Safety Assessment of Myristoyl Pentapeptide-4, Palmitoyl Pentapeptide-4, and Pentapeptide-4 as Used in Cosmetics

Status:	Scientific Literature Review for Public Comment
Release Date:	April 18, 2023
Panel Meeting Date:	September 11-12, 2023

*All interested persons are provided 60 days from the above release date (i.e., **June 17, 2023**) to comment on this safety assessment, and to identify additional published data that should be included or provide unpublished data which can be made public and included. Information may be submitted without identifying the source or the trade name of the cosmetic product containing the ingredient. All unpublished data submitted to CIR will be discussed in open meetings, will be available for review by any interested party, and may be cited in a peer-reviewed scientific journal. Please submit data, comments, or requests to the CIR Executive Director, Dr. Bart Heldreth.*

The Expert Panel for Cosmetic Ingredient Safety members are: Chair, Wilma F. Bergfeld, M.D., F.A.C.P.; Donald V. Belsito, M.D.; David E. Cohen, M.D.; Curtis D. Klaassen, Ph.D.; Allan E. Rettie, Ph.D.; David Ross, Ph.D.; Thomas J. Slaga, Ph.D.; Paul W. Snyder, D.V.M., Ph.D.; and Susan C. Tilton, Ph.D. The Cosmetic Ingredient Review (CIR) Executive Director is Bart Heldreth, Ph.D, and the Senior Director is Monice Fiume. This safety assessment was prepared by Preethi Raj, M.S., Senior Scientific Analyst/Writer, CIR.

1620 L Street, NW, Suite 1200 ◇ Washington, DC 20036-4702 ◇ ph 202.331.0651
cirinfo@cir-safety.org

ABBREVIATIONS

CAS	Chemical Abstracts Service
CIR	Cosmetic Ingredient Review
Council	Personal Care Products Council
CPSC	Consumer Product Safety Commission
FCA	Freund's Complete Adjuvant
Fmoc	fluorenylmethoxycarbonyl
FDA	Food and Drug Administration
HEPES	4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid
HET-CAM	hen's egg-chorioallantoic membrane
HRIPT	human repeated insult patch test
KTTKS	lysine-threonine-threonine-lysine-serine; Pentapeptide-4
LC-MS/MS	liquid chromatography with tandem mass spectrophotometry
LOQ	limit of quantification
LPPS	liquid-phase peptide synthesis
NR	none reported
OECD	Organisation for Economic Cooperation and Development
Pal-KTTKS	Palmitoyl Pentapeptide-4
Panel	Expert Panel for Cosmetic Ingredient Safety
PCI	primary cutaneous irritation
SLS	sodium lauryl sulfate
SPPS	solid-phase peptide synthesis
TG	test guideline
US	United States
VCRP	Voluntary Cosmetic Registration Program
wINCI; *Dictionary*	web-based International Cosmetic Ingredient Dictionary and Handbook

INTRODUCTION

This assessment reviews the safety of Myristoyl Pentapeptide-4, Palmitoyl Pentapeptide-4, and Pentapeptide-4 as used in cosmetic formulations. According to the web-based *International Cosmetic Ingredient Dictionary and Handbook* (wINCI *Dictionary*), these ingredients are reported to function in cosmetics as skin-conditioning agents (Table 1).[1]

The 3 ingredients included in this safety assessment are synthetic peptides which share the amino acid sequence lysine-threonine-threonine-lysine-serine, also represented as Lys-Thr-Thr-Lys-Ser, or, KTTKS.[2] Myristoyl Pentapeptide-4 and Palmitoyl Pentapeptide-4 have an additional saturated fatty acid group attached to the peptide structure, namely myristic acid and palmitic acid, respectively.

The safety of Palmitoyl Pentapeptide-4 was initially considered by the Expert Panel for Cosmetic Ingredient Safety (Panel) as part of a 2013 Draft Report on the safety of Palmitoyl Oligopeptides. Upon discussing the relevance of amino acid sequence in the grouping of these ingredients, the Palmitoyl Oligopeptides nomenclature was retired from the *Dictionary*, and the Panel decided to table the report and to re-group the ingredients. Accordingly, this ingredient family was formed.

The Panel has also previously reviewed the safety of the individual amino acids comprising these ingredients, as well as myristic acid and palmitic acid. In 2013, the Panel published a final report with the conclusion that α-amino acids are safe in the present practices of use and concentration in cosmetics as described in the safety assessment.[3] The safety of myristic acid and palmitic acid has been evaluated in several reviews.[4-7] Ultimately, in 2019, the Panel issued a final report on the safety of myristic acid and palmitic acid (as part of the safety assessment of fatty acids and fatty acid salts) with the conclusion that the ingredients are safe in cosmetics in the present practices of use and concentration described in the safety assessment when formulated to be non-irritating and non-sensitizing, which may be determined based on a quantitative risk assessment.[7]

This safety assessment includes relevant published and unpublished data that are available for each endpoint that is evaluated. Published data are identified by conducting an extensive search of the world's literature; a search was last conducted March 2023. A listing of the search engines and websites that are used and the sources that are typically explored, as well as the endpoints that the Panel typically evaluates, is provided on the Cosmetic Ingredient Review (CIR) website (https://www.cir-safety.org/supplementaldoc/preliminary-search-engines-and-websites; https://www.cir-safety.org/supplementaldoc/cir-report-format-outline). Unpublished data are provided by the cosmetics industry, as well as by other interested parties. Unpublished data which were submitted previously (when Palmitoyl Pentapeptide-4 was initially considered in the Palmitoyl Oligopeptides report) have been included for the Panel's review herein.

CHEMISTRY

Definition and Structure

Pentapeptide-4 is a synthetic peptide comprised of the amino acids, lysine, serine, and threonine, which are linked in the 5 amino acid sequence: lysine-threonine-threonine-lysine-serine (also represented as Lys-Thr-Thr-Lys-Ser or KTTKS; Figure 1).[1,2] Myristoyl Pentapeptide-4 and Palmitoyl Pentapeptide-4 (CAS No. 521091-64-5; 214047-00-4) each have a myristic acid or palmitic acid group, respectively, attached to the *N*-capped end of this sequence. The definitions and structures of the ingredients included in this review are provided in Table 1.

