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

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ABSTRACT: The Expert Panel for Cosmetic Ingredient Safety (Panel) reviewed the safety of Acetyl Hexapeptide-8 Amide (synonymous with Acetyl Hexapeptide-8 (sans "Amide")) in cosmetic products; this ingredient is reported to function as a skin conditioning agent - miscellaneous in cosmetics. The Panel reviewed data relevant to the safety of this ingredient in cosmetic formulations, and concluded that Acetyl Hexapeptide-8 Amide is safe in cosmetics in the present practices of use at concentrations up to 0.005%. The Panel further concluded that the available data are insufficient to make a determination that Acetyl Hexapeptide-8 Amide is safe under the intended conditions of use in cosmetic formulations at concentrations greater than 0.005%.

INTRODUCTION

The safety of Acetyl Hexapeptide-8 Amide (synonymous with Acetyl Hexapeptide-8 (sans "Amide")), as used in cosmetics, is reviewed in this safety assessment. According to the web-based *International Cosmetic Ingredient Dictionary and Handbook* (wINCI; *Dictionary*), Acetyl Hexapeptide-8 Amide functions as a skin conditioning agent - miscellaneous.[1] While Acetyl Hexapeptide-8 Amide is synonymous with in-use name, Acetyl Hexapeptide-8, and both are included in the *Dictionary*, the following synonyms have been retired or deleted from the *Dictionary*: Acetyl Hexapeptide-3, Acetyl Hexapeptide-24, and Acetyl Hexapeptide-24 Amide. Since the name, "Acetyl Hexapeptide-8 Amide," is more descriptive and its definition more accurate, this name was chosen for use throughout the report (i.e., instead of Acetyl Hexapeptide-8).

In 2018, the Expert Panel for Cosmetic Ingredient Safety (Panel) published a safety assessment of tripeptide-1, hexapeptide-12, their metal salts and fatty acyl derivatives, and palmitoyl tetrapeptide-7 as used in cosmetics.[2] The Panel concluded that these ingredients are safe in cosmetics in the present practices of use and concentration, as described in that safety assessment. (This report is available on the Cosmetic Ingredient Review (CIR) website. https://www.cir-safety.org/ingredients) Though the peptide sequences in those ingredients that have been reviewed differ from the peptide sequence in Acetyl Hexapeptide-8 Amide, it is important to note that the Panel has evaluated the safety of ingredients in which a distinct peptide sequence is part of the chemical structure.

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

CHEMISTRY

Definition and Structure

Acetyl Hexapeptide-8 Amide (CAS No. 616204-22-9, synthetic peptide also known as Acetyl Hexapeptide-8, Acetyl Hexapeptide-3, Acetyl Hexapeptide-24, and Acetyl Hexapeptide-24 Amide) is defined as the product obtained by the acetylation of hexapeptide-8, in which the C-terminus is an amide.[3] The sequence for this acetylated and amidated peptide is Ac-Glu-Glu-Met-Gln-Arg-Arg-NH_2 (acetyl group-glutamic acid-glutamic acid-methionine-glutamine-arginine-arginine-amino group).[4]

