


Safety Assessment of Tripeptide-1, Hexapeptide-12, Their Metal Salts and Fatty Acyl Derivatives, and Palmitoyl Tetrapeptide-7 as Used in Cosmetics

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Abstract

Tripeptide-1, Hexapeptide-12, their metal salts and fatty acyl derivatives, and Palmitoyl Tetrapeptide-7 function primarily as skin conditioning agents, and Palmitoyl Tripeptide-1, Palmitoyl Hexapeptide-12, Tripeptide-1, Copper Tripeptide-1, and Palmitoyl Tetrapeptide-7 are also used in cosmetic products. Typical use concentrations of these ingredients are <10 ppm. The Panel noted that the low use concentrations and negative safety test data reviewed obviate any concerns relating to the safety of these ingredients in cosmetic products. Thus, the Panel concluded that these ingredients are safe in cosmetics in the present practices of use and concentration described in this safety assessment.

Keywords

tripeptides, safety, cosmetics

Introduction

The safety of Tripeptide-1, Hexapeptide-12, their metal salts and fatty acyl derivatives, and Palmitoyl Tetrapeptide-7 as used in cosmetics (listed below) was reviewed by the Cosmetic Ingredient Review (CIR) Expert Panel (Panel). These ingredients function primarily as skin conditioning agents in cosmetic products.¹ The ingredient name, palmitoyl oligopeptide, listed in the web-based *International Cosmetic Ingredient Dictionary and Handbook* (WINCI; *Dictionary*) has been retired, because it was vague and indeterminately represented 2 other ingredients. The definition for this ingredient also contained no indication of the peptide sequence(s), a point that the Expert Panel deemed critical to a declaration of safety. Palmitoyl oligopeptide now represented by the name Palmitoyl Tripeptide-1 (Gly-His-Lys [GHK] peptide sequence) or Palmitoyl Hexapeptide-12 (Val-Gly-Val-Ala-Pro-Gly [VGVPAG] peptide sequence) in the *Dictionary*. Unfortunately, the dictionary recites 2 possible sequences for “Hexapeptide-12,” and relevant safety data be found for only one of these. Accordingly, this safety assessment only addresses Hexapeptide-12 and derivative ingredients (eg, Palmitoyl Hexapeptide-12) which contain the peptide sequence Val-Gly-Val-Ala-Pro-Gly. Specifically, the safety of such ingredients, containing the peptide sequence Ala-Pro-Gly-Val-Gly-Val, is not addressed. Thus, the data or conclusions in this safety assessment are not

applicable to other peptide sequences. In this report, “Hexapeptide-12” only represents Val-Gly-Val-Ala-Pro-Gly.

This safety assessment also includes data on a trade name material containing Palmitoyl Tripeptide-1 (Gly-His-Lys [GHK] peptide sequence) and Palmitoyl Tetrapeptide-7 (Gly-Gln-Pro-Arg [GQPR] peptide sequence) and other trade name materials in which Palmitoyl Hexapeptide-12 or Palmitoyl Tripeptide-1 is the only oligopeptide component. The Val-Gly-Val-Ala-Pro-Gly sequence is an elastin peptide and the Gly-His-Lys sequence is a liver growth factor peptide and a fragment of type I collagen. Data on the biological activity of these peptides are also included herein.

Tripeptide-1 (GHK)
Palmitoyl Tripeptide-1 (GHK)
Myristoyl Tripeptide-1 (GHK)

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Copper Tripeptide-1 (GHK)
 Bis(Tripeptide-1) Copper Acetate (GHK)
 Manganese Tripeptide-1 (GHK)
 Hexapeptide-12 (VGVAPG)
 Palmitoyl Hexapeptide-12 (VGVAPG)
 Myristoyl Hexapeptide-12 (VGVAPG)
 Palmitoyl Tetrapeptide-7 (GQPR)

Chemistry

The ingredients in this report are related structurally by bearing one of 3 distinct peptide sequences, either Tripeptide-1 (GHK), Hexapeptide-12 (VGVAPG), or Tetrapeptide-7 (GQPR). The ingredients reviewed in this safety assessment include one of these 3 peptide sequences also having a fatty acyl group at the *N*-terminus, or as their metal salts. For example, the structures of these 3 peptides are depicted in Figure 1, each with the fatty acyl group resulting from the reaction of palmitic acid with the *N* terminus of the peptide (ie, Palmitoyl Tripeptide-1, Palmitoyl Hexapeptide-12 (Palmitoyl-Valine-Glycine-Valine-Alanine-Proline-Glycine *only*), and Palmitoyl Tetrapeptide-7).

The definitions, structures, and functions of the ingredients in this report are included in Table 1. For example, Palmitoyl Tripeptide-1 consists of a short chain of 3 amino acids (also known as Gly-His-Lys [GHK] peptide sequence [a fragment of type I collagen]) connected via an amide bond at its *N*-terminus to palmitic acid. As another example, Palmitoyl Tetrapeptide-7 (Pal-GQPR) consists of a short chain of 4 amino acids (also known as GQPR peptide or glycine-glutamine-proline-arginine) similarly *N*-acylated with palmitic acid. The tetrapeptide portion is a fragment of the natural IgG immunoglobulins.

Throughout this report, ingredient name subheadings will include the ingredient name and its abbreviation (ie palmitoyl group [pal] and the abbreviated peptide sequence, or the abbreviated peptide sequence only) in parentheses. For example, Palmitoyl Tripeptide-1 will be written as Palmitoyl Tripeptide-1 (GHK), Hexapeptide-12 will be written as Hexapeptide-12 (VGVAPG), and Palmitoyl Tetrapeptide-7 will be written as Palmitoyl Tetrapeptide-7 (GQPR).

Physical and Chemical Properties

A supplier provided properties data on palmitoyl oligopeptide, a trade name mixture comprising Palmitoyl Tripeptide-1 and Palmitoyl Tetrapeptide-7.² Properties of these 2 ingredients are also included below separately.

Palmitoyl Tripeptide-1 (Pal-GHK). Palmitoyl Tripeptide-1 is a white powder, has a molecular weight of 578.80 Da, and an estimated logP of 4.81.² Another trade name mixture, containing 100 ppm Palmitoyl Tripeptide-1, is reported to have a density of 1.13.

Palmitoyl Hexapeptide-12 (Pal-VGVAPG). Palmitoyl Hexapeptide-12 is a white powder and has a molecular weight of 737.00 Da and a logP of 5.09.²

Method of Manufacture

General Information. Peptides have been synthesized by solid-phase fluorenylmethoxycarbonyl chemistry (Fmoc protection) using an automated peptide synthesizer.³ Palmitic acid can be coupled to the deprotected amino-terminus of the resin-bound protected peptides either manually or using the peptide synthesizer, employing the same reaction conditions used in standard amino acid coupling. Peptides and mono-palmitic acid-peptide conjugates can then be cleaved from the resin, side-chain deprotected, and purified using standard procedures.

Several strategies for the synthesis of lipidated peptides, both in solution and on solid support have been developed.^{4,5} Solid support is most frequently used to synthesize peptides with longer peptide chains. Shorter peptides have been synthesized both in solution and on solid support. For example, hexa- and heptapeptides corresponding to the Ras-C-terminus and Rab-C-terminus, respectively, have been synthesized in solution.^{6,7}

Palmitoyl Tripeptide-1 (Pal-GHK). Palmitoyl Tripeptide-1 can be synthesized via standard stepwise peptide synthesis.² The C-terminal amino acid (Lys) is protected on its acidic function, after which each *N*-protected amino acid (Gly, His) is sequentially coupled, adding to the amino terminus, with deprotection and amidation of the peptide at each step to elongate by one amino acid. A last coupling procedure is accomplished with palmitic acid instead of an amino acid. The protected peptide is deprotected on the side-chains of lysine and histidine and on the C-terminal acid moiety of Lys.

