Safety Assessment of Palmitoyl Oligopeptides as Used in Cosmetics

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INTRODUCTION

The safety of palmitoyl oligopeptides in cosmetics is reviewed in this safety assessment. Most of these ingredients function as skin conditioning agents in cosmetic products.[1] Additionally, palmitoyl oligopeptide and palmitoyl oligopeptide-70 function as a surfactant-cleansing agent and as a nail conditioning agent, respectively, and palmitoyl hexapeptide-14 functions as a surface modifier. Furthermore, palmitoyl tetrapeptide-20 and palmitoyl hexapeptide-12 function only as antioxidants and palmitoyl hexapeptide-26 functions only as an antimicrobial agent.

CHEMISTRY

Definition and Structure

A generic structure for palmitoyl oligopeptides (palmitoyl = *N*-(1-oxohexadecyl); oligopeptides = a chain of 2 or more amino acids linked through a peptide bond (i.e., carboxylic acid of one amino acid reacts with the β-position amine of another amino acid to form an amide (with loss of water)) is included below:

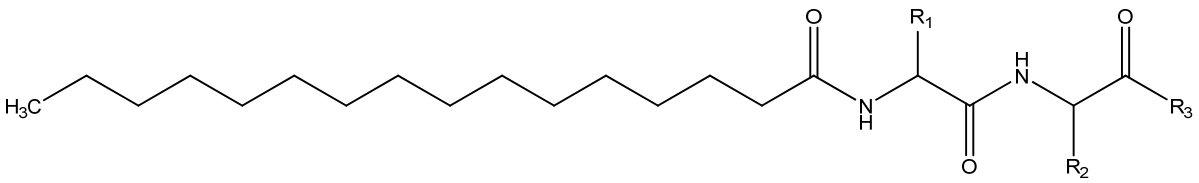

Figure1. Palmitoyl Oligopeptides – wherein R_1 and R_2 are each a residual amino side chain (eg, hydrogen in the case of glycine or methyl in the case of alanine) and R_3 is one or more amino acid residues (through traditional peptide linkage(s)), or is a hydroxyl group.

Both the definitions and functions of palmitoyl oligopeptides in cosmetics are included in Table 1. The results of a chemical substances search at the Organization for Economic Cooperation Development's eChemPortal, indicate that the following 2 CAS numbers are being used to identify palmitoyl oligopeptide: 147732-56-7 and 171263-26-6.[2] According to other sources, CAS No. 147732-56-7 is associated with the chemical name, L-Lysine,N-(1-oxohexadecyl)glycyl-L-histidyl (also known as palmitoyl tripeptide-5) and CAS No. 171263-26-6 is associated with the chemical name, Glycine, N-(1-oxohexadecyl)-L-valylglycyl-L-valyl-L-alanyl-L-propyl-.[3,4] Because these 2 CAS numbers are not listed in the monograph for palmitoyl oligopeptide in the *International Cosmetic Ingredient Dictionary and Handbook*,[1] whether or not they are being used to specifically identify palmitoyl oligopeptide needs to be confirmed.

According to one of the sources mentioned above, palmitoyl tripeptide-5 is the International Nomenclature Cosmetic Ingredient (INCI) name for L-Lysine,N-(1-oxohexadecyl)glycyl-L-histidyl (CAS No. 147732-56-7).[3] However, in the *International Cosmetic Ingredient Dictionary and Handbook (Dictionary)*, CAS No. 623172-56-5 is used to identify palmitoyl tripeptide-5, and L-Lysine,N-(1-oxohexadecyl)glycyl-L-histidyl is not listed as a technical name for this ingredient. Therefore, the CAS No. that is currently being used to identify palmitoyl tripeptide-5 needs to be confirmed. It is also noted that palmitoyl tripeptide-3 is listed as a technical name for palmitoyl tripeptide-5 in the *Dictionary* and that a chemical monograph on palmitoyl tripeptide-3 is not included.[1] Similarly, palmitoyl tetrapeptide-3 is listed as a technical name for palmitoyl tetrapeptide-7 and palmitoyl pentapeptide-3 is listed as a technical name for palmitoyl pentapeptide-4 in the *Dictionary*. Chemical monographs for palmitoyl tetrapeptide-3 and palmitoyl pentapeptide-3 are not included.

Reportedly, palmitoyl oligopeptide (Pal-GHK) is one of 2 active ingredients in the skin care ingredient Matrixyl 3000.[5] Palmitoyl oligopeptide 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), and it 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.

Physical and Chemical Properties

Information on the chemical and physical properties of palmitoyl oligopeptides were not found in the published literature.

Method of Manufacture

Palmitoyl Oligopeptides

The following general information relating to the synthesis of peptides coupled to palmitic acid was found in the published literature: Peptides have been synthesized by solid phase fluorenylmethoxycarbonyl (Fmoc) chemistry using an Advanced Chemtech MPS 350 synthesizer.[6] 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.[7,8] Regarding peptides with longer amino acid chains, synthesis on solid support has practically always been performed. 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.[9,10]

Palmitoyl Tripeptide

According to a publication on the stimulation of collagen synthesis summarized later in this report**,** palmitoyl tripeptide (pamitoyl-Gly-L-His-L-Lys) has been produced via solid phase synthesis, yielding a peptide of high purity (> 97%).[11]

Pamiltoyl Tetrapeptide

In a publication on mitogenic activity, also summarized later in this report, palmitoyl tetrapeptide (Pam-Ser-Ser-Asn-Ala) was obtained via the following process: Palmitic acid (Pam-OH) was coupled to O-tert-butyl-seryl-O-tert-butyl-seryl-asparaginyl-alanine-tert-butylester(H-L-Ser(Bu^t)-Ser(Bu^t)-Asn-Ala-Obu^t) with N,N'-dicyclohexylcarbodiimide in dimethylformamide/dichloromethane (2:1).[12] The resulting Pam-Ser(Bu^t)-Ser(Bu^t)-Asn-Ala-OBu^t was purified in dichloromethane/methanol (1:1). The tert-butyl groups were removed in trifluoroacetic acid to yield the compound Pam-Ser-Ser-Asn-Ala.

Impurities

Information on impurities in palmitoyl oligopeptides were not found in the published literature.

USE

Cosmetic

Most of the palmitoyl oligopeptides function as skin conditioning agents in cosmetic products.[1] In addition to this function, palmitoyl oligopeptide and palmitoyl oligopeptide-70 function as a surfactant-cleansing agent and a nail conditioning agent, respectively, and palmitoyl hexapeptide-14 functions as a surface modifier. Furthermore, palmitoyl tetrapeptide-20 and palmitoyl hexapeptide-12 function only as antioxidants and palmitoyl hexapeptide-26 functions only as an antimicrobial agent. According to information supplied to the Food and Drug Administration (FDA) by industry as part of the Voluntary Cosmetic Registration Program (VCRP) in 2012, the following palmitoyl oligopeptides are being used in cosmetic products:[13] palmitoyl oligopeptide, palmitoyl dipeptide-7, palmitoyl tripeptide-3, palmitoyl tripeptide-5, palmitoyl tripeptide-8, palmitoyl tripeptide-28, palmitoyl tripeptide-38, palmitoyl tetrapeptide-3, palmitoyl tetrapeptide-7, palmitoyl tetrapeptide-10, palmitoyl pentapeptide-3, palmitoyl pentapeptide-4, palmitoyl hexapeptide-14, and palmitoyl heptapeptide-5.

Results from a survey of ingredient use concentrations provided by the Personal Care Products Council in 2012 are anticipated. The VCRP data on ingredient use frequencies in cosmetics are summarized in Table 2.