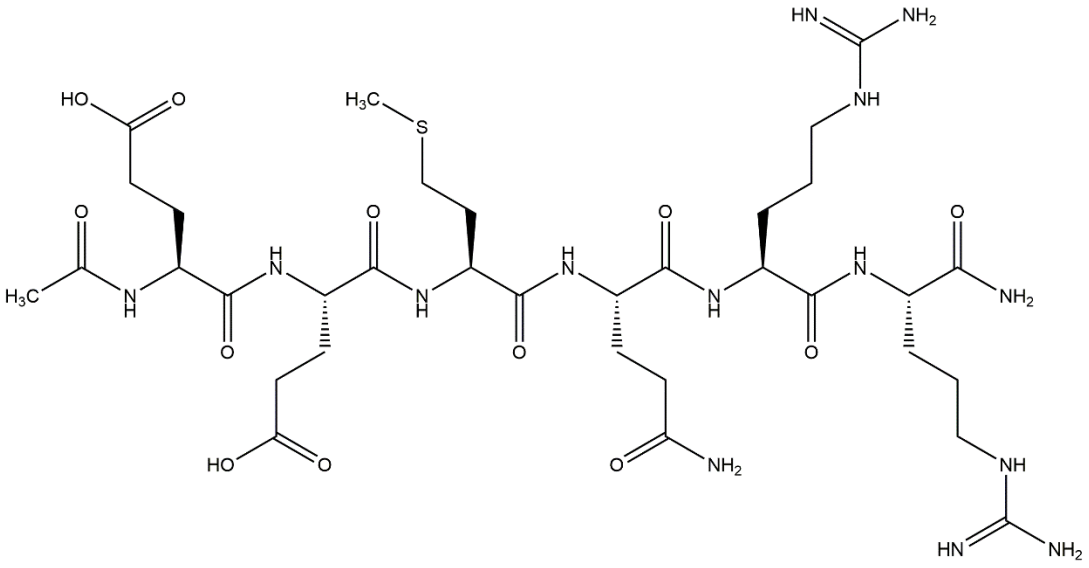

Figure 1. Acetyl Hexapeptide-8 Amide.

Chemical Properties

Acetyl Hexapeptide-8 Amide is a white powder with a molecular weight of 889.1 Da.[4,5] It is soluble in water, and has a log P of -6.3.[6,7]

Method of Manufacture

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

According to a manufacturer of Acetyl Hexapeptide-8 Amide, this ingredient is completely synthesized in the laboratory and no excipients, preservatives, or antioxidants are used during the manufacturing process.[4] Another source indicates that Acetyl Hexapeptide-8 Amide is synthesized in accordance with good manufacturing practice (GMP) guidelines, and involves a final freeze-drying step.[6] These freeze-dried products are commonly obtained as a polymorphous crystalline powder.

Composition/Impurities

According to a manufacturer of Acetyl Hexapeptide-8 Amide, no excipients, preservatives, or antioxidants are present.[4] Furthermore, according to this manufacturer's product specification, Acetyl Hexapeptide-8 Amide is > 95% pure and contains < 5% water. Another manufacturer has stated that the peptide purity of Acetyl Hexapeptide-8 Amide is > 80%, and that the results of an amino acid analysis indicate the presence of glutamic acid (2.7 to 3.3%), methionine (0.6 to 1%), and arginine (1.8 to 2.2%).[6]

Furthermore, Acetyl Hexapeptide-8 Amide is supplied either as a powder or provided as a tradename mixture that is an aqueous solution containing 0.5 g/l of the powder (i.e., 0.05% aqueous solution; pure active peptide in solution estimated at ~0.56 mM).[5,6] A 0.05% aqueous tradename mixture also contains 0.3% phenonip, which is a broad spectrum preservative with the following composition: phenoxyethanol, methylparaben, ethylparaben, propylparaben, butylparaben, and isobutylparaben.[6] According to another source, a tradename mixture contains Acetyl Hexapeptide-8 Amide (0.5 g/l), phenonip (0.5%), and water (99.45%).[10]

USE

Cosmetic

The safety of the cosmetic ingredient addressed in this safety assessment is evaluated based, in part, on data received from the United States (US) Food and Drug Administration (FDA) and the cosmetics industry on the expected use of this ingredient in cosmetics. Use frequencies of individual ingredients in cosmetics are collected from manufacturers and reported by cosmetic product category in FDA's Voluntary Cosmetic Registration Program (VCRP) database. 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.

According to 2021 VCRP data, Acetyl Hexapeptide-8 is reported to be used in 333 cosmetic products (311 leave-on and 22 rinse-off), and an additional 21 uses are reported with the name Acetyl Hexapeptide-3 (20 leave-on and 1 rinse-off; Table 1).[11] According to the *Dictionary*,[1] Acetyl Hexapeptide-3 is listed as a technical name for Acetyl Hexapeptide-8; therefore data for both of these ingredients are captured in the table. The results of a concentration of use survey conducted by the Council in 2019 indicate that Acetyl Hexapeptide-8 is used at concentrations up to 0.005% (in eye lotions and face and neck products; not spray), which is the highest reported maximum use concentration for leave-on formulations.[12] In rinse-off products, Acetyl Hexapeptide-8 is reported to be used at concentrations up to 0.000005% (skin cleansing products). The Panel is aware of products potentially being marketed at higher use concentrations claiming a non-cosmetic use, and, as such, these products are not under the purview of the Panel.[8,13]

