GREEN

Safety Assessment of Palmitoyl Oligopeptides Ingredients as Used in Cosmetics

CIR EXPERT PANEL MEETING

MARCH 18-19, 2013

Cosmetic Ingredient Review
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February 22, 2013

Memorandum

To: CIR Expert Panel

From: Wilbur Johnson, Jr.
Manager/Lead Specialist

Subject: Draft Report on Palmitoyl Oligopeptides

This is the first time that the Panel is seeing this report addressing 45 palmitoyl oligopeptides. A Scientific Literature Review (SLR) was announced for public comment last year.

Included for your review is a copy of the Draft Report, the CIR report history, Literature search strategy, Ingredient Data profile, and 2012 FDA VCRP data. Comments on the Scientific Literature Review (SLR) have been addressed and are included for the Panel's review (See pcpc1 pdf file).

The following unpublished data on Palmitoyl Oligopeptides were received from the Personal Care Products Council: chemistry (UV-visible spectral analysis, logP, and impurities data included) , methods of production, use concentration data, acute oral toxicity, ocular irritation, skin irritation/sensitization (animal and human) , and genotoxicity. These data are included in the attached pdf files (data1, data2, etc.) and have been incorporated into the draft report.

After reviewing this Draft Report and unpublished data received, the Expert Panel needs to determine whether the available data are sufficient for issuing a tentative report with a safe/safe with qualifications conclusion. If additional data are needed, the Panel should issue an insufficient data announcement listing those data needs.

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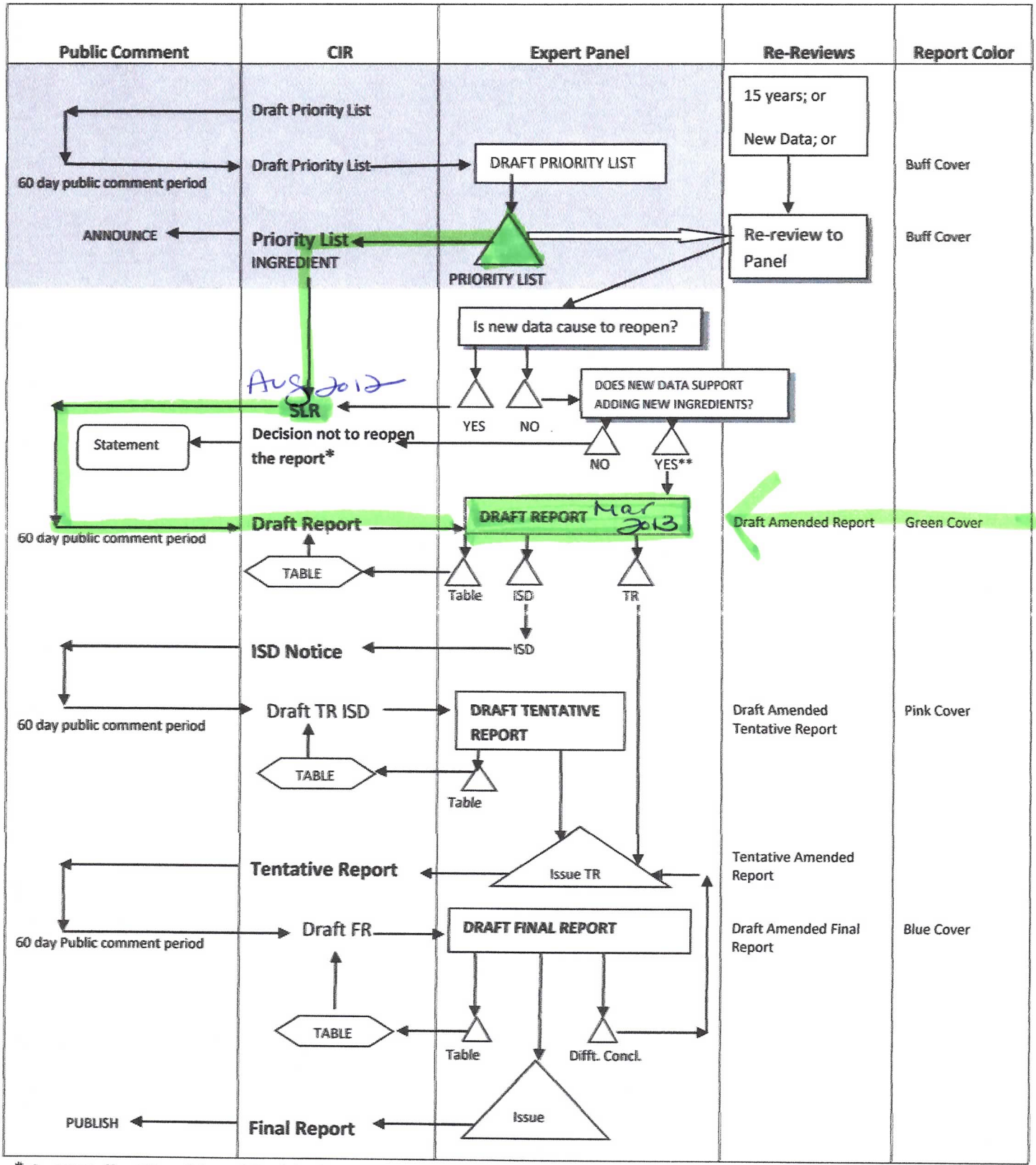
Palmitoyl Oligopeptides - Mar 2013

SAFETY ASSESSMENT FLOW CHART



*The CIR Staff notifies of the public of the decision not to re-open the report and prepares a draft statement for review by the Panel. After Panel review, the statement is issued to the Public.

**If Draft Amended Report (DAR) is available, the Panel may choose to review; if not, CIR staff prepares DAR for Panel Review.

Expert Panel Decision

Document for Panel Review

Option for Re-review

CIR History of:

Palmitoyl Oligopeptides

The Scientific Literature Review on Palmitoyl Oligopeptides was announced in August of 2012.

1st Review, Belsito and Marks Teams/Panel: March 18-19, 2013

The following data on palmitoyl oligopeptides , received from the Personal Care Products Council, are included in the draft report: chemistry (UV-visible spectral analysis, logP, and impurities data included) , methods of production, use concentration data, acute oral toxicity, ocular irritation, skin irritation/sensitization (animal and human) , and genotoxicity. These data have been incorporated into the SLR. Comments from the Council were also received.

Palmitoyl Oligopeptides Check List for March, 2013. Analyst – Wilbur Johnson																				
			Acute toxicity					Repeated dose toxicity				Irritation			Sensitization					
	Skin Penetration	Penetration Enhancement	ADME	Oral	Parenteral	Dermal	Inhale	Oral	Parenteral	Dermal	Inhale	Ocular Irritation	Dermal Irr. Animal	Dermal Irr Human	Sensitization Animal	Sensitization Human	Repro/Devel toxicity	Genotoxicity	Carcinogenicity	Phototoxicity
Palmitoyl Oligopeptide				X						X		X	X	X	X	X		X		
Palmitoyl Dipeptide-7																				
Palmitoyl Dipeptide-10	X																			
Palmitoyl Dipeptide-13																				
Palmitoyl Dipeptide-17																				
Palmitoyl Dipeptide-18				X						X		X	X	X	X			X		X
Palmitoyl Tripeptide-1																				
Palmitoyl Tripeptide-4																				
Palmitoyl Tripeptide-5																				
Palmitoyl Tripeptide-8																				
Palmitoyl Tripeptide-28																				
Palmitoyl Tripeptide-29																				
Palmitoyl Tripeptide-31																				
Palmitoyl Tripeptide-36																				
Palmitoyl Tripeptide-37																				
Palmitoyl Tripeptide-38												X		X		X		X		
Palmitoyl Tripeptide-40																				
Palmitoyl Tripeptide-42																				
Palmitoyl Tetrapeptide-7																				
Palmitoyl Tetrapeptide-10																				
Palmitoyl Tetrapeptide-20																				
Palmitoyl Pentapeptide-4				X						X		X	X	X	X	X		X		
Palmitoyl Pentapeptide-5																				
Palmitoyl Hexapeptide-12																				
Palmitoyl Hexapeptide-14																				
Palmitoyl Hexapeptide-15																				
Palmitoyl Hexapeptide-19																				
Palmitoyl Hexapeptide-26																				
Palmitoyl Hexapeptide-32																				
Palmitoyl Hexapeptide-36																				
Palmitoyl Hexapeptide-27 Acetate																				
Palmitoyl Heptapeptide-5																				

Palmitoyl Oligopeptides Check List for March, 2013. Analyst – Wilbur Johnson																				
			Acute toxicity					Repeated dose toxicity				Irritation			Sensitization					
	Skin Penetration	Penetration Enhancement	ADME	Oral	Parenteral	Dermal	Inhale	Oral	Parenteral	Dermal	Inhale	Ocular Irritation	Dermal Irr. Animal	Dermal Irr Human	Sensitization Animal	Sensitization Human	Repro/Devel toxicity	Genotoxicity	Carcinogenicity	Phototoxicity
Palmitoyl Nonapeptide-6																				
Palmitoyl Decapeptide-21																				
Palmitoyl Oligopeptide-70																				
Palmitoyl Hydrolyzed Collagen																				
Palmitoyl Hydrolyzed Milk Protein																				
Palmitoyl Hydrolyzed Wheat Protein																				
Potassium Palmitoyl Hydrolyzed Corn Protein																				
Potassium Palmitoyl Hydrolyzed Oat Protein																				
Potassium Palmitoyl Hydrolyzed Rice Protein																				
Potassium Palmitoyl Hydrolyzed Sweet Almond Protein																				
Potassium Palmitoyl Hydrolyzed Wheat Protein																				
Sodium Palmitoyl Hydrolyzed Collagen																				
Sodium Palmitoyl Hydrolyzed Wheat Protein																				

Ingredients	PubMed	Toxline	ChemIDplus	Multidatabase (See legend*)	DART	SciFinder	RTECS
PO	3 (272, with all below)	0 (316, with all below)	1	0	0	2(49)	
PO-70	0	0	0	0	0	0	
PP			0	0	0	1(34)	
PD			1	0	0	0(3)	
PTri			0	0	0	0(16)	
PTet			1	0	0	1(20)	
PHex			0	0	0	1(3)	
PHep			0	0	0	0(2)	
POct			0	0	0	0(12)	
PNon			0	0	0	0(7)	
PDec			0	0	0	0(1)	
PUndeca			0	0	0	0	
PDodeca			0	0	0	0(2)	
PTrideca			0	0	0	0	
PTetradeca			0	0	0	0	
PPentadeca			0	0	0	0(2)	
PHexadeca			0	0	0	0(1)	
PHeptadeca			0	0	0	0	
POctadeca			0	0	0	0	
PNonadeca			0	0	0	0	
PIcosa			0	0	0	0(2)	
PLP			0	0	0	1(35)	
PFAP			0	0	0	0(12)	
PM			0	0	0	1	

*Data in Table: Publications found; Multidatabase = HSDB, CCRIS, ITER, IRIS, Genetox, and LacMed

Searches Performed on 5/14-15/2012

Ingredients/Search Terms

Palmitoyl oligopeptide (PO)
Palmitoyl pentapeptide (PP)
Palmitoyl dipeptide (PD)
Palmitoyl tripeptide (PTri)
Palmitoyl tetrapeptide (PTet)
Palmitoyl hexapeptide (PHex)
Palmitoyl heptapeptide (PHep)
Palmitoyl octapeptide (POct)
Palmitoyl nonapeptide (PNon)
Palmitoyl decapeptide (PDec)
Palmitoyl undecapeptide (PUndeca)
Palmitoyl dodecapeptide (PDodeca)
Palmitoyl tridecapeptide (PTrideca)
Palmitoyl tetradecapeptide (PTetradeca)
Palmitoyl pentadecapeptide (PPentadeca)
Palmitoyl hexadecapeptide (PHexadeca)
Palmitoyl heptadecapeptide (PHeptadeca)
Palmitoyl octadecapeptide (POctadeca)
Palmitoyl nonadecapeptide (PNonadeca)
Palmitoyl icosapeptide (PIcosa)
Palmitoyl lipidated peptides (PLP)
Palmitoyl fatty acylated peptides (PFAP)
Palmitoyl matrikine (PM)
Palmitoyl oligopeptide-70 (PO-70)

Search Strings (NLM databases)

"Palmitoyl Oligopeptide" OR "Palmitoyl pentapeptide" OR "Palmitoyl dipeptide" OR "Palmitoyl tripeptide" OR "Palmitoyl tetrapeptide" OR "Palmitoyl hexapeptide" OR "Palmitoyl heptapeptide" OR "Palmitoyl octapeptide" OR "Palmitoyl nonapeptide" OR "Palmitoyl decapeptide" OR "Palmitoyl undecapeptide" OR "Palmitoyl dodecapeptide" OR "Palmitoyl tridecapeptide" OR "Palmitoyl tetradecapeptide" OR "Palmitoyl pentadecapeptide" OR "Palmitoyl hexadecapeptide" OR "Palmitoyl heptadecapeptide" OR "Palmitoyl octadecapeptide" OR "Palmitoyl nonadecapeptide" OR "Palmitoyl icosapeptide" OR "Palmitoyl lipidated peptides" OR "Palmitoyl fatty acylated peptides" OR "Palmitoyl matrikine"

2 CAS Nos. for Palmitoyl Oligopeptide: 171263-26-6 **(Not in ChemID)** and 1477732-56-7 **(in ChemID)**
171263-26-6 → 230,128 hits (PubMed)
147732-56-7 → 99,966 hits (PubMed)
"Palmitoyl Oligopeptide" → 183 hits (PubMed)*
171263-26-6 OR "Palmitoyl Oligopeptide" → 230,320 hits (PubMed)*
147732-56-7 OR "Palmitoyl Oligopeptide" → 100,148 hits (PubMed)*

*These results indicate no publications in common between chemical name AND either CAS No. Therefore, need to determine the chemical names with which these CAS Nos. are associated. Doesn't appear that these are CAS Nos. for Palmitoyl oligopeptide.

When used advance search screen, found that neither of above CAS Nos. yielded hits in PubMed. The 2 CAS Nos. yielded 0 hits in Multidatabase, DART, and Toxline on-line databases, and CDC, NTP, NTIS, ECETOC, and IARC websites.

1 CAS No. for Palmitoyl Tripeptide-5 in **Dictionary**: 623172-56-5 (**found in ChemID**)
According to ChemIDplus, this is the CAS No. for Palmitoyl tripeptide-5 bistrifluoracetate salt. [**Need to check with Bart because this CAS No. is included for palmitoyl tripeptide-5 in Dictionary. Recall also that, at Guidechem website, palmitoyl tripeptide-5 is associated with CAS No. 147732-56-7.]**

0 hits in PubMed and Toxline. CAS No. yielded 0 hits in Multidatabase, DART, and Toxline on-line databases, and 1 hit in ChemIDplus.

SciFinder Search Terms
See Table for Search Terms.

In SciFinder, 1st 2 CAS Nos. below (for palmitoyl oligopeptide) yielded hits, but no useful hits. The third CAS No., for palmitoyl tripeptide-5, yielded hits, but no useful hits.

CAS No. 171263-26-6 (Not in Table) → 29 hits (all patents; none useful)
CAS No. 147732-56-7 (Not in Table) → 56 hits (2 [non-Patents] ordered; remainder = not useful patents)
CAS No. 623172-56-5 (Not in Table) → 5 hits (all patents, none useful)

- Palmitoyl Decapeptide-21 (No CAS No. in **Dictionary**)
- Palmitoyl Dipeptide-7 (No CAS No.) (**FDA data**)
- Palmitoyl Dipeptide-10 (No CAS No.)
- Palmitoyl Dipeptide-13 (No CAS No.)
- Palmitoyl Dipeptide-17 (No CAS No.)
- Palmitoyl Dipeptide-18 (No CAS No.)
- Palmitoyl Heptapeptide-5 (No CAS No.) (**FDA data**)
- Palmitoyl Hexapeptide-12 (No CAS No.)
- Palmitoyl Hexapeptide-14 (No CAS No.) (**FDA data**)
- Palmitoyl Hexapeptide-15 (No CAS No.)
- Palmitoyl Hexapeptide-19 (No CAS No.)
- Palmitoyl Hexapeptide-26 (No CAS No.)
- Palmitoyl Hexapeptide-32 (No CAS No.)
- Palmitoyl Hexapeptide-36 (No CAS No.)
- Palmitoyl Nonapeptide-6 (No CAS No.)
- Palmitoyl Oligopeptide (**FDA data**)
- Palmitoyl Oligopeptide-70
- **Palmitoyl Pentapeptide-3** (**FDA data; listed as another name for Palmitoyl Pentapeptide-4 in Dictionary**)
- Palmitoyl Pentapeptide-4 (No CAS No.) **(FDA data**)
- Palmitoyl Pentapeptide-5 (No CAS No.)
- **Palmitoyl Tetrapeptide-3** (**FDA data; listed as another name for Palmitoyl Tetrapeptide-7 in Dictionary**)
- Palmitoyl Tetrapeptide-7 (No CAS No.) (**FDA data**)
- Palmitoyl Tetrapeptide-10 (No CAS No.) (**FDA data**)
- Palmitoyl Tetrapeptide-20 (No CAS No.)
- Palmitoyl Tripeptide-1 (No CAS No.)
- **Palmitoyl Trieptide-3** (**FDA data; listed as another name for Palmitoyl Tripeptide-5 in Dictionary**)
- Palmitoyl Tripeptide-4 (No CAS No.)
- Palmitoyl Tripeptide-5 (CAS No. 623172-56-5) (**FDA data**)
- Palmitoyl Tripeptide-8 (No CAS No.) (**FDA data**)
- Palmitoyl Tripeptide-28 (No CAS No.) (**FDA data**)
- Palmitoyl Tripeptide-29 (No CAS No.)
- Palmitoyl Tripeptide-31 (No CAS No.)
- Palmitoyl Tripeptide-36 (No CAS No.)
- Palmitoyl Tripeptide-37 (No CAS No.)
- Palmitoyl Tripeptide-38 (No CAS No.) (**FDA data**)
- Palmitoyl Tripeptide-40 (No CAS No.)
- Palmitoyl Tripeptide-42 (No CAS No.)

At first glance, ingredients in preceding list could be included in SLR on Palmitoyl Oligopeptide.

- Palmitoyl Alanine
- Palmitoyl Arginine
- Palmitoyl Camellia Sinensis Extract
- Palmitoyl Carnitine
- Palmitoyl Carnosine
- Palmitoyl Cocoa Seed Extract
- Palmitoyl Coffee Bean Extract
- Palmitoyl Collagen Amino Acids
- Palmitoyl Decapeptide-21
- Palmitoyl Dipeptide-7
- Palmitoyl Dipeptide-10
- Palmitoyl Dipeptide-13
- Palmitoyl Dipeptide-17
- Palmitoyl Dipeptide-18
- Palmitoyl Dipeptide-5 Diaminobutyroyl Hydroxythreonine
- Palmitoyl Dipeptide-5 Diaminohydroxybutyrate
- Palmitoyl Ethyltrimonium Methosulfate
- Palmitoyl Glutamic Acid
- Palmitoyl Glycine
- Palmitoyl Glycitein
- Palmitoyl Gold of Pleasure Amino Acids
- Palmitoyl Grape Seed Extract
- Palmitoyl Grapevine Shoot Extract
- Palmitoyl Heptapeptide-5
- Palmitoyl Hexapeptide-12
- Palmitoyl Hexapeptide-14
- Palmitoyl Hexapeptide-15
- Palmitoyl Hexapeptide-19
- Palmitoyl Hexapeptide-26
- Palmitoyl Hexapeptide-32
- Palmitoyl Hexapeptide-36
- Palmitoyl Hexapeptide-27 Acetate
- Palmitoyl Hyaluronate
- Palmitoyl Hydrolyzed Collagen
- Palmitoyl Hydrolyzed Milk Protein
- Palmitoyl Hydrolyzed Wheat Protein
- Palmitoyl Hydroxypropylcellulose
- Palmitoyl Hydroxypropyltrimonium Amylopectin/Glycerin Crosspolymer
- Palmitoyl Inulin
- Palmitoyl Isoleucine
- Palmitoyl Keratin Amino Acids
- Palmitoyl Lysyl Aminovaleroyl Lysine
- Palmitoyl Mare Milk
- Palmitoyl Methoxytryptamine
- Palmitoyl Millet Amino Acids
- Palmitoyl Myristyl Serinate
- Palmitoyl Nonapeptide-6
- Palmitoyl Oat Amino Acids
- Palmitoyl Oligopeptide
- Palmitoyl Oligopeptide-70
- Palmitoyl Olive Leaf Extract
- Palmitoyl Pea Amino Acids
- Palmitoyl Pentapeptide-4
- Palmitoyl Pentapeptide-5

- Palmitoyl PG-Trimonium Chloride
- Palmitoyl Pine Bark Extract
- Palmitoyl Proline
- Palmitoyl Quinoa Amino Acids
- Palmitoyl Rheum Rhaponticum Root Extract
- Palmitoyl Serine/Silk Amino Acids Methyl Esters
- Palmitoyl Silk Amino Acids
- Palmitoyl Tetrapeptide-7
- Palmitoyl Tetrapeptide-10
- Palmitoyl Tetrapeptide-20
- Palmitoyl Tormentilla Erecta Root Extract
- Palmitoyl Tripeptide-1
- Palmitoyl Tripeptide-4
- Palmitoyl Tripeptide-5
- Palmitoyl Tripeptide-8
- Palmitoyl Tripeptide-28
- Palmitoyl Tripeptide-29
- Palmitoyl Tripeptide-31
- Palmitoyl Tripeptide-36
- Palmitoyl Tripeptide-37
- Palmitoyl Tripeptide-38
- Palmitoyl Tripeptide-40
- Palmitoyl Tripeptide-42
- Palmitoyl Tryptamine

Preceding list is list of all ingredients in Dictionary with Palmitoyl at beginning of ingredient name.

Safety Assessment of Palmitoyl Oligopeptides as Used in Cosmetics

Status:	Draft Report for CIR Expert Panel Review
Release Date:	February 22, 2013
Panel Meeting Date:	March 18-19, 2013

The 2012 Cosmetic Ingredient Review Expert Panel members are: Chair, Wilma F. Bergfeld, M.D., F.A.C.P.; Donald V. Belsito, M.D.; Curtis D. Klaassen, Ph.D.; Daniel C. Liebler, Ph.D.; Ronald A Hill, Ph.D. James G. Marks, Jr., M.D.; Ronald C. Shank, Ph.D.; Thomas J. Slaga, Ph.D.; and Paul W. Snyder, D.V.M., Ph.D. The CIR Director is F. Alan Andersen, Ph.D. This report was prepared by Wilbur Johnson, Jr., M.S., Manager/Lead Specialist and Bart Heldreth, Ph.D., Chemist.

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INTRODUCTION

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

CHEMISTRY

The ingredients in this report are preliminarily grouped together as they are related structurally by an identical fatty, hydrophobic tail connected to a variable sequence of peptides. Each ingredient, in and of itself, has *intra*-ingredient variability in the order and identity of the peptides in the more hydrophilic end of the molecule, and some *inter*-ingredient overlap may occur.

Definition and Structure

A generic structure for palmitoyl oligopeptides (palmitoyl = *N*-(1-oxohexadecyl); oligopeptides = a chain of 2 or more amino acids linked through a peptide bond (i.e., carboxylic acid of one amino acid reacts with the β-position amine of another amino acid to form an amide (with loss of water)) and the structures of specific palmitoyl di-, tri-, and penta-peptides are shown in Figures 1 and 2.

Both the definitions and functions of palmitoyl oligopeptides in cosmetics are included in Table 1. The results of a chemical substances search at the Organization for Economic Cooperation Development's eChemPortal, indicate that the following 2 CAS numbers are being used to identify palmitoyl oligopeptide: 147732-56-7 and 171263-26-6.

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

Physical and Chemical Properties

Palmitoyl Oligopeptide

A chemical supplier provided data on palmitoyl oligopeptide , identified as CAS No. 147732-56-7 and CAS No. 171263-26-6.[3] Palmitoyl oligopeptide (CAS No. 147732-56-7) is also known as Pal - GHK (Pal-Gly-His-Lys-OH) and L-Lysine,N-(1-oxohexadecyl)glycyl-L-histidyl. It is a white powder and has a molecular weight of 578.80 and a log P of 4.81. The ingredient BIOPEPTIDE-CL (contains 100 ppm palmitoyl oligopeptide, as Pal-Gly-His-Lys-OH [CAS No. 147732-56-7]) has a density of 1.13.

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

Palmitoyl Dipeptide-18

Palmitoyl dipeptide-18 (N-palmitoyl glycyl histidine; trade name: NANOFIBERGEL-CS [currently not being marketed]) has the structural character of a lipid dipeptide amphiphilic compound and the function of a low-molecular-weight gelator.[4] When dissolved in water or polar solvents, low-molecular-weight gelators form a stringy assembly, which intertwines and forms a network described as holding H_2O to the gelate.

In a spectral analysis of palmitoyl dipeptide-18 (NANOFIBERGEL-CS), there was no evidence of absorbance in the UV-visible spectrum (290 to 450 nm).[4]

Palmitoyl Tripeptide-38

Palmitoyl tripeptide-38 (CAS No.1101175-36-3) is also known as Pal KMOOK and VOLULIP[tradename][5], and Palmitoyl-KMO$_2$K-OH, 2HCl.[6] It is a white powder and has a molecular weight of 675.97 and a logP of 4.01.[5] Palmitoyl tripeptide-38 (CAS No. 1101175-36-3) has been described as a white powder, whereas, VOLULIP has been described as a clear pale yellow liquid. According to a tentative specification, the specific gravity (at 20˚C) of VOLULIP is in the 0.850 to 0.890 range and the refractive index (at 25˚C) is in the 1.435 to 1.455 range.[6] Information on the composition of VOLULIP is included in the section on Composition/Impurities. The supplier of this information noted that the Cosmetic Ingredient Review (CIR) has concluded that of cetearyl ethylhexanoate and sorbitan isostearate (2 components of VOLULIP) are safe as used in cosmetic products.

Palmitoyl Pentapeptide-4

Palmitoyl pentapeptide-4 (CAS No. 214047-00-4) is also known as Pal KTTKS (Pal Ly-Thr-Thr-Lys-Ser).[7] This ingredient has been described as a white powder with a molecular weight of 902.07 and a log P of 3.48.

Method of Manufacture

Palmitoyl Oligopeptides

The following general information relating to the synthesis of peptides coupled to palmitic acid was found in the published literature: Peptides have been synthesized by solid phase fluorenylmethoxycarbonyl chemistry using an Advanced Chemtech MPS 350 synthesizer.[8] Palmitic acid was coupled to the deprotected amino-terminus of the resin-bound protected peptides both manually and by using the peptide synthesizer employing the same reaction conditions used in standard amino acid coupling. Peptides and monopalmitic acid-peptide conjugates were cleaved from the resin, deprotected, and purified using standard procedures.

Several strategies for the synthesis of lipidated peptides, both in solution and on solid support, have been developed.[9,10] Regarding peptides with longer amino acid chains, synthesis on solid support has practically always been performed. Shorter peptides have been synthesized both in solution and on solid support. Particularly, hexa- and heptapeptides corresponding to the Ras- and Rab-C-termini, respectively, have been synthesized in solution.[11,12]

Specifically, palmitoyl oligopeptide (CAS No. 147732-56-7) is synthesized via stepwise peptide synthesis.[3] The C-terminal amino acid (Lys) is protected on its acidic function, after which each protected amino acid (Gly, His) is coupled. A last coupling procedure is realized with palmitic acid instead of an amino acid. The protected peptide deprotected to remove the protecting group presents on the lateral function of lysine and histidine and on the C-terminal acidic function of Lys.

Palmitoyl oligopeptide (CAS No. 171263-26-6) is produced via stepwise acid phase peptide synthesis. The C-terminal amino acid (Gly) is protected on its acid function, after which each protected amino acid (Pro-Ala-val-Gly-Val) is coupled . A last coupling procedure is realized with palmitic acid instead of an amino acid. The protected peptide is deprotected to remove the protecting groups present on the lateral function of proline, alanine, valine, glycine, and valine and on the C-terminal function of the amino acid (name of amino acid not included).[3]

Palmitoyl Dipeptide-17

Palmitoyl dipeptide-17 (Palmitoyl-Gly-Pro, molecular weight = 410.59) is produced using the solid phase peptide synthesis method.[13]

Palmitoyl Dipeptide-18

Palmitoyl dipeptide-18 (NANOFIBEDRGEL-CS) is manufactured via a 2-step production procedure, which consists of the bonding of palmitoyl chloride and glycine and methyl ester, followed by bonding of the resulting palmitoyl glycine methyl ester with histidine.[4] It has been confirmed that the raw materials contain no animal-derived components. Based on 3 production trials resulting from the preceding method, it was confirmed that NANOFIBEDRGEL-CS can be manufactured at a purity level of 97% or above, and impurities at a level of 3% or below are produced.

Palmitoyl Tripeptide

According to a publication on the stimulation of collagen synthesis summarized later in this report**,** palmitoyl tripeptide (pamitoyl-Gly-L-His-L-Lys) has been produced via solid phase synthesis, yielding a peptide of high purity (> 97%).[14]

Palmitoyl Tripeptide-38

Palmitoyl tripeptide-38 is produced using solid phase synthesis with derivatives of amino acids (lysine and methione sulfone, a non-natural amino acid).[5] A last coupling procedure is realized with palmitic acid. At the final stage, ion exchange chromatography enables to exchange hydrochloride of each lysine.

The process of manufacturing Volulip™ (contains 500 ppm palmitoyl tripeptide-38) is defined as an association of *Portulaca pilosa* extract and a peptide palmitoyl-KMO_2K-OH, 2HCl in a liposoluble solvent.[6] Additional information on the composition of Volulip™ is included in the section on Composition/Impurities.

Pamiltoyl Tetrapeptide

In a publication on mitogenic activity, also summarized later in this report**,** palmitoyl tetrapeptide (Pam-Ser-Ser-Asn-Ala) was obtained via the following process: Palmitic acid (Pam-OH) was coupled to O-tert-butyl-seryl-O-tert-butyl-seryl-asparaginyl-alanine-tert-butylester(H-L-Ser(Bu^t)-Ser(Bu^t)-Asn-Ala-Obu^t) with N,N'-dicyclohexylcarbodiimide in dimethylformamide/dichloromethane (2:1).[15] The resulting Pam-Ser(Bu^t)-Ser(Bu^t)-Asn-Ala-OBu^t was purified in dichloromethane/methanol (1:1). The tert-butyl groups were removed in trifluoroacetic acid to yield the compound Pam-Ser-Ser-Asn-Ala. [Comments received from the Personal Care Products Council indicate that the palmitoyl tetrapeptide does not have an INCI name and is not being reviewed in this safety assessment. The description is included in case the safety test data included later in the report are determined to be useful in supporting the safety of other ingredients that are included.]

Palmitoyl Pentapeptide-4

Palmitoyl pentapeptide-4 is produced using stepwise peptide synthesis. The C-terminal amino acid (Ser) is protected on its acidic function, after which each protected amino acid (Lys-Thr-Thr-Lys) is coupled. A final coupling procedure is realized with palmitic acid instead of an amino acid.

Composition/Impurities

Palmitoyl Oligopeptide

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

Palmitoyl Dipeptide-17

Palmitoyl dipeptide-17 is 97% pure, and the total amount of any impurity in this ingredient is ≤ 2%.[13]

Palmitoyl Dipeptide-18

Most of the palmitoyl dipeptide-18 (NANOFIBERGEL-CS) impurities are analogs of NANOFIBERGEL-CS derived from palmitoyl chloride.[4] It was noted that palmitoyl chloride is produced from botanical palmitic acid with a different carbon number, and that its content is stably controlled. The percentages (highest values) of the following impurities from 3 production lots were reported as follows: lauroyl-glycine-histidine (0.18%), myristoyl-glycine-histidine (0.82%), stearoyl-glycine-histidine (0.38%), palmitoyl-glycine (1.86%), palmitoyl-glycine-histidine-methyl ester (0.51%), and palmitoyl-glycine-glycine-histidine (0.14%).

Palmitoyl Tripeptide-38

The impurites content of palmitoyl tripeptide-38 (CAS No. 1101175-36-3) has been described as follows: palmitic acid (< 5%) and water (< 5%).[5]

VOLULIP™ (trade name for palmitoyl tripeptide-38) has the following composition: palmitoyl KMO_2K-OH, 2HCl (≈ 0.05%), sucrose cocoate (≈ 0.4%), portulaca pilosa extract (≈ 2%), sorbitan isostearate (≈ 8%), and cetearyl

ethylhexanoate (qsp 100%), and manufacturing additives (water [1% maximum] and ethanol [0.1% maximum]).[6] Tentative specifications for VOLULIP™ include: KMO_2K-OH, 2HCl (450 to 550 ppm), water (< 1%), bacteria (< 100 cfu/g), and yeasts and molds (< 10 cfu/g). The supplier of these data noted that the CIR Expert Panel has concluded that cetearyl ethylhexanonate and sorbitan isosrtearate are safe as used in cosmetic products.

Palmitoyl Pentapeptide-4

The impurities content of palmitoyl pentapeptide-4 has been described as follows: acetate content (< 10%), palmitic acid (< 5%), and water content (< 5%).[7]

USE

Cosmetic

Most of the palmitoyl oligopeptides function as skin conditioning agents in cosmetic products.[1] In addition to this function, palmitoyl oligopeptide and palmitoyl oligopeptide-70 function as a surfactant-cleansing agent and a nail conditioning agent, respectively, and palmitoyl hexapeptide-14 functions as a surface modifier. Furthermore, palmitoyl tetrapeptide-20 and palmitoyl hexapeptide-12 function only as antioxidants and palmitoyl hexapeptide-26 functions only as an antimicrobial agent. According to information supplied to the Food and Drug Administration (FDA) by industry as part of the Voluntary Cosmetic Registration Program (VCRP) in 2012, the following palmitoyl oligopeptides are being used in cosmetic products:[16] **palmitoyl oligopeptide**, **palmitoyl dipeptide-7**, palmitoyl tripeptide-3, **palmitoyl tripeptide-5**, **palmitoyl tripeptide-8**, **palmitoyl tripeptide-28, palmitoyl tripeptide-38**, palmitoyl tetrapeptide-3, **palmitoyl tetrapeptide-7**, palmitoyl tetrapeptide-10, palmitoyl pentapeptide-3, **palmitoyl pentapeptide-4**, **palmitoyl hexapeptide-14**, and palmitoyl heptapeptide-5.

Results from surveys of ingredient use concentrations provided by the Personal Care Products Council in 2012 and 2013 indicate that, collectively, the following ingredients are being used at concentrations up to 0.9% and 0.06% in leave-on and rinse-off products, respectively: **palmitoyl oligopeptide**, **palmitoyl dipeptide-7**, **palmitoyl tripeptide-5**, **palmitoyl tripeptide-8**, **palmitoyl tripeptide-28, palmitoyl tripeptide-38**, **palmitoyl tetrapeptide-7**, **palmitoyl pentapeptide-4**, palmitoyl hexapeptide-12, **palmitoyl hexapeptide-14**, palmitoyl hexapeptide-19, palmitoyl hydrolyzed wheat protein, potassium palmitoyl hydrolyzed oat protein, and potassium palmitoyl hydrolyzed wheat protein.[17,18] [Overlap between the FDA and industry survey data sets is represented in bold print.] The 0.06% maximum use concentration in rinse-off products relates to potassium palmitoyl hydrolyzed oat protein in skin cleansing products. For leave-on products, the 0.9% maximum use concentration relates to potassium palmitoyl hydrolyzed wheat protein in body and hand products. The VCRP data on ingredient use frequencies and use concentration data provided by the Council are summarized in Table 2.

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

Palmitoyl oligopeptide is used in face, neck, body, and hand powders, and in body and hand sprays (maximum use concentration = 0.02% [powders] and 0.001% [sprays]). Palmitoyl pentapeptide-3 and palmitoyl hexapeptide-14 are also used in face powders (maximum use concentration = 0.06%). Because these ingredients are used in sprays or powders, they could possibly be inhaled. In practice, 95% to 99% of the droplets/particles released from cosmetic sprays have aerodynamic equivalent diameters >10 µm, with propellant sprays yielding a greater fraction of droplets/particles below 10 µm, compared with pump sprays.[19,20,21,22] Therefore, most droplets/particles incidentally inhaled from cosmetic sprays would be deposited in the nasopharyngeal and bronchial regions and would not be respirable (i.e., they would not enter the lungs) to any appreciable amount.[19,20]

Non-Cosmetic

A palmitoyl-tailed sequential oligopeptide carrier (SOC_n-II) for engineering immunogenic conjugates has been developed.[23] The authors noted that the main guideline in designing effective immunogens as vaccine candidates capable of eliciting potent and specific immune responses is to combine B/T cell epitopes and adjuvants as immunostimulators on the same carrier that links the major histocompatibility complex with T cell receptors. With the goal of contributing to the development of carriers for human usage, SOC_n-II was formed by the repeating peptide unit $(Aib\text{-}Lys\text{-}Aib\text{-}Gly)_n$, n = 2-7,

elongated from the amino-terminus by the palmitoyl group, which is known for its adjuvanticity. Aib in the amino acid sequence represents α-aminoisobutyric acid.

TOXICOKINETICS

Other than percutaneous absorption data on one ingredient, data on the absorption, distribution, metabolism, and excretion of palmitoyl oligopeptides were not found in the published literature. Percutaneous absorption data on palmitoyl dipeptide-10 (also known as palmitoyl carnosin [palmitoyl-β-Ala-His] are included below.

Palmitoyl Dipeptide-10

Prior to the percutaneous absorption study, the dipeptide carnosin (alanine and histidine) was synthesized (classical peptide synthesis) and a palmitoyl fatty acid chain was attached to the terminal NH_2 group.[14] Aliquots of carnosin and palmitoyl carnosin were then labeled with radioactive iodine. The labeled aliquots were incorporated into solutions of the cold peptides as tracer molecules. Standard Franz diffusion cells were used to study the diffusion and penetration kinetics of the labeled peptides. A known amount (not stated) of peptide solution was applied to the surface of the skin (source not stated), and the amount of radioactivity distributed in layers of the skin and the amount recovered in the receptor fluid of the diffusion cell were analyzed. Carnosin had very low affinity for the skin and did not penetrate beyond the stratum corneum. However, palmitoyl carnosin (lipophilic) diffused into the epidermis and dermis. Neither carnosin nor palmitoyl carnosin diffused beyond the dermis, in that no significant amount of radioactivity was found in the receptor fluid. Less than 10 to 4% of the initial radioactivity accumulated below the dermis within 6 h. The authors concluded that there was no significant transcutaneous penetration, and, therefore, no uptake into the blood or lymphatic fluids is to be expected.

TOXICOLOGY

Acute Oral Toxicity

Palmitoyl Oligopeptide

The acute oral toxicity of the ingredient BIOPEPTIDE-CL (contains 100 ppm palmitoyl oligopeptide, as Pal-Gly-His-Lys-OH) was evaluated using 10 Sprague-Dawley rats (5 males, 5 females; ages not stated).[24] The test substance, in its original form, was administered by gavage at a dose of 2,000 mg/kg. Dosing was followed by a 14-day observation period, after which necropsy was performed. Dosing had no effect on general behavior or body weight gain, and none of the animals died. There were no apparent abnormalities at necropsy. BIOPEPTIDE-CL was classified as nontoxic (LD50 > 2,000 mg/kg).

Palmitoyl Dipeptide-18

The acute oral toxicity of palmitoyl dipeptide-18 (NANOFIBERGEL-CS; purity: 89.8%) evaluate using groups of 10 Sprague-Dawley SPF rats [Crl:CD(SD)] (5 males, 5 females; 6 weeks old).[4] A single dose (2,000 mg/kg) of the test material was administered to each animal by oral gavage. Control animals received vehicle (0.5% methylcellulose aqueous solution) only. Dosing was followed by a 14-day observation period. Mortalities were not observed in test or control groups. There were no test substance-related changes in body weight or test-substance-related necropsy findings. Transient soft feces was the only reported test-substance-related clinical finding. It was concluded that the minimal lethal dose was greater than 2,000 mg/kg in both sexes.

Palmitoyl Pentapepide-4

Palmitoyl pentapeptide-4 was administered by gavage (concentration = 0.01%; dose volume = 20 ml/kg) once to each of 10 Sprague-Dawley rats (5 males, 5 females).[25] The animals were observed for up to 14 days post-administration, after which necropsy was performed. None of the animals died, and general behavior and body weight gain were unaffected by dosing. Additionally, there were no apparent abnormalities at necropsy. It was concluded that 0.01% palmitoyl pentapeptide-4 did not induce any signs of toxicity.

Repeated Dose Toxicity

Data relating to repeated dose toxicity from skin irritation or sensitization studies summarized later in the report text are included in this section.

Palmitoyl Oligopeptide

There were no clinical signs or mortalities in a cumulative skin irritation study on BIOPEPTIDE CL (contains 100 ppm palmitoyl oligopeptide, as Pal-Gly-His-Lys-OH) involving guinea pigs.[26] The test substance was evaluated in its original form.

In the guinea pig maximization test on BIOPEPTIDE- CL (contains 100 ppm palmitoyl oligopeptide, as Pal-Gly-His-Lys-OH), the test substance was evaluated at a concentration of 75% in a saline vehicle.[27] Clinical signs were not observed and none of the animals died during the study. Additionally, body weight gain was unaffected by test substance administration.

Palmitoyl Dipeptide-18

There were no abnormalities relating to general condition or body weight changes in any of the female Japanese White rabbits (Jla:JW strain) evaluated in the cumulative skin irritation study on palmitoyl dipeptide-18 (NANOFIBERGEL-CS, concentrations up to 5%).[4]

In the guinea pig maximization test on palmitoyl dipeptide-18 (NANOFIBERGEL-CS), the test material was also evaluated at concentrations up to 5%.[4] Observations for clinical signs and body weight changes revealed no test substance-related abnormalities.

Palmitoyl Pentapeptide-4

There were no clinical signs or treatment-related deaths in a cumulative skin irritation study on 0.01% palmitoyl pentapeptide-4 involving guinea pigs.[28]

In the guinea pig maximization test, palmitoyl pentapeptide-4 was injected/applied at concentrations of 0.0025% and 0.0075%.[29] Neither clinical signs nor mortalities were observed during the study.

Ocular Irritation

In Vivo

Palmitoyl Oligopeptide

The ocular irritation potential of the ingredient BIOPEPTIDE-CL (contains 100 ppm palmitoyl oligopeptide, as Pal-Gly-His-Lys-OH) was evaluated using 3 male New Zealand White rabbits.[30] The test substance (0.1 ml) was instilled into the conjunctival sac of the left eye of each animal, and the eyes were not rinsed. Ocular reactions were scored on at approximately 1 h, 24 h, 48 h, and 72 h post-instillation, and then on days 5 and 8. On day 1, very slight conjunctival reactions (chemosis and redness) were observed in all 3 animals. No other ocular reactions were observed for the duration of the study. It was concluded that BIOPEPTIDE-CL was a slight irritant in this study (maximum ocular irritation index = 4.7).

BIOPEPTIDE EL (contains 100 ppm palmitoyl oligopeptide, as Pal-Val-Gly-Val-Ala-Pro-Gly-OH) was instilled as a single dose (0.1 ml) into the left eye of each of 3 male New Zealand White rabbits.[31] Eyes were not rinsed, and reactions were scored at 24 h, 48 h, and 72 h post-instillation. Moderate or slight conjunctival irritation (chemosis [score = 2] and redness [score = 1 or 2]) was observed in all animals for up to 4 days post-instillation. Neither iridial irritation nor corneal opacity was observed. BIOPEPTIDE EL was considered a non-irritant when instilled into the eyes of rabbits. This conclusion was based on the observation that the mean scores for chemosis, redness, and degree of corneal opacity in 2 of the 3 animals did not reach the criteria values for irritation under the experimental conditions of the testing facility.

Palmitoyl Dipeptide-18

The ocular irritation potential of palmitoyl dipeptide-18 (NANOFIBERGEL-CS; purity: 89.8%) in 0.5% methylcellulose was studied using 18 female Japanese White rabbits (Jla:JW strain; 15 weeks old).[4] Concentrations of 1%, 2%, and 5% were tested using 3 groups of 6 rabbits, respectively. The test substance was instilled (0.1 ml) into the left eyes (unrinsed) of 3 rabbits per group, and instillation was followed by rinsing with water in the remaining 3 rabbits per group. Control right eyes were treated with 0.5% methylcellulose. Ocular reactions were evaluated using Draize methodology. At 1 h post-instillation, reddening of the conjunctiva (Draize maximum mean total score [MMTS] = 2) was observed in unrinsed eyes of all animals in 2% and 5% palmitoyl dipeptide-18 treatment groups. All reactions had cleared by 24 h post-instillation. Palmitoyl dipeptide-18 (1%) was non-irritating to unrinsed eyes. In groups subjected to ocular rinsing, reddening of the conjunctiva was observed at 1 h post-instillation in 1 of 3 rabbits treated with 5% palmitoyl dipeptide-18, but not in rabbits treated with lower concentrations. Ocular irritation also was not observe d in rinsed and unrinsed control eyes treated with 0.5% methylcellulose. It was concluded that concentrations of 2% and 5% were practically non-irritating and that the 1% concentration was non-irritating. Furthermore, reduced ocular irritation was observed after ocular rinsing.

Palmitoyl Pentapeptide-4

Palmitoyl pentapeptide-4 was evaluated at a concentration of 0.01% in an ocular irritation study involving 3 male New Zealand White rabbits.[32] The test substance (0.1 ml) was instilled into the left eye of each animal, and the right eye served as the untreated control. Eyes were not rinsed after instillation. The animals were observed for ocular reactions at 1 h, 24 h, 48 , and 72 h post-instillation. Ocular reactions were not observed during the study, and 0.01% palmitoyl pentapeptide-4 was classified as a non-irritant.

In Vitro

Palmitoyl Oligopeptide

The ocular irritation potential of the ingredient MAXI-LIP (contains 1,000 ppm palmitoyl oligopeptide, as Pal-Gly-His-Lys-OH) was evaluated in the hen's egg chorioallantoic membrane *in vitro* assay.[33] Details relating to the assay protocol were not included. Sodium dodecyl sulfate (0.5% w/v) served as the positive control. MAXI-LIP was classified as slightly irritating, but was considered "well tolerated". The positive control was classified as an ocular irritant.

The hen's egg chorioallantoic membrane *in vitro* assay was also used to evaluate the ocular irritation potential of Dermaxyl (contains 200 ppm palmitoyl oligopeptide, as Pal-Val-Gly-Val-Ala-Pro-Gly-OH).[34] The test substance was diluted to 50% (w/v) in distilled water prior to testing. The score for each egg was determined by the sum of the notations of hyperemia, hemorrhage, and coagulation (coagulation = opacity and/or thrombosis). The notation for the test substance corresponded to the arithmetic mean, rounded off to one decimal of the scores obtained for 4 eggs. Sodium dodecyl sulfate (0.5% w/v) served as the positive control. The mean irritation index was 0.8 for diluted Dermaxyl and 12.0 for the positive control. The test substance was classified as practically non-irritating.

Dermaxyl ocular irritation potential was also evaluated in the SIRC fibroblastic cell line using the neutral red releasing method.[34] Sodium dodecyl sulfate and sodium chloride served as positive and negative controls, respectively. The IC50 , defined as the test substance concentration that inhibited 50% of the cell survival and growth, was > 50%, and the % mortality at 50% dilution was 37.9%. It was concluded that the test substance caused "unimportant cytotoxicity".

