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

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*All interested persons are provided 60 days from the above release date **(i.e., until June 9, 2024)** to comment on this safety assessment, and to identify additional published data that should be included or provide unpublished data which can be made public and included.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 All unpublished data submitted to CIR will be discussed in open meetings, will be available for review by any interested party, and may be cited in a peer-reviewed scientific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.*

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ABBREVIATIONS

ARE	antioxidant/electrophile response element
CAS	Chemical Abstracts Service
CIR	Cosmetic Ingredient Review
Council	Personal Care Products Council
CPSC	Consumer Product Safety Commission
DHT	5α-dihydrotestosterone
DMSO	dimethyl sulfoxide
DPBS	Dulbecco's phosphate buffer solution
DPRA	direct peptide reactivity assay
E2	17-β estradiol
EC_{10}	10% effect concentration
ECVAM DB-ALM	European Centre for Validation of Alternative Methods Database on Alternative Methods
FCA	Freund's complete adjuvant
Fmoc	fluorenylmethoxycarbonyl
Fmoc-Lys(Boc)-OH	N_α-fluorenylmethoxycarbonyl-N_ε-(*t*-butoxycarbonyl)-lysine
Fmoc-Ser(tBu)-OH	N_α-fluorenylmethoxycarbonyl-*O*-(*t*-butyl)-L-serine
Fmoc-Thr(tBu)-OH	N_α-fluorenylmethoxycarbonyl-*O*-(*t*-butyl)-L-threonine
FDA	Food and Drug Administration
GLP	good laboratory practices
HEPES	4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid
hERα	human estrogen receptor α
hAR	human androgen receptor
HET-CAM	hen's egg-chorioallantoic membrane
HRIPT	human repeated insult patch test
I_{max}	maximal response
ICH Q3C	International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use Guideline for Residual Solvents
KTTKS	lysine-threonine-threonine-lysine-serine; Pentapeptide-4
KTSKS	lysine-threonine-serine-lysine-serine; Pentapeptide-4
LC-MS/MS	liquid chromatography with tandem mass spectrophotometry
LoD	limit of detection
LOQ	limit of quantification
LPPS	liquid-phase peptide synthesis
MTT	3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide
NR	none reported
OD	optical density
OECD	Organisation for Economic Cooperation and Development
Pal-KTTKS	Palmitoyl Pentapeptide-4
Panel	Expert Panel for Cosmetic Ingredient Safety
PBS	phosphate-buffered solution
PCI	primary cutaneous irritation
SDS	sodium dodecyl sulfate
SLS	sodium lauryl sulfate
SPPS	solid-phase peptide synthesis
TG	test guideline
US	United States
UVA/UVB	ultraviolet light A/ultraviolet light B
VCRP	Voluntary Cosmetic Registration Program
YAS	Yeast Androgen Screen
YES	Yeast Estrogen Screen
wINCI; *Dictionary*	web-based *International Cosmetic Ingredient Dictionary and Handbook*

ABSTRACT

The Expert Panel for Cosmetic Ingredient Safety (Panel) assessed the safety of Myristoyl Pentapeptide-4, Palmitoyl Pentapeptide-4, and Pentapeptide-4 (KTTKS and KTSKS sequences), which are reported to function as skin-conditioning agents in cosmetic products. The Panel reviewed the available data to determine the safety of these ingredients and concluded these ingredients are safe in cosmetics in the present practices of use and concentration described in this safety assessment.

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 comprise a 5-amino-acid-sequence (pentapeptide) containing lysine, serine, and threonine. One such sequence is 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 amino acid sequence of the pentapeptide portion of these ingredients can vary; thus, data for two variations of Pentapeptide-4, namely, KTTKS and KTSKS (Lys-Thr-Ser-Lys-Ser), are included in this report.

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 January 2024. 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.

CHEMISTRY

Definition and Structure

Pentapeptide-4 (CAS No. 149128-48-3) is a synthetic peptide comprised of the amino acids, lysine, serine, and threonine (forming a pentapeptide), in either the lysine-threonine-threonine-lysine-serine (also represented as Lys-Thr-Thr-Lys-Ser; i.e, KTTKS) or, lysine-threonine-serine-lysine-serine (also represented as Lys-Thr-Ser-Lys-Ser, i.e. KTSKS) sequence (Figure 1).[1,2] Myristoyl Pentapeptide-4 (CAS No. 1392416-25-9) 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.

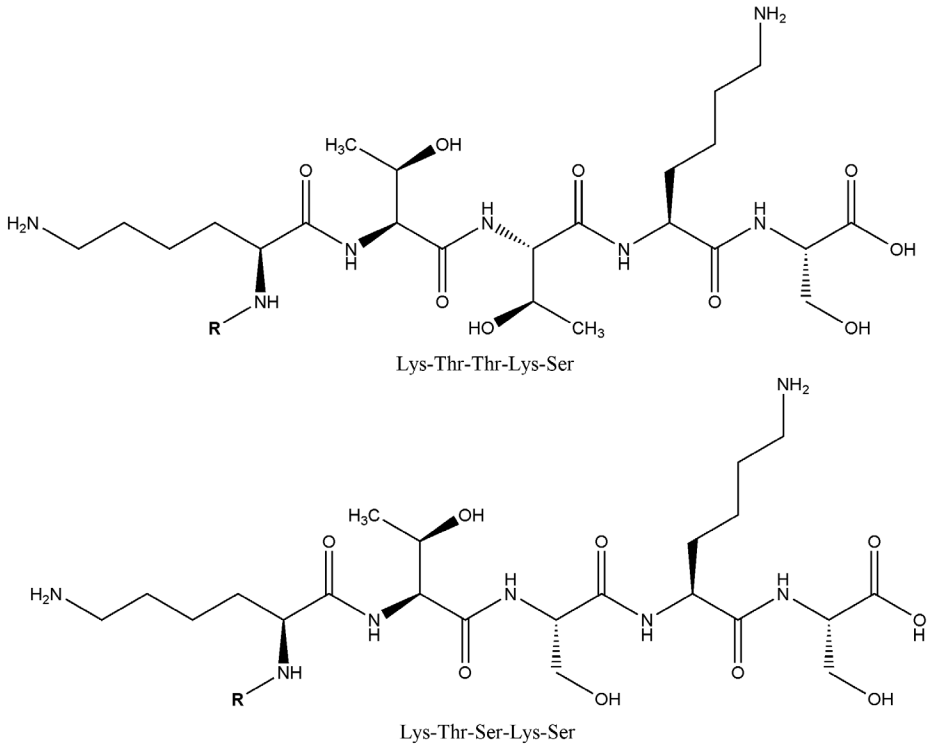


Figure 1. Pentapeptide-4 (when R is hydrogen) and *N*-capped derivatives (when R is the residue of myristic or palmitic acid)

Pentapeptide-4 is a subfragment of type I collagen propeptide, and is regarded as a signal peptide and a matrikine, which possesses 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 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 more 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. Additionally, these ingredients have the following predicted log p values for the KTTKS and KTSKS sequences, respectively: Myristoyl Pentapeptide-4 (1.85; 1.6), Palmitoyl Pentapeptide-4 (2.72; 2.52) , and Pentapeptide-4 (-4.12; -4.39).[18] Chemical properties for ingredients in this report are further outlined in Table 2.