Cosmetic products containing palmitoyl oligopeptides 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, palmitoyl tetrapeptide-3, and palmitoyl tetrapeptide-7 are used in suntan preparations that may be sprayed, and palmitoyl oligopeptide and palmitoyl pentapeptide-3 are used in face powders. Therefore, these ingredients 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 when compared with pump sprays.[14,15] Therefore, most droplets/particles incidentally inhaled from cosmetic sprays would be deposited in the nasopharyngeal region and would not be respirable (i.e., they would not enter the lungs) to any appreciable amount.[16,17] However, the potential for inhalation toxicity is not limited to respirable droplets/particles deposited in the lungs. Inhaled droplets/particles deposited in the nasopharyngeal and thoracic regions of the respiratory tract may cause toxic effects, depending on their chemical and other properties.

Non-Cosmetic

A palmitoyl-tailed sequential oligopeptide carrier (SOC_n-II) for engineering immunogenic conjugates has been developed.[18] The authors noted that the main guideline in designing effective immunogens as vaccine candidates capable of eliciting potent and specific immune responses is to combine 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

Other than percutaneous absorption data, data on the absorption, distribution, metabolism, and excretion of palmitoyl oligopeptides were not found in the published literature. Percutaneous absorption data on the palmitoyl dipeptide, palmitoyl carnosin (palmitoyl-β-Ala-His) are included below.

Palmitoyl Dipeptide

Prior to the percutaneous absorption study, carnosin was synthesized (classical peptide synthesis) and a palmitoyl fatty acid chain was attached to the terminal NH_2 group.[11] Aliquots of carnosin and palmitoyl carnosin were then labeled with radioactive iodine. The labeled aliquots were incorporated into solutions of the cold peptides as tracer molecules. Standard Franz diffusion cells were used to study the diffusion and penetration kinetics of the labeled peptides. A known amount (not stated) of peptide solution was applied to the surface of the skin (source not stated), and the amount of radioactivity distributed in layers of the skin and the amount recovered in the receptor fluid of the diffusion cell were analyzed. Carnosin had very low affinity for the skin and did not penetrate beyond the stratum corneum. However, palmitoyl carnosin (lipophilic) diffused into the epidermis and dermis. Neither peptide diffused beyond the dermis, in that no significant amount of radioactivity was found in the receptor fluid. Less than 10 to 4% of the initial radioactivity accumulated below the dermis within 6 h. Thus, there was no significant transcutaneous penetration, and, therefore, no uptake into the blood or lymphatic fluids is to be expected.

TOXICOLOGY

Acute and Repeated Dose Toxicity

Data on the acute and repeated dose toxicity of palmitoyl peptides were not found in the published literature.

Skin Irritation and Sensitization

Skin irritation and sensitization studies on palmitoyl peptides were not found in the published literature. However the absence of skin irritation is reported in the randomized clinical study on palmitoyl pentapeptide discussed in the following section.

Other Skin Studies

Studies relating to palmitoyl oligopeptide-induced skin rejuvenation are included in Table 3.

Palmitoyl Tripeptide-1

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).[19] Essentially, wrinkles are due to a lack of 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: total length of wrinkles, depth of wrinkles, and roughness amplitudes. A 39% decrease in wrinkle length, 23% decrease in wrinkle depth, and a 17% decrease in overall skin roughness were reported 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 (pamitoyl-Gly-L-His-L-Lys, 4 ppm in vehicle) were applied to the skin of 23 healthy female volunteers (ages not stated) for 4 weeks.[11] 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 Oligopeptide and Palmitoyl Pentapeptide-3

The peptides palmitoyl oligopeptide and palmitoyl pentapeptide-3, both modeled on repair signaling sequences, have been developed as cosmetic ingredients that enhance skin rejuvenation.[20] To further explain this function, 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. Therefore, peptides modeled on repair signaling sequences have been developed as cosmetic ingredients that enhance skin rejuvenation.

Palmitoyl Oligopeptide, Palmitoyl Tetrapeptide-7, and Palmitoyl Pentapeptide-4

An *in vivo* study on the skin rejuvenating effect of Matrixyl™ 3000 (palmitoyl oligopeptide + palmitoyl tetrapeptide-7 and Matrixyl™ (palmitoyl pentapeptide-4) was performed.[5] Panel 1 (Matrixyl™ 3000 vs. placebo) consisted of 24 volunteers with a mean age of 56.1 years. Panel 2 (Matrixyl™ 3000 vs. Matrixyl™) consisted of 25 volunteers with a mean age of 55.6 years. The test substances 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 ($p < 0.01$) and Matrixyl™ ($P < 0.05$) 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. The results (wrinkle reduction or skin roughness) were not found to be statistically significantly different when both test substances were compared. Also, after 56 days, a statistically significant increase in skin elasticity and tone resulted from application of Matrixyl™ 3000 ($p < 0.01$) and Matrixyl™ ($P < 0.05$) when compared to results at day 0.

Palmitoyl Tetrapeptide-7

In a cytometric study, 17 subjects (ages not stated) applied a cream formulated with 15 ppm palmitoyl tetrapeptide-7 to the face and neck for 1 month.[5] A significant increase in firmness was noted for the face (+19%) and neck (+40%). An increase in elasticity (face, 17%; neck, 27%) was also observed. These changes were not observed following treatment with placebo formulation on contralateral sides. Further study of the skin surface (observation of the microdepression network) revealed enhanced isotropy (+23%), a decrease in the deepest wrinkles (-56%), and an overall reduction in roughness (14%) after 15 days of palmitoyl tetrapeptide-7 application. The end result of these studies was an image of smoother, rejuvenated skin.

Palmitoyl Pentapeptide-3

Reportedly, in a randomized study, palmitoyl pentapeptide-3 (50 ppm) produced a significant benefit to lines and wrinkles around the eyes when compared to a vehicle control. Details relating to this study were not included.[21]

Palmitoyl Pentapeptide

A total of 93 female subjects (35 to 55 years old) participated in what was described as a double-blind, placebo-controlled , split-face, left-right randomized clinical study.[22] Both a moisturizer control product and the same product containing 3 ppm of the palmitoyl pentapeptide, palmitoyl-lysine-threonine-threonine-lysine-serine (pal-KTTKS) were applied (~ 0.4 g, each product) to each side of the face twice daily for 12 weeks. This study was designed to determine whether pal-KTTKS reduces wrinkles/fine lines. Both quantitative technical and expert grader image analysis were used. Pal-KTTKS was well-tolerated by the skin (i.e., no skin irritation) and provided significant improvement in terms of wrinkles/fine lines reduction, when compared to the control product. This effect was described as small, but was significant at weeks 8 and 12. In self-assessments, the subjects reported significant fine line/wrinkle improvements and noted improvements in the following other skin benefit areas: reduction in age spots, reduction in dark circles, and increased skin firmness. The latter 3 effects were described as significant at week 12.

CELLULAR EFFECTS

Studies on cellular effects of palmitoyl oligopeptides are summarized in Table 4.

Stimulation of Collagen and Fibronectin Synthesis

Palmitoyl Tripeptide-1

The stimulation of collagen synthesis by palmitoyl tripeptide-1 (pamitoyl-Gly-L-His-L-Lys) in human fibroblasts *in vitro* was studied.[11] 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 renewal (i.e., high density of collagen) of the tissue collagen.