Palmitoyl Tripeptide-38

In the neutral red release assay, the irritation potential of VOLULIP™ (contains 500 ppm palmitoyl tripeptide-38) was evaluated using the SIRC fibroblastic cell line.[35] The diluted test substance (diluted to 10% in cetearyl ethylhexanoate) was placed in contact with cells marked with neutral red for a defined period of time (not stated). The negative control (not stated) had an optical density of > 0.5. The parameters for assessment of cytotoxicity were % of cell death and IC50. The IC50 was estimated to be > 50% for the test substance. The positive control, sodium dodecyl sulfate, had an IC50 that was between 0.01% and 0.2%. It was concluded that diluted palmitoyl tripeptide-38 caused negligible cytotoxicity.

The ocular irritation potential of VOLULIP™ was also studied using the hen's egg chorioallantoic membrane *in vitro* assay.[36] The test substance was diluted to a concentration of 10% in cetearyl ethylhexanoate before testing. According to this assay, irritant effects (hyperemia, hemorrhage, and coagulation) that occurred up to 5 min after test substance application to the chorioallantoic membrane on day 10 of incubation were assessed. A mean irritation score of 5 (4 eggs) was reported for diluted VOLULIP™, classifying it as moderately irritating to the chorioallantoic membrane.

Palmitoyl Pentapeptide-4

The hen's egg chorioallantoic membrane *in vitro* assay was also used to evaluate the ocular irritation potential of MATRIXYL (contains 100 ppm palmitoyl pentapeptide-4), as supplied.[37] This ingredient was classified as a moderate ocular irritant (mean irritation index = 6). The positive control, sodium dodecyl sulfate (0.5% w/v) yielded a mean irritation index of 12 and was classified as an ocular irritant.

Skin Irritation and Sensitization

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

Animal

Palmitoyl Oligopeptide

The ingredient BIOPEPTIDE CL (contains 100 ppm palmitoyl oligopeptide, as Pal-Gly-His-Lys-OH) was evaluated for its skin irritation potential using 3 male New Zealand White rabbits (ages not stated). [38] BIOPEPTIDE CL was applied to scarified or non-scarified skin of the flank (0.5 ml on 6 cm^2 area, clipped free of hair), using an occlusive hypoallergenic dressing, for 24 h. Reactions were scored at 24 h and 72 h post-application. At 24 h post-application, slight erythema was observed on both flanks of 2 rabbits. These were the only reactions observed during the study. BIOPEPTIDE CL was classified as a non-irritant (PII = 0.3).

A cumulative skin irritation study on BIOPEPTIDE CL (contains 100 ppm palmitoyl oligopeptide, as Pal-Gly-His-Lys-OH) was performed using 10 guinea pigs (5 males, 5 females; ages not stated).[26] The test substance in its original form was applied to the left flank (0.05 ml on 2 cm x 2 cm area, clipped free of hair) once daily for 14 consecutive days. The right flank was treated with purified water (control). The test site was not covered with a dressing during the application period. Reactions were evaluated immediately prior to each application and approximately 24 h after the last application by comparing the reactions on both flanks. The animals were killed and cutaneous samples were removed from treated sites. Cutaneous reactions were not observed during the study. However, a very slight beige coloration of the skin was observed in each animal. It was concluded that BIOPEPTIDE CL was a non-irritant in guinea pigs (maximum weekly mean irritation index = 0).

The skin sensitization potential of BIOPEPTIDE- CL (contains 100 ppm palmitoyl oligopeptide, as Pal-Gly-His-Lys-OH) was studies using a total of 30 guinea pigs (ages not stated) in the maximization test.[27] The test group consisted of 20 animals (10 males, 10 females) and the control group consisted of 10 animals (5 males, 5 females). During induction day 1, test animals were injected intradermally with the test substance (1% in 0.9% isotonic saline vehicle [injection volume = 0.1 ml]) in the presence of Freund's complete adjuvant. The test substance in its original form (0.5 ml) was cutaneously applied to test animals on induction day 8. The control group was treated with vehicle only during induction. The challenge phase was initiated after a 12-day non-treatment period. The test substance (75% in saline vehicle [0.5 ml]) was applied to the right flank, and vehicle only (0.5 ml) was applied to the left flank of all animals. Next, the test substance was prepared on a dry compress and applied to the skin for 24 h using an occlusive dressing. Challenge reactions were evaluated at 24 h and 48 h after removal of the dressing. The animals were then killed and cutaneous samples were obtained from challenge sites. Microscopic examination was not performed on cutaneous samples. Cutaneous reactions were not observed during the challenge phase. It was concluded that no cutaneous reaction attributable to the sensitization potential of BIOPEPTIDE- CL at the maximal non-irritant concentration of 75% was observed in guinea pigs.

BIOPEPTIDE EL (contains 100 ppm palmitoyl oligopeptide, as Pal-Val-Gly-Val-Ala-Pro-Gly-OH) was evaluated in a skin irritation study involving 3 male New Zealand White rabbits (ages not stated).[39] The test substance, in its original form, was prepared on a dry compress and then applied (0.5 ml on 6 cm^2 area, clipped free of hair) for 4 h using a semi-occlusive dressing. Reactions were scored at 24 h, 48 h, and 72 h post-removal. Moderate cutaneous reactions (erythema, but no edema) were observed, and these reactions were reversible within 24 h or 48 h. Cutaneous reactions were not observed on days 3 and 4. BIOPEPTIDE EL was considered a non-irritant (mean erythema score < 1.0).

Palmitoyl Dipeptide-18

The skin irritation potential of palmitoyl dipeptide-18 (NANOFIBERGEL-CS; purity:89.8%) was studied using 12 female Japanese White rabbits (Jla:JW strain; 18 weeks old).[4] Concentrations of 1%, 2%, and 5% (in 0.5% methylcellulose solution) were tested. Each concentration (0.5 ml) was applied to a 2.5 x 2.5 cm lint patch, and 2 patches per concentration

were applied to non-abraded and abraded dorsal skin (clipped free of hair), respectively, for 24 h. The vehicle 0.5% methylcellulose was applied to control sites. Reactions were evaluated according to the Draize method after patch removal. Skin irritation was not observed following application of the vehicle control or each concentration of the test substance to intact or abraded skin (primary irritation index [PII] = 0). Palmitoyl dipeptide-18 was classified as a non-irritant in this study.

The skin irritation potential of palmitoyl dipeptide-18 (NANOFIBERGEL-CS; purity:89.8%) was also evaluated in a 14-day cumulative skin irritation test involving 12 female Japanese White rabbits (Jla:JW strain; 18 weeks old).[4] The concentrations applied were identical to those stated in the preceding study, and, except for the study duration, the application procedure was identical. Six rabbits each were treated with test substance solutions and vehicle, respectively. Reactions were evaluated according to the Draize method after patch removal daily. Skin irritation was not observed following application of the vehicle control or each concentration of the test substance to intact or abraded skin (PII = 0). It was concluded that repeated dermal application of palmitoyl dipeptide-18 for 14 consecutive days caused neither skin irritation nor cumulative skin irritation.

Palmitoyl dipeptide-18 (NANOFIBERGEL-CS; purity:89.8%) was evaluated in a maximization test involving a total of 40 female Hartley White guinea pigs.[4] Three groups of 10 animals were treated with the test material, and 2 groups of 5 animals served as negative and positive controls, respectively. A test concentration of 2% was selected as the highest concentration for intradermal induction, and 5% was the highest concentration for percutaneous induction. Test substance concentrations of 1%, 2%, and 5% were used during the challenge phase. In the negative control group, 0.5% methylcellulose was used for intradermal and percutaneous induction and challenge. In the positive control group, 0.1% 1-chloro-2,4-dinitrobenzene (DNCB) (vehicle:olive oil) was used for intradermal and percutaneous induction; challenge with 0.1% DNCB and vehicle (acetone) was performed. There were no challenge reactions to the test substance (1%, 2%, or 5%) or vehicle control (0.5% methylcellulose solution). In the positive control group, 0.1% DNCB solution induced sensitization, but challenge with the acetone vehicle did not. It was concluded that palmitoyl dipeptide-18 (NANOFIBERGEL-CS) did not have dermal sensitization potential in this study.

Palmitoyl Pentapeptide-4

In a skin irritation study, 0.01% palmitoyl pentapeptide-4 (0.5 ml) was applied to the left flank of each of 3 male New Zealand White rabbits.[32] The test site was clipped free of hair prior to application, and the ingredient was maintained in contact with the skin for 4 h using a semi-occlusive dressing. Reactions were scored at approximately 1 h, 24 h, 48 h, and 72 h after patch removal. The only reaction reported, very slight erythema, was observed in one animal on day 1. Palmitoyl pentapeptide-4 (0.01%) was classified as a non-irritant.

The cumulative skin irritation potential of palmitoyl pentapeptide-4 was evaluated using 10 guinea pigs (5 males, 5 females; strain and ages not stated). The test substance (0.01% w/w in formulation [0.05 ml volume]) was applied to a 2 cm x 2 cm area on the left flank once daily for 14 consecutive days.[40] The test site was clipped free of hair, and was not covered during the application period. The right flank was treated with distilled water only. Both flanks were scored for reactions prior to each application and at approximately 24 h after the last application. The animals were killed at the end of the observation period. Internal organs were not examined and skin samples were not excised. Very slight erythema was observed on the left flank of one animal on days 12 and 13. These were the only reactions reported during the study. Palmitoyl pentapeptide-4 (0.01%) was classified as a non-irritant (maximum weekly mean irritatioin index = 0).

Palmitoyl pentapeptide-4 (0.01% in formulation) was evaluated in the maximization test using 30 guinea pigs (strain not stated).[29] Test and control groups consisted of 20 animals (10 males, 10 females) and 10 animals (5 males, 5 females), respectively. Saline solution and mercaptobenzothiazole served as negative and positive controls, respectively. For intradermal injection, a test substance concentration of 75% w/w in saline was used (effective concentration = 0.01% x 75% = 0.0075%). On day 1, the test substance (mixed with Freund's complete adjuvant) was injected intradermally into the interscapular region. Sodium lauryl sulfate (10% w/w) was applied topically on day 7 to induce irritation. On day 8, the test substance (undiluted) was applied under an occlusive dressing for 48 h. For topical challenge, a test substance concentration of 25% w/w in saline was used [effective concentration = 0.01% x 25% = 0.0025%). Following a 12-day non-treatment period, test and control animals were challenged on day 22 by topical application of the test substance (under occlusive dressing) to the right flank for 24 h. Reactions were scored at approximately 24 h and 48 h after patch removal. The animals were killed at the end of the study. Internal organs were not examined and skin samples were not excised. Reactions were not observed during the challenge phase. The positive control induced sensitization. It was concluded that palmitoyl pentapeptide-4 in formulation did not induce delayed contact hypersensitivity in guinea pigs.

Human

Palmitoyl Oligopeptide

The skin irritation potential of the ingredient MAXI-LIP (contains 1,000 ppm palmitoyl oligopeptide, as Pal-Gly-His-Lys-OH) was evaluated using 10 healthy adult volunteers (ages not stated).[33] The ingredient was applied to dorsal skin (~ 0.02 ml on 50 mm^2 area), using an occlusive patch (Finn chamber on Scanpor), for 48 h. Untreated sites (covered with occlusive patch) served as negative controls. Reactions were scored 30 min after patch removal. Neither pathological irritation nor significant cutaneous intolerance was observed (primary irritation index [PII] = 0). There was also no evidence of a secondary effect. MAXI-LIP was classified as "very well tolerated".

The skin sensitization potential of MAXI-LIP was evaluated in a human repeated insult patch test (HRIPT) using 52 subjects.[41] The study was initiated with 57 subjects (16 to 79 years old), 5 of whom withdrew for reasons unrelated to ingredient application. During induction, patches (type not stated) were applied 3 times per week for a total of nine 24-h induction applications. Non-treatment periods during the induction phase were described as 24 h following each Tuesday and Thursday removal and 48 h following each Saturday removal. The challenge phase was initiated following a 2-week non-treatment period . Challenge patches were appliedfor 24 h to a new test site that was adjacent to the induction patch site. Reactions were scored at 24 h and 72 h after patch application. Barely perceptible (+ reaction) to moderate (2 reaction) reactions were observed during induction and/or challenge phases. However, it was noted that these transient, low-level responses were considered clinically insignificant. It was concluded that MAXI-LIP did not indicate a clinically significant potential for dermal irritation or allergic contact sensitization.

The ingredient DERMAXYL (contains 200 ppm palmitoyl oligopeptide, as Pal-Val-Gly-Val-Ala-Pro-Gly-OH) was evaluated for skin irritation potential using 10 adult volunteers.[34] A single 48-h application of the test substance (diluted to 50%) was made, under an occlusive patch, to dorsal skin. Neither pathological irritation nor significant cutaneous intolerance was observed (primary irritation index [PII] = 0). There was also no evidence of a secondary effect. Diluted Dermaxyl was considered very well tolerated.

An HRIPT on DERMAXYL was performed using 53 healthy adult volunteers.[42] The test substance was diluted to a concentration of 50% prior to application. Repeated, 48-h occlusive patch applications of the diluted test substance were made to each subject (area of application not stated). Eight induction applications were made, followed by challenge patch application. Neither pathological skin irritation (mean irritation index[induction] = 0.04) nor sensitization indicative of cutaneous intolerance was observed.

Palmitoyl Dipeptide-18

The skin irritation potential of palmitoyl dipeptide-18 (NANOFIBERGEL-CS; purity:89.8%) was evaluated using 40 male and female subjects (24 to 60 years old).[4] Initially, white petrolatum was applied to the test site (dorsal skin), after which the test substance was applied for 24 h under a closed dressing. Physiological saline and petrolatum served as controls. Reactions were scored at 60 minutes and 24 h after patch removal. Results were negative for palmitoyl dipeptide-18 and the control (PII = 0) at 60 minutes and 24 h after patch removal.

Palmitoyl Tripeptide-38

VOLULIP™ (contains 500 ppm palmitoyl tripeptide-38) was evaluated in a skin irritation study involving 11 adult female volunteers (phototypes I to IV).[43] The test substance was diluted to 10% in cetearyl ethylhexanoate and applied under an occlusive patch for 48 h. Skin reactions were not observed after patch removal, and it was concluded that the test substance had very good skin compatibility.

The skin irritation and sensitization potential of VOLULIP™ was studied in an HRIPT involving 106 subjects (males and females, 17 to 70 years old), 103 of whom completed the study.[44] Eleven subjects discontinued for reasons unrelated to application of the test substance. The test substance was diluted to 10% in cetearyl ethyhexanoate, and testing was performed using occlusive patches. Additional details relating to the test protocol were not provided. Distilled water and cetearyl ethylhexanoate served as negative and vehicle controls, respectively. Neither skin irritation nor allergic reactions were observed following repeated applications of the test substance or controls.

Palmitoyl Pentapeptide-4

MATRIXYL (contains 100 ppm palmitoyl pentapeptide-4) was evaluated for skin irritation potential in a study involving 10 adult volunteers (ages not stated).[37] The test substance was applied to the back (0.02 ml on 50 mm^2 area) of

each subject. Application sites were covered with an occlusive patch (Finn chamber on Scanpor) for 48 h. Untreated sites covered with an occlusive patch served as negative controls. Reactions sere scored at 30 min after patch removal. Very slight erythema was observed in 1 subject, and no other reactions were observed during the study. It was concluded that MATRIXYL was well-tolearated (PII = 0.10).

The skin irritation and sensitization potential of MATRIXYL was studied in an HRIPT (protocol not stated) using 59 male and female subjects (19 to 78 years old).[45] A total of 51 subjects completed the study, and 8 subjects withdrew for reasons unrelated to test substance administration. Positive reactions were not observed, and it was concluded that MATRIXYL did not have dermal irritation or allergic contact sensitization potential in this study.

Phototoxicity/Photosensitization

Palmitoyl Dipeptide-18

Based on the results of a spectral analysis on palmitoyl dipeptide-18 (i.e., no absorbance in the UV-visible range [290 to 450 nm]), it was considered that the test substance has no photosensitivity or phototoxicity and, thus, neither a photosensitization nor phototoxicity study was performed.[4]

Other Skin Studies

Studies relating to palmitoyl oligopeptide-induced skin rejuvenation are included in Table 4.

Palmitoyl Tripeptide-1

The anti-wrinkle effect, due to increased collagen synthesis, of palmitoyl tripeptide-1 (palmitoyl-Gly-His-Lys) was evaluated in a blind, vehicle-controlled test involving 15 female subjects (44 to 59 years old).[46] Essentially, wrinkles are due to a lack of collagen packing in the dermis. Both a cream containing the tripeptide (3 ppm) and a placebo cream were applied around the eye zones twice daily for 4 weeks. On days 0 and 28, skin replicas were taken on both sides of the face and analyzed using an image analysis system. The following measurements were made, and their variations analyzed with respect to day 0 and the placebo: total length of wrinkles, depth of wrinkles, and roughness amplitudes. A 39% decrease in wrinkle length, 23% decrease in wrinkle depth, and a 17% decrease in overall skin roughness were reported at the end of the 4-week period. The placebo cream had no significant effect. All differences between skin treated with the tripeptide versus the placebo cream were statistically significant.

Both a vehicle (not identified) and palmitoyl tripeptide-1 (pamitoyl-Gly-L-His-L-Lys, 4 ppm in vehicle) were applied to the skin of 23 healthy female volunteers (ages not stated) for 4 weeks.[14] Skin layer thickness was monitored using ultrasound echography. A small, but statistically significant, increase in skin thickness (~ 4%, compared to vehicle alone) was observed at the site treated with palmitoyl tripeptide. This value was not considered negligible, because it was noted that the thinning of aging skin occurs at a rate of approximately 6% every 10 years.

Palmitoyl Oligopeptide and Palmitoyl Pentapeptide-3

The peptides palmitoyl oligopeptide and palmitoyl pentapeptide-3, both modeled on repair signaling sequences, have been developed as cosmetic ingredients that enhance skin rejuvenation.[47] To further explain this function, the extracellular matrix (ECM) in the basement membrane that separates the epidermis from the dermis also serves as a mediator of receptor-induced interactions between cells, guiding growth and differentiation. Damage to the ECM leads to repair that is initiated through processes such as protein synthesis and cell differentiation and proliferation. Most of these functions are related to signaling by peptides that are released from the ECM to cells through cell membrane receptors. Over time, aged skin is characterized by decreased production of new collagen and increased proteolytic activity, resulting in increased collagen degradation. In senescent fibroblasts, there is decreased synthesis of type I collagen, and these cells proliferate at a much slower rate when compared to fibroblasts in young skin. Therefore, peptides modeled on repair signaling sequences have been developed as cosmetic ingredients that enhance skin rejuvenation.

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

An *in vivo* study on the skin rejuvenating effect of Matrixyl™ 3000 (palmitoyl oligopeptide + palmitoyl tetrapeptide-7 and Matrixyl™ (palmitoyl pentapeptide-4) was performed.[2] Panel 1 (Matrixyl™ 3000 vs. placebo) consisted of 24 volunteers with a mean age of 56.1 years. Panel 2 (Matrixyl™ 3000 vs. Matrixyl™) consisted of 25 volunteers with a

mean age of 55.6 years. The test substances and excipient were tested at a concentration of 3% in a cream formulation. Each cream formulation was applied to one-half of the face (on different sides) in the morning and at night for 2 months, in the absence of all other anti-wrinkle, reparative, restructuring, or regenerating products. Skin rejuvenation was assessed using profilometry and image analysis, photography, and cutometry. After 56 days, a statistically significant decrease in deep wrinkles and skin roughness resulted from application of Matrixyl™ 3000 ($p < 0.01$) and Matrixyl™ ($P < 0.05$) when compared to results at day 0. For a similar comparison involving the excipient cream, there were no statistically significant differences in results at day 0 vs. those at day 56. The results (wrinkle reduction or skin roughness) were not found to be statistically significantly different when both test substances were compared. Also, after 56 days, a statistically significant increase in skin elasticity and tone resulted from application of Matrixyl™ 3000 ($p < 0.01$) and Matrixyl™ ($P < 0.05$) when compared to results at day 0.

Palmitoyl Tetrapeptide-7

In a cytometric study, 17 subjects (ages not stated) applied a cream formulated with 15 ppm palmitoyl tetrapeptide-7 to the face and neck for 1 month.[2] A significant increase in firmness was noted for the face (+19%) and neck (+40%). An increase in elasticity (face, 17%; neck, 27%) was also observed. These changes were not observed following treatment with placebo formulation on contralateral sides. Further study of the skin surface (observation of the microdepression network) revealed enhanced isotropy (+23%), a decrease in the deepest wrinkles (-56%), and an overall reduction in roughness (14%) after 15 days of palmitoyl tetrapeptide-7 application. The end result of these studies was an image of smoother, rejuvenated skin.

Palmitoyl Pentapeptide-3

Reportedly, in a randomized study, palmitoyl pentapeptide-3 (50 ppm) produced a significant benefit to lines and wrinkles around the eyes when compared to a vehicle control. Details relating to this study were not included.[48]

Palmitoyl Pentapeptide-4

A total of 93 female subjects (35 to 55 years old) participated in what was described as a double-blind, placebo-controlled , split-face, left-right randomized clinical study.[49] Both a moisturizer control product and the same product containing 3 ppm of palmitoyl pentapeptide-4 (palmitoyl-lysine-threonine-threonine-lysine-serine [pal-KTTKS]) were applied (~ 0.4 g, each product) to each side of the face twice daily for 12 weeks. This study was designed to determine whether pal-KTTKS reduces wrinkles/fine lines. Both quantitative technical and expert grader image analysis were used. Pal-KTTKS was well-tolerated by the skin (i.e., no skin irritation) and provided significant improvement in terms of wrinkles/fine lines reduction, when compared to the control product. This effect was described as small, but was significant at weeks 8 and 12. In self-assessments, the subjects reported significant fine line/wrinkle improvements and noted improvements in the following other skin benefit areas: reduction in age spots, reduction in dark circles, and increased skin firmness. The latter 3 effects were described as significant at week 12.

CELLULAR EFFECTS

Studies on cellular effects of palmitoyl oligopeptides are summarized in Table 5.

Stimulation of Collagen and Fibronectin Synthesis

Palmitoyl Tripeptide-1

The stimulation of collagen synthesis by palmitoyl tripeptide-1 (pamitoyl-Gly-L-His-L-Lys) in human fibroblasts *in vitro* was studied.[14] Collagen synthesis was monitored by the incorporation of tritiated proline, considered to be a strong signal of collagen synthesis. Results indicated that this strong signal of collagen synthesis was observed at a concentration of 0.5 µM/liter. In another experiment, skin samples (human biopsies [abdominal tissue]) from plastic surgery were irradiated with daily doses of UVA light for one week. Microscopic examination revealed strong degradation of dermal collagen. Following irradiation, the skin samples were treated with retinoic acid (500 ppm) or palmitoyl tripeptide (5 ppm) during the same week. Treatment with either compound resulted in almost total preservation and/or renewal (i.e., high density of collagen) of the tissue collagen.

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

Normal human fibroblasts were cultured in Dulbecco's modified eagle medium in the presence of fetal calf serum.[2] After cell confluence was achieved, the culture medium was replaced and the fibroblasts were incubated (without serum) for 72 h in the presence of vitamin C and the following palmitoyl oligopeptides: palmitoyl oligopeptide (up to 7.5 ppm), palmitoyl tetrapeptide-7 (up to 3.5 ppm), Matrixyl™ 3000 (palmitoyl oligopeptide + palmitoyl tetrapeptide-7) (up to 11 ppm), and palmitoyl pentapeptide-4 (up to 8 ppm). Control media consisted of the culture medium alone or with a positive control (transforming growth factor beta (TGFβ). Matrix proteins (collagen 1 and fibronectin) were assayed using the enzyme-linked immunosorbant assay (ELISA) method and hyaluronic acid was assayed using a colorimetric method. Except for palmitoyl oligopeptide, a dose response for collagen 1 synthesis was observed following incubation with all of the test substances. Matrixyl™ 3000 (palmitoyl oligopeptide + palmitoyl tetrapeptide-7) yielded values for collagen 1 synthesis greater than those that would be expected on the basis of simple addition. Incubation with the positive control resulted in 102% stimulation of collagen synthesis.

Except for palmitoyl oligopeptide, a dose response for de novo synthesis of fibronectin and hyaluronic acid was observed in the presence of all of the test substances. The greatest increase in de novo fibronectin synthesis (119%) associated with a single oliogopeptide was observed in the presence of palmitoyl pentapeptide-4. However, Matrixyl™ 3000 (palmitoyl oligopeptide + palmitoyl tetrapeptide-7) induced a 164% increase in fibronectin synthesis. Palmitoyl pentapeptide-4 stimulated the de novo synthesis of hyaluronic acid by 30%, with no dose response. Matrixyl™ 3000 stimulated hyaluronic acid synthesis by 179%, whereas values for palmitoyl oligopeptide and palmitoyl tetrapeptide-7 were 14% and 16%, respectively.[2]

Stimulation of Collagen Synthesis and Fibroblast Proliferation

Palmitoyl Hexapeptide-14

Study results have indicated that palmitoyl hexapeptide-14, peptide designed using an innate immunity peptide template, stimulated cell migration, collagen synthesis, and fibroblast proliferation and scaffolding.[47] Details relating to the test protocol and study results were not included.

Down-regulation of Interleukin-6

Palmitoyl Tetrapeptide-7

The ability of palmitoyl tetrapeptide-7 (Rigin™) to down-regulate Interleukin-6 (IL-6, a cytokine) in both resting and inflamed cells *in vitro* was compared to results for dehydroepiandrosterone (DHEA), a secretory product of the human adrenal gland.[47] DHEA has been characterized as having a wide array of therapeutic benefits, one of which is reducing inflammation via the IL-6 pathway. Palmitoyl tetrapeptide-7 is among the group of peptides (referred to as rigins) derived from DHEA. The results for palmitoyl tetrapeptide-7 and DHEA were said to have been comparable in terms of the ability of each to down-regulate IL-6 in resting and in inflamed cells. Supposedly, this reduction in IL-6 can produce increased skin firmness, smoothness, and elasticity. Details relating to the test protocol and study results were not included.

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

Stimulation of Angiogenesis

Palmitoyl Oligopeptide

Palmitoyl oligopeptide, an elastin sequence, enhanced angiogenesis in the chick chorioallantoic membrane (in an *in vivo* model) by promoting endothelial cell migration and tubulogenesis through upregulation of membrane-type metalloproteinase-1 (MT1-MMP), a matrix metalloproteinase (MMP).[50] In the *in vivo* angiogenesis assay, the chick

chorioallantoic membrane was exposed to allow direct access. On day 6 of embryonic development, angiogenic areas were delimited with a silicon ring containing phosphate-buffered saline (PBS, control) or palmitoyl oligopeptide (50 ng) in a final volume of 20 µl. Embryos were then placed in an incubator to induce spontaneous angiogenesis and were treated daily. Treated areas were photographed daily on days 6 to 10 of embryonic development.

Mitogenic and Immune Adjuvant Activity

Palmitoyl Tetrapeptide

The palmitoyl tetrapeptide used in this study was identified as N-palmitoyl-(S)-seryl-(S)-seryl-(S)-asparaginyl- (S)-alanine, an analogue of the N-terminal part of the lipoprotein from the outer membrane of *Escherichia coli*.[15] This tetrapeptide was tested for biological activity *in vitro* using lymphocyte culture systems. The induction of DNA synthesis by palmitoyl tetrapeptide, as measured by the incorporation of ^{3}H-thymidine and ^{3}H-uridine, was followed in mouse splenocytes. Spleen cells were from the following inbred mouse (female mice, 8 to 12 weeks old) strains: C3H/HeJ, C3H/He/Bom/nunu, and Balb/c. The ability of palmitoyl tetrapeptide to polyclonically stimulate B-lymphocytes into immunoglobulin secretion was assessed using a hemolytic plaque assay. In another test, the ability of palmitoyl tetrapeptide to activate the BCL1 lymphoid B-cell line (tumor cell line) *in vitro* was examined.

In all strains, palmitoyl tetrapeptide had a stimulatory effect on B-lymphocytes that was comparable to the effect of native lipoprotein, as measured by the incorporation of ^{3}H-thymidine and ^{3}H-uridine, and by a hemolytic plaque assay. After 72 h of cultivation, an increase in ^{3}H-thymidine incorporation was observed starting at concentrations below 1 µg/ml, and the concentration that was optimal for stimulation amounted to 20-30 µg/ml. A marked increase in uridine incorporation was noted at concentrations ranging from 2.1 to 137 µg/ml. The number of plaque-forming cells against densely trinitrophenylated sheep red blood cells increased markedly after stimulation of mouse spleen cells. The B-lymphocyte tumor cell line BCL1 was also activated by palmitoyl tetrapeptide *in vitro*. In this cell line, palmitoyl tetrapeptide markedly enhanced the incorporation of ^{3}H-thymidine at concentrations > 2 µg/ml. Optimal stimulation was obtained at a concentration of ~ 30 µg/ml, and concentrations > 100 µg/ml had only a marginal effect. The results of this study demonstrated that the N-terminal tetrapeptide moiety of lipoprotein, linked to a lipophilic molecule, constitutes, by itself, a novel B-lymphocyte mitogen.[15]

Tripalmitoyl Pentapeptide

The adjuvant activity of tripalmitoyl pentapeptide (S-(2,3-bis-(palmitoyloxy)-(2RS)-propyl)-N-palmitoyl-(R)-cysteinyl-(S)-seryl-(S)-seryl(S)-asparaginyl-(S)-alanine) *in vitro* was studied.[51] In a direct hemolytic plaque assay, the stimulation of the primary antibody response toward underivatized sheep red blood cells (SRBC) and toward trinitrophenylated (TNP-) SRBC was markedly enhanced in the presence of tripalmitoyl pentapeptide (3.3 to 33.3 µg/ml). Plaque formation was increased up to 100-fold at optimal mitogen-and antigen-doses. At suboptimal doses (0.03 to 0.3 µg/ml), a 10- to 60-fold increase in plaque formation was reported. As measured by the enzyme-linked immunosorbent assay (ELISA), the antigen-specific IgM response was increased by ~ 7-fold and the IgG response was augmented by ~ 10-fold in the presence of tripalmitoyl pentapeptide. In the secondary *in vitro* response to TNP-SRBC, a 7- to 10-fold enhancement of the antibody titer was observed in the presence of tripalmitoyl pentapeptide. It was noted that the application of tripalmitoyl pentapeptide and antigen had to occur concurrently in order to produce a strong adjuvant effect. The addition of tripalmitoyl pentapeptide either a day before or a day after antigen application did not induce a significant positive effect. Actually, in several instances, decreased antibody production was observed. It was concluded that tripalmitoyl pentapeptide constitutes a potent immune adjuvant. [Comments received from the Council indicate that the tripalmitoyl pentapeptide is not one of the ingredients that is being reviewed in this safety assessment.]

Structure-Activity Relationships

Palmitoyl Dipeptide-18 and Impurities

Structural formulas for palmitoyl dipeptide-18 and the following palmitoyl dipeptide-18 impurities were entered into *Derek for Windows* for evaluation of all endpoints: lauroyl-glycine-histidine, myristoyl-glycine-histidine, stearoyl-glycine-histidine, palmitoyl-glycine, palmitoyl-glycine-histidine-methyl ester, and palmitoyl-glycine-glycine-histidine.[4] No alerts for palmitoyl dipeptide-18 or its impurities were shown. After considering these results along with the toxicity data on palmitoyl dipeptide-18 summarized earlier in this safety assessment, it was determined that these palmitoyl dipeptide-18 impurities have no problematic toxicity.

REPRODUCTIVE AND DEVELOPMENTAL TOXICITY

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

GENOTOXICITY

Genotoxicity data on palmitoyl oligopeptides are summarized in Table 6. Ames test results for the following ingredients were negative with and without metabolic activation in *Salmonella typhimurium* and *E. coli* bacterial strains: palmitoyl oligopeptide (MAXI-LIP), palmitoyl oligopeptide (BIOPEPTIDE-CL), palmitoyl tripeptide-38 (VOLULIP™), and palmitoyl pentapeptide-4. In other studies, *umu* test (using *umu*-test Umlac AT mutagenicity test kit) results for palmitoyl dipeptide-18 (NANOFIBERGEL-CS) were negative in *Salmonella typhimurium* and *E. coli* strains with and without metabolic activation, and results were negative for NANOFIBERGEL-CS in a chromosome aberrations test (with and without metabolic activation) involving human lymphocytes. However, NANOFIBERGEL-CS was genotoxic with, but not without, metabolic activation in *Salmonella typhimurium* strains TA97 and TA100. These positive results were thought to have been due to the presence of free histidine. Because Pal-G (palmitoyl dipeptide-18 impurity without histidine) was not genotoxic with or without metabolic activation in these strains, it was assumed that palmitoyl dipeptide-18 is not a genotoxic substance.

GENE ACTIVATION

In addition to the genotoxicity data summarized in the preceding section, data on gene activation are summarized below.

Reportedly, molecular biology methods have enabled access to intracellular, functional, and morphological changes induced by substances after cell layer (fibroblasts or keratinocytes) or tissue (epidermis and synthetic epidermis) exposure.[2] With this in mind, it is possible to define the profile of the method of action of a substance in relation to the genes activated or repressed, and compare the findings with those for a control cell culture or tissue. The gene activation profiles for palmitoyl oligopeptide and palmitoyl tetrapeptide-7 have been determined using a bank of 450 genes. Palmitoyl tetrapeptide-7 and palmitoyl pentapeptide-4 have very similar gene activation profiles. The genes regulated in the same way are those for functions associated with cell proliferation (platelet-derived growth factor [PDGF] associated protein and subunit, and ethylene response factor 1[ERF1]), matrix remodeling (urokinase inhibitor, metallothioneins, and lysyl oxidase), cell migration (heat shock protein 90 [HSP 90], Rho [Ras-homology], and GTPase), and cell attachment (fibronectin receptor).

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

Palmitoyl oligopeptide (Pal-glycine-histidine-lysine) activated fewer genes, however, its profile was more specifically oriented toward keratinocyte anchoring (alpha-catenin and laminin receptor) and differentiation (keratin 10). Additionally, this oligopeptide increased the synthesis of extracellular matrix (syndecan and heparin sulfate glycoprotein). The profile characterized by the genes activated in fibroblasts indicated that palmitoyl oligopeptide stimulated numerous genes more strongly when compared to palmitoyl pentapeptide-4. Additional details were not provided.[2]

CARCINOGENICITY

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

SUMMARY

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

Collectively, data supplied to the Food and Drug Administration (FDA) by industry as part of the Voluntary Cosmetic Registration Program (VCRP) and results from surveys of ingredient use concentrations provided by the Personal Care Products Council indicate that the following 19 palmitoyl oligopeptides are being used in cosmetic products: palmitoyl oligopeptide, palmitoyl dipeptide-7, palmitoyl tripeptide-3, palmitoyl tripeptide-5, palmitoyl tripeptide-8, palmitoyl tripeptide-28, palmitoyl tripeptide-38, palmitoyl tetrapeptide-3, palmitoyl tetrapeptide-7, palmitoyl tetrapeptide-10, palmitoyl pentapeptide-3, palmitoyl pentapeptide-4, palmitoyl hexapeptide-12, palmitoyl hexapeptide-14, palmitoyl hexapeptide-19, palmitoyl heptapeptide-5, palmitoyl hydrolyzed wheat protein, potassium palmitoyl hydrolyzed oat protein, and potassium palmitoyl hydrolyzed wheat protein. These ingredients are being used at concentrations up to 0.9% (potassium palmitoyl hydrolyzed wheat protein) and 0.06% (potassium palmitoyl hydrolyzed oat protein) in leave-on and rinse-off products, respectively.

Palmitoyl oligopeptide is used in face, neck, body, and hand powders, and in body and hand sprays (maximum use concentration = 0.02% [powders] and 0.001% [sprays]). Palmitoyl pentapeptide-3 and palmitoyl hexapeptide-14 are also used in face powders (maximum use concentration = 0.06%). Because these ingredients are used in sprays or powders, they could possibly be inhaled.

Some of the methods of manufacturing palmitoyl peptides include stepwise peptide synthesis for the production of palmitoyl oligopeptide and palmitoyl pentapeptide-4 and solid phase peptide synthesis for the production of palmitoyl dipeptide-17 and palmitoyl tripeptide-38.

In a spectral analysis of palmitoyl dipeptide-18 (NANOFIBERGEL-CS), there was no evidence of absorbance in the UV-visible spectrum (290 to 450 nm). Based on these results, it was considered that this ingredient has no photosensitivity or phototoxicity.

Other than percutaneous absorption data on palmitoyl dipeptide-10, data on the absorption, distribution, metabolism, and excretion of palmitoyl oligopeptides were not found in the published literature. In an *in vitro* skin penetration study, palmitoyl dipeptide-10 (also known as palmitoyl carnosin [palmitoyl-β-Ala-His], labeled with radioactive iodine] penetrated into the epidermis and dermis. It did not penetrate beyond the dermis, in that no significant amount of radioactivity was found in the receptor fluid. In the absence of skin penetration data on the ingredients reviewed in this safety assessment, it should be noted that palmitoyl oligopeptide (Pal-Gly-His-Lys-OH) has a molecular weight of 578.80 and a logP of 4.81, and that palmitoyl oligopeptide (Pal-Val-Gly-Val-Ala-Pro-Gly-OH) has a molecular weight of 737 and a logP of 5.09.

BIOPEPTIDE-CL (contains 100 ppm palmitoyl oligopeptide, as Pal-Gly-His-Lys-OH) and palmitoyl dipeptide-18 (NANOFIBERGEL-CS) were nontoxic (LD50 > 2,000 mg/kg) in acute oral toxicity studies involving rats. In another acute study, 0.01% palmitoyl pentapeptide-4 (dose volume = 20 ml/kg) was also nontoxic when administered orally to rats. Studies designed to evaluate the repeated dose toxicity of palmitoyl oligopeptides were not found in the published literature. However, neither treatment-related clinical signs/mortalities were observed in cumulative skin irritation/sensitization studies on the following ingredients: BIOPEPTIDE-CL (contains 100 ppm palmitoyl oligopeptide, as Pal-Gly-His-Lys-OH; guinea pigs tested), 75% BIOPEPTIDE-CL (guinea pigs tested), up to 5% palmitoyl dipeptide-18 (NANOFIBERGEL-CS; rabbits tested), up to 5% NANOFIBERGEL-CS (guinea pigs tested), 0.01% palmitoyl pentapeptide-4 (guinea pigs tested), and up to 0.0075% palmitoyl pentapeptide-4.

Palmitoyl oligopeptide (BIOPEPTIDE-CL, contains 100 ppm palmitoyl oligopeptide, as Pal-Gly-His-Lys-OH) was slightly irritating and Palmitoyl dipeptide-18 (NANOFIBERGEL-CS) was practically non-irritating (at 2% and 5%) and nonirritating (at 1%) to the eyes of rabbits. BIOPEPIDE EL (contains 100 ppm palmitoyl oligopeptide, as Pal-Val-Gly-Val-Ala-Pro-Gly-OH) and palmitoyl pentapeptide-4 (0.01%) were non-irritating to the eyes of rabbits. In the hen's egg chorioallantoic membrane *in vitro* assay for evaluating ocular irritation potential, MAXI-LIP (contains 1,000 ppm palmitoyl oligopeptide, as Pal-Gly-His-Lys-OH) and palmitoyl pentapeptide-4 were classified as irritants, VOLULIP™ (contains 500

ppm palmitoyl tripeptide-38) was classified as moderately irritating, and DERMAXYL (contains 200 ppm palmitoyl oligopeptide, as Pal-Val-Gly-Val-Ala-Pro-Gly-OH) was practically non-irritating. In the *in vitro* neutral red release assay for evaluating ocular irritation potential, DERMAXYL caused "unimportant cytotoxicity" and VOLULIP™ caused negligible cytotoxicity.

In skin irritation studies (single application) involving rabbits, the following palmitoyl oligopeptides were classified as non-irritants: BIOPEPTIDE CL, BIOPEPTIDE EL, NANOFIBERGEL-CS (up to 5%), and palmitoyl pentapeptide-4 (0.01%). Two of these ingredients were also classified as non-irritants in cumulative skin irritation studies involving guinea pigs (BIOPEPTIDE CL and palmitoyl pentapeptide-4 [0.01% in formulation]) and rabbits (NANOFIBERGEL-CS [up to 5%]). BIOPEPTIDE CL did not induce skin sensitization at a challenge concentration of 75% in the maximization test. Other maximization test results were negative in guinea pigs challenged with NANOFIBERGEL-CS (up to 5%) and palmitoyl pentapeptide-4 (0.0025%).

In human skin irritation studies (single application), the following ingredients were classified as non-irritants: MAXI-LIP, DERMAXYL (50%), NANOFIBERGEL-CS, VOLULIP™ (10%), and MATRIXYL (contains 100 ppm palmitoyl pentapeptide-4). The absence of skin irritation was also reported in a randomized clinical study on palmitoyl pentapeptide-4 (3 ppm palmitoyl-lysine-threonine-threonine-lysine-serine [pal-KTTKS, ~ 0.4 g] in moisturizer) involving 93 female subjects. The moisturizer was applied to the face twice daily for 12 weeks and resulted in significant improvement in terms of reduction of wrinkles/fine lines. The following ingredient HRIPT results (all available studies) were negative for skin irritation and sensitization: MAXI-LIP, DERMAXYL (50%), VOLULIP™ (10%), and MATRIXYL .

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

The stimulation of collagen synthesis by palmitoyl tripeptide-1 in human fibroblasts *in vitro* was studied. A strong signal of collagen synthesis was noted at a concentration of 0.5 µM/liter. In the same study, human skin samples were irradiated with daily doses of UVA light for one week, resulting in degradation of dermal collagen. Treatment with palmitoyl tripeptide-1 (5 ppm) during the same week caused almost total preservation and/or renewal of collagen. In another study, normal human fibroblasts were incubated in the presence of vitamin C and the following peptides: palmitoyl oligopeptide (up to 7.5 ppm), palmitoyl tetrapeptide-7 (up to 3.5 ppm), palmitoyl oligopeptide + palmitoyl tetrapeptide-7 (up to 11 ppm]), and palmitoyl pentapeptide-4 (up to 8 ppm). Except for palmitoyl oligopeptide, a dose response for collagen 1 synthesis and the *de novo* synthesis of fibronectin and hyaluronic acid was observed following incubation with all of the test substances. Palmitoyl hexapeptide-14 has been reported to stimulate cell migration, collagen synthesis, and fibroblast proliferation and scaffolding.

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

Palmitoyl oligopeptide enhanced angiogenesis in the chick chorioallantoic membrane (in an *in vivo* model) by promoting endothelial cell migration and tubulogenesis through upregulation of membrane-type metalloproteinase-1 (MT1-MMP), a matrix metalloproteinase.

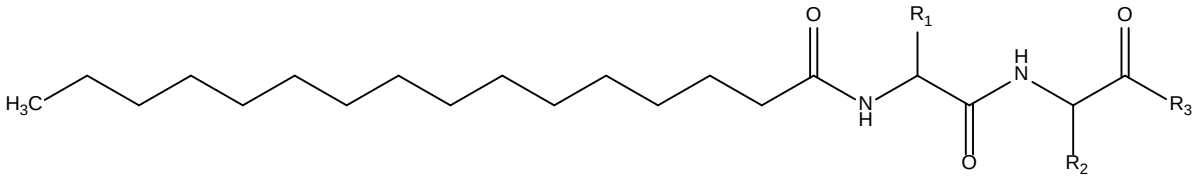
Study results have established palmitoyl tetrapeptide (Pam-Ser-Ser-Asn-Ala) as a novel B-lymphocyte mitogen and tripalmitoyl pentapeptide (S-(2,3-bis-(palmitoyloxy)-(2RS)-propyl)-N-palmitoyl-(R)-cysteinyl-(S)-seryl-(S)-seryl(S)-asparaginyl-(S)-alanine) as a potent immune adjuvant. [Comments received from the Council indicate that these compounds are not cosmetic ingredients.]

Ames test results for the following ingredients were negative with and without metabolic activation in *Salmonella typhimurium* and *E. coli* bacterial strains: palmitoyl oligopeptide (MAXI-LIP), palmitoyl oligopeptide (BIOPEPTIDE-CL), palmitoyl tripeptide-38 (VOLULIP™), and palmitoyl pentapeptide-4. In other studies, *umu* test (using *umu*-test Umlac AT mutagenicity test kit) results for palmitoyl dipeptide-18 (NANOFIBERGEL-CS) were negative in *Salmonella typhimurium* and *E. coli* strains with and without metabolic activation, and results were negative for NANOFIBERGEL-CS in a chromosome aberrations test (with and without metabolic activation) involving human lymphocytes. However, NANOFIBERGEL-CS was genotoxic with, but not without, metabolic activation in *Salmonella typhimurium* strains TA97 and TA100. These positive results were thought to have been due to the presence of free histidine. Because Pal-G (palmitoyl dipeptide-18 impurity without histidine) was not genotoxic with or without metabolic activation in these strains, it was assumed that palmitoyl dipeptide-18 is not a genotoxic substance.