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.[14-16]

Acetyl Hexapeptide-8 is not included on the European Union's list of substances that are restricted or list of substances that are prohibited in cosmetic products.[17]

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.[18] (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.[19] The composition of the multiple water-in-oil-in-water emulsion was described as follows: isopropyl myristate (20%), distilled water (75.99%), octyldodecanol and octyldodecyl xyloside and PEG-30 dipolyhydroxystearate (1.5%), and sucrose stearate (2.5%). Five parallel experiments for each formulation were performed. Porcine skin was cut with a dermatome set at 700 µm. Cut skin pieces were clamped between the donor and receptor chambers of the diffusion cells. The permeation area of the diffusion cell was 0.95 cm^2. The acceptor compartment was filled with 2 ml of 0.1% formic acid. An infinite dose (250 mg/cm^2) of Acetyl Hexapeptide-8 Amide (in emulsion) was applied onto the skin in the donor chamber. Samples (5 µl) for the analysis of permeated Acetyl Hexapeptide-8 Amide were taken after 2, 4, 6, and 8 h, and permeation was quantified using liquid chromatography with tandem mass spectrometry (LC-MS/MS). Acetyl Hexapeptide-8 Amide permeated more rapidly and to a statistically significantly higher extent from the multiple water-in-oil-in-water and the oil-in-water emulsions, while skin permeation of Acetyl Hexapeptide-8 Amide from the water-in-oil emulsion was undetectable. After 8 h, skin permeation was ranked in the order of multiple water-in-oil-in-water emulsion > oil-in-water emulsion > water-in-oil emulsion. A statistically significant difference ($p < 0.01$) between the cumulative permeated amount of Acetyl Hexapeptide-8 Amide after 8 h from the multiple water-in-oil-in-water emulsion (755 ± 149 ng/cm^2) and the oil-in-water emulsion (456 ± 120 ng/cm^2) was found.

In the same study, tape-stripping experiments using full-thickness porcine ear skin were also performed. The same emulsions were used, and 4 experiments for each formulation were performed. An Acetyl Hexapeptide-8 Amide emulsion (5 mg/cm^2) was applied and distributed with a saturated gloved finger, and the tape-stripping procedure was initiated after an exposure time of 1 h. After a residence time of 1 h, 46.7 ± 6.2 ng/cm^2 of applied Acetyl Hexapeptide-8 Amide penetrated into the stratum corneum from the multiple emulsion. The amounts that entered the stratum corneum from the oil-in-water and the water-in-oil emulsions were 24.7 ± 4.9 ng/cm^2 and 9.5 ± 2.1 ng/cm^2, respectively. Therefore, the multiple water-in-oil-in-water emulsion led to 4.91 ± 0.66-fold and 1.89 ± 0.25-fold higher skin deposition of Acetyl Hexapeptide-8 Amide than the water-in-oil and oil-in-water emulsion, respectively. The oil-in-water emulsion showed 2.61 ± 0.52-fold increased skin penetration of Acetyl Hexapeptide-8 Amide when compared to the water-in-oil emulsion. According to the results of these experiments, the penetration of Acetyl Hexapeptide-8 Amide from the different emulsions was in the order of multiple water-in-oil-in-water emulsion > oil-in-water emulsion > water-in-oil emulsion.

