The skin penetration of Acetyl Hexapeptide-8 Amide was evaluated using hairless guinea pig skin and human cadaver skin assembled in vitro diffusion cells. The composition of the receptor fluid was: anhydrous calcium chloride (140 mg/ml), dextrose (1000 mg/ml), 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES, 5960 mg/l), magnesium sulfate heptahydrate (200 mg/ml), potassium chloride (400 mg/ml), monobasic potassium dihydrogen phosphate (60 mg/ml), sodium bicarbonate (350 mg/ml), sodium chloride (7000 mg/ml), sodium phosphate dibasic (50 mg/ml), and gentamicin sulfate (50 mg/ml). An oil-in-water emulsion containing 10% Acetyl Hexapeptide-8 Amide (dose = 2 mg/cm²) was applied and distributed with a saturated gloved finger, and the tape-stripping procedure was initiated after an exposure time of 1 h. After a residence time of 1 h, 46.7 ± 6.2 ng/cm² of applied Acetyl Hexapeptide-8 Amide penetrated into the stratum corneum from the multiple emulsion. The amounts that entered the stratum corneum from the oil-in-water and the water-in-oil emulsions were 24.7 ± 4.9 ng/cm² and 9.5 ± 2.1 ng/cm², respectively. Therefore, the multiple water-in-oil-in-water emulsion led to 4.91 ± 0.66-fold and 1.89 ± 0.25-fold higher skin deposition of Acetyl Hexapeptide-8 Amide than the water-in-oil and oil-in-water emulsion, respectively. The oil-in-water emulsion showed 2.61 ± 0.52-fold increased skin penetration of Acetyl Hexapeptide-8 Amide when compared to the water-in-oil emulsion. According to the results of these experiments, the penetration of Acetyl Hexapeptide-8 Amide from the different emulsions was in the order of multiple water-in-oil-in-water emulsion > oil-in-water emulsion > water-in-oil emulsion.

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In another study, the skin penetration of 0.05% aqueous Acetyl Hexapeptide-8 Amide was studied using human skin that had been obtained from different donors who had undergone cosmetic surgery. Experiments were performed using a glass cell with an upper chamber (donor chamber) and a lower chamber (receptor chamber). The average diffusion area was 1.3 cm², and the receptor chamber volume was 4 ml. Skin disks (stratum corneum, ~ 2 cm²) were mounted between the 2 chambers. Isotonic phosphate buffer (pH = 7.4) with 0.01% sodium azide as preservative, was used as the receptor fluid. Samples (0.5 ml) of 0.05% aqueous Acetyl Hexapeptide-8 Amide were poured into the donor chamber and 100 µl aliquots of receptor fluid were periodically withdrawn for analysis. The concentration of the Acetyl Hexapeptide-8 Amide in the receptor fluid was quantified at 2 h using high-performance liquid chromatography. The total content of peptide in the receptor reservoir was 30% of the amount that was deposited onto the membrane in the donor chamber. The authors noted that these results indicate that the Acetyl Hexapeptide-8 Amide is capable of permeating through the skin.

Absorption, Distribution, Metabolism, and Excretion (ADME)

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

TOXICOLOGICAL STUDIES

Acute Toxicity Studies

The acute oral toxicity of Acetyl Hexapeptide-8 Amide was evaluated using rats (number and strain not stated). It was concluded that the test substance was non-toxic when administered orally (LD₅₀ > 2500 mg/kg).

Short-Term, Subchronic, and Chronic Toxicity Studies

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

DEVELOPMENTAL AND REPRODUCTIVE TOXICITY STUDIES

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

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

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

Effect on Skin Histology

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

After 6 weeks, all of the animals were alive and did not exhibit any side effects. The following changes were reported after 6 weeks for the aged model group, as compared to the normal control group: thinner skin, significant reduction in amount of collagen fibers in the dermis, and fibers were bound more loosely. When compared to the aged model group, the skin of mice treated with Acetyl Hexapeptide-8 Amide was thicker with a greater number of collagen fibers, and the fibers were dense and compact. The difference between the aged model group and the placebo control group was insignificant. Additionally, when compared to the aged model group, type I collagen fibers increased (p < 0.01) and type III collagen fibers decreased (p < 0.05) in the Acetyl Hexapeptide-8 Amide treatment group.