According to another source, Palmitoyl Tripeptide-1 can be produced via solid-phase synthesis, yielding a substituted peptide of high purity (>97%).⁸

Palmitoyl Hexapeptide-12 (Pal-VGVAPG). Palmitoyl Hexapeptide-12 can be produced via stepwise acid-phase peptide synthesis. The C-terminal amino acid (Gly) is protected on its acid function, after which each protected amino acid (Val-Gly-Val-Ala-Pro-) is sequentially coupled, adding to the amino terminus of the peptide at each step to elongate by one amino acid. A last coupling procedure is accomplished with palmitic acid instead of an amino acid. Finally, the protected peptide is deprotected at the C-terminal function (Gly) of the peptide.²

Hexapeptide-12 (VGVAPG). The synthetic peptide valine-glycine-valine-alanine-proline-glycine, which contains the recognition sequence for the elastin receptor, has been produced using an automated synthesizer.⁹ Reverse-phase high-performance liquid chromatography (HPLC) was used for further purification.

Copper Tripeptide-1 (GHK-Cu²⁺). Copper Tripeptide-1 is prepared by combining purified glycyl-L-histidyl-L-lysine with an equimolar amount of cupric acetate, followed by neutralization with 0.1 N sodium hydroxide and centrifugation (at 5,000g for 30 minutes at 3°C) to remove insoluble material, usually excess copper (II) as its hydroxide.¹⁰ The supernatant (in a

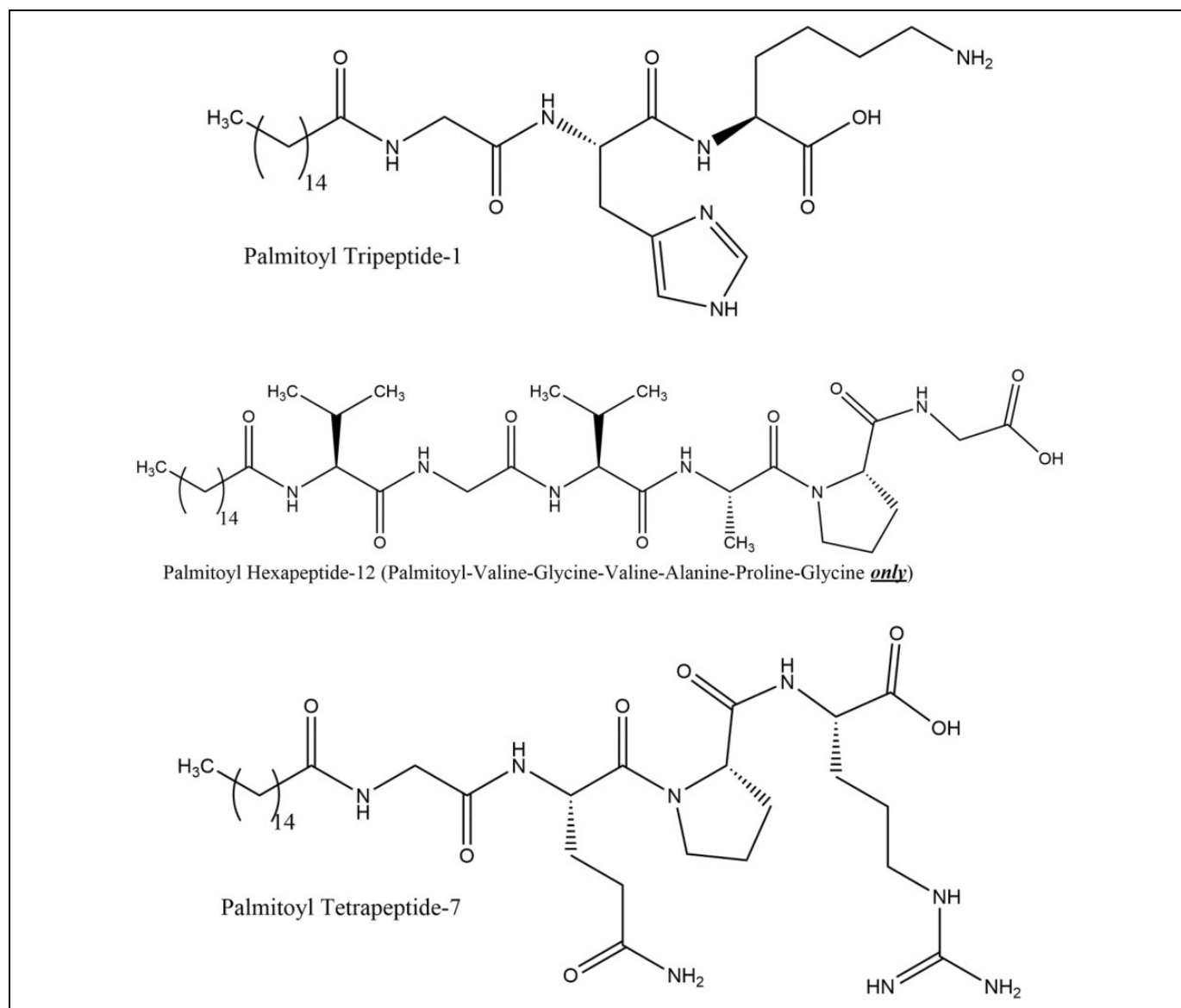


Figure 1. Example structures.

solvent of glass-distilled water) is passed through a G-10 column, and the elution peak absorbing at 600 nm is collected and lyophilized to obtain Copper Tripeptide-1.

Crystalline Copper Tripeptide-1 is prepared by dissolving glycyl-L-histidyl-L-lysine (30 mg, 88 μ mol) in an aqueous copper(II) acetate solution (0.3 mL, 0.3 M). Ethanol (1.26 mL) is added and the vessel walls are then scratched to initiate crystallization of dark blue-purple crystals. The mother liquor is decanted and the crystals are dissolved by adding distilled water. Ethanol (0.4 mL) is then introduced to reach a cloud point. After standing, dark purple-blue octahedral crystals are formed.¹⁰

Composition/Impurities

Palmitoyl Tripeptide-1 (Pal-GHK) and Palmitoyl Hexapeptide-12 (Pal-VGVAPG). According to one supplier, the impurities content

of Palmitoyl Tripeptide-1 and Palmitoyl Hexapeptide-12 has been described as follows: acetate (<5%), palmitic acid (<5%), and water (<5%).²

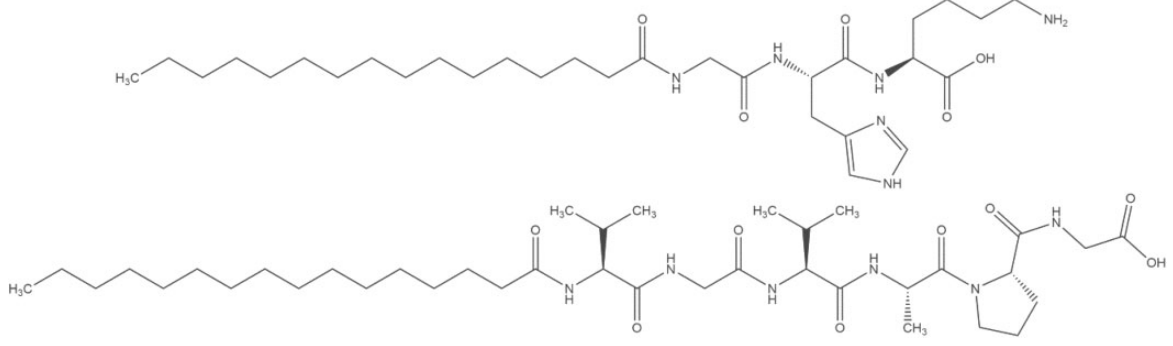
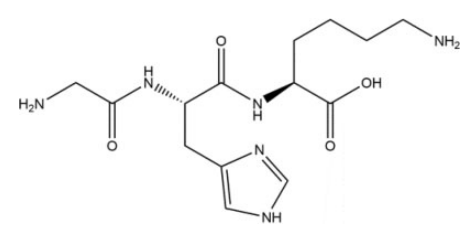
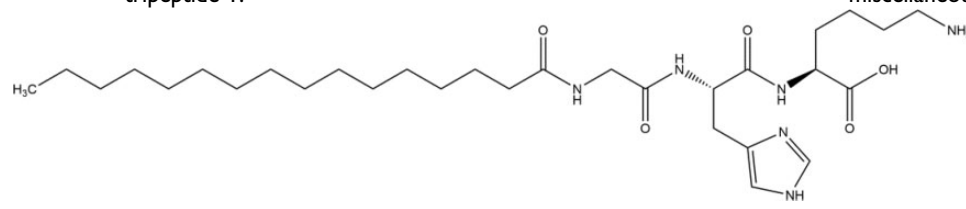
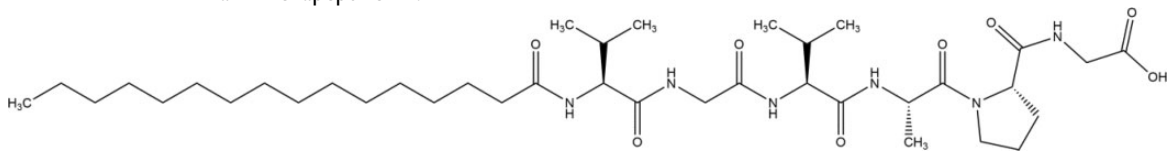
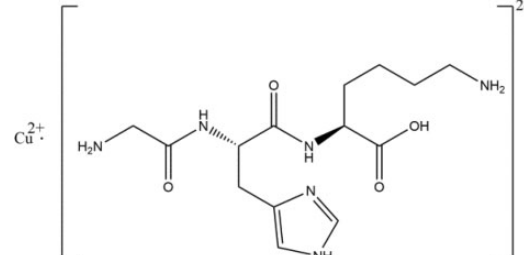
Tripeptide-1 (GHK). Crude Tripeptide-1 is approximately 95% pure, likely containing some incompletely deprotected peptides.¹⁰ However, these impurities can be removed in glass-distilled water (50 mg/mL), centrifuging at 20 000g for 1 hour at 3°C, and then lyophilizing the supernatant.

