# 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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*All interested persons are provided 60 days from the above date to comment on this Tentative Report and to identify additional published data that should be included or provide unpublished data which can be made public and included. Information may be submitted without identifying the source or the trade name of the cosmetic product containing the ingredient. All unpublished data submitted to CIR will be discussed in open meetings, will be available at the CIR office for review by any interested party and may be cited in a peer-reviewed scientific journal. Please submit data, comments, or requests to the CIR Director, Dr. Lillian J. Gill.*

The 2014 Cosmetic Ingredient Review Expert Panel members are: Chair, Wilma F. Bergfeld, M.D., F.A.C.P.; Donald V. Belsito, M.D.; Curtis D. Klaassen, Ph.D.; Daniel C. Liebler, Ph.D.; Ronald A Hill, Ph.D.; James G. Marks, Jr., M.D.; Ronald C. Shank, Ph.D.; Thomas J. Slaga, Ph.D.; and Paul W. Snyder, D.V.M., Ph.D. The CIR Director is Lillian J. Gill, D.P.A. This report was prepared by Wilbur Johnson, Jr., M.S., Senior Scientific Analyst and Bart Heldreth, Ph.D., Chemist.



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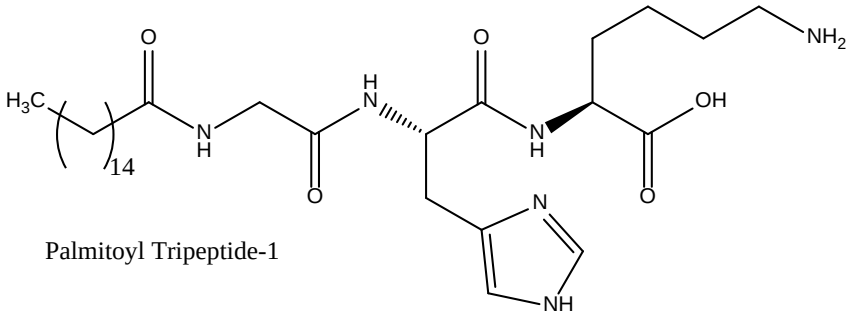
**ABSTRACT:** Tripeptide-1, hexapeptide-12, their metal salts and fatty acyl derivatives, and palmitoyl tetrapeptide-7 function primarily as skin conditioning agents, and palmitoyl tripeptide-1, palmitoyl hexapeptide-12, tripeptide-1, and copper tripeptide-1 are being used in cosmetic products. Typical 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 the present practices of use and concentration in cosmetics.

## INTRODUCTION

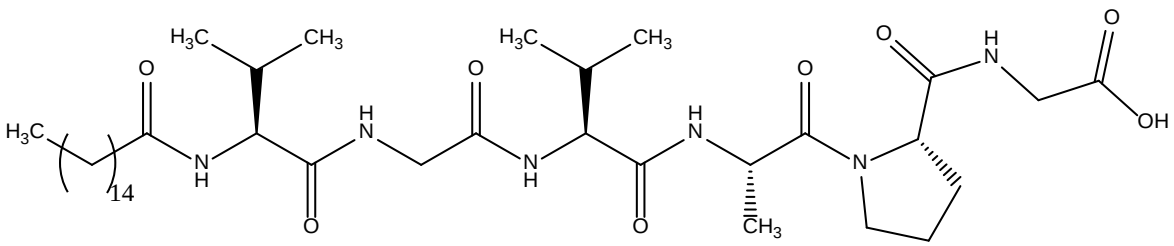
The safety of tripeptide-1, hexapeptide-12, their metal salts and fatty acyl derivatives, and palmitoyl tetrapeptide-7 as used in cosmetics is reviewed in this safety assessment. These ingredients function primarily as skin conditioning agents in cosmetic products.[1] The ingredient name, palmitoyl oligopeptide listed in the *International Cosmetic Ingredient Dictionary and Handbook* has been retired, and is now represented by the palmitoyl tripeptide-1 (Gly-His-Lys peptide sequence) or palmitoyl hexapeptide-12 (Val-Gly-Val-Ala-Pro-Gly peptide sequence) ingredient name in the dictionary. Most of the toxicity data included in this safety assessment are on a trade name material (Matrixyl 3000) containing palmitoyl tripeptide-1 (Gly-His-Lys peptide sequence) and palmitoyl tetrapeptide-7 (Gly-Gln-Pro-Arg 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 peptide sequences are also included.

## CHEMISTRY

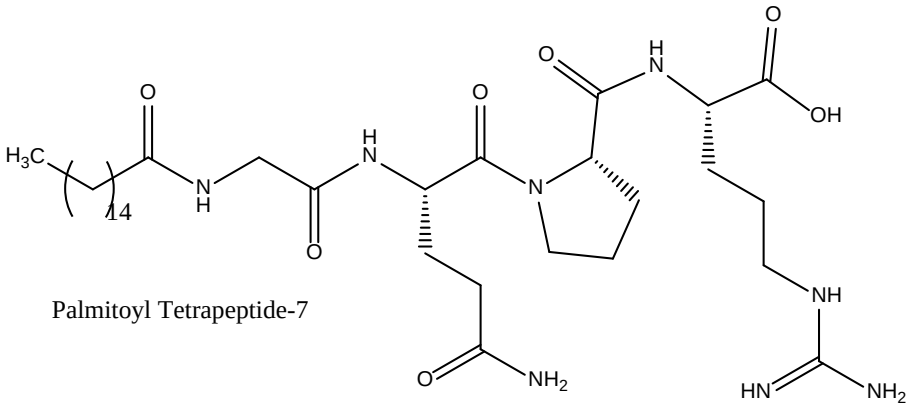
The ingredients in this report are related structurally by bearing one of three distinct peptide sequences, either tripeptide-1 (Glycine-Histidine-Lysine), hexapeptide-12 (Valine-Glycine-Valine-Alanine-Proline-Glycine), not Alanine-Proline-Glycine-Valine-Glycine-Valine), or tetrapeptide-7. Some of the ingredients reviewed in this safety assessment include not only one of these three peptide sequences but also have a fatty acyl group or metal salt at the *N*-terminus. For example, the structures of these three 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 (i.e., Palmitoyl Tripeptide-1, Palmitoyl Hexapeptide-12 (Palmitoyl-Valine-Glycine-Valine-Alanine-Proline-Glycine *only*), and Palmitoyl Tetrapeptide-7).




Palmitoyl Tripeptide-1



Palmitoyl Hexapeptide-12 (Palmitoyl-Valine-Glycine-Valine-Alanine-Proline-Glycine ***only***)



Palmitoyl Tetrapeptide-7

**Figure 1. Example Structures**

The ingredient name, palmitoyl oligopeptide in the *International Cosmetic Ingredient Dictionary and Handbook* has been retired and is represented by the palmitoyl tripeptide-1 (palmitoyl-glycine-histidine-lysine) or palmitoyl hexapeptide-12 (palmitoyl-valine-glycine-valine-alanine-proline-glycine or *palmitoyl-alanine-proline-glycine-valine-glycine-valine***)** ingredient name.[1] In addition to the palmitoyl tripeptide-1, this report only addresses the hexapeptide-12 ingredients with the valine-glycine-valine-alanine-proline-glycine sequence. Accordingly, the sequences from the retired ingredient name palmitoyl oligipeptide, specifically glycine-histidine-lysine (a.k.a. tripeptide-1) and alanine-proline-glycine-valine-glycine-valine (a.k.a. hexapeptide-12), and the fatty acyl and metal salt derivatives therein, and palmitoyl tetrapeptide-7 (a.k.a. pal-glycine-glutamine-proline-arginine) are all included in this report. The safety of the peptide sequence alanine-proline-glycine-valine-glycine-valine, and any fatty acyl or salt deriviatives thereof, is *not* addressed in this report. These ingredients are not grouped herein for the purpose of reading across one peptide to the other, but because of their historical relationship within the nomenclature. The old definition of palmitoyl oligopeptide (retired, amino acid sequence not stated) was: Palmitoyl oligopeptide is the product obtained by the reaction of palmitic acid with a synthetic peptide consisting of two or more of the following amino acids: alanine, arginine, aspartic acid, glycine, histidine, lysine, proline, serine, or valine.

The definitions, structures, and functions of the ingredients in this report are included in Table 1.

Palmitoyl oligopeptide (Pal-GHK; palmitoyl tripeptide-1) is one of 2 peptide derived ingredients in the skin care ingredient Matrixyl 3000.[2] Data on Matrixyl 3000 are included in this safety assessment. Palmitoyl tripeptide-1 consists of a short chain of 3 amino acids (also known as GHK peptide [fragment of type I collagen] or glycine-histidine-lysine) that is connected to palmitic acid. The other active ingredient is palmitoyl tetrapeptide-7 (Pal-GQPR), which consists of a short chain of four amino acids (also known as GQPR peptide or glycine-glutamine-proline-arginine) connected to palmitic acid. The tetrapeptide portion is a natural fragment of the IgG immunoglobulin.

Throughout the report text, ingredient name subheadings will include the 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 (Pal-Gly-His-Lys) and hexapeptide-12 will be written as hexapeptide-12 (Val-Gly-Val-Ala-Pro-Gly**)**. Other abbreviations include VGVAPG for hexapeptide-12 and GHK for tripeptide-1.

## Physical and Chemical Properties

A chemical supplier provided data on palmitoyl oligopeptide, identified as CAS No. 147732-56-7 and CAS No. 171263-26-6.[3] Properties of these 2 ingredients are included below.

### Palmitoyl Tripeptide-1 (Pal-Gly-His-Lys)

Palmitoyl tripeptide-1(CAS No. 147732-56-7) is also known as Pal - GHK (Pal-Gly-His-Lys-OH) and L-Lysine,N-(1-oxohexadecyl)glycyl-L-histidyl.[3] It is a white powder and has a molecular weight of 578.80 and an estimated log P of 4.81. The ingredient BIOPEPTIDE-CL (contains 100 ppm palmitoyl tripeptide-1) has a density of 1.13.