The skin penetration of Acetyl Hexapeptide-8 Amide was evaluated using hairless guinea pig skin and human cadaver skin assembled in in vitro diffusion cells.[20] The composition of the receptor fluid was: anhydrous calcium chloride (140 mg/ml), dextrose (1000 mg/ml), 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES, 5960 mg/l), magnesium sulfate heptahydrate (200 mg/ml), potassium chloride (400 mg/ml), monobasic potassium dihydrogen phosphate (60 mg/ml), sodium bicarbonate (350 mg/ml), sodium chloride (7000 mg/ml), sodium phosphate dibasic (50 mg/ml), and gentamicin sulfate (50 mg/ml). An oil-in-water emulsion containing 10% Acetyl Hexapeptide-8 Amide (dose = 2 mg/cm^2) was applied to the skin for 24 h. Skin disks were tape stripped to determine the amount of peptide in the stratum corneum. Skin penetration was measured in skin layers using hydrophilic interaction LC-MS/MS and electrospray ionization. Stable isotopically-labeled hexapeptides were used as internal standards for the quantitation of native hexapeptides to correct for matrix effects that are associated with electrospray ionization. Study results indicated that the majority of Acetyl Hexapeptide-8 Amide was washed from the surface of both skin types (guinea pig and human). For the Acetyl Hexapeptide-8 Amide that actually penetrated the skin, it remained mostly in the stratum corneum of hairless guinea pig skin (0.54% of applied dose) and human skin (0.22% of applied dose). Peptide levels were found to decrease as each layer was removed by tape stripping. The total amount of Acetyl Hexapeptide-8 Amide that was found in the epidermis was similar (at 0.01%) when hairless guinea pig skin and human skin were compared. Also, for both skin types, no peptide was detected in the dermis or buffer collected underneath the skin. No hexapeptide metabolite was detected in any layers of hairless guinea pig or human skin, or buffer collected underneath the skin.

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

Absorption, Distribution, Metabolism, and Excretion (ADME)

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

TOXICOLOGICAL STUDIES

Acute Toxicity Studies

Oral

The acute oral toxicity of Acetyl Hexapeptide-8 Amide was evaluated using rats (number and strain not stated).[6] It was concluded that the test substance was non-toxic when administered orally (LD_{50} > 2500 mg/kg).

Short-Term, Subchronic, and Chronic Toxicity Studies

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

DEVELOPMENTAL AND REPRODUCTIVE TOXICITY STUDIES

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

GENOTOXICITY STUDIES

In Vitro

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

CARCINOGENICITY STUDIES

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

OTHER RELEVANT STUDIES

Effect on Skin Histology

The effect of Acetyl Hexapeptide-8 Amide on skin histology was studied using groups of 10 Kunming mice, described as follows: normal control group, aged model group, placebo control group, and Acetyl Hexapeptide-8 Amide treatment group (aged mice only).[13] Aged models of the mice were established, and the histological changes before and after treatment were compared. Each vial of the test substance contained 10% Acetyl Hexapeptide-8 Amide in an oil-in-water emulsion without preservatives. The placebo control solution was a non-active oil-in-water emulsion without Acetyl Hexapeptide-8 Amide. Initially, 3 groups were injected s.c. with 0.1 ml/10 g of 10% D-galactose in skin of the nape and back daily for 6 weeks to establish the subacute aged models. The aging model induced by D-galactose was a common laboratory tool that was used to simulate senescence. Mice of the normal control group were injected s.c. with 0.1 ml/10 g saline (same areas). At the same time, the Acetyl Hexapeptide-8 Amide emulsion was applied to a shaved 2 x 2 cm site on the back of each test animal twice per day. The placebo solution was applied (shaved 2 x 2 cm site) to animals of the placebo control group. After 6 weeks, skin tissues (~ 1 x 1 cm) from the application sites of placebo control and test mice were removed. Skin tissues (~ 1 x 1 cm, from same site) were also removed from mice of the other 2 groups. Paraffin sections from all tissues were made and stained with hematoxylin-eosin (HE) stain and picrosirius-polarization (PSP) stain for microscopic examination.