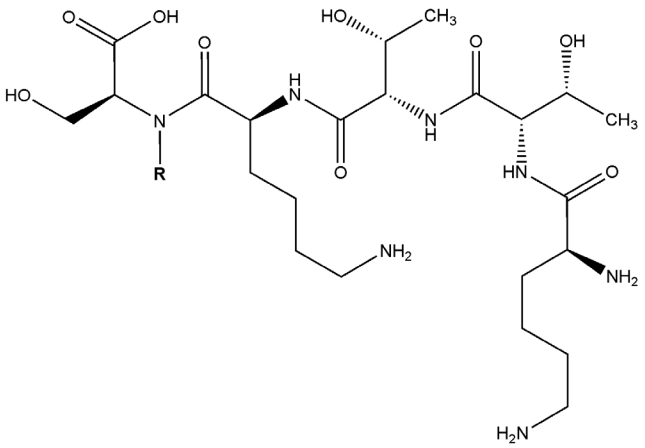


Figure 1. Pentapeptide-4 (when R is hydrogen) and *N*-capped derivatives (when R is the residue of myristic or palmitic acid)

Pentapeptide-4 is a subfragment of type I collagen propeptide, and is regarded as a signal peptide and a matrikine, which possess the ability to enhance dermal remodeling by triggering cellular processes, such as inhibiting collagenase activity and increasing extracellular matrix production.[2,8-11] The hydrophilic and ion charged nature of Pentapeptide-4 makes it difficult for it to pass through the intact stratum corneum.[12] However, through the attachment of a fatty acid, such as

palmitic acid, which has a 16-carbon chain, the peptide is rendered lipophilic and is more easily able to penetrate into the skin.[13]

Chemical Properties

Myristoyl Pentapeptide-4, Palmitoyl Pentapeptide-4, and Pentapeptide-4 have molecular weights of 774 g/mol,[14] 802.1 g/mol,[15,16] and 563.6 g/mol,[17] respectively. Palmitoyl Pentapeptide-4 has an estimated log p value of 3.32, while Pentapeptide-4 has an estimated log p value of -3.27.[2] Chemical properties for ingredients in this report are further outlined in Table 2.

Method of Manufacture

The methods of manufacturing detailed here are general to the production of peptide synthesis, and it is unknown whether they are specific to ingredients that are used in cosmetics. Synthetic peptides are commonly produced using solid-phase peptide synthesis (SPPS) or liquid-phase peptide synthesis (LPPS).[18,19] In the SPSS method, a resin (such as polystyrene, Merrifield, hydroxymethyl, phenylacetamidomethyl, Wang and 4-methylbenzhydrylamine) is used as a support to which the growing peptide is anchored. First, an amino acid with temporary protecting groups (e.g. fluorenylmethoxy-carbonyl (Fmoc) groups) on the reactive side chain and the alpha amino group is attached to the resin via its C-terminus. After addition of an amino acid, the protecting group is removed and the resin is washed with solvents (such as dimethylformamide or *N*-methylpyrrolidone) prior to subsequent additions. This process is repeated until the amino acid sequence is complete, upon which, the desired peptide is cleaved from the resin. In the LPPS method, single amino acids undergo coupling in solution to form short fragments of the desired peptide, which are then coupled to form a long peptide.

Palmitoyl Pentapeptide-4

A supplier described that a sample of Palmitoyl Pentapeptide-4 is produced using stepwise peptide synthesis.[15] Specifically, the C-terminal amino acid serine (Ser) is protected on its acidic function, after which each subsequent amino acid in the sequence, lysine-threonine-threonine-lysine (Lys-Thr-Thr-Lys), is coupled. Lastly, the final coupling procedure occurs with palmitic acid instead of an amino acid.

Impurities

Palmitoyl Pentapeptide-4

The impurities found in a sample of Palmitoyl Pentapeptide-4, as described by a supplier, were: acetate (< 10%), palmitic acid (< 5%), water (< 5%), and residual solvents (amounts not provided).[15] No further impurities data were found in the published literature.

USE

Cosmetic

The safety of the cosmetic ingredients addressed in this assessment is evaluated based on data received from the US Food and Drug Administration (FDA) and the cosmetics industry on the expected use of these ingredients in cosmetics and does not cover their use in airbrush delivery systems. Data are submitted by the cosmetic industry via the FDA's Voluntary Cosmetic Registration Program (VCRP) database (frequency of use) and in response to a survey conducted by the Personal Care Products Council (Council) (maximum use concentrations). The data are provided by cosmetic product categories, based on 21CFR Part 720. For most cosmetic product categories, 21CFR Part 720 does not indicate type of application and, therefore, airbrush application is not considered. Airbrush delivery systems are within the purview of the US Consumer Product Safety Commission (CPSC), while ingredients, as used in airbrush delivery systems, are within the jurisdiction of the FDA. Airbrush delivery system use for cosmetic application has not been evaluated by the CPSC, nor has the use of cosmetic ingredients in airbrush technology been evaluated by the FDA. Moreover, no consumer habits and practices data or particle size data are publicly available to evaluate the exposure associated with this use type, thereby preempting the ability to evaluate risk or safety.

According to the 2023 VCRP survey data, Palmitoyl Pentapeptide-4 has the greatest reported frequency of use; it is reported to be used in 239 formulations, 223 of which are leave-on products (Table 3).[20] Myristoyl Pentapeptide-4 is reported to have 4 uses, while Pentapeptide-4 has 1 reported use (Table 4). The results of the concentration of use survey conducted by the Council in 2022 indicate Myristoyl Pentapeptide-4 has the highest reported concentration of use in a leave-on formulation, at up to 0.05% in other eye makeup preparations.[21]

Since the last inquiry in 2013, the reported frequency and concentrations of use have increased for Palmitoyl Pentapeptide-4 (Table 3). Notably, total reported uses for Palmitoyl Pentapeptide-4 have increased from 51 to 239.[20,22] The highest reported maximum concentration of use for Palmitoyl Pentapeptide-4 in a leave-on formulation reported in 2022 is 0.0012% in eye lotions; the maximum reported concentration of use for this use category was 0.00061% at the time of the previous inquiry.[21,23]

Some of these ingredients are reported to be used in products that are applied near the eye; for example Palmitoyl Pentapeptide-4 is used as up to 0.0012% in eye lotions. Palmitoyl Pentapeptide-4 is reported to be used in a face powder (concentration not provided), and could possibly be inhaled. In practice, as stated in the Panel's respiratory exposure

resource document (https://www.cir-safety.org/cir-findings), most droplets/particles incidentally inhaled from cosmetics would be deposited in the nasopharyngeal and tracheobronchial regions and would not be respirable (i.e., they would not enter the lungs) to any appreciable amount. Conservative estimates of inhalation exposures to respirable particles during the use of loose powder cosmetic products are 400-fold to 1000-fold less than protective regulatory and guidance limits for inert airborne respirable particles in the workplace.