### Palmitoyl Hexapeptide-12 (Pal-Val-Gly-Val-Ala-Pro-Gly)

Palmitoyl hexapeptide-12 (CAS No. 171263-26-6) is also known as Pal VGVAPG (Pal-Val-Gly-Val-Ala-Pro-Gly-OH) and Glycine, N-(1-oxohexadecyl)-L-valylglycyl-L-valyl-L-alanyl-L-prolyl. It is also a white powder and has a molecular weight of 737.00 and a logP of 5.09.[3]

## Method of Manufacture

### General Information

Peptides have been synthesized by solid phase fluorenylmethoxycarbonyl chemistry (FMOC protection) using an Advanced Chemtech MPS 350 synthesizer.[4] Palmitic acid was coupled to the deprotected amino-terminus of the resin-bound protected peptides both manually and by using the peptide synthesizer employing the same reaction conditions used in standard amino acid coupling. Peptides and monopalmitic acid-peptide conjugates were cleaved from the resin, deprotected, and purified using standard procedures.

Several strategies for the synthesis of lipidated peptides, both in solution and on solid support, have been developed.[5,6] Solid support is most frequently used to synthesize peptides with longer amino acid chains. Shorter peptides have been synthesized both in solution and on solid support. Particularly, hexa- and heptapeptides corresponding to the Ras- and Rab-C-termini, respectively, have been synthesized in solution.[7,8]

### Palmitoyl Tripeptide-1 (Pal-Gly-His-Lys)

Palmitoyl oligopeptide (CAS No. 147732-56-7) is synthesized via stepwise peptide synthesis (specifically, palmitoyl tripeptide-1).[3] 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 (palmitoyl-Gly-L-His-L-Lys) has been produced via solid phase synthesis, yielding a peptide of high purity (> 97%).[9]

### Palmitoyl Hexapeptide-12 (Pal-Val-Gly-Val-Ala-Pro-Gly)

Palmitoyl oligopeptide (CAS No. 171263-26-6; one of the palmitoyl hexapeptide-12 sequences) is 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. The protected peptide is deprotected to remove the protecting group present on the C-terminal function (Gly) of the peptide.[3]

**Hexapeptide-12 (Val-Gly-Val-Ala-Pro-Gly)**

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.[10] Reverse-phase HPLC was used for further purification.

**Copper Tripeptide-1 (Gly-His-Lys-$Cu^{2+}$)**

Glycyl-L-histidyl-L-lysine-$Cu^{2+}$ is prepared by the combination of purified glycyl-L-histidyl-L-lysine with equimolar cupric acetate, followed by neutralization with 0.1 N sodium hydroxide and centrifugation (at 5000 g for 30 minutes at 3˚) to remove insoluble material, usually excess copper (II) as its hydroxide.[11] The supernatant (in a 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 glycyl-L-histidyl-L-lysine-$Cu^{2+}$.

Crystalline glycyl-L-histidyl-L-lysine-$Cu^{2+}$ is prepared by dissolving glycyl-L-histidyl-L-lysine-$Cu^{2+}$ (30 mg, 88 µmol) 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.[11]

## Composition/Impurities

**Palmitoyl Tripeptide-1 (Pal-Gly-His-Lys)**
**Palmitoyl Hexapeptide-12 (Pal-Val-Gly-Val-Ala-Pro-Gly)**

The impurities content of palmitoyl tripeptide-1 (CAS No. 147732-56-7) and palmitoyl hexapeptide-12 (CAS No. 171263-26-6) has been described as follows: acetate (< 5%), palmitic acid (< 5%), and water (< 5%).[3]

**Tripeptide-1 (Gly-His-Lys)**

Commercial glycyl-L-histidyl-L-lysine-$Cu^{2+}$ is approximately 95% pure, but often includes small amounts of mildly neurotoxic materials, as measured by behavior after intracranial injection, tail flick assays, and gripping ability of mice on spinning disks.[11] Most of the neurotoxic materials can be removed by dissolving glycyl-L-istidyl-L-lysine in glass-distilled water (50 mg/ml), centrifuging at 20,000 g for 1 h at 3˚, 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 Food and Drug Administration (FDA) by industry as part of the Voluntary Cosmetic Registration Program (VCRP) in 2014, the following palmitoyl oligopeptides are being used in cosmetic products:[12] palmitoyl oligopeptide (name retired, peptide sequence not stated) palmitoyl tripeptide-1, palmitoyl hexapeptide-12, tripeptide-1and copper tripeptide-1. The peptide sequence for palmitoyl oligopeptide is not stated in the VCRP database or in the survey of ingredient use concentrations mentioned below; however, the sequence could be either gly-his-lys (tripeptide-1) or valine-glycine-valine-alanine-proline-glycine (hexapeptide-12).

Results from a survey of ingredient use concentrations provided by the Personal Care Products Council (Council) in 2013 indicate that, collectively, the ingredients reviewed in this safety assessment are being used at concentrations ranging from 0.0000001% (palmitoyl oligopeptide (glycine-histidine-lysine-OH [GHK]) and palmitoyl oligopeptide (valine-glycine-valine-alanine-proline-glycine-OH [VGVAPG] ) to 1% (palmitoyl oligopeptide [GHK]). The highest concentration of 1% relates to ingredient use in leave-on products. VCRP 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 of < 10 ppm is customary.[13] Therefore, the 1% use concentration reported for tripeptide-1 needs to be confirmed.

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 oligopeptide (name retired, sequence unstated) is used in body and hand sprays (maximum use concentration = 0.001%). Because this ingredient (which is either palmitoyl tripeptide-1 or palmitoyl hexapeptide-12) 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.[14,15,16,17] Therefore, most droplets/particles incidentally inhaled from cosmetic sprays would be deposited in the nasopharyngeal and bronchial regions and would not be respirable (i.e., they would not enter the lungs) to any appreciable amount.[14,15]

### Non-Cosmetic

A palmitoyl-tailed sequential oligopeptide carrier ($SOC_n$-II) for engineering immunogenic conjugates has been developed.[18] These immunogens are designed to be effective vaccine candidates capable of eliciting potent and specific immune responses by combining B/T cell epitopes and adjuvants as immunostimulators on the same carrier that links the major histocompatibility complex with T cell receptors. With the goal of contributing to the development of carriers for human usage, $SOC_n$-II was formed by the repeating peptide unit $(Aib\text{-}Lys\text{-}Aib\text{-}Gly)_n$, $n$ = 2-7, elongated from the amino-terminus by the palmitoyl group, which is known for its adjuvanticity. Aib in the amino acid sequence represents α-aminoisobutyric acid.

## TOXICOKINETICS

### In Vivo Studies

#### Tripeptide-1 (Gly-His-Lys)

Glycyl-L-histidyl-L-lysine (1% in saline; dose = 10 mg/kg) was injected into the tail vein of male rats (number and ages not stated).[19] Blood samples were collected prior to dosing and for up to 60 minutes post-dosing. Plasma concentration-time profiles of glycyl-L-histidyl-L-lysine and its L-histidyl-L-lysine metabolite indicated that both were not detected in pre-dose plasma samples. However, after i.v. injection, glycyl-L-histidyl-L-lysine was rapidly degraded to L-histidyl-L-lysine, which was rapidly eliminated from circulating blood. It has been reported that glycyl-L-histidyl-L-lysine is unstable in human plasma and is rapidly degraded by aminopeptidases.[20,21]

### In Vitro Studies

#### Tripeptide-1 (Gly-His-Lys)

In an enzyme assay, the liver growth factor Gly-His-Lys was hydrolyzed by an aminotripeptidase purified from rat brain cytosol.[22]

## TOXICOLOGY

### Acute Oral Toxicity

**Palmitoyl Tripeptide-1 (Pal-Gly-His-Lys)**

The acute oral toxicity of the ingredient BIOPEPTIDE-CL (contains 100 ppm palmitoyl oligopeptide, as Pal-Gly-His-Lys-OH) was evaluated using 10 Sprague-Dawley rats (5 males, 5 females; ages not stated).[23] 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. BIOPEPTIDE-CL was classified as nontoxic (LD50 > 2,000 mg/kg).

### Repeated Dose Toxicity

**Palmitoyl Tripeptide-1 (Pal-Gly-His-Lys)**

There were no clinical signs or mortalities in a cumulative skin irritation study on BIOPEPTIDE CL (contains 100 ppm palmitoyl oligopeptide, as Pal-Gly-His-Lys-OH) involving guinea pigs.[24]

In the guinea pig maximization test on BIOPEPTIDE- CL (contains 100 ppm palmitoyl oligopeptide, as Pal-Gly-His-Lys-OH), the test substance was evaluated at a concentration of 75% in a saline vehicle.[25] 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-Gly-His-Lys)**

The ocular irritation potential of the ingredient BIOPEPTIDE-CL (contains 100 ppm palmitoyl oligopeptide, as Pal-Gly-His-Lys-OH) was evaluated using 3 male New Zealand White rabbits.[26] 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 h, 24 h, 48 h, and 72 h post-instillation, 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 BIOPEPTIDE-CL was a slight irritant in this study (maximum ocular irritation index = 4.7).

BIOPEPTIDE EL (contains 100 ppm palmitoyl oligopeptide, as Pal-Val-Gly-Val-Ala-Pro-Gly-OH) was instilled as a single dose (0.1 ml) into the left eye of each of 3 male New Zealand White rabbits.[27] Eyes were not rinsed, and reactions were scored at 24 h, 48 h, and 72 h post-instillation. Moderate or slight conjunctival irritation (chemosis [score = 2] and redness [score = 1 or 2]) was observed in all animals for up to 4 days post-instillation. Neither iridial irritation nor corneal opacity was observed. BIOPEPTIDE EL was considered a non-irritant 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 values for irritation under the experimental conditions of the testing facility.

**In Vitro**

**Palmitoyl Tripeptide-1 (Pal-Gly-His-Lys)**

The ocular irritation potential of the ingredient MAXI-LIP (contains 1,000 ppm palmitoyl oligopeptide, as Pal-Gly-His-Lys-OH) was evaluated in the hen's egg chorioallantoic membrane *in vitro* assay.[28] Details relating to the assay protocol were not included. Sodium dodecyl sulfate (0.5% w/v) served as the positive control. MAXI-LIP

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

The hen's egg chorioallantoic membrane *in vitro* assay was also used to evaluate the ocular irritation potential of Dermaxyl (contains 200 ppm palmitoyl oligopeptide, as Pal-Val-Gly-Val-Ala-Pro-Gly-OH).[29] 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 off to one decimal 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 diluted Dermaxyl and 12.0 for the positive control. The test substance was classified as practically non-irritating.

Dermaxyl ocular irritation potential was also evaluated in the SIRC fibroblastic cell line using the neutral red releasing method.[29] 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 the 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-Gly-Gln-Pro-Arg)**

The hen's egg chorioallantoic membrane *in vitro* assay was used to evaluate the ocular irritation potential of Rigin™, a trade name mixture that contains 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).[30]

## Skin Irritation and Sensitization

The following skin irritation and sensitization data on palmitoyl oligopeptide are summarized in Table 3.