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

Cytotoxicity

The cytotoxicity of Acetyl Hexapeptide-8 Amide was evaluated in an in vitro proliferation assay using the formazan-based antiproliferation assay (EZ4U assay).[5] Human embryonic kidney (HEK)-293 and neuroblastoma (IMR-32) cell lines, as well as human epidermal fibroblasts, were incubated for 48 h with test substance concentrations ranging from 0.01 µM to 100 µM. Doxorubicin, a commonly used drug in cancer chemotherapy, served as the reference compound. Significant antiproliferative activity of Acetyl Hexapeptide-8 Amide was observed at concentrations above 10 µM. Calculated half-maximal inhibitory concentration (IC_{50}) values for Acetyl Hexapeptide-8 Amide were 34.862 µM (in HEK-293 cells) and 64.458 (in IMR-32 cells). In human epidermal fibroblasts, a dose-dependent antiproliferative effect was observed; 67% inhibition was observed at 100 µM Acetyl Hexapeptide-8 Amide (highest test concentration). The data showed very strong antiproliferative effect of doxorubicin against the IMR-32 cell line (IC_{50} = 0.0051 µM) and the HEK-293 cell line (IC_{50} = 0.455 µM). The authors noted that the IC_{50} value of Acetyl Hexapeptide-8 Amide (34.862 µM) was approximately 75-fold higher than the IC_{50} of doxorubicin against the HEK-293 cell line, and more than 10,000-fold higher against the IMR-32 cell line. The authors also noted that the significant effect of Acetyl Hexapeptide-8 Amide in human epidermal fibroblasts was observed at 100 µM, whereas the significant effect of doxorubicin (at 5.628 µM) was at an 18-fold lower concentration. An IC_{50} value for Acetyl Hexapeptide-8 Amide in human epidermal fibroblasts was not reported, but it was noted that the test substance exhibited a 67% antiproliferative effect after 48 h of incubation at a concentration of 100 µM. Finally, the authors stated that, given the cytotoxic activity of Acetyl Hexapeptide-8 Amide against human epidermal fibroblasts, the use of Acetyl Hexapeptide-8 Amide at very high doses or for a very long period of time must be considered potentially dangerous for patients.

Inhibition of Catecholamine Release

The inhibitory activity of Acetyl Hexapeptide-8 Amide (tested at 100 µM) on calcium-evoked neurotransmitter release from digitonin-permeabilized chromaffin cells was studied.[8] Detergent-permeabilized chromaffin cells release both noradrenaline and adrenaline in response to an increase in intracellular calcium. Acetyl Hexapeptide-8 Amide (100 µM), caused 30% inhibition of the total catecholamine exocytosis. Botulinum neurotoxin A (BoNT A) (20 nM) caused up to 60% inhibition of catecholamine release. A 26-mer peptide (1 µM) derived from the C-terminal end of SNAP-25 (ESUP-E) caused up to 55% inhibition of catecholamine release. Dose response curves indicated an IC_{50} of 110 µM for the test substance, which was 5000 x higher than the characteristic of BoNT A, and 400 x higher than that of ESUP-E.

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

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

DERMAL IRRITATION AND SENSITIZATION STUDIES

Irritation

Animal

The skin irritation potential of a tradename mixture containing 0.05% aqueous Acetyl Hexapeptide-8 Amide was evaluated using albino male rabbits (number not stated).[6] The test protocol was not provided. There were no signs of erythema or edema at 7 d after removal of the test substance.

Sensitization

Human

The skin sensitization potential of a tradename mixture containing 0.05% aqueous Acetyl Hexapeptide-8 Amide was evaluated in a human repeated insult patch test (HRIPT) involving 50 subjects.[6] The test substance did not cause skin sensitization in any of the subjects tested. Details relating to the test protocol and study results were not included.

OCULAR IRRITATION STUDIES

In Vitro

The ocular irritation potential of a solution of Acetyl Hexapeptide-8 Amide (concentrations not stated) was evaluated using the neutral red uptake test.[6] Details relating to the test protocol were not included. It was concluded that the test substance is potentially not irritating to the eyes.

CLINICAL STUDIES

Other Clinical Reports

Ten healthy women applied an oil-in-water emulsion containing 10% Acetyl Hexapeptide-8 Amide twice per day for 30 d.[8] The emulsion without Acetyl Hexapeptide-8 Amide was applied to the contralateral side. Skin topography analysis was performed by obtaining silicon imprints from the lateral preorbital region of each subject. Silicon imprints, obtained after 0, 15, and 30 d, were analyzed by confocal laser scanning microscopy to assess the evolution of the skin surface before and after treatment. Topical application of 10% Acetyl Hexapeptide-8 Amide (in oil-in-water emulsion) resulted in significant attenuation of the depth and roughness of the wrinkles. The oil-in-water emulsion did not cause significant changes in skin topography. Quantitative analysis and normalization of the silicon replicas showed that the oil-in-water emulsion reduced by 10% the depth of the skin wrinkles (not identified as a statistically significant finding). The oil-in-water emulsion containing 10% Acetyl Hexapeptide-8 Amide decreased the depth of skin wrinkles by 20% by day 15 and by 30% by day 30.