Although products containing some of these ingredients may be marketed for use with airbrush delivery systems, this information is not available from the VCRP or the Council survey. Without information regarding the frequency and concentrations of use of these ingredients (and without consumer habits and practices data or particle size data related to this use technology), the data are insufficient to evaluate the exposure resulting from cosmetics applied via airbrush delivery systems.

The Pentapeptide-4 ingredients named in the report are not restricted from use in any way under the rules governing cosmetic products in the European Union.[24]

Non-Cosmetic

Palmitoyl Pentapeptide-4 has been tested for its wound-healing effects in a patch and cream formulation.[25]

TOXICOKINETIC STUDIES

Dermal Permeation

In Vitro

Palmitoyl Pentapeptide-4; Pentapeptide-4

The permeability of Palmitoyl Pentapeptide-4 and Pentapeptide-4 was evaluated in an in vitro study using 3 replicate skin samples of CrlOri: SKH1-hr strain hairless mice.[26] Intact hairless mouse skin was mounted on Franz diffusion cells with the epidermal side facing the donor compartment. In the receptor compartment, 4-(2-hydroxyethyl)-1-piperazineethane-sulfonic acid (HEPES) buffer was mixed with 15% ethanol containing phenylmethanesulfonylfluoride and 1,10-phenanthroline at final concentrations of 5 mM and 1mM, respectively, as proteolytic enzyme inhibitors. The donor compartment was loaded with a 1 ml solution of Palmitoyl Pentapeptide-4 or Pentapeptide-4 (100 µg/ml in 15% ethanol). After 24-h incubation, skin was removed from the diffusion cell and the remaining donor solution on skin surface was washed four times with 1 ml of distilled water. Upon drying, separation, and mincing of the skin layers (stratum corneum, epidermis, and dermis), the amount of Palmitoyl Pentapeptide-4 or Pentapeptide-4 distributed in each skin layer was extracted using 1 ml of methanol for 24 h with continuous shaking. The extracted samples were centrifuged and the supernatants were analyzed using liquid chromatography with tandem mass spectrometry (LC-MS/MS). No detectable level of Pentapeptide-4 was observed in the receptor solution over an observation period of 48 h; a trace amount of Palmitoyl Pentapeptide-4 was detected in the receptor solution after 24 h by LC-MS/MS; however, it was below the limit of quantification (LOQ) at < 0.5 µg/ml. No amount of Pentapeptide-4 was detected in any of the skin layers over a period of 24 h. Palmitoyl Pentapeptide-4 was observed in every skin layer: 4.2 ± 0.7 µg/cm^2 in the stratum corneum, 2.8 ± 0.5 µg/cm^2 in the epidermis, and 0.3 ± 0.1 µg/cm^2 in the dermis. Overall, 14.6% of the applied Palmitoyl Pentapeptide-4 was retained in the skin: 8.3% in the stratum corneum, 5.6% in the epidermis, and 0.6% in the dermis. Therefore, the researchers concluded that neither Palmitoyl Pentapeptide-4 nor Pentapeptide-4 could permeate through full-thickness hairless mouse skin over the time period used in these experiments.

In another portion of this study, the in vitro dermal stability of Palmitoyl-Pentapeptide-4 and Pentapeptide-4 was evaluated in epidermal and dermal skin extracts, and whole skin homogenate, prepared from hairless mice skin.[26] A portion (200 µl) of Pentapeptide-4 or Palmitoyl Pentapeptide-4 (40 µg/ml in 10 mM HEPES buffer, pH 7.4, as peptide concentration) was incubated with 200 µl of the epidermal skin extract, dermal skin extract, or skin homogenates at 37 °C for 120 min. At predetermined times, the amount of Palmitoyl Pentapeptide-4 and Pentapeptide-4 present in the incubated mixtures was sampled and analyzed by LC-MS/MS. Pentapeptide-4 was almost fully degraded in the dermal skin extract and skin homogenate, with 3.2% remaining in the dermal skin extract at 30 min and 1.5% remaining in the skin homogenate at 60 min. Palmitoyl Pentapeptide-4 was more stable in the skin extracts over time, compared to Pentapeptide-4. The concentration of Palmitoyl Pentapeptide-4 detected in the epidermal skin extract after 120 min was similar to the initial concentration. After 60 min, 11.2% Palmitoyl Pentapeptide-4 remained in the skin homogenate, and, after 120 min, 9.7% Palmitoyl Pentapeptide-4 remained in the dermal extract.

In parallel, the effects of various proteolytic enzyme inhibitors on enzymatic degradation was also investigated; five kinds of protease inhibitors, namely, thimerosal (0.5 mM), DL-thiorpan (0.25 mM), ethylenediaminetetraacetic acid (0.5 mM), phenylmethanesulfonylfluoride (5 mM), 1,10-phenanthroline (1 mM), or a combination of phenylmethanesulfonylfluoride and 1,10-phenanthroline, were added to Pentapeptide-4 or Palmitoyl Pentapeptide-4 in skin extracts or homogenates at 37 °C for 120 min. Phenylmethanesulfonylfluoride and 1,10-phenanthroline, separately and in combination, showed strong protective effects against the degradation of Pentapeptide-4 in the dermal skin extract and skin homogenates. With the exception of thimerosal, the other protease inhibitors had a substantial inhibitory effect on the degradation of Palmitoyl Pentapeptide-4.

TOXICOLOGICAL STUDIES

Acute Toxicity Studies

Oral

Palmitoyl Pentapeptide-4

The acute oral toxicity of Palmitoyl Pentapeptide-4, tested at 0.01% (vehicle not specified), was evaluated in Sprague-Dawley rats (5/sex), in accordance with Organisation for Economic Cooperation and Development (OECD) test guideline (TG) 401.[27] A single dose of the test substance (20 ml/kg) was administered via gavage. Mortality, clinical abnormalities, and body weight gain were monitored for a period of up to 14 d; all animals were killed at the end of the study. No deaths occurred during the study and no apparent changes or abnormalities were observed in general behavior, in body weights gain, or upon necropsy.

Short-Term, Subchronic, and Chronic Toxicity Studies

No short-term, subchronic, or chronic toxicity studies were found in the published literature, and unpublished data were not submitted.