Method of Manufacture

Palmitoyl Pentapeptide-4

Two samples of Palmitoyl Pentapeptide-4 (Pal-KTTKS and Pal-KTSKS) are described by a supplier as being obtained via solid phase synthesis at room temperature using Fmoc-amino acid derivatives.[19] An N_α-fluorenylmethoxycarbonyl-N_ε-(*t*-butoxycarbonyl)-lysine (Fmoc-Lys(Boc)-OH) complex is first activated with a coupling agent and reacted on serine-protected resin. Deprotection of the Fmoc residue with a base produces a dipeptide on the resin. For the Pal-KTTKS sequence, both activation and coupling are achieved using the N_α-fluorenylmethoxycarbonyl-*O*-(*t*-butyl)-L-threonine (Fmoc-Thr(tBu)-OH) complex, and deprotection is achieved with the Fmoc-Lys(Boc)-OH group. For the Pal-KTSKS sequence, the N_α-fluorenyl-methoxycarbonyl-*O*-(*t*-butyl)-L-serine (Fmoc-Ser(tBu)-OH), Fmoc-Thr(tBu)-OH, and Fmoc-Lys(Boc)-OH groups are utilized for activation, coupling, and deprotection, respectively. After the last Fmoc-deprotection step, palmitic acid is reacted in the same manner in each process and the resulting products are fully deprotected and purified to yield the final amino acid sequences (Pal-Lys-Thr-Thr-Lys-Ser-OH and Pal-Lys-Thr-Ser-Lys-Ser-OH).

Impurities

Palmitoyl Pentapeptide-4

The impurities found in a sample of Palmitoyl Pentapeptide-4 (Pal-KTTKS), as described by a supplier, were: acetate (< 10%), palmitic acid (< 5%), water (< 5%), and residual solvents (in accordance with the International Council for Harmonisation Of Technical Requirements for Pharmaceuticals for Human Use Guideline for Residual Solvents (ICH Q3C)).[15] Two distinct samples of Palmitoyl Pentapeptide-4, each comprising the Pal-KTTKS or Pal-KTSKS sequence, were described by a supplier as having ≥ 90% purity at 210 nm.[19] The supplier described the impurities in the first sample of Palmitoyl Pentapeptide-4 (Pal-KTTKS) as stereoisomers of Pal-KTTKS-OH, myristine-lysine-threonine-threonine-lysine-serine-OH, and stearyl-lysine-threonine-threonine-lysine-serine-OH. The impurities in the second Palmitoyl Pentapeptide-4

sample (Pal-KTSKS) were described by the supplier as stereoisomers of Pal-KTSKS-OH, myristyl-lysine-threonine-serine-lysine-serine-OH, and stearyl-lysine-threonine-serine-lysine-serine-OH.

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 included herein were obtained from the FDA's Voluntary Cosmetic Registration Program (VCRP) database in 2023 (frequency of use) and in response to a survey conducted by the Personal Care Products Council (Council) (maximum use concentrations). The data were provided by cosmetic product categories, based at that time 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 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. The results of the concentration of use survey conducted by the Council in 2022, and revised in 2023, indicate Palmitoyl Pentapeptide-4 has the highest maximum reported concentration of use, at up to 0.0035% in hair conditioners.[21] The highest leave-on maximum concentration of use reported is 0.0012% Palmitoyl Pentapeptide-4 in face and neck preparations. Concentration of use data were not reported for the other 2 ingredients.

Some of these ingredients are reported to be used in products that are applied near the eye; Palmitoyl Pentapeptide-4 is used at 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.[22]

Non-Cosmetic

Palmitoyl Pentapeptide-4 (Pal-KTTKS) has been tested male albino Wistar rats for its wound-healing effects.[23] Palmitoyl Pentapeptide-4 applied in a patch (0.1 and 1 mg) and cream (1 mg) form had a larger impact on wound healing in animals, compared to negative controls (untreated) and positive controls (ready-to-wear dressing; $p < 0.05$).

TOXICOKINETIC STUDIES

Dermal Penetration

In Vitro

Palmitoyl Pentapeptide-4; Pentapeptide-4

The permeability of Palmitoyl Pentapeptide-4 (Pal-KTTKS) and Pentapeptide-4 (KTTKS) was evaluated in an in vitro study using 3 replicate skin samples of CrlOri: SKH1-hr strain hairless mice.[24] 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-piperazineethanesulfonic acid (HEPES) buffer was mixed with 15% ethanol containing phenylmethane-sulfonylfluoride and 1,10-phenanthroline at final concentrations of 5 and 1 mM, respectively, as proteolytic enzyme inhibitors. The donor compartment was loaded with a 1 ml of Palmitoyl Pentapeptide-4 or Pentapeptide-4 (100 µg/ml in 15% ethanol) solution. After 24-h incubation, the skin was removed from the diffusion cell and the remaining donor solution on the skin surface was washed 4 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; < 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.

Absorption, Distribution, Metabolism, and Excretion (ADME)

In Vitro

Palmitoyl Pentapeptide-4; Pentapeptide-4

The dermal stability of Palmitoyl-Pentapeptide-4 (Pal-KTTKS) and Pentapeptide-4 (KTTKS) was evaluated in vitro in epidermal and dermal skin extracts and whole skin homogenate prepared from hairless mouse skin.[24] Pentapeptide-4 (200 µl) 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 whole 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 whole skin homogenate, with 3.2% remaining in the dermal skin extract at 30 min and 1.5% remaining in the whole skin homogenate at 60 min. The degradation of Pentapeptide-4 in the epidermal skin extract was slower than that seen in the dermal skin extract and whole skin homogenate, which was potentially attributed to lower amounts of proteolytic enzymes. 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 whole skin homogenate, and, after 120 min, 9.7% Palmitoyl Pentapeptide-4 remained in the dermal extract.

TOXICOLOGICAL STUDIES

Acute Toxicity Studies

Oral

Palmitoyl Pentapeptide-4

The acute oral toxicity of Palmitoyl Pentapeptide-4 (Pal-KTTKS), 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.[15,25] 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, body weight gain, or upon necropsy.

Short-Term Toxicity Studies

Dermal

Palmitoyl Pentapeptide-4

Groups of guinea pigs (5/sex; strain not specified) were treated with 0.01% Palmitoyl Pentapeptide (0.05 ml; vehicle not specified; Pal-KTTKS) in a 2-wk dermal irritation study.[15,26] No deaths or clinical signs related to treatment were noted during the study; internal organs were not examined. No further details were provided.

Subchronic, and Chronic Toxicity Studies

No 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

Details of the in vitro genotoxicity studies summarized below are provided in Table 4.

A solution of 0.5% Palmitoyl Pentapeptide-4 (Pal-KTTKS) in distilled water and ethanol (75/25), tested at 2% in distilled water, was not mutagenic in an Ames test at concentrations up to 5000 µg/plate using *Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537 and *Escherichia coli* WP2*uvr*A.[15,27] In another Ames test, performed in accordance with OECD TG 471, Palmitoyl Pentapeptide-4 (81.6% pure, Pal-KTSKS) in dimethyl sulfoxide (DMSO) was not mutagenic when tested at concentrations up to 5000 µg/plate using *S. typhimurium* strains TA98, TA100, TA102, TA1535, and TA1537,

with or without metabolic activation; signs of cytotoxic activity were observed under test conditions.[19,28] The genotoxic potential of Palmitoyl Pentapeptide-4 (> 96% pure, Pal-KTSKS) in water was evaluated in an in vitro mammalian cell micronucleus test in accordance with OECD TG 487 using cultured human lymphocytes.[19,29] Cells were treated with 250, 500, or 1000 µg/ml of the test article in the presence of metabolic activation for 4-h, followed by a 24-h recovery period; cells were also treated with 375, 500, or 750 µg/ml of the test article in the absence of metabolic activation for 4 h, followed by a 24-h recovery period (short treatment). In an additional assay, cells were treated with concentrations of 250, 320, or 400 µg/ml Palmitoyl Pentapeptide-4 for 24 h without a recovery period (continuous treatment). Neither statistically nor biologically significant increases in the number of micronucleated cells were observed with either treatment period; the test article was deemed not genotoxic.

