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

Status: Scientific Literature Review for Public Comment
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All interested persons are provided 60 days from the above date (i.e., March 15, 2020) to comment on this safety assessment and to identify additional published data that should be included or provide unpublished data which can be made public and included.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 All unpublished data submitted to CIR will be discussed in open meetings, will be available at the CIR office for review by any interested party and may be cited in a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 2020 Cosmetic Ingredient Review Expert Panel 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 CIR Executive Director is Bart Heldreth, Ph.D. This report was prepared by Wilbur Johnson, Jr., M.S., Senior Scientific Analyst.
INTRODUCTION

The safety of Acetyl Hexapeptide-8 and Acetyl Hexapeptide-8 Amide, as used in cosmetics, is reviewed in this Cosmetic Ingredient Review (CIR) 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-micellaneous.1

In 2018, the Expert Panel (Panel) published a safety assessment of tripeptide-1, hexapeptide-12, their metal salts and fatty acyl derivatives, and palmitoyl tetrapeptide-7 as used in cosmetics.2 The Panel concluded that these ingredients are safe in the present practices of use and concentration in cosmetics, as described in that safety assessment. 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 CIR 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 (CAS No. 616204-22-9; also known as acetyl hexapeptide-3) is defined as the product obtained by the acetylation of hexapeptide-8.1 The sequence for this acetylated peptide is Ac-Glu-Glu-Met-Gln-Arg-Arg-NH₂.3-5 Acetyl Hexapeptide-8 Amide is defined as the product obtained by the acetylation of hexapeptide-8 in which the C-terminus is an amide.3 According to the Dictionary, hexapeptide-8 is defined as a synthetic peptide containing arginine, glutamic acid, glutamine, and methionine.

Physical and Chemicals Properties

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

Method of Manufacture

One method of manufacture of Acetyl Hexapeptide-8 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.9,10 This
ingredient has been also been synthesized by solid phase on a \( p \)-methylbenzhydramine 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-\textit{tert}-butylation at 60°C and then purified.

According to a manufacturer of Acetyl Hexapeptide-8, this ingredient is completely synthesized in the laboratory and no excipients, preservatives, or antioxidants are used during the manufacturing process. Acetyl Hexapeptide-8 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 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, no excipients, preservatives, or antioxidants are present. Furthermore, according to this manufacturer’s product specification, Acetyl Hexapeptide-8 is > 98% pure and contains < 5% water. Another manufacturer has stated that the peptide purity of Acetyl Hexapeptide-8 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 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). The 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 (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 2019 VCRP data, Acetyl Hexapeptide-8 is reported to be used in 379 cosmetic products (350 leave-on and 29 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 0.015% (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 0.00005%. 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 0.015% (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 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 is not currently approved for drug use in the US (as an anti-wrinkle active), a National Institutes of Health (NIH) study purporting the safety of a topical drug use has been published. (These studies are included in the Other Clinical Studies section of this safety assessment.)
The skin penetration of Acetyl Hexapeptide-8 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 (dose = 2 mg/cm²) 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 liquid chromatography with tandem mass spectrometry 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 was washed from the surface of both skin types (guinea pig and human). For the Acetyl Hexapeptide-8 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 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 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 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 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 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

Oral

The acute oral toxicity of Acetyl Hexapeptide-8 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.

GENOTOXICITY STUDIES

In Vitro

Acetyl Hexapeptide-8 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

Carcinogenicity studies of Acetyl Hexapeptide-8 and 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 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 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 in an oil-in-water emulsion without preservatives. The placebo control solution was a non-active oil-in-water emulsion without the trade mixture. 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 trade mixture 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 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 treatment group.

Cytotoxicity

The cytotoxicity of Acetyl Hexapeptide-8 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, 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 (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.
The authors noted that the IC_{50} value of Acetyl Hexapeptide-8 was approximately 75-fold higher than the IC_{50} of doxorubicin against the HEK-293 cell line, and more than 10,000-fold higher against the IMR-32 cell line. The authors also noted that the significant effect of Acetyl Hexapeptide-8 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 against human epidermal fibroblasts, the use of Acetyl Hexapeptide-8 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 (tested at 100 µM) on Ca^{2+}-evoked neurotransmitter release from digitonin-permeabilized chromaffin cells was studied. Detergent-permeabilized chromaffin cells release both noradrenaline and adrenaline in response to an increase in intracellular Ca^{2+}. Acetyl Hexapeptide-8 (100 µM), caused 30% inhibition of the total catecholamine exocytosis. Botulinum neurotoxin A (BoNT A) (20 nM) caused up to 60% inhibition of catecholamine release. A 26-mer peptide (1 µM) derived from the C-terminal end of SNAP-25 (ESUP-E) caused up to 55% inhibition of catecholamine release. Dose response curves indicated an IC_{50} of 110 µM for the test substance, which was 5000 x higher than the characteristic of BoNT A, and 400 x higher than that of ESUP-E.