DEVELOPMENTAL AND REPRODUCTIVE TOXICITY STUDIES

No developmental and reproductive toxicity studies were found in the published literature, and unpublished data were not submitted.

GENOTOXICITY STUDIES

In Vitro

Palmitoyl Pentapeptide-4

The mutagenic potential of a 0.5% solution of Palmitoyl Pentapeptide-4 prepared in distilled water and ethanol was determined using an Ames test.[28] The preparation was tested at 2% using *Salmonella typhimurium* TA98, TA100, TA1535, and TA1537 and *Escherichia coli* WP2*uvr*A, with doses of 312.5, 625, 1250, 2500, and 5000 µg/plate in the presence and absence of metabolic activation. For positive controls, sodium azide, 9-aminoacridine, 2-nitrofluorene, and 4-nitroquinoline were tested in the absence of metabolic activation, while 2-anthramine was tested in the presence of metabolic activation. Revertant colonies were scored after 48 to 72 h of incubation at 37 °C. A 0.5% solution of Palmitoyl Pentapeptide-4 tested at 2% was not mutagenic under the conditions of the study. Results for the vehicle and positive controls were as expected.

CARCINOGENICITY STUDIES

No carcinogenicity studies were found in the published literature, and unpublished data were not submitted.

DERMAL IRRITATION AND SENSITIZATION STUDIES

Details on the dermal irritation and sensitization data summarized below can be found in Table 5.

Palmitoyl Pentapeptide-4, tested at 0.01% (vehicle not specified), was not irritating in an acute dermal irritation test performed in accordance with OECD TG 404 using New Zealand white rabbits nor in a 2-wk dermal irritation study using guinea pigs.[29,30] A trade name mixture containing 100 ppm Palmitoyl Pentapeptide-4 was tested for acute skin irritation using 10 subjects.[31] Very slight erythema was observed in 1 of the subjects and the primary cutaneous irritation (PCI) score was determined to be 0.10. The test substance was considered to be well-tolerated. A guinea pig maximization test was performed in accordance with OECD TG 406, to evaluate the sensitization potential of Palmitoyl Pentapeptide-4, tested at 0.01%.[32] Thirty guinea pigs (test animals: 10/sex; controls: 5/sex), received the test substance at an effective concentration of 0.0075% (w/w; in saline) followed by an undiluted epicutaneous application during induction, and a dermal application of the test substance at an effective concentration of 0.0025%, in saline, during challenge. No skin reactions were observed during evaluation of the test sites 24 and 48 h after patch removal; the test substance was deemed non-sensitizing. A trade name mixture containing 100 ppm Palmitoyl Pentapeptide-4 did not cause irritation or sensitization in a human repeated insult patch test (HRIPT) using 51 subjects.[33] No further details were provided.

OCULAR IRRITATION STUDIES

In Vitro

Palmitoyl Pentapeptide-4

A trade name mixture containing 100 ppm Palmitoyl Pentapeptide-4 was tested for ocular irritation potential in an in vitro hen's egg-chorioallantoic membrane (HET-CAM) assay, following the 1996 HET CAM protocol published in the *Journal Officiel Republique Francaise*.[31] The test substance was tested as supplied. The mean irritation index for the

positive control, sodium dodecyl sulfate (0.5% (w/v)), was 12.0, while the mean irritation index for the test substance was 6.0. Thus, the test substance was deemed a moderate ocular irritant. No further details were provided.

Animal

Palmitoyl Pentapeptide-4

Palmitoyl Pentapeptide-4 tested at 0.01% (vehicle not specified) was assessed for ocular irritation in 3 male New Zealand white rabbits, in accordance with OECD TG 405.[34] A single dose of 0.1 ml was instilled into the conjunctival sac of the left eye, and the eye was not rinsed. The right eye was untreated and served as a control. Ocular reactions were evaluated 1, 24, 48, and 72 h after instillation, and mean values for chemosis, redness of the conjunctiva, iris lesions, and corneal opacity were calculated for each animal. All mean values were 0 at each time interval and the test substance was deemed non-irritating to rabbit eyes under the conditions of this study.

CLINICAL STUDIES

Use Studies

Palmitoyl Pentapeptide-4 has been tested in several clinical studies for its use as an anti-wrinkle agent. A moisturizer containing 3 ppm Palmitoyl Pentapeptide-4 was well tolerated in a 12-wk, double-blind, placebo-controlled, split face, left-right randomized clinical study performed in 93 female subjects.[35] In an 8-wk, randomized parallel-group study conducted in 196 women, a cosmetic product regimen containing niacinamide, Palmitoyl Pentapeptide-4 (concentration not provided), palmitoyl-lysine-threonine, retinyl propionate, and carnosine in a moisturizing base was well tolerated compared to a moisturizer containing 0.02% tretinoin.[36] Palmitoyl Pentapeptide-4 was also well tolerated in another 8-wk, double-blind randomized trial evaluating the effectiveness of 3 cream formulations containing either acetylhexapeptide-3, Pentapeptide-4, or placebo (concentrations not provided).[37]

SUMMARY

This assessment reviews the safety of Myristoyl Pentapeptide-4, Palmitoyl Pentapeptide-4, and Pentapeptide-4 as used in cosmetic formulations. These 3 synthetic peptides share the amino acid sequence lysine-threonine-threonine-lysine-serine. According to the *Dictionary*, these ingredients are reported to function in cosmetics as skin-conditioning agents. As reported in 2023 VCRP data, Palmitoyl Pentapeptide-4 is used in 239 formulations. Myristoyl Pentapeptide-4 had the highest concentration of use reported in 2022 in a leave-on formulation, at up to 0.05% in other eye makeup preparations.

The permeability of Palmitoyl Pentapeptide-4 and Pentapeptide-4 was evaluated in an in vitro study using hairless mice skin. Either 1 ml of Palmitoyl Pentapeptide-4 or Pentapeptide-4 was incubated with skin samples for 24 h; the amount of each substance distributed in each skin layer was extracted using methanol and analyzed using LC-MS/MS. Pentapeptide-4 was not detected in the receptor solution after an observation period of 48 h; a trace amount of Palmitoyl Pentapeptide-4 was detected after 24 h, but it was below the LOQ at < 0.5 µg/ml. No amount of Pentapeptide-4 was detected in any of the skin layers over a period of 24 h. Palmitoyl Pentapeptide-4 was observed in every skin layer at: 4.2 ± 0.7 µg/cm^2 in the stratum corneum, 2.8 ± 0.5 µg/cm^2 in the epidermis, and 0.3 ± 0.1 µg/cm^2 in the dermis. Overall, 14.6% of the applied Palmitoyl Pentapeptide-4 was retained in the skin: 8.3% in the stratum corneum, 5.6% in the epidermis, and 0.6% in the dermis. The researchers concluded that Palmitoyl Pentapeptide-4 and Pentapeptide-4 did not permeate through full-thickness mouse skin.