The effect of Acetyl Hexapeptide-8 Amide on the skin was evaluated using 8 subjects.[21] Skin properties were studied using skin microtopography and transepidermal water loss. Four subjects were each given a 50 g vessel containing an Acetyl Hexapeptide-8 Amide (10% w/w) cream. The other 4 subjects were each given a 50 g vessel containing a placebo cream that did not contain Acetyl Hexapeptide-8 Amide. The subjects were instructed to apply the cream twice daily for 2 months (60 d). Skin surface evaluation and measurement of transepidermal water loss were performed before treatment, day 0, and then on days 20, 40, and 60. Self-evaluation was performed after the 2-month treatment (day 60). Side effects were also evaluated by the volunteers. To evaluate the tolerability and potential irritant power of the Acetyl Hexapeptide-8 Amide (10% w/w) cream, the subjects were asked to answer whether they experienced the following effects on the skin: warmth, dryness, stinging, redness, desquamation, dryness, itching, or ocular irritation. These variables were scored on a scale of 1 (slight) to 4 (great). Also, when compared to the placebo group, a statistically significant decrease in transepidermal water loss was observed after 20 d ($p = 0.025$) and 40 d ($p = 0.028$) of application of the Acetyl Hexapeptide-8 Amide (10% w/w) cream. At 60 d, the decrease in transepidermal water loss was not statistically significant. None of the following effects was reported after application of the Acetyl Hexapeptide-8 Amide (10% w/w) cream: warmth, dryness, stinging, redness, desquamation, dryness, itching, or ocular irritation.

Eyelid Irritation

A double-blind, placebo-controlled randomized trial on topically applied Acetyl Hexapeptide-8 Amide was conducted using 24 blepharospasm patients who were receiving botulinum neurotoxin therapy (orbicularis oculi muscle injections) at regular 3-mo intervals.[18] On the day after injection of botulinum neurotoxin, 12 patients applied an emulsion containing 0.005% Acetyl Hexapeptide-8 Amide twice daily to the eyelids. Topical application (repeated daily for ~ 7 mo) was standardized and targeted the eyelids only, independent of involvement of the orbital orbicularis oculi or surrounding muscles. A placebo (emulsion without Acetyl Hexapeptide-8 Amide) was applied topically to another 12 blepharospasm patients according to the same procedure. No severe adverse events were observed during the study. Four subjects (2 test and 2 placebo) experienced minor, self-limiting eyelid irritation. The irritation reactions observed did not necessitate any modifications of the test procedure.

SUMMARY

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

According to 2021 VCRP data, Acetyl Hexapeptide-8 is reported to be used in 333 cosmetic products 311 leave-on and 22 rinse-off); an additional 21 uses (20 leave-on and 1 rinse-off) are reported under the name acetyl hexapeptide-3. The

results of a concentration of use survey conducted by the Council in 2019 indicate that Acetyl Hexapeptide-8 is being used at concentrations up to 0.005% (in eye lotions and face and neck products; not spray), which is the highest reported maximum use concentration for leave-on formulations. In rinse-off products, Acetyl Hexapeptide-8 is reported to be used at concentrations up to 0.000005%. The Panel is aware of products potentially being marketed at higher use concentrations claiming a non-cosmetic use, and, as such, these products are not under the purview of the Panel.

The skin penetration of Acetyl Hexapeptide-8 Amide has been studied using porcine skin in vitro. Differences in the skin penetration of Acetyl Hexapeptide-8 Amide through porcine skin were observed when various vehicles for the test substance were used. For example, statistically significant difference ($p < 0.01$) between the cumulative permeated amount of Acetyl Hexapeptide-8 Amide after 8 h from the multiple water-in-oil-in-water emulsion (755 ± 149 ng/cm^2) and the oil-in-water emulsion (456 ± 120 ng/cm^2) was found. Overall, the penetration of Acetyl Hexapeptide-8 Amide from the different emulsions was in the order of multiple water-in-oil-in-water emulsion > oil-in-water emulsion > water-in-oil emulsion.

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

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

In an acute oral toxicity study, Acetyl Hexapeptide-8 Amide was evaluated using rats (number and strain not stated). The test substance was non-toxic when administered orally ($LD_{50} > 2500$ mg/kg).

Acetyl 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 (aged mice only) twice daily for 6 wk. 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 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, a significant antiproliferative effect of Acetyl Hexapeptide-8 Amide in human epidermal fibroblasts was observed at 100 µM.