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

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

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



Example structures of the ingredient Palmitoyl Oligopeptide:

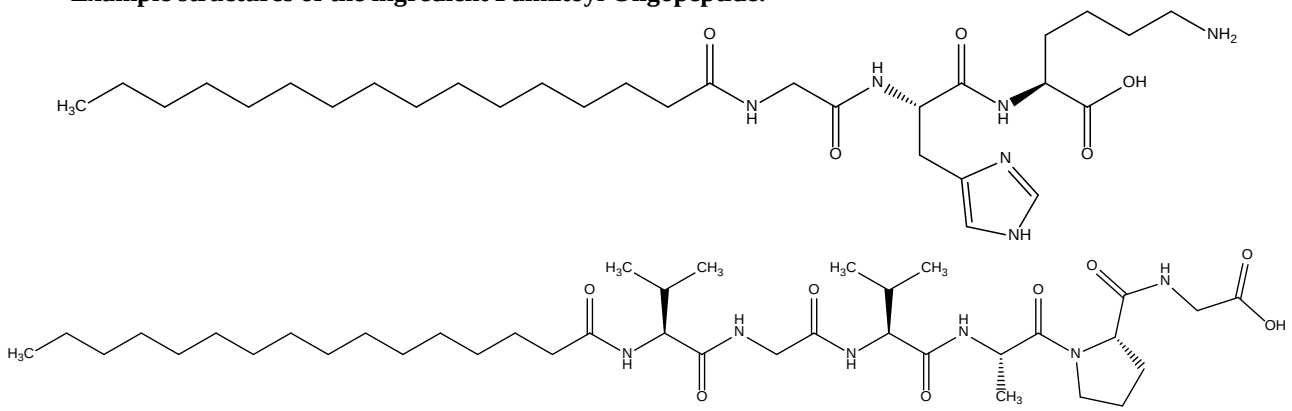
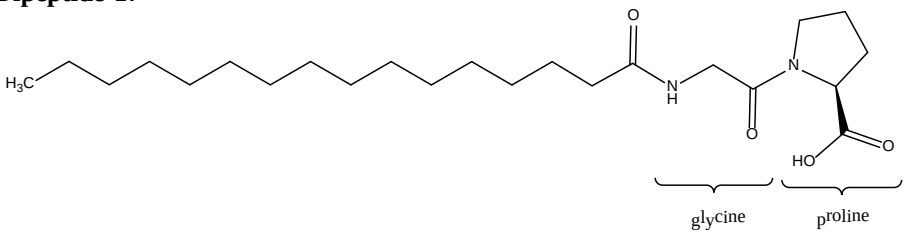
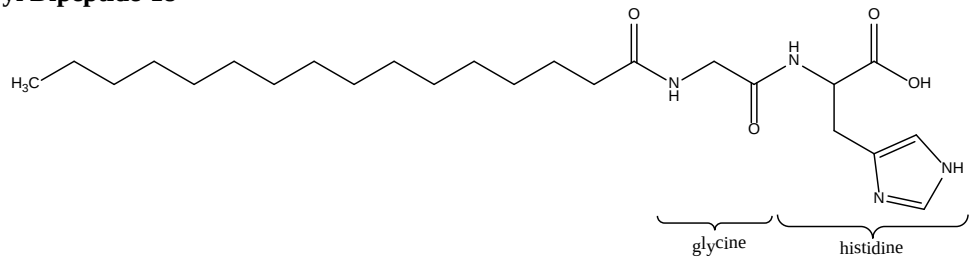


Figure 1. Palmitoyl Oligopeptide

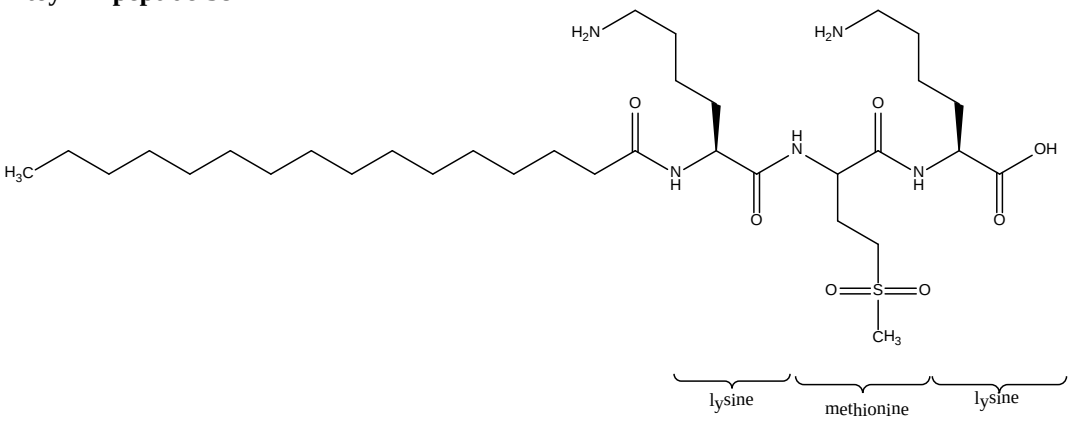
Palmitoyl Dipeptide-17



Palmitoyl Dipeptide-18



Palmitoyl Tripeptide-38



Palmitoyl Pentapeptide-4

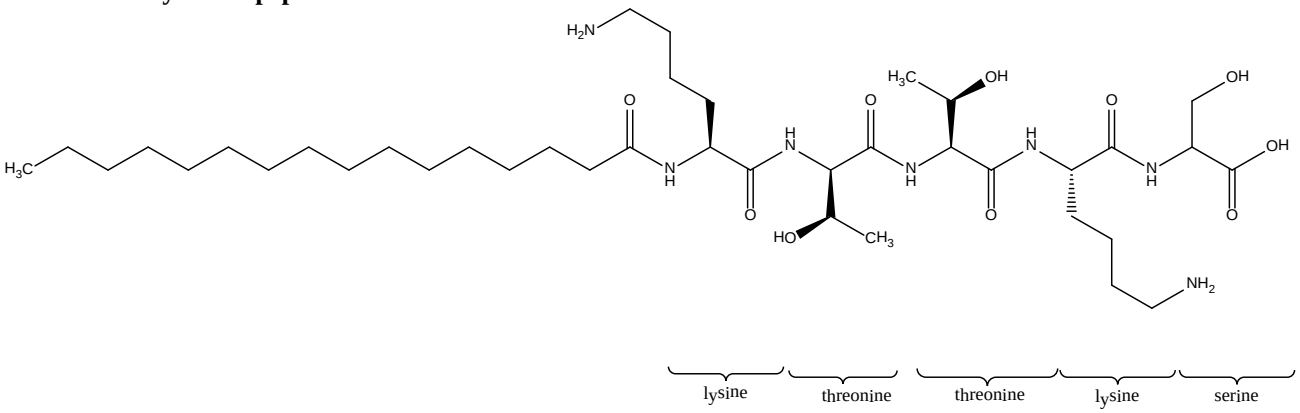


Figure 2. Palmitoyl oligopeptides – examples of specific palmitoyl di-, tri-, and penta-peptides.

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

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

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

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

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

Ingredient CAS No.	Definition	Function
Palmitoyl Decapeptide-21	Palmitoyl Decapeptide-21 is the product obtained by the reaction of palmitic acid and Decapeptide-21, *wherein Decapeptide-21 is the ten-residue synthetic peptide consisting of at least one each of arginine, aspartic acid, glutamine, glycine and proline, in any order.*	Skin-Conditioning Agents - Miscellaneous
Palmitoyl Oligopeptide-70	Palmitoyl Oligopeptide-70 is the product of the reaction of palmitic acid and Oligopeptide-70, *wherein Oligopeptide-70 is the eleven-residue synthetic peptide (undecapeptide) consisting of at least one each of alanine, cysteine, glycine, histidine, lysine, proline and serine, in any order.*	Nail Conditioning Agents; Skin-Conditioning Agents - Emollient; Skin-Conditioning Agents - Miscellaneous
Palmitoyl Hydrolyzed Collagen [68915-45-7]	Palmitoyl Hydrolyzed Collagen is the condensation product of palmitic acid chloride and Hydrolyzed Collagen, *wherein Hydrolyzed Collagen is the partial hydrolysate of animal or fish collagen derived by acid, enzyme or other method of hydrolysis. Hydrolyzed Collagen is characterized by a significant level of hydroxyproline residues. (**This oligopeptide contains an amino acid residue that is not one of the standard α-amino acids**.)*	Hair Conditioning Agents; Skin-Conditioning Agents - Miscellaneous; Surfactants - Cleansing Agents
Palmitoyl Hydrolyzed Milk Protein	Palmitoyl Hydrolyzed Milk Protein is the condensation product of palmitic acid chloride and Hydrolyzed Milk Protein, *wherein Hydrolyzed Milk Protein is the partial hydrolysate of milk protein derived by acid, enzyme or other method of hydrolysis.*	Hair Conditioning Agents; Skin-Conditioning Agents - Miscellaneous; Surfactants - Cleansing Agents
Palmitoyl Hydrolyzed Wheat Protein	Palmitoyl Hydrolyzed Wheat Protein is the condensation product of palmitic acid chloride and Hydrolyzed Wheat Protein, *wherein Hydrolyzed Wheat Protein is the partial hydrolysate of wheat protein derived by acid, enzyme or other method of hydrolysis.*	Hair Conditioning Agents; Skin-Conditioning Agents - Miscellaneous; Surfactants - Cleansing Agents
Potassium Palmitoyl Hydrolyzed Corn Protein	Potassium Palmitoyl Hydrolyzed Corn Protein is the potassium salt of the condensation product of palmitic acid chloride and Hydrolyzed Corn Protein, *wherein Hydrolyzed Corn Protein is the partial hydrolysate of corn protein derived by acid, enzyme or other method of hydrolysis.*	Hair Conditioning Agents; Skin-Conditioning Agents - Miscellaneous; Surfactants - Cleansing Agents
Potassium Palmitoyl Hydrolyzed Oat Protein	Potassium Palmitoyl Hydrolyzed Oat Protein is the potassium salt of the condensation product of palmitic acid chloride and Hydrolyzed Oat Protein, *wherein Hydrolyzed Oat Protein is the partial hydrolysate of oat protein derived by acid, enzyme or other method of hydrolysis.*	Hair Conditioning Agents; Skin-Conditioning Agents - Miscellaneous; Surfactants - Cleansing Agents
Potassium Palmitoyl Hydrolyzed Rice Protein	Potassium Palmitoyl Hydrolyzed Rice Protein is the potassium salt of the condensation product of palmitic acid chloride and Hydrolyzed Rice Protein, *wherein Hydrolyzed Rice Protein is the partial hydrolysate of rice protein derived by acid, enzyme or other method of hydrolysis.*	Emulsion Stabilizers; Hair Conditioning Agents; Skin-Conditioning Agents - Miscellaneous; Surfactants - Cleansing Agents

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

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

Table 2. Current Frequency and Concentration of Use According to Duration and Type of Exposure[16,17,18]

	Palmitoyl Oligopeptide		**Palmitoyl Dipeptide-7**		**Palmitoyl Tripeptide-3**	
	# of Uses	Conc. (%)	# of Uses	Conc. (%)	# of Uses	Conc. (%)
Exposure Type						
Eye Area	102	0.00001 to 0.02	1	0.002 to 0.5	4	NR
Incidental Ingestion	100	0.00001 to 0.003	NR	NR	NR	NR
Incidental Inhalation- Sprays	1	0.001	NR	NR	NR	NR
Incidental Inhalation- Powders	1	NR	NR	NR	NR	NR
Dermal Contact	366	0.0000001 to 0.2	8	0.002 to 0.5	14	NR
Deodorant (underarm)	NR	NR	NR	NR	NR	NR
Hair - Non-Coloring	2	NR	NR	NR	NR	NR
Hair-Coloring	NR	NR	NR	NR	NR	NR
Nail	3	NR	NR	NR	NR	NR
Mucous Membrane	100	0.00001 to 0.003	NR	NR	NR	NR
Baby Products	NR	NR	NR	NR	NR	NR
Duration of Use						
Leave-On	467	0.0000001 to 0.2	8	0.002 to 0.5	14	NR
Rinse off	4	0.00001 to 0.0008	NR	NR	NR	NR
Diluted for (bath) Use	NR	NR	NR	NR	NR	NR
Totals/Conc. Range	471	0.0000001 to 0.2	8	0.002 to 0.5	14	NR
	Palmitoyl Tripeptide-5		**Palmitoyl Tripeptide-8**		**Palmitoyl Tripeptide-28**	
	# of Uses	Conc. (%)	# of Uses	Conc. (%)	# of Uses	Conc. (%)
Exposure Type						
Eye Area	8	0.001 to 0.013	NR	NR	NR	NR
Incidental Ingestion	NR	NR	NR	NR	NR	NR
Incidental Inhalation- Sprays	NR	NR	NR	NR	NR	NR
Incidental Inhalation- Powders	NR	NR	NR	NR	NR	NR
Dermal Contact	39	0.001 to 0.013	4	0.001 to 0.05	1	0.0015
Deodorant (underarm)	NR	NR	NR	NR	NR	NR
Hair - Non-Coloring	NR	NR	NR	NR	NR	NR
Hair-Coloring	NR	NR	NR	NR	NR	NR
Nail	NR	NR	NR	NR	NR	NR
Mucous Membrane	NR	NR	NR	NR	NR	NR
Baby Products	NR	NR	NR	NR	NR	NR
Duration of Use						
Leave-On	39	0.001 to 0.013	4	0.001 to 0.05	1	0.0015
Rinse off	NR	NR	NR	NR	NR	NR
Diluted for (bath) Use	NR	NR	NR	NR	NR	NR
Totals/Conc. Range	39	0.001 to 0.013	4	0.001 to 0.05	1	0.0015
	Palmitoyl Tripeptide-38		**Palmitoyl Tetrapeptide-3**		**Palmitoyl Tetrapeptide-7**	
	# of Uses	Conc. (%)	# of Uses	Conc. (%)	# of Uses	Conc. (%)
Exposure Type						
Eye Area	NR	NR	14	NR	79	0.00005 to 0.02
Incidental Ingestion	1	0.00001 to 0.001	NR	NR	1	NR
Incidental Inhalation- Sprays	NR	NR	1	NR	3	0.001
Incidental Inhalation- Powders	NR	NR	NR	NR	NR	NR
Dermal Contact	1	0.0005	39	NR	193	0.000005 to 0.2
Deodorant (underarm)	NR	NR	NR	NR	NR	NR
Hair - Non-Coloring	NR	NR	NR	NR	NR	NR
Hair-Coloring	NR	NR	NR	NR	NR	NR
Nail	NR	NR	NR	NR	NR	NR
Mucous Membrane	1	0.00001 to 0.001	NR	NR	1	NR
Baby Products	NR	NR	NR	NR	NR	NR
Duration of Use						
Leave-On	2	0.00001 to 0.001	39	NR	191	0.000025 to 0.2
Rinse off	NR	NR	NR	NR	3	0.000005 to 0.0009
Diluted for (bath) Use	NR	NR	NR	NR	NR	NR
Totals/Conc. Range	2	0.00001 to 0.001	39	NR	194	0.000005 to 0.2

Table 2. Current Frequency and Concentration of Use According to Duration and Type of Exposure[16,17,18]

	Palmitoyl Tetrapeptide-10		**Palmitoyl Pentapeptide-3**		**Palmitoyl Pentapeptide-4**	
	# of Uses	Conc. (%)	# of Uses	Conc. (%)	# of Uses	Conc. (%)
Exposure Type						
Eye Area	2	NR	8	NR	11	0.00001 to 0.00061
Incidental Ingestion	NR	NR	NR	NR	NR	NR
Incidental Inhalation- Sprays	NR	NR	NR	NR	NR	NR
Incidental Inhalation- Powders	NR	NR	2	NR	NR	NR
Dermal Contact	11	NR	44	NR	51	0.00001 to 0.00061
Deodorant (underarm)	NR	NR	NR	NR	NR	NR
Hair - Non-Coloring	NR	NR	NR	NR	NR	NR
Hair-Coloring	NR	NR	NR	NR	NR	NR
Nail	NR	NR	NR	NR	NR	NR
Mucous Membrane	NR	NR	NR	NR	NR	NR
Baby Products	NR	NR	NR	NR	NR	NR
Duration of Use						
Leave-On	10	NR	42	NR	50	0.00001 to 0.00061
Rinse off	1	NR	2	NR	1	0.000085
Diluted for (bath) Use	NR	NR	NR	NR	NR	NR
Totals/Conc. Range	11	NR	44	NR	51	0.00001 to 0.00061

	Palmitoyl Hexapeptide-12		**Palmitoyl Hexapeptide-14**		**Palmitoyl Hexapeptide-19**	
	# of Uses	Conc. (%)	# of Uses	Conc. (%)	# of Uses	Conc. (%)
Exposure Type						
Eye Area	NR	NR	2	NR	NR	NR
Incidental Ingestion	NR	NR	NR	NR	NR	NR
Incidental Inhalation- Sprays	NR	NR	NR	NR	NR	NR
Incidental Inhalation- Powders	NR	NR	NR	0.06	NR	NR
Dermal Contact	NR	0.002	3	0.0018 to 0.06	NR	0.00025
Deodorant (underarm)	NR	NR	NR	NR	NR	NR
Hair - Non-Coloring	NR	NR	NR	NR	NR	NR
Hair-Coloring	NR	NR	NR	NR	NR	NR
Nail	NR	NR	NR	NR	NR	NR
Mucous Membrane	NR	NR	NR	NR	NR	NR
Baby Products	NR	NR	NR	NR	NR	NR
Duration of Use						
Leave-On	NR	0.002	3	0.0018 to 0.06	NR	0.00025
Rinse off	NR	NR	NR	NR	NR	NR
Diluted for (bath) Use	NR	NR	NR	NR	NR	NR
Totals/Conc. Range	NR	0.002	3	0.0018 to 0.06	NR	0.00025

	Palmitoyl Heptapeptide-5		**Palmitoyl Hydrolyzed Wheat Protein**		**Potassium Palmitoyl Hydrolyzed Oat Protein**	
	# of Uses	Conc. (%)	# of Uses	Conc. (%)	# of Uses	Conc. (%)
Exposure Type						
Eye Area	NR	NR	NR	NR	NR	NR
Incidental Ingestion	NR	NR	NR	NR	NR	NR
Incidental Inhalation- Sprays	NR	NR	NR	NR	NR	NR
Incidental Inhalation- Powders	NR	NR	NR	NR	NR	NR
Dermal Contact	2	NR	NR	0.37 to 0.42	NR	0.06
Deodorant (underarm)	NR	NR	NR	NR	NR	NR
Hair - Non-Coloring	NR	NR	NR	NR	NR	NR
Hair-Coloring	NR	NR	NR	NR	NR	NR
Nail	NR	NR	NR	NR	NR	NR
Mucous Membrane	NR	NR	NR	NR	NR	NR
Baby Products	NR	NR	NR	NR	NR	NR
Duration of Use						
Leave-On	2	NR	NR	0.37 to 0.42	NR	NR
Rinse off	NR	NR	NR	NR	NR	0.06

Table 2. Current Frequency and Concentration of Use According to Duration and Type of Exposure[16,17,18]

	Palmitoyl Heptapeptide-5		Palmitoyl Hydrolyzed Wheat Protein		Potassium Palmitoyl Hydrolyzed Oat Protein	
	# of Uses	Conc. (%)	# of Uses	Conc. (%)	# of Uses	Conc. (%)
Exposure Type						
Diluted for (bath) Use	NR	NR	NR	NR	NR	NR
Totals/Conc. Range	2	NR	NR	0.37 to 0.42	NR	0.06
	Potassium Palmitoyl Hydrolyzed Wheat Protein					
	# of Uses	Conc. (%)				
Exposure Type						
Eye Area	NR	NR				
Incidental Ingestion	NR	NR				
Incidental Inhalation- Sprays	NR	NR				
Incidental Inhalation- Powders	NR	0.05 to 0.9				
Dermal Contact	NR	NR				
Deodorant (underarm)	NR	NR				
Hair - Non-Coloring	NR	NR				
Hair-Coloring	NR	NR				
Nail	NR	NR				
Mucous Membrane	NR	NR				
Baby Products	NR	NR				
Duration of Use						
Leave-On	NR	0.05 to 0.9				
Rinse off	NR	NR				
Diluted for (bath) Use	NR	NR				
Totals/Conc. Range	NR	0.05 to 0.9				

MG = Methyl Glucose; NR = Not Reported; NS = Not Surveyed; Totals = Rinse-off + Leave-on Product Uses.
Note: Because each ingredient may be used in cosmetics with multiple exposure types, the sum of all exposure type uses may not equal the sum total uses.

Table 3. Skin Irritation and Sensitization Studies

Test Substance	Animals/Subjects	Doses/Concentrations Tested	Procedure	Results
BIOPEPTDE CL (contains 100 ppm palmitoyl oligo-peptide, as Pal-Gly-His-Lys-OH)	3 male New Zealand White rabbits (ages not stated)	0.5 ml on 6 cm^2 area of flank	Applied for 24 h under occlusive hypoallergenic dressing	Slight erythema in 2 rabbits (both flanks). Calssified as non-irritant (primary irri-tation index [PII] = 0.3)[38]
BIOPEPTDE CL	10 male and female guinea pigs (strain not stated)	0.05 ml on 4 cm^2 area on left flank	Applied (uncovered) once daily for 14 consecutive days	Non-irritant (maximum weekly mean irritation index = 0)[26]
BIOPEPTDE CL	20 male and female guinea pigs (strain and ages not stated)	Intradermal injection with 1% (0.1 ml) and cutaneous application of undiluted ingredient during induction. 24-h challenge with 75% [maximal non-irritant concentration] under occlusive dressing	Maximization test	Non-sensitizer[27]
BIOPEPTIDE EL (contains 100 ppm palmitoyl oligopeptide, as Pal-Val-glu-Val-Ala-Pro-Gly-OH)	3 male New Zealand White rabbits (ages not stated)	0.5 ml on 6 cm^2 area of flank	Applied for 4 h under semi-occlusive dressing	Moderate erythema, reversible within 24 h or 48 h. Classified as non-irritant (mean erythema score of < 1)[39]
NANOFIBERGEL-CS (palmitoyl dipeptide-18)	12 female Japanese White rabbits (Jla:JW strain; 18 weeks old)	Concentrations up to 5% (0.5 ml) on abraded or intact skin	Applied for 24 h under lint patch	Non-irritant, abraded and intact skin (PII = 0)[4]
NANOFIBERGEL-CS	12 female Japanese White rabbits (Jla:JW strain; 18 weeks old)	Concentrations up to 5% (0.5 ml) on abraded or intact skin	Applied for 24 h under lint patch for 14 consecutive days	Non-irritant, abraded and intact skin (PII = 0)[4]
NANOFIBERGEL-CS	30 female Hartley White guinea pigs	Intradermal injection with up to 2% and cutaneous application of up to 5% during induction. 24-h challenge with up to 5%	Maximization test	Non-sensitizer[4]
Palmitoyl Pentapeptide-4 (0.01% in formulation)	3 male New Zeraland White rabbits (ages not stated)	0.01% (0.5 ml) on left flank	Applied for 4 h under semi-occlusive dressing	Very slight erythema in 1 rabbit. Classified as non-irritant[32]
Palmitoyl Pentapeptide-4 (0.01% in formulation)	10 guinea pigs (strain and ages not stated	0.01% on 4 cm^2 area of left flank	Applied (uncovered) once daily for 14 consecutive days	Very slight erythema in 1 animal. Classified as non-irritant (PII = 0)[40]
Palmitoyl Pentapeptide-4 (0.01% in formulation)	20 guinea pigs (ages and strain not stated)	Intradermal injection with 0.0075% and cutaenous application of 0.01% during induction. Challenge with 0.0025% under occlusive dressing	Maximization test	Non-sensitizer[29]
MAXI-LIP (contains 1,000 ppm palmitoyl oligopeptide, as Pal-Gly-His-Lys-OH)	10 adults (ages not stated)	~ 0.02 ml on 50 mm^2 area of dorsal skin	Applied for 48 h under occlusive patch (Finn chamber)	Non-irritant (PII = 0)[33]

Table 3. Skin Irritation and Sensitization Studies

Test Substance	Animals/Subjects	Doses/Concentrations Tested	Procedure	Results
MAXI-LIP	52 subjects (16 to 79 years old)	Undiluted ingredient applied during induction and challenge	Human repeated insult patch test (HRIPT). 24-h induction applications. 24-h challenge.	Barely perceptible (+ reaction) to moderate (2 reaction) during induction and/or challenge phases. No clinically significant potential for skin irritation or sensitization[41]
DERMAXYL (contains 200 ppm palmitoyl oligopeotide, as Pal-Val-Gly-Val-ala-Pro-Gly-OH)	10 adults (ages not stated)	Test concentration of 50% on dorsal skin	Applied for 48 h under occlusive patch	Non-irritant when diluted to 50%[34]
DERMAXYL	53 adults (ages not stated)	Test concentration of 50% applied during induction and challenge	HRIPT. Eight 48-h induction applications, followed by challenge	Non-irritant (mean irritation index = 0.04) and non-sensitizer[42]
NANOFIBERGEL-CS (palmitoyl dipeptide-18)	40 male and female subjects (24 to 60 years old)	Undiluted ingredient applied to dorsal skin	Applied for 24 h under closed dressing	Non-irritant (PII = 0)[4]
VOLULIP™ (contains 500 ppm palmitoyl tripeptide-38)	11 adult female subjects (phototypes I to IV; ages not stated)	Diluted ingredient (10% in cetearyl ethyl-hexanoate) applied to skin	Applied for 48 h under occlusive patch	Non-irritant[43]
VOLULIP™	103 male and female subjects (17 to 70 years old)	Diluted ingredient (10% in cetearyl ethyl-hexanoate) applied to skin	HRIPT involving occlusive patches (protocol not stated)	Non-irritant and non-sensitizer[44]
MATRIXYL (contains 100 ppm palmitoyl pentapeptide-4)	10 adult subjects (ages not stated)	0.02 ml on 50 m^2 area on dorsal skin	Applied for 48 h under occlusive patch (Finn chamber)	Very slight erythema in 1 subject. Classified as non-irritant (PII = 0.10)[37]
MATRIXYL	51 male and female subjects (19 to 78 years old)	Undiluted ingredient applied during induction and challenge	HRIPT (protocol not stated)	Non-irritant and non-sensitizer[45]

Table 4. Skin Studies

Test Substance	Subjects	Test Concentration	Procedure	Results
Palmitoyl Tripeptide-1 (palmitoyl-gly-his-lys)	15 female subjects (44 to 59 years old)	3 ppm in a cream	Applied around eye zones twice daily for 4 weeks. Skin replicas from the face obtained on days 0 and 28 and analyzed using an image analysis system	Decreases in wrinkle length and depth, and skin roughness. Placebo cream had no effect[46]
Palmitoyl Oligopeptide + Palmitoyl Pentapeptide-7	24	3% in a cream formulation	Applied to the face in morning and at night for 2 months. Skin rejuvenation assessed usijng profilometry, and image analysis, photography, and cutometry.	Statistically significant decrease ($p < 0.01$) in deep wrinkles and skin roughness after 56 days, compared to results at day 0. Statistically significant increase ($p < 0.01$) in skin elasticity and tone[2]
Palmitoyl Pentapeptide-4	25	3% in cream formulation	Same procedure	Statistically significant decrease ($p < 0.05$) in deep wrinkles and skin roughness after 56 days, compared to results at day 0. Statistically significant increase ($p < 0.05$) in skin elasticity and tone[2]
Palmitoyl Tetrapeptide-7	17	15 ppm in cream	Applied to face and neck for 1 month	Significant increase in firmness (face and neck). Increase in elasticity, and decrease in deepest wrinkles and skin roughness[2]
Palmitoyl Pentapeptide-3	Number not stated	50 ppm	Applied to eye area. Study details not included	Significant benefit to lines and wrinkles around the eye when compared to vehicle control[48]
Palmitoyl Pentapeptide-4 (palmitoyl-lysine-threonine-threonine-lysine-serine)	93 female subjects (35 to 55 yeards old)	3 ppm in moisturizer	Applied (~0.4 g) to the face twice daily for 12 weeks. Quantitative technical and expert grader image analysis used	Significant improvement in terms of wrinkles/fine lines reduction (at weeks 8 and 12) when compared to moisturizer control product. No skin irritation. Results of self assessments yielded significant reductions in age spots and dark circles and increased skin firmness at week 12[49]

Table 5. Biological Activity

Test Substance	Test Concentration(s)	Procedure	Results
Palmitoyl Tripeptide-1 (palmitoyl-gly-L-his-L-lys)	0.5 µM/liter	Collagen synthesis monitored by incorporation of tritiated proline into human fibroblasts *in vitro*	Strong signal of collagen synthesis observed at 0.5 µM/liter[14]
Palmitoyl Tripeptide-1 (palmitoyl-gly-L-his-L-lys)	5 ppm	Human skin samples (abdominal tissue) from biopsy irradiated with daily doses of UVA for 1 week. Irradiation followed by treatment with oligopeptide	Irradiation caused strong collagen degradation. Treatment with 5 ppm resulted in almost total preservation and/or renewal (high density of collagen) of tissue collagen. Same results for 500 ppm retinoic acid[14]
Palmitoyl Oligopeptide, Palmitoyl Tetrapeptide-7, Palmitoyl Oligopeptide + Palmitoyl Tetrapeptide-7, and Palmitoyl Pentapeptide-4	Palmitoyl Oligopeptide (up to 7.5 ppm), Palmitoyl Tetrapeptide-7 (up to 3.5 ppm), Palmitoyl Oligopeptide + Palmitoyl Tetrapeptide-7 (up to 11 ppm), and Palmitoyl Pentapeptide-4 (up to 8 ppm)	Human fibroblasts incubated with each of the oligopeptides in the presence of vitamin C. Matrix proteins (collagen 1 and fibronectin) assayed using ELISA method. Hyaluronic acid assayed using a colorimetric method	Except for palmitoyl oligopeptide, a dose response for collagen 1, fibronectin, and hyaluronic acid synthesis was associated with each oligopeptide[2]
Palmitoyl Hexapeptide-14	Not stated	Not stated	Stimulated cell migration, collagen synthesis, and fibroblast proliferation and scaffolding[47]
Palmitoyl Tetrapeptide-7	Not stated	Assay to evaluate ability of oligopeptide to down-regulate IL-6 in resting and inflammed cells *in vitro.*	Results for palmitoyl oligopeptide and DHEA were comparable in terms of the ability of each to down-regulate IL-6 in resting and inflammed cells[47]
Palmitoyl Tetrapeptide-7	Not stated	Keratinocytes and fibroblasts exposed to oligopeptide in the presence and absence of UVB irradiation	Palmitoyl tetrapeptide-7 caused decrease in IL-6 secretion in the presence and absence of UVB[2]
Palmitoyl Oligopeptide	50 ng in 20 µl phosphate buffered saline (PBS)	*In vivo* angiogenesis assay using chick chorioallantoic membrane. On day 6, angiogenic areas delimited with a silicon ring and PBS or palmitoyl oligopeptide (50 ng) in a final volume of 20 µl placed inside the rings. Treated areas photographed daily on days 6 to 10 of development	Palmitoyl oligopeptide enhanced angiogenesis by promoting endothelial cell migration and tubulogenesis through upregulation of MT1-MMP[50]

Table 5. Biological Activity

Test Substance	Test Concentration(s)	Procedure	Results
Palmitoyl Tetrapeptide (N-palmitoyl-(S)-seryl-(S)-seryl-(S)-asparaginyl-(S)-alanine)	<1 to 137 µg/ml	Induction of DNA synthesis measured by incorporation of ^{3}H-thymidine and ^{3}H-uridine in mouse splenocytes from following mouse strains: C3H/HeJ, C3H/He/Bom/nunu, and Balb/c	In all strains, palmitoyl tetrapeptide had stimulatory effect on B-lymphocytes. Increase in ^{3}H-thymidine incorporation optimal in 20 to 30 µg/ml range. Marked increase in ^{3}H-uridine incorporation in 2.1 to 137 µg/ml range[15]
Palmitoyl Tetrapeptide (N-palmitoyl-(S)-seryl-(S)-seryl-(S)-asparaginyl-(S)-alanine)	<1 to 137 µg/ml	Hemolytic plaque assay used to assess ability of palmitoyl tetrapeptide to polyclonically stimulate B-lymphocytes into immunoglobulin secretion	The number of plaque-forming cells against densely trinitrophenylated sheep red blood cells increased markedly after stimulation of mouse spleen cells[15]
Palmitoyl Tetrapeptide (N-palmitoyl-(S)-seryl-(S)-seryl-(S)-asparaginyl-(S)-alanine)	<1 to 137 µg/ml	Ability of palmitoyl tetrapeptide to activate the BCL1 lymphoid B-cell line (tumor cell line) evaluted *in vitro*	Marked enhancement of ^{3}H-thymidine incorporation at concentrations > 2 µg/ml. Optimal stimulation at ~ 30 µg/ml[15]
Tripalmitoyl Pentapeptide (S-(2,3-bis-(palmitoyloxy)-(2RS)-propyl)-N-palmitoyl-(R)-cysteinyl-(S)-seryl-(S)-seryl(S)-asparaginyl-(S)-alanine)	0.03 to 33.3 µg/ml	Hemolytic plaque assay	Stimulation of the primary antibody response toward underivatized sheep red blood cells (SRBC) and toward trinitrophenylated (TNP-) SRBC was markedly enhanced in the presence of tripalmitoyl pentapeptide (3.3 to 33.3 µg/ml)[51]
Tripalmitoyl Pentapeptide (S-(2,3-bis-(palmitoyloxy)-(2RS)-propyl)-N-palmitoyl-(R)-cysteinyl-(S)-seryl-(S)-seryl(S)-asparaginyl-(S)-alanine)	0.03 to 33.3 µg/ml	Enzyme-linked immunosorbent assay (ELISA)	Antigen-specific IgM response increased by ~ 7-fold and IgG response augmented by ~ 10-fold in presence of tripalmitoyl pentapeptide. Application of tripalmitoyl pentapeptide and antigen had to occur concurrently in order to produce strong adjuvant effect[51]

Table 6. Genotoxicity of Palmitoyl Oligopeptides

Ingredient Name	Chemical Tested	Strain/cell type	Assay	Dose/Concentration	Results
Bacterial Systems					
Palmitoyl Oligopeptide	MAXI-LIP (contains 1,000 ppm palmitoyl oligopeptide, as Pal-Gly-His-Lys-OH)	*Salmonella typhimurium* strains TA98, TA100, TA1535, and TA1538	Ames test, with and without metabolic activation	0.1 ml in ethanol solution	Non-genotoxic[52]
Plmitoyl Oligopeptide	BIOPTPTIDE-CL (contains 100 ppm palmitoyl oligo-peptide, as Pal-Gly-His-Lys-OH)	*Salmonella typhimurium* strains TA98, TA102, TA1535, and TA1537	Ames test, with and without metabolic activation	Doses up to 5,000 µg/plate	Non-genotoxic[53]
Palmitoyl Dipeptide-18	NANOFIBERGEL-CS	*Salmonella typhimurium* strains TA98, TA100, TA1535, and TA1537 and *Escherichia coli* strain WP2*uvr*A	Ames test, with and without metabolic activation	Doses up to 4820 µg/plate (without activation) and up to 2410 µg/plate (with activation)	Genotoxic with, but not without, activation in strains TA97 and TA100. Positive results thought to be due to free histidine derived from test substance[4]
Palmitoyl Dipeptide-18	Palmitoyl-glycine (Pal-G, palmitoyl dipeptide-18 impurity; produced by eliminating histidine from palmitoyl dipep-tide-18)	*Salmonella typhimurium* strains TA98, TA100, TA1535, and TA1537 and *Escherichia coli* strain WP2*uvr*A	Ames test, with and without metabolic activation	Doses up to 5,000 µg/plate	Non-genotoxic. Because assay results for Pal-G (absence of free histidine) negative, it was assumed that palmitoyl dipeptide-18 is not genotoxic[4]
Palmitoyl Dipeptide-18	NANOFIBERGEL-CS	*Salmonella typhimurium* strains TA98, TA100, TA1535, and TA1537 and *Escherichia coli* strain WP2*uvr*A	*umu* test (using umu-test Umlac AT mutagenicity test kit), with and without metabolic activation	Doses up to 0.0754 mg/well	No DNA-damaging activity[4]
Palmitoyl Tripeptide-38	VOLULIP™ (contains 500 ppm palmitoyl tripeptide-38)	*Salmonella typhimurium* strains TA98, TA100, TA1535, and TA1537 and *Escherichia coli* strain WP2 strain (pKM 101)	Ames test, with and without metabolic activation	Doses up to 0.06 µl/plate	No evidence of cytotoxicity. Non-mutagenic and non-promutagenic[54]
Palmitoyl Pentapeptide-4	Palmitoyl Pentapeptide-4	*Salmonella typhimurium* strains TA98, TA100, TA1535, and TA1537 and *Escherichia coli* strain WP2*uvr*A	Ames test, with and without metabolic activation	Doses up to 5,000 µg/plate	No evidence of cytotoxicity. Non-genotoxic[55]

Table 6. Genotoxicity of Palmitoyl Oligopeptides

Ingredient Name	Chemical Tested	Strain/cell type	Assay	Dose/Concentration	Results
Mammalian System					
Palmitoyl Dipeptide-18	NANOFIBERGEL-CS	Cultured human lymphocytes	Chromosome aberrations test, with and without metabolic activation	Concentrations up to 61.4 µg/ml (without activation) and up to 96 µg/ml (with activation)	Non-genotoxic[4]

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2012 FDA VCRP Data

Palmitoyl Oligopeptide

03B - Eyeliner	1
03C - Eye Shadow	3
03D - Eye Lotion	62
03G - Other Eye Makeup Preparations	36
05G - Tonics, Dressings, and Other Hair Grooming Aids	2
07B - Face Powders	1
07C - Foundations	14
07E - Lipstick	100
07F - Makeup Bases	3
07H - Makeup Fixatives	1
07I - Other Makeup Preparations	25
08B - Cuticle Softeners	1
08C - Nail Creams and Lotions	1
08G - Other Manicuring Preparations	1
11A - Aftershave Lotion	2
12A - Cleansing	4
12C - Face and Neck (exc shave)	65
12D - Body and Hand (exc shave)	12
12F - Moisturizing	78
12G - Night	25
12I - Skin Fresheners	3
12J - Other Skin Care Preps	30
13C - Other Suntan Preparations	1
Total	**471**

Palmitoyl Dipeptide-7

03G - Other Eye Makeup Preparations	1
12C - Face and Neck (exc shave)	4
12F - Moisturizing	3
Total	**8**

Palmitoyl Tripeptide-3

03D - Eye Lotion	4
12C - Face and Neck (exc shave)	6
12F - Moisturizing	3
12G - Night	1
Total	**14**

Palmitoyl Tripeptide-5

03D - Eye Lotion	3
03G - Other Eye Makeup Preparations	5
07C - Foundations	2
07I - Other Makeup Preparations	2
12C - Face and Neck (exc shave)	12
12D - Body and Hand (exc shave)	2

12F - Moisturizing	8
12G - Night	5
Total	**39**

Palmitoyl Tripeptide-8

12C - Face and Neck (exc shave)	1
12F - Moisturizing	1
12G - Night	1
12J - Other Skin Care Preps	1
Total	**4**

Palmitoyl Tripeptide-28

12I - Skin Fresheners	1
Total	**1**

Palmitoyl Tripeptide-38

07E - Lipstick	1
Total	**1**

2012 FDA VCRP Data

Palmitoyl Tetrapeptide-3

03D - Eye Lotion	12
03G - Other Eye Makeup Preparations	2
07I - Other Makeup Preparations	1
12C - Face and Neck (exc shave)	5
12D - Body and Hand (exc shave)	1
12F - Moisturizing	8
12G - Night	5
12J - Other Skin Care Preps	4
13A - Suntan Gels, Creams, and Liquids	1
Total	**39**

Palmitoyl Tetrapeptide-7

03B - Eyeliner	1
03D - Eye Lotion	46
03G - Other Eye Makeup Preparations	32
07C - Foundations	10
07E - Lipstick	1
07F - Makeup Bases	2
07G - Rouges	1
07I - Other Makeup Preparations	2
12A - Cleansing	2
12C - Face and Neck (exc shave)	34
12D - Body and Hand (exc shave)	7
12F - Moisturizing	28
12G - Night	14
12H - Paste Masks (mud packs)	1
12I - Skin Fresheners	1
12J - Other Skin Care Preps	9
13B - Indoor Tanning Preparations	3
Total	**194**

Palmitoyl Tetrapeptide-10

03D - Eye Lotion	2
12A - Cleansing	1
12C - Face and Neck (exc shave)	5
12G - Night	3
Total	**11**

Palmitoyl Pentapeptide-3

03D - Eye Lotion	2
03G - Other Eye Makeup Preparations	6
07B - Face Powders	2
11G - Other Shaving Preparation Products	1
12A - Cleansing	1
12C - Face and Neck (exc shave)	17

12D - Body and Hand (exc shave)	2
12F - Moisturizing	5
12G - Night	4
12J - Other Skin Care Preps	4
Total	**44**

Palmitoyl Pentapeptide-4

03D - Eye Lotion	6
03G - Other Eye Makeup Preparations	5
07F - Makeup Bases	1
12A - Cleansing	1
12C - Face and Neck (exc shave)	19
12F - Moisturizing	12
12G - Night	4
12J - Other Skin Care Preps	3
Total	**51**

Palmitoyl Hexapeptide-14

03C - Eye Shadow	1
03D - Eye Lotion	1
12C - Face and Neck (exc shave)	1
Total	**3**

Palmitoyl Heptapeptide-5

12C - Face and Neck (exc shave)	2
Total	**2**

Personal Care Products Council
Committed to Safety, Quality & Innovation

Memorandum

TO: F. Alan Andersen, Ph.D.
Director - COSMETIC INGREDIENT REVIEW (CIR)

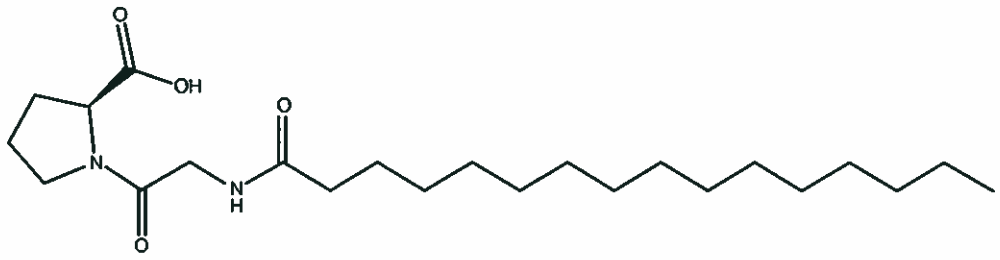
FROM: Halyna Breslawec, Ph.D.
Industry Liaison to the CIR Expert Panel

DATE: September 17, 2012

SUBJECT: Information on a Palmitoyl Dipeptide-17

A supplier has provided the following information on Palmitoyl Dipeptide-17

Chemical Formula: $C_{23}H_{42}N_2O_4$
Molecular Weight: 410.59



Palmitoyl-Gly-Pro

Method of Manufacture
Palmitoyl Dipeptide-17 is made using the Solid Phase Peptide Synthesis (SPPS) method.

The ingredient is at least 97% pure with the total amount of any individual impurity ≤2%.

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Memorandum

TO: F. Alan Andersen, Ph.D.
Director - COSMETIC INGREDIENT REVIEW (CIR)

FROM: Halyna Breslawec, Ph.D.
Industry Liaison to the CIR Expert Panel

DATE: September 24, 2012

SUBJECT: Concentration of Use by FDA Product Category: Palmitoyl Oligopeptide and Palmitoyl Oligopeptide-70

1101 17th Street, N.W., Suite 300 | Washington, D.C. 20036-4702 | 202.331.1770 | 202.331.1969 (fax) | www.personalcarecouncil.org

Concentration of Use by FDA Product Category*

Palmitoyl Oligopeptide
Palmitoyl Oligopeptide-70

Ingredient	Product Category	FDA Code†	Maximum Concentration of Use
Palmitoyl Oligopeptide	Eye liner	03B	0.005-0.01%
Palmitoyl Oligopeptide	Eye shadow	03C	0.0004-0.01%
Palmitoyl Oligopeptide	Eye lotion	03D	0.0001-0.02%
Palmitoyl Oligopeptide	Mascara	03F	0.00001-0.00002%
Palmitoyl Oligopeptide	Other eye makeup preparations	03G	0.0002%
Palmitoyl Oligopeptide	Foundations	07C	0.0002-0.1%
Palmitoyl Oligopeptide	Lipstick	07E	0.00001-0.003%
Palmitoyl Oligopeptide	Makeup bases	07F	0.001%
Palmitoyl Oligopeptide	Makeup fixatives	07H	0.2%
Palmitoyl Oligopeptide	Other makeup preparations	07I	0.0004-0.01%
Palmitoyl Oligopeptide	Aftershave lotions	11A	0.00002%
Palmitoyl Oligopeptide	Skin cleansing (cold creams, cleansing lotions, liquids and pads)	12A	0.00001-0.0008%
Palmitoyl Oligopeptide	Face and neck creams, lotions and powders not spray	12C	 0.00001-0.02%
Palmitoyl Oligopeptide	Body and hand creams lotions and powders not spray spray	12D	 0.0000001-0.005% 0.001%
Palmitoyl Oligopeptide	Moisturizing creams, lotions and powders not spray	12F	 0.0003%
Palmitoyl Oligopeptide	Night creams, lotions and powders not spray	12G	 0.0001-0.0002%

Palmitoyl Oligopeptide	Paste masks and mud packs	12H	0.0002%
Palmitoyl Oligopeptide	Other skin care preparations	12I	0.0001-0.0005%

*Ingredients included in the title of the table but not found in the table were included in the concentration of use survey, but no uses were reported.
†Product category codes used by FDA

Information collected in 2012
Table prepared September 24, 2012

Personal Care Products Council
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Memorandum

TO: F. Alan Andersen, Ph.D.
Director - COSMETIC INGREDIENT REVIEW (CIR)

FROM: Halyna Breslawec, Ph.D.
Industry Liaison to the CIR Expert Panel

DATE: October 22, 2012

SUBJECT: Information on Palmitoyl Dipeptide-18

Nissan Chemical Industries, Ltd. 2012. Nanofibergel-CS (Palmitoyl Dipeptide-18): General information for Cosmetic Ingredient Review.

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NANOFIBERGEL-CS

General Information for Cosmetic Ingredient Review

October, 22, 2012

Nissan Chemical Industries, Ltd.
7-1,3-chome, Kanda-Nishiki-cho
Chiyoda-ku, Tokyo 101-0054, Japan

Person Reporting the Information

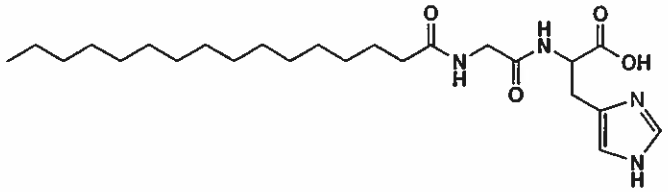
- Representative: Katsuaki Miyaj
- Managerial Position: Board of Director General Manager
- Department: Advanced Materials and Planning depertment
- Nissan Chemical Industries, Ltd.
- 7-1,3-chome, Kanda-Nishiki-cho, Chiyoda-ku, Tokyo 101-0054, Japan
- 81-03-3296-8391

Contents

A. Details of Development

1. NANOFIBERGEL-CS

1) Structural Formula



2) Trade Name: NANOFIBERGEL-CS
3) INCI Name: Palmitoyl Dipeptide18
4) Internal Development Code: PLG

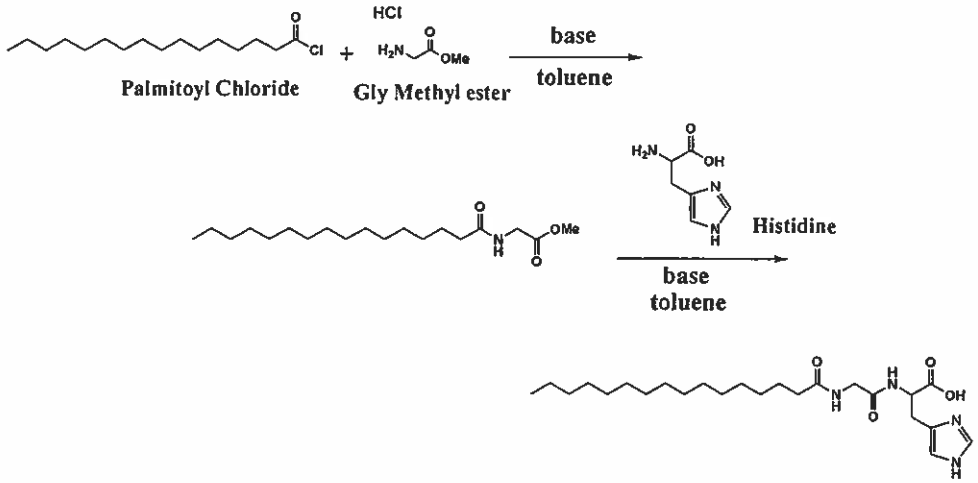
2. Details of Development

NANOFIBERGEL-CS is N-Palmitoyl Glycyl Histidine, has structural character of lipid dipeptide amphiphilic compound, and has function of low molecular gelator. Low-molecular-weight gelators form stringy assembly when they are dissolved in H_2O or polar solvents, and such stringy assembly intertwines and forms network with holding H_2O to gelate. When dissolved in solvent, ordinary amphiphilic compound is assembled spherically and is emulsified to function as surfactant. NANOFIBERGEL-CS, however, is different from ordinary amphiphilic compound since it does not function as surfactant while functions as gelator.

Such low-molecular-weight gelator which gelates H_2O or polar solvents is novel gelator which is not marketed at this moment. And, we found that gels which are obtained using such low molecular gelator have different physical properties from gels which are obtained using inorganic thickener or high molecular thickener. Furthermore, gels which is prepared with NANOFIBERGEL-CS allows free recombination of network structure by stress since its network structure is formed with wormlike micelle, and, therefore, it allows immediate conversion from gel state to liquid state (sol state). It, therefore, is sprayable gel base material and has watery sense of touch when it is liquefied by stress of fingers. And it allows preparation at the low viscosity range at which preparation using high molecular thickener or inorganic thickener is difficult. Our interests in functions and physical properties which are different from existing thickener have led us to develop such low molecular gelator as a product.