The in vitro dermal stability of Palmitoyl Pentapeptide-4 and Pentapeptide-4 was evaluated in several mice skin extracts. Either 200 µl Pentapeptide-4 or 40 µg/ml Palmitoyl Pentapeptide-4 (in 10 mM HEPES buffer) were incubated with 200 µl of the epidermal skin extract, dermal skin extract, or skin homogenates at 37 °C for 120 min. The amounts of each substance present in the incubated mixtures was sampled and analyzed by LC-MS/MS. Pentapeptide-4 was almost fully degraded in the dermal skin extract and skin homogenate, with 3.2% remaining in the dermal skin extract at 30 min and 1.5% remaining in the skin homogenate at 60 min. Palmitoyl Pentapeptide-4 was more stable in the skin extracts over time; the amount detected in the epidermal skin extract after 120 min was similar to the initial concentration. After 60 min, 11.2% Palmitoyl Pentapeptide-4 remained in the skin homogenate and after 120 min, 9.7% Palmitoyl Pentapeptide-4 remained in the dermal extract. When the inhibitory effect of 5 protease inhibitors (thimerosal, DL-thiorpan, ethylenediaminetetraacetic acid, phenylmethanesulfonylfluoride, and 1,10-phenanthroline) upon the enzymatic degradation of Palmitoyl Pentapeptide-4 and Pentapeptide-4 in the epidermal skin, dermal skin, or skin homogenates was evaluated, phenylmethanesulfonylfluoride and 1,10-phenanthroline were shown to have strong protective effects against the degradation of Pentapeptide-4 in the dermal skin extract and skin homogenates. With the exception of thimerosal, the other protease inhibitors had a substantial inhibitory effect on the degradation of Palmitoyl Pentapeptide-4.

In an acute oral toxicity study, performed in accordance with OECD TG 401, groups of Sprague-Dawley rats (5/sex) received a single dose of Palmitoyl Pentapeptide-4 (20 ml/kg), tested at 0.01%, via gavage. No deaths occurred during the study and no abnormalities were observed in the general behavior, body weight gain, or upon necropsy. The test substance was deemed non-toxic in rats.

A 0.5% solution of Palmitoyl Pentapeptide-4 tested at 2% was not mutagenic in an Ames test at up to 5000 µg/plate, with or without activation. The test was performed with *S. typhimurium* TA98, TA100, TA1535, TA1537 and *E. coli* WP2*uvr*A.

Palmitoyl Pentapeptide-4, tested at 0.01%, was not irritating to rabbit skin in an acute dermal irritation study nor to guinea pigs in a 2-wk dermal irritation study. In an acute irritation study using 10 subjects, a trade name mixture containing Palmitoyl Pentapeptide-4 was well tolerated; very slight erythema was seen in 1 of the subjects and the PCI = 0.10. Palmitoyl Pentapeptide-4 was not sensitizing in a guinea pig maximization test, when injected at 0.0075% in saline, and applied at 0.01% during intradermal induction, and at 0.0025% in saline during challenge. No irritation or sensitization was observed in an HRIPT in which 51 subjects were treated with a trade name mixture containing 100 ppm Palmitoyl Pentapeptide-4.

The ocular irritation potential of a trade name mixture containing 100 ppm Palmitoyl Pentapeptide-4 was evaluated in an in vitro HET-CAM assay. The mean irritation index for the test substance, when tested as supplied, was 6.0, compared to the sodium dodecyl sulfate value of 12.0. Thus, the test substance was deemed a moderate ocular irritant. In an acute ocular irritation study, a single, 0.1 ml dose of Palmitoyl Pentapeptide-4 tested at 0.01% was not irritating to New Zealand white rabbit eyes.

Clinically, a moisturizer containing 3 ppm Palmitoyl Pentapeptide-4 was well-tolerated in a 12-wk, double blind placebo-controlled, split face, left-right randomized clinical study performed in 93 female subjects. Palmitoyl Pentapeptide-4 has also been shown to be well tolerated in other randomized trials where it was tested in cosmetic formulations (concentrations not provided).

INFORMATION SOUGHT

The following information on these ingredients is being sought, with specific application to cosmetics, for use in the resulting safety assessment:

1. Method of manufacture and impurities data for these ingredients as used in cosmetics
2. Toxicological data, and any other information that would inform this safety assessment

TABLES

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

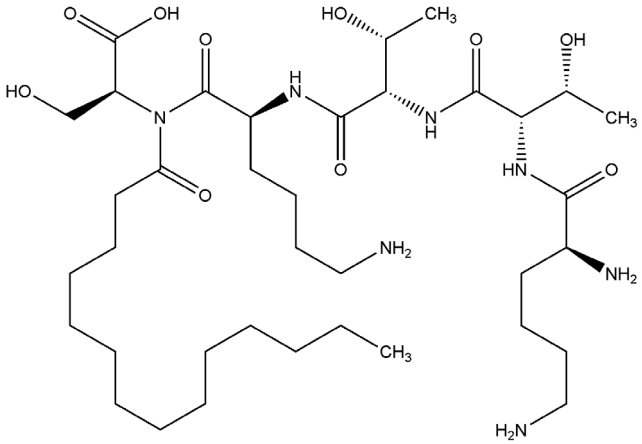
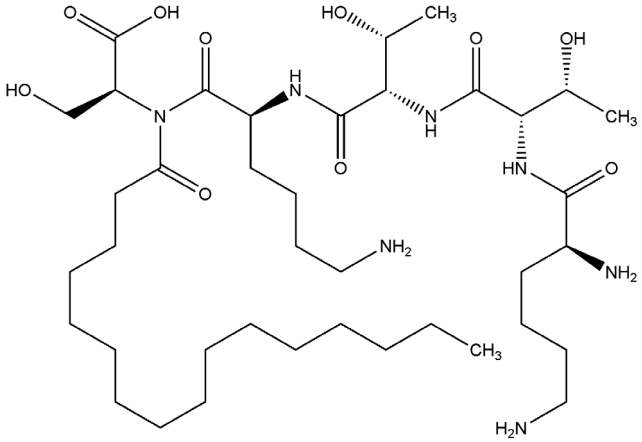
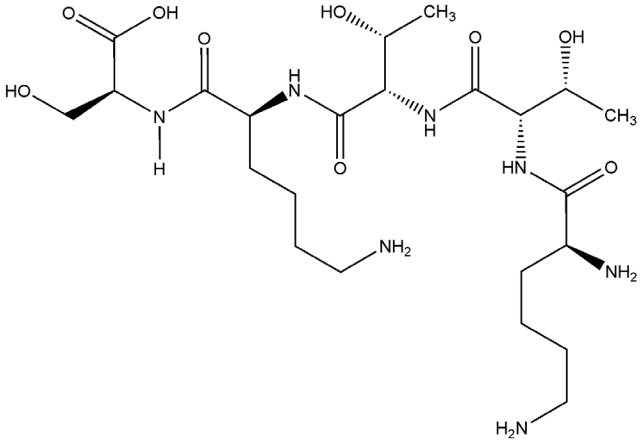
Ingredient	Definition	Function
Myristoyl Pentapeptide-4	Myristoyl Pentapeptide-4 is the reaction product of myristic acid and Pentapeptide-4. 	Skin-conditioning agent - miscellaneous
Palmitoyl Pentapeptide-4 521091-64-5 214047-00-4	Palmitoyl Pentapeptide-4 is the reaction product of palmitic acid and Pentapeptide-4. 	Skin-conditioning agent - miscellaneous
Pentapeptide-4	Pentapeptide-4 is a synthetic peptide containing lysine, serine, and threonine. 	Skin-conditioning agent - miscellaneous