### Animal

**Palmitoyl Tripeptide-1 (Pal-Gly-His-Lys)**

The ingredient BIOPEPTIDE CL (contains 100 ppm palmitoyl oligopeptide, as Pal-Gly-His-Lys-OH) was evaluated for its skin irritation potential using 3 male New Zealand White rabbits.[31] BIOPEPTIDE CL was applied to scarified or non-scarified skin of the flank (0.5 ml on 6 $cm^2$ area, clipped free of hair), using an occlusive hypoallergenic dressing, for 24 h. Reactions were scored at 24 h and 72 h post-application. At 24 h post-application, slight erythema was observed on both flanks of 2 rabbits. These were the only reactions observed during the study. BIOPEPTIDE CL was classified as a non-irritant (PII = 0.3).

A cumulative skin irritation study on BIOPEPTIDE CL was performed using 10 guinea pigs (5 males, 5 females; ages not stated).[24] The test substance was applied to the left flank (0.05 ml on 2 cm x 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 h 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 BIOPEPTIDE CL was a non-irritant in guinea pigs (maximum weekly mean irritation index = 0).

The skin sensitization potential of BIOPEPTIDE CL was studied using 30 guinea pigs (ages not stated) in the maximization test.[25] 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 non-treatment 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-h application period. Challenge reactions were evaluated at 24 h and 48 h 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 no cutaneous reaction attributable to the sensitization potential of BIOPEPTIDE- CL at the maximal non-irritant concentration of 75% was observed in guinea pigs.

BIOPEPTIDE EL (contains 100 ppm palmitoyl oligopeptide, as Pal-Val-Gly-Val-Ala-Pro-Gly-OH) was evaluated in a skin irritation study involving 3 male New Zealand White rabbits (ages not stated).[32] A dry compress containing the test substance was applied (0.5 ml on 6 $cm^2$ area, clipped free of hair) for 4 h under a semi-occlusive dressing. Reactions were scored at 24 h, 48 h, and 72 h post-removal. Moderate cutaneous reactions (erythema, but no edema) were observed, and these reactions were reversible within 24 h or 48 h. Cutaneous reactions were not observed on days 3 and 4. BIOPEPTIDE EL was considered a non-irritant (mean erythema score < 1.0).

**Human**

**Palmitoyl Tripeptide-1 (Pal-Gly-His-Lys)**

The skin irritation potential of the ingredient MAXI-LIP (contains 1,000 ppm palmitoyl oligopeptide, as Pal-Gly-His-Lys-OH) was evaluated using 10 healthy adult volunteers.[28] The ingredient was applied to dorsal skin (~ 0.02 ml on 50 $mm^2$ area), using an occlusive patch (Finn chamber on Scanpor), for 48 h. Untreated sites (covered with occlusive patch) served as negative controls. Reactions were scored 30 min after patch removal. Neither irritation nor significant cutaneous intolerance was observed (primary irritation index [PII] = 0). There was also no evidence of a secondary effect. MAXI-LIP was classified as "very well tolerated".

The skin sensitization potential of MAXI-LIP was evaluated in a human repeated insult patch test (HRIPT) using 52 subjects.[33] 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-h induction applications. Non-treatment periods during the induction phase were described as 24 h following each Tuesday and Thursday patch removal and 48 h following each Saturday removal. The challenge phase was initiated following a 2-week non-treatment period. Challenge patches were applied for 24 h to a new test site that was adjacent to the induction patch site. Reactions were scored 24 h and 72 h 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 MAXI-LIP did not indicate a clinically significant potential for dermal irritation or allergic contact sensitization.

**Palmitoyl Hexapeptide-12 (Pal-Val-Gly-Val-Ala-Pro-Gly)**

The ingredient DERMAXYL (contains 200 ppm palmitoyl oligopeptide, as Pal-Val-Gly-Val-Ala-Pro-Gly-OH) was evaluated for skin irritation potential using 10 adult volunteers.[29] A single 48-h application of the test substance (diluted to 50%) was made, under an occlusive patch, to dorsal skin. Neither irritation nor significant cutaneous intolerance was observed (primary irritation index [PII] = 0). There was also no evidence of a secondary effect. Diluted Dermaxyl was considered very well tolerated.

An HRIPT on DERMAXYL was performed using 53 healthy adult volunteers.[34] The test substance was diluted to a concentration of 50% prior to application. The test procedure involved 48-h occlusive patch applications of the diluted test substance (area of application not stated). Eight induction applications were made, followed by challenge patch application. Neither skin irritation (mean irritation index[induction] = 0.04) nor sensitization indicative of cutaneous intolerance was observed.

**Manganese Tripeptide-1 (Gly-His-Lys-$Mn^{2+}$)**

The use of manganese tripeptide-1 in the treatment of various signs of cutaneous facial photodamage was evaluated using 14 female subjects (40 to 70 years old) with moderate photodamage and hyperpigmentation of the face.[35] Individuals with a history of reactions to skin care products or who were undergoing concurrent topical

and/or systemic drug therapy for skin disorders were excluded from the study. All participants were required to discontinue use of retinoids, alpha and beta hydroxyl acids, and other topical skin care products. At 4 weeks prior to initiation of the study, the participants were required to discontinue direct facial sun exposure. A facial serum formulation containing manganese tripeptide-1 (formulated in a non-irritating facial serum base; concentration not stated) was applied by each subject twice daily for up to 12 weeks. The formulation was well tolerated. Only one of the 14 subjects had mild erythema, and there was one instance of tightness and drying associated with application of the formulation. According to the clinical evaluator, treatment with the manganese peptide complex produced a significant improvement in the appearance of mottled hyperpigmentation, sallowness, lentigines, and surface roughness/dryness.

**Palmitoyl Tetrapeptide-7 (Pal-Gly-Gln-Pro-Arg)**

The skin irritation and sensitization potential of Rigin™, a trade name mixture that contains 500 ppm palmitoyl tetrapeptide-7, was evaluated in an HRIPT involving 52 healthy male and female subjects (age range: 18 to 79 years).[36] The test material (0.2 ml) was applied to a 3/4" x 3/4" occlusive patch that was placed on the upper back, between the scapulae. During the induction phase, patches were applied (24 h) 3 times per week for a total of 9 induction applications. After a 2-week non-treatment period, a 24-h 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 h and 72 h post-application. It was concluded that results for the test material did not indicate a potential for dermal irritation or allergic contact sensitization.

**Other Skin Studies**

**Palmitoyl Tripeptide-1 (Pal-Gly-His-Lys)**

The anti-wrinkle effect, due to increased collagen synthesis, of palmitoyl tripeptide-1 (palmitoyl-Gly-His-Lys) was evaluated in a blind, vehicle-controlled test involving 15 female subjects (44 to 59 years old).[37] Essentially, wrinkles are due to reduced collagen-packing in the dermis. Both a cream containing the tripeptide (3 ppm) and a placebo cream were applied around the eye zones twice daily for 4 weeks. On days 0 and 28, skin replicas were taken on both sides of the face and analyzed using an image analysis system. The following measurements were made, and their variations analyzed with respect to day 0 and the placebo: 39% decrease in wrinkle length, 23% decrease in wrinkle depth, and a 17% decrease in overall skin roughness at the end of the 4-week period. The placebo cream had no significant effect. All differences between skin treated with the tripeptide versus the placebo cream were statistically significant.

Both a vehicle (not identified) and palmitoyl tripeptide-1 (palmitoyl-Gly-L-His-L-Lys, 4 ppm in vehicle) were applied to the skin of 23 healthy female volunteers for 4 weeks.[9] Skin layer thickness was monitored using ultrasound echography. A small, but statistically significant, increase in skin thickness (~ 4%, compared to vehicle alone) was observed at the site treated with palmitoyl tripeptide. This value was not considered negligible, because it was noted that the thinning of aging skin occurs at a rate of approximately 6% every 10 years.

**Palmitoyl Tripeptide-1 (Pal-Gly-His-Lys)**
**Palmitoyl Tetrapeptide-7 (Pal-Gly-Gln-Pro-Arg)**

The peptide palmitoyl oligopeptide, modeled on repair signaling sequences, has been marketed as a cosmetic ingredient that enhances skin rejuvenation.[38] The extracellular matrix (ECM) in the basement membrane that separates the epidermis from the dermis also serves as a mediator of receptor-induced interactions between cells, guiding growth and differentiation. Damage to the ECM leads to repair that is initiated through processes such as protein synthesis and cell differentiation and proliferation. Most of these functions are related to signaling by peptides that are released from the ECM to cells through cell membrane receptors. Over time, aged skin is characterized by decreased production of new collagen and increased proteolytic activity, resulting in increased collagen degradation. In senescent fibroblasts, there is decreased synthesis of type I collagen, and these cells proliferate at a much slower rate when compared to fibroblasts in young skin. Peptides modeled on repair signaling sequences have been claimed to be cosmetic ingredients that enhance skin rejuvenation.

An *in vivo* study on the skin rejuvenating effect of Matrixyl™ 3000 (palmitoyl oligopeptide + palmitoyl tetrapeptide-7 was performed.[2] Panel 1 (Matrixyl™ 3000 vs. placebo) consisted of 24 volunteers with a mean age of 56.1 years. The test substance and excipient were tested at a concentration of 3% in a cream formulation. Each cream formulation was applied to one-half of the face (on different sides) in the morning and at night for 2 months, in the absence of all other anti-wrinkle, reparative, restructuring, or regenerating products. Skin rejuvenation was assessed using profilometry and image analysis, photography, and cutometry. After 56 days, a statistically significant decrease in deep wrinkles and skin roughness resulted from application of Matrixyl™ 3000 ($p < 0.01$) when compared to results at day 0. For a similar comparison involving the excipient cream, there were no statistically significant differences in results at day 0 vs. those at day 56. Also, after 56 days, a statistically significant increase in skin elasticity and tone resulted from application of Matrixyl™ 3000 ($p < 0.01$) when compared to results at day 0.