Based on above, NANOFIBERGEL-CS will be developed as cosmetic raw material, additive for quasi-drug and pharmaceutical additive.

3. Production



NANOFIBERGEL-CS is made by 2 step production procedure which is consisted of bonding of palmitoyl chloride and glycine methyl ester, and then bonding of obtained palmitoyl glycine methyl ester and histidine. Raw materials were confirmed to contain no animal derived components.

B. Information of Impurities

1. Composition and Source of Impurities

As a result of 3 serial production trials based on above production method, it is confirmed that NANOFIBERGEL-CS can be manufactured at the purity of 97% or above and 3% or below impurities are produced.

Most of the impurities are analogs of NANOFIBERGEL-CS derived from palmitoyl chloride which is produced from botanical palmitic acid with different carbon number and its content is stably controlled. Profiles of the impurities in the lot which were obtained by 3 serial production trials are summarized as in the following Table 1.

Table 1 Profiles of the impurities

		Lau-GH	Myr-GH	Ste-GH	Pal-G	Pal-GHOMe	Pal-GGH
Production Lot	1st	0.16%	0.82%	0.17%	1.86%	0.46%	0.12%
	2nd	0.16%	0.81%	0.16%	0.81%	0.51%	0.13%
	3rd	0.18%	0.79%	0.38%	0.86%	0.40%	0.14%

Abbreviation: Lau=lauroyl, Myr=myristoyl, Ste=stearoyl, Pal= palmitoyl, G=glycine, H = histidine, OMe=methyl ester

C. Dermal Permeability

No dermal permeability test is conducted. Conducted tests related to the skin effect at this stage are primary skin irritation study, 14-day cumulative skin irritation study and human patch test (See D. Toxicity Data).

D. Toxicity Data

1. Summary

For NANOFIBERGEL-CS, the toxicity studies which are indicated in Table 2 were conducted for the purpose of application of additive for quasi-drug. Based on these results, NANOFIBERGEL-CS was determined to have minimum lethal dose of higher than 2000 mg/kg with single oral dose in rats, to have no genotoxicity, to have no sensitizing potential and irritancy with 5% of physically suspending concentration and to be graded as safe product for human based on closed patch test. In addition, no alerts were shown for any impurities during *in silico* safety evaluation using *Derek for Windows* with 6 impurities of NANOFIBERGEL-CS. These impurities of NANOFIBERGEL-CS, therefore, are determined to have no critical toxicity. Results of each toxicity study are summarized in sections 2 – 7 and result tables are shown in Addenda 1 – 12.

Since indication of "PLG", which is the internal development code of NANOFIBERGEL-CS, was used during these toxicity studies, NANOFIBERGEL-CS is referred as PLG as follows.

2. Acute Toxicity

1) An oral single-dose toxicity study of PLG in rats (GLP study)

(1) Study Number: B-7159

(2) Testing Facility: Bozo Research Center Inc.

(3) Outline of Test

i) Test Substance: PLG (Purity: 89.8%)

ii) Test Animal: Crl:CD(SD) Rat, 6 weeks old, male and female

iii) Test Method: Single dose of 2000 mg/kg was chosen for PLG, and single oral gavage administration was conducted for Sprague-Dawley SPF rats (Crl:CD(SD), 5 males and 5 females for each group and then rats were observed for 14 days. The control group which was administered vehicle only (0.5% MC aqueous solution) was also used.

iv) Results: No death was observed for both the control and 2000 mg/kg groups, and minimum lethal dose was estimated to be higher than 2000 mg/kg for both sexes. No abnormalities due to test substance administration were observed on body weight measurement and necropsy. For clinical signs, each one male and female in the 2000 mg/kg group showed soft feces at 2 hours after treatment, however, there were no abnormalities on the day following administration or thereafter (Addendum 1).

(4) Conclusion: It was estimated that the minimum lethal dose by single oral administration of PLG by gavage to rats was higher than 2000 mg/kg in both sexes. Administration of PLG at 2000 mg/kg was associated with transient soft feces.

Table 2 List of Toxicity Studies of NANOFIBERGEL-CS (PLG)

Study Title	Study Number	Study Schedule			GLP/ Non-GLP	Test Animal	Result
		Study Initiation	Observation Completion	Study Completion			
An oral single-dose toxicity study of PLG in rats	B-7159	2011.09.01	2011.09.29	2012.09.14	GLP	Rat	Minimum lethal dose: Higher than 2000mg/kg
A skin sensitization study of PLG in guinea pigs (Maximization Test Method)	I-3886	2011.08.09	2011.09.17	2012.09.28	GLP	Guinea Pig	Not skin sensitizer
Photosensitization Study	Not Determined						No absorption was observed at 290-400nm by absorbance measurement
Bacterial reverse mutation test of PLG	M-11-027	2011.09.05	2011.11.10	2012.10.31[a)]	GLP	-	Positive due to free histidine derived from PLG
Bacterial reverse mutation test of Pal-G (Impurity of PLG) -Pal-G is produced by eliminating histidine from PLG-	M-11-029	2011.09.05	2011.11.10	2012.10.31[a)]	Non-GLP	-	Negative
Umu Test of PLG	M-11-028	2011.09.28	2011.09.29	2012.10.31[a)]	Non-GLP	-	Negative
Chromosome aberration test of PLG using cultured human lymphocytes	D451 (080-105)	2011.06.17	2011.08.26	2012.09.26	GLP	-	Negative
A primary skin irritation study of PLG in rabbits	I-3883	2011.08.09	2011.08.27	2012.09.28	GLP	Rabbit	5, 2 and 1%: " Non-stimulant"
A 14-day cumulative skin irritation study of PLG in rabbits	I-3884	2011.08.09	2011.09.06	2012.09.28	GLP	Rabbit	5, 2 and 1%: No cumulative skin irritation reaction
Phototoxicity Study	Not Determined						No absorption was observed at 290-400nm by absorbance measurement
A primary eye irritation study of PLG in rabbits	I-3885	2011.08.09	2011.08.29	2012.09.28	GLP	Rabbit	5 and 2%: "Practically not irritant" 1%: "Not irritant" Eye wash is effective
Closed patch test for [PLG (Lot No. TS-2197-104)] and 2 controls (24-Hour Continuous Application)	IWSK_ p-7932	2011.09.12	2011.09.14	2011.09.21	Non-GLP	Human	Skin Irritation Index: 0 "Safe product"

heduled end date

3. Immunogenicity

1) A skin sensitization study of PLG in guinea pigs (Maximization Test Method) (GLP study)

(1) Study Number: I-3886

(2) Testing Facility: Bozo Research Center Inc.

(3) Outline of Test

i) Test Substance: PLG (Purity: 89.8%)

ii) Test Animal: Hartley White Guinea Pigs, 20 animals, 6 weeks old, female

iii) Test Method: Test group was consisted of 3 groups of the test substance group (10 animals), the negative control group (5 animals) and the positive control group (5 animals). PLG concentration for the test substance group was chosen as 2% which was the highest concentration for intradermal induction for sensitization, and as 5% which was physically preparable highest concentration for percutaneous induction for sensitization. Challenge was conducted at the concentrations of 5%, 2% and 1%, and vehicle of 0.5% methylcellulose solution. For the negative control group, 0.5% methylcellulose solution was treated by intradermal and percutaneous induction for sensitization, and then challenge exposure was conducted with similar method as the test substance group. For the positive control group, 0.1% solution of 1-Chloro-2,4-dinitrobenzene (DNCB) (vehicle: olive oil) was treated by intradermal and percutaneous induction for sensitization, and then challenge exposure was conducted with 0.1% solution and vehicle (acetone). 24 and 48 hours after removal of dressing, dermal reaction was observed and dermal sensitization was evaluated.

iv) Result: For both the test substance group and negative control group, no dermal reactions were observed on challenge exposure site with 5, 2 and 1% test substance solution and 0.5% methylcellulose solution, and positive rate of all treatment sites for each group was 0%. On challenge site with 0.1% DNCB solution of the positive control group, erythema and edema of grade 3 was observed in all animals (5/5) and eschar formation as other change was observed in 3 animals (3/5) on observations at 24 and 48 hours after removal of dressing. These changes were obvious sensitizing reaction. For the positive control group, no dermal reaction was observed on challenge site with vehicle of acetone. On both clinical sign and body weight, no abnormalities were observed in any animals of all test groups (Addendum 2).

(4) Conclusion: It was concluded that PLG did not show dermal sensitization potential under the conditions of this study.

2) Photosensitization

On absorbance measurement (Study Number: 12-PLGARD-013, non-GLP study), no absorption was observed at the range of UV-VIS spectrum (290 – 450 nm) (Addendum 3). According to the result, it was considered that PLG has no photosensitivity, and, therefore, no photosensitization study was conducted.

4. Genotoxicity

1) Bacterial Reverse Mutation Test

(1) Bacterial reverse mutation test of PLG (GLP study)

i) Study Number: M-11-027

ii) Testing Facility: Hatano Research Institute, Food and Drug Safety Center

iii) Outline of Test

a) Test Substance: PLG (Purity: 89.8%)

b) Test Method: Test was conducted using tester strains of *Salmonella typhimurium* TA100, TA1535, TA98, TA1537 and *Escherichia coli* WP2 *uvrA* with and without S9 mix by pre-incubation method. Based on dose finding study, the following doses were selected and 2 main studies were conducted.

Without S9mix

- TA100 and TA1535: 18.8, 37.7, 75.4, 150, 302, 1210 and 4820 μg/plate
- WP2 uvrA and TA1537: 302, 602, 1210, 2410 and 4820 μg/plate
- TA98: 75.4, 150, 302, 602, 1210, 2410 and 4820 μg/plate

With S9mix

- TA100 and TA98: 37.7, 75.4, 150, 302, 602, 1210 and 2410 μg/plate
- TA1535 and WP2 uvrA: 150, 302, 602, 1210 and 2410 μg/plate
- TA1537: 18.8, 37.7, 75.4, 150, 302, 602 and 2410 μg/plate

c) Result: In the main studies, no growth inhibition was observed for all tester strains used. Before incubation, precipitate derived from the test substance on plate agar was observed at the dose levels of 1210 μg/plate and above without S9 mix and at the dose levels of 150 μg/plate and above with S9 mix. After incubation, such precipitate was observed at the dose levels of 302 μg/plate and above without S9 mix and at the dose levels of 150 μg/plate and above with S9 mix. In the main studies, increase of revertant colonies 2 times or higher than negative control value was observed for TA100 and TA98 with S9 mix, and such increase showed dose dependency and repeatability. In the main studies, increase of revertant colonies 2 times or higher than negative control value was not observed for TA100 and TA98 without S9 mix and other tester strains.

Validation study to assess the effects of the test substance to growth of tester strains was conducted using TA100 with and without S9 mix. With S9 mix, increase of bacteria flora accompanied with increase of the dose level of test substance was observed (Addendum 4).

d) Discussion: In this bacterial reverse mutation test, a few times of growth is possible for the bacteria since low amount of free histidine contained in top agar (0.05mmol/L) is brought onto the agar. If, however, higher amount of free histidine is brought, it is known that bacterial flora and number of spontaneous revertant colony are increased since frequency of bacterial growth is

increased. In this validation test using TA100, dose dependent increase of bacterial flora was observed with S9 mix. With S9 mix, therefore, it was suggested that free histidine derived from test substance is brought onto the agar. Regardless of type of top agar used (for no addition of amino acid or for Salmonella typhimurium), dose dependent increase of revertant colony was observed. Therefore, it is suggested that free histidine derived from test substance which is brought to agar affect the number of spontaneous revertant colony. Bacterial reverse mutation test of Pal-G, impurity of PLG (compound which is produced by eliminating histidine from test substance) indicated negative result (See 4, 1), (2).). Validation test which was conducted as a part of above test with impurity, no increase of bacterial flora and revertant colony was observed both with and without S9 mix. Negative result was also obtained from *umu* test of PLG and no DNA injury potential of PLG was observed.

e) Conclusion: It was speculated that increase of revertant colony caused by PLG was assumed to be strongly affected by free histidine derived from PLG.

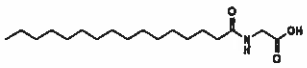
(2) Bacterial reverse mutation test of Pal-G (Impurity of PLG) (non-GLP study)

i) Study Number: M-11-029

ii) Testing Facility: Hatano Research Institute, Food and Drug Safety Center

iii) Outline of Test

a) Test Substance: Pal-G (Purity: 92.2%) It is compound which is produced by eliminating histidine from PLG and is contained in PLG as impurity (see following figure).



b) Test Method: *Salmonella typhimurium* TA100, TA1535, TA98, TA1537 and *Escherichia coli* WP2 *uvrA* were used as tester strain, and were submitted for test by pre-incubation method with and without S9 mix. Based on the results of dose finding study, 2 main studies were conducted at the following doses.

With/without S9mix

- All tester strains: 313, 625, 1250, 2500 and 5000 µg/plate

c) Result: In the main studies, no growth inhibition was observed in any tester strains. Precipitate on plate agar which derived from the test substance was observed at all dose levels with and without S9 mix before and after incubation. In the main studies, the number of revertant colonies in the test substance was not increased twice or more than that in the negative control in any strain with or without S9 mix. Validation study using TA100 was conducted with and without S9 mix to assess the effect of the test substance to growth of tester strain. As a result of the study, no increase of bacteria flora by effect of test substance was observed (Addendum

5).

d) Conclusion: It was concluded that the test substance, Pal-G (Impurity of PLG), had no mutagenic activity (negative) in this test system.

2) *Umu* test of PLG (non-GLP study)

(1) Study Number: M-11-028

(2) Testing Facility: Hatano Research Institute, Food and Drug Safety Center

(3) Outline of Test

i) Test Substance: PLG (Purity: 89.8%)

ii) Test Method: Test was conducted using "Mutagenicity test kit *umu*-test Umlac AT" with and without S9 mix. Dose finding study was conducted with setting of treatment groups, blank test groups, a negative control group and a positive control group, at 7 dose levels of 0.0750, 0.150, 0.300, 0.600, 1.20, 2.40 and 4.83 mg/well for both treatment groups and blank test groups. Based on the result of dose finding study, main study was conducted at 7 dose levels of 0.00118, 0.00236, 0.00471, 0.00942, 0.0188, 0.0377 and 0.0754 mg/well with and without S9 mix.

iii) Result: No growth inhibition of tester strains was observed for both with and without S9 mix. Precipitate derived from test substance was observed with dispersed state in wells at the doses of 0.0377 and 0.0754 mg/well for both with and without S9 mix (treatment group and blank test group). For OD_{620} value (after correction), no increase which is twice or more than that in negative group (after correction) was observed both with and without S9 mix (Addendum 6).

(4) Conclusion: It was concluded that PLG had no DNA-damaging activity (negative) in this test system.

3) Chromosome aberration test of PLG using cultured human lymphocytes (GLP study)

(1) Study Number: D451(080－105)

(2) Testing Facility: BioSafety Research Center, Foods, Drugs and Pesticides

(3) Outline of Test

i) Test Substance: PLG (Purity: 89.8%)

ii) Test Method: Based on the result of preliminary test (mitotic index measurement), treatment concentrations were chosen for *in vitro* chromosome aberration test using cultured human lymphocytes from healthy human. 24.6, 49.2, and 61.4 μg/mL were chosen for short-time treatment method without metabolic activation (-S9 treatment), 61.4, 76.8 and 96.0 μg/mL for that with metabolic activation (+S9 treatment) and 24.6, 49.2 and 61.4 μg/mL for continuous treatment method with 24 hours treatment (24 hours treatment), and for each sample of 3 concentrations, microscopy was conducted.

iii) Result: For the PLG treatment group, no obvious induction of chromosome aberration (structural and numerical aberration) was observed at any concentrations in any treatment method (Addendum 7).

(4) Conclusion: It was concluded that PLG did not induce chromosomal aberrations (negative) in human

lymphocytes under the test conditions employed.

4) Conclusion of Genotoxicity: PLG showed increase in the number of revertant colonies associate with the increase of test substance dose level with S9 mix on bacterial reverse mutation test, and it was caused by free histidine which is derived from PLG. Since the bacterial reverse mutation test with compound which is produced by eliminating histidine from PLG (Pal-G) indicated negative result, PLG was assumed to have no gene mutagenicity. Furthermore, PLG was considered to have no initial DNA injury potential since *umu* test of PLG was negative, and was also considered to have no chromosome damaging potential since the chromosome aberration test using cultured human lymphocytes was negative. Based on above, PLG was determined to have no genotoxicity.

5. Local Irritation

1) A primary skin irritation study of PLG in rabbits (GLP study)

(1) Study Number: I-3883

(2) Testing Facility: Bozo Research Center Inc.

(3) Outline of Test

i) Test Substance: PLG (Purity: 89.8%)

ii) Test Animal: Japanese White Rabbit (Jla:JW) , 12 animals, 18 weeks old, female

iii) Test Method: In selecting dose concentrations of the test substance, the highest concentration of 5% was chosen as maximum concentration which is physically preparable, and then lower concentrations of 2% and 1% were selected. The test substance (0.5 mL) was applied to a patch of 2.5 cm × 2.5 cm (lint patch) and two patches applied as closed patch to non-abraded and abraded skin on the clipped dorsal area of each rabbit. The vehicle of 0.5% methylcellulose solution was also applied by same method. After application for 24 hours, patches were removed and the skin observed over time for irritation changes according to the Draize method.

iv) Result: For the non-abraded and abraded skin to which 5, 2 or 1% test solution or 0.5% methylcellulose solution, the vehicle, was applied, dermal reactions such as erythema and edema were not observed and the primary skin irritation index (P.I.I.) was all 0. In the general condition and body weight, there were no abnormalities in any animals (Addendum 8).

(4) Conclusion: It was concluded that PLG caused no irritation effects on the rabbit skin and thus PLG was judged to be "non-irritant" at 5, 2 and 1%.

2) A 14-day cumulative skin irritation study of PLG in rabbits (GLP study)

(1) Study Number: I-3884

(2) Testing Facility: Bozo Research Center Inc.

(3) Outline of Test

i) Test Substance: PLG (Purity: 89.8%)

ii) Test Animal: Japanese White Rabbit (Jla:JW), 12 animals, 17 weeks old, female

iii) Test Method: In selecting treatment concentrations of the test substance, the highest concentration of 5% was chosen as the maximum concentration which is physically preparable, and then lower concentrations of 2% and 1% were selected. The test substance (each 0.1 mL) was applied open to intact and abraded skin areas (each 2.5 cm × 2.5 cm) on the clipped dorsal area of each rabbit. The vehicle of 0.5% methylcellulose solution was also applied by same method. This operation was done for 14 consecutive days and the skin was observed daily for irritation changes according to the Draize method. 12 rabbits were used for the main study in total and 6 rabbits each were treated with test substance solutions and vehicle, respectively.

iv) Result: For the non-abraded and abraded skin to which 5, 2 or 1% test solution or 0.5% methylcellulose solution, the vehicle, was applied, dermal reactions such as erythema and edema were not observed and the total mean score during the observation period was all 0.. In the general condition and body weight, there were no abnormalities in any animals (Addendum 9).

(4) Conclusion: It was concluded that 14-day repeated dermal application of PLG caused no irritation effects on the rabbit skin and there were no cumulative dermal irritation reactions at concentrations of 5, 2 or 1%.

3) Phototoxicity

On absorption measurement (Study Number: 12-PLGARD-013, non-GLP study), no absorption was observed in the range of UV-VIS spectrum (290 – 450 nm) (Addendum 3). According to the result, it was considered that PLG has no phototoxicity, and, therefore, no phototoxicity study was conducted.

4) A primary eye irritation study of PLG in rabbits (GLP study)

(1) Study Number: I-3885

(2) Testing Facility: Bozo Research Center Inc.

(3) Outline of Test

i) Test Substance: PLG (Purity: 89.8%)

ii) Test Animal: Japanese White Rabbit (Jla:JW), 18 animals, 15 weeks old, female

iii) Test Method: In selecting treatment concentrations of the test substance, the highest concentration of 5% was chosen as the maximum concentration which was physically preparable, and then lower concentrations of 2% and 1% were selected. The test substance (0.1 mL) was applied to the left eye of 3 animals in each group (unwashed eye group). In addition, the test substance suspension was applied to the eye of 3 different animals and the treated eye was washed with 100 mL of water for injection for 30 seconds from 30 seconds after application (washed eye group). After application, eyes were observed over time for changes in the cornea, iris and conjunctiva by the Draize method

and irritation effects classified by the Kay and Calandra method. For all animals of each group, right eye was served as control and vehicle of 0.5% methylcellulose solution was treated.

iv) Result: In the unwashed eye group, in the eyes of the animals in the 5 and 2% groups, reddening of conjunctiva was observed in all animals (3/3) at 1 hour after application, but it was no longer observed in any animals at 24 hours after application. The maximum mean total score (MMTS) was 2.0 at 1 hour after application and the final evaluation was "practically non-irritant" for 5% and 2% test suspensions. For the 1% test suspension, the final evaluation was "non-irritant" since there were no changes during the observation period. In the observation of the washed eye group where the eye was washed 30 seconds after application, redness in conjunctiva was observed only at 1 hour after application in the 5% group in 1/3 animals, and thus irritation effects were decreased in comparison with the unwashed eye group. For the eyes to which 2 or 1% test suspension were applied, there were no irritation reactions. On observation of control eyes to which 0.5% methylcellulose solution was treated, no change was observed in all animals of both non-irrigation and irrigation groups. In the general condition and body weight, there were no abnormalities in any animals (Addendum 10).

(4) Conclusion: Irritation effects of PLG on the rabbit eye were judged to be "practically non-irritant" at 5 and 2% while they were "non-irritant" at 1%. It was concluded that eye-washing reduced eye-irritation.

6. Human Patch Test

1) Closed patch test for [PLG (Lot No. TS-2197-104)] and 2 controls (24-Hour Continuous Application)

(1) Testing Facility: SOUKEN Co. Ltd, Shiba Palace Clinic

(2) Study Number: I WSK_p-7932

(3) Outline of Test

i) Test Substance: PLG (Purity: 89.8%)

ii) Test Animal: 40 humans, 24 – 60 years old, Japanese male and female

iii) Test Method: To dorsal area of 40 subjects who met with selection criteria, patch tester to which white petrolatum was applied in advance and PLG was smeared as thin layer was applied for 24 hours with closed dressing. The skin reactions at 60 minutes and 24 hours after removal of patch tester were visually inspected according to Closed Patch Test Grading Criteria and Skin Irritation Index. Physiological saline and white petrolatum were used as controls.

iv) Result: Results of closed patch test evaluation at 60 minutes and 24 hours after removal of patch tester were negative both for PLG application and control. Skin irritation index was 0 and it is safe product (Addendum 11).

(4) Conclusion: The skin irritation index was evaluated by 0 as for 'PLG(Lot No. TS-2197-104)' as the Safe product, it can be judged that there is no problem on safety.

7. Safety Evaluation for Impurities

1) *In silico* safety evaluation of impurities of PLG

(1) Outline of Test

i) Test Substance: Lau-GH, Myr-GH, Ste-GH, Pal-G, Pal-GHOMe, Pal-GGH and PLG

ii) Used Software: *Derek for Windows* (version 13.0.0)

iii) Test Method: Structural formula was entered to *Derek for Windows* for evaluation of all endpoints.

iv) Result: No alerts were raised for all test substances (Addendum 12).

(2) Conclusion: Since no alerts were shown for all tested impurities of PLG and PLG, and since PLG which has similar structure with these impurities showed no toxicity in toxicity study, impurities of PLG were determined to have no problematic toxicity.

8. Test Completion Dates of Toxicity Studies

Study initiation date, observation completion date and test completion date are shown in Table 2.

After the completion of the studies, purity of the standard product was found to have been decreased by approximately 1% due to technical error of purity determination of the standard product of the test substance for quantification. Due to this deviation, schedule of final report completion was delayed since reissue of COA of the standard product and recalculation of test concentration data were necessary. Change from the draft test report to the final report was slight change of the concentrations of test substance only and it was confirmed that there are no changes in the toxicity study result. INCI name, therefore, was registered to Personal Care Products Council at the phase of draft test report.

E. Others

NANOFIBERGEL-CS can produce gel base material which has targeted viscosity at the concentration below 1%. NANOFIBERGEL-CS currently is under development for marketing as premix, not as powder.

We are planning to market two types of premixes; one is used by 10-fold dilution containing 7.5% of NANOFIBERGEL-CS, and the other is used by 4-fold dilution containing 4% of NANOFIBERGEL-CS. Biologically active substances shall not be added in premix and we will be manufacturing and marketing premix as additive of cosmetics.

NANOFIBERGEL-CS will be contained in the final product only at 1% or below.

Addendum 1-1 An oral single-dose toxicity study of PLG in rats (B-7159) - Mortality and minimum lethal dose

B-7159

Table 1 An oral single-dose toxicity study of PLG in rats
Mortality and Minimum lethal dose

Sex	Dose mg/kg	Number of animals	Distribution of death: 1-5 min 15	30	1 hour 1	2	4	6	1 day 1	2	3	4	5	6	7	8	9	10	11	12	13	14	Mortality	Minimum lethal dose mg/kg
Male	0	5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0/5	
	2000	5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0/5	> 2000
Female	0	5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0/5	
	2000	5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0/5	> 2000

i : Immediately after dosing

Addendum 1-2-1 An oral single-dose toxicity study of PLG in rats (B-7159) - Clinical signs

B-7159

Table 2-2 An oral single-dose toxicity study of PLG in rats
Clinical signs
Male

Dose mg/kg	Findings	minutes			hours				days													
		1-5	15	30	1	2	4	6	1	2	3	4	5	6	7	8	9	10	11	12	11	14
0	No. of animals	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
	No abnormality	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
2000	No. of animals	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
	No abnormality	5	5	5	5	4	4	4	5	5	5	5	5	5	5	5	5	5	5	5	5	5
	Soft feces	0	0	0	0	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0

1 : Immediately after dosing

Addendum 1-2-2 An oral single-dose toxicity study of PLG in rats (B-7159) - Clinical signs

B-7159

Table 2-2 An oral single-dose toxicity study of PLG in rats
Clinical signs
Female

Dose mg/kg	Findings	minutes			hours				days															
		1-5	15	30	1	2	4	6	1	2	3	4	5	6	7	8	9	10	11	12	13	14
0	No. of animals	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
	No abnormality	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
2000	No. of animals	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
	No abnormality	5	5	5	5	4	4	4	5	5	5	5	5	5	5	5	5	5	5	5	5	5
	Soft feces	0	0	0	0	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0

1 : Immediately after dosing

Addendum 1-3-1 An oral single-dose toxicity study of PLG in rats (B-7159) -- Body weight

Table 3 - 1 An oral single-dose toxicity study of PLG in rats B-7159

Item : Body weight
Sex : Male
Unit : g

Test Article Dose	Day	0	1	2	3	7	10	14
0 mg/kg	Mean	167	194	204	215	250	276	304
	S.D.	4	3	4	4	7	8	12
	n	5	5	5	5	5	5	5
2000 mg/kg	Mean	166	193	201	216	256	287	319
	S.D.	3	5	6	4	6	9	11
	n	5	5	5	5	5	5	5

No significant difference between treated group and control group.

Addendum 1-3-2 An oral single-dose toxicity study of PLG in rats (B-7159) – Body weight

Table 3 - 2 An oral single-dose toxicity study of PLG in rats B-7159

Item : Body weight
Sex : Female

Unit : g

Test Article Dose	Day	0	1	2	3	7	10	14
0 mg/kg	Mean	125	143	149	156	170	180	185
	S.D.	3	3	5	8	10	12	12
	n	5	5	5	5	5	5	5
2000 mg/kg	Mean	124	143	149	154	166	174	184
	S.D.	2	4	6	6	7	11	10
	n	5	5	5	5	5	5	5

No significant difference between treated group and control group.

Addendum 1-4-1 An oral single-dose toxicity study of PLG in rats (B-7159)
– Gross pathological findings

B-7159

Table 4-1 An oral single-dose toxicity study of PLG in rats
Gross pathological findings
Male

Organs	Findings	Dose (mg/kg)	0		2000	
			a	b	a	b
		No. of animals	5 (0)		5 (0)	
External appearance	No abnormality		5 (0)		5 (0)	
Viscera of cranial cavity	No abnormality		5 (0)		5 (0)	
Viscera of thoracic cavity	No abnormality		5 (0)		5 (0)	
Viscera of abdominal cavity	No abnormality		5 (0)		5 (0)	

a : Survived
b : Died

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Addendum 1-4-2 An oral single-dose toxicity study of PLG in rats (B-7159) – Gross pathological findings

B-7159

Table 4-2 An oral single-dose toxicity study of PLG in rats
Gross pathological findings
Female

Organs	Findings	Dose (mg/kg)	0		2000	
			a	b	a	b
		No. of animals	5 (0)		5 (0)	
External appearance	No abnormality		5 (0)		5 (0)	
Viscera of cranial cavity	No abnormality		5 (0)		5 (0)	
Viscera of thoracic cavity	No abnormality		5 (0)		5 (0)	
Viscera of abdominal cavity	No abnormality		5 (0)		5 (0)	

a : Survived
b : Died

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Addendum 2-1-1 A skin sensitization study of PLG in guinea pigs (Maximization Test Method) (I-3886) – Skin reactions

Table 1-1 A skin sensitization study of PLG in guinea pigs (Maximization Test) I-3886
Skin reactions

Test group	Substance for induction: Intradermal (concentration)	Substance for induction: Topical (concentration)	Substance for challenge (concentration)	Score of skin reactions	Number of animals: 24hours [a]	Number of animals: ≥48hours [a]	Number of animals with positive reactions [b]	Positive reaction rate (%)
Test article group	PLG (2%)	PLG (5%)	PLG (5%)	0	10	10		
				1	0	0		
				2	0	0	0/10	0
				3	0	0		
				Mean score	0	0		
			PLG (2%)	0	10	10		
				1	0	0		
				2	0	0	0/10	0
				3	0	0		
				Mean score	0	0		
			PLG (1%)	0	10	10		
				1	0	0		
				2	0	0	0/10	0
				3	0	0		
				Mean score	0	0		
			0.5% MC solution	0	10	10		
				1	0	0		
				2	0	0	0/10	0
				3	0	0		
				Mean score	0	0		

a): Hours after removal of the application for challenge
b): The number of animals with individual score of 1 or above / Number of animals examined

Addendum 2-1-2 A skin sensitization study of PLG in guinea pigs (Maximization Test Method) (I-3886) – Skin reactions

Table 1-2 A skin sensitization study of PLG in guinea pigs (Maximization Test) Skin reactions

I-3886

Test group	Substance for induction: Intradermal	Substance for induction: Topical	Substance for challenge (concentration)	Score of skin reactions	Number of animals: 24hours [a]	Number of animals: 48hours [a]	Number of animals with positive reactions [b]	Positive reaction rate (%)
				0	5	5		
				1	0	0		
			PLG (5%)	2	0	0	0/5	0
				3	0	0		
				Mean score	0	0		
				0	5	5		
				1	0	0		
			PLG (2%)	2	0	0	0/5	0
				3	0	0		
				Mean score	0	0		
Negative control group	0.5% MC solution	0.5% MC solution		0	5	5		
				1	0	0		
			PLG (1%)	2	0	0	0/5	0
				3	0	0		
				Mean score	0	0		
				0	5	5		
				1	0	0		
			0.5% MC solution	2	0	0	0/5	0
				3	0	0		
				Mean score	0	0		

a): Hours after removal of the application for challenge

b): The number of animals with individual score of 1 or above / Number of animals examined

Addendum 2-1-3 A skin sensitization study of PLG in guinea pigs (Maximization Test Method) (I-3886) – Skin reactions

Table 1-3 A skin sensitization study of PLG in guinea pigs (Maximization Test) 1-3886
Skin reactions

Test group	Substance for induction		Substance for challenge (concentration)	Score of skin reactions	Number of animals		Number of animals with positive reactions [b]	Positive reaction rate (%)
	Intradermal (concentration)	Topical (concentration)			24hours [a]	48hours [a]		
Positive control group	DNCB in olive oil (0.1%)	DNCB in olive oil (0.1%)	DNCB in acetone (0.1%)	0	0	0	5/5	100
				1	0	0		
				2	0	0		
				3	5 (3) [c]	5 (3) [c]		
				Mean score	3.0	3.0		
			Acetone	0	5	5	0/5	0
				1	0	0		
				2	0	0		
				3	0	0		
				Mean score	0	0		

a): Hours after removal of the application for challenge
b): The number of animals with individual score of 1 or above / Number of animals examined
c): The number of animals revealed eschar formation

Addendum 2-2 A skin sensitization study of PLG in guinea pigs (Maximization Test Method) (I-3886) – Clinical signs

Table 2 A skin sensitization study of PLG in guinea pigs (Maximization Test) I-3886

Clinical signs

Test group	Animal number	Days after the start of induction																							
		0[a]	1	2	3	4	5	6	7[b]	8	9	10	11	12	13	14	15	16	17	18	19	20	21[c]	22	23	24[d]
Test article group	1101	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	1102	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	1103	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	1104	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	1105	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	1106	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	1107	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	1108	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	1109	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	1110	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Negative control group	2101	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	2102	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	2103	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	2104	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	2105	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Positive control group	3101	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	3102	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	3103	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	3104	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	3105	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–

–: No abnormal findings
a): Starting day of induction
b): Day of topical induction
c): Day of challenge
d): Final day of observation

Addendum 2-3 A skin sensitization study of PLG in guinea pigs (Maximization Test Method) (I-3886) – Body weight

Table 3 A skin sensitization study of PLG in guinea pigs (Maximization Test) I-3886

Body weight

Test group	Animal number	Days after the start of induction		
		0 [a]	7 [b]	21 [c]	24 [d]
Test article group	1101	343	375	423	445
	1102	370	394	456	466
	1103	360	387	453	458
	1104	329	358	415	419
	1105	390	427	516	519
	1106	356	384	458	460
	1107	355	357	390	400
	1108	372	409	463	468
	1109	372	374	475	485
	1110	370	376	465	469
	Mean	362	384	451	459
	S.D.	17	22	35	33
Negative control group	2101	362	404	458	463
	2102	330	349	417	420
	2103	380	434	518	522
	2104	367	413	470	490
	2105	353	368	425	432
	Mean	358	394	458	465
	S.D.	19	35	40	42
Positive control group	3101	387	422	501	517
	3102	390	428	512	526
	3103	378	405	499	513
	3104	401	437	521	533
	3105	358	392	473	476
	Mean	383	417	501	513
	S.D.	16	18	18	22

Unit : g

a): Starting day of induction
b): Day of topical induction
c): Day of challenge
d): Final day of observation

Addendum 3 UV-VIS Spectrum of PLG (12-PLGARD-013)

Study Number: 12-PLGARD-013

Study Report Summary

Study Title: UV-VIS Spectrum of PLG
Study Number: 12-PLGARD-013

1. Study Substance

Table 1 Test substance used in this test

Name	Lot No.	Manufacturing Site
PLG	C-02	NARD CHEMICALS, LTD.
PLG Reference Standard	HY-2130-177	Synthesis Research Department, Nissan Chemical Industries, Ltd.

2. Study Method

Approximately 10 mg of PLG was weighed and dissolved in methanol to make exactly 200 mL to obtain sample solution (concentration: 50 μg/mL). Sample solution was prepared for three times from weighing. UV-VIS spectrum of the sample solution was measured by UV-VIS spectrophotometer (UV-2400PC, SHIMADZU CORP.). Before measurement of the sample solution, baseline was corrected with methanol. Range of wavelength measured was from 190 nm to 450 nm, and scan speed was intermediate.

3. Study Result

Measurement results of UV-VIS spectrum of PLG are shown in Table 2 and Figure 1. As a result of measurement, the absorption maximum was shown near 204 nm, and no absorption was shown in the range of 280~ 450 nm. PLG (Lot No. C-02) also indicated similar result.

Table 2 UV-VIS Spectrum Measurement Result of PLG Reference Standard (Lot No.HY-2130-177)

	Concentration (mol/L)	Wavelenght (nm)	Absorption	Molar Extinction Coefficient
#1	1.118×10^{-4}	203.8	1.019	9.11×10^{3}
#2	1.115×10^{-4}	203.8	1.024	9.18×10^{3}
#3	1.121×10^{-4}	203.9	1.027	9.16×10^{3}
Mean	–	204	1.02	9.2×10^{3}

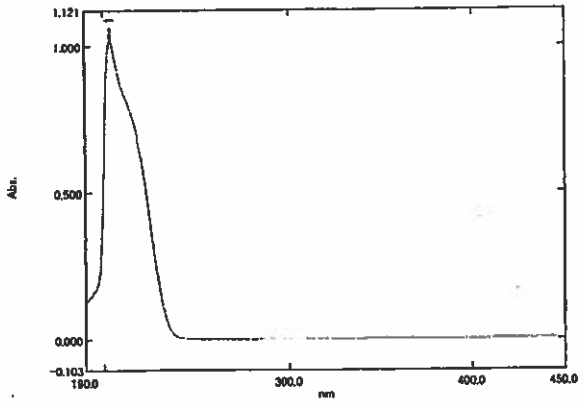


Figure 1 UV-VIS Spectrum of Methanol Solution of PLG Reference Standard (Lot No. HY-2130-177)

Addendum 4-1 Bacterial reverse mutation test of PLG (M-11-027) – Dose-finding test

Study No. M-11-027

Table 1 Results of dose-finding test in bacterial reverse mutation test of PLG

With (+) or without (-) S9 mix	Test article dose* (μg/plate)	Experimental period: September 6, 2011 - September 9, 2011									
		Number of revertants (number of colonies / plate, Mean ± S.D.)									
		Base - pair substitution type						Frameshift type			
		TA100			TA1535			WP2 uvrA		TA98		TA1537	
S9 mix (-)	0 (Negative control)	103	82	90	9	12	13	30	24	28	14	19	19	6	7	6
		(92 ± 11)			(11 ± 2)			(27 ± 1)			(13 ± 3)			(6 ± 1)		
	14.4	89			9			16			18			4		
	48.1	77			11			32			17			6		
	144	95			15			22			16			3		
	483 †	50			5			20			14			4		
	1440 (†) †	67			2			24			10			3		
	4830 (†) †	40			3			31			6			4		
S9 mix (+)	0 (Negative control)	93	90	74	11	10	16	34	28	34	25	30	29	13	19	12
		(86 ± 10)			(14 ± 3)			(32 ± 1)			(28 ± 3)			(15 ± 4)		
	14.4	101			11			38			39			17		
	48.1	131			23			42			44			18		
	144 (†) †	207			21			38			58			16		
	483 (†) †	170			16			32			84			4		
	1440 (†) †	121			19			34			61			3		
	4830 (†) ††	76			4			19			14			2		
Positive control	Chemical	AF-2			SA			AF-2			AF-2			9AA		
	Dose (μg / plate)	0.01			0.5			0.01			0.1			80		
S9 mix (-)	Number of colonies / plate	321	309	351	595	612	517	99	82	87	407	423	416	331	220	307
		(327 ± 11)			(574 ± 50)			(89 ± 9)			(415 ± 8)			(286 ± 58)		
Positive control	Chemical	B[a]P			2AA			2AA			B[a]P			B[a]P		
	Dose (μg / plate)	1			2			10			5			5		
S9 mix (+)	Number of colonies / plate	879	885	842	355	360	341	633	650	687	272	277	265	142	127	123
		(869 ± 23)			(353 ± 9)			(657 ± 28)			(271 ± 6)			(131 ± 10)		

*, Doses are adjusted for purity of the test article (correction factor: 1.12).
Negative control, Dimethyl sulfoxide
AF-2, 2-(2-Furyl)-3-(5-nitro-2-furyl)acrylamide; SA, Sodium azide; 9AA, 9-Aminoacridine; B[a]P, Benzo(a)pyrene; 2AA, 2-Aminoanthracene
(†), Precipitate was observed on the surface of agar plates in all strains used just before the incubation.
†, Precipitate was observed on the surface of agar plates in all strains used just after the incubation.
††, the precipitate derived from the test article on the surface of agar plate which interferes with colony count was observed in all strains used just after the incubation.

Addendum 4-2 Bacterial reverse mutation test of PLG (M-11-027) – Test I

Study No. M-11-027

Table 2 Results of bacterial reverse mutation test I of PLG

With (+) or without (-) S9 mix	Test article dose[#] (μg/ plate)	Experimental period: September 12, 2011 - September 15, 2011 Number of revertants (number of colonies / plate, Mean ± S.D.)														
		Base - pair substitution type										Frameshift type				
		TA100			TA1535			WP2 uvrA				TA98			TA1537	
S9 mix (-)	6 (Negative control)	78	87	83	13	6	7	21	22	17		13	56	18	10	7	5
		(82 ± 1)			(10 ± 4)			(27 ± 9)				(33 ± 2)			(5 ± 5)	
	18.8	83	113	109	8	9	7	NT				NT			NT	
		(102 ± 18)			(8 ± 1)											
	52.5	90	90	95	10	6	4	NT				NT			NT	
		(92 ± 3)			(7 ± 3)											
	35.1	78	11	11	5	4	4	NT				17	21	19	NT	
		(79 ± 3)			(4 ± 3)							(20 ± 4)				
	150	80	89	90	8	7	5	NT				16	18	24	NT	
		(86 ± 6)			(3 ± 2)							(19 ± 4)				
	302 †	64	71	82	7	5	2	30	26	30		23	18	15	3	5	5
		(73 ± 9)			(5 ± 1)			(25 ± 5)				(19 ± 4)			(5 ± 1)	
	602 †	NT			NT			24	35	22		16	19	15	6	4	5
								(28 ± 5)				(16 ± 3)			(4 ± 2)	
	1210 (†) †	77	11	67	6	2	7	28	25	25		13	38	14	4	2	1
		(65 ± 12)			(5 ± 3)			(26 ± 2)				(14 ± 2)			(3 ± 1)	
	2410 (†) †	NT			NT			34	23	23		18	17	14	6	5	5
								(27 ± 6)				(16 ± 2)			(5 ± 1)	
	4820 (†) †	72	58	67	5	4	6	28	33	29		23	15	10	6	1	5
		(66 ± 7)			1 (5 ± 1)			(27 ± 3)				(16 ± 7)			(6 ± 3)	
S9 mix (+)	0 (Negative control)	86	70	84	9	10	11	22	31	30		37	23	31	8	18	37
		(80 ± 9)			(10 ± 1)			(28 ± 5)				(30 ± 8)			(14 ± 6)	
	18.8	NT			NT			NT				NT			13	14	16
															(14 ± 3)	
	37.7	127	107	113	NT			NT				34	38	38	14	11	13
		(116 ± 10)										(37 ± 2)			(14 ± 1)	
	75.4	148	131	139	NT			NT				58	47	65	33	13	16
		(143 ± 7)										(57 ± 9)			(11 ± 3)	
	150 (†) †	205	213	194	14	8	16	35	32	33		54	81	62	12	10	13
		(205 ± 11)			(13 ± 4)			(33 ± 2)				(69 ± 10)			(11 ± 2)	
	303 (†) †	208	194	177	12	12	11	22	35	24		70	64	69	36	7	10
		(193 ± 16)			(12 ± 1)			(27 ± 7)				(68 ± 3)			(11 ± 5)	
	602 (†) †	143	143	155	9	6	9	36	26	34		61	81	70	5	1	2
		(147 ± 7)			(9 ± 1)			(33 ± 5)				(71 ± 10)			(2 ± 1)	
	1210 (†) †	148	128	182	13	5	11	33	38	26		57	16	48	NT		
		(136 ± 11)			(9 ± 5)			(32 ± 6)				(54 ± 5)				
	1410 (†) †	76	93	78	6	14	9	25	29	25		48	56	52	1	2	2
		(82 ± 9)			(10 ± 4)			(26 ± 2)				(52 ± 4)			(2 ± 1)	
Positive control	Chemical	AF-2			9A			AF-2				AF-2			9AA		
	Dose (μg / plate)	0.01			0.5			0.01				0.1			40		
S9 mix (-)	Number of colonies / plate	352	356	352	601	595	585	113	111	96		459	449	396	234	281	343
		(353 ± 3)			(594 ± 8)			(105 ± 16)				(429 ± 29)			(286 ± 55)	
Positive control	Chemical	B(a)P			2AA			2AA				B(a)P			B(a)P		
	Dose (μg / plate)	5			2			10				5			5		
S9 mix (+)	Number of colonies / plate	892	781	788	515	589	553	485	593	681		320	281	253	349	158	169
		(821 ± 62)			(569 ± 26)			1 (586 ± 46)				1 (285 ± 55)			(163 ± 6)	

#, Doses are adjusted for purity of the test article (conversion factor: 1.121.

Negative control, Dimethyl sulfoxide

AF-2, 2-(2-Furyl)-3-(5-nitro-2-furyl)acrylamide; SA, Sodium azide; 9AA, 9-Aminoacridine; B(a)P, Benzo(a)pyrene; 2AA, 2-Aminoanthracene

(†), Precipitate was observed on the surface of agar plates in all strains used just before the incubation.

†, Precipitate was observed on the surface of agar plates in all strains used just after the incubation.

NT, Not tested

Addendum 4-3 Bacterial reverse mutation test of PLG (M-11-027) – Test II

Study No. M-11-027

Table 3 Results of bacterial reverse mutation test II of PLG

With (+) or without (-) S9 mix	Test article dose[#] (μg/plate)	Experimental period: September 19, 2011 – September 22, 2011 Number of revertant (number of colonies / plate. Mean ± S.D.)														
		Base - pair substitution type									Frameshift type					
		TA100			TA1535			WP2 uvrA			TA98			TA1111		
S9 mix (-)	0 (Negative control)	103	86	86	8	18	7	28	11	22	28	20	15	7	8	8
		(92 ± 10)			(9 ± 1)			(22 ± 7)			(20 ± 5)			(8 ± 1)		
	18.8	100	90	76	9	12	8	NT			NT			NT		
		(89 ± 12)			(10 ± 1)											
	37.7	68	64	72	5	9	5	NT			NT			NT		
		(68 ± 11)			(6 ± 1)											
	75.4	81	77	81	10	6	9	NT			15	14	16	NT		
		(80 ± 2)			(8 ± 2)						(15 ± 1)					
	150	79	82	89	4	6	6	NT			17	22	24	NT		
		(83 ± 6)			(7 ± 1)						(21 ± 4)					
(-)	302 †	60	62	61	5	2	5	21	19	22	18	10	19	8	4	1
		(61 ± 1)			(5 ± 2)			(21 ± 2)			(16 ± 5)			(6 ± 2)		
	602 †	NT			NT			22	26	27	28	21	11	4	9	1
								(25 ± 3)			(23 ± 4)			(5 ± 1)		
	1210 (†) †	84	78	74	5	5	2	26	25	23	12	11	13	4	1	4
		(79 ± 5)			(4 ± 1)			(25 ± 2)			(12 ± 1)			(4 ± 2)		
	2410 (†) †	NT			NT			26	21	18	18	13	11	4	1	6
								(22 ± 4)			(14 ± 4)			(4 ± 1)		
	4820 (†) †	84	11	74	1	2	5	25	22	25	15	15	20	4	1	4
		(90 ± 5)			(5 ± 2)			(24 ± 2)			(17 ± 3)			(3 ± 1)		
S9 mix (+)	0 (Negative control)	101	104	82	14	9	11	24	86	52	35	30	12	12	15	16
		(92 ± 9)			(11 ± 3)			(54 ± 6)			(31 ± 3)			(14 ± 2)		
	18.8	NT			NT			NT			NT			15	17	14
														(16 ± 1)		
	37.7	134	110	119	NT			NT			40	33	61	16	15	14
		(118 ± 7)									(38 ± 5)			(15 ± 1)		
	75.4	135	130	143	NT			NT			44	58	48	12	18	16
		(153 ± 24)									(50 ± 7)			(15 ± 3)		
	150 (†) †	194	222	240	16	12	16	34	31	29	62	71	73	6	15	12
		(219 ± 23)			(15 ± 2)			(31 ± 3)			(71 ± 3)			(11 ± 5)		
	302 (†) †	211	207	218	17	13	12	29	22	26	94	85	80	5	1	3
		(212 ± 6)			(14 ± 3)			(26 ± 4)			(86 ± 7)			(1 ± 2)		
	602 (†) †	163	149	171	11	14	10	27	18	21	80	65	70	4	4	4
		(161 ± 11)			(12 ± 2)			(22 ± 5)			(78 ± 8)			(4 ± 0)		
	1210 (†) †	145	148	151	12	12	11	30	29	28	51	58	59	NT		
		(148 ± 3)			(12 ± 1)			(29 ± 1)			(55 ± 5)					
	2410 (†) †	134	110	95	11	12	14	20	28	28	41	46	48	5	5	2
		(110 ± 17)			(12 ± 2)			(25 ± 4)			(45 ± 4)			(3 ± 1)		
Positive control	Chemical	AF-1			5A			AF-2			AF-2			9AA		
	Dose (μg/plate)	0.01			0.5			0.01			0.1			80		
S9 mix (-)	Number of colonies / plate	339	306	334	581	118	501	86	81	95	416	179	368	281	730	327
		(326 ± 11)			(533 ± 43)			(87 ± 7)			(198 ± 19)			(513 ± 27)		
Positive control	Chemical	B[a]P			2AA			2AA			B[a]P			B[a]P		
	Dose (μg/plate)	5			2			10			5			5		
S9 mix (+)	Number of colonies / plate	1018	906	823	368	369	340	769	731	767	725	264	281	154	154	150
		(914 ± 98)			(359 ± 16)			(756 ± 21)			(257 ± 24)			(153 ± 2)		

#, Doses are adjusted for purity of the test article (correction factor: 1.125).

