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

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Status: Tentative Report for Public Comment  
Release Date: September 25, 2020  
Panel Date: December 7-8, 2020

*All interested persons are provided 60 days from the above release date (i.e., November 24, 2020) to comment on this safety assessment and to identify additional published data that should be included or provide unpublished data which can be made public and included. Information may be submitted without identifying the source or the trade name of the cosmetic product containing the ingredient. All unpublished data submitted to the Cosmetic Ingredient Review (CIR) will be discussed in open meetings, will be available at the CIR office for review by any interested party and may be cited in a peer-reviewed scientific journal. Please submit data, comments, or requests to the CIR Executive Director, Dr. Bart Heldreth*

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

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**ABSTRACT:** The Expert Panel for Cosmetic Ingredient Safety (Panel) reviewed the safety of Acetyl Hexapeptide-8 Amide in cosmetic products; this ingredient is reported to function as a skin-conditioning agent-miscellaneous in cosmetics. The Panel reviewed data relevant to the safety of this ingredient in cosmetic formulations, and concluded that Acetyl Hexapeptide-8 Amide is safe in cosmetics in the present practices of use and concentration described in this safety assessment.

## **INTRODUCTION**

The safety of Acetyl Hexapeptide-8 Amide, as used in cosmetics, is reviewed in this safety assessment. According to the web-based *International Cosmetic Ingredient Dictionary and Handbook* (wINCI; *Dictionary*), Acetyl Hexapeptide-8 Amide functions as a skin-conditioning agent-miscellaneous.<sup>1</sup>

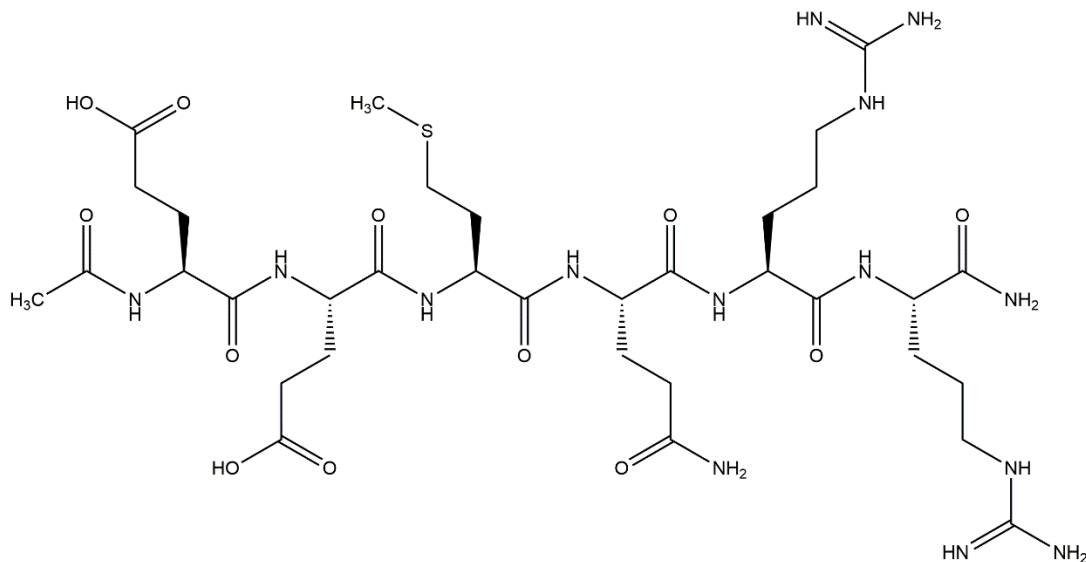
In 2018, the Expert Panel for Cosmetic Ingredient Safety (Panel) published a safety assessment of tripeptide-1, hexapeptide-12, their metal salts and fatty acyl derivatives, and palmitoyl tetrapeptide-7 as used in cosmetics.<sup>2</sup> The Panel concluded that these ingredients are safe in the present 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 Amide (CAS No. 616204-22-9; also known as Acetyl Hexapeptide-8, Acetyl Hexapeptide-3, Acetyl Hexapeptide-24, and Acetyl Hexapeptide-24 Amide) is defined as the product obtained by the acetylation of hexapeptide-8 in which the C-terminus is an amide.<sup>3</sup> The sequence for this acetylated and amidated peptide is Ac-Glu-Glu-Met-Gln-Arg-Arg-NH<sub>2</sub>.