Cytotoxicity

The cytotoxicity of Acetyl Hexapeptide-8 Amide was evaluated in an in vitro proliferation assay using the formazan-based antiproliferation assay (EZ4U assay). Human embryonic kidney (HEK)-293 and neuroblastoma (IMR-32) cell lines, as well as human epidermal fibroblasts, were incubated for 48 h with test substance concentrations ranging from 0.01 µM to 100 µM. Doxorubicin, a commonly used drug in cancer chemotherapy, served as the reference compound. Significant antiproliferative activity was observed at concentrations above 10 µM. Calculated half-maximal inhibitory concentration (IC50) values were 34.862 µM (in HEK-293 cells) and 64.458 µM (in IMR-32 cells). In human epidermal fibroblasts, a dose-dependent antiproliferative effect was observed; 67% inhibition was observed at 100 µM (highest test concentration). The data showed very strong antiproliferative effect of doxorubicin against the IMR-32 cell line (IC50 = 0.0051 µM) and the HEK-293 cell line (IC50 = 0.455 µM). The authors noted that the IC50 value of Acetyl Hexapeptide-8 Amide was approximately 75-fold higher than the IC50 of doxorubicin against the HEK-293 cell line and 64.458 (in IMR-32 cells). In human epidermal fibroblasts, a dose-dependent antiproliferative effect was observed; 67% inhibition was observed at 100 µM (highest test concentration). The data showed very strong antiproliferative effect of doxorubicin against the IMR-32 cell line (IC50 = 0.0051 µM) and the HEK-293 cell line (IC50 = 0.455 µM). The authors noted that the IC50 value of Acetyl Hexapeptide-8 Amide was approximately 75-fold higher than the IC50 of doxorubicin against the HEK-293 cell line, and more than 10,000-fold higher against the IMR-32 cell line. The authors also noted that the significant effect of Acetyl Hexapeptide-8 Amide in human epidermal fibroblasts was observed at 100 µM, whereas the significant effect of doxorubicin (at 5.628 µM) was at an 18-fold lower concentration. Finally, the authors stated that, given the cytotoxic activity of Acetyl Hexapeptide-8 Amide against human epidermal fibroblasts, the use of Acetyl Hexapeptide-8 Amide at very high doses or for a very long period of time must be considered potentially dangerous for patients.

Inhibition of Catecholamine Release

The inhibitory activity of Acetyl Hexapeptide-8 Amide (tested at 100 µM) on Ca2+-evoked neurotransmitter release from digitonin-permealized chromaffin cells was studied. Detergent-permealized chromaffin cells release both noradrenaline and adrenaline in response to an increase in intracellular Ca2+. Acetyl Hexapeptide-8 Amide (100 µM), caused 30% inhibition of the total catecholamine exocytosis. Botulinum neurotoxin A (BoNT A) (20 nM) caused up to 60% inhibition of catecholamine release. A 26-mer peptide (1 µM) derived from the C-terminal end of SNAP-25 (ESUP-E) caused up to 55% inhibition of catecholamine release. Dose response curves indicated an IC50 of 110 µM for the test substance, which was 5000 x higher than the characteristic of BoNT A, and 400 x higher than that of ESUP-E.
Effect on N-Ethylmaleimide-Sensitive Factor Attachment Protein Receptor (SNARE) Complex Formation