Table 2. Chemical properties

Property	Value	Reference
	Myristoyl Pentapeptide-4	
Molecular Weight (g/mol)	774	14
Topological Polar Surface Area (Å^2)	296 (estimated)	14
	Palmitoyl Pentapeptide-4	
Physical Form	powder	15
Color	white	15
Molecular Weight (g/mol)	802.1	15,16
Topological Surface Area (Å^2)	296 (estimated)	16
log p	3.32 (estimated)	2
	Pentapeptide-4	
Molecular Weight (g/mol)	563.6	17
Topological Polar Surface Area (Å^2)	292 (estimated)	17
log p	-3.27 (estimated)	2

Table 3. Frequency (2023/2012) and concentration (2022/2013) of use according to duration and exposure for Palmitoyl Pentapeptide-4

	# of Uses		*Max Conc of Use (%)*	
	2023[20]	**2012[22]**	**2022[21]**	**2013[23]**
Totals	**239**	**51**	**0.000005-0.0035**	**0.00001 – 0.00061**
summarized by likely duration and exposure*				
Duration of Use				
Leave-On	*223*	*50*	*0.00036-0.0012*	*0.00001 – 0.00061*
Rinse-Off	*16*	*1*	*0.000005-0.0035*	*0.000085*
Diluted for (Bath) Use	*NR*	*NR*	*NR*	*NR*
Exposure Type*				
Eye Area	31	11	0.0012	0.00001 – 0.00061
Incidental Ingestion	NR	NR	NR	NR
Incidental Inhalation-Spray	117[a]; 64[b]	16[a]; 19[b]	NR	NR
Incidental Inhalation-Powder	1; 64[b]	19[b]	0.00036-0.0012[c]	0.00001 – 0.00061[c]
Dermal Contact	236	51	0.000005-0.0012	0.00001 – 0.00061
Deodorant (underarm)	NR	NR	NR	NR
Hair - Non-Coloring	3	NR	0.00035-0.0035	NR
Hair-Coloring	NR	NR	NR	NR
Nail	NR	NR	NR	NR
Mucous Membrane	2	NR	0.000005	NR
Baby Products	NR	NR	NR	NR
as reported by product category				
Eye Makeup Preparations				
Eye Lotion	21	6	0.0012	0.00001 – 0.00061
Other Eye Makeup Preparations	10	5	NR	NR
Hair Preparations (non-coloring)				
Hair Conditioner	1	NR	0.0035	NR
Rinses (non-coloring)	1	NR	NR	NR
Shampoos (non-coloring)	1	NR	0.00035	NR
Makeup Preparations				
Face Powders	1	NR	NR	NR
Foundations	4	NR	NR	0.00005 – 0.00011
Makeup Bases	NR	1	NR	NR
Personal Cleanliness Products				
Bath Soaps and Detergents	1	NR	0.000005	NR
Other Personal Cleanliness Products	1	NR	NR	NR
Skin Care Preparations				
Cleansing	10	1	0.000005	0.000085
Face and Neck (exc shave)	59	19	0.0012 (not spray)	0.00001 – 0.00061 (not spray)
Body and Hand (exc shave)	5	NR	0.00036 (not spray)	0.00003 – 0.00011 (not spray)
Moisturizing	101	12	0.00059 (not spray)	NR
Night	8	4	NR	0.00001 – 0.00031 (not spray)
Paste Masks (mud packs)	1	NR	NR	NR
Skin Fresheners	8	NR	NR	NR
Other Skin Care Preparations	6	3	NR	0.00031

NR – not reported

*likely duration and exposure is derived based on product category (see Use Categorization https://www.cir-safety.org/cir-findings)

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

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

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

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

Table 4. Frequency (2023)[20] and concentration (2022)[21] of use according to likely duration and exposure by product category

	Myristoyl Pentapeptide-4		Pentapeptide-4	
	# of Uses	Max Conc of Use (%)	# of Uses	Max Conc of Use (%)
Totals	**4**	**0.05**	**1**	**NR**
summarized by likely duration and exposure*				
Duration of Use				
Leave-On	*4*	*0.05*	*1*	*NR*
Rinse-Off	*NR*	*NR*	*NR*	*NR*
Diluted for (Bath) Use	*NR*	*NR*	*NR*	*NR*
Exposure Type*				
Eye Area	4	0.05	NR	NR
Incidental Ingestion	NR	NR	NR	NR
Incidental Inhalation-Spray	NR	NR	1[a]	NR
Incidental Inhalation-Powder	NR	NR	NR	NR
Dermal Contact	4	0.05	1	NR
Deodorant (underarm)	NR	NR	NR	NR
Hair - Non-Coloring	NR	NR	NR	NR
Hair-Coloring	NR	NR	NR	NR
Nail	NR	NR	NR	NR
Mucous Membrane	NR	NR	NR	NR
Baby Products	NR	NR	NR	NR
as reported by product category				
Eye Makeup Preparations				
Eye Lotion				
Other Eye Makeup Preparations	4	0.05		
Hair Preparations (non-coloring)				
Hair Conditioner				
Rinses (non-coloring)				
Shampoos (non-coloring)				
Makeup Preparations				
Face Powders				
Foundations				
Makeup Bases				
Personal Cleanliness Products				
Bath Soaps and Detergents				
Other Personal Cleanliness Products				
Skin Care Preparations				
Cleansing				
Face and Neck (exc shave)				
Body and Hand (exc shave)				
Moisturizing			1	NR
Night				
Paste Masks (mud packs)				
Skin Fresheners				
Other Skin Care Preparations				