### Immunosuppression and Hepatocellular Effects

**Tripeptide-1 (Gly-His-Lys)**

The immunosuppressive activity of the Gly-His-Lys tripeptide was evaluated using CBA mice and Wistar rats (animal numbers not stated).[39] The tripeptide (in sterile isotonic NaCl) was administered i.p. ten times at the following doses before, during and after immunization: 0.5, 1.5, 5, 50, 150 and 450 mg/kg. The dose volumes were 0.1 ml (mice) and 0.2 ml (rats), and the interval between doses was 24 h. The animals were killed one day after the last injection. Liver sections were examined morphologically and the mitotic index of hepatocytes was calculated. Sheep erythrocytes served as the antigen. Humoral response intensity was estimated by the number of antibody-producing cells in the spleen at 5 days after immunization. The delayed-type hypersensitivity (DTH) reaction in rats was assayed by the difference between the weights of regional (site of antigen administration) and contralateral (popliteal) lymph nodes and counts of nucleated cells in these lymph nodes. A marked increase in the mitotic index of hepatocytes was observed at doses of $\geq 1.5$ mg/kg. The 0.5 mg/kg dose had no effect on the mitotic index. Signs of liver degeneration were observed at doses of 150 and 450 mg/kg, and these changes were more pronounced at the higher dose. Doses of the tripeptide $\geq 1.5$ mg/kg also suppressed the humoral immune response; however, this effect was not observed at a dose of 0.5 mg/kg. This immunosuppressive effect was described as dose-dependent. The effects of the tripeptide on the DTH and humoral immune response were similar.

## CELLULAR EFFECTS

### Stimulation of Angiogenesis

**Palmitoyl Hexapeptide-12 (Pal-Val-Gly-Val-Ala-Pro-Gly)**

Palmitoyl Hexapeptide-12 (Pal-Val-Gly-Val-Ala-Pro-Gly), an elastin sequence, enhanced angiogenesis in the chick chorioallantoic membrane by promoting endothelial cell migration and tubulogenesis through upregulation of membrane-type metalloproteinase-1 (MT1-MMP), a matrix metalloproteinase (MMP).[40] In the *in vivo* angiogenesis assay, the chick chorioallantoic membrane was exposed to allow direct access. On day 6 of embryonic development, angiogenic areas were delimited with a silicon ring containing phosphate-buffered saline (PBS, control) or palmitoyl hexapeptide-12 (50 ng) in a final volume of 20 µl. Embryos were then placed in an incubator to induce spontaneous angiogenesis and were treated daily. Treated areas were photographed daily on days 6 to 10 of embryonic development.

**Hexapeptide-12 (Val-Gly-Val-Ala-Pro-Gly)**

*In vitro* organ culture with rings from normal human coronary artery was used to demonstrate the angiogenic role of the elastin peptide Val-Gly-Val-Ala-Pro-Gly in human vascular smooth muscle cells.[41] After 3 days in culture (Val-Gly-Val-Ala-Pro-Gly, 100 µg/ml), the vascular rings in the collagen gel containing elastin peptide elaborated metalloproteinase activity and sprouted and grew. The authors noted that these results suggest that Val-Gly-Val-Ala-Pro-Gly peptide generated at the site of proteolysis during vascular injury may have angiogenic activity.

## Stimulation of Collagen and Fibronectin Synthesis

**Palmitoyl Oligopeptide (peptide sequence not stated)**
**Palmitoyl Tripeptide-1 (Pal-Gly-His-Lys)**
**Palmitoyl Tetrapeptide-7 (Pal-Gly-Gln-Pro-Arg)**

Normal human fibroblasts were cultured in Dulbecco's modified eagle medium in the presence of fetal calf serum.[2] After cell confluence was achieved, the culture medium was replaced and the fibroblasts were incubated (without serum) for 72 h in the presence of vitamin C and palmitoyl oligopeptide (peptide sequence not stated, up to 7.5 ppm) or Matrixyl™ 3000 (palmitoyl oligopeptide + palmitoyl tetrapeptide-7) (up to 11 ppm). Control media consisted of the culture medium alone or with a positive control (transforming growth factor beta (TGFβ). Matrix proteins (collagen 1 and fibronectin) were assayed using the enzyme-linked immunosorbant assay (ELISA) method and hyaluronic acid was assayed using a colorimetric method. A dose response for collagen 1 synthesis was observed following incubation with Matrixyl™ 3000, but not palmitoyl oligopeptide. Matrixyl™ 3000 yielded values for collagen 1 synthesis greater than those that would be expected on the basis of simple addition. Incubation with the positive control resulted in 102% stimulation of collagen synthesis.

A dose response for *de novo* synthesis of fibronectin and hyaluronic acid was observed in the presence of Matrixyl™ 3000, but not palmitoyl oligopeptide. Matrixyl™ 3000 induced a 164% increase in fibronectin synthesis; this ingredient also stimulated hyaluronic acid synthesis by 179%, whereas the value for palmitoyl oligopeptide was 14%.[2]

**Palmitoyl Tripeptide-1 (Pal-Gly-His-Lys)**

The stimulation of collagen synthesis by palmitoyl tripeptide-1 (pamitoyl-Gly-L-His-L-Lys) in human fibroblasts *in vitro* was studied.[9] Collagen synthesis was monitored by the incorporation of tritiated proline, considered to be a strong signal of collagen synthesis. Results indicated that this strong signal of collagen synthesis was observed at a concentration of 0.5 µM/liter. In another experiment, skin samples (human biopsies [abdominal tissue]) from plastic surgery were irradiated with daily doses of UVA light for one week. Microscopic examination revealed strong degradation of dermal collagen. Following irradiation, the skin samples were treated with retinoic acid (500 ppm) or palmitoyl tripeptide (5 ppm) during the same week. Treatment with either compound resulted in almost total preservation and/or recovery of high density of collagen in the skin samples.

## Cell Proliferation

**Tripeptide-1(Gly-His-Lys)**

It has been found that the human plasma tripeptide**,** glycyl-L-histidyl-L-lysine alters the growth rate or state of differentiation of a wide variety of cultured cells and organisms (e.g., hepatocytes, neurons, mycoplasma, fungi, and *Ascaris* larvae), and may mediate a functional or nutritional need that is common to diverse organisms.[42,43,44,45] Studies on the growth-promotion activity of glycyl-L-histidyl-L-lysine are summarized below.

## Effect on Growth Factor Production

**Copper Tripeptide-1 (Gly-His-Lys-$Cu^{2+}$)**

The effect of copper tripeptide-1 on normal and keloid-producing dermal fibroblasts was studied using a serum-free *in vitro* model.[46] Primary cultures of dermal fibroblasts were established from excisional biopsies of 3 different keloid and 3 different normal facial skin specimens obtained from 5 patients. Copper tripeptide-1 was added to cultures at a concentration of $1 \times 10^{-9}$ mol/L. The cellular response was described in terms of viability and secretion of basic fibroblast growth factor (bFGF) and transforming growth factor-β1 (TGF-β1). Cell viability was between 86% and 96% (mean = 92%) throughout the experiment. Phosphate-buffered saline served as the vehicle control. No different bFGF secretion pattern was observed in normal fibroblasts or keloid-producing fibroblasts when compared to controls. At 24 h, normal and keloid-producing dermal fibroblasts treated with copper tripeptide-1 secreted less TGF-β1when compared to controls ($p < 0.05$), suggesting a possible clinical use for decreasing excessive scar formation.

## Chemotactic Activity

### Hexapeptide-12 (Val-Gly-Val-Ala-Pro-Gly)

The extracellular matrix contains elastic fibers, which provide elasticity and resilience to tissues that require the ability to deform repetitively and reversibly. The valine-glycine-valine-alanine-proline-glycine hexapeptide (Val-Gly-Val-Ala-Pro-Gly or VGVAPG) is known for its chemotactic activity against monocytes, fibroblasts, and tumor cells.[47,48,49]

The Val-Gly-Val-Ala-Pro-Gly peptide (VGVAPG), amino acid sequence is a repeating peptide in tropoelastin, and study results have demonstrated that tropoelastin and elastin-derived peptides are chemotactic for fibroblasts and monocytes.[50,51,52] A study was performed to identify the chemotactic activity of VGVAPG using fetal bovine ligament nuchae fibroblasts and human mononuclear peripheral blood cells.[47] Chemotaxis was assayed using a double micropore membrane system in modified Boyden chambers. Study results indicated that VGVAPG was chemotactic for fibroblasts and monocytes, and optimal activity was noted at a concentration of ~$10^{-8}$ M. The authors noted that the following results support the possibility that at least part of the chemotactic activity of tropoelastin and elastin peptides is contained in VGVAPG sequences: (1) polyclonal antibody to bovine elastin selectively blocked the fibroblast and monocyte chemotactic activity of both elastin-derived peptides and VGVAPG; (2) monocyte chemotaxis to VGVAPG was selectively blocked by preexposing the cells to elastin peptides; and (3) undifferentiated (nonelastin producing) bovine ligament fibroblasts, capable of chemotaxis to platelet-derived growth factor, did not show chemotactic responsiveness to either VGVAPG or elastin peptides until after matrix-induced differentiation and the onset of elastin synthesis.

## Enzyme Upregulation/Release

### Hexapeptide-12 (Val-Gly-Val-Ala-Pro-Gly)

The valine-glycine-valine-alanine-proline-glycine hexapeptide is also known for its ability to activate metalloproteinases.[53,54]

Soluble kappa-elastin peptides have been shown to stimulate the expression of matrix metalloproteinase-2 (gelatinase A, MMP-2), but not metalloproteinase-9 (MmP-9) by human fibrosarcoma HT-1080 cells, both at the protein and mRNA levels; the maximal effect was observed at 25 µg/ml of kappa-elastin.[53] The valine-glycine-valine-alanine-proline-glycine hexapeptide was found to mimick this stimulatory effect on kappa elastin MMP-2 secretion, described as 1.6-fold over the control value, at a concentration of 200 µg/ml.

The treatment of cultured human skin fibroblasts with tropoelastin or with heterogenic peptides, obtained after organo-alkaline or leukocyte elastase hydrolysis of insoluble elastin, induced a high expression of pro-collagenase-1 (pro-matrix metalloproteinase-1 (pro-MMP-1)).[54] The identical effect was achieved after stimulation with a valine-glycine-valine-alanine-proline-glycine synthetic hexapeptide (200 µg/ml).