Use

Cosmetic

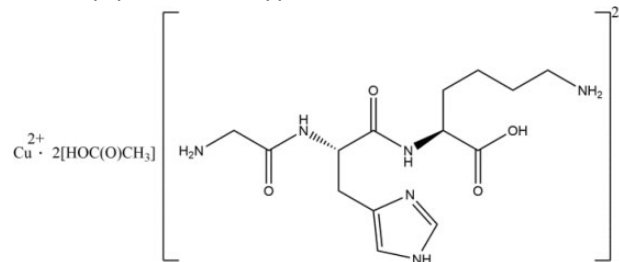
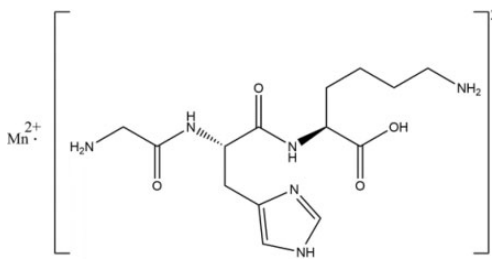
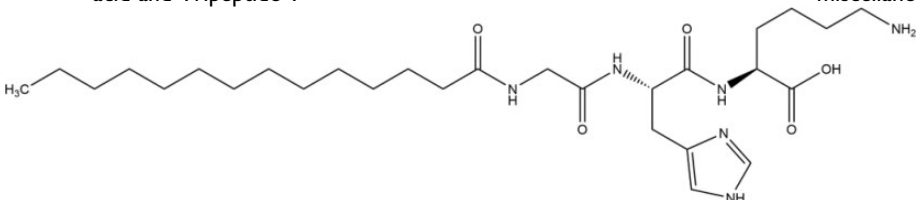
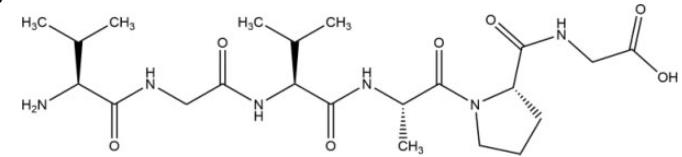
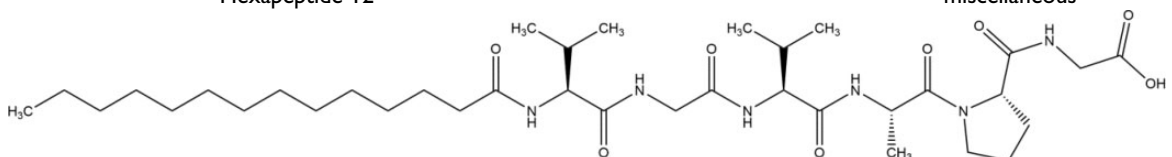
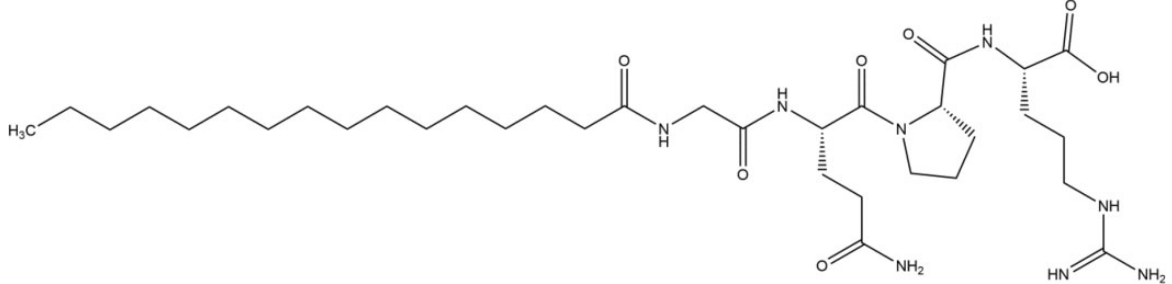
The ingredients reviewed in this safety assessment function primarily as skin conditioning agents in cosmetic products.¹ According to information supplied to the U.S. Food and Drug

Table 1. Definitions, Structures and Functions of the Ingredients in This Safety Assessment.^{1,a}

Ingredient name and CAS no.	Definition and structure	Function
Palmitoyl Oligopeptide [171263-26-6 and 147732-56-7]	<p>Palmitoyl oligopeptide is the product obtained by the reaction of palmitic acid with either a tripeptide consisting of gly-his-lys, or a hexapeptide consisting of val-gly-val-ala-pro-gly.</p> <p>The INCI name, Palmitoyl Oligopeptide, originally developed in 1994, was designated with a retired status in 2013. Trade name assignments formerly published with the name Palmitoyl Oligopeptide will be retained in the retired monograph, and also published with the new name assignment as either Palmitoyl Tripeptide-I or Palmitoyl Hexapeptide-12, for an interim period.</p>	Skin-conditioning agents - miscellaneous; surfactants - cleansing agents
		
Tripeptide-I [1269107-24-5]	Tripeptide-I is the synthetic peptide consisting of gly-his-lys.	Skin protectants; skin-conditioning agents – miscellaneous
		
Palmitoyl Tripeptide-I	Palmitoyl Tripeptide-I is the reaction product of palmitic acid and tripeptide-I.	Skin-conditioning agents - miscellaneous
		
Palmitoyl Hexapeptide 12	Palmitoyl Hexapeptide-12 is the product of the reaction of palmitic acid and Hexapeptide-12.	Antioxidants
		
Copper Tripeptide-I [89030-95-5]	Copper Tripeptide-I is a complex formed by copper and Tripeptide-I	Skin-conditioning agents - miscellaneous
		

(continued)

Table I. (continued)

Ingredient name and CAS no.	Definition and structure	Function
Bis(Tripeptide-I) Copper acetate [130120-57-9]	<p>Bis(Tripeptide-I) Copper acetate is acetate salt of the product of the reaction of Tripeptide-I with copper chloride</p> 	Skin-conditioning agents - miscellaneous
Manganese Tripeptide-I [611182-15-1]	<p>Manganese Tripeptide-I is a complex of manganese and Tripeptide-I</p> 	Skin-conditioning agents - miscellaneous
Myristoyl Tripeptide-I	<p>Myristoyl Tripeptide-I is the product obtained by the reaction of myristic acid and Tripeptide-I</p> 	Skin-conditioning agents - miscellaneous
Hexapeptide-12	<p>Hexapeptide-12 is the synthetic peptide consisting of val-gly-val-ala-pro-gly</p> 	Skin-conditioning agents - miscellaneous
Myristoyl Hexapeptide 12	<p>Myristoyl Hexapeptide-12 is the reaction product of myristic acid and Hexapeptide 12</p> 	Skin-conditioning agents - miscellaneous
Palmitoyl Tetrapeptide-7	<p>Palmitoyl Tetrapeptide-7 is the reaction product of palmitic acid and tetrapeptide-7, wherein tetrapeptide-7 is the synthetic peptide consisting of gly-gln-pro-arg.</p> 	Skin-Conditioning Agents - Miscellaneous

^aSome chemical structures and definitions from the INCI dictionary were supplemented by CIR Staff.