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

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

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

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

DISCUSSION

The Panel noted the absence of systemic toxicity and detailed genotoxicity data on Acetyl 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 that was reported in the Council survey. On the subject of potential percutaneous absorption, the Panel also noted differing degrees of reported skin penetration by Acetyl Hexapeptide-8 Amide with in vitro models. The Panel felt that studies that employed LC-MS/MS to measure the peptide were most reliable, and noted that these indicated minimal skin penetration.

The Panel was aware of reports of products containing higher use concentrations of Acetyl Hexapeptide-8 than what was reported in the Council survey, and acknowledged that whether these products are drugs or cosmetics remains unknown. In dermal studies, 10% Acetyl Hexapeptide-8 Amide may have drug activity (i.e., an anti-wrinkle), as indicated by an effect on type I and type III collagen in the dermis. However, the Panel noted that whether the mechanism of action of this product is via hydration of the skin or a biological effect on collagen synthesis is unclear. The Panel was of the understanding that Acetyl Hexapeptide-8 Amide would not be likely to produce a drug (anti-wrinkle) effect when used at a low concentration (i.e., 0.005%) in leave-on cosmetic products. However, the Panel acknowledges that the drug effect may be apparent at higher use concentrations, but the threshold is not known. Additionally, use concentrations > 0.005% are unsupported by the available safety test data. Therefore, the Panel determined the available data were insufficient to determine safety at concentrations > 0.005%, and the following data are needed:

- an NOEL for type I and type III collagen synthesis.

The Panel also 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 concluded that Acetyl Hexapeptide-8 Amide is safe in cosmetics in the present practices of use and concentration described in this safety assessment when the concentration does not exceed 0.005%, and that the available data are insufficient to make a determination that Acetyl Hexapeptide-8 Amide is safe under the intended conditions of use in cosmetic formulations at concentrations > 0.005%.

TABLE

Table 1. Frequency (2021) and concentration of use (2019) of Acetyl Hexapeptide-8 Amide according to duration and type of exposure.[11,12]

	# of Uses Reported as Acetyl Hexapeptide-8	# of Uses Reported as acetyl hexapeptide-3	Conc. (%)
Totals*	**333**	**21**	**0.000005-0.005**
Duration of Use			
Leave-On	*311*	*20*	*0.00005-0.005*
Rinse off	*22*	*1*	*0.000005*
Diluted for (bath) Use	*NR*	*NR*	*NR*
Exposure Type			
Eye Area	35	7	0.00005-0.005
Incidental Ingestion	2	NR	0.00025
Incidental Inhalation- Sprays	127[a]; 102[b]	2[a]; 6[b]	NR
Incidental Inhalation- Powders	102[b];	6[b]	0.0001; 0.00026-0.005[c]
Dermal Contact	329	21	0.000005-0.005
Deodorant (underarm)	NR	NR	NR
Hair - Non-Coloring	2	NR	NR
Hair-Coloring	NR	NR	NR
Nail	NR	NR	NR
Mucous Membrane	10	NR	0.00025
Baby Products	NR	NR	NR

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

NR = Not Reported

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

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

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

REFERENCES

1. Nikitakis J, Kowcz A. International Cosmetic Ingredient Dictionary and Handbook Online Version (wINCI). http://webdictionary.personalcarecouncil.org/jsp/Home.jsp 2019. Accessed: 10/30/2019.

2. Johnson W, Bergfeld WF, Belsito DV, et al. Safety assessment of tripeptide-1, hexapeptide-12, their metal salts and fatty acyl derivatives, and palmitoyl tetrapeptide-7 as used in cosmetics. *International Journal of Toxicology*. 2018;37(3):90S-102S.

3. European Union. 2019. Cosmetic Ingredient Database (Cosing) - Ingredients and Fragrance Inventory. https://data.europa.eu/euodp/en/data/dataset/cosmetic-ingredient-database-ingredients-and-fragrance-inventory/resource/6f1e1fc0-2cf2-4426-adf9-d6a3734fa3a6. https://data.europa.eu/euodp/en/data/dataset/cosmetic-ingredient-database-ingredients-and-fragrance-inventory/resource/6f1e1fc0-2cf2-4426-adf9-d6a3734fa3a6. Accessed: 10-30-2019.