The effects of the Val-Gly-Val-Ala-Pro-Gly hexapeptide on polymorphonuclear leukocytes (PMNLs) were studied *in vitro*.[55] PMNLs were obtained from 20 healthy volunteers (< 30 years old). Val-Gly-Val-Ala-Pro-Gly is a repeated sequence in the elastin molecule, and polymorphonuclear leukocyte (PMNL) stimulation by elastin peptides results in several responses that are normally associated with inflammation, such as, migration, aggregation, degranulation, and generation of oxygen radicals. The results of this study indicated that, when compared to non-treated cells, Val-Gly-Val-Ala-Pro-Gly stimulated superoxide anion production ($p < 0.001$); 2.5 x $10^{-5}$ M was the most effective concentration. Val-Gly-Val-Ala-Pro-Gly also had the following effects: stimulatory effect on $H_2O_2$ production ($p < 0.01$), when compared to the non-stimulated basic value, significantly ($p < 0.05$) enhanced the release of elastase, significantly ($p < 0.01$) increased intracellular free calcium ($Ca^{++}$), when compared to the basic value of 6 µmol/min, and significantly ( $p < 0.01$) increased the release of myeloperoxidase (enzyme of neutrophilic granulocytes) activity.

### Effect on Cell Adhesion

**Hexapeptide-12 (Val-Gly-Val-Ala-Pro-Gly)**

The synthetic peptide Val-Gly-Val-Ala-Pro-Gly inhibited the adhesion of freshly isolated rabbit vascular smooth muscle cells to α-elastin at concentrations of 0.01 to 1 mM.[10] There was no inhibitory activity on the adhesion of cells in the synthetic state at a concentration of 0.01 mM. It was noted that, during the early stages of atherosclerosis, arterial smooth muscle cells undergo a transition from a contractile to a synthetic phenotype that is characterized by the loss of myofilaments and the formation of extensive rough endoplasmic reticulum and a large Golgi complex.

## OTHER EFFECTS

### Effect on Wound Healing

**Copper Tripeptide-1 (Gly-His-Lys-$Cu^{2+}$)**

Glycyl-L-histidyl-L-lysine-$Cu^{2+}$ is a growth factor that has been isolated from human plasma. The peptide portion of this complex has an amino acid structure that is similar to the copper ion transport site on human albumin, and, thus, has an affinity for copper(II) that is equivalent to that of the copper transport site on albumin.[56] This peptide sequence is that of the peptide moiety of palmitoyl oligopeptide (Pal-GHK).

The term, matrikine is proposed to designate extracellular matrix-derived peptides that regulate connective tissue cell activity, and glycyl-histidyl-lysine complexed with copper is a well-studied matrikine.[57] The expression and activation of matrix metalloproteinases in a model of experimental wound healing in rats and their modulation by glycyl-L-histidyl-L-lysine-Cu(II), a potent activator of wound repair, were investigated using groups of 6 male Sprague-Dawley rats.[58] The rats were anesthetized by i.p. injection with sodium pentobarbital (40 mg/kg), and dorsal skin was clipped free of hair. Full thickness skin incisions were made perpendicular to the spine through the panniculus carnosus to the fascial plane. Wound chambers were inserted under the skin, and the incisions were closed. Glycyl-L-histidyl-L-lysine-$Cu^{2+}$ (2 g, in phosphate-buffered saline) (3 test groups) or the same volume of saline (3 control groups) was injected serially into the chambers. The animals were killed on day 3, 7, 12, 18, or 22 after chamber implantation. Wound fluid was immediately collected by aspiration with a 1-ml syringe, centrifuged, and then stored until zymography. The solid material deposited in the chambers was collected and remained frozen until the time of analysis. The wound fluid and the neosynthetized connective tissue deposited in the chambers were collected and analyzed for matrix metalloproteinase expression and/or activity. For histologic examination, wound chambers were immediately immersed, after collection in 10% formalin (in phosphate-buffered saline). After fixation of chamber contents (no specific definition of tissues), tissues were embedded in paraffin and 4 µm thick sections were stained.

Throughout the experiment, interstitial collagenase activity increased progressively in the wound fluid; L-histidyl-L-lysine-$Cu^{2}$ treatment did not alter the activity of this enzyme. Matrix metalloproteinase-9 (gelatinase B) and matrix metalloproteinase-2 (gelatinase A) were the 2 main gelatinolytic activities expressed during the healing process. During the early stages of wound healing (day 3) pro-matrix metalloproteinase (pro-form of matrix metalloproteinase-9) was strongly expressed. In the wound fluid, pro-matrix metalloproteinase decreased rapidly and disappeared after day 18. Pro-matrix metalloproteinase-2 was expressed at low levels at the beginning of the healing process, having increased progressively until day 7 and decreased until day 18. In the wound tissue, matrix metalloproteinase-9 expression persisted in the glycyl-L-histidyl-L-lysine-$Cu^{2+}$-injected chamber until day 22. Activated matrix metalloproteinase-2 was present in the wound fluid and wound tissue, having increased until day 12 and then decreased progressively. Glycyl-L-histidyl-L-lysine-$Cu^{2+}$ injections increased pro-matrix metalloproteinase-2 and activated matrix metalloproteinase-2 during the later stages of healing (days 18 and/or 22). Together with biochemical analysis, histologic examination of chamber contents confirmed that glycyl-L-histidyl-L-lysine-Cu(II) injection increased cell invasion and extracellular matrix deposition in the wound chambers. The authors noted that these study results demonstrated that various types of matrix metalloproteinases are selectively expressed or activated at various periods of wound healing. They also noted that glycyl-L-histidyl-L-lysine-$Cu^{2+}$ modulated the expression of these enzymes and may, thereby, significantly affect wound remodeling.[58]

## 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 reviewed in this safety assessment were not found in the published literature.

## GENOTOXICITY

### Palmitoyl Tripeptide-1 (Pal-Gly-His-Lys)

Ames test results for palmitoyl tripeptide-1 (MAXI-LIP and BIOPEPTIDE-CL trade name materials) were negative with and without metabolic activation in *Salmonella typhimurium* bacterial strains.

The genotoxicity of MAXI-LIP (contains 1,000 ppm palmitoyl oligopeptide, as Pal-Gly-Lys-OH) was evaluated in the Ames test, with and without metabolic activation, using the following *Salmonella typhimurium* strains: TA98, TA100, TA1535, and TA1538.[59] The test material (0.1 ml in ethanol solution) was non-genotoxic. In another assay, the genotoxicity of BIOPEPTIDE-CL (contains 100 ppm palmitoyl oligopeptide, as Pal-Gly-His-Lys-OH) was evaluated, with and without metabolic activation, using the following *Salmonella typhimurium* strains: TA98, TA102, TA1535, and TA1537.[60] At doses up to 5,000 µg/plate, the test material was classified as non-genotoxic.

### Palmitoyl Tetrapeptide-7 (Pal-Gly-Gln-Pro-Arg)

The Ames test was used to evaluate the genotoxicity of Rigin™, a trade name mixture that contains 500 ppm palmitoyl tetrapeptide-7palmitoyl tetrapeptide-7, in the following *Salmonella typhimurium* strains: TA98, TA100, TA1535, TA1537, and TA1538.[61] The test material (1 ml, in 9 ml of DMSO) was evaluated with and without metabolic activation. The test material was considered non-mutagenic in all bacterial strains.

### Effect on DNA

### Tripeptide-1(Gly-His-Lys)

The effects of glycyl-L-histidyl-L-lysine on Morris hepatoma 7777 cells were studied. The cells were incubated with glycyl-L-histidyl-L-lysine at concentrations ranging from 0.2 ng/ml to 20 ng/ml.[62] A glycyl-L-histidyl-L-lysine concentration of 2 ng/ml had the greatest stimulatory effect on $^{3}$H-thymidine and $^{3}$H-leucine incorporation. The incorporation of $^{3}$H-thymidine into DNA in randomly proliferating cells increased by 50%. Also, in randomly proliferating cells, the incorporation of $^{3}$H-leucine into protein increased by 29%. Additionally, synergistic effects were noted when insulin and glucagon were included in the incubation mixture along with glycyl-L-histidyl-L-lysine. The results of experiments involving cells rendered quiescent by serum starvation indicated that cells in the G1 phase of the cell cycle were more sensitive to glycyl-L-histidyl-L-lysine stimulation. Also, in experiments involving quiescent cells, $^{3}$H-thymidine incorporation increased earlier and peaked at a higher value when compared to control cells. The authors noted that this finding suggests that glycyl-L-histidyl-L-lysine may play a role in stimulating quiescent cells to re-enter the cell cycle.

### Gene Activation

### Palmitoyl Tripeptide-1 (Pal-Gly-His-Lys)

Reportedly, molecular biology methods have enabled access to intracellular, functional, and morphological changes induced by substances after cell layer (fibroblasts or keratinocytes) or tissue (epidermis and synthetic epidermis) exposure.[2] With this in mind, it is possible to define the profile of the method of action of a substance in relation to the genes activated or repressed, and compare the findings with those for a control cell culture or tissue. The gene activation profile for palmitoyl oligopeptide (Pal-glycine-histidine-lysine) has been determined using a bank of 450 genes. Palmitoyl oligopeptide activated few genes, however, its profile was more specifically oriented

toward keratinocyte anchoring (alpha-catenin and laminin receptor) and differentiation (keratin 10). Additionally, this oligopeptide increased the synthesis of extracellular matrix (syndecan and heparin sulfate glycoprotein). The profile characterized by the genes activated in fibroblasts indicated that palmitoyl oligopeptide stimulated numerous genes. Additional details were not provided.[2]

## CARCINOGENICITY

Data on the carcinogenicity of tripeptide-1, hexapeptide-12, their metal salts and fatty acyl derivatives, and palmitoyl tetrapeptide-7 reviewed in this safety assessment were not found in the published literature.

### Effect on Normal and Cancer Cell Growth

**Tripeptide-1 (Gly-His-Lys)**

Glycyl-L-histidyl-L-lysine was studied to determine its growth-promoting potential using human KB cells (subline of human HeLa tumor cell line), HeLa cells, and WI-38 cells (human diploid cell line, derived from normal embryonic lung tissue) in serum-free medium, serum-limited medium (dialyzed fetal calf serum [DFCS]), and cell medium supplemented with bovine serum albumin (BSA).[63] Glycyl-L-histidyl-L-lysine stimulated the growth of KB and HeLa cells, but not WI-38 cells. When compared to cells grown in serum-free medium, there was no significant difference in the cellular growth ratio between cells grown in media supplemented with glycyl-L-histidyl-L-lysine or BSA. However, when a combination of BSA and GHL was present in the 0.5% DFCS medium, the growth-promoting activity of GHL was observed. The rate of growth of cells in the serum-limited medium containing BSA and glycyl-L-histidyl-L-lysine was not significantly different when compared to cells grown in medium containing 5% DFCS. The concentration of glycyl-L-histidyl-L-lysine that was required for optimal growth of cells in serum-limited medium containing BSA (6 mg/l) was in the range of 250 to 500 ng/ml. The concentration of BSA that was required for optimal growth in serum-limited media containing glycyl-L-histidyl-L-lysine (500 ng/ml) was 6 mg/ml. BSA concentrations of > 6 mg/ml caused a decrease in the growth-promoting activity of the medium.