NR – not reported

*likely duration and exposure is derived based on product category (see Use Categorization https://www.cir-safety.org/cir-findings)

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

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

Table 5 . Dermal irritation and sensitization studies

Test Article	Vehicle	Concentration/Dose	Test Population	Procedure	Results	Reference
IRRITATION						
ANIMAL						
Palmitoyl Pentapeptide-4	not specified	0.01%; 0.5 ml	3 male New Zealand white rabbits	OECD TG 404. Acute dermal irritation study. Semi-occlusive application of the test substance was made to shaved skin for 4 h. Skin reactions were observed 1, 24, 48, and 72 h after patch removal. Mean values for erythema and edema were calculated for each animal.	Not irritating. Very slight erythema was observed in 1 animal, only on day 1. All erythema and edema mean scores over 24, 48, and 72 h were 0.	[29]
Palmitoyl Pentapeptide-4	not specified	0.01%; 0.05 ml	Guinea pigs (5/sex; strain not specified)	2-wk dermal irritation study. Open application to a shaved, 2 cm^2 area of the left flank for 14 d. Purified water applied to the right flank served as controls. Skin reactions were evaluated before and approximately 24 h after each application; these values were used to calculate daily irritation and weekly mean irritation indices. The animals were killed at the end of the study; no internal organ examination or excision of skin was performed.	Non-irritating. No treatment-related deaths or skin reactions in controls were observed. Very slight erythema was noted in 1 animal on days 12 and 13, which was not attributed to the test substance.	[30]
HUMAN						
Trade name mixture containing 100 ppm Palmitoyl Pentapeptide-4	not specified	0.02 ml	10 subjects	Acute skin irritation study. Occlusive, neat application of the test substance was made to a 50 mm^2 area of the back for 48 h using Finn chambers. Untreated sites covered with an occlusive patch served as negative controls. Skin reactions were scored 30 min after patch removal.	Well -tolerated. Very slight erythema (hardly visible) in 1 of the subjects. PCI = 0.10.	[31]
SENSITIZATION						
ANIMAL						
Palmitoyl Pentapeptide-4	saline	0.01%; Induction: 75% (effective concentration 0.0075%); Intradermal challenge: applied neat (effective concentration 0.01%) Challenge: 25% (effective concentration: 0.0025%)	Guinea pigs (strain not specified) test animals: 10/sex controls: 5/sex	OECD TG 406. Guinea pig maximization test. Saline solution and mercaptobenzothiazole in corn oil served as negative and positive controls, respectively. On day 1, the test substance was mixed with FCA and injected intradermally in the back. After pretreatment with 10% SLS, the test substance was applied on day 8, under occlusion, to the same region for 48 h. After a rest period of 12 d, both test and control animals received an occlusive challenge application of the test substance to the right flank, as well as an occlusive application of the vehicle control to the left flank, both for 48 h. Skin reactions were evaluated 24 and 48 h after patch removal.	Not sensitizing. Controls yielded expected results.	[32]
HUMAN						
Trade name mixture containing 100 ppm Palmitoyl Pentapeptide-4	not specified	not specified	51 subjects	HRIPT	Non-irritating and non-sensitizing	[33]

Abbreviations: FCA – Freund's Complete Adjuvant; HRIPT – human repeat insult patch test; OECD – Organisation for Economic Cooperation and Development; PCI – primary cutaneous irritation; SLS – sodium lauryl sulfate; TG – test guideline

REFERENCES

1. Nikitakis J, Kowcz A. Web-Based *International Cosmetic Ingredient Dictionary and Handbook* (wINCI *Dictionary*). https://incipedia.personalcarecouncil.org/winci/. Washington, D.C.: Personal Care Products Council. Last Updated 2023. Accessed 4/14/2023.

2. Abu Samah NH, Heard CM. Topically applied KTTKS: a review. *Int J Cosmet Sci*. 2011;33(6):483-490.

3. Burnett CL, Heldreth B, Bergfeld WF, et al. Safety assessment of α-amino acids as used in cosmetics. *Int J Toxicol*. 2013;32(6 Suppl):41s-64s.

4. Andersen FA, (ed.). Final Report on the Safety Assessment of Oleic Acid, Lauric Acid, Palmitic Acid, Myristic Acid, and Stearic Acid. *Int J Toxicol*. 1987;6(3):321-401.

5. Andersen FA, (ed.). Annual review of cosmetic ingredient safety assessments-2004/2005. *Int J Toxicol*. 2006; 2(25 Suppl):1-89.

6. Becker LC, Bergfeld WF, Belsito DV, et al. Final report of the amended safety assessment of myristic acid and its salts and esters as used in cosmetics. *Int J Toxicol*. 2010;29(4 Suppl):162s-186s.

7. Burnett CL, Bergfeld WF, Belsito DV, et al. Safety Assessment of Fatty Acids & Fatty Acid Salts as Used in Cosmetics 2019. Available from CIR (https://www.cir-safety.org/ingredients).

8. Lupo MP, Cole AL. Cosmeceutical peptides. *Dermatol Ther*. 2007;20(5):343-349.

9. Katayama K, Armendáriz-Borunda J, Raghow R, Kang AH, Seyer JM. A pentapeptide from Type I procollgen promotes extracellular matrix production. *J Biol Chem*. 1993;268:9941-9944.

10. Aldag C, Nogueira Teixeira D, Leventhal PS. Skin rejuvenation using cosmetic products containing growth factors, cytokines, and matrikines: a review of the literature. *Clin Cosmet Investig Dermatol*. 2016;9:411-419.

11. Tsai WC, Hsu CC, Chung CY, Lin MS, Li SL, Pang JH. The pentapeptide KTTKS promoting the expressions of type I collagen and transforming growth factor-beta of tendon cells. *J Orthop Res*. 2007;25(12):1629-1634.