Administration (FDA) by industry as part of the Voluntary Cosmetic Registration Program (VCRP), the following palmitoyl oligopeptides or oligopeptides are being used in cosmetic products:¹¹ palmitoyl oligopeptide (name retired, peptide sequence not stated), Palmitoyl Tripeptide-1, Palmitoyl Hexapeptide-12, Tripeptide-1, Copper Tripeptide-1, and Palmitoyl Tetrapeptide-7. The peptide sequence for palmitoyl oligopeptide is not stated in the VCRP database or in the survey of ingredient use concentrations mentioned below; however, this designation could refer to either GHK (Tripeptide-1) or VGVAPG (Hexapeptide-12).

Results from a survey of ingredient use concentrations conducted by the Personal Care Products Council (Council) in 2013 and updated in 2014 indicate that, collectively, the ingredients reviewed in this safety assessment are being used at maximum concentrations ranging from 0.0000001% (Palmitoyl Tripeptide-1 and Palmitoyl Hexapeptide-12) to 0.002% (Palmitoyl Hexapeptide-12).^{12,13} The highest concentration of 0.002% relates to ingredient use in leave-on products. Voluntary Cosmetic Registration Program data on ingredient use frequencies and use concentration data provided by the Council are summarized in Table 2. In addition to the data included in the survey of ingredient use concentrations, one submission indicated that peptides are being used in cosmetic products at concentrations between 1 ppm and 30 ppm, and that their use at concentrations <10 ppm is customary.¹⁴

Cosmetic products containing Tripeptide-1, Hexapeptide-12, and related amides may be applied to the skin and hair, or, incidentally, may come in contact with the eyes and mucous membranes. Products containing these ingredients may be applied as frequently as several times per day and may come in contact with the skin or hair for variable periods following application. Daily or occasional use may extend over many years.

Palmitoyl Hexapeptide-12 is used in body and hand sprays (maximum use concentration = 0.002%). Because this ingredient is used in products that are sprayed, the ingredient could possibly be inhaled. In practice, 95% to 99% of the droplets/particles released from cosmetic sprays have aerodynamic equivalent diameters >10 μm , with propellant sprays yielding a greater fraction of droplets/particles below 10 μm , compared with pump sprays.¹⁵⁻¹⁸ Therefore, most droplets/particles incidentally inhaled from cosmetic sprays would be deposited in the nasopharyngeal and bronchial regions and would not be respirable (ie, they would not enter the lungs) to any appreciable amount.^{15,16}

Toxicokinetics

In Vivo Studies

Tripeptide-1 (GHK). Tripeptide-1 (1% in saline; dose = 10 mg/kg) was injected into the tail vein of male rats (number not specified).¹⁹ Blood samples were collected prior to dosing and for up to 60 minutes post-dosing. Plasma concentration–time profiles of Tripeptide-1 and its L-histidyl-L-lysine metabolite

indicated that neither were detected in predose plasma samples. However, after intravenous (IV) injection, Tripeptide-1 was rapidly degraded to L-histidyl-L-lysine, which was rapidly eliminated from circulating blood. It has been reported that Tripeptide-1 is unstable in human plasma and is rapidly degraded by aminopeptidases.^{20,21}

In Vitro Studies

Tripeptide-1 (GHK). In an enzyme assay, the liver growth factor, Tripeptide-1, was hydrolyzed by an aminotripeptidase purified from rat brain cytosol.²⁰

Toxicology

Acute Oral Toxicity

Palmitoyl Tripeptide-1 (Pal-GHK). The acute oral toxicity of a trade name mixture containing 100 ppm Palmitoyl Tripeptide-1 was evaluated using 10 Sprague-Dawley rats (5 males, 5 females).²² The test substance was administered by gavage at a dose of 2,000 mg/kg. Dosing was followed by a 14-day observation period, after which necropsy was performed. Dosing had no effect on general behavior or body weight gain, and none of the animals died. There were no apparent abnormalities at necropsy. This mixture was classified as nontoxic ($\text{LD}_{50} > 2,000 \text{ mg/kg}$).

Repeated Dose Toxicity

Palmitoyl Tripeptide-1 (Pal-GHK). There were no clinical signs or mortalities in a cumulative skin irritation study on a trade name mixture containing 100 ppm Palmitoyl Tripeptide-1 involving guinea pigs. Details relating to the test protocol were not provided.²³

In the guinea pig maximization test on a trade name mixture containing 100 ppm Palmitoyl Tripeptide-1, the test substance was evaluated at a concentration of 75% in a saline vehicle.²⁴ Clinical signs were not observed and none of the animals died during the study. Additionally, body weight gain was unaffected by test substance administration.

Ocular Irritation

In Vivo

Palmitoyl Tripeptide-1 (Pal-GHK). The ocular irritation potential of the ingredient, a trade name mixture containing 100 ppm Palmitoyl Tripeptide-1, was evaluated using 3 male New Zealand white rabbits.²⁵ The test substance (0.1 mL) was instilled into the conjunctival sac of the left eye of each animal, and the eyes were not rinsed. Ocular reactions were scored at approximately 1 hour, 24 hours, 48 hours, and 72 hour-postinstillation, and then on days 5 and 8. On day 1, very slight conjunctival reactions (chemosis and redness) were observed in all 3 animals. No other ocular reactions were observed for the duration of the study. It was concluded that this mixture was a slight irritant (maximum ocular irritation index = 4.7).

Table 2. Current Frequency and Concentration of Use According to Duration and Type of Exposure.^{11-13,a}

	Palmitoyl oligopeptide (sequence not indicated)		Palmitoyl tripeptide-1		Palmitoyl hexapeptide-12	
	# of Uses	Conc. (%)	# of Uses	Conc. (%)	# of Uses	Conc. (%)
Totals/conc. range	519	0.00001-0.002	1	0.0000001-0.001	NR	0.0000001-0.002
Duration of use						
Leave-on	515	0.00001-0.002	1	0.0000001-0.001	NR	0.0000001-0.002
Rinse off	4	NR	NR	0.0001-0.0008	NR	0.001
Diluted for (bath) use	NR	NR	NR	NR	NR	NR
Exposure type						
Eye area	117	0.00001-0.0002	NR	0.0001-0.0004	NR	0.001-0.002
Incidental ingestion	100	0.0015-0.0018	NR	0.001	NR	0.0005
Incidental inhalation - sprays	217	0.001	1 ^b	NR	NR	0.001 ^b
Incidental inhalation - powders	2	0.00001-0.0004 ^c	NR	0.0000001-0.0006 ^c	NR	0.0000001-0.002 ^c
Dermal contact	396	0.00001-0.002	1	0.0000001-0.001	NR	0.0000001-0.002
Deodorant (underarm)	NR	NR	NR	NR	NR	NR
Hair-noncoloring	NR	NR	NR	NR	NR	NR
Hair-coloring	NR	NR	NR	NR	NR	NR
Nail	2	NR	NR	NR	NR	0.001
Mucous membrane	100	0.0015-0.0018	NR	0.001	104	0.0005
Baby products	NR	NR	NR	NR	NR	NR
	Tripeptide-1		Copper Tripeptide-1		Palmitoyl Tetrapeptide-7	
	# of Uses	Conc. (%)	# of Uses	Conc. (%)	# of Uses	Conc. (%)
Totals/conc. range	36	0.00002-0.001	18	NR	249	0.000005-0.0015
Duration of use						
Leave-On	35	0.00002-0.001	17	NR	245	0.00001-0.0015
Rinse off	1	0.00003	1	NR	4	0.000005-0.0007
Diluted for (bath) use	NR	NR	NR	NR	NR	NR
Exposure type						
Eye area	3	0.00002	9	NR	102	0.00001-0.00021
Incidental ingestion	2	NR	NR	NR	1	NR
Incidental inhalation - sprays	18	NR	7 ^b	NR	114	0.00018 ^b
Incidental inhalation - powders	17	0.0001-0.001 ^c	6 ^c	NR	112	0.00015-0.0015 ^c
Dermal contact	34	0.00002-0.001	16	NR	248	0.000005-0.0015
Deodorant (underarm)	NR	NR	NR	NR	NR	NR
Hair-noncoloring	NR	0.0001	NR	NR	NR	NR
Hair-coloring	NR	NR	NR	NR	NR	NR
Nail	NR	NR	NR	NR	NR	NR
Mucous membrane	2	NR	NR	NR	1	NR
Baby products	NR	NR	NR	NR	NR	NR

Abbreviations: NR, not reported; NS, not surveyed; Totals, rinse-off + leave-on product uses.