### Chemotactic Activity and Metastasis

**Hexapeptide-12 (Val-Gly-Val-Ala-Pro-Gly)**

Tumor cell interactions with elastin and implications relating to pulmonary metastasis were studied using tumor cell lines of murine origin, namely, M27 Lewis lung carcinoma cells and H59 Lewis lung carcinoma cells.[48] Elastin surrounds microvessels in the pulmonary circulation and may pose a barrier to the extravasation of metastatic tumor cells. Lung-colonizing murine melanoma cells are the source of enzymatic activity that degrades elastin, and, additionally, the elastin fragments liberated by enzymatic digestion of insoluble elastin stimulate tumor cell chemotaxis. The results of this study indicated that Val-Gly-Val-Ala-Pro-Gly, a synthetic peptide that is a repeat sequence in the elastin molecule, displayed tumor cell chemotactic activity. It was postulated that the ability to migrate in response to elastin fragments may facilitate tumor cell invasion of elastin-rich pulmonary tissue.

In another study, it was noted that the M27and H59 variants of Lewis lung carcinoma differ in their responsiveness to Val-Gly-Val-Ala-Pro-Gly.[49] M27 cells, selected for metastasis to the lung, are highly responsive to a positive gradient of Val-Gly-Val-Ala-Pro-Gly. H59 cells, selected for metastasis to the liver, do not migrate in response to Val-Gly-Val-Ala-Pro-Gly.

## SUMMARY

The safety of tripeptide-1 and hexapeptide-12, and related amides in cosmetics is reviewed in this safety assessment.

The ingredients reviewed in this safety assessment function primarily as skin conditioning agents in cosmetic products. According to information supplied to the FDA by industry as part of the VCRP in 2014, the following palmitoyl oligopeptides are being used in cosmetic products: palmitoyl oligopeptide (name retired, peptide sequence not stated) palmitoyl tripeptide-1, tripeptide-1, and copper tripeptide-1. The peptide sequence for palmiotoyl oligopeptide is not stated in the VCRP database or in the Personal Care Product Council's survey of ingredient use concentrations; however, the sequence could be either gly-his-lys (tripeptide-1) or valine-glycine-valine-alanine-proline-glycine (hexapeptide-12).

Results from a survey of ingredient use concentrations provided by the Council in 2013 indicate that, collectively, the ingredients reviewed in this safety assessment are being used at concentrations ranging from 0.0000001% (palmitoyl tripeptide-1and palmitoyl hexapeptide-12) to 1% (palmitoyl tripeptide-1). The highest concentration of 1% relates to ingredient use in leave-on products. In addition to the data included in the survey of ingredient use concentrations, additional information shows 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.[13]

The peptide sequences in ingredients reviewed in this safety assessment have been produced by solid phase synthesis.

The impurities content of both palmitoyl tripeptide-1 and palmitoyl oligopeptide hexapeptide-12 has been described as follows: acetate (< 5%), palmitic acid (< 5%), and water (< 5%). Commercial glycyl-L-histidyl-L-lysine-$Cu^{2+}$ (copper tripeptide-1) is approximately 95% pure, but often includes small amounts of mildly neurotoxic materials. Most of the neurotoxic materials can be removed by dissolving glycyl-L-histidyl-L-lysine in glass-distilled water (50 mg/ml), centrifuging at 20,000 g for 1 h at 3˚, and then lyophilizing the supernatant.

After i.v. 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. In an enzyme assay, the liver growth factor tripeptide-1 was hydrolyzed by an aminotripeptidase purified from rat brain cytosol.

BIOPEPTIDE-CL (contains 100 ppm palmitoyltripeptide-1) was nontoxic (LD50 > 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 BIOPEPTIDE-CL and 75% BIOPEPTIDE-CL involving guinea pigs.

BIOPEPTIDE-CL (contains 100 ppm palmitoyl tripeptide-1) was slightly irritating to the eyes of rabbits. BIOPEPIDE EL (contains 100 ppm palmitoyl hexapeptide-12) was non-irritating to the eyes of rabbits. In the hen's egg chorioallantoic membrane *in vitro* assay for evaluating ocular irritation potential, MAXI-LIP (contains 1,000 ppm palmitoyl tripeptide-1) was classified as an irritant, DERMAXYL (contains 200 ppm palmitoyl hexapeptide-12) was practically non-irritating, and Rigin™ (contains 500 ppm palmitoyl tetrapeptide-7) was slightly irritating. In the *in vitro* neutral red release assay for evaluating ocular irritation potential, DERMAXYL caused "unimportant cytotoxicity".

In skin irritation studies (single application) involving rabbits, BIOPEPTIDE CL and BIOPEPTIDE EL were classified as non-irritants. BIOPEPTIDE CL was also classified as a non-irritant in a cumulative skin irritation study involving guinea pigs. BIOPEPTIDE CL did not induce skin sensitization at a challenge concentration of 75% in the maximization test.

In human skin irritation studies (single application), MAXI-LIP and DERMAXYL (50%) were classified as non-irritants. HRIPT results for MAXI-LIP, DERMAXYL (50%), and Rigin™ (contains 500 ppm palmitoyl tetrapeptide-7) were negative for skin irritation and sensitization.

A facial serum formulation containing manganese tripeptide-1 was applied by each of 14 subjects with moderate photodamage and hyperpigmentation twice daily for up to 12 weeks. The formulation was well tolerated; one subject had mild erythema.

A cream containing 3 ppm palmitoyl tripeptide-1) was applied around the eyes of 15 female subjects twice daily for 4 weeks. Application resulted in a statistically significant anti-wrinkle effect, in that decreased wrinkle length, and depth and a decrease in overall skin roughness were observed. The application of palmitoyl tripeptide-1 (4 ppm in vehicle) to the skin of 23 female subjects for 4 weeks caused a statistically significant increase (4%) in skin thickness. A study evaluating the skin rejuvenating effect of Matrixyl™ 3000 (palmitoyl tripeptide-1+ palmitoyl tetrapeptide-7) was performed using 24 subjects. The cream formulation was applied to the face twice daily for 2 months. A statistically significant decrease in both deep wrinkles and skin roughness and a statistically significant increase in skin elasticity and tone were reported.

Dose-dependent suppression of the humoral immune response was observed in CBA mice and Wistar rats at i.p. doses of $\geq$ 1.5 mg/kg tripeptide-1. The doses tested ranged from 0.5 to 450 mg/kg.

The stimulation of collagen synthesis by palmitoyl tripeptide-1 in human fibroblasts *in vitro* was studied. A strong signal of collagen synthesis was noted at a concentration of 0.5 µM/liter. In the same study, human skin samples were irradiated with daily doses of UVA light for one week, resulting in degradation of dermal collagen. Treatment with palmitoyl tripeptide-1 (5 ppm) during the same week caused almost total preservation and/or renewal of collagen. In another study, normal human fibroblasts were incubated in the presence of vitamin C and palmitoyl oligopeptide (up to 7.5 ppm) or palmitoyl oligopeptide + palmitoyl tetrapeptide-7 (up to 11 ppm]). A dose response for collagen 1 synthesis and the *de novo* synthesis of fibronectin and hyaluronic acid was not observed .

Palmitoyl hexapeptide-12 enhanced angiogenesis in the chick chorioallantoic membrane (in an *in vivo* model) by promoting endothelial cell migration and tubulogenesis through upregulation of membrane-type metalloproteinase-1 (MT1-MMP), a matrix metalloproteinase. Results from an *in vitro* assay using human vascular smooth muscle cells suggested that hexapeptide-12 may have angiogenic activity. After 3 days in culture, the vascular rings in the collagen gel containing the peptide elaborated metalloproteinase activity, sprouted, and grew. According to another study, various types of matrix metalloproteinases are selectively expressed or activated during various periods of wound healing. Other peptide-induced cellular effects were as follows: stimulation of collagen synthesis (palmitoyl oligopeptide and palmitoyl tripeptide-1), alteration of growth rate or state of differentiation of hepatocytes and neurons (tripeptide-1), reduced secretion of human dermal fibroblast growth factors (copper tripeptide-1), chemotactic activity for fetal bovine ligament nuchae fibroblasts and human monocytes (hexapeptide-12), stimulation of pro-collagenase-1 expression in human skin fibroblasts (hexapeptide-12), and stimulation of elastase and myeloperoxidase release from human polymorphonuclear leukocytes (hexapeptide-12).

Ames test results for palmitoyl tripeptide-1 (MAXI-LIP and BIOPEPTIDE-CL trade name materials) and Rigin™ (contains 500 ppm palmitoyl tetrapeptide-7) were negative with and without metabolic activation in *Salmonella typhimurium* bacterial strains. In another assay, a tripeptide-1 concentration of 2 ng/ml had the greatest stimulatory effect on $^{3}$H-thymidine and $^{3}$H-leucine incorporation into the DNA of proliferating Morris hepatoma 7777 cells. The gene activation profile for palmitoyl tripeptide has been determined using a bank of 450 genes. Palmitoyl tripeptide-1 activated few genes; however, its profile was more specifically oriented toward keratinocyte anchoring (alpha-catenin and laminin receptor) and differentiation (keratin 10). Additionally, palmitoyl tripeptide-1 increased the synthesis of extracellular matrix (syndecan and heparin sulfate glycoprotein).

Data on the carcinogenicity or reproductive and developmental toxicity of the ingredients reviewed in this safety were not found in the published literature. However, data from other studies indicated that tripeptide-1 stimulated the growth of human KB and HeLa tumor cells, but not normal human WI-38 cells, and that hexapeptide-12 displayed tumor cell chemotactic activity, which may facilitate metastasis.

## DISCUSSION

Use concentration data provided indicate that the ingredients reviewed in this safety assessment are being used at concentrations up to 1%, a value reported for palmitoyl tripeptide-1 in leave-on products. Information substantiating the use of peptides at concentrations between 1 ppm and 30 ppm in cosmetic products, and use at concentrations of < 10 ppm, as customary, 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

includes 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 also noted that this safe conclusion is applicable only to ingredient names associated with the following known peptide sequences: Glycine-Histidine-Lysine (Gly-His-Lys or GHK), Valine-Glycine-Valine-Alanine-Proline-Glycine (Val-Gly-Val-Ala-Pro-Gly or VGVAPG), and Glycine-Glutamine-Proline-Arginine (Gly-Gln-Pro-Arg or GQPR).