Palmitoyl Oligopeptide, Palmitoyl Tetrapeptide-7, and Palmitoyl Pentapeptide-4

Normal human fibroblasts were cultured in Dulbecco's modified eagle medium in the presence of fetal calf serum.[5] 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 the following palmitoyl oligopeptides: palmitoyl oligopeptide (up to 7.5 ppm), palmitoyl tetrapeptide-7 (up to 3.5 ppm), Matrixyl™ 3000 (palmitoyl oligopeptide + palmitoyl tetrapeptide-7) (up to 11 ppm), and palmitoyl pentapeptide-4 (up to 8 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. Except for palmitoyl oligopeptide, a dose response for collagen 1 synthesis was observed following incubation with all of the test substances. Matrixyl™ 3000 (palmitoyl oligopeptide + palmitoyl tetrapeptide-7) 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.

Except for palmitoyl oligopeptide, a dose response for de novo synthesis of fibronectin and hyaluronic acid was observed in the presence of all of the test substances. The greatest increase in de novo fibronectin synthesis (119%) associated with a single oliogopeptide was observed in the presence of palmitoyl pentapeptide-4. However, Matrixyl™ 3000 (palmitoyl oligopeptide + palmitoyl tetrapeptide-7) induced a 164% increase in fibronectin synthesis. Palmitoyl pentapeptide-4 stimulated the de novo synthesis of hyaluronic acid by 30%, with no dose response. Matrixyl™ 3000 stimulated hyaluronic acid synthesis by 179%, whereas values for palmitoyl oligopeptide and palmitoyl tetrapeptide-7 were 14% and 16%, respectively.[5]

Stimulation of Collagen Synthesis and Fibroblast Proliferation

Palmitoyl Hexapeptide-14

Study results have indicated that palmitoyl hexapeptide-14, peptide designed using an innate immunity peptide template, stimulated cell migration, collagen synthesis, and fibroblast proliferation and scaffolding.[20] Details relating to the test protocol and study results were not included.

Down-regulation of Interleukin-6

Palmitoyl Tetrapeptide-7

The ability of palmitoyl tetrapeptide-7 (Rigin™) to down-regulate Interleukin-6 (IL-6, a cytokine) in both resting and inflamed cells *in vitro* was compared to results for dehydroepiandrosterone (DHEA), a secretory product of the human adrenal gland.[20] DHEA has been characterized as having a wide array of therapeutic benefits, one of which is reducing inflammation via the IL-6 pathway. Palmitoyl tetrapeptide-7 is among the group of peptides (referred to as rigins) derived from DHEA. The results for palmitoyl tetrapeptide-7 and DHEA were said to have been comparable in terms of the ability of each to down-regulate IL-6 in resting and in inflamed cells. Supposedly, this reduction in IL-6 can produce increased skin firmness, smoothness, and elasticity. Details relating to the test protocol and study results were not included.

Palmitoyl tetrapeptide-7 was also shown to decrease IL-6 secretion by keratinocytes in a basal setting and after exposure to UVB irradiation (35 mJ/cm^2).[5] The level of IL-6 was also reduced in fibroblasts. However, the amplitude of the reduction was less, considering that the secretion level of fibroblasts is naturally lower. Details relating to the test protocol and study results were not included.

Stimulation of Angiogenesis

Palmitoyl Oligopeptide

Palmitoyl oligopeptide, an elastin sequence, enhanced angiogenesis in the chick chorio-allantoic 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 (MMP).[23] In the *in vivo* angiogenesis assay, the chick chorio-allantoic 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 oligopeptide (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.

Mitogenic and Immune Adjuvant Activity

Palmitoyl Tetrapeptide

The palmitoyl tetrapeptide used in this study was identified as N-palmitoyl-(S)-seryl-(S)-seryl-(S)-asparaginyl- (S)-alanine, an analogue of the N-terminal part of the lipoprotein from the outer membrane of *Escherichia coli*.[12] This tetrapeptide was tested for biological activity *in vitro* using lymphocyte culture systems. The induction of DNA synthesis by palmitoyl tetrapeptide, as measured by the incorporation of ^{3}H-thymidine and ^{3}H-uridine, was followed in mouse splenocytes. Spleen cells were from the following inbred mouse (female mice, 8 to 12 weeks old) strains: C3H/HeJ, C3H/He/Bom/nunu, and Balb/c. The ability of palmitoyl tetrapeptide to polyclonically stimulate B-lymphocytes into immunoglobulin secretion was assessed using a hemolytic plaque assay. In another test, the ability of palmitoyl tetrapeptide to activate the BCL1 lymphoid B-cell line (tumor cell line) *in vitro* was examined.

In all strains, palmitoyl tetrapeptide had a stimulatory effect on B-lymphocytes that was comparable to the effect of native lipoprotein, as measured by the incorporation of ^{3}H-thymidine and ^{3}H-uridine, and by a hemolytic plaque assay. After 72 h of cultivation, an increase in ^{3}H-thymidine incorporation was observed starting at concentrations below 1 µg/ml, and the concentration that was optimal for stimulation amounted to 20-30 µg/ml. A marked increase in uridine incorporation was noted at concentrations ranging from 2.1 to 137 µg/ml. The number of plaque-forming cells against densely trinitrophenylated sheep red blood cells increased markedly after stimulation of mouse spleen cells. The B-lymphocyte tumor cell line BCL1 was also activated by palmitoyl tetrapeptide *in vitro*. In this cell line, palmitoyl tetrapeptide markedly enhanced the incorporation of ^{3}H-thymidine at concentrations > 2 µg/ml. Optimal stimulation was obtained at a concentration of ~ 30 µg/ml, and concentrations > 100 µg/ml had only a marginal effect. The results of this study demonstrated that the N-terminal tetrapeptide moiety of lipoprotein, linked to a lipophilic molecule, constitutes, by itself, a novel B-lymphocyte mitogen.[12]

Tripalmitoyl Pentapeptide

The adjuvant activity of tripalmitoyl pentapeptide (S-(2,3-bis-(palmitoyloxy)-(2RS)-propyl)-N-palmitoyl-(R)-cysteinyl-(S)-seryl-(S)-seryl(S)-asparaginyl-(S)-alanine) *in vitro* was studied.[24] In a direct hemolytic plaque assay, the stimulation of the primary antibody response toward underivatized sheep red blood cells (SRBC) and toward trinitrophenylated (TNP-) SRBC was markedly enhanced in the presence of tripalmitoyl pentapeptide (3.3 to 33.3 µg/ml). Plaque formation was increased up to 100-fold at optimal mitogen-and antigen-doses. At suboptimal doses (0.03 to 0.3 µg/ml), a 10- to 60-fold increase in plaque formation was reported. As measured by the enzyme-linked immunosorbent assay (ELISA), the antigen-specific IgM response was increased by ~ 7-fold and the IgG response was augmented by ~ 10-fold in the presence of tripalmitoyl pentapeptide. In the secondary *in vitro* response to TNP-SRBC, a 7- to 10-fold enhancement of the antibody titer was observed in the presence of tripalmitoyl pentapeptide. It was noted that the application of tripalmitoyl pentapeptide and antigen had to occur concurrently in order to produce a strong adjuvant effect. The addition of tripalmitoyl pentapeptide either a day before or a day after antigen application did not induce a significant positive effect. Actually, in several instances, decreased antibody production was observed. It was concluded that tripalmitoyl pentapeptide constitutes a potent immune adjuvant.

REPRODUCTIVE AND DEVELOPMENTAL TOXICITY

Data on the reproductive and developmental toxicity palmitoyl oligopeptides were not found in the published literature.