CARCINOGENICITY STUDIES

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

OTHER RELEVANT STUDIES

Endocrine Activity

Palmitoyl Pentapeptide-4

The estrogenic and androgenic activity of a formulation containing 0.12% Palmitoyl Pentapeptide-4 (other contents not specified; Pal-KTSKS) was evaluated in transformed yeast cells using the XenoScreen Yeast Estrogen Screen (YES) and Yeast Androgen Screen (YAS) assays.[19,30] *Saccharomyces cerevisiae* cells were genetically transformed with human estrogen receptor α (hERα) and human androgen receptors (hAR) and, additionally, had an expression plasmid carrying the reporter gene lacZ inserted. Binding of the test article with hERα or hAR receptors resulted in the interaction of these receptors with the corresponding response elements on the expression plasmid, in turn affecting β-galactosidase gene expression. Thus, the amount of secreted β-galactosidase, which was correlated with colorimetric quantification of the conversion of the yellow substrate, chlorophenol red-β-D-galactopyranoside, into a red product at 570 nm (corrected for unspecific absorption and light scattering at 690 nm), indicated the estrogenic or androgenic activity of the test article. The difference between these optical density (OD) absorbance values (OD_{690} - OD_{570}) was used to calculate growth factor values and induction ratios. Eight serial dilutions of the test article (half-log steps) in DMSO, resulting in final concentrations of 3.16×10^{-6} - 1×10^{-2} M, were added to yeast cells in the agonist assays. For the agonist YES assay, 17-β estradiol (E2) was used as the positive control at 7 final concentrations between 1×10^{-11} – 1×10^{-8} M; 5α-dihydrotestosterone (DHT) was used as the positive control for the agonist YAS assay at 7 final concentrations between 1×10^{-9} – 1×10^{-6} M, using half-log dilution steps. DMSO (1%) was used as the solvent control. The inhibitory activity of the test article dilutions were evaluated in the presence of E2 (1.3×10^{-9} M) in an antagonistic YES assay and in the presence of DHT (3×10^{-8} M) in an antagonistic YAS assay. Serial dilutions of 4-hydroxytamoxifen and flutamide were used as antagonist positive controls. The test article showed estrogenic activity with an EC_{10} value of 6.9×10^{-3}; cellular toxicity (growth factors ≤ 0.5) at the two highest concentrations tested was observed in the YAS assay. No estrogenic antagonist, androgenic agonist, or androgenic antagonist activities were observed.

Similarly, the estrogen agonist effects of a formulation containing 0.12% Palmitoyl Pentapeptide-4 (other contents not specified; Pal-KTSKS), were assessed in a XenoScreen XL YES assay.[31] Lyticase and a detergent were used to facilitate the secretion of the intracellularly synthesized β-galactosidase. Test article samples were serially diluted in 8 steps (half-log steps) in water with 1% DMSO, with concentrations ranging from 5.21×10^{-5} – 6.7×10^{-3} M. E2 was used as the positive control in 8 final concentrations between 2.1×10^{-12} – 6.7×10^{-9} M, using half-log dilution steps; 1% DMSO served as the solvent control. The limit of detection (LoD) for estrogenic activity was 1.49×10^{-11} M E2. No inhibition of cellular growth or estrogenic agonist activity was observed at any concentration tested.

DERMAL IRRITATION AND SENSITIZATION STUDIES

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

A formulation containing 0.12% Palmitoyl Pentapeptide-4 (tested as supplied; Pal-KTSKS) did not cause irritation when applied to a reconstructed human epidermis model (EpiSkin®) in a cutaneous primary irritation test performed in accordance with OECD TG 439.[19,32] Palmitoyl Pentapeptide-4, tested at 0.01% (vehicle not specified; Pal-KTTKS), 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 performed in accordance with OECD TG 404 using guinea pigs.[15,26,33] A trade name mixture containing 0.01% Palmitoyl Pentapeptide-4 (applied neat; Pal-KTTKS) was tested for acute skin irritation using 10 subjects.[15,34] 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 formulation containing 0.12% Palmitoyl Pentapeptide-4 (tested at 15% in distilled water; Pal-KTSKS) was not irritating when applied for 48 h, under semi-occlusive conditions in a patch test using 11 subjects.[19,35]

Palmitoyl Pentapeptide-4 (81.6% pure, Pal-KTSKS) was predicted to be non-sensitizing when tested at 5 mM (5 µl) and 25 mM (250 µl) in water in a direct peptide reactivity assay (DPRA) performed in accordance with OECD TG 442C.[19,36]

Palmitoyl Pentapeptide-4 (81.6% pure; Pal-KTSKS) was tested at up to 200 µM (0.05 ml) in DMSO using the KeratinoSens™ cell line in an antioxidant/electrophile response element (ARE)-Nrf2 luciferase assay, performed in accordance with OECD TG 442D.[19,37] The test article yielded a maximal response value (I_{max}) of 1.35 compared to an I_{max} of 5.12 for the positive control, cinnamaldehyde; the test article was predicted to be non-sensitizing. A guinea pig maximization test was performed in accordance with OECD TG 406, to evaluate the sensitization potential of Palmitoyl Pentapeptide-4 (0.01%; Pal-KTTKS).[15,38] 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 formulation containing 0.12% Palmitoyl Pentapeptide-4 (tested at 15% in distilled water; Pal-KTSKS) was not irritating or sensitizing when applied under semi-occlusive conditions in a human repeated insult patch test (HRIPT) using 106 subjects.[19,39] The undiluted application of a trade name mixture containing 0.01% Palmitoyl Pentapeptide-4 (Pal-KTTKS) to a 3.61 cm^2 area, resulting in 5.54 $\mu g/cm^2$ applied Palmitoyl Pentapeptide-4, did not cause irritation or sensitization in an occlusive HRIPT using 51 subjects.[15,40,41]

Phototoxicity Studies

Palmitoyl Pentapeptide-4

The potential for a sample of Palmitoyl Pentapeptide-4 (tested at 0.0015%; Pal-KTSKS), in water, to absorb ultraviolet light A (UVA) and ultraviolet light B (UVB) was evaluated, in accordance with OECD TG 101.[19,42] The diluted article (1 ml) was placed in a calibrated spectrophotometer in order to read UVA/UVB absorption. No absorbance peak was observed between 290 and 400 nm, which was suggestive of a molar extinction coefficient (ε; a measure of how strongly a chemical species or substance absorbs light at a particular wavelength; is an intrinsic property of chemical species that is dependent on structure) < 1000 M^{-1} cm^{-1}. The test article was predicted to be non-phototoxic.

OCULAR IRRITATION STUDIES

Details on the ocular irritation studies summarized below can be found in Table 6.

A formulation containing 0.12% Palmitoyl Pentapeptide-4 (300 µl dose; Pal-KTSKS) was tested in an in vitro hens egg-chorioallantoic membrane (HET-CAM) assay, performed in agreement with French Good Laboratory Practices (GLP) and the European Directive 2004/10/EC.[19,43] The mean score calculated for hyperemia, hemorrhage, and coagulation, opacity, and/or thrombosis was 4.25; the test article was classified as slightly irritating. In another HET-CAM assay, a trade name mixture containing 0.01% Palmitoyl Pentapeptide-4 (Pal-KTTKS), which was tested as supplied, produced a mean irritation index of 6.0; the mean irritation index of the positive control, sodium dodecyl sulfate, was 12.0.[15,34] The test article was classified as moderately irritating. The ocular irritation potential of a formulation containing 0.12% Palmitoyl Pentapeptide-4 (tested at 30% in glycerin and water; Pal-KTSKS) was tested in a SkinEthic™ human corneal epithelial model, in accordance with OECD TG 492.[19,44] Mean cell viability when tested with the test article was 104.3%; the test article was considered not irritating. Palmitoyl Pentapeptide-4 tested at 0.01% (vehicle not specified; Pal-KTTKS) was assessed for ocular irritation in 3 male New Zealand white rabbits, in accordance with OECD TG 405.[15,45] A single dose of 0.1 ml was instilled into the conjunctival sac of the left eye, and the eye was not rinsed. All mean values for chemosis, redness of the conjunctiva, iris lesions, and corneal opacity were 0 at each tested time interval. 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.[46] In an 8-wk, randomized parallel-group study conducted in 196 women, a cosmetic product regimen containing niacinamide, Palmitoyl Pentapeptide-4, palmitoyl-lysine-threonine, retinyl propionate, and carnosine in a moisturizing base was well tolerated compared to a moisturizer containing 0.02% tretinoin;[47] although the concentration of Palmitoyl Pentapeptide-4 in the moisturizing base is not provided, it was reported to not exceed the maximum reported concentration of use of this ingredient in non-spray face and neck products that was reported to the Council in response to the use survey (i.e., 0.0012%).[48] 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).[49]

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 are comprised of a varied 5-amino-acid-sequence containing lysine, threonine, and serine; this report reviews the safety of two sequences, namely Pal-KTTKS and Pal-KTSKS. 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. Palmitoyl Pentapeptide-4 had the highest maximum

concentration of use reported in response to a 2022 concentration of use survey; it is used at up to 0.0035% in hair conditioners.