12. Lintner K. Promoting production in the extracellular matrix without compromising barrier. *Cutis*. 2002;70(6 Suppl):13-16; discussion 21-13.

13. Mortazavi SM, Kobarfard F, Maibach HI, Moghimi HR. Effect of palmitic acid conjugation on physicochemical properties of peptide KTTKS: a preformulation study. *J Cosmet Sci*. 2019;70(6):299-312.

14. National Library of Medicine. PubChem : Myristoyl pentapeptide-4 https://pubchem.ncbi.nlm.nih.gov/compound/Myristoyl-pentapeptide-4. Last Updated 2023 Mar 4. Accessed 02/06/2023.

15. Sederma. 2012. Summary of information on Palmitoyl Pentapeptide-4 (previously named Palmitoyl Pentapeptide-3). Unpublished data submitted by Personal Care Products Council on November 13, 2012.

16. National Library of Medicine. PubChem : L-Serine, N2-(1-oxohexadecyl)-L-lysyl-L-threonyl-L-threonyl-L-lysyl- (Compound) (Palmitoyl Pentapeptide-4; CAS No. 214047-00-4). https://pubchem.ncbi.nlm.nih.gov/compound/9897237#section=Structures. Last Updated 2023 Mar 4. Accessed 02/06/2023.

17. National Library of Medicine. PubChem : L-Serine, L-lysyl-L-threonyl-L-threonyl-L-lysyl- (Pentapeptide-4; CAS No. 149128-48-3). https://pubchem.ncbi.nlm.nih.gov/compound/9959565. Last Updated 2023 Mar 4. Accessed 02/06/2023.

18. Chandrudu S, Simerska P, Toth I. Chemical methods for peptide and protein production. *Molecules (Basel, Switzerland)*. 2013;18(4):4373-4388.

19. Isidro-Llobet A, Kenworthy MN, Mukherjee S, et al. Sustainability challenges in peptide synthesis and purification: from R&D to production. *J Org Chem*. 2019;84(8):4615-4628.

20. U.S. Food and Drug Administration Center for Food Safety & Applied Nutrition (CFSAN). 2023. Voluntary Cosmetic Registration Program - Frequency of Use of Cosmetic Ingredients (VCRP). Obtained under the Freedom of Information Act from CFSAN; requested as "Frequency of Use Data" January 4, 2023; received February 2, 2023.

21. Personal Care Products Council. 2022. Concentration of Use by FDA Product Category: Pentapeptide-4. Unpublished data submitted by the Personal Care Products Council on July 6, 2022.

22. U.S. Food and Drug Administration Center for Food Safety & Applied Nutrition (CFSAN). 2012. Voluntary Cosmetic Registration Program - Frequency of Use of Cosmetic Ingredients. Obtained under the Freedom of Information Act from CFSAN; requested as "Frequency of Use Data" January 2012, Received May 2012.

23. Personal Care Products Council. 2013. Concentration of Use by FDA product category: Palmitoyl peptide ingredients. Unpublished data submitted by the Personal Care Products Council on January 23, 2013.

24. European Union. EUR-Lex: Access to European Union law. https://eur-lex.europa.eu/homepage.html. Last Updated 2023. Accessed 04/11/2023.

25. Kachooeian M, Mousivand Z, Sharifikolouei E, et al. Matrixyl patch vs matrixyl cream: a comparative in vivo investigation of matrixyl (MTI) effect on wound healing. *ACS Omega*. 2022;7(28):24695-24704.

26. Choi YL, Park EJ, Kim E, Na DH, Shin YH. Dermal stability and in vitro skin permeation of collagen pentapeptides (KTTKS and palmitoyl-KTTKS). *Biomol Ther (Seoul)*. 2014;22(4):321-327.

27. CIT. 1999. Summary of acute oral toxicity in rats Palmitoyl Pentapeptide-4. Laboratory study number 18838 TAR. Unpublished data submitted by Personal Care Products Council on November 13, 2012.

28. CIT. 1999. Summary of bacterial reverse mutation test Palmitoyl Pentapeptide-4. Laboratory study number 18796 MMJ. Unpublished data submitted by Personal Care Products Council on November 13, 2012.

29. CIT. 1999. Summary of acute dermal irritation in rabbits Palmitoyl Pentapeptide-4. Laboratory study number 18839 TAL. Unpublished data submitted by Personal Care Products Council on November 13, 2012.

30. CIT. 1999. Sumary of local tolerance study after repeated topical application for 2 weeks in guinea pigs: Palmitoyl Pentapeptide-4. Laboratory study number 18842 TSG. Unpublished data submitted by Personal Care Products Council on November 13, 2012.

31. Institut D'Expertise Cliniqu. 1998. Summary of HET-CAM assay and human primary cutaneous tolerance of MATRIXYL (contains 100 pmm Palmitoyl Pentapeptide-4). Report No. 80503RD2. Unpublished data submitted by Personal Care Products Council on November 13, 2012.

32. CIT. 1999. Summary of skin sensitization test in guinea pigs Palmitoyl Pentapeptide-4. Laboratory study number 18841 TSG. Unpublished data submitted by Personal Care Products Council on November 13, 2012.

33. Consumer Product Testing Company. 1999. Summary of repeated insult patch test of MATRIXYL (contains 100 ppm Palmitoyl Pentapeptide-4). Experiment Reference Number. C99-0567-02. Unpublished data submitted by Personal Care Products Council on November 13, 2012.

34. CIT. 1999. Summary of acute eye irritation in rabbits Palmitoyl Pentapeptide-4. Laboratory study number 18840 TAL. Unpublished data submitted by Personal Care Products Council on November 13, 2012.

35. Robinson LR, Fitzgerald NC, Doughty DG, Dawes NC, Berge CA, Bissett DL. Topical palmitoyl pentapeptide provides improvement in photoaged human facial skin. *Int J Cosmet Sci*. 2005;27(3):155-160.

36. Fu JJ, Hillebrand GG, Raleigh P, et al. A randomized, controlled comparative study of the wrinkle reduction benefits of a cosmetic niacinamide/peptide/retinyl propionate product regimen vs. a prescription 0.02% tretinoin product regimen. *Br J Dermatol*. 2010;162(3):647-654.

37. Aruan RR, Hutabarat H, Widodo AA, Firdiyono M, Wirawanty C, Fransiska L. Double-blind, randomized trial on the effectiveness of acetylhexapeptide-3 cream and palmitoyl pentapeptide-4 cream for crow's feet. *J Clin Aesthet Dermatol*. 2023;16(2):37-43.