An experiment was performed to determine if 10% Acetyl Hexapeptide-8 Amide prevents or destabilizes formation of the SNARE complex in vitro. Recombinant synaptic proteins vesicle-associated membrane protein (VAMP), syntaxin, and in vitro transcribed and translated [35S]SNAP-25 were used. Incubation of the 3 synaptic proteins led to the formation of protein complex of 75 kDa that was resistant to the chaotropic detergent sodium dodecyl sulfate (SDS), but sensitive to heat. These are 2 well-known properties of the SNARE complex. When the proteins were incubated with Acetyl Hexapeptide-8 Amide (at 1 mM and 2 mM), formation of the SNARE complex was prevented in a dose-dependent manner. At 2 mM, the 75 Da band was undetectable, suggesting complete abrogation of complex formation by the small peptide. The authors noted that these results indicate that Acetyl Hexapeptide-8 Amide can prevent the assembly of the protein complex that drives Ca2+-dependent exocytosis in secretory cells, implying that this peptide may modulate neurotransmitter release from these cells.

DERMAL IRRITATION AND SENSITIZATION STUDIES

Irritation

Animal

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

Sensitization

Human

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

OCULAR IRRITATION STUDIES

In Vitro

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

CLINICAL STUDIES

Other Clinical Reports

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

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

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

SUMMARY

The safety of Acetyl Hexapeptide-8 and Acetyl Hexapeptide-8 Amide, as used in cosmetics, is reviewed in this safety assessment. According to the Dictionary, Acetyl Hexapeptide-8 is reported to function as a skin-conditioning agent-humectant and Acetyl Hexapeptide-8 Amide is reported to function as a skin-conditioning agent-miscellaneous. It seems that Acetyl Hexapeptide-8 and Acetyl Hexapeptide-8 Amide were, until recently, each labeled as Acetyl Hexapeptide-8 (i.e. the “Amide” name was not in use, though the chemical was). Accordingly, all of the data in the literature states Acetyl Hexapeptide-8 as the test material, when the ingredient under investigation therein was actually amidated, i.e. Acetyl Hexapeptide-8 Amide.

According to 2020 VCRP data, Acetyl Hexapeptide-8 is reported to be used in 452 cosmetic products (422 leave-on and 30 rinse-off); an additional 33 uses (32 leave-on and 1 rinse-off) are reported under the name acetyl hexapeptide-3. The results of a concentration of use survey conducted by the Council in 2019 indicate that Acetyl Hexapeptide-8 is being used at concentrations up to 0.005% (in eye lotions and face and neck products; not spray), which is the highest reported maximum use concentration for leave-on formulations. In rinse-off products, Acetyl Hexapeptide-8 is reported to be used at concentrations up to 0.000005%. According to VCRP and Council survey data, Acetyl Hexapeptide-8 Amide is not being used in cosmetic products. However, this is merely a confusion in the nomenclature, as Acetyl Hexapeptide-8 Amide = Acetyl Hexapeptide-8 in consumer formulations (and even in the published literature). Thus, there is certainly some misplacement of frequency of use counts and concentration of use values between these 2 ingredients.

The in vitro skin penetration of Acetyl Hexapeptide-8 Amide has been demonstrated using porcine skin. Differences in the skin penetration of Acetyl Hexapeptide-8 Amide through porcine skin were observed when various vehicles for the test substance were used. For example, statistically significant difference (p < 0.01) between the cumulative permeated amount of Acetyl Hexapeptide-8 Amide after 8 h from the multiple water-in-oil-in-water emulsion (755 ± 149 ng/cm²) and the oil-in-water emulsion (456 ± 120 ng/cm²) was found. Overall, the penetration of Acetyl Hexapeptide-8 Amide from the different emulsions was in the order of multiple water-in-oil-in-water emulsion > oil-in-water emulsion > water-in-oil emulsion.

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

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

In an acute oral toxicity study, Acetyl Hexapeptide-8 Amide was evaluated using rats (number and strain not stated). The test substance was non-toxic when administered orally (LD₅₀ > 2500 mg/kg).

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

The effect of Acetyl Hexapeptide-8 Amide (10% in oil-in-water emulsion without preservatives) on skin histology was studied using groups of 10 Kunming mice, one of which was an aged model group. The test substance was applied twice daily for 6 weeks. When compared to the normal control group, the following changes were observed in the aged model group: thinner skin, significant reduction in amount of collagen fibers in the dermis, and fibers were bound more loosely.