The permeability of Palmitoyl Pentapeptide-4 (Pal-KTTKS) and Pentapeptide-4 (KTTKS) 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 mouse skin extracts. Either 200 µl Pentapeptide-4 or 40 µg/ml Palmitoyl Pentapeptide-4 (in 10 mM HEPES buffer) was incubated with 200 µl of the epidermal skin extract, dermal skin extract, or whole skin homogenates at 37 °C for 120 min. The amounts of each substance present in the incubated mixtures were sampled and analyzed by LC-MS/MS. Pentapeptide-4 was almost fully degraded in the dermal skin extract and whole skin homogenate, with 3.2% remaining in the dermal skin extract at 30 min and 1.5% remaining in the whole skin homogenate at 60 min. Pentapeptide-4 degradation was slower in the epidermal skin extract which was attributed to lower amounts of proteolytic enzymes. 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 whole skin homogenate and after 120 min, 9.7% Palmitoyl Pentapeptide-4 remained in the dermal extract.

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; Pal-KTTKS), 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. No deaths or clinical signs related to treatment were noted in groups of guinea pigs (5/sex) treated with 0.01% Palmitoyl Pentapeptide (0.05 ml) in a 2-wk dermal irritation study.

A solution of 0.5% Palmitoyl Pentapeptide-4 (Pal-KTTKS) in distilled water and ethanol (75/25), tested at 2% in distilled water, was not mutagenic at up to 5000 µg/plate, with or without metabolic activation using *S. typhimurium* strains TA98, TA100, TA1535, TA1537 and *E. coli* WP2*uvr*A. Palmitoyl Pentapeptide-4 (Pal-KTSKS) in DMSO was not mutagenic to *S. typhimurium* strains TA98, TA100, TA1535, and TA1537, with or without metabolic activation, in another Ames test performed in accordance with OECD TG 471; signs of cytotoxic activity were observed under test conditions. In an in vitro mammalian cell micronucleus test, performed in accordance with OECD TG 487, cultured human lymphocytes were treated for 4 h with up to 1000 µg/ml Palmitoyl Pentapeptide-4 (Pal-KTSKS) in the presence of metabolic activation (24-h recovery), and for 4 h with up to 750 µg/ml Palmitoyl Pentapeptide-4 in the absence of metabolic activation (24-h recovery). Additionally, cells were treated continuously for 24 h (without a recovery period), in the absence of metabolic activation, with up to 400 µg/ml Palmitoyl Pentapeptide-4. Neither statistically nor biologically significant increases in the number of micronucleated cells were observed with the short-term or continuous treatments; the test article was deemed non-genotoxic.

When tested in XenoScreen YES and YAS agonist and antagonist assays, a formulation containing 0.12% Palmitoyl Pentapeptide-4 (Pal-KTSKS) showed estrogenic activity with an EC_{10} value of 6.9×10^{-3}; cellular toxicity (growth factors ≤ 0.5) at the two highest concentrations tested was observed in the YAS assay. No estrogenic antagonist, androgenic agonist, or androgenic antagonist activities were observed. The same test article did not exhibit inhibition of cellular growth or estrogen agonist activity at any concentration tested in another Xenoscreen XL YES assay; the LoD for estrogenic activity was 1.49×10^{-11} M E2.

A formulation containing 0.12% Palmitoyl Pentapeptide-4 in glycerin and water (tested as supplied; Pal-KTSKS) was not irritating to an EpiSkin® model in a cutaneous primary irritation test performed in accordance with OECD TG 439. Palmitoyl Pentapeptide-4, tested at 0.01% (Pal-KTTKS), was not irritating to rabbit skin in an acute dermal irritation study, nor was it irritating to guinea pig skin in a 2-wk dermal irritation study. In a clinical acute irritation study using 10 subjects, a trade name mixture containing Palmitoyl Pentapeptide-4 (0.01%) was well tolerated; very slight erythema was seen in 1 of the subjects, and the PCI was 0.10. A formulation containing 0.12% Palmitoyl Pentapeptide-4 (in distilled water; Pal-KTSKS) was not irritating in a human patch test using 11 subjects.

Palmitoyl Pentapeptide-4 (81.6% pure; Pal-KTSKS) was predicted to be non-sensitizing when tested in a DPRA (OECD TG 442C) and a ARE-Nrf2 luciferase assay (OECD 442D). In a guinea pig maximization test, Palmitoyl Pentapeptide-4 (0.01%; Pal-KTTKS) was not sensitizing when injected at effective test concentrations of 0.0075% in saline during intradermal induction, applied at 0.01% during epicutaneous induction, and applied at 0.0025% in saline during challenge. A formulation containing 0.12% Palmitoyl Pentapeptide-4 (tested at 15% in distilled water; Pal-KTSKS) was not irritating or sensitizing when tested under semi-occlusive conditions in an HRIPT using 106 subjects. No irritation or

sensitization was observed in an occlusive HRIPT in which 51 subjects were treated with a trade name mixture containing 0.01% Palmitoyl Pentapeptide-4 (Pal-KTTKS).

The potential for a sample of Palmitoyl Pentapeptide-4 (tested at 0.0015%; Pal-KTSKS) to cause phototoxicity was evaluated in an UVA/UVB spectrum test performed in accordance with OECD TG 101. No absorbance peak was observed between 290 and 400 nm, which was suggestive of a molar extinction coefficient < 1000 M^{-1} cm^{-1}; the test article was predicted to be non-phototoxic.

A formulation containing 0.12% Palmitoyl Pentapeptide-4 (300 µl dose; Pal-KTSKS) yielded a mean irritation score of 4.25 when tested in a HET-CAM assay and was classified as slightly irritating. Similarly, the ocular irritation potential of a trade name mixture containing 0.01% Palmitoyl Pentapeptide-4 (tested as supplied) was evaluated in another HET-CAM assay. The mean irritation index for the test substance, when tested as supplied, was 6.0, compared to a score of 12.0 for the positive control, sodium dodecyl sulfate. Thus, the test substance was classified as a moderate ocular irritant. Mean cell viability of a SkinEthic™ human corneal epithelial model when tested with a formulation containing 0.12% Palmitoyl Pentapeptide-4 (in glycerin and water; Pal-KTSKS) was 104.3%; the test article was considered non-irritating. In an acute ocular irritation study, a single, 0.1 ml dose of Palmitoyl Pentapeptide-4 tested at 0.01% (vehicle not specified; Pal-KTTKS) 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 (concentration did not exceed the maximum reported concentration of use in face and neck products).

DISCUSSION

This assessment reviews the safety of Myristoyl Pentapeptide-4, Palmitoyl Pentapeptide-4, and Pentapeptide-4 as used in cosmetic formulations. The Panel concluded these ingredients are safe in cosmetics in the present practices of use and concentration described in this safety assessment. The amino acid sequence of the pentapeptide portion of these ingredients can vary; one sequence is lysine-threonine-threonine-lysine-serine (i.e., Lys-Thr-Thr-Lys-Ser, or, KTTKS), and the other is Lys-Thr-Ser-Lys-Ser (or KTSKS). The Panel found the information in the report sufficient to apply the conclusion to both sequences.