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

### **Chemical Properties**

Acetyl Hexapeptide-8 Amide is a white powder with a molecular weight of 889.0 Da.<sup>4-6</sup> It is soluble in water and has a log P of -6.3.<sup>7,8</sup>

## 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.<sup>9,10</sup> 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/thioanisole/water (95/2.5/2.5, v/v/v, 7 ml/g resin) for 2 h. The crude peptides were precipitated by filtration into cold diethyl ether and vacuum-dried. The crude product was dissolved in 10% acetic acid for de-*tert*-butylation at 60 °C 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.<sup>4</sup> 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.<sup>7</sup> 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.<sup>4</sup> Furthermore, according to this manufacturer's product specification, Acetyl Hexapeptide-8 Amide is > 95% pure and contains < 5% water. Another manufacturer has stated that the peptide purity of Acetyl Hexapeptide-8 Amide is > 80%, and that the results of an amino acid analysis indicate the presence of glutamic acid (2.7 to 3.3%), methionine (0.6 to 1%), and arginine (1.8 to 2.2%).<sup>7</sup>

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).<sup>6,7</sup> 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.<sup>7</sup> According to another source, a tradename mixture contains Acetyl Hexapeptide-8 Amide (0.5 g/l), phenonip (0.5%), and water (99.45%).<sup>11</sup>

## 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.<sup>12</sup> 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.<sup>13</sup>

Acetyl Hexapeptide-3 is listed as a technical name for Acetyl Hexapeptide-8 Amide, 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 Amide is reported to be used in 452 cosmetic products (422 leave-on and 30 rinse-off) as Acetyl Hexapeptide-8 Amide, and an additional 33 uses are reported with the name Acetyl Hexapeptide-3 (32 leave-on and 1 rinse-off; Table 1).<sup>12</sup> The results of a concentration of use survey conducted by the Council in 2019 indicate that Acetyl Hexapeptide-8 Amide 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.<sup>13</sup> In rinse-off products, Acetyl Hexapeptide-8 Amide is reported to be used at concentrations up to 0.000005% (skin cleansing products).

Cosmetic products containing Acetyl Hexapeptide-8 Amide may be applied to the skin or near the eyes at concentrations up to 0.005% (stated above). Acetyl Hexapeptide-8 Amide also could be incidentally ingested during product use (e.g., use in lipsticks at concentrations up to 0.00025%). Products containing Acetyl Hexapeptide-8 Amide 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 Amide 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.<sup>14-16</sup>

Acetyl Hexapeptide-8 Amide is not included on the European Union's list of substances that are restricted or list of substances that are prohibited in cosmetic products.<sup>17</sup>

### 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.<sup>18</sup> (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.<sup>19</sup> The composition of the multiple water-in-oil-in-water emulsion was described as follows: isopropyl myristate (20%), distilled water (75.99%), octyldodecanol and octyldodecyl xyloside and PEG-30 dipolyhydroxystearate (1.5%), and sucrose stearate (2.5%). Five parallel experiments for each formulation were performed. Porcine skin was cut with a dermatome set at 700  $\mu\text{m}$ . 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  $\text{cm}^2$ . The acceptor compartment was filled with 2 ml of 0.1% formic acid. An infinite dose (250  $\text{mg}/\text{cm}^2$ ) of Acetyl Hexapeptide-8 Amide (in emulsion) was applied onto the skin in the donor chamber. Samples (5  $\mu\text{l}$ ) 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 ( $755 \pm 149 \text{ ng}/\text{cm}^2$ ) and the oil-in-water emulsion ( $456 \pm 120 \text{ ng}/\text{cm}^2$ ) was found.