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

The inhibitory activity of Acetyl Hexapeptide-8 Amide (tested at 100 µM) on Ca²⁺-evoked neurotransmitter release from digitonin-permealized chromaffin cells was studied. Acetyl Hexapeptide-8 Amide (100 µM), caused 30% inhibition of the total catecholamine exocytosis.

An experiment was performed to determine if Acetyl Hexapeptide-8 Amide prevents or destabilizes formation of the SNARE complex in vitro. Recombinant synaptic proteins VAMP, syntaxin, and in vitro transcribed and translated [35S]SNAP-25 were used. Incubation of the 3 synaptic proteins led to the formation of protein complex of 75 kDa that was resistant to the chaotropic detergent SDS, but sensitive to heat. When the proteins were incubated with Acetyl Hexapeptide-8 Amide (at 1 mM and 2 mM), formation of the SNARE complex was prevented in a dose-dependent manner. These results indicate that Acetyl Hexapeptide-8 Amide can prevent the assembly of the protein complex that drives Ca²⁺-dependent exocytosis in secretory cells, implying that this peptide may modulate neurotransmitter release from these cells.

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

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

**DISCUSSION**

To be developed…

**CONCLUSION**

To be determined…
Table 1. Frequency (2020) and concentration of use (2019) of Acetyl Hexapeptide-8 according to duration and type of exposure.\textsuperscript{13,14}

<table>
<thead>
<tr>
<th>Duration of Use</th>
<th># of Uses Reported as Acetyl Hexapeptide-8</th>
<th># of Uses Reported as acetyl hexapeptide-3</th>
<th>Conc. (%)</th>
</tr>
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<tbody>
<tr>
<td>Totals\textsuperscript{*}</td>
<td>452</td>
<td>33</td>
<td>0.000005-0.005</td>
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<tr>
<td>Leave-On</td>
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<td>32</td>
<td>0.00005-0.005</td>
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<td>Rinse off</td>
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<td>0.00005</td>
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<td>Diluted for (bath) Use</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
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<tr>
<td>Eye Area</td>
<td>61</td>
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<td>0.00005-0.005</td>
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<tr>
<td>Incidental Ingestion</td>
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<td>NR</td>
<td>0.00025</td>
</tr>
<tr>
<td>Incidental Inhalation- Sprays</td>
<td>154\textsuperscript{a}; 134\textsuperscript{b}</td>
<td>5\textsuperscript{a}; 16\textsuperscript{a}</td>
<td>NR</td>
</tr>
<tr>
<td>Incidental Inhalation- Powders</td>
<td>1; 134\textsuperscript{a}; 16\textsuperscript{a}</td>
<td>0.0001; 0.00026-0.005\textsuperscript{c}</td>
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<tr>
<td>Dermal Contact</td>
<td>447</td>
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<td>0.00005-0.005</td>
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<tr>
<td>Deodorant (underarm)</td>
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<td>NR</td>
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<td>Hair - Non-Coloring</td>
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<td>NR</td>
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<tr>
<td>Hair-Coloring</td>
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<td>NR</td>
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<tr>
<td>Nail</td>
<td>NR</td>
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<td>Mucous Membrane</td>
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<tr>
<td>Baby Products</td>
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<td>NR</td>
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</table>

\textsuperscript{*}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.

\textsuperscript{a}It is possible that these products may be sprays, but it is not specified whether the reported uses are sprays

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

\textsuperscript{c}It is possible that these products may be powders, but it is not specified whether the reported uses are powders