The Panel stated that although HRIPTs were not performed at maximum use concentrations, the negative results obtained in these studies, in conjunction with the negative results observed in chemico and in vitro, mitigated any concerns regarding sensitization. Additionally, the negative human dermal irritation studies at less than the maximum use concentration were supported by a negative in vitro study. The Panel noted the lack of developmental and reproductive toxicity and carcinogenicity data; however, the low reported maximum concentration of use for these ingredients, the limited percutaneous absorption evidenced in vitro, the negative genotoxicity studies, and the absence of endocrine disruption for a formulation containing 0.12% Palmitoyl Pentapeptide-4 (Pal-KTSKS) mitigated the need for such data. The Panel also noted some changes in the keratin profile of subjects treated with a facial cream containing Palmitoyl Pentapeptide-4, suggesting a potential biologic effect; however, these were not considered adverse effects based upon the lack of erythema, dryness, and transepidermal water loss. The Panel also considered the available method of manufacturing and impurities data for Palmitoyl Pentapeptide-4. Furthermore, the Panel acknowledged its previous safety review of the individual amino acids comprising these ingredients, as well as the safety assessments of myristic and palmitic acid.

The Panel also discussed the issue of incidental inhalation exposure that could result from the use of formulations containing these ingredients; for example, Palmitoyl Pentapeptide-4 is reported to be used in a face powder (concentration not provided) and could be possibly inhaled. Inhalation toxicity data were not available. Coupled with the small actual exposure in the breathing zone and the low concentrations at which these ingredients are used (or expected to be used) in potentially inhaled products, the available information indicates that incidental inhalation would not be a significant route of exposure that might lead to local respiratory or systemic effects. A detailed discussion and summary of the Panel's approach to evaluating incidental inhalation exposures to ingredients in cosmetic products is available at https://www.cir-safety.org/cir-findings.

The Panel's respiratory exposure resource document (see link above) notes that airbrush technology presents a potential safety concern, and that no data are available for consumer habits and practices thereof. As a result of deficiencies in these critical data needs, the safety of cosmetic ingredients applied by airbrush delivery systems cannot be assessed by the Panel. Therefore, the Panel has found the data insufficient to support the safe use of cosmetic ingredients applied via an airbrush delivery system.

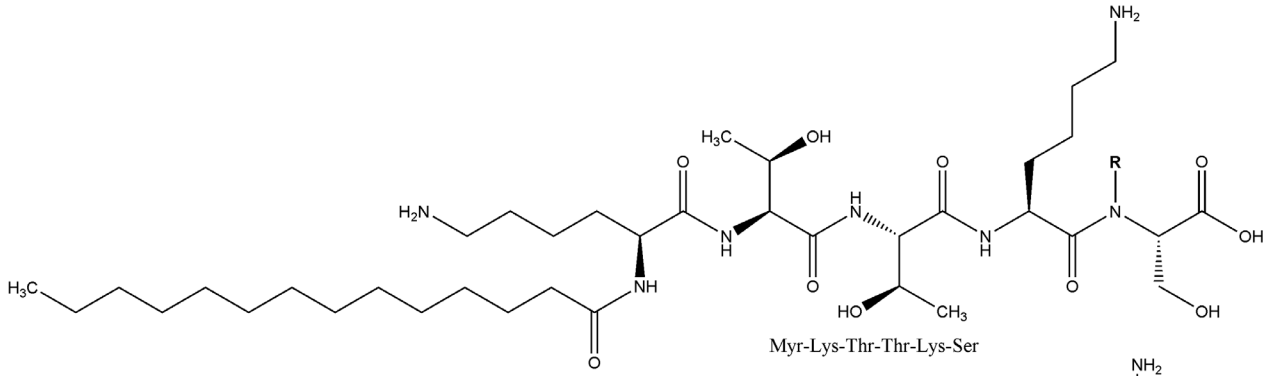
CONCLUSION

The Expert Panel for Cosmetic Ingredient Safety concluded that Myristoyl Pentapeptide-4, Palmitoyl Pentapeptide-4, and Pentapeptide-4 (KTTKS and KTSKS sequences) are safe in cosmetics in the present practices of use and concentration described in this safety assessment.

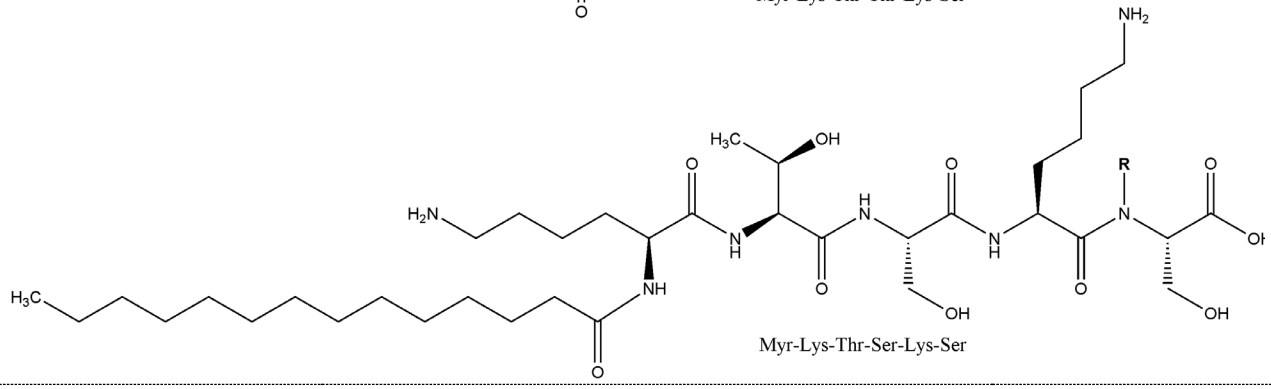
TABLES

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

Ingredient	Definition	Function
Myristoyl Pentapeptide-4 1392416-25-9	Myristoyl Pentapeptide-4 is the reaction product of myristic acid and Pentapeptide-4.	Skin-conditioning agent - miscellaneous