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

An experiment was performed to determine if 10% Acetyl Hexapeptide-8 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 [^{35}S]-SNAP-25 were used. Incubation of the 3 synaptic proteins led to the formation of protein complex of 75 kDa that was resistant to the chaotropic detergent sodium dodecyl sulfate (SDS), but sensitive to heat. These are 2 well-known properties of the SNARE complex. When the proteins were incubated with Acetyl Hexapeptide-8 (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 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.

DERMAL IRRITATION AND SENSITIZATION STUDIES

Irritation

Animal

The skin irritation potential of the tradename mixture containing 0.05% aqueous Acetyl Hexapeptide-8 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 days after removal of the test substance.

Sensitization

Human

The skin sensitization potential of the tradename mixture containing 0.05% aqueous Acetyl Hexapeptide-8 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 (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 twice per day for 30 days. The emulsion without Acetyl Hexapeptide-8 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 days, 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 (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 decreased the depth of skin wrinkles by 30%.
The effect of Acetyl Hexapeptide-8 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 (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. The subjects were instructed to apply the cream twice daily for 2 months (60 days). Skin surface evaluation and measurement of transepidermal water loss were performed before day 0, during day 20 and day 40, and day 60 after treatment. 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 (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 days (p = 0.025) and 40 days (p = 0.028) of application of the Acetyl Hexapeptide-8 (10% w/w) cream. At 60 days, the decrease in trans-epidermal water loss was not statistically significant. None of the following effects was reported after application of the Acetyl Hexapeptide-8 (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 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 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) 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 CIR safety assessment. According to the Dictionary, Acetyl Hexapeptide-8 functions as a skin-conditioning agent-humectant and Acetyl Hexapeptide-8 Amide functions as a skin-conditioning agent-micellaneous.

According to 2019 VCRP data, Acetyl Hexapeptide-8 is reported to be used in 379 cosmetic products (350 leave-on and 29 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.

The in vitro skin penetration of Acetyl Hexapeptide-8 has been demonstrated using porcine skin. Differences in the skin penetration of Acetyl Hexapeptide-8 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 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 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 was evaluated using hairless guinea pig skin and human cadaver skin in vitro. For the Acetyl Hexapeptide-8 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 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 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 is capable of permeating through the skin.

In an acute oral toxicity study, Acetyl Hexapeptide-8 was evaluated using rats (number and strain not stated). The test substance was non-toxic when administered orally (LD₅₀ > 2500 mg/kg).
Acetyl Hexapeptide-8 was evaluated for genotoxicity potential in the Ames test, using the following *Salmonella 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 (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 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 in human epidermal fibroblasts was observed at 100 µM.

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

An experiment was performed to determine if Acetyl Hexapeptide-8 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 (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 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 the tradename mixture containing 0.05% aqueous Acetyl Hexapeptide-8 was evaluated using albino male rabbits (number not stated). There were no signs or erythema or edema at 7 days after removal of the test substance. The tradename mixture containing Acetyl Hexapeptide-8 (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 (concentrations not stated) was evaluated using the neutral red uptake test. Results indicated that the test substance is potentially not irritating to the eyes.

**INFORMATION SOUGHT**

- Subchronic and chronic dermal toxicity data
- Dermal irritation and sensitization data
- Toxicokinetic data
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<th>Duration of Use</th>
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<td>374</td>
<td>33</td>
<td>0.000005-0.005</td>
</tr>
<tr>
<td>Deodorant (underarm)</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
</tr>
<tr>
<td>Hair - Non-Coloring</td>
<td>1</td>
<td>NR</td>
<td>NR</td>
</tr>
<tr>
<td>Hair-Coloring</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
</tr>
<tr>
<td>Nail</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
</tr>
<tr>
<td>Mucous Membrane</td>
<td>13</td>
<td>NR</td>
<td>0.00025</td>
</tr>
<tr>
<td>Baby Products</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
</tr>
</tbody>
</table>

<sup>a</sup>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.
<sup>b</sup>NR = Not Reported
<sup>c</sup>It is possible that these products may be sprays, but it is not specified whether the reported uses are sprays
<sup>d</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>e</sup>It is possible that these products may be powders, but it is not specified whether the reported uses are powders
REFERENCES