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

The skin penetration of Acetyl Hexapeptide-8 Amide was evaluated using hairless guinea pig skin and human cadaver skin assembled in in vitro diffusion cells.<sup>5</sup> The composition of the receptor fluid was: anhydrous calcium chloride (140  $\text{mg}/\text{ml}$ ), dextrose (1000  $\text{mg}/\text{ml}$ ), 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES, 5960  $\text{mg}/\text{l}$ ), magnesium sulfate heptahydrate (200  $\text{mg}/\text{ml}$ ), potassium chloride (400  $\text{mg}/\text{ml}$ ), monobasic potassium dihydrogen phosphate (60  $\text{mg}/\text{ml}$ ), sodium bicarbonate (350  $\text{mg}/\text{ml}$ ), sodium chloride (7000  $\text{mg}/\text{ml}$ ), sodium phosphate dibasic (50  $\text{mg}/\text{ml}$ ), and gentamicin sulfate (50  $\text{mg}/\text{ml}$ ). An oil-in-water emulsion containing 10% Acetyl Hexapeptide-8 Amide (dose = 2  $\text{mg}/\text{cm}^2$ ) was applied to the skin for 24 h. Skin disks were tape stripped to determine the amount of peptide in the stratum corneum. Skin penetration was measured in skin layers using hydrophilic interaction LC-MS/MS and electrospray ionization. Stable isotopically-labeled hexapeptides were used as internal standards for the quantitation of native hexapeptides to correct for matrix effects that are associated with electrospray ionization. Study results indicated that the majority of Acetyl Hexapeptide-8 Amide was washed from the surface of both skin types (guinea pig and human). For the Acetyl Hexapeptide-8 Amide that actually penetrated the skin, it remained mostly in the stratum corneum of hairless guinea pig skin (0.54% of applied dose) and human skin (0.22% of applied dose). Peptide levels were found to decrease as each layer was removed by tape stripping. The total amount of Acetyl Hexapeptide-8 Amide that was found in the epidermis was similar (at 0.01%) when hairless guinea pig skin and human skin were compared. Also, for both skin types, no peptide was detected in the dermis or buffer collected underneath the skin. No hexapeptide metabolite was detected in any layers of hairless guinea pig or human skin, or buffer collected underneath the skin.

In another study, the skin penetration of 0.05% aqueous Acetyl Hexapeptide-8 Amide was studied using human skin that had been obtained from different donors who had undergone cosmetic surgery.<sup>9</sup> 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  $\text{cm}^2$ , and the receptor chamber volume was 4 ml. Skin disks (stratum corneum,  $\sim 2 \text{ cm}^2$ ) were mounted between the 2 chambers. Isotonic phosphate buffer (pH = 7.4) with 0.01% sodium azide as preservative, was used as the receptor fluid. Samples (0.5 ml) of 0.05% aqueous Acetyl Hexapeptide-8 Amide were poured into the donor chamber and 100  $\mu\text{l}$  aliquots of receptor fluid were periodically withdrawn for analysis. The concentration of the Acetyl Hexapeptide-8 Amide in the

receptor fluid was quantified at 2 h using high-performance liquid chromatography. The total content of peptide in the receptor reservoir was 30% of the amount that was deposited onto the membrane in the donor chamber. The authors noted that these results indicate that the Acetyl Hexapeptide-8 Amide is capable of permeating through the skin.

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

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

### **TOXICOLOGICAL STUDIES**

#### **Acute Toxicity Studies**

##### **Oral**

The acute oral toxicity of Acetyl Hexapeptide-8 Amide was evaluated using rats (number and strain not stated).<sup>7</sup> It was concluded that the test substance was non-toxic when administered orally ( $LD_{50} > 2500$  mg/kg).

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

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

### **DEVELOPMENTAL AND REPRODUCTIVE TOXICITY STUDIES**

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

### **GENOTOXICITY STUDIES**

#### **In Vitro**

Acetyl Hexapeptide-8 Amide was evaluated for genotoxicity potential in the Ames test, using the following *Salmonella typhimurium* strains: TA97, TA98, TA100, TA102, and TA1537.<sup>6,7</sup> The primary reference for these data was unavailable. Over the range of concentrations tested, 0.05 to 5 mg/plate, the test substance was non-genotoxic.

### **CARCINOGENICITY STUDIES**

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

### **OTHER RELEVANT STUDIES**

#### **Effect on Skin Histology**

The effect of Acetyl Hexapeptide-8 Amide on skin histology was studied using groups of 10 Kunming mice, described as follows: normal control group, aged model group, placebo control group, and Acetyl Hexapeptide-8 Amide treatment group.<sup>20</sup> 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 picosirius-polarization (PSP) stain for microscopic examination.