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

^bIt is possible that these products may be sprays, but it is not specified whether the reported uses are sprays.

^cIt is possible that these products may be powders, but it is not specified whether the reported uses are powders.

Palmitoyl Hexapeptide-12 (Pal-VGVAPG). A trade name mixture containing 100 ppm Palmitoyl Tripeptide-12 was instilled as a single dose (0.1 mL) into the left eye of each of 3 male New Zealand white rabbits.²⁶ Eyes were not rinsed, and reactions were scored at 24 hours, 48 hours, and 72 hour-postinstillation. Moderate or slight conjunctival irritation (chemosis [score = 2] and redness [score = 1 or 2]) was observed in all animals for up to 4 days postinstillation. Neither iris irritation nor corneal opacity was observed. This mixture was considered a nonirritant when instilled into the eyes of rabbits. This conclusion was based on the observation that the

mean scores for chemosis, redness, and degree of corneal opacity in 2 of the 3 animals did not reach the criteria for irritation under the experimental conditions of this study.

In Vitro

Palmitoyl Tripeptide-1 (Pal-GHK). The ocular irritation potential of a trade name mixture containing 1,000 ppm Palmitoyl Tripeptide-1 was evaluated in the hen's egg chorioallantoic membrane in vitro assay.²⁷ Details relating to the assay protocol were not presented. Sodium dodecyl sulfate (0.5% w/v) served as the positive control. This trade name mixture was

classified as slightly irritating, but was considered “well tolerated.” The positive control was classified as an ocular irritant.

Palmitoyl Hexapeptide-12 (Pal-VGVAPG). The hen’s egg chorioallantoic membrane in vitro assay was also used to evaluate the ocular irritation potential of a trade name mixture containing 200 ppm Palmitoyl Hexapeptide-12.²⁸ The test substance was diluted to 50% (w/v) in distilled water prior to testing. The score for each egg was determined by the sum of the notations of hyperemia, hemorrhage, and coagulation (coagulation = opacity and/or thrombosis). The notation for the test substance corresponded to the arithmetic mean, rounded to the nearest tenth, of the scores obtained for 4 eggs. Sodium dodecyl sulfate (0.5% w/v) served as the positive control. The mean irritation index was 0.8 for the diluted trade name mixture and 12.0 for the positive control. The test substance was classified as practically nonirritating.

Ocular irritation potential of this trade name mixture was also evaluated in the Statens Seruminstiut Rabbit Cornea (SIRC) fibroblastic cell line using the neutral red releasing method.²⁸ Sodium dodecyl sulfate and sodium chloride served as positive and negative controls, respectively. The IC₅₀, defined as the test substance concentration that inhibited 50% of cell survival and growth, was >50%, and the mortality at 50% dilution was 37.9%. It was concluded that the test substance caused negligible cytotoxicity.

Palmitoyl Tetrapeptide-7 (Pal-GQPR). The hen’s egg chorioallantoic membrane in vitro assay was used to evaluate the ocular irritation potential of a trade name mixture containing 500 ppm Palmitoyl Tetrapeptide-7. The assay procedure stated in the preceding section was used. The test material was classified as slightly irritating (mean irritation index = 3.75).²⁹

Skin Irritation and Sensitization

The following skin irritation and sensitization data are also summarized in Table 3.

Animal

Palmitoyl Tripeptide-1 (Pal-GHK). A trade name mixture containing 100 ppm Palmitoyl Tripeptide-1 was evaluated for its skin irritation potential using 3 male New Zealand white rabbits.³⁰ This mixture was applied to scarified or nonscarified skin of the flank (0.5 mL on 6 cm² area, clipped free of hair), using an occlusive hypoallergenic dressing, for 24 hours. Reactions were scored at 24 hours and 72 hours postapplication. At 24 hour-postapplication, slight erythema was observed on both flanks of 2 rabbits. These were the only reactions observed during the study. This trade name mixture was classified as a nonirritant (PII = 0.3).

A cumulative skin irritation study on this mixture was performed using 10 guinea pigs (5 males, 5 females).²³ The test substance was applied to the left flank (0.05 mL on a 2 cm × 2 cm area, clipped free of hair) once daily for 14 consecutive days. The right flank was treated with purified water (control). The test site was not covered with a dressing during the

application period. Reactions were evaluated immediately prior to each application and approximately 24 hours after the last application by comparing the reactions on both flanks. The animals were killed and cutaneous samples were removed from treated sites. Cutaneous reactions were not observed during the study. However, a very slight beige coloration of the skin was observed in each animal. It was concluded that the test substance was a nonirritant in guinea pigs (maximum weekly mean irritation index = 0).

The skin sensitization potential of this mixture was studied using 30 guinea pigs (strain not stated) in the maximization test.²⁴ The test group consisted of 20 animals (10 males, 10 females) and the control group consisted of 10 animals (5 males, 5 females). During induction day 1, test animals were injected intradermally with the test substance (1% in 0.9% isotonic saline vehicle [injection volume = 0.1 mL]) in the presence of Freund’s complete adjuvant. The test substance (0.5 mL) was cutaneously applied to test animals on induction day 8. The control group was treated only with vehicle during the induction period. The challenge phase was initiated after a 12-day nontreatment period. A dry compress containing the test substance (75% in saline vehicle [0.5 mL]) was applied, under an occlusive dressing to the right flank, and vehicle only (0.5 mL) was applied to the left flank of all animals. The compress and occlusive dressing were removed at the end of the 24-hour application period. Challenge reactions were evaluated at 24 hours and 48 hours after removal. The animals were then killed and cutaneous samples were obtained from challenge sites. Microscopic examination was not performed on cutaneous samples. Cutaneous reactions were not observed during the challenge phase. It was concluded that test substance did not induce sensitization in guinea pigs.

Palmitoyl Hexapeptide-12 (Pal-VGVAPG). A trade name material containing 100 ppm Palmitoyl Hexapeptide-12 was evaluated in a skin irritation study involving 3 male New Zealand white rabbits.³¹ A dry compress containing the test substance was applied (0.5 mL on 6 cm² area, clipped free of hair) for 4 hours under a semioclusive dressing. Reactions were scored at 24 hour, 48 hour, and 72 hour postremoval. Moderate cutaneous reactions (erythema, but no edema) were observed, and these reactions were reversible within 24 hours or 48 hours. Cutaneous reactions were not observed on days 3 and 4. This trade name mixture was considered a nonirritant (mean erythema score <1.0).

Human

Palmitoyl Tripeptide-1 (Pal-GHK). The skin irritation potential of a trade name mixture containing 1,000 ppm Palmitoyl Tripeptide-1 was evaluated using 10 healthy adult volunteers.²⁷ The mixture was applied to dorsal skin (~0.02 mL on 50 mm² area), using an occlusive patch (Finn chamber on Scanpor), for 48 hours. Untreated sites (covered with occlusive patch) served as negative controls. Reactions were scored 30 minutes after patch removal. Neither irritation nor significant cutaneous intolerance was observed (primary irritation index [PII] = 0).

Table 3. Skin Irritation and Sensitization Studies.