GENOTOXICITY/GENE ACTIVATION

Data on the genotoxicity of palmitoyl oligopeptides were not found in the published literature. However, data on gene activation were found and are summarized below.

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.[5] 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 profiles for palmitoyl oligopeptide and palmitoyl tetrapeptide-7 have been determined using a bank of 450 genes. Palmitoyl tetrapeptide-7 and palmitoyl pentapeptide-4 have very similar gene activation profiles. The genes regulated in the same way are those for functions associated with cell proliferation (platelet-derived growth factor [PDGF] associated protein and subunit, and ethylene response factor 1[ERF1]), matrix remodeling (urokinase inhibitor, metallothioneins, and lysyl oxidase), cell migration (heat shock protein 90 [HSP 90], Rho [Ras-homology], and GTPase), and cell attachment (fibronectin receptor).

Palmitoyl tetrapeptide-7 induced marked expression of a gene coding for granulocyte chemotactic protein-2 (CGP-2) (recruits cleaning cells prior to wound healing) and the vascular endothelial growth factor (VEGF) and ephrin receptor genes. These 2 genes create conditions that are conducive to setup of cutaneous microvascularization and innervation, rendering the newly synthesized epidermis fully operational (integrin-a-6 for keratinocyte installation on the basal lamina and hemidesmosomal plaque protein for cohesion of the corneocytic layer).

Palmitoyl oligopeptide (Pal-glycine-histidine-lysine) activated fewer 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 more strongly when compared to palmitoyl pentapeptide-4. Additional details were not provided.[5]

CARCINOGENICITY

Data on the carcinogenicity of palmitoyl oligopeptides were not found in the published literature.

SUMMARY

The safety of palmitoyl oligopeptides in cosmetics is reviewed in this safety assessment. Most of these ingredients function as skin conditioning agents in cosmetic products. Additionally, palmitoyl oligopeptide and palmitoyl oligopeptide-70 function as a surfactant-cleansing agent and a nail conditioning agent, respectively, and palmitoyl hexapeptide-14 functions as a surface modifier. Furthermore, palmitoyl tetrapeptide-20 and palmitoyl hexapeptide-12 function only as antioxidants and palmitoyl hexapeptide-26 functions only as an antimicrobial agent. Reportedly, palmitoyl oligopeptide and palmitoyl pentapeptide-3, both modeled on repair signaling sequences, have been developed as cosmetic ingredients that enhance skin rejuvenation.

According to information supplied to the Food and Drug Administration (FDA) by industry as part of the Voluntary Cosmetic Registration Program (VCRP) in 2012, the following palmitoyl oligopeptides are being used in cosmetic products: palmitoyl oligopeptide, palmitoyl dipeptide-7, palmitoyl tripeptide-3, palmitoyl tripeptide-5, palmitoyl tripeptide-8, palmitoyl tripeptide-28, palmitoyl tripeptide-38, palmitoyl tetrapeptide-3, palmitoyl tetrapeptide-7, palmitoyl tetrapeptide-10, palmitoyl pentapeptide-3, palmitoyl pentapeptide-4, palmitoyl hexapeptide-14, and palmitoyl heptapeptide-5.

According to one method of manufacturing palmitoyl oligopeptides, palmitic acid is 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 as for a standard amino acid coupling. Peptides and monopalmitic acid-peptide conjugates are then cleaved from the resin, deprotected, and purified using standard procedures.

Other than percutaneous absorption data, data on the absorption, distribution, metabolism, and excretion of palmitoyl oligopeptides were not found in the published literature. In an *in vitro* skin penetration study, palmitoyl carnosin (palmitoyl-β-Ala-His, labeled with radioactive iodine) penetrated into the epidermis and dermis. It did not penetrate beyond the dermis, in that no significant amount of radioactivity was found in the receptor fluid.

Data on the acute and repeated dose toxicity of palmitoyl peptides were not found in the published literature, and the same was true for skin irritation and sensitization data. However, the absence of skin irritation was reported in a randomized

clinical study on palmitoyl pentapeptide involving 93 female subjects. A moisturizer containing 3 ppm palmitoyl-lysine-threonine-threonine-lysine-serine (pal-KTTKS, ~ 0.4 g) was applied to the face twice daily for 12 weeks. Application of the moisturizer also resulted in significant improvement in terms of reduction of wrinkles/fine lines.

A cream containing palmitoyl tripeptide-1 (3 ppm) was applied to 15 female subjects twice daily for 4 weeks. Statistically significant reductions in wrinkle length and depth, and skin roughness were reported. In another study, palmitoyl tripeptide-1 (4 ppm in vehicle) was applied to the skin of 23 healthy female volunteers for 4 weeks. A small, but statistically significant, increase in skin thickness (~ 4%) was observed at the application site. The skin rejuvenating effect of a trade name material identified as palmitoyl oligopeptide + palmitoyl tetrapeptide-7 and one identified as palmitoyl pentapeptide-4 was studied using 2 groups of 24 and 25 subjects, respectively. Each material was applied at a concentration of 3% in a cream formulation twice daily for 2 months. When compared to day 0, results on application day 56 (both formulations) indicated a statistically significant decrease in deep wrinkles, skin roughness, and skin elasticity and tone. Similar effects were observed in a study in which 17 subjects applied a cream, formulated with 15 ppm palmitoyl tetrapeptide-7, for 1 month. Information on the statistical significance of these findings was not included. Reportedly, the application of palmitoyl pentapeptide-3 (50 ppm) produced a significant benefit in terms of reducing lines and wrinkles.

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 the following peptides: palmitoyl oligopeptide (up to 7.5 ppm), palmitoyl tetrapeptide-7 (up to 3.5 ppm), palmitoyl oligopeptide + palmitoyl tetrapeptide-7 (up to 11 ppm]), and palmitoyl pentapeptide-4 (up to 8 ppm). Except for palmitoyl oligopeptide, a dose response for collagen 1 synthesis and the *de novo* synthesis of fibronectin and hyaluronic acid was observed following incubation with all of the test substances. Palmitoyl hexapeptide-14 has been reported to stimulate cell migration, collagen synthesis, and fibroblast proliferation and scaffolding.

Palmitoyl tetrapeptide-7 and dehydroepiandrosterone (DHEA) down-regulated interleukin-6 (IL-6) in both resting and inflamed cells *in vitro*. Reduction of inflammation via the IL-6 pathway is a therapeutic benefit associated with DHEA, and palmitoyl tetrapeptide-7 is among the group of peptides derived from DHEA. Supposedly, this reduction in IL-6 can produce increased skin firmness, smoothness, and elasticity. Palmitoyl tetrapeptide-7 has also been shown to decrease IL-6 secretion by keratinocytes in a basal setting and after exposure to UVB irradiation. The level of IL-6 in fibroblasts was also reduced.

Palmitoyl oligopeptide enhanced angiogenesis in the chick chorio-allantoic 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.

Study results have established palmitoyl tetrapeptide as a novel B-lymphocyte mitogen and tripalmitoyl pentapeptide as a potent immune adjuvant.

Data on the genotoxicity, carcinogenicity, or reproductive and developmental toxicity of palmitoyl oligopeptides were not found in the published literature. However, the gene activation profiles for palmitoyl oligopeptide and palmitoyl tetrapeptide-7 have been determined using a bank of 450 genes, and have been found to be very similar. The genes regulated in the same way are those for functions associated with cell proliferation (platelet-derived growth factor [PDGF] associated protein and subunit, and ethylene response factor 1[ERF1]), matrix remodeling (urokinase inhibitor, metallothioneins, and lysyl oxidase), cell migration (heat shock protein 90 [HSP 90], Rho [Ras-homology], and GTPase), and cell attachment (fibronectin receptor).