Negative control, Dimethyl sulfoxide

AF-2, 2-(2-Furyl)-3-(5-nitro-2-furyl)acrylamide; SA, Sodium azide; 9AA, 9-Aminoacridine; B[a]P, Benzo[a]pyrene; 2AA, 2-Anthramine

(†), Precipitate was observed on the surface of agar plates in all strains used just before the incubation.

†, Precipitate was observed on the surface of agar plates in all strains used just after the incubation.

NT, Not tested

Addendum 4-4 Bacterial reverse mutation test of PLG (M-11-027) – Confirmation test

Study No. M-11-027

Table 4 Results of confirmation test in bacterial reverse mutation test of PLG

With (+) or without (-) S9 mix	Experimental period: November 07, 2011 - November 10, 2011								
	Group	Tester strain suspension[#]	Top agar[##]	Test article dose[###] (μg/plate)					
				0	Vehicle	18.8	37.7	75.4	150
S9 mix (-)	1	+	For top agar without amino acid	45	--	--	--	--	--
	2			--	40	42	53	45	46
	3		For *Salmonella typhimurium*	134	--	--	--	--	--
	4			--	106	98	84	99	86
	5	-		--	0	0	0	0	0
S9 mix (+)	6	+	For top agar without amino acid	45	--	--	--	--	--
	7			--	56	66	78	120	171 (†) †
	8		For *Salmonella typhimurium*	123	--	--	--	--	--
	9			--	92	123	145	167	208 (†) †
	10	-		--	0	0	0	0	0 (†) †

#, *Salmonella typhimurium* TA100 was used.

##, Top agar without amino acid contains only biotin.
Top agar for *Salmonella typhimurium* contains biotin and histidine.

###, 0: None of the vehicle and the test article.
Vehicle, Dimethyl sulfoxide
Doses are adjusted for purity of the test article (correction factor: 1.05).

--, Not available

(†), Precipitate was observed on the surface of agar plates in all strains used just before the incubation.

†, Precipitate was observed on the surface of agar plates in all strains used just after the incubation.

Addendum 5-1 Bacterial reverse mutation test of Pal-G (Impurity of PLG) (M-11-029) - Dose-finding test

Study No. M-11-029

Table 1 Results of dose-finding test in bacterial reverse mutation test of Pal-G (Impurity of PLG)

With (+) or without (-) S9 mix	Test article dose# (μg/ plate)	Experimental period: September 6, 2011 - September 9, 2011 Number of revertants (number of colonies / plate, Mean ± S.D.)				
		Base - pair substitution type			Frameshift type	
		TA100	TA1535	WP2 *uvrA*	TA98	TA1537
S9 mix (-)	0 (Negative control)	100 77 81 (86 ± 12)	8 17 14 (13 ± 5)	25 31 19 (25 ± 6)	19 19 25 (21 ± 3)	7 9 5 (7 ± 2)
	15	70	12	28	20	5
	50 †	83	15	27	22	3
	150 †	86	11	20	23	8
	500 (†) †	96	11	23	26	11
	1500 (†) †	68	12	19	22	6
	5000 (†) †	85	10	21	17	8
S9 mix (+)	0 (Negative control)	83 87 79 (83 ± 4)	20 37 15 (17 ± 3)	32 29 41 (34 ± 6)	23 29 27 (26 ± 3)	17 12 14 (14 ± 3)
	15	75	17	23	30	15
	50	82	16	36	32	20
	150 (†) †	93	16	45	33	22
	500 (†) †	73	16	37	36	19
	1500 (†) †	79	16	32	26	14
	5000 (†) †	89	14	24	30	1
Positive control S9 mix (-)	Chemical	AF-2	SA	AF-2	AF-2	9AA
	Dose (μg / plate)	0.02	0.5	0.02	0.1	80
	Number of colonies / plate	315 299 319 (311 ± 11)	565 563 523 (550 ± 24)	105 109 92 (102 ± 9)	354 339 357 (350 ± 10)	209 358 408 (325 ± 104)
Positive control S9 mix (+)	Chemical	B[a]P	2AA	2AA	B[a]P	B[a]P
	Dose (μg / plate)	5	2	10	5	5
	Number of colonies / plate	961 869 816 (882 ± 73)	370 479 430 (426 ± 55)	659 695 678 (677 ± 18)	273 280 287 (280 ± 7)	127 134 131 (131 ± 4)

#, Doses are adjusted for purity of the test article (correction factor: 1.08).

Negative control, Dimethyl sulfoxide

AF-2, 2-(2-Furyl)-3-(5-nitro-2-furyl)acrylamide; SA, Sodium azide; 9AA, 9-Aminoacridine; B[a]P, Benzo[a]pyrene; 2AA, 2-Aminoanthracene

(†), Precipitate was observed on the surface of agar plates in all strains used just before the incubation.

†, Precipitate was observed on the surface of agar plates in all strains used just after the incubation.

Addendum 5-2 Bacterial reverse mutation test of Pal-G (Impurity of PLG) (M-11-029) - Test I

Study No. M-11-029

Table 2 Results of bacterial reverse mutation test I of Pal-G (Impurity of PLG)

With (+) or without (-) S9 mix	Test article dose# (μg/ plate)	Experimental period: September 12, 2011 - September 15, 2011														
		Number of revertants (number of colonies / plate, Mean ± S.D.)														
		Base - pair substitution type									Frameshift type					
		TA100			TA1535			WP2 uvrA			TA98			TA1537		
(-)	0 (Negative control)	90	91	95	6	11	15	38	28	25	23	27	28	10	9	9
		(92 ± 3)			(11 ± 5)			(30 ± 7)			(26 ± 3)			(9 ± 1)		
	313 (†) †	81	80	86	9	13	6	31	32	25	17	24	20	13	7	4
		(82 ± 3)			(9 ± 4)			(29 ± 4)			(20 ± 4)			(8 ± 5)		
	625 (†) †	95	87	81	3	8	7	33	27	27	16	19	27	5	8	7
		(88 ± 7)			(6 ± 3)			(29 ± 3)			(21 ± 6)			(7 ± 2)		
	1250 (†) †	90	98	75	12	11	9	23	29	28	23	21	22	4	5	3
		(88 ± 12)			(11 ± 2)			(27 ± 3)			(22 ± 1)			(4 ± 1)		
	2500 (†) †	80	94	74	3	8	5	18	27	22	18	23	20	2	5	6
		(83 ± 10)			(5 ± 3)			(22 ± 5)			(20 ± 3)			(4 ± 2)		
	5000 (†) †	82	81	80	9	9	9	27	17	33	18	12	21	5	6	2
		(81 ± 1)			(9 ± 0)			(26 ± 8)			(17 ± 5)			(4 ± 2)		
(+)	0 (Negative control)	81	82	109	6	12	9	29	26	40	25	36	27	12	13	19
		(91 ± 16)			(9 ± 3)			(32 ± 7)			(29 ± 6)			(15 ± 4)		
	313 (†) †	80	91	86	8	9	7	23	32	32	22	22	25	12	14	15
		(86 ± 6)			(8 ± 1)			(29 ± 5)			(23 ± 2)			(14 ± 2)		
	625 (†) †	81	92	84	7	5	6	21	36	29	30	32	20	13	19	10
		(86 ± 6)			(6 ± 1)			(29 ± 8)			(27 ± 6)			(14 ± 5)		
	1250 (†) †	90	98	88	7	9	10	31	34	21	31	31	24	15	17	14
		(92 ± 5)			(9 ± 2)			(29 ± 7)			(29 ± 4)			(15 ± 2)		
	2500 (†) †	86	90	81	7	5	9	31	20	40	26	29	31	20	16	13
		(86 ± 5)			(7 ± 2)			(30 ± 10)			(29 ± 3)			(16 ± 4)		
	5000 (†) †	84	77	80	7	5	5	33	27	36	23	33	28	7	7	9
		(80 ± 4)			(6 ± 1)			(32 ± 5)			(28 ± 5)			(8 ± 1)		
Positive control S9 mix (-)	Chemical	AF-2			SA			AF-2			AF-2			9AA		
	Dose (μg / plate)	0.01			0.5			0.01			0.1			80		
	Number of colonies / plate	363	336	351	545	572	545	87	84	119	458	476	491	318	261	340
		(350 ± 14)			(554 ± 16)			(97 ± 19)			(475 ± 17)			(306 ± 41)		
Positive control S9 mix (+)	Chemical	B[a]P			2AA			2AA			B[a]P			B[a]P		
	Dose (μg / plate)	5			2			10			5			5		
	Number of colonies / plate	941	851	836	350	341	351	583	545	533	350	327	286	158	172	170
		(876 ± 57)			(347 ± 6)			(554 ± 26)			(321 ± 32)			(167 ± 8)		

#, Doses are adjusted for purity of the test article (correction factor: 1.08).

Negative control, Dimethyl sulfoxide

AF-2, 2-(2-Furyl)-3-(5-nitro-2-furyl)acrylamide; SA, Sodium azide; 9AA, 9-Aminoacridine; B[a]P, Benzo[a]pyrene; 2AA, 2-Aminoanthracene

(†), Precipitate was observed on the surface of agar plates in all strains used just before the incubation.

†, Precipitate was observed on the surface of agar plates in all strains used just after the incubation.

Addendum 5-3 Bacterial reverse mutation test of Pal-G (Impurity of PLG) (M-11-029) - Test II

Study No. M-11-029

Table 3 Results of bacterial reverse mutation test II of Pal-G (Impurity of PLG)

With (+) or without (-) S9 mix	Test article dose# (μg/plate)	Experimental period: September 19, 2011 - September 22, 2011														
		Number of revertants (number of colonies / plate, Mean ± S.D.)														
		Base - pair substitution type										Frameshift type				
		TA100			TA1535			WP2 uvrA				TA98			TA1537		
(-)	0 (Negative control)	94	95	98	8	12	13	24	28	30		25	29	26	10	8	9
		(96 ± 2)			(11 ± 3)			(27 ± 3)				(27 ± 2)			(9 ± 1)		
	313 (†) †	90	89	96	9	10	11	30	32	25		26	22	24	7	9	9
		(92 ± 4)			(10 ± 1)			(29 ± 4)				(24 ± 2)			(8 ± 1)		
	625 (†) †	85	92	93	8	9	8	28	26	24		25	25	30	11	8	6
		(90 ± 4)			(8 ± 1)			(26 ± 2)				(27 ± 3)			(8 ± 3)		
	1250 (†) †	94	90	94	11	11	9	29	18	23		23	24	19	10	6	7
		(93 ± 2)			(10 ± 1)			(23 ± 6)				(22 ± 3)			(8 ± 2)		
	2500 (†) †	91	88	89	8	12	10	20	25	28		29	25	21	7	9	9
		(89 ± 2)			(10 ± 2)			(24 ± 4)				(25 ± 4)			(8 ± 1)		
	5000 (†) †	93	89	90	14	10	12	33	21	26		20	22	18	5	8	5
		(91 ± 2)			(12 ± 2)			(27 ± 6)				(20 ± 2)			(6 ± 2)		
(+)	0 (Negative control)	89	92	100	12	15	10	28	31	36		28	35	29	14	15	18
		(94 ± 6)			(12 ± 3)			(32 ± 4)				(31 ± 4)			(16 ± 2)		
	313 (†) †	94	85	90	8	8	10	31	35	30		30	27	25	12	16	10
		(90 ± 5)			(9 ± 1)			(32 ± 3)				(27 ± 3)			(13 ± 3)		
	625 (†) †	91	92	84	11	10	14	32	28	28		31	28	30	14	14	16
		(89 ± 4)			(12 ± 2)			(29 ± 2)				(30 ± 2)			(15 ± 1)		
	1250 (†) †	88	95	91	9	7	10	29	33	35		33	31	28	13	18	15
		(91 ± 4)			(9 ± 2)			(12 ± 3)				(31 ± 3)			(15 ± 3)		
	2500 (†) †	90	88	87	8	13	12	38	34	30		27	31	25	18	16	14
		(88 ± 2)			(11 ± 3)			(34 ± 4)				(28 ± 4)			(16 ± 2)		
	5000 (†) †	89	85	88	11	11	10	35	36	31		25	30	32	11	8	10
		(87 ± 2)			(11 ± 1)			(14 ± 3)				(29 ± 4)			(10 ± 2)		
Positive control S9 mix (-)	Chemical	AF-2			SA			AF-2				AF-2			9AA		
	Dose (μg / plate)	0.01			0.5			0.01				0.1			80		
	Number of colonies / plate	343	341	317	590	555	579	89	82	95		505	457	423	265	441	350
		(334 ± 14)			(575 ± 18)			(89 ± 7)				(462 ± 41)			(352 ± 88)		
Positive control S9 mix (+)	Chemical	B[a]P			2AA			2AA				B[a]P			B[a]P		
	Dose (μg / plate)	5			2			10				5			5		
	Number of colonies / plate	1061	900	892	407	442	450	614	548	563		374	113	350	134	131	135
		(951 ± 95)			(433 ± 23)			(575 ± 35)				(352 ± 21)			(133 ± 2)		

#, Doses are adjusted for purity of the test article (correction factor: 1.03).

Negative control, Dimethyl sulfoxide

AF-2, 2-(2-Furyl)-3-(5-nitro-2-furyl)acrylamide; SA, Sodium azide; 9AA, 9-Aminoacridine; B[a]P, Benzo[a]pyrene; 2AA, 2-Aminoanthracene

(†), Precipitate was observed on the surface of agar plates in all strains used just before the incubation.

†, Precipitate was observed on the surface of agar plates in all strains used just after the incubation.

Addendum 5-4 Bacterial reverse mutation test of Pal-G (Impurity of PLG) (M-11-029) - Confirmation test

Study No. M-11-029

Table 4 Results of confirmation test in bacterial reverse mutation test of Pal-G (Impurity of PLG)

With (+) or without (-) S9 mix	Experimental period: November 07, 2011 - November 10, 2011								
	Group	Tester strain suspension#	Top agar##	Test article dose### (μg/plate)					
				0	Vehicle	19.5	39.1	78.1	156
S9 mix (-)	1	+	For top agar without amino acid	50	–	–	–	–	–
	2			–	35	36	41	30 (†) †	37 (†) †
	3		For *Salmonella typhimurium*	114	–	–	–	–	–
	4			–	98	97	106	91 (†) †	82 (†) †
	5	–		–	0	0	0	0 (†) †	0 (†) †
S9 mix (+)	6	+	For top agar without amino acid	60	–	–	–	–	–
	7			–	42	48	44	38	38
	8		For *Salmonella typhimurium*	104	–	–	–	–	–
	9			–	94	119	92	96	89
	10	–		–	0	0	0	0 †	0 †

#, *Salmonella typhimurium* TA100 was used.

##, Top agar without amino acid contains only biotin.
Top agar for *Salmonella typhimurium* contains biotin and histidine.

###, 0: None of the vehicle and the test article.
Vehicle, Dimethyl sulfoxide
Doses are adjusted for purity of the test article (correction factor: 1.08).

–, Not available

(†), Precipitate was observed on the surface of agar plates in all strains used just before the incubation.

†, Precipitate was observed on the surface of agar plates in all strains used just after the incubation.

Addendum 6-1 *Umu* test of PLG (M-11-028) - Dose finding test

Study No. M-11-028

Table 1 Results of dose-finding test in *umu* test of PLG

Experimental date: September 28, 2011

Test article: PLG

Positive control: AF-2

With (+) or without (-) S9 mix	Test article dose* (mg/well)	Tester strain: NM2009; OD_{420} (mean): Treatment group	Blank	Corrected value
(-)	Negative control	0.361 0.366 (0.365)	0.026 0.025 (0.026)	0.238
	0.0750 †	0.275 0.291 (0.283)	0.063 0.062 (0.063)	0.150
	0.150 †	0.390 0.508 (0.449)	0.157 0.142 (0.150)	0.219
	0.300 ††	0.609 0.725 (0.712)	0.387 0.401 (0.394)	0.318
	0.600 ††	1.397 0.994 (1.696)	0.846 0.862 (0.854)	0.246
	1.20 ††	1.846 1.415 (1.631)	1.735 1.484 (1.610)	-0.329
	2.40 ††	1.968 1.629 (1.799)	2.109 1.964 (2.037)	-0.238
	4.83 ††	2.001 1.705 (1.863)	1.797 1.836 (1.817)	0.046

With (-) or without (-) S9 mix	Positive control: AF-2 dose (ng/well)	Tester strain: NM2009; OD_{420} (mean): Positive control
(-)	Negative control	0.349 0.352 (0.351)
	0.01	0.360 0.378 (0.369)
	0.03	0.402 0.371 (0.390)
	0.1	0.523 0.480 (0.502)
	0.3	0.797 0.706 (0.752)
	1	1.417 1.242 (1.329)
	3	2.069 1.847 (1.958)
	10	1.807 1.704 (1.756)

*, Doses are adjusted for purity of the test article (correction factor: 1.12).

Negative control, 10 vol% dimethyl sulfoxide Blank, mean of tester strain

Corrected value, [OD_{420} (mean) of treatment group] − [OD_{420} (mean) of blank of the same dose]

Negative control, 10 vol% dimethyl sulfoxide

AF-2, 2-(2-Furyl)-3-(5-nitro-2-furyl)acrylamide

†, Precipitate deemed from the test article dispersed homogeneously in the well [treatment group and blank].

††, Precipitate deemed from the test article uneven over the bottom of well [treatment group and blank].

Test article: PLG

Positive control: 2AA

With (+) or without (-) S9 mix	Test article dose* (mg/well)	Tester strain: NM2009; OD_{420} (mean): Treatment group	Blank	Corrected value
(+)	Negative control	0.359 0.365 (0.362)	0.034 0.036 (0.035)	0.327
	0.0750 †	0.427 0.302 (0.408)	0.107 0.091 (0.099)	0.309
	0.150 †	0.423 0.346 (0.385)	0.266 0.392 (0.329)	0.056
	0.300 ††	0.467 0.394 (0.431)	0.930 0.420 (0.675)	-0.244
	0.600 ††	0.607 0.687 (0.690)	0.790 0.848 (0.884)	-0.135
	1.20 ††	1.150 1.217 (1.274)	1.199 1.043 (1.202)	0.072
	2.40 ††	1.354 2.176 (1.865)	1.868 1.590 (1.729)	0.136
	4.83 ††	1.841 2.822 (3.632)	2.168 1.966 (2.066)	-0.434

With (+) or without (-) S9 mix	Positive control: 2AA dose (ng/well)	Tester strain: NM2009; OD_{420} (mean): Positive control
(+)	Negative control	0.344 0.348 (0.346)
	0.3	1.116 1.246 (1.231)
	1	1.643 1.771 (1.807)
	3	1.927 1.958 (1.943)
	10	1.123 1.115 (1.119)
	30	0.617 0.613 (0.615)
	100	0.796 0.823 (0.810)
	300	0.874 0.892 (0.883)

*, Doses are adjusted for purity of the test article (correction factor: 1.12).

Negative control, 10 vol% dimethyl sulfoxide Blank, mean of tester strain

Corrected value, [OD_{420} (mean) of treatment group] − [OD_{420} (mean) of blank of the same dose]

Negative control, 10 vol% dimethyl sulfoxide

2AA, 2-Aminoanthracene

†, Precipitate deemed from the test article dispersed homogeneously in the well [treatment group and blank].

††, Precipitate deemed from the test article uneven over the bottom of well [treatment group and blank].

¶: The corrected OD_{420} values of doses of 0.300 to 4.83 mg/well in the treatment group and blank without and with S9 mix, were not used for the judgment because it was judged that the measured values of these absorbance were not accurate.

Addendum 6-2 *Umu* test of PLG (M-11-028)

Study No. M-11-028

Table 2 Results of *umu* test of PLG

Experimental date: September 29, 2011

Test article

PLG

With (+) or without (-) S9 mix.	Test article dose* (mg/well)	Tester strain: NM2009; OD_{600} (mean): Treatment group	Blank	Corrected value
S9 mix. (-)	Negative control	0.434 0.434 (0.434)	0.026 0.026 (0.026)	0.408
	0.00118	0.430 0.423 (0.427)	0.032 0.032 (0.032)	0.395
	0.00236	0.422 0.423 (0.423)	0.035 0.035 (0.035)	0.388
	0.00471	0.406 0.398 (0.402)	0.036 0.051 (0.044)	0.358
	0.00942	0.362 0.349 (0.356)	0.038 0.037 (0.038)	0.318
	0.0188	0.351 0.342 (0.347)	0.037 0.031 (0.034)	0.313
	0.0377 †	0.363 0.312 (0.338)	0.040 0.050 (0.045)	0.293
	0.0754 †	0.457 0.386 (0.422)	0.068 0.040 (0.054)	0.368

*, Doses are adjusted for purity of the test article (correction factor: 1.12).

Negative control, 10 vol % dimethyl sulfoxide Blank, assay of tester strain

Corrected value, [OD_{600}(mean) of treatment group]−[OD_{600}(mean) of blank of the same dose]

†, Precipitate derived from the test article dispersed homogeneously in the well (treatment group and blank)

Positive control

AF-2

With (+) or without (-) S9 mix	Positive control dose (ng/well)	Tester strain: NM2009; OD_{600} (mean): Positive control
S9 mix (-)	Negative control	0.432 0.429 (0.426)
	0.01	0.427 0.430 (0.429)
	0.03	0.451 0.459 (0.455)
	0.1	0.618 0.581 (0.600)
	0.3	0.851 0.787 (0.819)
	1	1.464 1.375 (1.420)
	3	2.196 2.039 (2.118)
	10	2.362 2.272 (2.317)

Negative control, 10 vol% dimethyl sulfoxide

AF-2, 2-(2-Furyl)-3-(5-nitro-2-furyl)acrylamide

PLG

With (+) or without (-) S9 mix.	Test article dose* (mg/well)	Tester strain: NM2009; OD_{600} (mean): Treatment group	Blank	Corrected value
S9 mix. (+)	Negative control	0.419 0.404 (0.412)	0.038 0.037 (0.038)	0.374
	0.00118	0.417 0.391 (0.404)	0.037 0.036 (0.037)	0.367
	0.00236	0.429 0.382 (0.396)	0.034 0.034 (0.034)	0.362
	0.00471	0.394 0.364 (0.379)	0.038 0.038 (0.038)	0.341
	0.00942	0.395 0.349 (0.373)	0.045 0.039 (0.042)	0.330
	0.0188	0.423 0.341 (0.382)	0.048 0.052 (0.050)	0.332
	0.0377 †	0.450 0.438 (0.444)	0.057 0.073 (0.065)	0.379
	0.0754 †	0.512 0.520 (0.516)	0.250 0.332 (0.291)	0.225

*, Doses are adjusted for purity of the test article (correction factor: 1.12).

Negative control, 10 vol % dimethyl sulfoxide Blank, assay of tester strain

Corrected value, [OD_{600}(mean) of treatment group]−[OD_{600}(mean) of blank of the same dose]

†, Precipitate derived from the test article dispersed homogeneously in the well (treatment group and blank)

2AA

With (+) or without (-) S9 mix	Positive control dose (ng/well)	Tester strain: NM2009; OD_{600} (mean): Positive control
S9 mix (+)	Negative control	0.485 0.485 (0.485)
	0.3	2.073 2.069 (2.071)
	1	1.756 1.761 (1.759)
	3	2.196 2.197 (2.196)
	10	1.691 1.683 (1.687)
	30	0.692 0.706 (0.699)
	100	0.865 0.924 (0.895)
	300	0.959 1.034 (0.997)

Negative control, 10 vol% dimethyl sulfoxide

2AA, 2-Aminoanthracene

Addendum 7-1-1 Chromosome aberration test of PLG using cultured human lymphocytes (D451(080－105)) - Mitotic indices of human lymphocytes treated with PLG in dose-finding study [Short-term treatment]

Exp. No. D451 (080-105)

Table 1. Mitosic indices of human lymphocytes treated with PLG in dose-finding study [Short-term treatment]

Compound	[Short-term treatment : -S9] Conc. (μg/mL)	Number of cells	Mitosic index (%)	Percent of control	[Short-term treatment : +S9] Compound	Conc. (μg/mL)	Number of cells	Mitosic index (%)	Percent of control
DMSO	0	500	11.2	100.0	DMSO	0	500	8.6	100.0
		500	10.0				500	8.8	
			(10.6)					(8.7)	
PLG	3.50	500	11.0	92.5	PLG	3.50	500	9.6	100.0
		500	8.6				500	7.8	
			(9.8)					(8.7)	
	7.00	500	9.4	82.1		7.00	500	7.8	89.5
		500	8.0				500	7.6	
			(8.7)					(7.7)	
	14.0	500	7.4	67.0		14.0	500	8.8	86.5
		500	6.8				500	6.6	
			(7.1)					(7.7)	
	28.0	500	6.2	59.4		28.0	500	8.0	87.4
		500	6.4				500	7.2	
			(6.3)					(7.6)	
	56.0	500	5.6	49.1		56.0	500	5.8	65.3
		500	4.8				500	5.6	
			(5.2)					(5.7)	
	112	500	2.0	21.7		112	500	3.8	44.8
		500	2.6				500	4.0	
			(2.3)					(3.9)	
	224	Toxic				224	300	3.0	34.5
		Toxic					500	2.0	
								(2.0)	
	449 <	Toxic				449 <	Toxic		
		Toxic					Toxic		

DMSO: Negative control (Dimethyl sulfoxide,10 μL/mL)
+: Visible precipitation was observed at the end of treatment period.
( ): Mean

Fifty% mitosis inhibition concentration was as follows:
[Short-term treatment : -S9] ——— 28.2 (μg/mL)
[Short-term treatment : +S9] ——— 110 (μg/mL)

Addendum 7-1-2 Chromosome aberration test of PLG using cultured human lymphocytes (D451(080－105)) - Mitotic indices of human lymphocytes treated with PLG in dose-finding study [Continuous treatment]

Exp. No. D451 (080-105)

Table 2. Mitosic indices of human lymphocytes treated with PLG in dose-finding study [Continuous treatment]

	[Continuous treatment : 24 h]			
Compound	Conc. (μg/ml)	Number of cells	Mitosic index (%)	Percent of control
DMSO	0	500	6.8	100.0
		500	6.8	
			(6.8)	
PLG	1.75	500	5.8	91.2
		500	6.6	
			(6.2)	
	3.50	500	6.0	92.3
		500	6.6	
			(6.7)	
	7.00	500	5.6	91.2
		500	6.0	
			(6.2)	
	14.0	500	5.0	75.0
		500	5.2	
			(5.1)	
	28.0	500	4.6	62.2
		500	4.0	
			(4.2)	
	56.0	500	3.8	48.3
		500	2.8	
			(3.2)	
	112	500	1.0	19.1
		500	1.6	
			(1.2)	
	224	500	0.4	4.4
		500	0.0	
			(0.2)	

DMSO: Negative control (Dimethyl sulfoxide, 10 μl/ml)
( ): Mean

Fifty% mitosis inhibition concentration was as follows:
[Continuous treatment : 24 h] —— 41.7 (μg/ml)

Addendum 7-2-1 Chromosome aberration test of PLG using cultured human lymphocytes (D451(080－105)) - Mitotic indices of human lymphocytes treated with PLG in chromosome aberration test [Short-term treatment]

Table 8. Mitotic indices of human lymphocytes treated with PLG in chromosome aberration test [Short-term treatment]

Exp. No. D451 (080-105)

Compound	[Short-term treatment : -S9] Conc. (μg/mL)	Number of cells	Mitotic index (%)	Percent of control	Compound	[Short-term treatment : +S9] Conc. (μg/mL)	Number of cells	Mitotic index (%)	Percent of control
DMSO	0	500	12.0	100.0	DMSO	0	500	7.6	100.0
		500	10.4				500	8.6	
			(11.2)					(8.1)	
PLG	6.14	500	10.6	93.8	PLG	12.3	500	7.8	102.5
		500	10.4				500	8.0	
			(10.5)					(8.3)	
	12.3	500	11.4	100.0		24.6	500	9.2	103.7
		500	11.0				500	7.6	
			(11.2)					(8.4)	
	24.6	500	9.2	78.6		49.2	500	8.0	103.7
		500	8.4				500	8.5	
			(8.8)					(8.4)	
	49.2	500	5.6	50.0		61.4	500	6.8	84.0
		500	5.6				500	4.8	
			(5.6)					(6.8)	
	61.4	500	5.2	44.6		76.8	500	4.6	51.9
		500	4.8				500	3.8	
			(5.0)					(4.2)	
	76.8	500	3.8	32.1		96.0	500	3.6	39.3
		500	3.4				500	2.8	
			(3.6)					(3.2)	
	96.0	500	4.6	41.1		120	500	3.4	44.4
		500	4.6				500	2.8	
			(4.6)					(3.6)	
	120	500	2.4	21.4		241	Toxic		
		500	2.4				Toxic		
			(2.4)						
					CP	12.5	500	2.6	34.6
							500	3.0	
								(2.8)	
MMC	0.5	500	6.6	58.0		25.0	500	1.0	12.6
		500	6.4				500	1.2	
			(6.5)					(1.1)	

DMSO: Negative control (Dimethyl sulfoxide,10 μL/mL)
MMC: Positive control (Mitomycin C)
CP: Positive control (Cyclophosphamide)
( ): Mean

Addendum 7-2-2 Chromosome aberration test of PLG using cultured human lymphocytes (D451(080－105)) - Mitotic indices of human lymphocytes treated with PLG in chromosome aberration test [Continuous treatment]

Exp. No. D451 (080-105)

Table 4. Mitotic indices of human lymphocytes treated with PLG in chromosome aberration test [Continuous treatment]

Compound	Conc. (μg/mL)	[Continuous treatment : 24 h] Number of cells	Mitotic index (%)	Percent of control
DMSO	0	500	6.0	100.0
		500	6.0	
			(6.0)	
PLG	6.14	500	5.2	86.7
		500	5.2	
			(5.2)	
	12.2	500	5.8	95.0
		500	5.6	
			(5.7)	
	24.6	500	5.4	86.7
		500	5.0	
			(5.2)	
	49.2	500	4.0	65.0
		500	3.8	
			(3.9)	
	61.4	500	2.4	46.7
		500	2.2	
			(2.3)	
	76.8	500	1.6	26.7
		500	1.6	
			(1.6)	
	96.0	500	1.6	23.3
		500	1.2	
			(1.4)	
	120	500	1.4	30.0
		500	2.2	
			(1.8)	
MMC	0.25	500	4.0	66.7
		500	4.0	
			(4.0)	

DMSO: Negative control (Dimethyl sulfoxide,10 μL/mL)
MMC: Positive control (Mitomycin C)
( ): Mean

Addendum 7-3-1 Chromosome aberration test of PLG using cultured human lymphocytes (D451(080－105)) - Short-term treatment: –S9

Table 5. Chromosome aberration test in human lymphocytes treated with PLG (Short-term treatment: -S9)

Exp. No. D451(080-105)

Compound	Conc. (μg/mL)	Time of exposure (h)	Relative mitotic index (%)	Number of cells analyzed	Number of structural aberrations					Number of cells with aberrations -gap(%)	Number of cells analyzed for polyploid	Number of polyploid cells (%)
					gap	csb	cte	csh	cse	ech			
DMSO	0	3	100.0	200	0	0	0	0	0	0	0 (0.0)	200	0 (0.0)
PLG	24.6	3	78.6	200	0	1	0	0	0	0	2 (0.5)	200	0 (0.0)
	49.2	3	50.0	200	0	0	0	0	0	0	0 (0.0)	200	2 (1.0)
	61.4	3	44.6	200	0	0	0	0	0	0	0 (0.0)	200	1 (0.5)
	76.8	3	32.3	NA									
MMC	0.5	3	58.0	200	6	15	25	0	0	0	39 (19.5) *	200	0 (0.0)

Abbreviations: csb; chromatid break, cte: chromatid exchange, csh: chromosome break, cse: chromosome exchange, ech: others
-gap: total number of cells with aberrations except gap
DMSO: Negative control (Dimethyl sulfoxide;10 μL/mL)
MMC: Positive control (Mitomycin C)
NA: Not analyzed
*: Significant difference from control (Fisher's exact test):p≤0.025

Addendum 7-3-2 Chromosome aberration test of PLG using cultured human lymphocytes (D451(080－105)) - Short-term treatment: +S9

Table 6. Chromosome aberration test in human lymphocytes treated with PLG (Short-term treatment: +S9)

Exp. No. D451(080-105)

Compound	Conc. (μg/mL)	Time of exposure (h)	Relative mitotic index (%)	Number of cells analyzed	Number of structural aberrations: gap	csb	cse	csb	cse	oth	Number of cells with aberrations -gap(%)	Number of cells analyzed for polyploid	Number of polyploid cells (%)
DMSO	0	3	100.0	200	0	0	0	0	0	0	0 (0.0)	200	0 (0.0)
PLG	61.3	3	84.0	200	0	0	0	0	0	0	0 (0.0)	200	0 (0.0)
	76.8	3	81.9	200	0	0	0	0	0	0	0 (0.0)	200	0 (0.0)
	96.0	3	29.5	200	0	0	0	0	0	0	0 (0.0)	200	2 (0.5)
	120	3	44.4	NA									
CP	12.5	3	24.6	NA									
	25.0	3	13.6	200	11	38	30	9	0	0	52 (26.5) *	200	0 (0.0)

Abbreviation: csb; chromatid break, cse: chromatid exchange, csb: chromosome break, cse: chromosome exchange, oth: others
-gap: total number of cells with aberrations except gap
DMSO: Negative control (Dimethyl sulfoxide, 10 μL/mL)
CP: Positive control (Cyclophosphamide)
NA: Not analysed
*: Significant difference from control (Fisher's exact test):p≤0.025

Addendum 7-3-3 Chromosome aberration test of PLG using cultured human lymphocytes (D451(080−105)) - Continuous treatment: 24 h

Table 7. Chromosome aberration test in human lymphocytes treated with PLG (Continuous treatment: 24h)

Exp. No. D451(080-105)

Compound	Conc. (μg/mL)	Time of exposure (h)	Relative mitotic index (%)	Number of cells analysed	Number of cells with structural aberrations: gap	ctb	cre	csb	cse	exh	Number of cells with aberrations -gap(%)	Number of cells analyzed for polyploid	Number of polyploid cells (%)
DMSO	0	24	100.0	200	0	1	0	0	0	0	1 (0.5)	200	1 (0.5)
PLG	24.6	24	86.7	200	1	1	0	0	0	0	1 (0.5)	200	0 (0.0)
	49.2	24	65.0	200	0	1	0	0	0	0	1 (0.5)	200	1 (0.5)
	61.4	24	46.7	200	0	0	0	0	0	0	0 (0.0)	200	3 (1.5)
	76.8	24	26.7	NA									
MMC	0.25	24	66.7	200	4	12	24	0	0	0	33 (16.5) *	200	0 (0.0)

Abbreviation: ctb; chromatid break, cre: chromatid exchange, csb: chromosome break, cse: chromosome exchange, exh: others
-gap: total number of cells with aberrations except gap
DMSO: Negative control (Dimethyl sulfoxide,10 μL/mL)
MMC: Positive control (Mitomycin C)
NA: Not analysed
*: Significant difference from control (Fisher's exact test):p≤0.025

Addendum 8-1 A primary skin irritation study of PLG in rabbits (I-3883) – Skin irritation reactions

Table 1 A primary skin irritation study of PLG in rabbits
Skin irritation reactions: from 24 hours to 72 hours

I-3883

Applied substance (concentration)	Animal number	Score (Erythema and eschar / Edema) Hours after application						Individual primary irritation index [a]	Primary irritation index (P.I.I.) [b]
		Intact skin			Abraded skin				
		24 hrs	48 hrs	72 hrs	24 hrs	48 hrs	72 hrs		
PLG (5%)	1101	0 / 0	0 / 0	0 / 0	0 / 0	0 / 0	0 / 0	0	0
	1104	0 / 0	0 / 0	0 / 0	0 / 0	0 / 0	0 / 0	0	
	1105	0 / 0	0 / 0	0 / 0	0 / 0	0 / 0	0 / 0	0	
	1108	0 / 0	0 / 0	0 / 0	0 / 0	0 / 0	0 / 0	0	
	1109	0 / 0	0 / 0	0 / 0	0 / 0	0 / 0	0 / 0	0	
	1112	0 / 0	0 / 0	0 / 0	0 / 0	0 / 0	0 / 0	0	
	Total	0	0	0	0	0	0		
	Mean	0	0	0	0	0	0		
PLG (2%)	1101	0 / 0	0 / 0	0 / 0	0 / 0	0 / 0	0 / 0	0	0
	1102	0 / 0	0 / 0	0 / 0	0 / 0	0 / 0	0 / 0	0	
	1105	0 / 0	0 / 0	0 / 0	0 / 0	0 / 0	0 / 0	0	
	1106	0 / 0	0 / 0	0 / 0	0 / 0	0 / 0	0 / 0	0	
	1109	0 / 0	0 / 0	0 / 0	0 / 0	0 / 0	0 / 0	0	
	1110	0 / 0	0 / 0	0 / 0	0 / 0	0 / 0	0 / 0	0	
	Total	0	0	0	0	0	0		
	Mean	0	0	0	0	0	0		
PLG (1%)	1102	0 / 0	0 / 0	0 / 0	0 / 0	0 / 0	0 / 0	0	0
	1103	0 / 0	0 / 0	0 / 0	0 / 0	0 / 0	0 / 0	0	
	1106	0 / 0	0 / 0	0 / 0	0 / 0	0 / 0	0 / 0	0	
	1107	0 / 0	0 / 0	0 / 0	0 / 0	0 / 0	0 / 0	0	
	1110	0 / 0	0 / 0	0 / 0	0 / 0	0 / 0	0 / 0	0	
	1111	0 / 0	0 / 0	0 / 0	0 / 0	0 / 0	0 / 0	0	
	Total	0	0	0	0	0	0		
	Mean	0	0	0	0	0	0		
0.5% MC solution	1103	0 / 0	0 / 0	0 / 0	0 / 0	0 / 0	0 / 0	0	0
	1104	0 / 0	0 / 0	0 / 0	0 / 0	0 / 0	0 / 0	0	
	1107	0 / 0	0 / 0	0 / 0	0 / 0	0 / 0	0 / 0	0	
	1108	0 / 0	0 / 0	0 / 0	0 / 0	0 / 0	0 / 0	0	
	1111	0 / 0	0 / 0	0 / 0	0 / 0	0 / 0	0 / 0	0	
	1112	0 / 0	0 / 0	0 / 0	0 / 0	0 / 0	0 / 0	0	
	Total	0	0	0	0	0	0		
	Mean	0	0	0	0	0	0		

a): Individual primary irritation index was determined by the totaling the scores of reactions for erythema, eschar and edema formation observed at 24, 48 and 72 hours after application in the intact skin and abraded skin, and then dividing it by 6.
b): P.I.I. was determined by dividing the totaled individual P.I.I. by 6 (the number of rabbits) to obtain the average.

Addendum 8-2 A primary skin irritation study of PLG in rabbits (I-3883) – Clinical signs

I-3883

Table 2 A primary skin irritation study of PLG in rabbits

Clinical signs

Animal number	Hours after application	Days after application		
	0 a)	1	2	3
1101	-	-	-	-
1102	-	-	-	-
1103	-	-	-	-
1104	-	-	-	-
1105	-	-	-	-
1106	-	-	-	-
1107	-	-	-	-
1108	-	-	-	-
1109	-	-	-	-
1110	-	-	-	-
1111	-	-	-	-
1112	-	-	-	-

a): Immediately after application

-: No abnormal findings

Addendum 8-3 A primary skin irritation study of PLG in rabbits (I-3883) – Body weight

Table 3 A primary skin irritation study of PLG in rabbits 1-3883

Body weight

Animal number	Days after application	
	Day 0 [a]	Day 3 [b]
1101	3.05	3.08
1102	3.29	3.34
1103	3.26	3.28
1104	3.03	3.07
1105	3.10	3.20
1106	3.08	3.16
1107	3.47	3.57
1108	2.96	3.00
1109	3.16	3.18
1110	3.20	3.29
1111	3.02	3.08
1112	3.31	3.33

Unit: kg

a): Before application

b): Final day of observation

Addendum 9-1 A 14-day cumulative skin irritation study of PLG in rabbits (I-3884) – Skin reactions

Table 1 A 14-day cumulative skin irritation study of PLG in rabbits I-3884
Skin reactions

Applied substance (concentration)		Days after application														
		2	3	4	5	6	7	8	9	10	11	12	13	14	15
PLG (5%)	Intact skin	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Abraded skin	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Total mean score	0	0	0	0	0	0	0	0	0	0	0	0	0	0
PLG (2%)	Intact skin	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Abraded skin	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Total mean score	0	0	0	0	0	0	0	0	0	0	0	0	0	0
PLG (1%)	Intact skin	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Abraded skin	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Total mean score	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0.5% MC solution	Intact skin	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Abraded skin	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Total mean score	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Intact skin : The value obtained by dividing the individual scores (erythema, eschar + edema) in the intact skin at each observation by 6, the number of animals.
Abraded skin : The value obtained by dividing the individual scores (erythema, eschar + edema) in the abraded skin at each observation by 6, the number of animals
Total Mean Score : The value obtained by dividing the totaled individual scores (erythema, eschar + edema) at each observation by 6, the number of animals.