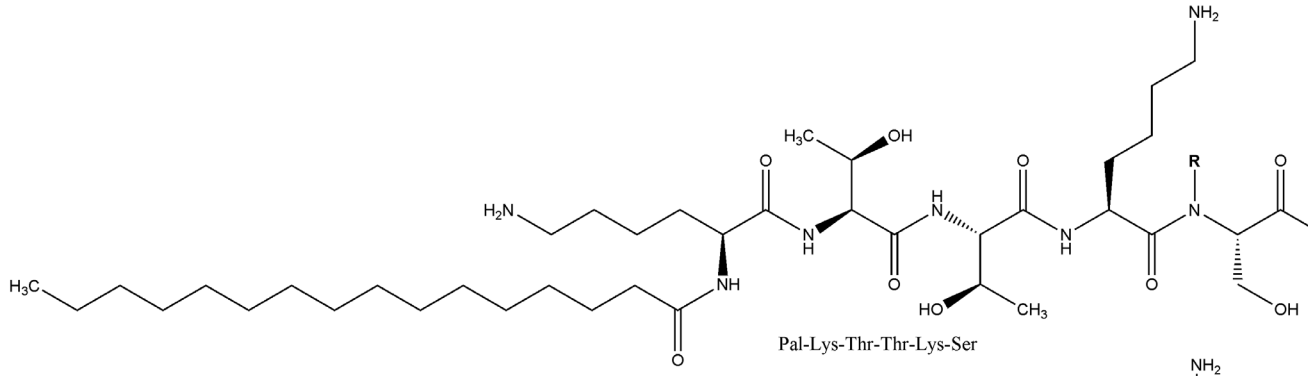


Myr-Lys-Thr-Thr-Lys-Ser

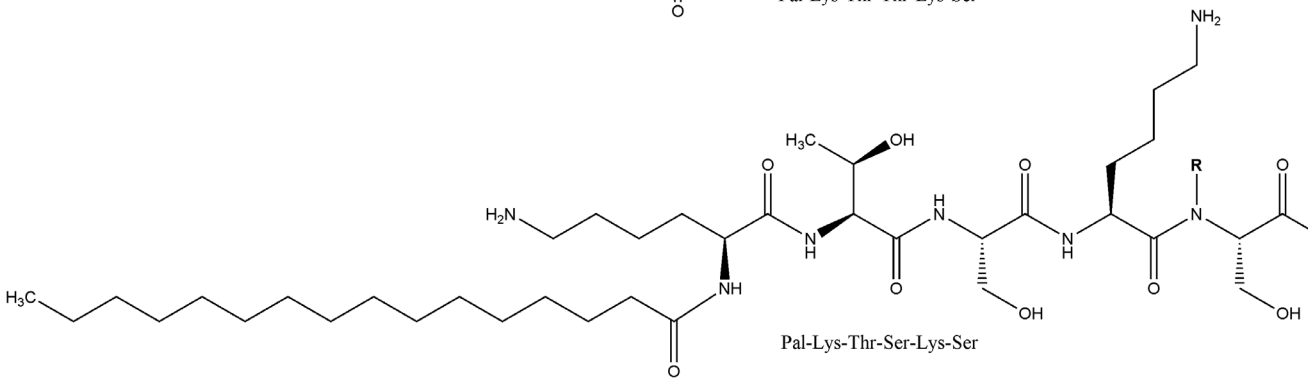


Myr-Lys-Thr-Ser-Lys-Ser

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



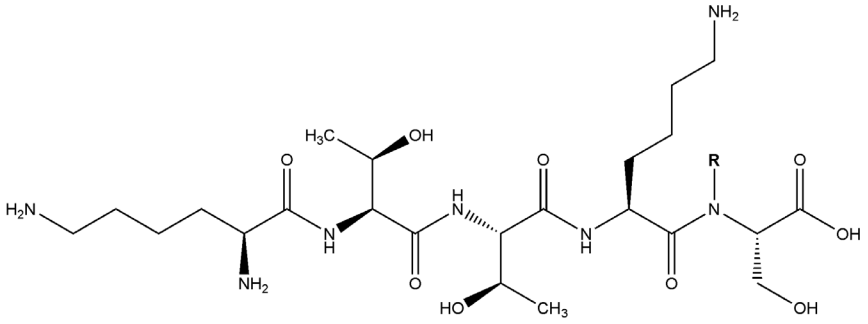
Pal-Lys-Thr-Thr-Lys-Ser



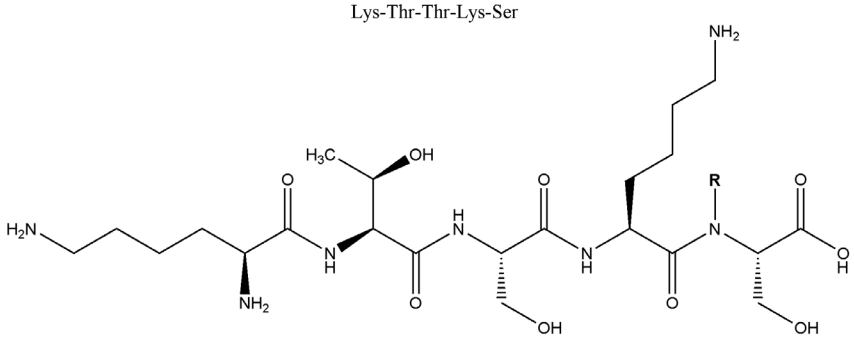
Pal-Lys-Thr-Ser-Lys-Ser

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

Ingredient	Definition	Function
Pentapeptide-4 149128-48-3	Pentapeptide-4 is a synthetic peptide containing lysine, serine, and threonine.	Skin-conditioning agent - miscellaneous



Lys-Thr-Thr-Lys-Ser



Lys-Thr-Ser-Lys-Ser

Table 2. Chemical properties

Property	Value	Reference
	Myristoyl Pentapeptide-4	
Molecular Weight (g/mol)	774 (Myr-Lys-Thr-Thr-Lys-Ser) 759.99 (Myr-Lys-Thr-Ser-Lys-Ser)	14
Topological Polar Surface Area (Å^2)	296 (estimated; Myr-Lys-Thr-Thr-Lys-Ser)	14
log p	1.85 (estimated; Myr-Lys-Thr-Thr-Lys-Ser) 1.6 (estimated; Myr-Lys-Thr-Ser-Lys-Ser)	18
	Palmitoyl Pentapeptide-4	
Physical Form	Powder	15
Color	White	15
Molecular Weight (g/mol)	802.1 (Pal-Lys-Thr-Thr-Lys-Ser) 788.04 (Pal-Lys-Thr-Ser-Lys-Ser)	15,16
Topological Surface Area (Å^2)	296 (estimated; Pal-Lys-Thr-Thr-Lys-Ser)	16
log p	2.72 (estimated; Pal-Lys-Thr-Thr-Lys-Ser) 2.52 (estimated; Pal-Lys-Thr-Ser-Lys-Ser)	18
	Pentapeptide-4	
Molecular Weight (g/mol)	563.65 (Lys-Thr-Thr-Lys-Ser) 549.63 (Lys-Thr-Ser-Lys-Ser)	17
Topological Polar Surface Area (Å^2)	292 (estimated; Lys-Thr-Thr-Lys-Ser)	17
log p	-4.12 (estimated; Lys-Thr-Thr-Lys-Ser) -4.39 (estimated; Lys-Thr-Ser-Lys-Ser)	18

Table 3. Frequency (2023)[20] and concentration (2022)[21] of use according to likely duration and exposure by product category

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

NR – not reported

*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.

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

[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. Genotoxicity studies

Test Article	Vehicle	Concentration/Dose	Test System	Procedure	Results	Reference
IN VITRO						
0.5% Palmitoyl Pentapeptide-4 in distilled water/ethanol (75/25) Pal-KTTKS	distilled water	tested at 2% 312.5, 625, 1250, 2500, and 5000 µg/plate, with or without metabolic activation	*S. typhimurium* TA98, TA100, TA1535, TA1537, and *E. coli* WP2*uvr*A	Ames test. 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.	Not mutagenic. Results for the vehicle and positive controls were as expected.	[15,27]
Palmitoyl Pentapeptide-4, 81.6% pure Pal-KTSKS	DMSO	1.6, 5, 16, 50, 160, 500, 1600, and 5000 µg/plate, with or without metabolic activation	*S. typhimurium* TA98, TA100, TA102, TA1535, and TA1537	Ames test. OECD TG471. In the absence of metabolic activation, sodium azide and mitomycin were tested in water, and 2-nitroflourene and 9-aminoacridine were tested in DMSO, for positive controls. In the presence of metabolic activation, 2-aminoanthracine was tested in DMSO as a positive control.	Not mutagenic. Signs of cytotoxic activity were observed under test conditions for the test article; controls produced expected results.	[19,28]
Palmitoyl Pentapeptide-4, > 96% pure Pal-KTSKS	sterile water	as supplied <u>with metabolic activation:</u> 4-h treatment, 24-h recovery: 250, 500, or 1000 µg/ml <u>without metabolic activation</u>: 4-h treatment, 24-h recovery: 375, 500, or 750 µg/ml 24-h, continuous treatment: 250, 320, or 400 µg/ml	Cultured human peripheral blood lymphocytes	Micronucleus test. OECD TG 487. Cells were treated for 4 h, with a 24-h recovery period, with and without metabolic activation (short treatment). In an additional assay, cells were treated for 24 h without a recovery period (continuous treatment). Cells treated were treated for 4 h followed by a 24-h recovery period, with cyclophosphamide in the presence of metabolic activation and with mitomycin in the absence of metabolic activation. Mitomycin and griseofulvin were used as positive controls in the 24-h, continuous assay.	Not genotoxic. Neither statistically or biologically significant increases in the number of micronucleated cells were observed with the short-term or continuous treatments.	[19,29]

DMSO – dimethyl sulfoxide; OECD – Organisation for Economic Cooperation and Development; TG – test guideline