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

## **Cytotoxicity**

The cytotoxicity of Acetyl Hexapeptide-8 Amide was evaluated in an in vitro proliferation assay using the formazan-based antiproliferation assay (EZ4U assay).<sup>6</sup> 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  $\mu\text{M}$  to 100  $\mu\text{M}$ . Doxorubicin, a commonly used drug in cancer chemotherapy, served as the reference compound. Significant antiproliferative activity of Acetyl Hexapeptide-8 Amide was observed at concentrations above 10  $\mu\text{M}$ . Calculated half-maximal inhibitory concentration ( $\text{IC}_{50}$ ) values for Acetyl Hexapeptide-8 Amide were 34.862  $\mu\text{M}$  (in HEK-293 cells) and 64.458 (in IMR-32 cells). In human epidermal fibroblasts, a dose-dependent antiproliferative effect was observed; 67% inhibition was observed at 100  $\mu\text{M}$  Acetyl Hexapeptide-8 Amide (highest test concentration). The data showed very strong antiproliferative effect of doxorubicin against the IMR-32 cell line ( $\text{IC}_{50} = 0.0051 \mu\text{M}$ ) and the HEK-293 cell line ( $\text{IC}_{50} = 0.455 \mu\text{M}$ ). The authors noted that the  $\text{IC}_{50}$  value of Acetyl Hexapeptide-8 Amide was approximately 75-fold higher than the  $\text{IC}_{50}$  of doxorubicin against the HEK-293 cell line, and more than 10,000-fold higher against the IMR-32 cell line. The authors also noted that the significant effect of Acetyl Hexapeptide-8 Amide in human epidermal fibroblasts was observed at 100  $\mu\text{M}$ , whereas the significant effect of doxorubicin (at 5.628  $\mu\text{M}$ ) 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  $\mu\text{M}$ ) on  $\text{Ca}^{2+}$ -evoked neurotransmitter release from digitonin-permeabilized chromaffin cells was studied.<sup>9</sup> Detergent-permeabilized chromaffin cells release both noradrenaline and adrenaline in response to an increase in intracellular  $\text{Ca}^{2+}$ . Acetyl Hexapeptide-8 Amide (100  $\mu\text{M}$ ), 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  $\mu\text{M}$ ) derived from the C-terminal end of SNAP-25 (ESUP-E) caused up to 55% inhibition of catecholamine release. Dose response curves indicated an  $\text{IC}_{50}$  of 110  $\mu\text{M}$  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.<sup>9</sup> Recombinant synaptic proteins vesicle-associated membrane protein (VAMP), syntaxin, and in vitro transcribed and translated [<sup>35</sup>S]SNAP-25 were used. Incubation of the 3 synaptic proteins led to the formation of protein complex of 75 kDa that was resistant to the chaotropic detergent sodium dodecyl sulfate (SDS), but sensitive to heat. These are 2 well-known properties of the SNARE complex. When the proteins were incubated with Acetyl Hexapeptide-8 Amide (at 1 mM and 2 mM), formation of the SNARE complex was prevented in a dose-dependent manner. At 2 mM, the 75 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  $\text{Ca}^{2+}$ -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).<sup>7</sup> 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.<sup>7</sup> 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.<sup>7</sup> 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.<sup>9</sup> 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.<sup>21</sup> 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.<sup>18</sup> 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 orbital orbicularis oculi or surrounding muscles. A placebo (emulsion without Acetyl Hexapeptide-8 Amide) was applied topically to another 12 blepharospasm patients according to the same procedure. No severe adverse events were observed during the study. Four subjects (2 test and 2 placebo) experienced minor, self-limiting eyelid irritation. The irritation reactions observed did not necessitate any modifications of the test procedure.

## **SUMMARY**

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

According to 2020 VCRP data, Acetyl Hexapeptide-8 Amide 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 Amide 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 Amide is reported to be used at concentrations up to 0.000005%.

The in vitro skin penetration of Acetyl Hexapeptide-8 Amide has been demonstrated using porcine skin. Differences in the skin penetration of Acetyl Hexapeptide-8 Amide through porcine skin were observed when various vehicles for the test substance were used. For example, statistically significant difference ( $p < 0.01$ ) between the cumulative permeated amount of Acetyl Hexapeptide-8 Amide after 8 h from the multiple water-in-oil-in-water emulsion ( $755 \pm 149$  ng/cm<sup>2</sup>) and the oil-in-water emulsion ( $456 \pm 120$  ng/cm<sup>2</sup>) 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 ( $LD_{50} > 2500$  mg/kg).