Addendum 9-2 A 14-day cumulative skin irritation study of PLG in rabbits (I-3884) – Clinical signs

Table 2 A 14-day cumulative skin irritation study of PLG in rabbits I-3884

Clinical signs

Animal number	Days after application																											
	1		2		3		4		5		6		7		8		9		10		11		12		13		14		15
	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	
1101	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
1102	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
1103	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
1104	-	-	-	-	-	-	-	~	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
1105	-	-	-	~	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
1106	-	-	-	-	-	-	-	~	-	~	-	-	-	-	-	-	-	-	-	~	-	-	-	-	-	-	-	-	-
1107	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
1108	~	-	-	-	-	-	-	-	-	~	-	-	-	-	-	-	-	~	-	-	-	-	-	-	-	~	-	~	-
1109	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	~	-	-	-	-	-	-	-	-	-	-	-	-	-
1110	~	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
1111	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	~	-	-	-	-	-	-	-	-	-	-	-
1112	-	-	-	-	-	~	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

A: Before application

B: One hour after application

-: No abnormal findings

Addendum 9-3 A 14-day cumulative skin irritation study of PLG in rabbits (I-3884) -- Body weight

I-3884

Table 3 A 14-day cumulative skin irritation study of PLG in rabbits

Body weight

Animal number	Days after application		
	Day 1 [a]	Day 8	Day 15 [b]
1101	3.25	3.30	3.35
1102	3.15	3.18	3.21
1103	2.90	3.04	3.08
1104	3.00	3.04	3.07
1105	3.05	3.08	3.10
1106	3.19	3.22	3.38
1107	2.87	2.94	3.09
1108	3.06	3.09	3.14
1109	3.02	3.06	3.12
1110	2.82	2.86	2.96
1111	3.00	3.04	3.19
1112	2.75	2.76	2.83

Unit : kg

a): Before application

b): Final day of observation

Addendum 10-1 A primary eye irritation study of PLG in rabbits (I-3885) – Eye irritation reactions

Table 1 An eye irritation study of PLG in rabbits I-3885

Eye irritation reactions

Test group		Number of animals examined	Mean total score (MTS)					MMTS
			1 hr	24 hrs	48 hrs	72 hrs	96 hrs	
High dose (5%) (Unwashed eye group)	L: PLG (5%)	3	2.0	0	0	0	0	2.0
	R: 0.5% MC solution	3	0	0	0	0	0	0
High dose (5%) (Washed eye group)	L: PLG (5%)	3	0.7	0	0	0	0	0.7
	R: 0.5% MC solution	3	0	0	0	0	0	0
Middle dose (2%) (Unwashed eye group)	L: PLG (2%)	3	2.0	0	0	0	0	2.0
	R: 0.5% MC solution	3	0	0	0	0	0	0
Middle dose (2%) (Washed eye group)	L: PLG (2%)	3	0	0	0	0	0	0
	R: 0.5% MC solution	3	0	0	0	0	0	0
Low dose (1%) (Unwashed eye group)	L: PLG (1%)	3	0	0	0	0	0	0
	R: 0.5% MC solution	3	0	0	0	0	0	0
Low dose (1%) (Washed eye group)	L: PLG (1%)	3	0	0	0	0	0	0
	R: 0.5% MC solution	3	0	0	0	0	0	0

L: Left eye, R: Right eye

MMTS : Maximum mean total score

Addendum 10-2 A primary eye irritation study of PLG in rabbits (I-3885) -- Other ocular changes

Table 2 An eye irritation study of PLG in rabbits I-3885

Other ocular changes

Test group		Other ocular changes (PLG treated eye / 0.5% MC solution treated eye)											
		Hours after application						Days after application					
		0 a)	1 hr	2 hrs	3 hrs	4 hrs	5 hrs	6 hrs	1 day	2 days	3 days	4 days	
High dose (5%) (Unwashed eye group)	Number of animals examined	3 / 3	3 / 3	3 / 3	3 / 3	3 / 3	3 / 3	3 / 3	3 / 3	3 / 3	3 / 3	3 / 3	
	No changes	3 / 3	3 / 3	3 / 3	3 / 3	3 / 3	3 / 3	3 / 3	3 / 3	3 / 3	3 / 3	3 / 3	
	Abnormal findings	0 / 0	0 / 0	0 / 0	0 / 0	0 / 0	0 / 0	0 / 0	0 / 0	0 / 0	0 / 0	0 / 0	
High dose (5%) (Washed eye group)	Number of animals examined	3 / 3	3 / 3	3 / 3	3 / 3	3 / 3	3 / 3	3 / 3	3 / 3	3 / 3	3 / 3	3 / 3	
	No changes	3 / 3	3 / 3	3 / 3	3 / 3	3 / 3	3 / 3	3 / 3	3 / 3	3 / 3	3 / 3	3 / 3	
	Abnormal findings	0 / 0	0 / 0	0 / 0	0 / 0	0 / 0	0 / 0	0 / 0	0 / 0	0 / 0	0 / 0	0 / 0	
Middle dose (2%) (Unwashed eye group)	Number of animals examined	3 / 3	3 / 3	3 / 3	3 / 3	3 / 3	3 / 3	3 / 3	3 / 3	3 / 3	3 / 3	3 / 3	
	No changes	3 / 3	3 / 3	3 / 3	3 / 3	3 / 3	3 / 3	3 / 3	3 / 3	3 / 3	3 / 3	3 / 3	
	Abnormal findings	0 / 0	0 / 0	0 / 0	0 / 0	0 / 0	0 / 0	0 / 0	0 / 0	0 / 0	0 / 0	0 / 0	
Middle dose (2%) (Washed eye group)	Number of animals examined	3 / 3	3 / 3	3 / 3	3 / 3	3 / 3	3 / 3	3 / 3	3 / 3	3 / 3	3 / 3	3 / 3	
	No changes	3 / 3	3 / 3	3 / 3	3 / 3	3 / 3	3 / 3	3 / 3	3 / 3	3 / 3	3 / 3	3 / 3	
	Abnormal findings	0 / 0	0 / 0	0 / 0	0 / 0	0 / 0	0 / 0	0 / 0	0 / 0	0 / 0	0 / 0	0 / 0	
Low dose (1%) (Unwashed eye group)	Number of animals examined	3 / 3	3 / 3	3 / 3	3 / 3	3 / 3	3 / 3	3 / 3	3 / 3	3 / 3	3 / 3	3 / 3	
	No changes	3 / 3	3 / 3	3 / 3	3 / 3	3 / 3	3 / 3	3 / 3	3 / 3	3 / 3	3 / 3	3 / 3	
	Abnormal findings	0 / 0	0 / 0	0 / 0	0 / 0	0 / 0	0 / 0	0 / 0	0 / 0	0 / 0	0 / 0	0 / 0	
Low dose (1%) (Washed eye group)	Number of animals examined	3 / 3	3 / 3	3 / 3	3 / 3	3 / 3	3 / 3	3 / 3	3 / 3	3 / 3	3 / 3	3 / 3	
	No changes	3 / 3	3 / 3	3 / 3	3 / 3	3 / 3	3 / 3	3 / 3	3 / 3	3 / 3	3 / 3	3 / 3	
	Abnormal findings	0 / 0	0 / 0	0 / 0	0 / 0	0 / 0	0 / 0	0 / 0	0 / 0	0 / 0	0 / 0	0 / 0	

a) : Immediately after application

Addendum 10-3 A primary eye irritation study of PLG in rabbits (I-3885) – Clinical sign

Table 3 An eye irritation study of PLG in rabbits I-3885

Clinical sign

Test group	Animal number	Hours after application						Days after application			
		0 a)	1 hr	2 hrs	3 hrs	4 hrs	5 hrs	6 hrs	1 day	2 days	3 days	4 days
High dose (5%) (Unwashed eye group)	1101	-	-	-	-	-	-	-	-	-	-	-
	1102	-	-	-	-	-	-	-	-	-	-	-
	1103	-	-	-	-	-	-	-	-	-	-	-
High dose (5%) (Washed eye group)	2101	-	-	-	-	-	-	-	-	-	-	-
	2102	-	-	-	-	-	-	-	-	-	-	-
	2103	-	-	-	-	-	-	-	-	-	-	-
Middle dose (2%) (Unwashed eye group)	3101	-	-	-	-	-	-	-	-	-	-	-
	3102	-	-	-	-	-	-	-	-	-	-	-
	3103	-	-	-	-	-	-	-	-	-	-	-
Middle dose (2%) (Washed eye group)	4101	-	-	-	-	-	-	-	-	-	-	-
	4102	-	-	-	-	-	-	-	-	-	-	-
	4103	-	-	-	-	-	-	-	-	-	-	-
Low dose (1%) (Unwashed eye group)	5101	-	-	-	-	-	-	-	-	-	-	-
	5102	-	-	-	-	-	-	-	-	-	-	-
	5103	-	-	-	-	-	-	-	-	-	-	-
Low dose (1%) (Washed eye group)	6101	-	-	-	-	-	-	-	-	-	-	-
	6102	-	-	-	-	-	-	-	-	-	-	-
	6103	-	-	-	-	-	-	-	-	-	-	-

a) : Immediately after application

- : No changes

Addendum 10-4 A primary eye irritation study of PLG in rabbits (I-3885) -- Body weight

I-3885

Table 4 An eye irritation study of PLG in rabbits

Body weight

Test group	Animal number	Days after application	
		0 a)	4 b)
High dose (5%) (Unwashed eye group)	1101	2.54	2.58
	1102	2.47	2.50
	1103	2.45	2.47
High dose (5%) (Washed eye group)	2101	2.46	2.48
	2102	2.69	2.73
	2103	2.56	2.59
Middle dose (2%) (Unwashed eye group)	3101	2.48	2.55
	3102	2.55	2.66
	3103	2.65	2.70
Middle dose (2%) (Washed eye group)	4101	2.60	2.62
	4102	2.47	2.56
	4103	2.44	2.53
Low dose (1%) (Unwashed eye group)	5101	2.43	2.45
	5102	2.67	2.74
	5103	2.34	2.42
Low dose (1%) (Washed eye group)	6101	2.52	2.61
	6102	2.51	2.66
	6103	2.81	2.88

Unit : kg

a) : Before application

b) : Final day of observation

Addendum 11-1 Closed patch test for [PLG (Lot No. TS-2197-104)] and 2 controls (24-Hour Continuous Application) – Ingredients and amounts contained in [PLG (Lot No. TS-2197-104)], Evaluation criteria and Skin irritation index

Table 1-1 Ingredients and amounts contained in [PLG (Lot No.TS-2197-104)]

PLG (Lot No.TS-2197-104)	W/W(%)
PLG	100.0000

Table 2-1 Evaluation criteria

Evaluation criteria	Evaluation	Score
No response	Negative(-)	0
Slight erythema	Weakly positive(±)	0.5
Obvious erythema	Positive (+)	1.0
Erythema + edema, papule	Strongly positive (++)	2.0
Erythema + edema, papule + vesicle	Strongly positive (+++)	3.0
Bulla	Strongly positive (++++)	4.0

* A subject was withdrawn at a point where a result of strongly positive (++) was confirmed.

Table 2-2 Skin irritation index

Skin irritation index	Classification
Less than 5.0	Safe product
5.0 - 15.0	Acceptable product
15.0 - 30.0	Product requiring improvements
Over 30.0	Unsafe product

* Skin irritation index = (total score sum for either 60 minutes or 24 hours after removal of investigational product, whichever had the stronger reaction/number of subjects) × 100

Addendum 11-2-1 Closed patch test for [PLG (Lot No. TS-2197-104)] and 2 controls (24-Hour Continuous Application) – Findings of trial [PLG (Lot No. TS-2197-104)]

Table 3-1 Findings of trial [PLG (Lot No.TS-2197-104)]

Subject number	Sex	Age	60 min after patch removal	24 hours after patch removal
1	♂	56	–	–
2	♂	56	–	–
3	♂	52	–	–
4	♀	45	–	–
5	♀	50	–	–
6	♂	30	–	–
7	♂	56	–	–
8	♀	25	–	–
9	♂	51	–	–
10	♀	35	–	–
11	♂	32	–	–
12	♂	24	–	–
13	♀	38	–	–
14	♀	39	–	–
15	♀	40	–	–
16	♂	49	–	–
17	♀	42	–	–
18	♀	42	–	–
19	♀	44	–	–
20	♂	48	–	–
21	♀	26	–	–
22	♂	53	–	–
23	♀	26	–	–
24	♀	44	–	–
25	♀	36	–	–
26	♀	30	–	–
27	♂	24	–	–
28	♂	49	–	–
29	♂	60	–	–
30	♂	31	–	–
31	♂	34	–	–
32	♂	48	–	–
33	♂	45	–	–
34	♂	24	–	–
35	♀	49	–	–
36	♂	29	–	–
37	♀	42	–	–
38	♀	34	–	–
39	♀	51	–	–
40	♂	47	–	–

Addendum 11-2-2 Closed patch test for [PLG (Lot No. TS-2197-104)] and 2 controls
(24-Hour Continuous Application) – Findings of trial [Physiological saline solution]

Table 3-2 Findings of trial [Physiological saline solution]

Subject number	Sex	Age	60 min after patch removal	24 hours after patch removal
1	♂	56	–	–
2	♂	56	–	–
3	♂	52	–	–
4	♀	45	–	–
5	♀	50	–	–
6	♂	30	–	–
7	♂	56	–	–
8	♀	25	–	–
9	♂	51	–	–
10	♀	35	–	–
11	♂	32	–	–
12	♂	24	–	–
13	♀	38	–	–
14	♀	39	–	–
15	♀	40	–	–
16	♂	49	–	–
17	♀	42	–	–
18	♀	42	–	–
19	♀	44	–	–
20	♂	48	–	–
21	♀	26	–	–
22	♂	53	–	–
23	♀	26	–	–
24	♀	44	–	–
25	♀	36	–	–
26	♀	30	–	–
27	♂	24	–	–
28	♂	49	–	–
29	♂	60	–	–
30	♂	31	–	–
31	♂	34	–	–
32	♂	48	–	–
33	♂	45	–	–
34	♂	24	–	–
35	♀	49	–	–
36	♂	29	–	–
37	♀	42	–	–
38	♀	34	–	–
39	♀	51	–	–
40	♂	47	–	–

Addendum 11-2-3 Closed patch test for [PLG (Lot No. TS-2197-104)] and 2 controls (24-Hour Continuous Application) -- Findings of trial [White petroleum]

Table 3-3 Findings of trial [White petroleum]

Subject number	Sex	Age	60 min after patch removal	24 hours after patch removal
1	♂	56	–	–
2	♂	56	–	–
3	♂	52	–	–
4	♀	45	–	–
5	♀	50	–	–
6	♂	30	–	–
7	♂	56	–	–
8	♀	25	–	–
9	♂	51	–	–
10	♀	35	–	–
11	♂	32	–	–
12	♂	24	–	–
13	♀	38	–	–
14	♀	39	–	–
15	♀	40	–	–
16	♂	49	–	–
17	♀	42	–	–
18	♀	42	–	–
19	♀	44	–	–
20	♂	48	–	–
21	♀	26	–	–
22	♂	53	–	–
23	♀	26	–	–
24	♀	44	–	–
25	♀	36	–	–
26	♀	30	–	–
27	♂	24	–	–
28	♂	49	–	–
29	♂	60	–	–
30	♂	31	–	–
31	♂	34	–	–
32	♂	48	–	–
33	♂	45	–	–
34	♂	24	–	–
35	♀	49	–	–
36	♂	29	–	–
37	♀	42	–	–
38	♀	34	–	–
39	♀	51	–	–
40	♂	47	–	–

Addendum 11-3 Closed patch test for [PLG (Lot No. TS-2197-104)] and 2 controls (24-Hour Continuous Application) – Skin Irritation Index

Table 4 Skin Irritation Index
<Results>

Investigational product	Skin irritation index	Skin irritation
PLG (Lot No.TS-2197-104)	0	Safe product

Addendum 12 *In silico* safety evaluation of Impurities of PLG – *Derek for Windows* Report Cover

¥¥Bill¥hp01¥安全性研究生月研¥PLG不純物¥Lau-GH.rtf

Derek for Windows Report

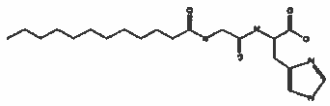
User name:	hagios
Date created:	2012年9月26日
Program version:	Derek for Windows_13.0.0
Filename of knowledge base:	C:¥Program Files¥Lhasa Ltd¥LPS 13¥DfW_2011i.mdb
Knowledge base version:	DfW13.0.0_14_03_2011
Knowledge base last modified date:	2011年7月20日
Testing a single alert:	Off
Species:	bacterium dog Escherichia coli guinea pig hamster human mammal monkey mouse primate rabbit rat rodent Salmonella typhimurium
Superendpoints:	Carcinogenicity Chromosome damage Genotoxicity Hepatotoxicity HERG channel inhibition Irritation Miscellaneous endpoints Mutagenicity Ocular toxicity Rapid prototypes: bladder disorders Rapid prototypes: blood in urine Rapid prototypes: bone marrow toxicity Rapid prototypes: bradycardia Rapid prototypes: chromosome damage in vitro Rapid prototypes: hepatotoxicity Rapid prototypes: kidney disorders Rapid prototypes: mitochondrial dysfunction Rapid prototypes: nephrotoxicity Rapid prototypes: splenotoxicity Rapid prototypes: thyroid toxicity Reproductive toxicity Respiratory sensitisation Skin sensitisation Thyroid toxicity
Perceive tautomers:	On
Hydrogen options:	Perceive implicit and explicit hydrogens
Autosave results (DRK file):	Off
Autosave results directory:	Not applicable
Name field:	Not specified

Addendum 12-1 *In silico* safety evaluation of impurities of PLG – Lau-GH (*Derek for Windows* Report)

Derek for Windows Report

Compound name: Lau-GH
Relative molecular mass: 394.516 Calculated by LPS
Exact molecular mass: 394.25801 Calculated by LPS
Log Kp: -2.896 cm/h [for Kp] Obtained from External Data Source
Molecular weight = 394.516
Log P value used in Log Kp calculation = 3.142001
Log P: 3.142 Obtained from External Data Source

Submitted compound:



List of alerts found:

Nothing to report

Addendum 12-1 *In silico* safety evaluation of impurities of PLG – Lau-GH (*Derek for Windows* Report)

LHASA PREDICTIONS

alpha-2-mu-Globulin nephropathy

mammal - Reasoning

alpha-2-mu-Globulin nephropathy in mammal is DOUBTED
[Molecular Weight > 350] is [CERTAIN]
Molecular Weight is 394.516 Calculated by LPS
[species rat] is [PLAUSIBLE]
[mammal other than rat] is [PLAUSIBLE]

rat - Reasoning

alpha-2-mu-Globulin nephropathy in rat is DOUBTED
[Molecular Weight > 350] is [CERTAIN]
Molecular Weight is 394.516 Calculated by LPS
[species rat] is [CERTAIN]

rodent - Reasoning

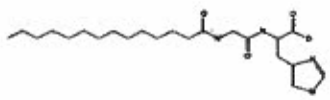
alpha-2-mu-Globulin nephropathy in rodent is DOUBTED
[Molecular Weight > 350] is [CERTAIN]
Molecular Weight is 394.516 Calculated by LPS
[species rat] is [PLAUSIBLE]
[mammal other than rat] is [PLAUSIBLE]

Addendum 12-2 *In silico* safety evaluation of impurities of PLG -- Myr-GH (*Derek for Windows* Report)

Derek for Windows Report

Compound name: Myr-GH
Relative molecular mass: 422.57 Calculated by LPS
Exact molecular mass: 422.28931 Calculated by LPS
Log Kp: -2.316 cm/h [for Kp] Obtained from External Data Source
Molecular weight = 422.57
Log P value used in Log Kp calculation = 4.2
Log P: 4.2 Obtained from External Data Source

Submitted compound:



List of alerts found:

Nothing to report

Addendum 12-2 *In silico* safety evaluation of impurities of PLG – Myr-GH (*Derek for Windows* Report)

LHASA PREDICTIONS

alpha-2-mu-Globulin nephropathy

mammal - Reasoning

alpha-2-mu-Globulin nephropathy in mammal is DOUBTED
[Molecular Weight > 350] is [CERTAIN]
Molecular Weight is 422.57 Calculated by LPS
[species rat] is [PLAUSIBLE]
[mammal other than rat] is [PLAUSIBLE]

rat - Reasoning

alpha-2-mu-Globulin nephropathy in rat is DOUBTED
[Molecular Weight > 350] is [CERTAIN]
Molecular Weight is 422.57 Calculated by LPS
[species rat] is [CERTAIN]

rodent - Reasoning

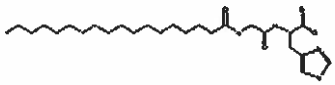
alpha-2-mu-Globulin nephropathy in rodent is DOUBTED
[Molecular Weight > 350] is [CERTAIN]
Molecular Weight is 422.57 Calculated by LPS
[species rat] is [PLAUSIBLE]
[mammal other than rat] is [PLAUSIBLE]

Addendum 12-3 *In silico* safety evaluation of impurities of PLG – Ste-GH (*Derek for Windows* Report)

Derek for Windows Report

Compound name: Ste-GH
Relative molecular mass: 478.678 Calculated by LPS
Exact molecular mass: 478.35191 Calculated by LPS
Log Kp: -1.156 cm/h [for Kp] Obtained from External Data Source
Molecular weight = 478.678
Log P value used in Log Kp calculation = 6.316
Log P: 6.316 Obtained from External Data Source

Submitted compound:



List of alerts found:

Nothing to report

Addendum 12-3 *In silico* safety evaluation of impurities of PLG -- Ste-GH (*Derek for Windows* Report)

LHASA PREDICTIONS

alpha-2-mu-Globulin nephropathy

mammal - Reasoning

alpha-2-mu-Globulin nephropathy in mammal is DOUBTED
[Molecular Weight > 350] is [CERTAIN]
Molecular Weight is 478.678 Calculated by LPS
[species rat] is [PLAUSIBLE]
[mammal other than rat] is [PLAUSIBLE]

rat - Reasoning

alpha-2-mu-Globulin nephropathy in rat is DOUBTED
[Molecular Weight > 350] is [CERTAIN]
Molecular Weight is 478.678 Calculated by LPS
[species rat] is [CERTAIN]

rodent - Reasoning

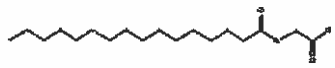
alpha-2-mu-Globulin nephropathy in rodent is DOUBTED
[Molecular Weight > 350] is [CERTAIN]
Molecular Weight is 478.678 Calculated by LPS
[species rat] is [PLAUSIBLE]
[mammal other than rat] is [PLAUSIBLE]

Addendum 12-4 *In silico* safety evaluation of impurities of PLG – Pal-G (*Derek for Windows* Report)

Derek for Windows Report

Compound name: Pal-G
Relative molecular mass: 313.482 Calculated by LPS
Exact molecular mass: 313.26169 Calculated by LPS
Log Kp: -0.018 cm/h [for Kp] Obtained from External Data Source
Molecular weight = 313.482
Log P value used in Log Kp calculation = 6.4986
Log P: 6.499 Obtained from External Data Source

Submitted compound:



List of alerts found:

Nothing to report

Addendum 12-4 *In silico* safety evaluation of impurities of PLG – Pal-G (*Derek for Windows* Report)

LHASA PREDICTIONS

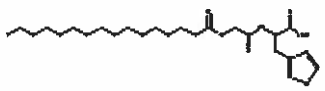
Nothing to report

Addendum 12-5 *In silico* safety evaluation of impurities of PLG – Pal-GHOMe (*Derek for Windows* Report)

Derek for Windows Report

Compound name: Pal-GHOMe
Relative molecular mass: 464.651 Calculated by LPS
Exact molecular mass: 464.33626 Calculated by LPS
Log Kp: -1.756 cm/h [for Kp] Obtained from External Data Source
Molecular weight = 464.651
Log P value used in Log Kp calculation = 5.377601
Log P: 5.378 Obtained from External Data Source

Submitted compound:



List of alerts found:

Nothing to report

Addendum 12-5 *In silico* safety evaluation of impurities of PLG – Pal-GHOMe (*Derek for Windows* Report)

LHASA PREDICTIONS

alpha-2-mu-Globulin nephropathy

mammal - Reasoning

alpha-2-mu-Globulin nephropathy in mammal is DOUBTED
[Molecular Weight > 350] is [CERTAIN]
Molecular Weight is 464.651 Calculated by LPS
[species rat] is [PLAUSIBLE]
[mammal other than rat] is [PLAUSIBLE]

rat - Reasoning

alpha-2-mu-Globulin nephropathy in rat is DOUBTED
[Molecular Weight > 350] is [CERTAIN]
Molecular Weight is 464.651 Calculated by LPS
[species rat] is [CERTAIN]

rodent - Reasoning

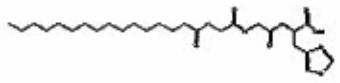
alpha-2-mu-Globulin nephropathy in rodent is DOUBTED
[Molecular Weight > 350] is [CERTAIN]
Molecular Weight is 464.651 Calculated by LPS
[species rat] is [PLAUSIBLE]
[mammal other than rat] is [PLAUSIBLE]

Addendum 12-6 *In silico* safety evaluation of impurities of PLG – Pal-GGH (*Derek for Windows* Report)

Derek for Windows Report

Compound name: Pal-GGH
Relative molecular mass: 521.703 Calculated by LPS
Exact molecular mass: 521.35772 Calculated by LPS
Log Kp: -2.487 cm/h [for Kp] Obtained from External Data Source
Molecular weight = 521.703
Log P value used in Log Kp calculation = 4.810999
Log P: 4.811 Obtained from External Data Source

Submitted compound:



List of alerts found:

Nothing to report

Addendum 12-6 *In silico* safety evaluation of impurities of PLG – Pal-GGH (*Derek for Windows* Report)

LHASA PREDICTIONS

alpha-2-mu-Globulin nephropathy

mammal - Reasoning

alpha-2-mu-Globulin nephropathy in mammal is DOUBTED
[Molecular Weight > 350] is [CERTAIN]
Molecular Weight is 521.703 Calculated by LPS
[species rat] is [PLAUSIBLE]
[mammal other than rat] is [PLAUSIBLE]

rat - Reasoning

alpha-2-mu-Globulin nephropathy in rat is DOUBTED
[Molecular Weight > 350] is [CERTAIN]
Molecular Weight is 521.703 Calculated by LPS
[species rat] is [CERTAIN]

rodent - Reasoning

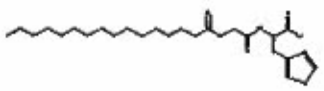
alpha-2-mu-Globulin nephropathy in rodent is DOUBTED
[Molecular Weight > 350] is [CERTAIN]
Molecular Weight is 521.703 Calculated by LPS
[species rat] is [PLAUSIBLE]
[mammal other than rat] is [PLAUSIBLE]

Addendum 12-7 *In silico* safety evaluation of impurities of PLG -- PLG (*Derek for Windows* Report)

Derek for Windows Report

Compound name: PLG
Relative molecular mass: 450.624 Calculated by LPS
Exact molecular mass: 450.32851 Calculated by LPS
Log Kp: -1.736 cm/h [for Kp] Obtained from External Data Source
Molecular weight = 450.624
Log P value used in Log Kp calculation = 5.258
Log P: 5.258 Obtained from External Data Source

Submitted compound:



List of alerts found:

Nothing to report

Addendum 12-7 *In silico* safety evaluation of impurities of PLG – PLG (*Derek for Windows* Report)

LHASA PREDICTIONS

alpha-2-mu-Globulin nephropathy

mammal - Reasoning

alpha-2-mu-Globulin nephropathy in mammal is DOUBTED
[Molecular Weight > 350] is [CERTAIN]
Molecular Weight is 450.624 Calculated by LPS
[species rat] is [PLAUSIBLE]
[mammal other than rat] is [PLAUSIBLE]

rat - Reasoning

alpha-2-mu-Globulin nephropathy in rat is DOUBTED
[Molecular Weight > 350] is [CERTAIN]
Molecular Weight is 450.624 Calculated by LPS
[species rat] is [CERTAIN]

rodent - Reasoning

alpha-2-mu-Globulin nephropathy in rodent is DOUBTED
[Molecular Weight > 350] is [CERTAIN]
Molecular Weight is 450.624 Calculated by LPS
[species rat] is [PLAUSIBLE]
[mammal other than rat] is [PLAUSIBLE]

Personal Care Products Council
Committed to Safety, Quality & Innovation

Memorandum

TO: F. Alan Andersen, Ph.D.
Director - COSMETIC INGREDIENT REVIEW (CIR)

FROM: Halyna Breslawec, Ph.D. 10/31/12
Industry Liaison to the CIR Expert Panel

DATE: October 31, 2012

SUBJECT: Information on Palmitoyl Oligopeptide

Palmitoyl Oligopeptide is defined as: "the product obtained by the reaction of palmitic acid with a synthetic peptide consisting of two or more of the following amino acids: alanine, arginine, aspartic acid, glycine, histidine, lysine, proline, serine, or valine."

As outlined in the attached summary from Sederma, trade name mixtures containing Palmitoyl Oligopeptide contain either Palmitoyl glycine histidine lysine (Pal GHK) or palmitoyl valine glycine valine alanine proline glycine (Pal VGVAPG)

Sederma. 2012. Summary information Palmitoyl Oligopeptide.

Information on Mixtures Containing Pal GHK

NAMSA. 2000. Summary of genotoxicity *Salmonella typhimurium* reverse mutation study of MAXI-LIP (contains 1000 ppm Palmitoyl Oligopeptide as Pal GHK).

Institut D'Expertise Clinque. 2000. Summary of *in vitro* and tolerance studies of MAXI-LIP (contains 1000 ppm Palmitoyl Oligopeptide as Pal GHK).

Consumer Product Testing Co. 2000. Summary of repeated insult patch test of MAXI-LIP (contains 1000 ppm Palmitoyl Oligopeptide as Pal GHK).

Centre International de Toxicologie. 1997. Summary of evaluation of the cutaneous primary irritation index in rabbits of BIOPEPTIDE CL (contains 100 ppm Palmitoyl Oligopeptide as Pal GHK).

Anonymous. 1992. Summary of reverse mutation assay by the Ames test BIOPEPTIDE CL (contains 100 ppm Palmitoyl Oligopeptide as Pal GHK).

1101 17th Street, N.W., Suite 300 | Washington, D.C. 20036-4702 | 202.331.1770 | 202.331.1969 (fax) | www.personalcarecouncil.org

Centre International de Toxicologie. 1997. Summary of acute eye irritation in rabbits BIOPEPTIDE CL (contains 100 ppm Palmitoyl Oligopeptide as Pal GHK).

Centre International de Toxicologie. 1997. Summary of acute oral toxicity in rats BIOPEPTIDE CL (contains 100 ppm Palmitoyl Oligopeptide as Pal GHK).

Anonymous. 1993. Summary of skin sensitization test in guinea pigs BIOPEPTIDE CL (contains 100 ppm Palmitoyl Oligopeptide as Pal GHK).

Centre International de Toxicologie. 1997. Summary of local tolerance study after repeated topical application for 2 weeks in guinea pigs BIOPEPTIDE CL (contains 100 ppm Palmitoyl Oligopeptide as Pal GHK).

Information on Mixtures Containing Pal VGVAPG

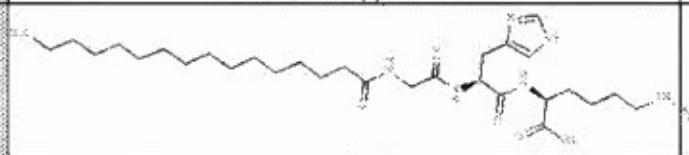
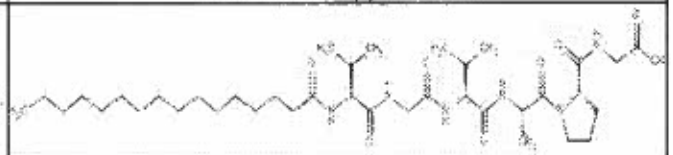
Institut D'Expertise Clinique. 2003. Ocular primary tolerance of DERMAXYL (contains 200 ppm Palmitoyl Oligopeptide as Pal VGVAPG).

Institut D'Expertise Clinique. 2004. Dermal tolerance stud of DERMAXYL (contains 200 ppm Palmitoyl Oligopeptide as Pal VGVAPG).

Centre International de Toxicologie. 1994. Acute dermal irritation in rabbits BIOPEPTIDE EL (contains 100 ppm Palmitoyl Oligopeptide as Pal VGVAPG).

Centre International de Toxicologie. 1994. Acute eye irritation in rabbits BIOPEPTIDE EL (contains 100 ppm Palmitoyl Oligopeptide as Pal VGVAPG).

Sederma, 2012

INCI name	Palmitoyl Oligopeptide	
INCI Monograph ID	7147	
Trade names of SEDERMA mixtures from PcPc website	Bio-Bustyl Biopeptide-CL Biopeptide-EL Dermaxyl Helioxyl Matrixyl 3000 MAXI-LIP REGESTRIL	
Technical name from PcPc website	/	
Trade Name	/	
Other Names	**Pal GHK** (Pal-Gly-His-Lys-OH)	**Pal VGVAPG** (Pal-Val-Gly-Val-Ala-Pro-Gly-OH)
Chemical Name	L-Lysine, N-(1-oxohexadecyl)glycyl-L-histidyl-	Glycine, N-(1-oxohexadecyl)-L-valylglycyl-L-valyl-L-alanyl-L-prolyl
Cas Number	147732-56-7	171263-26-6
Appearance	White Powder	White Powder
Formula	C30 H54 N8 O5	C38 H66 N8 O8
Molecular Weight	578.80	737.00
Log P (estimated)	4.81	5.09
EPI suite	KOWWIN v.1.68 estimates	KOWWIN v.1.68 estimates
Dermal absorption	The following criteria were proposed by De Heer (1999) to discriminate between chemicals with high and low dermal absorption: - 10% dermal absorption is used in case MW > 500 and log Pow is smaller than -1 or higher than 4, otherwise - 100% dermal absorption is used. De Heer C, Wilschut A, Stevenson H, Hakkert BC (1999): Guidance document on the estimation of dermal absorption according to a tiered approach. An update. TNO report No. V98.1237. TNO Nutrition and Food Research Institute, Zeist, The Netherlands.	
DA (%)	10	10
Manufacturing Process	This compound is synthesized by stepwise peptide synthesis. The C-terminal aminoacid (Lys) is protected on its acidic function, then each protected aminoacid (Gly, His) is coupled. A last coupling procedure is realised with palmitic acid instead of an aminoacid and the protected peptide is deprotected to remove the protecting group presents on the lateral function of Lysine and Histidine and on C-terminal acidic function of Lys.	This product is synthesized by stepwise solid phase peptide synthesis. The C-terminal aminoacid (Gly) is protected on its acidic function, then each protected aminoacid (Pro-Ala-Val-Gly-Val) is coupled. A last coupling procedure is realized with palmitic acid instead of an aminoacid and the protected peptide is deprotected to remove the protecting groups present on the lateral function of Proline, Alanine, Valine, Glycine and Valine and on C-terminal acidic function of
Impurities	Acetate content < 5% Palmitic acid < 5% Water content < 5% Residual solvents comply with ICH Q3C	Acetate content < 5% Palmitic acid < 5% Water content < 5% Residual solvents comply with ICH Q3C
Formula		
Safety data	Please find safety data packages for 2 mixtures MAXI-LIP and BIOPEPTIDE CL which contain 1000ppm and 100ppm of Pal GHK, respectively **MAXI-LIP (1000ppm) - Safety Data** - Toxicological assessment and certificate - Reverse Mutation Study - AMES test (Report n° 99T1557000), February 2000 : **Not mutagenic** on strains TA98, TA100, TA1535, TA1537 and TA1538. - Ocular Tolerance Assessment - HET CAM (Report n° 91178RD2), January 2000 : **Slightly irritant** - Primary Cutaneous Tolerance - Patch test (Report n° 91178RD2), January 2000 : **Very well tolerated** - Repeated Insult Patch Test - HRIPT (Report n° C99-1266.03), February 2000 : **No irritation and No sensitization** **BIOPEPTIDE CL (100ppm) - Safety Data** - Evaluation of the cutaneous primary irritation index in rabbits (Report n° 15129 TAL), March 1997 : **Non-irritant** - Acute eye irritation in rabbits (Report n° 15130TAL), March 1997 : **Slightly irritant.** - Local tolerance study after repeated topical application for 2 weeks in guinea-pigs (Report n° 15133TSG), June 1997 : **Non-irritant** - Skin sensitization test in guinea-pigs - Magnusson and Kligman (Report n° 9440TSG), January 1993 : **No sensitization** - Reverse Mutation Study - AMES test (Report n° 9484MMO), December 1997 : **Not mutagenic** on strains TA1535, TA1537, TA102, TA98 et TA100.	Please find safety data packages for 2 mixtures DERMAXYL and BIOPEPTIDE EL which contain 200ppm and 100ppm of Pal VGVAPG, respectively. **DERMAXYL (200ppm) - Safety Data** - Toxicological assessment and certificate - Ocular Primary Tolerance - Neutral Red (Report n° 031251RD), October 2003 : **Unimportant cytotoxicity** - Ocular Primary Tolerance - HET CAM (Report n° 031251RD), October 2003 : **Pratically non-irritant** - Primary Cutaneous Tolerance - Patch test (Report n° 031251RD), October 2003 : **Very well tolerated** - Repeated Insult Patch Test - HRIPT (Report n° B031337RD), January 2004 : **No irritation and No sensitization** **BIOPEPTIDE EL (100ppm) - Safety Data** - Acute Dermal Irritation in rabbits (Report n°11190 TAL), February 1994 : **non-irritant** - Acute eye irritation in rabbits (Report n° 11191 TAL), February 1994 : **non-irritant**
Other safety information	Pal GHK has been used up to 1000 ppm in several Sederma's products and widely supplied since 1992 in The EU, The US, Canada, Korea, Japan, Australia without any complaint concerning their innocuity.	Pal VGVAPG has been used up to 200 ppm in several Sederma's products and widely supplied since 1994 in The EU, The US, Canada, Korea, Japan, Australia without any complaint concerning their innocuity.

Lab No. 99T 15570 00

MG019-223

P.O. No. 00991475

ORIGINAL NUMBER 1

REVISED REPORT

REVISED PAGE

STUDY TITLE:

GENOTOXICITY: *SALMONELLA TYPHIMURIUM*

REVERSE MUTATION STUDY

TEST ARTICLE:

LEV 99122

Trade name : MAXI-LIP - contains 1000 ppm Palmitoyl Oligopeptide as Pal GHK

TEST FACILITY:

NAMSA
2261 Tracy Road
Northwood, OH 43619-1397

SPONSOR:

PIERRE FERRANDON
SEDERMA
29 RUE DU CHEMIN VERT BP 33
LE PERRAY EN YVELINES, FRANCE

NAMSA
Ensuring Medical Device Safety and Compliance™

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SUMMARY

A *Salmonella typhimurium* reverse mutation standard plate incorporation study was conducted to determine whether a ethanol test article solution would cause mutagenic changes in histidine-dependent *Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537, and TA1538 in the presence and absence of S9 metabolic activation. The methodology of Ames *et al* (1975) was followed using a ethanol test article solution.

The ethanol test article solution (prepared the day of the assay) was found to be non-inhibitory to growth of tester strains TA98, TA100, TA1535, TA1537, and TA1538. Separate tubes containing 2 ml of molten top agar supplemented with histidine-biotin solution were inoculated with 0.1 ml of culture for each of five tester strains, and 0.1 ml of the ethanol test article solution. A 0.5 ml aliquot of S9 homogenate simulating metabolic activation was added when necessary. The mixture was poured across triplicate Minimal E plates. Parallel testing was also conducted with a negative control, and four positive controls. The mean number of revertants of the triplicate test plates were compared to the mean number of revertants of the triplicate negative control plates for each of the five tester strains employed. The values (means) obtained for the positive controls were used as points of reference.

Under the conditions of this assay, the ethanol test article solution was not considered to be mutagenic to *Salmonella typhimurium* tester strains. The negative and positive controls performed as anticipated.

Study and Supervisory Personnel: Cherise M. McCoy, BS
Anthony M. Jackson, BA

Approved by: Angela Watson 2-25-00
Angela M. Watson, PhD
Manager, In Vitro Toxicology
Date Completed

This report has been revised to delete the 95% from the test solution or vehicle and issue original #1 and original #2 and to clarify information in the introduction and material section. The conclusions are not affected. This revision is authorized by signature above.

/las

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 1003U

IEC Japan

IEC France

IEC Singapour

INSTITUT D'EXPERTISE CLINIQUE

Trade Name : **MAXI-LIP** contains 1000ppm Palmitoyl Oligopeptide as Pal GHK

REPORT: IN VITRO AND TOLERANCE STUDIES

SPONSOR : **SEDERMA**

IN VITRO STUDY : **OCULAR TOLERANCE ASSESSMENT**

IN VITRO STUDY REALISED
ON HEN'S EGG CHORION-ALLANTOIC MEMBRANE
FOR ASSESSING OCULAR TOLERANCE
(According to the HET CAM protocol published in the J.O.R.F., dated 26 December 1996)

CLINICAL STUDY : **EVALUATION OF THE PRIMARY CUTANEOUS TOLERANCE**

VERIFICATION OF THE GOOD EPICUTANEOUS LOCAL
TOLERANCE, AFTER A SINGLE APPLICATION
TO THE SKIN OF THE BACK AND UNDER
OCCLUSIVE PATCH FOR 48 HOURS,
IN 10 HEALTHY ADULT VOLUNTEERS
(Single patch test)

TEST ARTICLE : **LEV 99122**

PROTOCOL : N° 90613PE, of 17 June 1999

REPORT : N° 91178RD2, of 31 January 2000

Study Monitor :
Mr. P. FERRANDON
SEDERMA
29 rue du Chemin Vert
B.P 33
78610 LE-PERRAY-EN-YVELINES - FRANCE

Clinical Investigator :
Dr. M. JONAS – BRUN, M.D.
Dermatologist
I.E.C.
88, boulevard des Belges
69006 LYON - France

Study Director :
Mr. J.R. CAMPOS
Doctor in Cellular Biology and Microbiology
Graduate in Dermocosmetology
I.E.C.
87, rue de Sèze
69006 LYON - France

12 page-document

AUTHENTICATION

The study subject of the present report was conducted under my responsibility, in compliance with the experimental protocol, the procedures of the Biological Research Facility and the regulations of the Good Laboratory Practices.

All observations and numerical data obtained during this study are reported in the present document. After reading, I certify that these data are an accurate reflexion of the results obtained.

Jean Robert CAMPOS
Study Director

I read this report and I agree with its content.

Etienne CAMEL
Technical Manager

As a conclusion,

According to the classification published in the J.O.R.F. :

- The positive control (Sodium Dodecyl Sulfate at 0.5% (W/V)) is **irritant at the ocular level.**

- The test article designated as **"LEV 99122"**, studied as supplied, is **slightly irritant at the ocular level.**

Lyon,
31 January 2000

J.P. GUILLOT
Senior Toxicologist - Pharmacologist
I.E.C. Manager

J.R. CAMPOS
Doctor in Cellular Biology and Microbiology
Graduate in Dermocosmetology
Study Director

PROTOCOL

The test article was applied as supplied, once only, at the dose level of about 0.02 ml per volunteer, on a surface of about 50 mm² of skin on the back of 10 volunteers. The test article was kept in contact with the skin under an occlusive patch (Finn Chambers on Scanpor) for 48 consecutive hours. This application was performed in parallel and under the same conditions with a patch alone (without test article), as "negative" control.

Cutaneous clinical examinations were performed about 30 minutes after removal of the patches. Evaluation of the reactions was made according to a given numerical scale.

The values obtained allowed interpretation of the results according to the type of test article.

RESULTS AND CONCLUSION

No reaction of pathological irritation and significant of a cutaneous intolerance was noted. No secondary effect was observed.

The index of Primary Cutaneous Irritation (P.C.I.) was equal to 0.

From the results obtained under the experimental conditions used, the single application of this test article to the skin of the back and under occlusive patch for 48 hours, in the healthy adult volunteer, may be considered as : VERY WELL TOLERATED.

Lyon,
31 January 2000

J.P. GUILLOT
Senior Toxicologist - Pharmacologist
I.E.C. Manager

Dr. M. JONAS - BRUN, M.D.
Post graduate in dermatology
Investigator
Study Director

CABINET DE CONSULTANT ET D'EXPERTISE

Jean-Pierre GUILLOT

Expert Toxicologue - Pharmacologue
Expert auprès de la D.G.C.C.R.F.
(Répression des Fraudes)
Expert national à l'O.C.D.E.
Eurotox Registered Toxicologist

TRADE NAME : **MAXI-LIP**

ATTESTATION

On request of the Company **SEDERMA**, we have examined the dossier for the evaluation of the primary tolerance of the test article designated :

"LEV 99122"

Examination of the information included in this dossier concerned principally :
- the normal conditions of use,
- the attestation of the manufacturer, stating that the formula to be studied was elaborated in conformity with the regulations in effect,
- the results of the cutaneous and ocular primary tolerance tests.

This examination allows us to ascertain that, to the best of our knowledge, this test article may be considered as **"WELL TOLERATED"**, as regards its ocular primary tolerance, and **"VERY WELL TOLERATED"**, as regards its cutaneous primary tolerance.

Bessenay, 31 January 2000

J.P. GUILLOT
Senior Toxicologist - Pharmacologist

Route de Bibost - 69690 BESSENAY - FRANCE - Tél. : (0)4 74 70 93 39 - Fax : (0)4 74 70 94 98
Tél. international : + 33 4 74 70 93 39 - Fax international : +33 4 70 94 98
e-mail : info@iec.fr - Internet : //www.iec.fr

Consumer Product Testing Co.

EST. 1975

FINAL REPORT

Trade Name : MAXI - LIP contains 1000 ppm Palmitoyl Oligopeptide as Pal GHK

CLIENT:
SEDERMA
29, Rue Du Chemin Vert – BP 33
78610 LE PERRAY-EN-YVELINES
CEDEX - FRANCE

ATTENTION:
Dr. Pierre Ferrandon, Ph.D.
Scientific Coordinator

TEST:
Repeated Insult Patch Test
Protocol No.: 1.01

TEST MATERIAL:
LEV 99122

EXPERIMENT REFERENCE NUMBER:
C99-1266.03

Richard R. Eisenberg, M.D.
Board Certified Dermatologist

Michael J. Frentzko, B.A.
Director, Clinical Evaluations

Robert W. Shanahan, Ph.D.
Principal Investigator

Joy Frank, R.N.
Study Director

70 New Dutch Lane • Fairfield, New Jersey 07004-2514 • (973) 808-7111 • Fax (973) 808-7234

Consumer Product Testing Co.

EST. 1975

QUALITY ASSURANCE UNIT STATEMENT

Study No.: C99-1266.03

The objective of the Quality Assurance Unit (QAU) is to monitor the conduct and reporting of clinical laboratory studies. The QAU maintains copies of study protocols and standard operating procedures and has inspected this study on the date(s) listed below. Studies lasting six months or more are inspected at time intervals to assure the integrity of the study. The findings of such inspections are reported to management and the Study Director. All materials and data pertinent to this study will be stored or disposed of in accordance with current Standard Operating Procedures.

Date(s) of inspection:
December 28, 1999
January 10, 2000
February 15, 2000
February 17, 2000

Senior personnel involved:

OnChi Cheung, B.S. - Quality Assurance Associate

Beatrice Ongige, B.S. - Quality Assurance Associate

Kathleen Alworth, B.A. 2/18/00
Director of Quality Assurance

The representative signature of the Quality Assurance Unit signifies that this study has been performed in accordance with standard operating procedures and study protocol as well as government regulations regarding such procedures and protocols as outlined in the Federal Register (Vol. 46, No. 17 of Tuesday, January 27, 1981).

70 New Dutch Lane • Fairfield, New Jersey 07004-2514 • (973) 808-7111 • Fax (973) 808-7234
Clinical • Toxicology • Analytical Chemistry • Microbiology

Methodology (continued):

Induction Phase:

Patches were applied three (3) times per week (e.g., Monday, Wednesday, and Friday) for a total of nine (9) applications. The site was marked to ensure the continuity of patch application. Following supervised removal and scoring of the first Induction patch, participants were instructed to remove all subsequent Induction patches at home, twenty-four hours after application. The evaluation of this site was made again just prior to re-application. If a participant was unable to report for an assigned test day, one (1) makeup day was permitted. This day was added to the Induction period.

With the exception of the first supervised Induction Patch reading, if any test site exhibited a moderate (2-level) reaction during the Induction Phase, application was moved to an adjacent area. Applications are discontinued for the remainder of this test phase, if a moderate (2-level) reaction was observed on this new test site. Applications would also be discontinued if marked (3-level) or severe (4-level) reactivity was noted.

Rest periods consisted of twenty-four hours following each Tuesday and Thursday removal, and forty-eight hours following each Saturday removal.

Challenge Phase:

Approximately two (2) weeks after the final Induction patch application, a Challenge patch was applied to a virgin test site adjacent to the original Induction patch site, following the same procedure described for Induction. The patch was removed and the site scored at the clinic twenty-four and seventy-two hours post-application.

Evaluation Key:

0 = No visible skin reaction
\+ = Barely perceptible or spotty erythema
1 = Mild erythema covering most of the test site
2 = Moderate erythema, possible presence of mild edema
3 = Marked erythema, possible edema
4 = Severe erythema, possible edema, vesiculation, bullae and/or ulceration

Results:

The results of each participant are appended (Table 1).

Barely perceptible (+) to moderate (2) patch test responses were observed during the Induction and/or Challenge test phases. None of these generally transient, low-level responses were considered clinically significant.

Summary: **Under the conditions of this study, test material, LEV 99122, did not indicate a clinically significant potential for dermal irritation or allergic contact sensitization.**

Fifty seven (57) subjects, male and female, ranging in age from 16 to 79 years, were selected for this evaluation. Fifty two (52) subjects completed this study. The remaining subjects discontinued their participation for various reasons, none of which were related to the application of the test material.

SPONSOR
Société Séderma
29 rue du Chemin Vert
B.P. 33
78610 Le-Perray-en-Yvelines CEDEX
France

STUDY TITLE
EVALUATION OF THE CUTANEOUS PRIMARY IRRITATION INDEX IN RABBITS

TEST SUBSTANCE
BIOPEPTIDE CL
Contains 100 ppm Palmitoyl Oligopeptide as Pal GHK

STUDY DIRECTOR
Xavier Manciaux

STUDY COMPLETION DATE
5 March 1997

PERFORMING LABORATORY
Centre International de Toxicologie (C.I.T.)
Miserey - 27005 Evreux - France

LABORATORY STUDY NUMBER
15129 TAL

SUMMARY

At the request of Société Séderma, Le-Perray-en-Yvelines, France, the potential of the test substance BIOPEPTIDE CL to induce dermal irritation was evaluated in rabbits. The study was conducted in compliance with the Principles of Good Laboratory Practice Regulations.

Methods

A single dose of 0.5 ml of the test substance in its original form was prepared on a dry gauze pad and then applied to a 6 cm^2 scarified or non-scarified clipped area of the skin of three male New Zealand White rabbits.

The test substance was held in contact with the skin for 24 hours by means of an occlusive hypoallergenic dressing. Cutaneous reactions were observed approximately 24 and 72 hours after application of the test substance.

No residual test substance was observed after removal of the dressing.

The mean score of the values for erythema and oedema recorded for all animals after 24 and 72 hours was calculated to obtain the Cutaneous Primary Irritation index (C.P.I.).

Results

Only a slight erythema was noted at the 24-hour reading on both flanks of two animals.

No other cutaneous reactions were observed during the study.

The C.P.I. index was: 0.3.

Conclusion

Under our experimental conditions, the test substance BIOPEPTIDE CL was considered non-irritant when administered by cutaneous route in rabbits.