Table 5. Dermal irritation and sensitization studies

Test Article	Vehicle	Test Concentration/Dose	Test Population/ System	Procedure	Results	Reference
IRRITATION						
IN VITRO						
Formulation containing 0.12% Palmitoyl Pentapeptide-4, glycerin, and water Pal-KTSKS	tested as supplied	10 µl; 100% (effective test concentration: 0.12% Palmitoyl Pentapeptide-4)	EpiSkin® reconstructed human epidermis model	Cutaneous primary irritation test. OECD TG 439. The test article, positive control (10 µl SDS), and negative control (10 µl PBS) were in contact with the epidermis model for 15 min, followed by a 42-h incubation period. Cell viability was evaluated via an MTT assay.	Predicted to be not irritating. The test article, as supplied did not stain the cells or interact with MTT.	[19,32]
ANIMAL						
Palmitoyl Pentapeptide-4 Pal-KTTKS	not specified	0.01%; 0.5 ml	3 male New Zealand white rabbits	Acute dermal irritation study. OECD TG 404. 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.	[15,33]
Palmitoyl Pentapeptide-4 Pal-KTTKS	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 daily for 14 d; the site was not rinsed. Purified water applied to the right flank served as the control. 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.	Non-irritating. Very slight erythema was noted in 1 animal on days 12 and 13. According to the researchers, these reaction were not attributed to an irritant effect of the test substance because they were very slight and only occurred in 1 animal.	[15,26]
HUMAN						
Trade name mixture containing 0.01% Palmitoyl Pentapeptide-4 Pal-KTTKS	tested as supplied	0.02 ml (effective test concentration: 0.01% Palmitoyl Pentapeptide-4)	10 subjects	Acute skin irritation study. A single 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.	[15,34]
Formulation containing 0.12% Palmitoyl Pentapeptide-4 Pal-KTSKS	distilled water	160 µl; 15% (effective test concentration: 0.018% Palmitoyl Pentapeptide-4)	11 subjects; phototype II - IV	Patch test; semi-occlusive application to 400 mm^2 for 48 h; test sites were scored before patching and 15 – 30 min after patch removal	Not irritating. No reactions were observed in either the test or control subjects.	[19,35]
SENSITIZATION						
IN CHEMICO/ IN VITRO						
Palmitoyl Pentapeptide-4, 81.6% pure Pal-KTSKS	water	5 (50 µl) and 25 mM (250 µl)	cysteine and leucine	DPRA; OECD TG 442C and ECVAM DB-ALM Protocol No 154; 24-h incubation period; each concentration was tested 3 times; mean percent depletion of cysteine and lysine was evaluated; positive control: cinnamaldehyde in acetonitrile; negative control: peptide in buffer	Prediction of non-sensitizing. Mean percent depletion of cysteine and lysine was 4.58%, reflecting no or minimal reactivity.	[19,36]
Palmitoyl Pentapeptide-4, 81.6% pure Pal-KTSKS	DMSO	0.98 – 2000 µM; 0.05 ml	KeratinoSens™ cell line	ARE-Nrf2 Luciferase test method; OECD TG 442D and ECVAM DB-ALM protocol 155; performed 2 times; positive control: cinnamaldehyde; negative controls: 1% DMSO in treatment medium	Prediction of non-sensitizing. I_{max} of 1.35, compared to 5.12 for positive control.	[19,37]

Table 5. **Dermal irritation and sensitization studies**

Test Article	Vehicle	Test Concentration/Dose	Test Population/ System	Procedure	Results	Reference
				ANIMAL		
Palmitoyl Pentapeptide-4, 0.01% Pal-KTTKS	saline	Induction: 75% (effective concentration 0.0075%); topical induction: 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 of the test site with 10% SLS (pet) on day 7, the test substance was applied on day 8 under occlusion to the same region for 48 h. After a non-treatment 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 24 h. Skin reactions were evaluated 24 and 48 h after patch removal.	Not sensitizing. Controls yielded expected results.	[15,38]
				HUMAN		
Formulation containing 0.12% Palmitoyl Pentapeptide-4 Pal-KTSKS	distilled water	160 µl; 15% (effective concentration: 0.018% Palmitoyl Pentapeptide-4)	106 subjects; phototype II - III	HRIPT; semi-occlusive conditions (400 mm^2); induction: 9 applications (48 – 72 h) were made to the upper back over a 3-wk period. Concurrent applications of distilled water under the same conditions served as control sites. Challenge: after a non-treatment period of 2 wk, a 48-h application was made to an induction site and an untreated site. Treated sites were scored before patching, 15 – 30 min after patch removal, and, additionally, 48 h after patch removal during the challenge phase	Not irritating or sensitizing. No reactions were induced during the induction or challenge phases.	[19,39]
Trade name mixture containing 0.01% Palmitoyl Pentapeptide-4 Pal-KTTKS	NA	applied undiluted: 55.4 mg/cm of the mixture this results in 5.54 µg/cm^2 of Palmitoyl Pentapeptide-4	51 subjects	HRIPT; The remaining ingredients in the mixture included: glycerin (qsp 100), water (25%), butylene glycol (20%), carbomer (1%), polysorbate 20 (0.5%), sodium lactate (max 1%); 2 g of the test material applied to a 3.61 cm^2 area under occlusive conditions.	Non-irritating and non-sensitizing	[15,40,41]

ARE – antioxidant/electrophile response element; DPRA – direct peptide reactivity assay; ECVAM DB-ALM - European Centre for Validation of Alternative Methods Database on Alternative Methods; FCA – Freund's Complete Adjuvant; HRIPT – human repeated insult patch test; MTT - 3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide; NA – not applicable; OECD – Organisation for Economic Cooperation and Development; PBS – phosphate-buffered solution; PCI – primary cutaneous irritation; SDS – sodium dodecyl sulfate; SLS – sodium lauryl sulfate; TG – test guideline

Table 6. Ocular irritation studies

Test Article	Vehicle	Test Concentration/Dose	Test Population	Procedure	Results	Reference
IN VITRO						
Formulation containing 0.12% Palmitoyl Pentapeptide-4 Pal-KTSKS	water	300 µl; 10% (effective test concentration: 0.012% Palmitoyl Pentapeptide-4)	4 eggs (test article); 2 eggs (reference controls)	In vitro HET-CAM assay; performed in agreement with French GLP, the European Directive 2004/10/EC, and the August 2004 decree from the *Journal Officiel Republique Francaise*; positive control: 0.4 and 3.2% lauryl sulfobetaine in saline solution; negative control: 0.05% lauryl sulfobetaine in saline solution	Classified as slightly irritating. The mean score calculated for hyperemia, hemorrhage, and coagulation, opacity, and/or thrombosis was 4.25.	[19,43]
Trade name mixture containing 0.01% Palmitoyl Pentapeptide-4 Pal-KTTKS	tested as supplied	dose not specified (effective test concentration: 0.01% Palmitoyl Pentapeptide-4)	HET-CAM	In vitro HET-CAM assay; 1996 HET CAM protocol published in the *Journal Officiel Republique Francaise*; positive control: SDS (0.05% (w/v))	Classified as moderately irritating. The mean irritation index for the SDS was 12, while the mean irritation index for the test substance was 6	[15,34]
Formulation containing 0.12% Palmitoyl Pentapeptide-4; glycerin, and water Pal-KTSKS	water	30 µl; 30% (effective test concentration: 0.036% Palmitoyl Pentapeptide-4)	human immortalized comeal epithelial cells	SkinEthic™ human corneal epithelial model. OECD TG 492, in agreement with French GLP, European Directive 2004/10/CE, and 2004 decree published in the *Journal Officiel Republique Francaise*. 2 epithelia were used as replicates; 30 min incubation period; positive control: methyl acetate; negative control: DPBS; cell viability evaluated via MTT assay	Not irritating. Mean cell viability for the test article was 104.3%. Positive controls yielded expected results.	[19,44]
ANIMAL						
Palmitoyl Pentapeptide-4 Pal-KTTKS	not specified	0.01%; 0.1 ml	3 male New Zealand white rabbits	OECD TG 405. A single dose was instilled into the conjunctival sac of the left eye. Treated eyes were not rinsed; right eyes served as control Ocular reactions were evaluated 1, 24, 48 and 72 h. Mean values for chemosis, redness of the conjunctiva, iris lesions, and corneal opacity were calculated for each animal	Classified as non-irritant. All mean values were 0 at each time interval.	[15,45]

DPBS – Dulbecco's phosphate buffer solution; GLP – good laboratory practices; HET-CAM – hen's egg-chorioallantoic membrane test; MTT – 3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide; OECD – Organisation for Economic Cooperation and Development; SDS – sodium dodecyl 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 2024. Accessed 01/24/2024.