Table 1. Definitions and functions of the ingredients in this safety assessment.[1]
(The italicized text below represents additions made by CIR staff.)

Ingredient CAS No.	Definition	Function
Palmitoyl Oligopeptide	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.	Skin-Conditioning Agents - Miscellaneous; Surfactants - Cleansing Agents
Palmitoyl Dipeptide-7 [911813-90-6]	Palmitoyl Dipeptide-7 is the reaction product of palmitic acid and Dipeptide-7, *wherein Dipeptide-7 is a two-residue synthetic peptide containing lysine and threonine*.	Skin-Conditioning Agents - Miscellaneous
Palmitoyl Dipeptide-10 [1206592-01-9]	Palmitoyl Dipeptide-10 is the product of the reaction of palmitic acid and Dipeptide-10, *wherein Dipeptide-10 is the two-residue synthetic peptide consisting of alanine and histidine*.	Skin-Conditioning Agents - Miscellaneous
Palmitoyl Dipeptide-13	Monograph development in progress. *Palmitoyl Dipeptide-13 is the product of the reaction of palmitic acid and Dipeptide-13, wherein Dipeptide-13 is the two-residue synthetic peptide consisting of tryptophan and glutamic acid.*	
Palmitoyl Dipeptide-17	Monograph development in progress. *Palmitoyl Dipeptide-17 is the product of the reaction of palmitic acid and Dipeptide-17, wherein the Dipeptide-17 monograph development is in progress.*	
Palmitoyl Dipeptide-18	Monograph development in progress. *Palmitoyl Dipeptide-18 is the product of the reaction of palmitic acid and Dipeptide-18, wherein the Dipeptide-17 monograph development is in progress.*	
Palmitoyl Tripeptide-1	Palmitoyl Tripeptide-1 is the reaction product of palmitic acid and Tripeptide-1, *wherein Tripeptide-1 is a three-residue synthetic peptide containing glycine, histidine, and lysine*.	Skin-Conditioning Agents - Miscellaneous
Palmitoyl Tripeptide-4	Palmitoyl Tripeptide-4 is the product of the reaction of palmitic acid and Tripeptide-4, *wherein Tripeptide-4 is a three-residue synthetic peptide containing arginine, glycine and histidine*.	Skin-Conditioning Agents - Miscellaneous
Palmitoyl Tripeptide-5 [623172-55-4]	Palmitoyl Tripeptide-5 is the reaction product of palmitic acid and Tripeptide-5, *wherein Tripeptide-5 is a three-residue synthetic peptide containing lysine and valine*.	Skin-Conditioning Agents - Miscellaneous
Palmitoyl Tripeptide-8	Palmitoyl Tripeptide-8 is the product obtained by the reaction of palmitic acid and Tripeptide-8, *wherein Tripeptide-8 is a three-residue synthetic peptide consisting of arginine, histidine and phenylalanine*.	Skin-Conditioning Agents - Miscellaneous
Palmitoyl Tripeptide-28	Palmitoyl Tripeptide-28 is the reaction product of palmitic acid and Tripeptide-28, *wherein Tripeptide-28 is the three-residue synthetic peptide consisting of arginine, lysine and phenylalanine*.	Skin-Conditioning Agents - Miscellaneous
Palmitoyl Tripeptide-29	Palmitoyl Tripeptide-29 is the product obtained by the reaction of palmitic acid and Tripeptide-29, *wherein Tripeptide-29 is the three-residue synthetic peptide consisting of glycine, proline and hydroxyproline. (**This tripeptide contains an amino acid residue that is not one of the standard α-amino acids**, which means this should have been named Palmitoyl Dipeptide-x Hydroxyproline.)*	Skin-Conditioning Agents - Miscellaneous
Palmitoyl Tripeptide-31	Palmitoyl Tripeptide-31 is the product obtained by the reaction of palmitic acid and Tripeptide-31, *wherein Tripeptide-31 is the three-residue synthetic peptide consisting of glycine, leucine and phenylalanine*.	Skin-Conditioning Agents - Miscellaneous
Palmitoyl Tripeptide-36	Palmitoyl Tripeptide-36 is the product of the reaction of palmitic acid and Tripeptide-36, *wherein Tripeptide-36 is the three-residue synthetic peptide consisting of lysine*.	Skin-Conditioning Agents - Miscellaneous
Palmitoyl Tripeptide-37	Palmitoyl Tripeptide-37 is the product obtained by the reaction of palmitic acid and Tripeptide-37, *wherein Tripeptide-37 is a three-residue synthetic peptide containing lysine and phenylalanine.*	Skin-Conditioning Agents - Miscellaneous
Palmitoyl Tripeptide-38	Palmitoyl Tripeptide-38 is the reaction product of palmitic acid and Tripeptide-38, *wherein Tripeptide-38 is a three-residue synthetic peptide containing lysine and methionine*.	Skin-Conditioning Agents - Miscellaneous
Palmitoyl Tripeptide-40	Palmitoyl Tripeptide-40 is the reaction product of palmitic acid and and Tripeptide-40, *wherein Tripeptide-40 is the three-residue synthetic peptide consisting of methionine and tyrosine*.	Skin-Conditioning Agents - Miscellaneous

Table 1. Definitions and functions of the ingredients in this safety assessment.[1]

(The italicized text below represents additions made by CIR staff.)