Palmitoyl oligopeptide (name retired, sequence unstated) is used in body and hand sprays (maximum use concentration = 0.001%). Because this ingredient, which is actually either palmitoyl tripeptide-1 or palmitoyl hexapeptide-12, 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. The 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% – 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

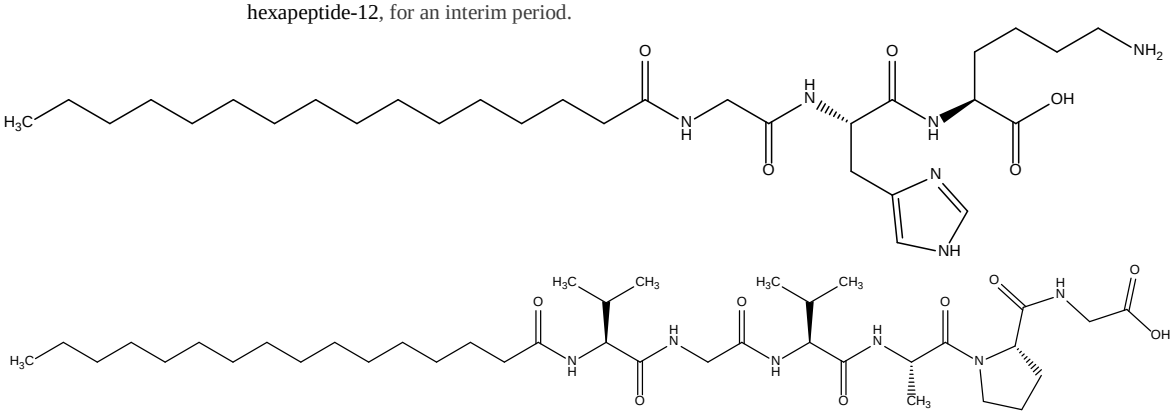
The CIR Expert Panel concluded that the following cosmetic ingredients are safe in the present practices of use and concentration in cosmetics, as described in this safety assessment.

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

*Not 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.

**Table 1.** Definitions, structures and functions of the ingredients in this safety assessment.[1, CIR staff]

| Ingredient Name and CAS No. | Definition & Structure | Function |
|---|---|---|
| Palmitoyl Oligopeptide [171263-26-6 and 147732-56-7] | 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, *but not ala-pro-gly-val-gly-val.*<br><br>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-1 or palmitoyl hexapeptide-12, for an interim period. | Skin-Conditioning Agents - Miscellaneous; Surfactants - Cleansing Agents |
| Tripeptide-1 [1269107-24-5] | Tripeptide-1 is the synthetic peptide consisting of gly-his-lys. | Skin Protectants; Skin-Conditioning Agents - Miscellaneous |



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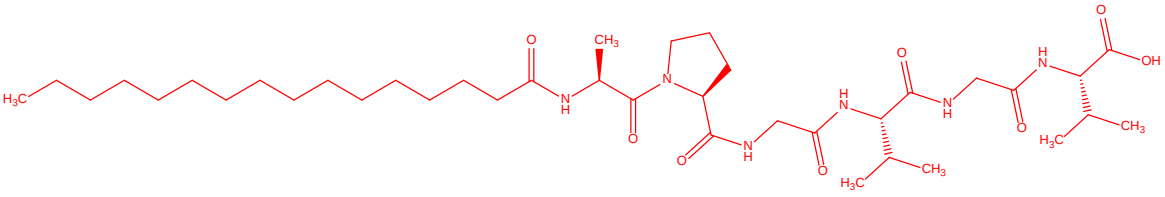


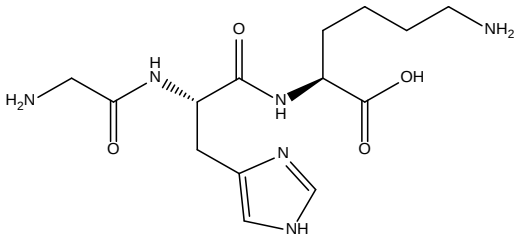


**Table 1.** Definitions, structures and functions of the ingredients in this safety assessment.[1, CIR staff]

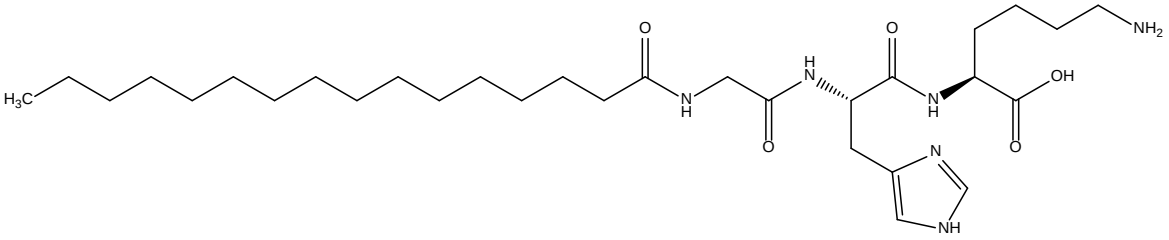
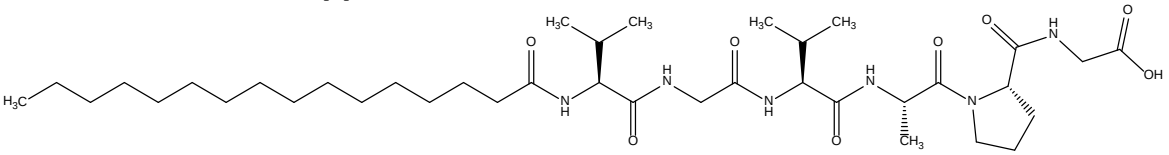
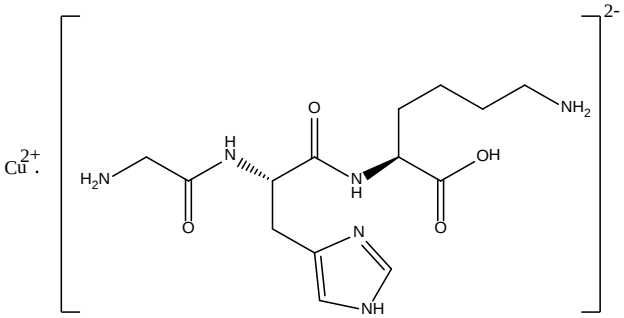
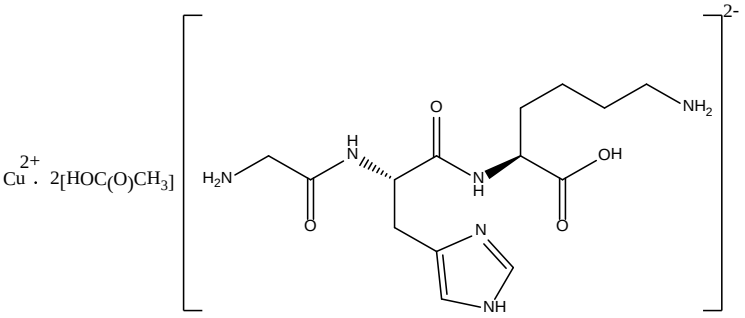
| Ingredient Name and CAS No. | Definition & Structure | Function |
|---|---|---|
| Palmitoyl Tripeptide-1 | Palmitoyl Tripeptide-1 is the reaction product of palmitic acid and tripeptide-1.  | Skin-Conditioning Agents - Miscellaneous |
| Palmitoyl Hexapeptide-12 | Palmitoyl Hexapeptide-12 is the product of the reaction of palmitic acid and hexapeptide-12.  | Antioxidants |
| Copper Tripeptide-1 [89030-95-5] | Copper Tripeptide-1 is a complex formed by copper and tripeptide-1.  | Skin-Conditioning Agents - Miscellaneous |
| Bis(Tripeptide-1) Copper Acetate [130120-57-9] | Bis(Tripeptide-1) Copper Acetate is acetate salt of the product of the reaction of tripeptide-1 with copper chloride.  | Skin-Conditioning Agents - Miscellaneous |

**Table 1.** Definitions, structures and functions of the ingredients in this safety assessment.[1, CIR staff]

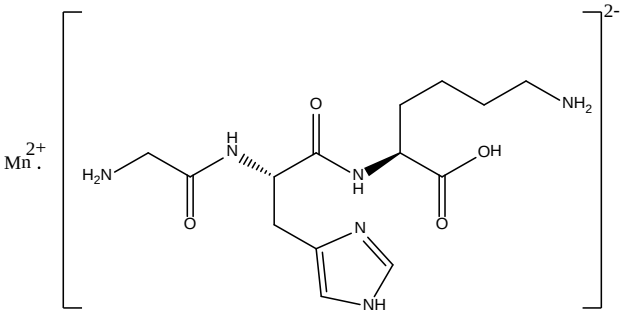
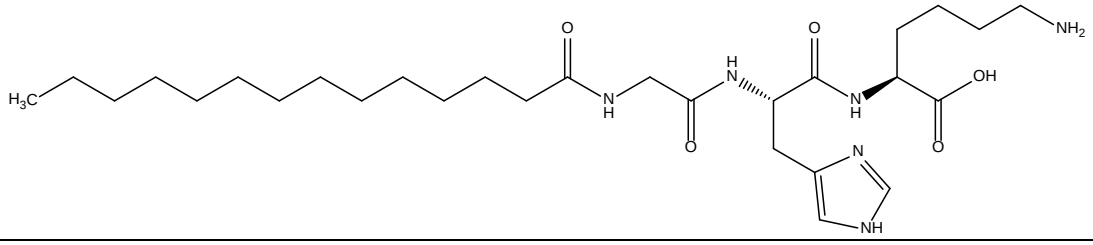
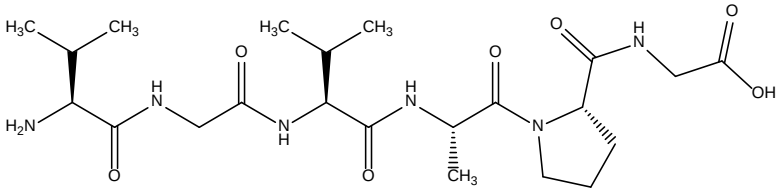
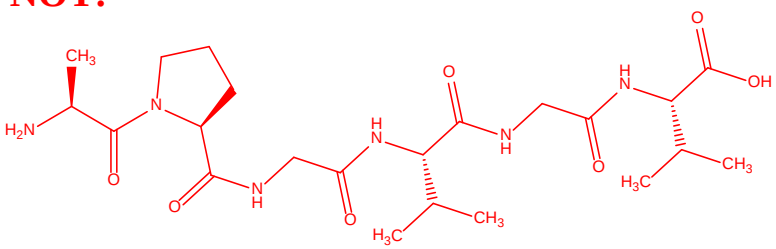
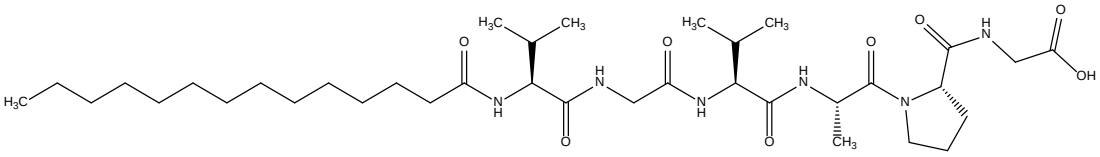
| Ingredient Name and CAS No. | Definition & Structure | Function |
|---|---|---|
| Manganese Tripeptide-1 [611182-15-1] | Manganese Tripeptide-1 is a complex of manganese and tripeptide-1.  | Skin-Conditioning Agents - Miscellaneous |
| Myristoyl Tripeptide-1 | Myristoyl Tripeptide-1 is the product obtained by the reaction of myristic acid and tripeptide-1.  | Skin-Conditioning Agents - Miscellaneous |
| Hexapeptide-12 | Hexapeptide-12 is the synthetic peptide consisting of either val-gly-val-ala-pro-gly, *but not ala-pro-gly-val-gly-val.*  **NOT:**  | Skin-Conditioning Agents - Miscellaneous |
| Myristoyl Hexapeptide-12 | Myristoyl Hexapeptide-12 is the reaction product of myristic acid and hexapeptide-12.  | Skin-Conditioning Agents - Miscellaneous |