STUDY No. 9484 MMO

BIOPEPTIDE-CL

contains 100ppm Palmitoyl oligopeptide as Pal GHK

REVERSE MUTATION ASSAY
BY THE AMES TEST

ADDRESSEE:
Sederma
Mr. Lintner
29 rue du Chemin Vert
B.P. 33
78610 Le Perray-en-Yvelines Cédex
France

DATE: 11.12.92

STUDY No. 9484 MMO

SUMMARY

The in vitro potential mutagenic activity of the test substance BIOPEPTIDE-CL was investigated by the Ames test using 5 strains of bacteria Salmonella typhimurium: TA 1535, TA 1537, TA 102, TA 98 and TA 100. This test enables the detection of base-pair substitution and frameshift mutagens.

After a preliminary assay to define the concentrations to be used for the mutagenicity study, the test substance was tested on two independent assays. Each assay was carried out both in the absence and in the presence of a metabolic activation system, the S9 mix, prepared from a liver microsomal fraction S9 of rats treated with Aroclor 1254. The methods used were:

- the direct plate incorporation method for the 2 assays without S9 mix and for the first assay with S9 mix,
- the preincubation method (1 h, 37°C) for the second assay with S9 mix.

The concentrations were with and without S9 mix: 312.5, 625, 1250, 2500 and 5000 µg/plate.

The negative and solvent control results were equivalent to those usually obtained in our Laboratory. The number of revertants induced by the positive controls was higher than the spontaneous one, which demonstrated the sensitivity of this test and the efficacy of the S9 mix throughout this study.

The test substance BIOPEPTIDE-CL did not induce any significant increase in the revertant number with or without S9 mix in any of the 5 strains.

In conclusion, under our experimental conditions, the test substance BIOPEPTIDE-CL did not show mutagenic activity in the Ames test.

SPONSOR
Société Séderma
29 rue du Chemin Vert
B.P. 33
78610 Le-Perray-en-Yvelines CEDEX
France

STUDY TITLE
ACUTE EYE IRRITATION
IN RABBITS

TEST SUBSTANCE
BIOPEPTIDE CL

Contains 100 ppm Palmitoyl oligopeptide as Pal GHK

STUDY DIRECTOR
Xavier Manciaux

STUDY COMPLETION DATE
14 March 1997

PERFORMING LABORATORY
Centre International de Toxicologie (C.I.T.)
Miserey - 27005 Evreux - France

LABORATORY STUDY NUMBER
15130 TAL

SUMMARY

At the request of Société Séderma, Le-Perray-en-Yvelines, France, the ocular irritation that could be induced by the test substance BIOPEPTIDE CL was evaluated in rabbits. The study was conducted in compliance with the Principles of Good Laboratory Practice Regulations.

Methods

A single dose of 0.1 ml of the test substance in its original form was instilled into the conjunctival sac of the left eye of three male New Zealand White rabbits.

The eyes were not rinsed after administration of the test substance. Ocular reactions were observed approximately 1 hour, 24, 48 and 72 hours after the administration and then on days 5 and 8.

The Maximum Ocular Irritation index (Ma.O.I.) was calculated.

Results

Only very slight or slight conjunctival reactions (chemosis and redness) were noted in all animals on day 1.

No other ocular reactions were observed during the study.

The Ma.O.I. index was 4.7 on day 1.

Conclusion

Under our experimental conditions, the test substance BIOPEPTIDE CL was considered slightly irritant when administered by ocular route in rabbits.

SPONSOR
Société Séderma
29 rue du Chemin Vert
B.P. 33
78610 Le-Perray-en-Yvelines CEDEX
France

STUDY TITLE
ACUTE ORAL TOXICITY
IN RATS

TEST SUBSTANCE
BIOPEPTIDE CL

contains 100 ppm Palmitoyl oligopeptide
as Pal GHK

STUDY DIRECTOR
Xavier Manciaux

STUDY COMPLETION DATE
5 March 1997

PERFORMING LABORATORY
Centre International de Toxicologie (C.I.T.)
Miserey - 27005 Evreux - France

LABORATORY STUDY NUMBER
15127 TAR

SUMMARY

At the request of Société Séderma, Le-Perray-en-Yvelines, France, the acute oral toxicity of the test substance BIOPEPTIDE CL was evaluated in rats according to O.E.C.D. (No. 401, 24th February 1987) and E.C. (92/69/E.E.C., B_1, 31st July 1992) guidelines. The study was conducted in compliance with the Principles of Good Laboratory Practice Regulations.

Methods

The test substance was administered by oral route to one group of ten fasted Sprague-Dawley rats (five males and five females).

The test substance was administered in its original form, by gavage, at a dose of 2000 mg/kg, taking into consideration that its density was 1.13.

Clinical signs, mortality and body weight gain were checked for a period of 14 days following the single administration of the test substance.

All animals were subjected to necropsy.

The interpretation of results was carried out according to the classification criteria laid down in Council Directive 93/21/E.E.C. (27th April 1993) adapting to technical progress for the eighteenth time Council Directive 67/548/E.E.C.

Results

The general behaviour and body weight gain of the animals were not affected by treatment with the test substance.

No death occurred at 2000 mg/kg.

No apparent abnormalities were observed at necropsy.

Conclusion

Under our experimental conditions, the oral LD_{50} of the test substance BIOPEPTIDE CL was higher than 2000 mg/kg in rats. No signs of toxicity were observed at this dose.

Classification

Concerning the potential toxicity by oral route, according to Commission Directive 93/21/E.E.C., the test substance BIOPEPTIDE CL should not be classified.

STUDY No. 9440 TSG

BIOPEPTIDE-CL
contains 100ppm Palmitoyl Oligopeptide as Pal GHK
SKIN SENSITIZATION TEST

IN GUINEA-PIGS

(Maximization method of Magnusson and Kligman)

Addressee

Société Séderma
Mr. Lintner
29, rue du Chemin Vert
B.P. 33
78610 Le-Perray-en-Yvelines
France

Date: 20.1.93

STUDY No. 9440 TSG

SUMMARY

At the request of Société Séderma, Le-Perray-en-Yvelines, France, the sensitization potential of the test substance BIOPEPTIDE-CL was evaluated in guinea-pigs by intradermal injection and cutaneous application, according to the maximization method of Magnusson and Kligman (1), O.E.C.D. Guideline No. 406 and the Principles of Good Laboratory Practice (O.E.C.D., 12th May 1981).

Methods

Thirty guinea-pigs (15 males and 15 females) were allocated to 2 groups: a control group (5 males and 5 females) and a treated group (10 males and 10 females).

The sensitization potential of the test substance was evaluated after a 10-day induction period during which time the animals were treated with an isotonic solution of 0.9% NaCl (control group) or the test substance (treated group). On day 1, in presence of Freund's adjuvant, 0.1 ml of the test substance at a concentration of 1% in the vehicle was administered by intradermal route. On day 8, 0.5 ml of the test substance in its original form was applied by cutaneous route. After a period of 12 days without treatment, a challenge cutaneous application of 0.5 ml of the vehicle (left flank) and 0.5 ml of the test substance at the maximal non-irritant concentration of 75% in the vehicle (right flank) were administered to all the animals. The test articles were prepared on a dry compress then applied to the skin and held in place for 24 hours by means of an occlusive dressing. Cutaneous reactions on the challenge application site were then evaluated 24 and 48 hours after removal of the dressing.

After the final scoring period, the animals were sacrificed and cutaneous samples were taken from the challenge application sites from all the animals. No histological examination was performed on the cutaneous samples.

Reference

(1) **Magnusson, B.; Kligman, A.M.:** The identification of contact allergens by animal assay. The guinea pig maximization test. J. Invest. Derm. 52: 268-276 (1969).

Results

No clinical signs or deaths occurred during the study.

The body weight gain of the treated animals was unaffected by administration of the test substance.

No cutaneous reactions were observed 24 and 48 hours after removal of the dressing of the challenge cutaneous application of the test substance.

Conclusion

Under our experimental conditions and according to the maximization method of Magnusson and Kligman, no cutaneous reaction attribuable to the sensitization potential of the test substance BIOPEPTIDE-CL at the maximal non-irritant concentration of 75% was observed in guinea-pigs.

SPONSOR
Société Séderma
29 rue du Chemin Vert
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78610 Le-Perray-en-Yvelines Cédex
France

STUDY TITLE
LOCAL TOLERANCE STUDY AFTER REPEATED TOPICAL APPLICATION FOR 2 WEEKS IN GUINEA-PIGS

TEST SUBSTANCE
BIOPEPTIDE CL

contains 100 ppm Palmitoyl oligopeptide as Pal GHK

STUDY DIRECTOR
Xavier Manciaux

STUDY COMPLETION DATE
25 June 1997

PERFORMING LABORATORY
Centre International de Toxicologie (C.I.T.)
Miserey - 27005 Evreux - France

LABORATORY STUDY NUMBER
15133 TSG

SUMMARY

At the request of Société Séderma, Le-Perray-en-Yvelines, France, the effects of repeated application of the test substance BIOPEPTIDE CL to the skin was evaluated in guinea-pigs. The study was conducted in compliance with the Principles of Good Laboratory Practice Regulations.

Methods

A volume of 0.05 ml of the test substance in its original form was applied to the left flank of ten guinea-pigs (five males and five females) once daily, at approximately the same time each day, for 14 consecutive days (days 1 to 14).
The test substance was applied over the same area of clipped skin, measuring approximately 2 cm x 2 cm. The test site was not covered by a dressing.
The right flank received purified water under the same experimental conditions.
Cutaneous reactions were evaluated in each animal immediately before each application and approximately 24 hours after the last application by comparing the reactions on both flanks.
Photographs of the treated application sites of each animal were performed immediately before treatment on day 1 then on days 5, 9, 12 and 15.

At the end of the observation period, the animals were killed and cutaneous samples were taken from the treated sites.

Results

No clinical signs and no mortality were noted during the study.

No cutaneous reactions were observed during the study. Only a very slight beige colouration of the skin was noted in all animals.

The Maximum Weekly Mean Irritation Index was 0.0.

Conclusion

Under our experimental condition, the repeated application for 14 days of the test substance BIOPEPTIDE CL failed to induce any skin irritation in guinea-pigs.

Classification

According to the obtained Maximum Weekly Mean Irritation Index, the test substance BIOPEPTIDE CL could be classified as Non-Irritant.

IEC France

INSTITUT D'EXPERTISE CLINIQUE

IEC JAPAN - IEC SINGAPORE - IEC KOREA - IEC BULGARIE - IEC ARGENTINA

REPORT : IN VITRO AND CLINICAL COMPATIBILITY STUDIES

SPONSOR : **SEDERMA**

IN VITRO STUDIES : **OCULAR PRIMARY TOLERANCE**

HET CAM

STUDY REALISED
ON HEN'S EGG CHORION-ALLANTOIC MEMBRANE
(According to the protocol published in the J.O.R.F. of 26 December 1996)

NEUTRAL RED RELEASING METHOD

STUDY REALISED ON THE SIRC FIBROBLASTIC CELL LINE
(According to the protocol published in the J.O.R.F. of 30 December 1999)

CLINICAL STUDY : **CUTANEOUS COMPATIBILITY**

VERIFICATION OF THE GOOD CUTANEOUS COMPATIBILITY, AFTER A SINGLE APPLICATION TO THE SKIN OF THE BACK AND UNDER OCCLUSIVE PATCH FOR 48 HOURS, IN 10 ADULT VOLUNTEERS
(Single patch test)

TEST ARTICLE : **DERMAXYL / FON 01178** (batch n° DERXYLV 1), as supplied or diluted to 50% Contains 200 ppm Palmitoyl oligopeptide as Pal VGVAPG

PROTOCOL : N° 031782D, of 24 March 2003

REPORT : N° 031251RD, of 21 October 2003

Study Monitor :
Mrs. C. MAS-CHAMBERLAIN
Scientific Manager
SEDERMA
29, rue du Chemin Vert
B.P. 33
78610 LE PERRAY-EN-YVELINES - FRANCE

Clinical Investigator :
Dr. B. BISBAL, M.D.
Dermatologist
I.E.C.
88, boulevard des Belges
69006 LYON - FRANCE

Head of in vitro :
(Study Director)
Mr. J.R. CAMPOS
Doctor in Cellular Biology and Microbiology
Graduate in Dermocosmetology
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69006 LYON - FRANCE

21 page-document

The observed phenomena were quantified according to the following table, according to their time of coming :

	TIME OF COMING		
PHENOMENON	t ≤ 30 sec	30 sec < t ≤ 2 min	2 min < t ≤ 5 min
Hyperaemia	5	3	1
Haemorrhage	7	5	3
Coagulation*	9	7	5

* Coagulation = opacity and/or thrombosis.

The score for each egg was determined by the sum of the notations of hyperaemia, haemorrhage and coagulation. The notation of the studied test article corresponded to the arithmetic mean, rounded off to one decimal of the scores obtained for 4 eggs ; the maximum notation being 21.

The irritant potential on the CAM of the test article (as supplied or diluted) was given by the Classification below :

NOTATION	ASSESSMENT
N < 1	Practically Non Irritant
1 ≤ N < 5	Slightly Irritant
5 ≤ N < 9	Moderately Irritant
N ≥ 9	Irritant

RESULTS AND CONCLUSION

According to the experimental conditions adopted, the study aiming at assessing ocular primary tolerance by HET CAM allowed to obtain the following results :

Positive Control : Sodium Dodecyl Sulfate (0.5% (W/V))

Mean Irritation Index = 12.0

Test article : diluted to 50% (W/V) in distilled water

Mean Irritation Index = 0.8

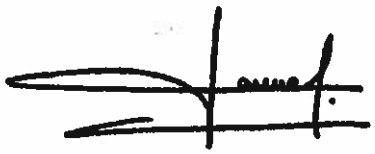
In conclusion,

According to the classification published in the J.O.R.F. :

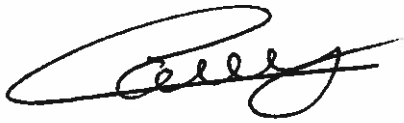
- The positive control (Sodium Dodecyl Sulfate at 0.5% (W/V)) is **irritant at ocular level.**

- The test article, diluted to 50% (W/V) in distilled water, is **practically non irritant at ocular level.**

Lyon,
21 October 2003



E. CAMEL
Pharm. D., D.E.A.
Senior Toxicologist
(Eurotox Registered Toxicologist)
General Manager
(Director of the Trial Installation)



J.R. CAMPOS
Doctor in Cellular Biology
and Microbiology
Graduate in Dermocosmetology
Head of in vitro
(Study Director)

This study was conducted by INSTITUT D'EXPERTISE CLINIQUE (I.E.C.),
managed by Mr. J.P. GUILLOT, Senior Toxicologist (Eurotox Registered Toxicologist).

RESULTS AND CONCLUSION

According to the experimental conditions used, the study aiming at assessing ocular primary tolerance by the Neutral Red Releasing Test allowed to obtain the following results :

Quality Control : Sodium Dodecyl Sulfate (0.01 – 0.05 – 0.2% (W/W))

- 1st step : C.I. 50 (in %) = 0.12

Negative control : Sodium Chloride at 0.9% (W/V)

- 1st step : D.O. (540 nm) = 0.959

The C.I. 50 of the Quality Control and the D.O. of the Negative Control enabled validation of the study.

Test article : as supplied

- 1st step : estimation of the C.I. 50 :
C.I. 50 (in %) = > 50
% of mortality at dilution 50% = 37.9

In conclusion,

According to the classification published in the J.O.R.F. :

The test article, as supplied, presents with a **"unimportant cytotoxicity".**

Lyon,
21 October 2003

E. CAMEL
Pharm. D., D.E.A.
Senior Toxicologist
(Eurotox Registered Toxicologist)
General Manager
(Director of the Trial Installation)

J.R. CAMPOS
Doctor in Cellular Biology and Microbiology
Graduate in Dermocosmetology
Head of in vitro
(Study Director)

This study was conducted by INSTITUT D'EXPERTISE CLINIQUE (I.E.C.), managed by Mr. J.P. GUILLOT, Senior Toxicologist (Eurotox Registered Toxicologist).

RESULTS AND CONCLUSION

No reaction of pathological irritation and significant of a cutaneous intolerance was noted.
No secondary effect was observed.

The index of Primary Cutaneous Irritation (P.C.I.) was equal to 0.

The individual results are presented in the table page 19.

From the results obtained under the experimental conditions used, the single application of this test article, diluted to 50%, to the skin of the back and under occlusive patch for 48 hours, in the adult volunteer, can be considered as VERY WELL TOLERATED.

Lyon,
21 October 2003

E. CAMEL **Pharm. D., D.E.A.** **Senior Toxicologist** **(Eurotox Registered Toxicologist)** **General Manager**	**E. GRACIANNETTE** **Post graduate in Industrial Biology (E.B.I.)** **Responsible for the Study**	**Dr. B. BISBAL, M.D.** **Dermatologist** **Clinical Investigator**

This study was conducted by INSTITUT D'EXPERTISE CLINIQUE (I.E.C.),
registered by the French Ministry of Health, under number 22056 MHC,
and managed by Mr. J.P. GUILLOT, Senior Toxicologist (Eurotox Registered Toxicologist).

CABINET DE CONSULTANT ET D'EXPERTISE

Jean-Pierre GUILLOT

Expert Toxicologue - Pharmacologue
Membre de la C.G. d'U.M.A. de la D.G.C.C.R.F.
(Répression des Fraudes)
Expert national à l'O.C.D.E.
Eurotox Registered Toxicologist

ATTESTATION

On request of the Company **SEDERMA**, we have examined the dossier to verify the ocular and cutaneous compatibility of the test article designated :

"DERMAXYL / FON 01178 (batch n° DERXYLV 1)" as supplied or diluted to 50%

Examination of the information included in this dossier concerned principally :
- the attestation of the manufacturer, stating that this formula was elaborated in conformity with the regulations in effect,
- the normal conditions of use,
- the results of the in vitro tests of ocular primary tolerance,
- the results of the clinical test of cutaneous compatibility.

This examination allows us to ascertain that, to the best of our knowledge, this test article may be considered as :

. **"WELL TOLERATED"**, as regards its ocular primary tolerance
. **"VERY WELL TOLERATED"**, as regards its clinical cutaneous compatibility.

These results allow to plan a more specific verification of the acceptability of the test article at the ocular and cutaneous levels (in-use test : discomfort, cumulative irritation), of sensitisation, of photo-irritation (...), and/or of the justification of the effects claimed.

Bessenay, 21 October 2003

J.P. GUILLOT
Senior Toxicologist - Pharmacologist
(Eurotox Registered Toxicologist)

Route de Bibost - 69690 BESSENAY - FRANCE - Tél. : (0)4 74 70 93 39 - Fax : (0)4 74 70 94 98

IEC France

INSTITUT D'EXPERTISE CLINIQUE

IEC JAPAN - IEC SINGAPORE - IEC KOREA - IEC BULGARIE - IEC ARGENTINA

BULGARIA

REPORT : TOLERANCE STUDY

MANUFACTURER : **SEDERMA**

TEST ARTICLE : **DERMAXYL** (ref. : FON01178 - batch n° DERXYLV1), diluted to 50%
(contains 200ppm Palmitoyl Ohjopeptide as Pal VGVAPG)

CLINICAL STUDY : VERIFICATION OF THE ABSENCE OF **IRRITANT AND SENSITISING POTENTIALS** BY REPEATED EPICUTANEOUS 48 HOUR APPLICATIONS UNDER OCCLUSIVE PATCH, IN 53 HEALTHY ADULT VOLUNTEERS (Marzulli and Maibach's method : Repeated Insult Patch Test)

SPONSOR : Consultancy and Expertise Office J.P. GUILLOT

REPORT : N° B031337RD, of 13 January 2004

Study Request : Protocol n° PF2056, of 24 March 2003
(Order form n° 20031382)

Study Timetable :
- Start of the study : 20 October 2003
- End of observations : 28 November 2003
- End of the study (signature of final report by the Study Director) : 13 January 2004

Study Monitor :
Mme C. MAS-CHAMBERLAIN
Scientific Manager
SEDERMA
29, rue du Chemin Vert
78610 LE PERRAY-EN-YVELINES
- FRANCE

Study Coordinator :
Mr. J.R. CAMPOS
Graduate in Dermocosmetology
Doctor in Cellular Biology
and Microbiology
I.E.C. France
88, boulevard des Belges
69006 LYON - FRANCE

Clinical Investigator
Coordinator :
(Study Director)
Dr. A. POPOVA, M.D.
Dermatologist
I.E.C. Bulgarie
Lozenetz
16A, Kichinev street
1407 SOFIA - BULGARIA

25 page-document

QUALITY CONTROL

This study was conducted in conformity with the standard operating procedures of the Clinical Research Center, the general procedures of I.E.C., the signed protocol and the general principles of the Good Clinical Practices published by I.C.H. (Guideline of 1st March 1996).

The control of the clinical studies is carried out to ensure that all critical phases (test article applications and examinations) of a particular study type are controlled, at least once quarterly, for the studies performed during this time period.

The results of these controls were reported to the Study Director, the Coordinator and the General Management.

This report has been audited by the Quality Control Unit of I.E.C. France and is an accurate account of the procedures followed, and accurately records the original raw laboratory data generated in this study.

	Date of control	**Date of report to the Study Director and the Coordinator**	**Date of report to the General Management**
Report (vs. raw data) :	13 January 2004	13 January 2004	13 January 2004

Signature :

Nicole GUILLOT
Head of Quality Control Unit

Date : 13 January 2004

These examinations were performed, for the 1st, 8th (induction) and 10th (challenge) applications, by comparison to the reactions possibly obtained with a patch alone (without test article), applied in parallel under the same conditions, as "negative" control.
Analysis and interpretation of the results were carried out as a function of the data obtained under the experimental conditions adopted.

- **As regards local cutaneous tolerance,** this analysis was completed by a calculation of the Mean Irritation Index (M.I.I.) equal to the total of the quotations of the 8 readings corresponding to induction, divided by the number of panellists included in this study and the number of readings performed (maximum M.I.I. = 4).

- **As regards evaluation of the sensitising potential,** a reaction whose intensity is equal to 3 (erythema with infiltration, papulae, vesicles) was considered as "positive". If an individual irritation reaction had been noted during the 1st application, or after those corresponding to induction, or if an erythema was observed to the control area (right side), the test article was considered as "positive" if the challenge application had provoked a reaction whose intensity was clearly higher, and/or if it had tended to increase as the readings were performed.

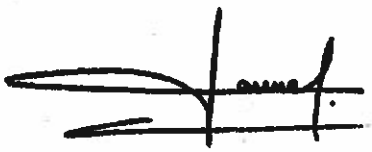
RESULTS AND CONCLUSION

No pathological irritation, nor sensitization reaction significant of a cutaneous intolerance was noted.

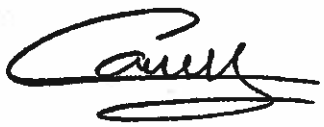
The Mean Irritation Index (M.I.I.), obtained during induction, was equal to 0.04, thus enabling arbitrary classification of the test article applications as "non irritant".

In conclusion and given the results obtained under the experimental conditions adopted, the single and repeated epicutaneous applications of this test article, diluted to 50%, under occlusive patch, in the healthy adult volunteer, did not provoke any primary or cumulative irritation reaction, nor any cutaneous sensitisation.

Lyon and Sofia,
13 January 2004



E. CAMEL
Pharm. D., D.E.A.
Senior Toxicologist
(Eurotox Registered Toxicologist)
General Manager



J.R. CAMPOS
Graduate in Dermocosmetology
Doctor in Cellular Biology and Microbiology
Study Coordinator
I.E.C. France /I.E.C. Bulgarie

Dr. A. POPOVA, M.D.
Dermatologist
Clinical Investigator
Coordinator
Study Director

This study was conducted by INSTITUT D'EXPERTISE CLINIQUE - BULGARIE,
registered by the Bulgarian Health Authorities
Scientific Member of the Board of Directors of I.E.C. Bulgarie :
Professor Rumyana YANKOVA, MD., Ph. D., Head of the Dermatology
and Allergology Department of Plovdiv Medical University.

SPONSOR

Société Séderma
29 rue du Chemin Vert
78610 Le Perray-en-Yvelines Cédex
France

STUDY TITLE

ACUTE DERMAL IRRITATION IN RABBITS

TEST SUBSTANCE

BIOPEPTIDE EL

contains 100 ppm Palmitoyl oligopeptide as Pal VGVAPG

STUDY DIRECTOR

Jack Clouzeau

STUDY COMPLETION DATE

10th February 1994

PERFORMING LABORATORY

Centre International de Toxicologie (C.I.T.)
Miserey - 27005 Evreux - France

LABORATORY STUDY NUMBER

11190 TAL

SUMMARY

At the request of Société Séderma, Le Perray-en-Yvelines, France, potential of the test substance, BIOPEPTIDE EL, to induce dermal irritation was evaluated in rabbits according to O.E.C.D. (No. 404, 12th May 1981) and E.C. (92/69/E.E.C.) guidelines. The study was conducted in compliance with the Principles of Good Laboratory Practice Regulations.

Methods

A single dose of 0.5 ml of the test substance in its original form was prepared on a dry compress and then applied to a 6 cm^2 clipped area of the skin of 3 male New Zealand White rabbits.

The test substance was held in contact with the skin for 4 hours by means of a semi-occlusive dressing. Cutaneous reactions were observed approximately 1, 24, 48 and 72 hours after removal of the dressing.

No residual test substance was observed after removal of the dressing.

The mean score of the values for erythema and oedema recorded for each animal after 24, 48 and 72 hours was calculated.

The interpretation of results was carried out according to the classification criteria laid down in Directive 91/325/E.E.C. Commission Directive of 1st March 1991 adapting to technical progress for the twelfth time Council Directive 67/548/E.E.C.

Results

Moderate cutaneous reactions, which were reversible within 24 or 48 hours after the removal of the dressing, were noted.

On days 3 and 4, no cutaneous reactions were observed during the study.

The mean score for erythema is < 1.0.

No oedema was noted.

Conclusion

As the mean scores for erythema, oedema for 2 out of the 3 animals did not reach the criteria values for irritation, under our experimental conditions, the test substance, BIOPEPTIDE EL, was considered as non-irritant when administered by cutaneous route in rabbits.

Labelling

Commission Directive 91/325/E.E.C.

Labelling not indicated for the test substance.

SPONSOR

Société Séderma
29 rue du Chemin Vert
78610 Le Perray-en-Yvelines Cédex
France

STUDY TITLE

ACUTE EYE IRRITATION IN RABBITS

TEST SUBSTANCE

BIOPEPTIDE EL

contains 100 ppm Palmitoyl Oligopeptide as Pal VGVAPG

STUDY DIRECTOR

Jack Clouzeau

STUDY COMPLETION DATE

10th February 1994

PERFORMING LABORATORY

Centre International de Toxicologie (C.I.T.)
Miserey - 27005 Evreux - France

LABORATORY STUDY NUMBER

11191 TAL

SUMMARY

At the request of Société Séderma, Le Perray-en-Yvelines, France, the ocular irritation that could be induced by the test substance, BIOPEPTIDE EL, was evaluated in rabbits according to O.E.C.D. (No. 405, 24th February 1987) and E.C. (92/69/E.E.C.) guidelines. The study was conducted in compliance with the Principles of Good Laboratory Practice Regulations.

Methods

Having confirmed that the test substance was not irritant or corrosive when administered by cutaneous route, a single dose of 0.1 ml of the test substance in its original form was instilled into the conjunctival sac of the left eye of 3 male New Zealand White rabbits.

The eyes were not rinsed after administration of the test substance. Ocular reactions were observed approximately 1, 24, 48, 72 and 96 hours after the administration.

The mean score of the values recorded for each animal after 24, 48 and 72 hours was calculated.

The interpretation of results was carried out according to the classification criteria laid down in Directive 91/325/E.E.C. Commission Directive of 1st March 1991 adapting to technical progress for the twelfth time Council Directive 67/548/E.E.C.

Results

Moderate or slight conjunctival irritation (chemosis: score of 2; redness: score of 1 or 2) occurred in all 3 animals for up to 4 days post-instillation.

No iridial irritation and corneal opacity were noted.

Conclusion

As the mean scores for chemosis, redness and iris, degree of corneal opacity for 2 out of the 3 animals did not reach criteria values for irritation, under our experimental conditions, the test substance, BIOPEPTIDE EL, was considered as non-irritant when administered by ocular route in rabbits.

Labelling

Commission Directive 91/325/E.E.C.

Labelling not indicated for the test substance.

Personal Care Products Council
Committed to Safety, Quality & Innovation

Memorandum

TO: F. Alan Andersen, Ph.D.
Director - COSMETIC INGREDIENT REVIEW (CIR)

FROM: Halyna Breslawec, Ph.D.
Industry Liaison to the CIR Expert Panel

DATE: November 13, 2012

SUBJECT: Information on Palmitoyl Tripeptide-38

Sederma. 2012. Summary of information on Palmitoyl Tripeptide-38

Vivotecnia. 2008. Summary of bacterial reverse mutation test of VOLULIP (contains 500 ppm Palmitoyl Tripeptide-38). Final Report B-00695.

Evic France. 2008. Summary of assessment of the irritant potential of a test element (VOLULIP (contains 500 ppm Palmitoyl Tripeptide-38)) by direct application to the SIRC fibroblastic cell line by the neutral red release method. Bo 1242/08-2368.

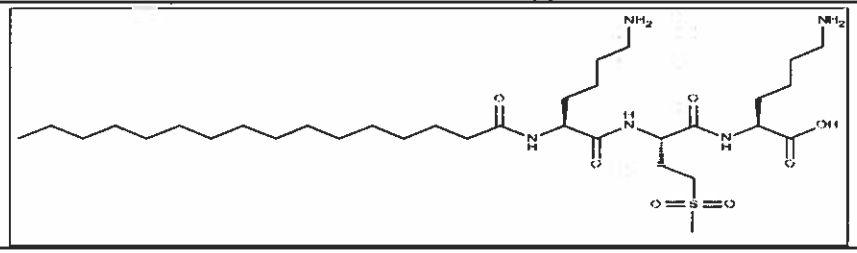
Evic France. 2008. Summary of assessment of the irritant potential of a test element (VOLULIP (contains 500 ppm Palmitoyl Tripeptide-38)) after application to the embryonic hen's egg chorioallantoic membrane. Bo 1241/08-2368.

Evic France. 2008. Summary of checking in human of the skin compatibility of a cosmetic raw material (VOLULIP (contains 500 ppm Palmitoyl Tripeptide-38)) after single application under patch. Io 535/08.2368.

Evic Romania. 2008. Summary of confirmation in human of the skin compatibility and absence of allergenic potential of one mixture of ingredients (VOLULIP (contains 500 ppm Palmitoyl Tripeptide-38)) after repeated application under patch. EF Po 183/08-2368.

Sederma. 2009. Toxciological assessment of a cosmetic ingredient: VOLULIP (contains 500 ppm Palmitoyl Tripeptide-38).

1101 17th Street, N.W., Suite 300 | Washington, D.C. 20036-4702 | 202.331.1770 | 202.331.1969 (fax) | www.personalcarecouncil.org

INCI name	**Palmitoyl Tripeptide-38**
INCI Monograph ID	24136
Trade names of SEDERMA mixtures from PcPc website	VOLULIP MATRIXYL SYNTHE'6
Technical name from PcPc website	-
Trade Name	-
Other Names	**Pal KMOOK**
Chemical Name	-
Cas Number	1101175-36-3
Appearance	White Powder
Formula	C33 H65 N5 O7 S1
Molecular Weight	675,97
Log P (estimated)	4,01
EPI suite	KOWWIN v.1.68 estimates
Dermal absorption	The following criteria were proposed by De Heer (1999) to discriminate between chemicals with high and low dermal absorption: - 10% dermal absorption is used in case MW > 500 and log Pow is smaller than -1 or higher than 4, otherwise - 100% dermal absorption is used. De Heer C, Wilschut A, Stevenson H, Hakkert BC (1999): Guidance document on the estimation of dermal absorption according to a tiered approach. An update. TNO report No. V98.1237. TNO Nutrition and Food Research Institute, Zeist, The Netherlands.
DA (%)	10
Manufacturing Process	Palmitoyl Tripeptide-38 is synthesized by solid phase synthesis with derivatives of aminoacids (lysine and methione sulfone, a non-natural amino acid) A last coupling procedure is realized with palmitic acid. At a final stage, a ions exchange chromatography enables to exchange hydrochloride of each lysine.
Impurities	Palmitic acid < 5% Water content < 5% Residual solvents comply with ICH Q3C
Formula	
Safety data	Please find safety data package for a mixture VOLULIP which contains 500ppm of Pal KMOOK. <u>VOLULIP (500ppm) - Safety Data</u> - Toxicological assessment and certificate - Reverse Mutation Study - AMES test (Report n° B-00695), November 2008 :**Non mutagenic and Non promutagenic.** - Ocular Primary Tolerance - Neutral Red (Report n° Bo 1242/08-2368), October 2008 : **Negligible Cytotoxicity** - Ocular Tolerance Assessment - HET CAM (Report n° Bo 1241/08-2368), October 2008 : **Moderately Irritant** - Primary Cutaneous Tolerance - Patch test (Report n° lo 535), September 2008 :**Very good compatibility** - Repeated Insult Patch Test - HRIPT (Report n° Po 183), November 2008 :**No allergic reaction**
Other safety information	Pal KMOOK has been used up to 500 ppm in several Sederma's products and widely supplied since 2008 in The EU, The US, Canada, Korea, Japan, Australia without any complaint concerning their innocuity

vivotecnia

Final Report B-00695

BACTERIAL REVERSE MUTATION TEST

B-00695 FINAL REPORT

X-LIP 07265

BATCH: XLIP TOX/01 E1

Final report date: 3 November 2008

TRADE NAME : VOLULIP - contains 500 ppm Palmitoyl Tripeptide-38

Test facility
VIVOTECNIA Research S.L.
Parque Científico de Madrid
C/Santiago Grisolía, 2
28760 Tres Cantos (Madrid)
Spain

3. QUALITY ASSURANCE STATEMENT

Inspections of the Ames test are performed routinely according to a pre-established schedule to ensure that the tests are performed according to the study protocol and standard operating procedures (SOPs). This is a control process of different main technical phases concerning Ames tests. An in depth inspection is performed every 20 tests or more. No inspection was performed in the present test.

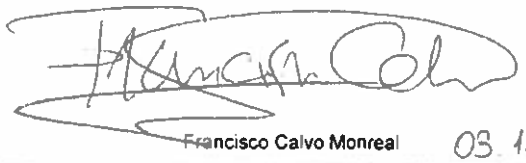
According to the European Directive 2004/10/CE and the Good Laboratory Practice (GLP) principles of Spain (RD 1369/2000), I herewith state:

1. The last inspection performed on an Ames test was as follows:

Date of inspection	Inspected process	Report date to the Laboratory and Study Director
20.08.08	Agar medium and bacteria mixture	20.08.08
20.08.08	Colony counting	20.08.08

2. The study report contains all the necessary information needed to perform the study according to GLPs.
3. Results shown on the final report describe accurately the data collected during the study.

Date of inspection	Inspected document	Report date to the Laboratory and Study Director
03.09.08	Study plan	03.09.08
15.10.08	Final report	15.10.08



Francisco Calvo Monreal 03.11.2008

Quality assurance unit Date

4. SUMMARY

The present bacterial reverse mutation test (Ames test) was performed in order to evaluate the mutagenic potential of the test item.

The test was performed in accordance with OECD Guideline 471 for the Testing of Chemicals (Bacterial Reverse Mutation Test. Adopted 21st July 1997) and the test Method B13/B14 of Commission Directive 2000/32/EC.

Doses ranging from 5µL to 0.06µL per plate were tested. No cytotoxicity was observed at any dose.

Suspensions of 4 amino-acid requiring strains of *Salmonella typhimurium* (TA98, TA100, TA1535, TA1537) and one *Escherichia coli* WP2 strain (pKM 101) auxotroph for an amino acid were exposed by the direct plate incorporation method to five doses of the test item in the presence and in the absence of an exogenous metabolic activation system. Both tests were repeated with the pre-incubation method.

Revertant bacteria due to point or frameshift-mutations at specific locus are able to grow, forming colonies. These colonies were counted and compared to the number of spontaneous revertant colonies on solvent control plate (negative control). Similarly, specific standard mutagens were tested and used as positive controls.

Based on the results obtained in this study, the test item **X-LIP 07265** was found to be NON MUTAGENIC and NON-PROMUTAGENIC under the test conditions.

STUDY/TEST ELEMENT REFERENCES : **Bo 1242/08-2368**

SPONSOR:

SEDERMA
29 rue du Chemin Vert
BP 33
78612 LE PERRAY EN YVELINES

TEST ELEMENT:

X-LIP 07265 – Réf. X-LIP/07 batch E1

TRADE NAME : VOLULIP

Contains 500ppm Palmitoyl Tripeptide-38

ASSESSMENT OF THE IRRITANT POTENTIAL OF A TEST ELEMENT BY DIRECT APPLICATION TO THE SIRC FIBROBLASTIC CELL LINE BY THE NEUTRAL RED RELEASE METHOD

NRR

Final report

Blanquefort, October 6, 2008

12 pages in this report including 1 page appendix

LABORATOIRES DE RECHERCHE ET D'EXPÉRIMENTATION

48, rue Jean Ouvert - F33290 BLANQUEFORT - Tél. 33 (0)5 56 95 59 95 - Fax 33 (0)5 56 95 05 22 - E-mail : evicfrance-blanquefort@evic.fr
57, rue Ulysse Gayon - F33000 BORDEAUX - Tél. 33 (0)5 57 14 00 80 - Fax 33 (0)5 56 48 72 49 - E-mail : evicfrance-idac@evic.fr
51, avenue de Paris - F94300 VINCENNES - Tél. 33 (0)1 41 74 40 23 - Fax 33 (0)1 41 74 40 24 - E-mail : evicfrance-paris@evic.fr
SA au capital de 385 878 € - RC 70870 Bordeaux - SIRET 470 200 700 00015 - FR 794 70200700

EVALUATION DU POTENTIEL IRRITANT D'UN ELEMENT D'ESSAI PAR APPLICATION DIRECTE SUR UNE LIGNEE DE CELLULE FIBROBASTIQUE SIRC PAR LA METHODE DE RELARGAGE DU ROUGE NEUTRE (RRN)

ASSESSMENT OF THE IRRITANT POTENTIAL OF A TEST ELEMENT BY DIRECT APPLICATION TO THE SIRC FIBROBLASTIC CELL LINE BY THE NEUTRAL RED RELEASE METHOD (NRR)

RESUME/ *SUMMARY*

- **PRINCIPE DE L'ETUDE/ *PRINCIPLE OF THE STUDY***

L'élément d'essai, dilué, a été mis directement en contact des cellules marquées par un colorant vital : le rouge neutre, pendant un temps défini. Les paramètres d'appréciation de la cytotoxicité retenus ont été le pourcentage de mortalité cellulaire et la CI50 ou concentration de l'élément d'essai inhibant de 50% la survie et la croissance cellulaires.
La cytotoxicité de l'élément d'essai a été donnée par l'échelle ci-dessous.

The test element diluted was put in contact with cells marked by a vital dye : the neutral red, for a defined time. The parameters of assessment of the cytotoxicity retained were the percentage of cell death and the IC50 or concentration of the test element that inhibited of 50% the cell survival and growth.
The cytotoxicity of the product was given by the following scale.

(CI 50) concentration inhibitrice 50% *(IC 50) Inhibitory concentration 50%*	**% de mortalité observé à la dilution 50%** *% of mortality observed at the 50% dilution*	**Classification** *Classification*
> 50	≤ 20	cytotoxicité négligeable / *negligible cytotoxicity*
> 50	> 20 et / *and* < 50	cytotoxicité peu importante / *not very important cytotoxicity*
> 25 et / *and* ≤ 50		cytotoxicité modérée / *moderate cytotoxicity*
≤ 25		cytotoxicité importante / *important cytotoxicity*

- **DATE(S) DE DÉBUT ET DE FIN D'EXPÉRIMENTATION / *EXPERIMENTAL STARTING DATE AND EXPERIMENTAL COMPLETION DATE* :** du 2 au 4 septembre 2008/ *from September 2 to 4, 2008*

- **RESULTATS/ *RESULTS* :**

Elément d'essai *Test element*	**Temps de contact (sec)** *Contact time (sec)*	**CI_{50} (%) estimée** *Estimated IC_{50} (%)*	**% de mortalité observé à la dilution 50%** *% of mortality observed at the 50% dilution*	**CI_{50} (%) déterminée** *Determined IC_{50} (%)*	**Classification** *Classification*	**Comparaison par rapport à des éléments d'essais appartenant à la même catégorie** *Comparison with test elements belonging to the same category*
X-LIP 07265 – Réf. X-LIP/07 batch E1 Dilué à 10 % dans du Cetearyl Ethylhexanoate/ *diluted at 10 % with Cetearyl Ethylhexanoate*	60	> 50 %	0 %	/	Cytotoxicité négligeable/ *negligible cytotoxicity*	*

* néant/*none*

The test complied because the IC50 of the Sodium Dodecyl Sulfate was between 0.01% and 0.2%, the negative control had a mean optical density higher than 0.6.

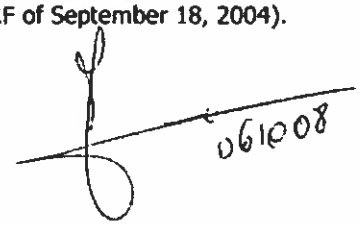
According to the defined grading scale, the cytotoxicity of the test element **X-LIP 07265 – Réf. X-LIP/07** diluted at 10% with Cetearyl Ethylhexanoate was judged **negligible.**

The response obtained for the test element (cosmetic ingredients) cannot be compared, due to a lack of historical data in that category of product.

XI . STUDY RESPONSIBLE PERSONNEL'S STATEMENT

Study Director

I the undersigned, **Sarah JULIENNE**, declare that the overall conduct of the study was carried out under my responsibility and it complies with Good Laboratory Practices (decreed of August 10th, 2004 published in the OJRF of September 18, 2004).



Quality Assurance

I the undersigned, **Michèle DARRICAU**, declare that:
- this type of study was audited (revelation : reading of the OD) according to the procedure of the Test Facility on August 19, 2008,
- the audit report was transmitted to the Management and the Study Director on August 25, 2008,
- the draft report was audited and its conformity was brought to the knowledge of the Study Director on September 30, 2008 and of the Management Direction on October 3, 2008,
- the final report was examined and its conformity was brought to the knowledge of the Study Director on October 6, 2008 and of the Management Direction on October 10, 2008
- the results reported accurately and completely reflect the raw data of the study.

EVIC france

STUDY/TEST ELEMENT REFERENCES : **Bo 1241/08-2368**

SPONSOR:

SEDERMA
29 rue du Chemin Vert
BP 33
78612 LE PERRAY EN YVELINES

TEST ELEMENT:

X-LIP 07265 – Réf. X-LIP/07 batch E1

TRADE NAME : VOLULIP

Contains 500 ppm Palmitoyl Tripeptide-38

ASSESSMENT OF THE IRRITANT POTENTIAL OF A TEST ELEMENT AFTER APPLICATION TO THE EMBRYONIC HEN'S EGG CHORIOALLANTOIC MEMBRANE

HET-CAM

Final report

Blanquefort, October 6, 2008

10 pages in this report including 1 appendix

LABORATOIRES DE RECHERCHE ET D'EXPÉRIMENTATION

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51, avenue de Paris - F94300 VINCENNES - Tél. 33 (0)1 41 74 40 23 - Fax 33 (0)1 41 74 40 24 - E-mail : evicfrance-paris@evic.fr
SA au capital de 305 878 € - RC 78878 Bordeaux - SIRET 470 200 700 00018 - FR 794 70200700

EVALUATION DU POTENTIEL IRRITANT D'UN ELEMENT D'ESSAI PAR APPLICATION SUR LA MEMBRANE CHORIO-ALLANTOÏDIENNE DE L'OEUF DE POULE EMBRYONNE - HET-CAM

ASSESSMENT OF THE IRRITANT POTENTIAL OF A TEST ELEMENT AFTER APPLICATION TO THE EMBRYONIC HEN'S EGG CHORIOALLANTOIC MEMBRANE - HET-CAM

RESUME/ *SUMMARY*

- **PRINCIPE DE L'ETUDE/ *PRINCIPLE OF THE STUDY***

L'étude a été basée sur l'observation, par une personne qualifiée des effets irritants (hyperhémie, hémorragie, coagulation) pouvant survenir dans les cinq minutes suivant le dépôt d'un élément d'essai sur la membrane chorioallantoïdienne (MCA) d'oeufs de poule embryonnés au dixième jour d'incubation.
Le potentiel irritant a été scoré selon une échelle allant de 0 à 21. L'élément d'essai a été classé dans l'une des catégories définies en fonction du score moyen obtenu.

The study was based on the observation, by a trained person, of the irritant effects (hyperhemia, haemorrhage and coagulation) occurring during the five minutes after application of test element to the chorioallantoic membrane (CAM) of embryonic hen's eggs on the tenth day of incubation.
The irritant potential was scored according to a scale from 0 to 21. The test element was classified in one of the categories defined according to the mean score obtained.

Score moyen/ *Mean Score* (Scm/ *MSc*)	**Classification/ *Classification***
Scm/ *MSc* < 1	Pratiquement non irritant/ *Practically non irritant*
1 ≤ Scm/ *MSc* < 5	Faiblement irritant/ *Weakly irritant*
5 ≤ Scm/ *MSc* < 9	Modérément Irritant/ *Moderately irritant*
Scm/ *MSc* ≥ 9	Irritant/ *Irritant*

- **DATE(S) DE DEBUT ET DE FIN D'EXPERIMENTATION / *EXPERIMENTAL STARTING DATE AND EXPERIMENTAL COMPLETION DATE :*** 2 septembre 2008/ *September 2, 2008*

- **RESULTATS/ *RESULTS* :**

Elément d'essai *Test element*	**Concentration testée** *Tested concentration*	**Score moyen sur 4 œufs** *Mean score on 4 eggs*	**Classification** *Classification*	**Comparaison par rapport à des éléments d'essai appartenant à la même catégorie** *Comparison with test elements belonging to the same category*
X-LIP 07265 – Réf. X-LIP/07 batch E1	Dilué à 10 % dans du Cetearyl Ethylhexanoate/ *diluted at 10 % with Cetearyl Ethylhexanoate*	**5**	Modérément Irritant/ *moderately irritant*	/

/néant/ *none*

X . RESULTS

Eggs		1	2	3	4
Hyperhemia *Quotation according to time ≤ 30 sec = 5 ≤ 2 min = 3 ≤ 5 min = 1*	Type	Observed	Observed	Observed	Observed
	Times In sec	25	26	26	25
	Note 1	5	5	5	5
Haemorrhage *Quotation according to time ≤ 30 sec = 7 ≤ 2 min = 5 ≤ 5 min = 3*	Times in sec	/	/	/	/
	Note 2	0	0	0	0
Coagulation (opacity and/or thrombosis) *Quotation according to time ≤ 30 sec = 9 ≤ 2 min = 7 ≤ 5 min = 5*	Type	/	/	/	/
	Times in sec	/	/	/	/
	Note 3	0	0	0	0
Score = note 1 + note 2 + note 3		5	5	5	5

XI . CONCLUSION

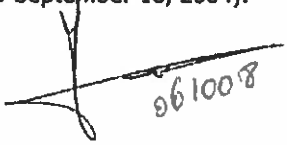
Mean score obtained on 4 eggs (M Sc)	**5**
Classification of the test element X-LIP 07265 – Réf. X-LIP/07 Diluted at 10 % with Cetearyl Ethylhexanoate	**Moderately irritant against the chorioallantoic membrane of the embryonic hens' egg**

The response obtained for the test element (cosmetic ingredient) cannot be compared, due to a lack of historical data in that category of product.