Ingredient CAS No.	Definition	Function
Palmitoyl Tripeptide-42	Palmitoyl Tripeptide-42 is the product obtained by the reaction of palmitic acid chloride and Tripeptide-42, *wherein Tripeptide-42 is the three-residue synthetic peptide consisting of lysine and proline*.	Skin-Conditioning Agents - Miscellaneous
Palmitoyl Tetrapeptide-7	Palmitoyl Tetrapeptide-7 is the reaction product of palmitic acid and Tetrapeptide-7, *wherein Tetrapeptide-7 is a four-residue synthetic peptide containing arginine, glutamine, glycine and proline*.	Skin-Conditioning Agents - Miscellaneous
Palmitoyl Tetrapeptide-10 [887140-79-6]	Palmitoyl Tetrapeptide-10 is the product obtained by the reaction of palmitic acid and Tetrapeptide-10, *wherein Tetrapeptide-10 is the four-residue synthetic peptide composed of lysine, threonine and phenylalanine*.	Skin-Conditioning Agents - Miscellaneous
Palmitoyl Tetrapeptide-20	Palmitoyl Tetrapeptide-20 is the product obtained by the reaction of palmitic acid and Tetrapeptide-20, *wherein Tetrapeptide-20 is the four-residue synthetic peptide consisting of arginine, histidine, phenylalanine and tryptophan*.	Antioxidants
Palmitoyl Pentapeptide-4 [521091-64-5] [214047-00-4]	Palmitoyl Pentapeptide-4 is the reaction product of palmitic acid and Pentapeptide-4, *wherein Pentapeptide-4 is a five-residue synthetic peptide containing lysine, serine and threonine*.	Skin-Conditioning Agents - Miscellaneous
Palmitoyl Pentapeptide-5	Palmitoyl Pentapeptide-5 is the reaction product of palmitic acid and Pentapeptide-5, *wherein Pentapeptide-5 is a five-residue synthetic peptide containing glycine, leucine, phenylalanine and tyrosine*.	Skin-Conditioning Agents - Miscellaneous
Palmitoyl Hexapeptide-12	Palmitoyl Hexapeptide-12 is the product of the reaction of palmitic acid and Hexapeptide-12, *wherein Hexapeptide-12 is a six-residue synthetic peptide containing alanine, glycine, proline and valine*.	Antioxidants
Palmitoyl Hexapeptide-14	Palmitoyl Hexapeptide-14 is the product of the reaction of palmitic acid and Hexapeptide-14, *wherein Hexapeptide-14 is a six-residue synthetic peptide containing alanine, leucine, lysine and phenylalanine*.	Skin-Conditioning Agents - Miscellaneous; Surface Modifiers
Palmitoyl Hexapeptide-15	Palmitoyl Hexapeptide-15 is the product obtained by the reaction of palmitic acid and Hexapeptide-15, *wherein Hexapeptide-15 is a six-residue synthetic peptide containing glycine, lysine and threonine*.	Skin-Conditioning Agents - Miscellaneous
Palmitoyl Hexapeptide-19	Palmitoyl Hexapeptide-19 is the reaction product of palmitic acid and Hexapeptide-19, *wherein Hexapeptide-19 is the six-residue synthetic peptide consisting of asparagine, aspartic acid, lysine and methionine*.	Skin-Conditioning Agents - Miscellaneous
Palmitoyl Hexapeptide-26	Palmitoyl Hexapeptide-26 is the product of the reaction of palmitic acid and Hexapeptide-26, *wherein Hexapeptide-26 is the six-residue synthetic peptide consisting of alanine, arginine, glutamine, lysine and phenylalanine*.	Antimicrobial Agents
Palmitoyl Hexapeptide-32	Palmitoyl Hexapeptide-32 is the product obtained by the reaction of palmitic acid and Hexapeptide-32, *wherein Hexapeptide-32 is a six-residue synthetic peptide consisting of alanine, glycine, hydroxyproline, and proline. (**This hexapeptide contains an amino acid residue that is not one of the standard α-amino acids**, which means this should have been named Palmitoyl Pentapeptide-x Hydroxyproline.)*	Skin-Conditioning Agents - Miscellaneous
Palmitoyl Hexapeptide-36	Palmitoyl Hexapeptide-36 is the palmitic acid ester of Hexapeptide-36, *wherein Hexapeptide-36 is the six-residue synthetic peptide consisting of aspartic acid, isoleucine, phenylalanine and tryptophan*.	Skin-Conditioning Agents - Miscellaneous
Palmitoyl Hexapeptide-27 Acetate [1181365-35-4]	Palmitoyl Hexapeptide-27 Acetate is the acetate salt of the product obtained by the reaction of Hexapeptide-27 with palmitic acid, *wherein Hexapeptide-27 is the six-residue synthetic peptide consisting of alanine, arginine, phenylalanine, serine, and tyrosine*.	Skin-Conditioning Agents - Humectant
Palmitoyl Heptapeptide-5	Palmitoyl Heptapeptide-5 is the reaction product of palmitic acid and Heptapeptide-5, *wherein Heptapeptide-5 is the seven-residue synthetic peptide consisting of glycine, hydroxyproline, isoleucine and leucine. (**This heptapeptide contains an amino acid residue that is not one of the standard α-amino acids**, which means this should have been named Palmitoyl Hexapeptide-x Hydroxyproline.)*	Skin-Conditioning Agents - Miscellaneous
Palmitoyl Nonapeptide-6	Palmitoyl Nonapeptide-6 is the reaction product of palmitic acid and Nonapeptide-6, *wherein Nonapeptide-6 is the nine-residue synthetic peptide consisting of alanine, asparagine, glutamic acid, leucine, methionine and proline*.	Skin-Conditioning Agents - Miscellaneous
Palmitoyl Decapeptide-21	Palmitoyl Decapeptide-21 is the product obtained by the reaction of palmitic acid and Decapeptide-21, *wherein Decapeptide-21 is the ten-residue synthetic peptide consisting of arginine, aspartic acid, glutamine, glycine and proline.*	Skin-Conditioning Agents - Miscellaneous

Table 1. Definitions and functions of the ingredients in this safety assessment.[1]

(The italicized text below represents additions made by CIR staff.)

Ingredient CAS No.	Definition	Function
Palmitoyl Oligopeptide-70	Palmitoyl Oligopeptide-70 is the product of the reaction of palmitic acid and Oligopeptide-70, *wherein Oligopeptide-70 is the eleven-residue synthetic peptide (undecapeptide) consisting of alanine, cysteine, glycine, histidine, lysine, proline and serine.*	Nail Conditioning Agents; Skin-Conditioning Agents - Emollient; Skin-Conditioning Agents - Miscellaneous
Palmitoyl Hydrolyzed Collagen [68915-45-7]	Palmitoyl Hydrolyzed Collagen is the condensation product of palmitic acid chloride and Hydrolyzed Collagen, *wherein Hydrolyzed Collagen is the partial hydrolysate of animal or fish collagen derived by acid, enzyme or other method of hydrolysis. Hydrolyzed Collagen is characterized by a significant level of hydroxyproline residues. (**This oligopeptide contains an amino acid residue that is not one of the standard α-amino acids**.)*	Hair Conditioning Agents; Skin-Conditioning Agents - Miscellaneous; Surfactants - Cleansing Agents
Palmitoyl Hydrolyzed Milk Protein	Palmitoyl Hydrolyzed Milk Protein is the condensation product of palmitic acid chloride and Hydrolyzed Milk Protein, *wherein Hydrolyzed Milk Protein is the partial hydrolysate of milk protein derived by acid, enzyme or other method of hydrolysis.*	Hair Conditioning Agents; Skin-Conditioning Agents - Miscellaneous; Surfactants - Cleansing Agents
Palmitoyl Hydrolyzed Wheat Protein	Palmitoyl Hydrolyzed Wheat Protein is the condensation product of palmitic acid chloride and Hydrolyzed Wheat Protein, *wherein Hydrolyzed Wheat Protein is the partial hydrolysate of wheat protein derived by acid, enzyme or other method of hydrolysis.*	Hair Conditioning Agents; Skin-Conditioning Agents - Miscellaneous; Surfactants - Cleansing Agents
Potassium Palmitoyl Hydrolyzed Corn Protein	Potassium Palmitoyl Hydrolyzed Corn Protein is the potassium salt of the condensation product of palmitic acid chloride and Hydrolyzed Corn Protein, *wherein Hydrolyzed Corn Protein is the partial hydrolysate of corn protein derived by acid, enzyme or other method of hydrolysis*.	Hair Conditioning Agents; Skin-Conditioning Agents - Miscellaneous; Surfactants - Cleansing Agents
Potassium Palmitoyl Hydrolyzed Oat Protein	Potassium Palmitoyl Hydrolyzed Oat Protein is the potassium salt of the condensation product of palmitic acid chloride and Hydrolyzed Oat Protein, *wherein Hydrolyzed Oat Protein is the partial hydrolysate of oat protein derived by acid, enzyme or other method of hydrolysis*.	Hair Conditioning Agents; Skin-Conditioning Agents - Miscellaneous; Surfactants - Cleansing Agents
Potassium Palmitoyl Hydrolyzed Rice Protein	Potassium Palmitoyl Hydrolyzed Rice Protein is the potassium salt of the condensation product of palmitic acid chloride and Hydrolyzed Rice Protein, *wherein Hydrolyzed Rice Protein is the partial hydrolysate of rice protein derived by acid, enzyme or other method of hydrolysis*.	Emulsion Stabilizers; Hair Conditioning Agents; Skin-Conditioning Agents - Miscellaneous; Surfactants - Cleansing Agents

Table 1. Definitions and functions of the ingredients in this safety assessment.[1]

(The italicized text below represents additions made by CIR staff.)