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

The effect of Acetyl Hexapeptide-8 Amide (10% in oil-in-water emulsion without preservatives) on skin histology was studied using groups of 10 Kunming mice, one of which was an aged model group. The test substance was applied 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  $\mu$ M to 100  $\mu$ M. Significant antiproliferative activity was observed at concentrations above 10  $\mu$ M. Particularly, the significant effect of Acetyl Hexapeptide-8 Amide in human epidermal fibroblasts was observed at 100  $\mu$ M.

The inhibitory activity of Acetyl Hexapeptide-8 Amide (tested at 100  $\mu$ M) on  $Ca^{2+}$ -evoked neurotransmitter release from digitonin-permealized chromaffin cells was studied. Acetyl Hexapeptide-8 Amide (100  $\mu$ M), caused 30% inhibition of the total catecholamine exocytosis.

An experiment was performed to determine if Acetyl Hexapeptide-8 Amide prevents or destabilizes formation of the SNARE complex in vitro. Recombinant synaptic proteins VAMP, syntaxin, and in vitro transcribed and translated [ $^{35}S$ ]SNAP-25 were used. Incubation of the 3 synaptic proteins led to the formation of protein complex of 75 kDa that was resistant to the chaotropic detergent SDS, but sensitive to heat. When the proteins were incubated with Acetyl Hexapeptide-8 Amide (at 1 mM and 2 mM), formation of the SNARE complex was prevented in a dose-dependent manner. These results indicate that Acetyl Hexapeptide-8 Amide can prevent the assembly of the protein complex that drives  $Ca^{2+}$ -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.<sup>7</sup> Results indicated that the test substance is potentially not irritating to the eyes.

## **DISCUSSION**

The Panel noted the absence of systemic toxicity and genotoxicity data on Acetyl Hexapeptide-8 Amide. However, concern over the lack of these data was mitigated, after considering the peptide structure of this ingredient, the associated low log P value of -6.3 (percutaneous absorption unlikely), and the low maximum use concentration of 0.005% in leave-on cosmetic products.

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

## **CONCLUSION**

The Expert Panel for Cosmetic Ingredient Safety (Panel) concluded that Acetyl Hexapeptide-8 Amide is safe in cosmetics in the present practices of use and concentration described in this safety assessment.



**TABLES**

**Table 1.** Frequency (2020) and concentration of use (2019) of Acetyl Hexapeptide-8 Amide according to duration and type of exposure.<sup>12,13</sup>

	<b># of Uses Reported as Acetyl Hexapeptide-8 Amide</b>	<b># of Uses Reported as acetyl hexapeptide-3</b>	<b>Conc. (%)</b>
<b>Totals*</b>	<b>452</b>	<b>33</b>	<b>0.000005-0.005</b>
<b><i>Duration of Use</i></b>			
<i>Leave-On</i>	422	32	0.00005-0.005
<i>Rinse off</i>	30	1	0.000005
<i>Diluted for (bath) Use</i>	NR	NR	NR
<b><i>Exposure Type</i></b>			
Eye Area	61	1	0.00005-0.005
Incidental Ingestion	4	NR	0.00025
Incidental Inhalation- Sprays	154 <sup>a</sup> ; 134 <sup>b</sup>	5 <sup>a</sup> ; 16 <sup>b</sup>	NR
Incidental Inhalation- Powders	1; 134 <sup>b</sup>	16 <sup>b</sup>	0.0001; 0.00026-0.005 <sup>c</sup>
Dermal Contact	447	33	0.000005-0.005
Deodorant (underarm)	NR	NR	NR
Hair - Non-Coloring	1	NR	NR
Hair-Coloring	NR	NR	NR
Nail	NR	NR	NR
Mucous Membrane	13	NR	0.00025
Baby Products	NR	NR	NR

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

NR = Not Reported

<sup>a</sup>It is possible that these products may be sprays, but it is not specified whether the reported uses are sprays

<sup>b</sup>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

<sup>c</sup>It is possible that these products may be powders, but it is not specified whether the reported uses are powders

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