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(Suppl 6):41-64.

4. Elder RL, (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; 25(Suppl 2):40-47.

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(Suppl 4):162-186.

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. Anonymous. 2023. ACD Percepta Version 2022.2.3 Predicted log P values for Pentapeptide-4 ingredients. Unpublished data submitted by Personal Care Products Council on September 18, 2023.

19. Sederma. 2023. Summary Information on Palmitoyl Pentapeptide-4. Unpublished data submitted to the Cosmetic Ingredient Review on July 21, 2023.

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. 2023. Concentration of Use by FDA Product Category: Pentapeptide-4 Ingredients Revised. Unpublished data submitted by the Personal Care Products Council on September 21, 2023.

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

23. 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.

24. 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.

25. CIT. 1999. Summary of acute oral toxicity of 0.01% Palmitoyl Pentapeptide-4 in rats. Laboratory study number 18838 TAR. Unpublished data submitted by Personal Care Products Council on November 13, 2012.

26. CIT. 1999. Sumary of local tolerance study after repeated topical application for 2 weeks in guinea pigs: 0.01% Palmitoyl Pentapeptide-4. Laboratory study number 18842 TSG. Unpublished data submitted by Personal Care Products Council on November 13, 2012.

27. CIT. 1999. Summary of bacterial reverse mutation test of 0.5% Palmitoyl Pentapeptide-4. Laboratory study number 18796 MMJ. Unpublished data submitted by Personal Care Products Council on November 13, 2012.

28. IDEA Lab. 2019. Bacterial reverse mutation assay: determination of the mutagenic activity of a test item containing 81.6% Palmitoyl Pentapeptide-4 on *Salmonella typhimurium* (Ames test) according to the OECD #471. Report No. 6.46_5S-53451-ID-19/09966. Unpublished data submitted to the Cosmetic Ingredient Review on May 30, 2023.

29. Insitut Pasteur de Lille. 2021. In vitro mammalian cell micronucleus test on cultured human lymphocytes of a test item containing 81.6% Palmitoyl Pentapeptide-4. Report No. FSR-IPL 210103. Unpublished data submitted to the Cosmetic Ingredient Review on May 30, 2023.

30. Xenometrix. 2020. XenoScreen YES/YAS endocrine disruptor testing of a formulation containing 0.12% Palmitoyl Pentapeptide-4. Study No. SEDE2010. Unpublished data submitted to the Cosmetic Ingredient Review on May 30, 2023.

31. Xenometrix. 2021. XenoScreen XL YES endocrine disruptor testing of a formulation containing 0.12% Palmitoyl Pentapeptide-4. Study No. SEDE2105. Unpublished data submitted to the Cosmetic Ingredient Review on May 30, 2023.

32. IEC France. 2020. Study performed on an EpiSKIN® reconstructed human epidermis model: Evaluation of the interaction of a formulation containing 0.12% Palmitoyl Pentapeptide-4 with MTT and staining power. Report No. 20080RD Unpublished data submitted to the Cosmetic Ingredient Review on May 30, 2023.

33. CIT. 1999. Summary of acute dermal irritation of 0.01% Palmitoyl Pentapeptide-4 in rabbits. Laboratory study number 18839 TAL. Unpublished data submitted by Personal Care Products Council on November 13, 2012.

34. Institut D'Expertise Cliniqu. 1998. Summary of HET-CAM assay and human primary cutaneous tolerance of MATRIXYL (a trade name mixture containing 0.01% Palmitoyl Pentapeptide-4. Report No. 80503RD2. Unpublished data submitted by Personal Care Products Council on November 13, 2012.

35. Eurofins EVIC Product Testing Romania SRL. 2021. Human patch test under dermatological control of a formulation containing 0.12% Palmitoyl Pentapeptide-4. Report No ER 21/049 P21 0044. Unpublished data submitted to the Cosmetic Ingredient Review on May 30, 2023.

36. IDEA Lab. 2019. *In chemico* skin sensitization: Direct Peptide Reactivity (DPRA) according to the OECD 442C guideline of a test item containing 81.6% Palmitoyl Pentapeptide-4. Study No. 6.53-52287-ID-19/09966. Unpublished data submitted to the Cosmetic Ingredient Review on May 30, 2023.

37. IDEA Lab. 2019. In vitro sensitization test: KeratinoSens™ keratinocytes test based on the signaling pathway Keap1-Nrf2-ARE coupled to the luciferase reporter gene; test item containing 81.6% Palmitoyl Pentapeptide-4. Study No. 6.52-52291-ID-19/09966. Unpublished data submitted to the Cosmetic Ingredient Review on May 30, 2023.

38. CIT. 1999. Summary of skin sensitization test in guinea pigs using 0.01% Palmitoyl Pentapeptide-4. Laboratory study number 18841 TSG. Unpublished data submitted by Personal Care Products Council on November 13, 2012.

39. Eurofins EVIC Product Testing Romania SRL. 2021. Human repeated insult patch test with challenge; Formulation containing 0.12% Palmitoyl Pentapeptide-4. Report No. ER 21/048-14 P21 0045. Unpublished data submitted to the Cosmetic Ingredient Review on May 30, 2023.

40. Consumer Product Testing Company. 1999. Summary of repeated insult patch test of MAXTRIXYL (a trade name mixture containing 0.01% Palmitoyl Pentapeptide-4). Experiment Reference Number. C99-0567-02. Unpublished data submitted by Personal Care Products Council on November 13, 2012.

41. Sederma. 2023. Product information and summary of HRIPT conducted by Consumer Product Testing Co. nº C99-0567.02. Unpublished data submitted by Sederma on October 9, 2023.

42. Sederma. 2021. Test report: UVA spectrum on Palmitoyl Pentapeptide-4 (0.0015%). Unpublished data submitted to the Cosmetic Ingredient Review on July 21, 2023.

43. IDEA Lab. 2020. Evaluation of the ocular irritant potential of a formulation containing 0.12% Palmitoyl Pentapeptide-4 by application onto the hen egg chorio-allantoic membrane (HET-CAM). Study No. 6.02-54075-ID-20/00404. Unpublished data submitted to the Cosmetic Ingredient Review on May 30, 2023.

44. IDEA Lab. 2020. Ocular irritation evaluation of a formulation containing 0.12% Palmitoyl Pentapeptide-4 on human corneal epithelial model according to the OECD guideline nº492 - SkinEthic™ model. Study No. 6.49_S-54682-ID-20/00404. Unpublished data submitted to the Cosmetic Ingredient Review on May 30, 2023.

45. CIT. 1999. Summary of acute eye irritation in rabbits using 0.01% Palmitoyl Pentapeptide-4. Laboratory study number 18840 TAL. Unpublished data submitted by Personal Care Products Council on November 13, 2012.

46. 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.

47. 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.

48. Personal Care Products Council. 2023. Clarification that the amount of Palmitoyl Pentapeptide-4 in a test article described in a published study (Fu et al., *Br J Dermatol.* 2010;162(3):647-654) does not exceed the concentration of this ingredient in face and neck products reported to the PCPC in response to the concentration of use survey. Memorandum submitted by Personal Care Products Council on April 28, 2023.

49. 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.