Ingredient CAS No.	Definition	Function
Potassium Palmitoyl Hydrolyzed Sweet Almond Protein	Potassium Palmitoyl Hydrolyzed Sweet Almond Protein is the potassium salt of the condensation product of palmitic acid chloride and Hydrolyzed Sweet Almond Protein, *wherein Hydrolyzed Sweet Almond Protein is the partial hydrolysate of sweet almond protein derived by acid, enzyme or other method of hydrolysis*.	Emulsion Stabilizers; Hair Conditioning Agents; Skin-Conditioning Agents - Miscellaneous; Surfactants - Cleansing Agents
Potassium Palmitoyl Hydrolyzed Wheat Protein	Potassium Palmitoyl Hydrolyzed Wheat Protein is the potassium salt of the condensation product of palmitic acid chloride and Hydrolyzed Wheat Protein, *wherein Hydrolyzed Wheat Protein is the partial hydrolysate of wheat protein derived by acid, enzyme or other method of hydrolysis*.	Hair Conditioning Agents; Skin-Conditioning Agents - Miscellaneous; Surfactants - Cleansing Agents
Sodium Palmitoyl Hydrolyzed Collagen	Sodium Palmitoyl Hydrolyzed Collagen is the sodium salt of the condensation product of palmitic acid chloride and Hydrolyzed Collagen, *wherein Hydrolyzed Collagen is the partial hydrolysate of animal or fish collagen derived by acid, enzyme or other method of hydrolysis. Hydrolyzed Collagen is characterized by a significant level of hydroxyproline residues. (**This oligopeptide contains an amino acid residue that is not one of the standard α-amino acids**.)*	Hair Conditioning Agents; Skin-Conditioning Agents - Miscellaneous; Surfactants - Cleansing Agents
Sodium Palmitoyl Hydrolyzed Wheat Protein	Sodium Palmitoyl Hydrolyzed Wheat Protein is the sodium salt of the condensation product of palmitic acid chloride and Hydrolyzed Wheat Protein, *wherein Hydrolyzed Wheat Protein is the partial hydrolysate of wheat protein derived by acid, enzyme or other method of hydrolysis.*	Hair Conditioning Agents; Skin-Conditioning Agents - Miscellaneous; Surfactants - Cleansing Agents

Table 2. Current Frequency of Use According to Duration and Type of Exposure Provided in 2012.[13]

	Palmitoyl Oligopeptide		**Palmitoyl Dipeptide-7**		**Palmitoyl Tripeptide-3**	
	# of Uses	Conc. (%)	# of Uses	Conc. (%)	# of Uses	Conc. (%)
Exposure Type						
Eye Area	102		1		4	
Incidental Ingestion	100		0		0	
Incidental Inhalation- Sprays	1		0		0	
Incidental Inhalation- Powders	1		0		0	
Dermal Contact	366		8		14	
Deodorant (underarm)	0		0		0	
Hair - Non-Coloring	2		0		0	
Hair-Coloring	0		0		0	
Nail	3		0		0	
Mucous Membrane	100		0		0	
Baby Products	0		0		0	
Duration of Use						
Leave-On	467		8		14	
Rinse off	4		0		0	
Diluted for (bath) Use	0		0		0	
Totals/Conc. Range	471		8		14	
	Palnitoyl Tripeptide-5		**Palmitoyl Tripeptide-8**		**Palmitoyl Tripeptide-28**	
	# of Uses	Conc. (%)	# of Uses	Conc. (%)	# of Uses	Conc. (%)
Exposure Type						
Eye Area	8		0		0	
Incidental Ingestion	0		0		0	
Incidental Inhalation- Sprays	0		0		0	
Incidental Inhalation- Powders	0		0		0	
Dermal Contact	39		4		1	
Deodorant (underarm)	0		0		0	
Hair - Non-Coloring	0		0		0	
Hair-Coloring	0		0		0	
Nail	0		0		0	
Mucous Membrane	0		0		0	
Baby Products	0		0		0	
Duration of Use						
Leave-On	39		4		1	
Rinse off	0		0		0	
Diluted for (bath) Use	0		0		0	
Totals/Conc. Range	39		4		1	
	Palmitoyl Tripeptide-38		**Palmitoyl Tetrapeptide-3**		**Palmitoyl Tetrapeptide-7**	
	# of Uses	Conc. (%)	# of Uses	Conc. (%)	# of Uses	Conc. (%)
Exposure Type						
Eye Area	0		14		79	
Incidental Ingestion	1		0		1	
Incidental Inhalation- Sprays	0		1		3	
Incidental Inhalation- Powders	0		0		0	
Dermal Contact	0		39		193	
Deodorant (underarm)	0		0		0	
Hair - Non-Coloring	0		0		0	
Hair-Coloring	0		0		0	
Nail	0		0		0	
Mucous Membrane	1		0		1	
Baby Products	0		0		0	
Duration of Use						
Leave-On	1		39		191	
Rinse off	0		0		3	
Diluted for (bath) Use	0		0		0	
Totals/Conc. Range	1		39		194	

Table 2. Current Frequency of Use According to Duration and Type of Exposure Provided in 2011.[13]

	Palmitoyl Tetrapeptide-10		**Palmitoyl Pentapeptide-3**		**Palmitoyl Pentapeptide-4**	
	# of Uses	Conc. (%)	# of Uses	Conc. (%)	# of Uses	Conc. (%)
Exposure Type						
Eye Area	2		8		11	
Incidental Ingestion	0		0		0	
Incidental Inhalation- Sprays	0		0		0	
Incidental Inhalation- Powders	0		2		0	
Dermal Contact	11		44		51	
Deodorant (underarm)	0		0		0	
Hair - Non-Coloring	0		0		0	
Hair-Coloring	0		0		0	
Nail	0		0		0	
Mucous Membrane	0		0		0	
Baby Products	0		0		0	
Duration of Use						
Leave-On	10		42		50	
Rinse off	1		2		1	
Diluted for (bath) Use	0		0		0	
Totals/Conc. Range	11		44		51	
	Palmitoyl Hexapeptide-14		**Palmitoyl Heptapeptide-5**			
	# of Uses	Conc. (%)	# of Uses	Conc. (%)		
Exposure Type						
Eye Area	2		0			
Incidental Ingestion	0		0			
Incidental Inhalation- Sprays	0		0			
Incidental Inhalation- Powders	0		0			
Dermal Contact	3		2			
Deodorant (underarm)	0		0			
Hair - Non-Coloring	0		0			
Hair-Coloring	0		0			
Nail	0		0			
Mucous Membrane	0		0			
Baby Products	0		0			
Duration of Use						
Leave-On	3		2			
Rinse off	0		0			
Diluted for (bath) Use	0		0			
Totals/Conc. Range	3		2			

MG = Methyl Glucose; NR = Not Reported; NS = Not Surveyed; Totals = Rinse-off + Leave-on Product Uses.