Test substance	Animals/subjects	Doses/concentrations tested	Procedure	Results
Palmitoyl Tripeptide-I 100 ppm	Three male New Zealand white rabbits	0.5 mL on 6 cm ² area of flank	Applied for 24 hours under occlusive hypoallergenic dressing	Slight erythema in 2 rabbits (both flanks). Classified as nonirritant (primary irritation index [PII] = 0.3) ³⁰
Palmitoyl Tripeptide-I 100 ppm	Ten male and female guinea pigs (strain not stated)	0.05 mL on 4 cm ² area on left flank	Applied (uncovered) once daily for 14 consecutive days	Nonirritant (maximum weekly mean irritation index = 0) ²³
Palmitoyl Tripeptide-I 100 ppm	20 male and female guinea pigs (strain not stated)	Intradermal injection with 1% (0.1 mL) and cutaneous application of undiluted ingredient during induction. 24-hour challenge with 75% [maximal nonirritant concentration] under occlusive dressing	Maximization test	Non-sensitizer ²⁴
Palmitoyl Hexapeptide-I 12 100 ppm	Three male New Zealand white rabbits	0.5 mL on 6 cm ² area of flank	Applied for 4 hours under semi-occlusive dressing	Moderate erythema, reversible within 24 hours or 48 hours. Classified as nonirritant (mean erythema score of <1) ³¹
Palmitoyl Tripeptide-I 1,000 ppm	Ten adults	~0.02 mL on 50 mm ² area of dorsal skin	Applied for 48 hours under occlusive patch (Finn chamber)	Nonirritant (PII = 0) ²⁷
Palmitoyl Tripeptide-I 1,000 ppm	Fifty-two subjects (16 to 79 years old)	Undiluted ingredient applied during induction and challenge	Human repeated insult patch test (HRIPT). 24-hour induction applications. 24-hour challenge	Barely perceptible (+ reaction) to moderate (2 reaction) during induction and/or challenge phases. No clinically significant potential for skin irritation or sensitization ³²
Palmitoyl Hexapeptide-I 12 200 ppm	Ten adults	Test concentration of 50% on dorsal skin	Applied for 48 hours under occlusive patch	Nonirritant when diluted to 50% ²⁸
Palmitoyl Hexapeptide-I 12 200 ppm	Fifty-three adults	Test concentration of 50% applied during induction and challenge	HRIPT. Eight 48-hour induction applications, followed by challenge	Nonirritant (mean irritation index = 0.04) and nonsensitizer ³³
Palmitoyl Tetrapeptide-7 (contains 500 ppm pal GQPR)	Fifty-two subjects (18 to 79 years old)	0.2 mL on 3/4' × 3/4' occlusive patch	HRIPT. Nine 24-h induction applications, followed by challenge	Nonirritant and nonsensitizer ³⁴

Abbreviation: HRIPT, human repeated insult patch test.

There was also no evidence of a secondary effect. The test substance was classified as “very well tolerated”.

The skin sensitization potential of this trade name mixture was evaluated in a human repeated insult patch test (HRIPT) using 52 subjects.³² The study was initiated with 57 subjects (16 to 79 years old), 5 of whom withdrew for reasons unrelated to ingredient application. During induction, patches (type not stated) were applied 3 times per week for a total of nine 24-hour induction applications. Non-treatment periods during the induction phase were described as 24 hours following each Tuesday and Thursday patch removal and 48 hours following each Saturday removal. The challenge phase was initiated following a 2-week nontreatment period. Challenge patches were applied for 24 hours to a new test site that was adjacent to the induction patch site. Reactions were scored 24 hours and 72 hours after patch application. Barely perceptible (+) to moderate (2+) reactions were observed during induction and/or challenge phases. However, it was noted that these transient, low-level responses were considered clinically insignificant. It was concluded that the test substance did not indicate a clinically significant potential for dermal irritation or allergic contact sensitization.

Palmitoyl Hexapeptide-12 (Pal-VGVAPG). A trade name mixture containing 200 ppm Palmitoyl Hexapeptide-12 was evaluated for skin irritation potential using 10 adult volunteers.²⁸ A single 48-hour application of the test substance (diluted to 50%) was made, under an occlusive patch, on dorsal arm skin. Neither irritation nor significant cutaneous intolerance was observed (PII = 0). There was also no evidence of a secondary effect. Diluted test material was considered very well tolerated.

An HRIPT on this trade name mixture was performed using 53 healthy adult volunteers.³³ The test substance was diluted to a concentration of 50% prior to application. The test procedure involved 48-hour occlusive patch applications of the diluted test substance (area of application not specified). Eight induction applications were made, followed by challenge patch application. Neither skin irritation (mean irritation index [induction] = 0.04) nor sensitization was observed.

Palmitoyl Tetrapeptide-7 (Pal-GQPR). The skin irritation and sensitization potential of a trade name mixture containing 500 ppm Palmitoyl Tetrapeptide-7, was evaluated in an HRIPT involving 52 healthy male and female subjects (age range: 18 to 79 years).³⁴ The test material (0.2 mL) was applied to a 3/4" × 3/4" occlusive patch that was placed on the upper back between the scapulae. During the induction phase, patches were applied (24 hours) 3 times per week for a total of 9 induction applications. After a 2-week nontreatment period, a 24-hour challenge patch was applied to a new site that was adjacent to the original application site. Reactions were scored at the time of patch removal and at 24 hour and 72 hour-postapplication. It was concluded that results for the test material did not indicate a potential for dermal irritation or allergic contact sensitization.

Reproductive and Developmental Toxicity

Data on the reproductive and developmental toxicity of Tripeptide-1, Hexapeptide-12, their metal salts and fatty acyl derivatives, and Palmitoyl Tetrapeptide-7 were neither found in the published literature nor submitted.

Genotoxicity

Palmitoyl Tripeptide-1 (Pal-GHK)

The genotoxicity of a trade name mixture containing 1,000 ppm Palmitoyl Tripeptide-1 was evaluated in the Ames test, with and without metabolic activation, using the following *Salmonella typhimurium* strains: TA98, TA100, TA1535, and TA1538.³⁵ The test material (0.1 mL in ethanol solution) was nongenotoxic. In another assay, the genotoxicity of a trade name mixture containing 100 ppm Palmitoyl Tripeptide-1 was evaluated, with and without metabolic activation, using the following *S typhimurium* strains: TA98, TA102, TA1535, and TA1537.³⁶ At doses up to 5,000 µg/plate, the test material was classified as nongenotoxic.

Palmitoyl Tetrapeptide-7 (Pal-GQPR)

The Ames test was used to evaluate the genotoxicity of a trade name mixture containing 500 ppm Palmitoyl Tetrapeptide-7, in the following *S typhimurium* strains: TA98, TA100, TA1535, TA1537, and TA1538.³⁷ The test material (1 mL, in 9 mL of DMSO) was evaluated with and without metabolic activation. The test material was considered nonmutagenic in all bacterial strains.

Carcinogenicity

Data on the carcinogenicity of Tripeptide-1, Hexapeptide-12, their metal salts and fatty acyl derivatives, and Palmitoyl Tetrapeptide-7 were neither found in the published literature nor submitted.

Summary

The safety of the following ingredients in cosmetics is reviewed in this safety assessment: Tripeptide-1, Palmitoyl Tripeptide-1, Myristoyl Tripeptide-1, Hexapeptide-12, Palmitoyl Hexapeptide-12, Myristoyl Hexapeptide-12, Copper Tripeptide-1, Bis(Tripeptide-1) Copper Acetate, Manganese Tripeptide-1, and Palmitoyl Tetrapeptide-7.

The ingredients reviewed in this safety assessment function primarily as skin conditioning agents in cosmetic products. According to information supplied to the USFDA by industry as part of the VCRP, the following palmitoyl oligopeptides are being used in cosmetic products: palmitoyl oligopeptide (name retired, peptide sequence not stated), Palmitoyl Tripeptide-1, Tripeptide-1, Copper Tripeptide-1, and Palmitoyl Tetrapeptide-7. The peptide sequence for palmitoyl oligopeptide is not stated in the VCRP database or in the Council's

survey of ingredient use concentrations; however, the sequence could be either GHK (Tripeptide-1) or VGVAPG (Hexapeptide-12).