4. Active Peptide Company. 2019. Acetyl hexapeptide-8. https://www.activepeptide.com/product/acetyl-hexapeptide-8/. Accessed: 10-30-2019.

5. Grosicki M, Latacz G, Szopa A, Cukier A, Kie, Kononowicz K. The study of cellular cytotoxicity of argireline - an anti-aging peptide. *Acta Biochim Pol* 2014;61(1):29-32.

6. Lipotec. 2006. Argiline. An anti-aging peptide. https://wrinklesystem.com/laboratorydata/argireline.pdf. Accessed: 10-31-2019.

7. Lim SH, Sun Y, Thiruvallur Madanagopal T, Rosa V, Kang L. Enhanced Skin Permeation of Anti-wrinkle Peptides via Molecular Modification. *Sci Rep*. 2018;8:1596.

8. Blanes-Mira C, Clemente J, Jodas G, et al. A synthetic hexapeptide (Argireline) with antiwrinkle activity. *Int J Cosmet Sci*. 2002;24(5):303-310.

9. Gutierrez LM, Canaves J, Ferrer-Montiel A, Reig JA, Montal M, Viniegra S. A peptide that mimics the carboxy-terminal domain of SNAP-25 blocks Ca^{2+}-dependent exocytosis in chromaffin cells. *FEBS Lett*. 1995;372(1):39-43.

10. Ruiz MA, Clares B, Morales ME, Cazalla S, Gallardo V. Preparation and stability of cosmetic formulations with an anti-aging peptide. *J Cosmet Sci*. 2007;58(2):157-171.

11. U.S. Food and Drug Administration Center for Food Safety & Applied Nutrition (CFSAN). Voluntary Cosmetic Registration Program - Frequency of use of Cosmetic Ingredients. College Park, MD. 2021. (Obtained under the Freedom of Information Act from CFSAN; requested as "Frequency of Use Data" January 4, 2021; received January 21, 2021.

12. Personal Care Products Council. 2019. Concentration of Use by FDA Product Category: Acetyl Hexapeptide-8 Amide. Unpublished data submitted by the Personal Care Products Council on 7-24-2019.

13. Wang Y, Wang M, Xiao XS, Huo J, Zhang WD. The anti-wrinkle efficacy of Argireline. *J Cosmet Laser Ther*. 2013;15(4):237-241.

14. Aylott RI, Byrne GA, Middleton JD, Roberts ME. Normal use levels of respirable cosmetic talc: preliminary study. *Int J Cosmet Sci*. 1979;1(3):177-186.

15. Russell RS, Merz RD, Sherman WT, Sivertson JN. The determination of respirable particles in talcum powder. *Food Cosmet Toxicol*. 1979;17(2):117-122.

16. CIR Science and Support Committee of the Personal Care Products Council (CIR SSC). 11-3-2015. Cosmetic Powder Exposure. Unpublished data submitted by the Personal Care Products Council.

17. European Commission. CosIng database; following Cosmetic Regulation No. 1223/2009. Last Updated 2009. http://ec.europa.eu/growth/tools-databases/cosing/. Accessed: 10-28-2019.

18. Lungu C, Considine E, Zahir S, Ponsati B, Arrastia S, Hallett M. Pilot study of topical acetyl hexapeptide-8 in the treatment for blepharospasm in patients receiving botulinum toxin therapy. *Eur J Neurol*. 2013;20(3):515-518.

19. Hoppel M, Reznicek G, Kählig H, Kotisch H, Resch GP, Valenta C. Topical delivery of acetyl hexapeptide-8 from different emulsions: influence of emulsion composition and internal structure. *Eur J Pharm Sci*. 2015;68(Feb 20):27-35.

20. Kraeling ME, Zhou W, Wang P, Ogunsola OA. In vitro skin penetration of acetyl hexapeptide-8 from a cosmetic formulation. *Cutan Ocul Toxicol*. 2015;34(1):46-52.

21. Raikou V, Varvaresou A, Panderi I, Papageorgiou E. The efficacy study of the combination of tripe[ptide-10-citrulline and acetyl hexapeptide-3. A prospective, randomized controlled study. *J Cosmet Dermatol*. 2017;16(2):271-278.