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 Studies

Test Substance	Subjects	Test Concentration	Procedure	Results
Palmitoyl Tripeptide-1 (palmitoyl-gly-his-lys)	15 female subjects (44 to 59 years old)	3 ppm in a cream	Applied around eye zones twice daily for 4 weeks. Skin replicas from the face obtained on days 0 and 28 and analyzed using an image analysis system	Decreases in wrinkle length and depth, and skin roughness. Placebo cream had no effect[19]
Palmitoyl Oligopeptide + Palmitoyl Pentapeptide-7	24	3% in a cream formulation	Applied to the face in morning and at night for 2 months. Skin rejuvenation assessed usijng profilometry, and image analysis, photography, and cutometry.	Statistically significant decrease ($p < 0.01$) in deep wrinkles and skin roughness after 56 days, compared to results at day 0. Statistically significant increase ($p < 0.01$) in skin elasticity and tone[5]
Palmitoyl Pentapeptide-4	25	3% in cream formulation	Same procedure	Statistically significant decrease ($p < 0.05$) in deep wrinkles and skin roughness after 56 days, compared to results at day 0. Statistically significant increase ($p < 0.05$) in skin elasticity and tone[5]
Palmitoyl Tetrapeptide-7	17	15 ppm in cream	Applied to face and neck for 1 month	Significant increase in firmness (face and neck). Increase in elasticity, and decrease in deepest wrinkles and skin roughness[5]
Palmitoyl Pentapeptide-3	Number not stated	50 ppm	Applied to eye area. Study details not included	Significant benefit to lines and wrinkles around the eye when compared to vehicle control[21]
Palmitoyl Pentapeptide (palmitoyl-lysine-threonine-threonine-lysine-serine)	93 female subjects (35 to 55 yeards old)	3 ppm in moisturizer	Applied (~0.4 g) to the face twice daily for 12 weeks. Quantitative technical and expert grader image analysis used	Significant improvement in terms of wrinkles/fine lines reduction (at weeks 8 and 12) when compared to moisturizer control product. No skin irritation. Results of self assessments yielded significant reductions in age spots and dark circles and increased skin firmness at week 12[22]

Table 4. Biological Activity

Test Substance	Test Concentration(s)	Procedure	Results
Palmitoyl Tripeptide-1 (palmitoyl-gly-L-his-L-lys)	0.5 µM/liter	Collagen synthesis monitored by incorporation of tritiated proline into human fibroblasts *in vitro*	Strong signal of collagen synthesis observed at 0.5 µM/liter[11]
Palmitoyl Tripeptide-1 (palmitoyl-gly-L-his-L-lys)	5 ppm	Human skin samples (abdominal tissue) from biopsy irradiated with daily doses of UVA for 1 week. Irradiation followed by treatment with oligopeptide	Irradiation caused strong collagen degradation. Treatment with 5 ppm resulted in almost total preservation and/or renewal (high density of collagen) of tissue collagen. Same results for 500 ppm retinoic acid[11]
Palmitoyl Oligopeptide, Palmitoyl Tetrapeptide-7, Palmitoyl Oligopeptide + Palmitoyl Tetrapeptide-7, and Palmitoyl Pentapeptide-4	Palmitoyl Oligopeptide (up to 7.5 ppm), Palmitoyl Tetrapeptide-7 (up to 3.5 ppm), Palmitoyl Oligopeptide + Palmitoyl Tetrapeptide-7 (up to 11 ppm), and Palmitoyl Pentapeptide-4 (up to 8 ppm)	Human fibroblasts incubated with each of the oligopeptides in the presence of vitamin C. Matrix proteins (collagen 1 and fibronectin) assayed using ELISA method. Hyaluronic acid assayed using a colorimetric method	Except for palmitoyl oligopeptide, a dose response for collagen 1, fibronectin, and hyaluronic acid synthesis was associated with each oligopeptide[5]
Palmitoyl Hexapeptide-14	Not stated	Not stated	Stimulated cell migration, collagen synthesis, and fibroblast proliferation and scaffolding[20]
Palmitoyl Tetrapeptide-7	Not stated	Assay to evaluate ability of oligopeptide to down-regulate IL-6 in resting and inflammed cells *in vitro.*	Results for palmitoyl oligopeptide and DHEA were comparable in terms of the ability of each to down-regulate IL-6 in resting and inflammed cells[20]
Palmitoyl Tetrapeptide-7	Not stated	Keratinocytes and fibroblasts exposed to oligopeptide in the presence and absence of UVB irradiation	Palmitoyl tetrapeptide-7 caused decrease in IL-6 secretion in the presence and absence of UVB[5]
Palmitoyl Oligopeptide	50 ng in 20 µl phosphate buffered saline (PBS)	*In vivo* angiogenesis assay using chick chorio-allantoic membrane. On day 6, angiogenic areas delimited with a silicon ring and PBS or palmitoyl oligopeptide (50 ng) in a final volume of 20 µl placed inside the rings. Treated areas photographed daily on days 6 to 10 of development	Palmitoyl oligopeptide enhanced angiogenesis by promoting endothelial cell migration and tubulogenesis through upregulation of MT1-MMP[23]

Table 4. Biological Activity

Test Substance	Test Concentration(s)	Procedure	Results
Palmitoyl Tetrapeptide (N-palmitoyl-(S)-seryl-(S)-seryl-(S)-asparaginyl-(S)-alanine)	<1 to 137 µg/ml	Induction of DNA synthesis measured by incorporation of ^{3}H-thymidine and ^{3}H-uridine in mouse splenocytes from following mouse strains: C3H/HeJ, C3H/He/Bom/nunu, and Balb/c	In all strains, palmitoyl tetrapeptide had stimulatory effect on B-lymphocytes. Increase in ^{3}H-thymidine incorporation optimal in 20 to 30 µg/ml range. Marked increase in ^{3}H-uridine incorporation in 2.1 to 137 µg/ml range[12]
Palmitoyl Tetrapeptide (N-palmitoyl-(S)-seryl-(S)-seryl-(S)-asparaginyl-(S)-alanine)	<1 to 137 µg/ml	Hemolytic plaque assay used to assess ability of palmitoyl tetrapeptide to polyclonically stimulate B-lymphocytes into immunoglobulin secretion	The number of plaque-forming cells against densely trinitrophenylated sheep red blood cels increased markedly after stimulation of mouse spleen cells[12]
Palmitoyl Tetrapeptide (N-palmitoyl-(S)-seryl-(S)-seryl-(S)-asparaginyl-(S)-alanine)	<1 to 137 µg/ml	Ability of palmitoyl tetrapeptide to activate the BCL1 lymphoid B-cell line (tumor cell line) evaluted *in vitro*	Marked enhancement of ^{3}H-thymidine incorporation at concentrations > 2 µg/ml. Optimal stimulation at ~ 30 µg/ml[12]
Tripalmitoyl Pentapeptide (S-(2,3-bis-(palmitoyloxy)-(2RS)-propyl)-N-palmitoyl-(R)-cysteinyl-(S)-seryl-(S)-seryl(S)-asparaginyl-(S)-alanine)	0.03 to 33.3 µg/ml	Hemolytic plaque assay	Stimulation of the primary antibody response toward underivatized sheep red blood cells (SRBC) and toward trinitrophenylated (TNP-) SRBC was markedly enhanced in the presence of tripalmitoyl pentapeptide (3.3 to 33.3 µg/ml)[24]
Tripalmitoyl Pentapeptide (S-(2,3-bis-(palmitoyloxy)-(2RS)-propyl)-N-palmitoyl-(R)-cysteinyl-(S)-seryl-(S)-seryl(S)-asparaginyl-(S)-alanine)	0.03 to 33.3 µg/ml	Enzyme-linked immunosorbent assay (ELISA)	Antigen-specific IgM response increased by ~ 7-fold and IgG response augmented by ~ 10-fold in presence of tripalmitoyl pentapeptide. Application of tripalmitoyl pentapeptide and antigen had to occur concurrently in order to produce strong adjuvant effect[24]

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