Results from a survey of ingredient use concentrations conducted by the Council in 2013 and updated in 2014 indicate that, collectively, the ingredients reviewed in this safety assessment are being used at maximum concentrations ranging from 0.0000001% (Palmitoyl Tripeptide-1 and Palmitoyl Hexapeptide-12) to 0.002% (Palmitoyl Hexapeptide-12). The highest concentration of 0.002% relates to ingredient use in leave-on products. In addition to the data included in the survey of ingredient use concentrations, one submission indicated that peptides are being used in cosmetic products at concentrations between 1 ppm and 30 ppm, and that their use at concentrations of <10 ppm is customary.

The impurity content of both Palmitoyl Tripeptide-1 and Palmitoyl Hexapeptide-12 has been described as follows: acetate (<5%), palmitic acid (<5%), and water (<5%). Crude Copper Tripeptide-1 is reported to be approximately 95% pure, but can be further washed to remove residual, nondeprotected peptides, resulting from incomplete synthesis.

After IV injection, Tripeptide-1 was rapidly degraded to L-histidyl-L-lysine, which was rapidly eliminated (in minutes) from circulating blood. It has been reported that Tripeptide-1 is unstable in human plasma and is rapidly degraded by aminopeptidases. In an enzyme assay, the liver growth factor, Tripeptide-1, was hydrolyzed by an aminotripeptidase purified from rat brain cytosol.

A trade name mixture containing 100 ppm Palmitoyl Tripeptide-1 was nontoxic ($LD_{50} > 2,000$ mg/kg) in an acute oral toxicity study involving rats. Studies designed to evaluate the repeated dose toxicity of the ingredients reviewed in this safety assessment were not found in the published literature. However, neither treatment-related clinical signs/mortalities were reported in cumulative skin irritation/sensitization studies on this mixture in a guinea pig maximization test.

A trade name mixture containing 100 ppm Palmitoyl Tripeptide-1 was slightly irritating to the eyes of rabbits. A trade name mixture containing 100 ppm Palmitoyl Tripeptide-12 was nonirritating to the eyes of rabbits. In the hen's egg chorioallantoic membrane in vitro assay for evaluating ocular irritation potential, a trade name mixture containing 1,000 ppm Palmitoyl Tripeptide-1 was classified as an irritant, a trade name mixture containing 200 ppm Palmitoyl Hexapeptide-12 was practically nonirritating, and a trade name mixture containing 500 ppm Palmitoyl Tetrapeptide-7 was slightly irritating. In the in vitro neutral red release assay (SIRC fibroblastic cell line) for evaluating ocular irritation potential, a trade name mixture containing 200 ppm Palmitoyl Hexapeptide-12 caused "unimportant cytotoxicity."

In skin irritation studies (single application) involving rabbits, a trade name mixture containing 100 ppm Palmitoyl Tripeptide-1 and a trade name mixture containing 100 ppm Palmitoyl Hexapeptide-12 were classified as non-irritants. A trade name mixture containing 100 ppm Palmitoyl Tripeptide-1 was classified as a nonirritant in a cumulative skin irritation

study involving guinea pigs. This mixture also did not induce skin sensitization at a challenge concentration of 75% in the guinea pig maximization test.

In human skin irritation studies (single application), a trade name mixture containing 1,000 ppm Palmitoyl Tripeptide-1 and a different mixture containing 200 ppm Palmitoyl Hexapeptide-12 (50%) were classified as non-irritants. Human repeated insult patch test results for these 2 trade name mixtures, and another trade name mixture containing 500 ppm Palmitoyl Tetrapeptide-7, were negative for skin irritation and sensitization.

Ames test results for Palmitoyl Tripeptide-1 and Palmitoyl Tetrapeptide-7 were negative with and without metabolic activation in *S typhimurium* bacterial strains.

Discussion

Use concentration data indicate that the ingredients reviewed in this safety assessment are being used at concentrations up to 0.002%, a value reported for Palmitoyl Hexapeptide-12 in leave-on products (in night products [not spray] and in body and hand sprays). Information substantiating the use of peptides at concentrations between 1 ppm and 30 ppm in cosmetic products, and customary use at concentrations of <10 ppm, was also evaluated. The Panel agreed that the data on peptide use should be relied upon as typical use concentrations for all of the ingredients reviewed in this safety assessment, which include Tripeptide-1, Hexapeptide-12, their metal salts and fatty acyl derivatives, and Palmitoyl Tetrapeptide-7. Thus, given the low use concentrations of these ingredients, together with the negative repeated dose toxicity, skin irritation and sensitization, and genotoxicity data, it was determined that the available data support the safe use of these ingredients in cosmetic products. The Panel noted that this safe conclusion is applicable only to ingredient names associated with the following known peptide sequences: GHK, VGVAPG, and GQPR.

Palmitoyl Hexapeptide-12 is used in body and hand sprays (maximum use concentration = 0.002%). Because this ingredient is used in products that are sprayed, the ingredient could possibly be inhaled. The Panel discussed the issue of incidental inhalation exposure from propellant and pump sprays and powders, and considered pertinent data indicating that incidental inhalation exposures to this ingredient in such cosmetic products would not cause adverse health effects. These data considered include data characterizing the potential for this ingredient to cause repeated dose toxicity, dermal irritation or sensitization, and genotoxicity. The Panel noted that 95% to 99% of droplets/particles produced in cosmetic aerosols would not be respirable to any appreciable amount. Coupled with the small actual exposure in the breathing zone and the concentrations at which the ingredients are used, the available information indicates that incidental inhalation would not be a significant route of exposure that might lead to local respiratory or systemic effects. A detailed discussion and summary of the Panel's approach to evaluating incidental inhalation exposures

to ingredients in cosmetic products is available at <http://www.cir-safety.org/cir-findings>.

Conclusion

The CIR Panel concluded that the following cosmetic ingredients are safe in cosmetics in the present practices of use and concentration described in this safety assessment.

Tripeptide-1 (GHK)
 Palmitoyl Tripeptide-1 (GHK)
 Myristoyl Tripeptide-1 (GHK)^a
 Copper Tripeptide-1 (GHK)
 Bis(Tripeptide-1) Copper Acetate (GHK)^a
 Manganese Tripeptide-1 (GHK)^a
 Hexapeptide-12 (VGVAPG)^a
 Palmitoyl Hexapeptide-12 (VGVAPG)
 Myristoyl Hexapeptide-12 (VGVAPG)^a
 Palmitoyl Tetrapeptide-7 (GQPR)

^aNot reported to be in current use. Were ingredients in this group not in current use to be used in the future, the expectation is that they would be used in product categories and at concentrations comparable to others in this group.

Authors' Note

Unpublished sources cited in this report is available from the Executive Director, Cosmetic Ingredient Review, 1620L Street, NW, Suite 1200, Washington, DC 20036, USA.

Authors' Contributions

Wilbur Johnson contributed to conception and design, acquisition, analysis, and interpretation, and drafted the manuscript; Bart Heldreth contributed to conception and design, acquisition, analysis, and interpretation, drafted the manuscript, and critically revised the manuscript; Lillian Gill, Wilma Bergfeld, Donald Belsito, Ronald Hill, Curtis Klaassen, Daniel Liebler, James Marks, Ronald Shank, Thomas Slaga, and Paul Snyder contributed to conception and design, analysis and interpretation, and critically revised the manuscript. All authors gave final approval and agree to be accountable for all aspects of work ensuring integrity and accuracy.