NR = Not Reported
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<table>
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<th>Description</th>
<th>Code</th>
<th>Count</th>
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<tr>
<td>Eye Lotion</td>
<td></td>
<td>03D</td>
<td>33</td>
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<tr>
<td>Other Eye Makeup Preparations</td>
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<td>03G</td>
<td>28</td>
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<tr>
<td>Shampoos (non-coloring)</td>
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<td>05F</td>
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<tr>
<td>Face Powders</td>
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<td>Foundations</td>
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<td>07C</td>
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<td>10A</td>
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<td>Aftershave Lotion</td>
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<td>11A</td>
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<tr>
<td>Cleansing</td>
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<td>12A</td>
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<tr>
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<tr>
<td>Night</td>
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<td>12G</td>
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<td>Paste Masks (mud packs)</td>
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<td>Skin Fresheners</td>
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<td>Suntan Gels, Creams, and Liquids</td>
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\[452\]

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<td>Other Skin Care Preps</td>
<td></td>
<td>12J</td>
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\[33\]
Memorandum

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

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

DATE: July 24, 2019

SUBJECT: Concentration of Use by FDA Product Category: Acetyl Hexapeptide-8
### Concentration of Use by FDA Product Category – Acetyl Hexapeptide-8*

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Product Category</th>
<th>Maximum Concentration of Use</th>
</tr>
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<tbody>
<tr>
<td>Acetyl Hexapeptide-8</td>
<td>Eye shadows</td>
<td>0.00005%</td>
</tr>
<tr>
<td>Acetyl Hexapeptide-8</td>
<td>Eye lotions</td>
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</tr>
<tr>
<td>Acetyl Hexapeptide-8</td>
<td>Face powders</td>
<td>0.0001%</td>
</tr>
<tr>
<td>Acetyl Hexapeptide-8</td>
<td>Foundations</td>
<td>0.0005%</td>
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<td>Acetyl Hexapeptide-8</td>
<td>Aftershave lotions</td>
<td>0.0001%</td>
</tr>
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<td>Acetyl Hexapeptide-8</td>
<td>Skin cleansing (cold creams, cleansing lotions, liquids and pads)</td>
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</tr>
<tr>
<td>Acetyl Hexapeptide-8</td>
<td>Face and neck products</td>
<td>0.00026-0.005%</td>
</tr>
<tr>
<td>Acetyl Hexapeptide-8</td>
<td>Body and hand products</td>
<td>0.0015%</td>
</tr>
<tr>
<td>Acetyl Hexapeptide-8</td>
<td>Moisturizing products</td>
<td>0.0009%</td>
</tr>
<tr>
<td>Acetyl Hexapeptide-8</td>
<td>Other skin care preparations</td>
<td>0.0013%</td>
</tr>
<tr>
<td>Acetyl Hexapeptide-8</td>
<td>Suntan products</td>
<td>0.0005%</td>
</tr>
</tbody>
</table>

*Ingredients included in the title of the table but not found in the table were included in the concentration of use survey, but no uses were reported.

Information collected in 2019
Table prepared: July 23, 2019
Memorandum

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

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

DATE: January 30, 2020

SUBJECT: Scientific Literature Review: Safety Assessment of Acetyl Hexapeptide-8 and Acetyl Hexapeptide-8 Amide as Used in Cosmetics (release date: January 15, 2020)

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

Key Issues
Non-Cosmetic Use - Although this section mentions that NIH conducted a study, it is not possible to determine which study in the Other Clinical Studies section was done by NIH without looking up the references (reference 22). It should be made clear that the NIH did not look at Acetyl Hexapeptide-8 for its anti-wrinkle activity. NIH looked to see if topical Acetyl Hexapeptide-8 could extend the time between injections of botulinum neurotoxin used to control blepharospasm.

Additional Considerations
Cosmetic Use - Please identify the rinse-off product category associated with the highest use concentration.
Dermal Penetration - The emulsions used in the study described in reference 18 are not clear. The first sentence says: “(multiple water-in-oil-in-water, oil-in-water, and oil-in-water)”. How many emulsions does “multiple water-in-oil-in-water” represent? Later in the description of the study, it states “oil-in-water” and “water-in-oil” suggesting that perhaps the second “oil-in-water” in the first sentence should be “water-in-oil”.
Effect on Skin Histology - If correct, it should be made clear that the “trade mixture” contains Acetyl Hexapeptide-8.