**Table 1.** Definitions, structures and functions of the ingredients in this safety assessment.[1, CIR staff]

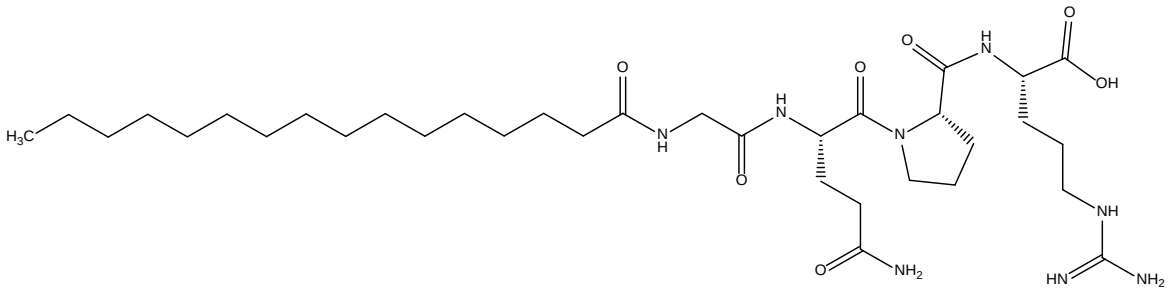
| Ingredient Name and CAS No. | Definition & Structure | Function |
|---|---|---|
| Palmitoyl Tetrapeptide-7 | Palmitoyl Tetrapeptide-7 is the reaction product of palmitic acid and tetrapeptide-7, *wherein tetrapeptide-7 is the synthetic peptide consisting of gly-gln-pro-arg.*  | Skin-Conditioning Agents - Miscellaneous |

**Table 2**. Current Frequency and Concentration of Use According to Duration and Type of Exposure[12,64,65]

| | **Palmitoyl Oligopeptide (no sequence)** | | **Palmitoyl Tripeptide-1** | | **Palmitoyl Hexapeptide-12** | |
|---|---|---|---|---|---|---|
| | # of Uses | Conc. (%) | # of Uses | Conc. (%) | # of Uses | Conc. (%) |
| **Totals/Conc. Range** | 519 | 0.00001-0.02 | 1 | 0.0000001-1 | NR | 0.0000001-0.5 |
| **Duration of Use** | | | | | | |
| *Leave-On* | 515 | 0.00001-0.02 | 1 | 0.0000001-1 | NR | 0.0000001-0.5 |
| *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 | 0.0001-0.2 | NR | 0. 01-0.2 |
| *Incidental Ingestion* | 100 | 0.0015-0.009 | NR | 0.001-1 | NR | 0.5 |
| *Incidental Inhalation- Sprays* | 217 | 0.001 | 1** | NR | NR | 0.01** |
| *Incidental Inhalation- Powders* | 2 | 0.00001-0.0004* | NR | 0.00001-0.02* | NR | 0.0000001-0.1* |
| *Dermal Contact* | 396 | 0.00001-0.02 | 1 | 0.0000001-1 | NR | 0.0000001-0.2 |
| *Deodorant (underarm)* | NR | NR | NR | NR | NR | NR |
| *Hair - Non-Coloring* | NR | NR | NR | NR | NR | NR |
| *Hair-Coloring* | NR | NR | NR | NR | NR | NR |
| *Nail* | 2 | NR | NR | NR | NR | 0.01 |
| *Mucous Membrane* | 100 | 0.0015-0.009 | NR | 0.001-1 | 104 | 0.5 |
| *Baby Products* | NR | NR | NR | NR | NR | NR |
| | **Tripeptide-1** | | **Copper Tripeptide-1** | | | |
| | # of Uses | Conc. (%) | # of Uses | Conc. (%) | | |
| **Totals/Conc. Range** | 36 | 0.00002-.0.001 | 18 | NR | | |
| **Duration of Use** | | | | | | |
| *Leave-On* | 35 | 0.00002-0.001 | 17 | NR | | |
| *Rinse off* | 1 | 0.00003 | 1 | NR | | |
| *Diluted for (bath) Use* | NR | NR | NR | NR | | |
| **Exposure Type** | | | | | | |
| *Eye Area* | 3 | 0.00002 | 9 | NR | | |
| *Incidental Ingestion* | 2 | NR | NR | NR | | |
| *Incidental Inhalation- Sprays* | 18 | NR | 7** | NR | | |
| *Incidental Inhalation- Powders* | 17 | 0.0001-0.001* | 6* | NR | | |
| *Dermal Contact* | 34 | 0.00002-0.001 | 16 | NR | | |
| *Deodorant (underarm)* | NR | NR | NR | NR | | |
| *Hair - Non-Coloring* | NR | 0.0001 | NR | NR | | |
| *Hair-Coloring* | NR | NR | NR | NR | | |
| *Nail* | NR | NR | NR | NR | | |
| *Mucous Membrane* | 2 | NR | NR | NR | | |
| *Baby Products* | NR | NR | NR | NR | | |

NR = Not Reported; NS = Not Surveyed; Totals = Rinse-off + Leave-on Product Uses.
*It is possible that these products may be powders, but it is not specified whether the reported uses are powders.
**It is possible that these products may be sprays, but it is not specified whether the reported uses are sprays.
Note: Because 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.

**Table 3**. Skin Irritation and Sensitization Studies

| Test Substance | Animals/Subjects | Doses/Concentrations Tested | Procedure | Results |
|---|---|---|---|---|
| BIOPEPTDE CL (contains 100 ppm palmitoyl oligo-peptide, as Pal-Gly-His-Lys-OH) | 3 male New Zealand White rabbits (ages not stated) | 0.5 ml on 6 $cm^2$ area of flank | Applied for 24 h under occlusive hypoallergenic dressing | Slight erythema in 2 rabbits (both flanks). Classified as non-irritant (primary irritation index [PII] = 0.3)[31] |
| BIOPEPTDE CL | 10 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 | Non-irritant (maximum weekly mean irritation index = 0)[24] |
| BIOPEPTDE CL | 20 male and female guinea pigs (strain and ages not stated) | Intradermal injection with 1% (0.1 ml) and cutaneous application of undiluted ingredient during induction. 24-h challenge with 75% [maximal non-irritant concentration] under occlusive dressing | Maximization test | Non-sensitizer[25] |
| BIOPEPTIDE EL (contains 100 ppm palmitoyl oligopeptide, as Pal-Val-Gly-Val-Ala-Pro-Gly-OH) | 3 male New Zealand White rabbits (ages not stated) | 0.5 ml on 6 $cm^2$ area of flank | Applied for 4 h under semi-occlusive dressing | Moderate erythema, reversible within 24 h or 48 h. Classified as non-irritant (mean erythema score of < 1)[32] |
| MAXI-LIP (contains 1,000 ppm palmitoyl oligopeptide, as Pal-Gly-His-Lys-OH) | 10 adults (ages not stated) | ~ 0.02 ml on 50 $mm^2$ area of dorsal skin | Applied for 48 h under occlusive patch (Finn chamber) | Non-irritant (PII = 0)[28] |
| MAXI-LIP | 52 subjects (16 to 79 years old) | Undiluted ingredient applied during induction and challenge | Human repeated insult patch test (HRIPT). 24-h induction applications. 24-h challenge. | Barely perceptible (+ reaction) to moderate (2 reaction) during induction and/or challenge phases. No clinically significant potential for skin irritation or sensitization[33] |
| DERMAXYL (contains 200 ppm palmitoyl oligopeotide, as Pal-Val-Gly-Val-Ala-Pro-Gly-OH) | 10 adults (ages not stated) | Test concentration of 50% on dorsal skin | Applied for 48 h under occlusive patch | Non-irritant when diluted to 50%[29] |
| DERMAXYL | 53 adults (ages not stated) | Test concentration of 50% applied during induction and challenge | HRIPT. Eight 48-h induction applications, followed by challenge | Non-irritant (mean irritation index = 0.04) and non-sensitizer[34] |
| MATRIXYL (contains 100 ppm palmitoyl pentapeptide-4) | 10 adult subjects (ages not stated) | 0.02 ml on 50 $m^2$ area on dorsal skin | Applied for 48 h under occlusive patch (Finn chamber) | Very slight erythema in 1 subject. Classified as non-irritant (PII = 0.10)[66] |
| MATRIXYL | 51 male and female subjects (19 to 78 years old) | Undiluted ingredient applied during induction and challenge | HRIPT (protocol not stated) | Non-irritant and non-sensitizer[67] |

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