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References

1. Nikitakis J, Breslawec HP. *International Cosmetic Ingredient Dictionary and Handbook*. 14 ed. Washington, DC: Personal Care Products Council; 2014.
2. Sederma. Summary information on palmitoyl oligopeptide. Unpublished data submitted by the Personal Care Products Council on 10-31-2012. 2012. pp.1.
3. Verheul AF, Udhayakumar V, Jue DL, Wohlhueter RM, Lal AA. Monopalmitic acid-peptide conjugates induce cytotoxic T cell responses against malarial epitopes: importance of spacer amino acids. *J Immunol Methods*. 1995;182(2):219-226.
4. Naider FR, Becker JM. Synthesis of prenylated peptides and peptide esters. *Biopolymers*. 1997;43(1):3-14.
5. Kadereit D, Waldmann H. Invited review chemoenzymatic synthesis of lipidated peptides. *Monatshfte Chemie*. 2000;131(6):571-584.
6. Kuhn K, Owen DJ, Bader B, Wittinghofer A, Kuhlmann J, Waldmann H. Synthesis of functional Ras lipoproteins and fluorescent derivatives. *J Am Chem Soc*. 2001;123(6):1023-1035.
7. Kuhlmann J, Tebbe A, Völkert M, et al. Photoactivatable synthetic Ras proteins: "baits" for the identification of plasma-membrane-bound binding partners of Ras. *Angewandte Chemie* 2002;41(14):2546-2550.
8. Lintner K, Peschard O. Biologically active peptides: from a laboratory bench curiosity to a functional skin care product. *Int J Cosmetic Sci*. 2000;22(3):207-218.
9. Yamamoto K, Aoyagi M, Yamamoto M. Changes in elastin-binding proteins during the phenotypic transition of rabbit arterial smooth muscle cells in primary culture. *Exp Cell Res*. 1995; 218(1):339-345.
10. Pickart L, Lovejoy S. Biological activity of human plasma copper-binding growth factor glycyl-L-histidyl-L-lysine. *Methods Enzymology* 1987;147:314-328.
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: MD FDA; 2014. Obtained under the Freedom of Information Act from CFSAN; requested as "Frequency of Use Data" January 8 2014; received February 25 2014).
12. Personal Care Products Council. Concentration of use by FDA product category. Palmitoyl oligopeptide, tripeptide-1 ingredients, and hexapeptide-12 ingredients. Unpublished data submitted by the Personal Care Products Council on 9-27-2013 and updated on 4-23-2014. 2014. pp.1-3.
13. Personal Care Products Council. Concentration of use by FDA product category: palmitoyl tetrapeptide-7. Unpublished data submitted by the Personal Care Products Council on 10-3-2014. 2014. pp.1.
14. Lintner K. Personal Communication. Comments on use concentrations of peptides in cosmetic products, made at the March 17-18, 2014 Cosmetic Ingredient Review (CIR) Expert Panel Meeting. Washington, D.C., 2014.
15. Rothe H, Fautz R, Gerber E, et al. Special aspects of cosmetic spray safety evaluations: principles on inhalation risk assessment. *Toxicol Lett*. 2011;205(2):97-104.
16. Bremmer HJ, Prud'homme de Lodder LCH, van Engelen JGM. Cosmetics fact sheet: to assess the risks for the consumer; Updated version for ConsExpo 4. Bilthoven, Netherlands: Netherlands National Institute for Public Health and the Environment. 2006. <http://www.rivm.nl/bibliotheek/rapporten/320104001.pdf>.

- Accessed August 24, 2011. Report No. RIVM 320104001/2006. 1-77.
17. Rothe H. Special aspects of cosmetic spray evaluation. 2011. Unpublished information presented to the 26 September CIR Expert Panel. Washington D.C.
 18. Johnsen MA. The Influence of particle size. *Spray Technol Marketing* 2004;14(11):24-27.
 19. Endo T, Miyagi M, Ujiie A. Simultaneous determination of glycyl-L-histidyl-L-lysine and its metabolite, L-histidyl-L-lysine, in rat plasma by high-performance liquid chromatography with post-column derivatization. *J Chromatogr B*. 1997;692(1):37-42.
 20. Sachs L, Marks N. A highly specific aminotripeptidase of rat brain cytosol. Substrate specificity and effects of inhibitors. *Biochimica et Biophysica Acta*. 1982;706(2):229-238.
 21. Dalpozzo A, Kanai K, Kereszturi G, Calabrese G. H-Gly-His psi (NHCO)Lys-OH, partially modified retro-inverso analogue of the growth factor glycyl-L-histidyl-L-lysine with enhanced enzymatic stability. *J Peptide Protein Res*. 1993;41(6):561-566.
 22. Centre International de Toxicologie. BIOPEPTIDE CL (contains 100 ppm palmitoyl oligopeptide as Pal GHK). Unpublished data submitted by the Personal Care Products Council on 10-31-2012. 1997. pp.1-2.
 23. Centre International de Toxicologie. Summary of local tolerance study after repeated topical application for 2 weeks in guinea pigs. BIOPEPTIDE CL (contains 100 ppm palmitoyl oligopeptide as Pal GHK). Unpublished data submitted by the Personal Care Products Council on 10-31-2012. 1997. pp.1-2.
 24. Anonymous. Summary of skin sensitization test in guinea pigs. BIOPEPTIDE CL (contains 100 ppm palmitoyl oligopeptide as Pal GHK). Unpublished data submitted by the Personal Care Products Council on 10-31-2012. 1993. pp.1-3.
 25. Centre International de Toxicologie. Summary of acute eye irritation in rabbits. BIOPEPTIDE CL (contains 100 ppm palmitoyl oligopeptide as Pal GHK). Unpublished data submitted by the Personal Care Products Council on 10-31-2012. 1997. pp.1-2.
 26. Centre International de Toxicologie. Acute eye irritation in rabbits. BIOPEPTIDE EL (contains 100 ppm palmitoyl oligopeptide as Pal VGVAPG). Unpublished data submitted by the Personal Care Products Council on 10-31-2012. 2013. pp.1-2.
 27. Institut D'Expertise Clinique. Summary of in vitro and tolerance studies of MAXI-LIP (contains 1000 ppm palmitoyl oligopeptide as Pal GHK). Unpublished data submitted by the Personal Care Products Council on 10-31-2012. 2000. pp.1-4.
 28. Institut D'Expertise Clinique. Ocular primary tolerance and cutaneous compatibility of DERMAYL (contains 200 ppm palmitoyl oligopeptide, as Pal VGVAPG). Unpublished data submitted by the Personal Care Products Council on 10-31-2012. 2003. pp. 1-6.
 29. Institut D'Expertise Clinique. Hen's egg chorion-allantoic membrane for assessing ocular tolerance of RiginTM. Unpublished data submitted by Prof. Karl Lintner, Ph.D. on 3-17-2014. 1999. pp.1-6.
 30. Centre International de Toxicologie. Summary of evaluation of the cutaneous primary irritation index in rabbits. BIOPEPTIDE CL (contains 100 ppm palmitoyl oligopeptide as Pal GHK). Unpublished data submitted by the Personal Care Products Council on 10-31-2012. 1997. pp.1-2.
 31. Centre International de Toxicologie. Acute dermal irritation in rabbits. BIOPEPTIDE EL (contains 100 ppm palmitol oligopeptide, as Pal VGVAPG). Unpublished data submitted by the Personal Care Products Council on 10-31-2012. 2013. pp.1-2.
 32. Consumer product Testing Co. Summary of repeated insult patch test of MAXI-LIP (contains 1000 ppm palmitoyl oligopeptide as Pal GHK). Unpublished data submitted by the Personal Care Products Council on 10-31-2012. 2000. pp.1-4.
 33. Institut D'Expertise Clinique. Dermal tolerance study of DERMAYL (contains 200 ppm palmitoyl oligopeptide as Pal VGVAPG). Unpublished data submitted by the Personal Care Products Council on 10-31-2012. 2004. pp.1-3.
 34. Consumer Product Testing Co. Repeated insult patch test on RiginTM. Unpublished data submitted by Prof. Karl Lintner, Ph.D. on 3-17-2014. 1999. pp.1-3.
 35. North American Science Associates, Inc. (NAMSA). Summary of genotoxicity Salmonella reverse mutation study of MAXI-LIP (contains 1000 ppm palmitoyl oligopeptide as Pal GHK). Unpublished data submitted by the Personal Care Products Council on 10-31-2012. 2000. pp.1.
 36. Anonymous. Summary of reverse mutation assay by the Ames test on BIOPEPTIDE-CL (contains 100 ppm palmitoyl oligopeptide as Pal GHK). Unpublished data submitted by the Personal Care Products Council on 10-31-2012. 1992. pp.1-2.
 37. North American Science Associates, Inc. (NAMSA). Salmonella typhimurium reverse mutation study on RiginTM. Unpublished data submitted by Prof. Karl Lintner, Ph.D. on 3-17-2014. 1999. pp.1-2.