XII . STUDY RESPONSIBLE PERSONNEL'S STATEMENT

Study Director

I the undersigned, **Sarah JULIENNE** declare that the overall conduct of the study was carried out under my responsibility and it complies with the Good Laboratory Practices (decreed of August 10, 2004 published in the OJRF of September 18, 2004).



Quality Assurance

I the undersigned, **Michèle DARRICAU**, declare that:
- this type of study was audited (test element preparation) according to the procedure of the Test Facility on August 28, 2008,
- the audit report was transmitted to the Management and the Study Director on September 1st, 2008,
- the draft report was audited and its conformity was brought to the knowledge of the Study Director on September 30, 2008 and of the Management Direction on October 3, 2008,
- the final report was examined and its conformity was brought to the knowledge of the Study Director on October 6, 2008 and of the Management Direction on October 10, 2008
- the results reported accurately and completely reflect the raw data of the study.

EVIC france

STUDY/PRODUCT REFERENCES : Io 535 / 08.2368

CHECKING IN HUMAN OF THE SKIN COMPATIBILITY OF A COSMETIC RAW MATERIAL AFTER SINGLE APPLICATION UNDER PATCH

Patch test under dermatological control

SPONSOR : SEDERMA

TEST PRODUCT : X-LIP 07265 – Reference X-LIP/07 diluted at 10% with Cetearyl Ethyl Hexanoate

TRADE NAME : VOLULIP

contains 500ppm Palmitoyl Tripeptide-38

Study report

Bordeaux, September 17th, 2008

AK/FO

16 pages in this report including 6 in Appendices

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51, avenue de Paris - F94300 VINCENNES - Tél. 33 (0)1 41 74 40 23 - Fax 33 (0)1 41 74 40 24 - E-mail : evicfrance-paris@evic.fr
SA au capital de 365 878 € – RC 70B70 Bordeaux – SIRET 470 200 700 00016 – FR 79470200700

IX . RESULTS

The individual data of the skin examination and questioning of the volunteers are enclosed in **Appendices 3**.

In brief :

Control time after patch removal	Number of reactive volunteers	Types of reaction	Mean daily irritation score Mdls	% of reactive volunteers
T 15 minutes	0	None	0	0%

X . CONCLUSION

Under the experimental conditions adopted, the cosmetic raw material **X-LIP 07265 – Reference X-LIP/07,** diluted at 10% with Cetearyl Ethyl Hexanoate, **has a very good skin compatibility.**

Signatures and dates

Investigator : Doctor Andreea KÖLÖNTE (dermatologist) 26/09/08

I the undersigned, Andreea KÖLÖNTE, declare that the overall conduct of the study was carried out under my responsibility and in accordance with the principles of Good Clinical Practices (Iinternational recommendations ICH E 6, step 4, of 1/5/1996).

Quality Assurance Personnel : Danièle PICARD 26/09/08

I the undersigned, Danièle PICARD, declare that:
- this kind of study was audited according to the procedure of the investigator centre on August 04th, 2008,
- the report of the audits was transmitted to the Management of Evic France and to the Investigator,
- the final report was examined on September 23rd, 2008,
- the results reported accurately and completely reflect the raw data of the study.

EVIC france

Study: Io S35 / 08.2368 – SUMMARY OF THE STUDY REPORT

Reference of the accepted quotation : 08-0891/1

TITLE OF THE STUDY: CHECKING IN HUMAN OF THE SKIN COMPATIBILITY OF A COSMETIC RAW MATERIAL AFTER SINGLE APPLICATION UNDER PATCH – Patch test under dermatological control.

AIM OF THE STUDY: Checking of the skin compatibility of the raw material **X-LIP 07265 – Reference X-LIP/07**, diluted at 10 % in Cetearyl ethyl hexanoate, after single application to the skin, under exaggerated experimental conditions (under occlusive patch for 48 hours).

Clinical examination 15 minutes after patch removal.

DATES OF THE STUDY: from September 08th to 10th, 2008

NUMBER OF VOLUNTEERS whose data are exploitable: **11** women with phototype I to IV (11 included, neither withdrawal nor exclusion)

RESULTS

Number of reactive volunteers	Types of reaction	Mean daily irritation score Mdis	% of reactive volunteers
0	None	0	**0%**

Conclusion

Very good compatibility ☒

Signatures and dates

Investigator : Doctor Andreea KÖLÖNTE (dermatologist) 26/09/08

Quality Assurance Personnel : Danièle PICARD 26/09/08

STUDY/MIXTURE OF INGREDIENTS REFERENCES: EF Po 183/08-2368/ER 08/155-1/08-1360

TRADE NAME : VOLULIP

contains 500 ppm Palmitoyl Tripeptide-38

CONFIRMATION IN HUMAN OF THE SKIN COMPATIBILITY AND ABSENCE OF ALLERGENIC POTENTIAL OF ONE MIXTURE OF INGREDIENTS AFTER REPEATED APPLICATION UNDER PATCH

Human Repeated Insult Patch Test (HRIPT)

SPONSOR: SEDERMA
29, Rue du Chemin Vert
BP 33
78162 LE PERRAY EN YVELINES
For: Mrs Sophie DUBUC

TEST MIXTURE OF INGREDIENTS: X-LIP 07265 – Réf. X-LIP/07 diluted at 10% with Cetearyl Ethylhexanoate

Study report

Bucharest, November 26th, 2008

34 pages in this report including 21 in Appendices

SC BIO HIGH TECH SRL – 15, Constantin Bosianu street, S 4, Bucharest
Phone : 004021 335 70 90; Fax 004021 335 70 91 Mobile phone: 0040 728 302 244 E-mail: evicromania@evic.ro
RO 16679189, J40/13128/2004

For VOLULIP:
One hundred six (106) qualified subjects, male and female, ranging in age from 19 to 70 years, were selected for this evaluation. One hundred three (103) subjects completed this study. The remaining subjects discontinued their participation for various reasons, none of which were related to the application of the test material.

RESULTS

Denomination	Induction phase	
	Type of reactivity on the induction site	Number and percentage of reactive volunteers
Test mixture of ingredients: X-LIP 07265	None	0 / 0%
Control: Cetearyl Ethylhexanoate	None	0 / 0%
Control: Distilled water	None	0 / 0%

Denomination	Challenge	
	Type of reactivity on the induction site and virgin site	Number and percentage of reactive volunteers
Test mixture of ingredients: X-LIP 07265	None	0 / 0%
Control: Cetearyl Ethylhexanoate	None	0 / 0%
Control: Distilled water	None	0 / 0%

CONCLUSION

Under the experimental conditions adopted the repeated applications of the mixture of ingredients **X-LIP 07265 - Réf. X-LIP/07 diluted at 10% with Cetearyl Ethylhexanoate** under occlusive patch induced no reaction of irritation and the mixture of ingredients **has a very good skin compatibility.**

Moreover, the repeated applications **induced no allergic reaction.**

Signatures and dates

Investigator: Doctor Rozalia OLSAVSZKY (dermatologist) 3.12.2005

Quality Control Personnel: Lucia BOSCA 2.12.2005

Head manager of the investigator centre: Alina NANU 2.12.2005

3/3

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www.sederma.fr

Study: X-LIP 07265

TOXICOLOGICAL ASSESSMENT OF A COSMETIC INGREDIENT:

VOLULIP™

Date : February 2009

Study: X-LIP 07265

sederma

TOXICOLOGICAL ASSESSMENT OF A COSMETIC INGREDIENT

VOLULIP™

Date: February, 2009

1. PRODUCT DEFINITION

- Manufacturing process:

  Association of *Portulaca pilosa* extract and a peptide Palmitoyl-KMO2K-OH, 2HCl in a liposoluble excipient.

- Form

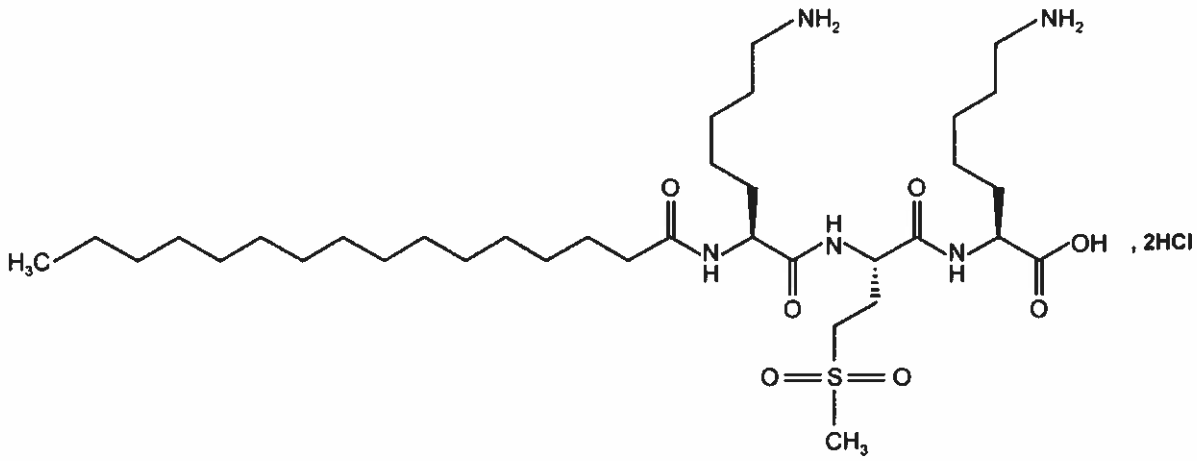
  ⇨ Physical presentation:
  Clear pale yellow liquid

  ⇨ Carrier or vehicle solvent: nature and proportions:
  Cetearyl Ethylhexanoate ≈ 89%
  Sorbitan Isostearate ≈ 8%
  Sucrose Cocoate ≈ 0.4%

- Chemical formula of the relevant components:

  Portulaca pilosa extract

  Palmitoyl-KMO2K-OH, 2HCl



sederma

TOXICOLOGICAL ASSESSMENT OF A COSMETIC INGREDIENT

VOLULIP™

Date: February, 2009

- Specifications: (tentative)

This information contained below is not contractual; it is subject to change.
The specifications published on the corresponding certificate of analysis will apply.

⇨ Physical:

Specific gravity (20°C)	0.850 -0.890
Refractive index (25°C)	1.435 - 1.455

⇨ Chemical:

Water content (%)	< 1%
KMO_2K-OH, 2HCl content (HPLC)	450 - 550 ppm

⇨ Microbiological:

Bacteria	< 100 cfu/g
Yeasts and molds	< 10 cfu/g

- Composition:

INCI	%	CAS Nr	EINECS Nr
Cetearyl Ethylhexanoate	qsp 100	90411-68-0	291-445-1
Sorbitan Isostearate	≈ 8	71902-01-7	276-171-2
Portulaca Pilosa Extract (*)	≈ 2	**	/
Sucrose Cocoate	≈ 0.4	91031-88-8	292-993-4
Palmitoyl KMO2K-OH, 2HCl (*)	≈ 0.05	**	/

(*): INCI name: PCPC pending ; (**): CAS number pending

Manufacturing additives:
Water: max. 1%
Ethanol: max. 0.1%

2. RECOMMENDED CONDITIONS OF USE

- Method of application: Topical
- Concentration of tested efficacy: 1 %
- Recommended use: For every cosmetic application, in particular lipcare products.
- Frequency of use: Several times a day.

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TOXICOLOGICAL ASSESSMENT
OF A COSMETIC INGREDIENT
VOLULIP™

Date: February, 2009

3. HISTORICAL AND BIBLIOGRAPHICAL DATA IN TOXICOLOGY (as of 05/02/2009)

Cetearyl Ethylhexanoate

The safety of Cetearyl Ethylhexanoate has been assessed by the Cosmetic Ingredient Review Expert Panel (CIREP). They evaluated the scientific data and concluded that Cetearyl Ethylhexanoate was safe as a cosmetic ingredient in the present practices of use. In 2003, as part of the scheduled re-evaluation of ingredients, the CIR Expert Panel considered available new data on this ingredient and reaffirmed the above conclusion.
They reported that the acute oral toxicity of Cetearyl Ethylhexanoate was low and that the ingredient produced no significant acute, subchronic or dermal skin or eye irritation. The ingredient produced no evidence of skin sensitization. Similar studies with product formulations containing Cetearyl Ethylhexanoate confirmed these results, as well as indicated the ingredient was not phototoxic.[1] Safety datasheet indicates: LD50 > 16ml/kg / Moderate skin irritation (rabbit) – no irritant (human) / slight irritation on eye (rabbit) / No sensitisation. [2]

Portula pilosa:

Portulaca is the type genus of the purslane family Portulacaceae, comprising about 40-100 species. Purslane can be eaten raw or cooked, and lends itself to stir fry dishes. Some say it has a slight lemon-like taste and mushroom-like texture. [3]

Portulaca pilosa is also describe as an edible plant in NUTTAB 2006 that is an food composition publication containing data on the nutrient content of foods available in Australia. [4]

Moreover, *Portulaca pilosa* has been used in Brazil as a traditional remedy to cause diuresis, antipyresis and analgesia. [5]

Palmitoyl KMO2K-OH:

The palmitoyl peptide is a lipopeptide manufactured by SEDERMA.

Sorbitan Isostearate

The safety of Sorbitan Esters has been assessed by the CIREP. They evaluated the scientific data and concluded that Sorbitan Isostearate was safe for use in cosmetic and personal care products.
As a class, sorbitan esters were relatively nontoxic via ingestion in acute and long-term studies. They were generally minimal to mild skin irritants, except that Sorbitan Isostearate applied to the skin was a moderate irritant in one study. Sorbitan esters did not act as sensitizing agents. The fatty acid component, tested alone, typically caused only slight irritation and sensitization, and was not photosensitizing. Sorbitan esters were not ocular irritants. These esters and their corresponding fatty acids were not mutagenic. In clinical tests, sorbitan esters were generally minimal to mild skin irritants and were non sensitizing.
Sorbitan isosterarate is considered safe for use in cosmetic formulations under the present practices of use. [6] [7]
Safety datasheet indicates: LD50 > 16ml/kg and it is moderately irritant for the skin (rabbit) [8]

Sucrose cocoate

This mixture contains a mixture of sucrose esters of coconut fatty acids in aqueous ethanol solution. It is an emulsifier employed in emollient, skin-moisturizing cosmetic formulations, It is non irritant for skin (rabbit), non irritant for eyes (15% aqueous solution - rabbit), non sensitizing (HRIPT) [9]
Sucrose cocoate is a sucrose fatty acid ester. The FDA has approved the use of sucrose fatty acid esters as direct food additives. The Joint FAO/WHO Expert Committee on Food Additives (JECFA) has reviewed the safety of the sucrose fatty acid esters. They identified an acceptable daily intake (ADI) of up to 30 mg/kg, [10]

[1] http://www.cosmeticsinfo.org/ingredient_details.php?ingredient_id=218

[2] MSDS

[3] http://en.wikipedia.org/wiki/Portulaca

[4] NUTTAB 2006
http://www.foodstandards.gov.au/monitoringandsurveillance/nuttab2006/onlineversionintroduction/onlineversion.cfm?&action=getFood&foodID=15A10212

[5] Rocha, M., Fulgencio, S., Rabetti, A., Nicolau, M., Poli, A., Simões, C. M. and Ribeiro-do-Valle, R. M., 1994, Effects of hydroalcoholic extracts of Portulaca pilosa and Achyroline satureioides on urinary sodium and potassium excretion. J. Ethnopharmacol., 43(3): 179-183

[6] Lanigan RS; Yamarik TA; - Final report on the safety assessment of sorbitan caprylate, sorbitan cocoate, sorbitan diisostearate, sorbitan dioleate, sorbitan distearate, sorbitan isostearate, sorbitan olivate, sorbitan sesquiisostearate, sorbitan sesquistearate, and sorbitan triisostearate. Cosmetic Ingredient Review Expert panel Int J Toxicol. 2002; 21 Suppl 1:93-112.

[7] http://www.cosmeticsinfo.org/ingredient_details.php?ingredient_id=712

[8] MSDS and safety report

[9] MSDS and Product Information Data Sheet

[10] http://www.cosmeticsinfo.org/ingredient_details.php?ingredient_id=1761

4. TOXICOLOGICAL TESTS

4.1. Local toxicity (on commercial product = X-LIP/07)

- Cutaneous primary tolerance

Report **E**vic n° Io 535/08.2368 - September 17th, 2008: diluted at 10%
Single patch test on humans (10 adult volunteers): **cutaneous compatibility may be judged very good.**

- Ocular irritation:

HET CAM - Report **E**vic n° Bo 1241/08.2368 - October 6th, 2008: diluted at 10%
- HET CAM = **Moderately Irritant.**

Neutral Red Release method - Report Evic n° Bo 1242/08.2368 – October 6th, 2008: diluted at 10%
- NRR = **negligible cytotoxicity**.

4.2. Allergenicity (on commercial product = X-LIP/07)

- Sensitisation:

Report Evic n° Po 183/08.2368 - November 26th, 2008: diluted at 10% application, on 100 volunteers under occlusive patch: the product induced no reaction of irritation and has very good skin compatibility. The repeated applications induced **no allergic reaction.**

4.3. Systemic toxicity (on X-LIP/TOX 01 E1 = 500ppm of peptide in an ethanolic solution)

- Mutagenesis according to OECD guideline n°471:

Report Phycher B-00695 – November 3rd, 2008: pure application
Ames test: **non mutagenic**.

5. SPECIFIC PRECAUTIONS KNOWN: None.

6. RISK ASSESSMENT – CERTIFICATE

X-LIP 07265

sederma

TOXICOLOGICAL ASSESSMENT CERTIFICATE :

VOLULIP ™

Preamble : The company SEDERMA manufactures and markets a cosmetic ingredient named **VOLULIP ™** which is an association of Portulaca pilosa extract and a peptide Palmitoyl-KMO2K-OH, 2HCl in a liposoluble excipient.
It provides a toxicological assessment file for this product that is displayed in appendix and is composed of 5 chapters (product description, recommendations of use, bibliographical review, tests and toxicological results, precautions of use).

Study : The product can be used in every cosmetic application, skin care and toiletries, in particular lipcare products in a concentration of 1 %. Skin and ocular tolerance tests, allergenic risk test (sensitisation according to RIPT method) made on a representative batch of **VOLULIP ™** of SEDERMA, and systemic toxicity tests (mutagenesis test according to AMES), do not indicate any contraindication to the cosmetic use of the product. The manufacturer does not mention any precaution of use.

Conclusion : On the basis of the file studied, and within the present state of our knowledge, it is possible to conclude that the use of the **VOLULIP ™** of SEDERMA in recommended conditions of cosmetic use does not present any reasonably foreseeable risk in case of introduction as such into a cosmetic product (as long as no chemical modification occurs during this process).

Besides, as with all new cosmetic ingredients, the principles of cosmeto-vigilance should be applied: any abnormally high number or severe cases of adverse effects for human health should lead to new evaluation of its suitability for use in cosmetics.

Expert's Name and Signature:
M. Dominique SABOUREAU

Date: February 16nth 2009

Address: 47 avenue Jean Moulin
33610 Cestas Gazinet
France

Qualification: Expert Toxicologue
(Eurotox Registered Toxicologist)

Personal Care Products Council
Committed to Safety, Quality & Innovation

Memorandum

TO: F. Alan Andersen, Ph.D.
Director - COSMETIC INGREDIENT REVIEW (CIR)

FROM: Halyna Breslawec, Ph.D.
Industry Liaison to the CIR Expert Panel

DATE: November 13, 2012

SUBJECT: Information on Palmitoyl Pentapeptide-4

Sederma. 2012. Summary of information on Palmitoyl Pentapeptide-4 (previously named Palmitoyl Pentapeptide-3).

CIT. 1999. Summary of acute dermal irritation in rabbits Palmitoyl Pentapeptide-4. Laboratory study number 18839 TAL.

CIT. 1999. Summary of acute eye irritation in rabbits Palmitoyl Pentapeptide-4. Laboratory study number 18840 TAL.

CIT. 1999. Summary of acute oral toxicity in rats Palmitoyl Pentapeptide-4. Laboratory study number 18838 TAR.

CIT. 1999. Summary of local tolerance study after repeated topical application for 2 weeks in guinea pigs Palmitoyl Pentapeptide-4. Laboratory study number 18842 TSG.

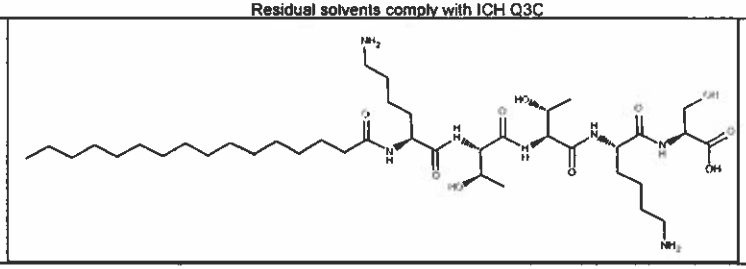
CIT. 1999. Summary of skin sensitization test in guinea pigs Palmitoyl Pentapeptide-4. Laboratory study number 18841 TSG.

CIT. 1999. Summary of bacterial reverse mutation test Palmitoyl Pentapeptide-4. Laboratory study number 18796 MMJ.

Institut D'Expertise Cliniqu. 1998. Summary of HET-CAM assay and human primary cutaneous tolerance of MATRIXYL (contains 100 ppm Palmitoyl Pentapeptide-4). Report No. 80503RD2.

Consumer Product Testing Co. 1999. Summary of repeated insult patch test of MATRIXYL (contains 100 ppm Palmitoyl Pentapeptide-4). Experiment Reference Number: C99-0567.02.

1101 17th Street, N.W., Suite 300 | Washington, D.C. 20036-4702 | 202.331.1770 | 202.331.1969 (fax) | www.personalcarecouncil.org

INCI name	Palmitoyl Pentapeptide-4
INCI Monograph ID	12108
Trade names of SEDERMA mixtures from PcPc website	MATRIXYL
Technical name from PcPc website	Palmitoyl Pentapeptide-3 L-Serine, N2-(1-oxohexadecyl)-L-lysyl-L-threonyl-L-seryl-L-lysyl- L-Serine, N2-(1-oxohexadecyl)-L-lysyl-L-threonyl-L-threonyl-L-lysy
Trade Name	Lipopentapeptide 3
Other Names	Pal KTTKS (Pal Lys-Thr-Thr-Lys-Ser'
Chemical Name	-
Cas Number	214047-00-4
Appearance	White Powder
Formula	C39 H75 N7 O10
Molecular Weight	902,07
Log P (estimated)	3,48
EPI suite	KOWWIN v.1.68 estimates
Dermal absorption	The following criteria were proposed by De Heer (1999) to discriminate between chemicals with high and low dermal absorption: - 10% dermal absorption is used in case MW > 500 and log Pow is smaller than -1 or higher than 4, otherwise - 100% dermal absorption is used. De Heer C, Wilschut A, Stevenson H, Hakkert BC (1999): Guidance document on the estimation of dermal absorption according to a tiered approach. An update. TNO report No. V98.1237. TNO Nutrition and Food Research Institute, Zeist. The Netherlands.
DA (%)	100
Manufacturing Process	This compound is synthesized by stepwise peptide synthesis. The C-terminal aminoacid (Ser) is protected on its acidic function, then each protected aminoacid (Lys-Thr-Thr-Lys) is coupled. A last coupling procedure is realised with palmitic acid instead of an aminoacid.
Impurities	Acetate content < 10% Palmitic acid < 5% Water content < 5% Residual solvents comply with ICH Q3C
Formula	
Safety data	Please find Safety data package on Palmitoyl Pentapeptide-4 (previously named Palmitoyl Pentapeptide-3) at the concnentration of 0,01%: - Acute Dermal Irritation in Rabbits (Repot n° 18839 TAL), September 1999: Non Irritant - Acute Eye Irritation in Rabbits (Report n° 18840 TAL), October 1999: Non Irritant - Acute Oral Toxicity in Rats (Report n° 18838 TAR), October 1999: a single administration of a dose-volume of 20 ml/Kg does not induce any signs of toxicity - Local Tolerance after Repeated Topical Application for 2 weeks in Guinea-pigs (Report n° 18842 TSG), October 1999:Non Irritant - Skin Sensitization Test in Guinea-pigs - Magnusson & Kligman (Report n° 18841 TSG), October 1999: Does not induced delayed contact Hypersensitivity in guinea-pigs - Reverse Mutation Study - AMES test (Report n° 18796 MMJ), October 1999: Non mutagenic Please find safety data package for a mixture MATRIXYL which contains 100ppm of Pal KTTKS: MATRIXYL (100ppm) - Safety Data - Toxicological assessment and certificate - Ocular Tolerance Assessment - HET CAM (Report n° 80503RD2), June 1998: Moderately Irritant - Primary Cutaneous Tolerance - Patch test (Report n° 80503RD2), June 1998: Well tolerated - Repeated Insult Patch Test - HRIPT (Report n° C99-0597.02), August 1998: No Irritation and No sensitization

SPONSOR
Société Séderma
29 rue du Chemin Vert
B.P. 33
78610 Le-Perray-en-Yvelines CEDEX
France

TEST SUBSTANCE
PALMITOYL-PENTAPEPTIDE 3 4

STUDY TITLE
ACUTE DERMAL IRRITATION IN RABBITS

STUDY DIRECTOR
Xavier Manciaux

STUDY COMPLETION DATE
29 September 1999

PERFORMING LABORATORY
CIT
Centre International de Toxicologie
BP 563 - 27005 Evreux - France

LABORATORY STUDY NUMBER
18839 TAL

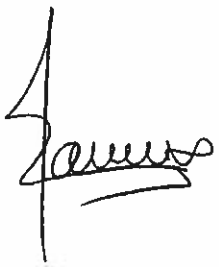
STATEMENT OF THE STUDY DIRECTOR

The study was performed in compliance with the principles of Good Laboratory Practice as described in:

- OECD Principles on Good Laboratory Practice (as revised in 1997), ENV/MC/CHEM (98) 17.
- Décret N° 90-206 du 7 mars 1990 concernant les Bonnes Pratiques de Laboratoire (Journal Officiel du 9 mars 1990), Ministère de l'Industrie et de l'Aménagement du Territoire, Annexe du 1er janvier 1999.
- Council Directive 87/18/EEC of 18 December 1986 on the harmonization of laws, regulations or administrative provisions relating to the application of the Principles of Good Laboratory Practice and the verification of their applications for tests on chemical substances (OJ No. L 15 of 17.1.87).
- Japanese Ministry of Health and Welfare, Good Laboratory Practice Standards, Pharmaceutical Affairs Bureau, Ordinance No. 21, March 26, 1997.

I declare that this report constitutes a true and faithful record of the procedures undertaken and the results obtained during the performance of the study.

This study was performed at CIT, Centre International de Toxicologie, BP 563, 27005 Evreux, France.



Toxicology

X. Manciaux Date: 29 September 1999
Study Director
Doctor of Pharmacy

OTHER SCIENTISTS INVOLVED IN THIS STUDY

For Pharmacy: P.O. Guillaumat
Doctor of Pharmacy

For Toxicology: C. Pelcot
Study Supervisor

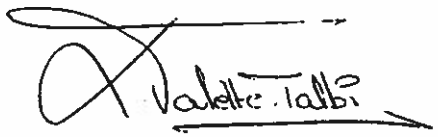
STATEMENT OF QUALITY ASSURANCE UNIT

Type of inspections	Dates		
	Inspections	Reported to Study Director (*)	Reported to Management (*)
Protocol	1 July 1999	2 July 1999	2 July 1999
Report	20 September 1999	24 September 1999	24 September 1999

In addition to the above-mentioned inspections, at about the same time as the study described in the present report, "process-based" and routine facility inspections of critical procedures relevant to this study type were also made by the Quality Assurance Unit.
The findings of these inspections were reported to the Study Director and to CIT Management.

The inspections were performed in compliance with CIT Quality Assurance Unit procedures and the Good Laboratory Practice.

The reported methods and procedures were found to describe those used and the results to constitute an accurate and complete reflection of the study raw data.



L. Valette-Talbi Date: 29 September 1999
Doctor of Biochemistry
Head of Quality Assurance Unit
and Scientific Archives

(*) The dates indicated correspond to the dates of signature of audit reports by Study Director and Management.

SUMMARY

At the request of Société Séderma, Le-Perray-en-Yvelines, France, the potential of the test substance PALMITOYL-PENTAPEPTIDE 4 to induce skin irritation was evaluated in rabbits according to OECD (No. 404, 17th July 1992) and EC (92/69/EEC, B.4, 31st July 1992) guidelines.
The study was conducted in compliance with the principles of Good Laboratory Practice Regulations.

Methods

The study design was established according to available information on the test substance and the above guidelines.

The test substance was prepared at the concentration of 0.01% according to a procedure provided by the Sponsor.

A single dose of 0.5 ml of the test substance formulation was applied for 4 hours to the closely-clipped skin of one flank of three male New Zealand White rabbits.

The test substance was held in contact with the skin by means of a semi-occlusive dressing. Cutaneous reactions were observed approximately 1 hour, 24, 48 and 72 hours after removal of the dressing.

The mean values of the scores for erythema and oedema were calculated for each animal.

Results

A very slight erythema was noted in one animal on day 1 only.
No other cutaneous reactions were observed during the study.

Mean scores over 24, 48 and 72 hours for each animal were 0.0, 0.0 and 0.0 for erythema and 0.0, 0.0 and 0.0 for oedema.

Conclusion

Under our experimental conditions, the test substance PALMITOYL-PENTAPEPTIDE 4 is non-irritant when applied topically to rabbits at the concentration of 0.01%.

SPONSOR
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29 rue du Chemin Vert
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78610 Le-Perray-en-Yvelines CEDEX
France

TEST SUBSTANCE
PALMITOYL-PENTAPEPTIDE 4

STUDY TITLE
ACUTE EYE IRRITATION
IN RABBITS

STUDY DIRECTOR
Xavier Manciaux

STUDY COMPLETION DATE
1 October 1999

PERFORMING LABORATORY
CIT
Centre International de Toxicologie
BP 563 - 27005 Evreux - France

LABORATORY STUDY NUMBER
18840 TAL

CENTRE INTERNATIONAL DE TOXICOLOGIE

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E-mail : CIT@compuserve.com

STATEMENT OF THE STUDY DIRECTOR

The study was performed in compliance with the principles of Good Laboratory Practice as described in:

- OECD Principles on Good Laboratory Practice (as revised in 1997), ENV/MC/CHEM (98) 17.
- Décret N° 90-206 du 7 mars 1990 concernant les Bonnes Pratiques de Laboratoire (Journal Officiel du 9 mars 1990), Ministère de l'Industrie et de l'Aménagement du Territoire, Annexe du 1er janvier 1999.
- Council Directive 87/18/EEC of 18 December 1986 on the harmonization of laws, regulations or administrative provisions relating to the application of the Principles of Good Laboratory Practice and the verification of their applications for tests on chemical substances (OJ No. L 15 of 17.1.87).
- Japanese Ministry of Health and Welfare, Good Laboratory Practice Standards, Pharmaceutical Affairs Bureau, Ordinance No. 21, March 26, 1997.

I declare that this report constitutes a true and faithful record of the procedures undertaken and the results obtained during the performance of the study.

This study was performed at CIT, Centre International de Toxicologie, BP 563, 27005 Evreux, France.

Toxicology

X. Manciaux Date: 1 October 1999
Study Director
Doctor of Pharmacy

OTHER SCIENTISTS INVOLVED IN THIS STUDY

For Pharmacy: P.O. Guillaumat
Doctor of Pharmacy

For Toxicology: C. Pelcot
Study Supervisor

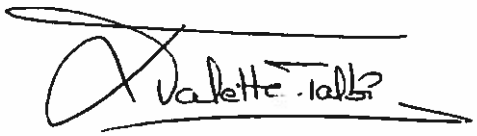
STATEMENT OF QUALITY ASSURANCE UNIT

Type of inspections	Dates		
	Inspections	Reported to Study Director (*)	Reported to Management (*)
Protocol	1 July 1999	2 July 1999	2 July 1999
Study	7 July 1999	8 July 1999	8 July 1999
Report	27 September 1999	28 September 1999	28 September 1999

In addition to the above-mentioned inspections, at about the same time as the study described in the present report, "process-based" and routine facility inspections of critical procedures relevant to this study type were also made by the Quality Assurance Unit.
The findings of these inspections were reported to the Study Director and to CIT Management.

The inspections were performed in compliance with CIT Quality Assurance Unit procedures and the Good Laboratory Practice.

The reported methods and procedures were found to describe those used and the results to constitute an accurate and complete reflection of the study raw data.



L. Valette-Talbi Date: 1 October 1999
Doctor of Biochemistry
Head of Quality Assurance Unit
and Scientific Archives

(*) The dates indicated correspond to the dates of signature of audit reports by Study Director and Management.

SUMMARY

At the request of Société Séderma, Le-Perray-en-Yvelines, France, the potential of the test substance PALMITOYL-PENTAPEPTIDE 4 to induce ocular irritation was evaluated in rabbits according to OECD (No. 405, 24th February 1987) and EC (92/69/EEC, B.5, 31st July 1992) guidelines.
The study was conducted in compliance with the principles of Good Laboratory Practice Regulations.

Methods

The study design was established according to available information on the test substance and the above guidelines.

The test substance was prepared at the concentration of 0.01% according to a procedure provided by the Sponsor.

As no irritant effects were anticipated, a single dose of 0.1 ml of the test substance formulation was instilled into the conjunctival sac of the left eye of three male New Zealand White rabbits. The right eye was not treated and served as control.
The eyes were not rinsed after administration of the test substance.

Ocular reactions were observed approximately 1 hour, 24, 48 and 72 hours after the administration.

The mean values of the scores for chemosis, redness of the conjunctiva, iris lesions and corneal opacity were calculated for each animal.

Results

No ocular reactions were observed during the study.

Mean scores calculated for each animal over 24, 48 and 72 hours were 0.0, 0.0 and 0.0 for chemosis, 0.0, 0.0 and 0.0 for redness of the conjunctiva, 0.0, 0.0 and 0.0 for iris lesions and 0.0, 0.0 and 0.0 for corneal opacity.

Conclusion

Under our experimental conditions, the test substance PALMITOYL-PENTAPEPTIDE 4 at the concentration of 0.01% is non-irritant when administered by ocular route to rabbits.

SPONSOR
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France

TEST SUBSTANCE
PALMITOYL-PENTAPEPTIDE

STUDY TITLE
ACUTE ORAL TOXICITY
IN RATS

STUDY DIRECTOR
Xavier Manciaux

STUDY COMPLETION DATE
5 October 1999

PERFORMING LABORATORY
CIT
Centre International de Toxicologie
BP 563 - 27005 Evreux - France

LABORATORY STUDY NUMBER
18838 TAR

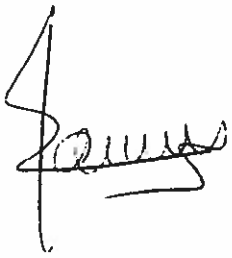
STATEMENT OF THE STUDY DIRECTOR

The study was performed in compliance with the principles of Good Laboratory Practice as described in:

- OECD Principles on Good Laboratory Practice (as revised in 1997), ENV/MC/CHEM (98) 17.
- Décret N° 90-206 du 7 mars 1990 concernant les Bonnes Pratiques de Laboratoire (Journal Officiel du 9 mars 1990), Ministère de l'Industrie et de l'Aménagement du Territoire, Annexe du 1er janvier 1999.
- Council Directive 87/18/EEC of 18 December 1986 on the harmonization of laws, regulations or administrative provisions relating to the application of the Principles of Good Laboratory Practice and the verification of their applications for tests on chemical substances (OJ No. L 15 of 17.1.87).
- Japanese Ministry of Health and Welfare, Good Laboratory Practice Standards, Pharmaceutical Affairs Bureau, Ordinance No. 21, March 26, 1997.

I declare that this report constitutes a true and faithful record of the procedures undertaken and the results obtained during the performance of the study.

This study was performed at CIT, Centre International de Toxicologie, BP 563, 27005 Evreux, France.



Toxicology

X. Manciaux Date: 5 October 1999
Study Director
Doctor of Pharmacy

OTHER SCIENTISTS INVOLVED IN THIS STUDY

For Pharmacy: P.O. Guillaumat
Doctor of Pharmacy

For Toxicology: C. Pelcot
Study Supervisor

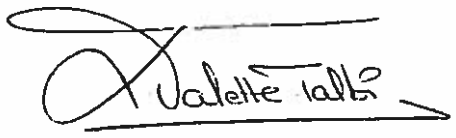
STATEMENT OF QUALITY ASSURANCE UNIT

Type of inspections	Dates		
	Inspections	Reported to Study Director (*)	Reported to Management (*)
Protocol	1 July 1999	2 July 1999	2 July 1999
Report	27 September 1999	28 September 1999	28 September 1999

In addition to the above-mentioned inspections, at about the same time as the study described in the present report, "process-based" and routine facility inspections of critical procedures relevant to this study type were also made by the Quality Assurance Unit.
The findings of these inspections were reported to the Study Director and to CIT Management.

The inspections were performed in compliance with CIT Quality Assurance Unit procedures and the Good Laboratory Practice.

The reported methods and procedures were found to describe those used and the results to constitute an accurate and complete reflection of the study raw data.



L. Valette-Talbi Date: 5 October 1999
Doctor of Biochemistry
Head of Quality Assurance Unit
and Scientific Archives

(*) The dates indicated correspond to the dates of signature of audit reports by Study Director and Management.

SUMMARY

At the request of Société Séderma, Le-Perray-en-Yvelines, France, the acute oral toxicity of the test substance PALMITOYL-PENTAPEPTIDE 3 was evaluated in rats according to OECD (No. 401, 24th February 1987) and EC (92/69/EEC, B.1, 31st July 1992) guidelines.
The study was conducted in compliance with the principles of Good Laboratory Practice Regulations.

Methods

The test substance was prepared at the concentration of 0.01% according to a procedure provided by the Sponsor.

The test substance formulation was administered by oral route (gavage) to one group of ten fasted Sprague-Dawley rats (five males and five females), under a volume of 20 ml/kg.

Clinical signs, mortality and body weight gain were checked for a period of up to 14 days following the single administration of the test substance.

All animals were subjected to necropsy.

Results

No deaths occurred during the study.

The general behaviour and body weight gain of the animals were not affected by treatment with the test substance.

No apparent abnormalities were observed at necropsy in all animals.

Conclusion

Under our experimental conditions, a single oral administration of a dose-volume of 20 ml/kg of the test substance PALMITOYL-PENTAPEPTIDE 3 at the concentration of 0.01% does not induce any signs of toxicity in rats.

SPONSOR
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29 rue du Chemin Vert
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78610 Le-Perray-en-Yvelines CEDEX
France

TEST SUBSTANCE
PALMITOYL-PENTAPEPTIDE 4

STUDY TITLE
LOCAL TOLERANCE STUDY AFTER REPEATED TOPICAL APPLICATION FOR 2 WEEKS IN GUINEA-PIGS

STUDY DIRECTOR
Xavier Manciaux

STUDY COMPLETION DATE
11 October 1999

PERFORMING LABORATORY
CIT
Centre International de Toxicologie
BP 563 - 27005 Evreux - France

LABORATORY STUDY NUMBER
18842 TSG

STATEMENT OF THE STUDY DIRECTOR

The study was performed in compliance with the principles of Good Laboratory Practice as described in:

- OECD Principles on Good Laboratory Practice (as revised in 1997), ENV/MC/CHEM (98) 17.
- Décret N° 90-206 du 7 mars 1990 concernant les Bonnes Pratiques de Laboratoire (Journal Officiel du 9 mars 1990), Ministère de l'Industrie et de l'Aménagement du Territoire, Annexe du 1er janvier 1999.
- Council Directive 87/18/EEC of 18 December 1986 on the harmonization of laws, regulations or administrative provisions relating to the application of the Principles of Good Laboratory Practice and the verification of their applications for tests on chemical substances (OJ No. L 15 of 17.1.87).
- Japanese Ministry of Health and Welfare, Good Laboratory Practice Standards, Pharmaceutical Affairs Bureau, Ordinance No. 21, March 26, 1997.

The study was also conducted in compliance with Animal Health regulation, in particular:

- Council Directive 86/609/EEC of 24th November 1986 on the harmonization of laws, regulations or administrative provisions relating to the protection of animals used for experimental or other scientific purposes.

I declare that this report constitutes a true and faithful record of the procedures undertaken and the results obtained during the performance of the study.

This study was performed at CIT, Centre International de Toxicologie, BP 563, 27005 Evreux, France.

Toxicology

X. Manciaux Date: 11 October 1999
Study Director
Doctor of Pharmacy

OTHER SCIENTISTS INVOLVED IN THIS STUDY

For Pharmacy: P.O. Guillaumat
Doctor of Pharmacy

For Toxicology: C. Pelcot
Study Supervisor

STATEMENT OF QUALITY ASSURANCE UNIT

Type of inspections	Dates		
	Inspections	Reported to Study Director (*)	Reported to Management (*)
Protocol	24 June 1999	24 June 1999	24 June 1999
Report	5 October 1999	8 October 1999	11 October 1999

In addition to the above-mentioned inspections, at about the same time as the study described in the present report, "process-based" and routine facility inspections of critical procedures relevant to this study type were also made by the Quality Assurance Unit.
The findings of these inspections were reported to the Study Director and to CIT Management.

The inspections were performed in compliance with CIT Quality Assurance Unit procedures and the Good Laboratory Practice.

The reported methods and procedures were found to describe those used and the results to constitute an accurate and complete reflection of the study raw data.

L. Valette-Talbi Date: 11 October 1999
Doctor of Biochemistry
Head of Quality Assurance Unit
and Scientific Archives

(*) The dates indicated correspond to the dates of signature of audit reports by Study Director and Management.

SUMMARY

At the request of Société Séderma, Le-Perray-en-Yvelines, France, the local tolerance of the test substance PALMITOYL-PENTAPEPTIDE 3 after repeated cutaneous applications for 2 weeks was evaluated in guinea-pigs. 4
The study was conducted in compliance with the principles of Good Laboratory Practice Regulations.

Methods

The test substance was prepared at the concentration of 0.01% according to a procedure provided by the Sponsor.

A volume of 0.05 ml of the test substance formulation was applied to the left flank of ten guinea-pigs (five males and five females) once daily, at approximately the same time each day, for 14 consecutive days.

The test substance formulation was applied over the same area of clipped skin, measuring approximately 2 cm x 2 cm. No rinsing of the test site was performed. The test site was not covered by a dressing.
The right flank received purified water under the same experimental conditions.

Cutaneous reactions were evaluated on both flanks of each animal before each application and approximately 24 hours after the last application.

The cutaneous reactions recorded were used to calculate Daily Irritation and Weekly Mean Irritation indices. The Maximum Weekly Mean Irritation Index was used to classify the test substance.

Photographs of the treated application sites of each animal were performed before treatment on days 1, 5, 9, 12 and 15.

At the end of the observation period, the animals were killed without examination of internal organs. No skin samples were taken.

Results

No clinical signs and no deaths related to treatment were noted during the study.

No cutaneous reactions were observed on the right control flank.
On the left treated flank, a very slight erythema was noted in one animal only, on days 12 and 13.
No other cutaneous reactions were observed during the study.

As these cutaneous reactions were very slight and as they occurred in only one animal on days 12 and 13 only, they were not attributed to an irritant effect of the test substance.

The Maximum Weekly Mean Irritation Index obtained was 0.0.

Conclusion

Under our experimental conditions, the repeated cutaneous application for 14 days of the test substance PALMITOYL-PENTAPEPTIDE 3 at the concentration of 0.01% (w/w) does not induce skin irritation in guinea-pigs. 4

According to the obtained Maximum Weekly Mean Irritation Index, the test substance should be classified as non-irritant.

SPONSOR
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29 rue du Chemin Vert
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78610 Le-Perray-en-Yvelines CEDEX
France

TEST SUBSTANCE
PALMITOYL-PENTAPEPTIDE 3

STUDY TITLE
SKIN SENSITIZATION TEST
IN GUINEA-PIGS
(Maximization method of Magnusson and Kligman)

STUDY DIRECTOR
Xavier Manciaux

STUDY COMPLETION DATE
11 October 1999

PERFORMING LABORATORY
CIT
Centre International de Toxicologie
BP 563 - 27005 Evreux - France

LABORATORY STUDY NUMBER
18841 TSG

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STATEMENT OF THE STUDY DIRECTOR

The study was performed in compliance with the principles of Good Laboratory Practice as described in:

- OECD Principles on Good Laboratory Practice (as revised in 1997), ENV/MC/CHEM (98) 17.
- Décret N° 90-206 du 7 mars 1990 concernant les Bonnes Pratiques de Laboratoire (Journal Officiel du 9 mars 1990), Ministère de l'Industrie et de l'Aménagement du Territoire, Annexe du 1er janvier 1999.
- Council Directive 87/18/EEC of 18 December 1986 on the harmonization of laws, regulations or administrative provisions relating to the application of the Principles of Good Laboratory Practice and the verification of their applications for tests on chemical substances (OJ No. L 15 of 17.1.87).
- Japanese Ministry of Health and Welfare, Good Laboratory Practice Standards, Pharmaceutical Affairs Bureau, Ordinance No. 21, March 26, 1997.

I declare that this report constitutes a true and faithful record of the procedures undertaken and the results obtained during the performance of the study.

This study was performed at CIT, Centre International de Toxicologie, BP 563, 27005 Evreux, France.

Toxicology

X. Manciaux Date: 11 October 1999
Study Director
Doctor of Pharmacy

OTHER SCIENTISTS INVOLVED IN THIS STUDY

For Pharmacy: P.O. Guillaumat
Doctor of Pharmacy

For Toxicology: C. Pelcot
Study Supervisor

STATEMENT OF QUALITY ASSURANCE UNIT

Type of inspections	Dates		
	Inspections	Reported to Study Director (*)	Reported to Management (*)
Protocol	1 July 1999	2 July 1999	2 July 1999
Report	5 October 1999	8 October 1999	11 October 1999

In addition to the above-mentioned inspections, at about the same time as the study described in the present report, "process-based" and routine facility inspections of critical procedures relevant to this study type were also made by the Quality Assurance Unit.
The findings of these inspections were reported to the Study Director and to CIT Management.

The inspections were performed in compliance with CIT Quality Assurance Unit procedures and the Good Laboratory Practice.

The reported methods and procedures were found to describe those used and the results to constitute an accurate and complete reflection of the study raw data.

L. Valette-Talbi Date: 11 October 1999
Doctor of Biochemistry
Head of Quality Assurance Unit
and Scientific Archives

(*) The dates indicated correspond to the dates of signature of audit reports by Study Director and Management.

SUMMARY

At the request of Société Séderma, Le-Perray-en-Yvelines, France, the potential of the test substance PALMITOYL-PENTAPEPTIDE 4 to induce delayed contact hypersensitivity was evaluated in guinea-pigs according to the maximization method of Magnusson and Kligman and to OECD (No. 406, 17th July 1992) and EC (96/54/EEC, B.6, 30 July 1996) guidelines.
The study was conducted in compliance with the principles of Good Laboratory Practice Regulations.

Methods

At the request of the Sponsor, the test substance was formulated at the concentration of 0.01%. All the test substance formulations prepared for the study were dilutions from this 0.01% formulation.

Thirty guinea-pigs were allocated to two groups: a control group 1 (five males and five females) and a treated group 2 (ten males and ten females).

On day 1, intradermal injections of Freund's complete adjuvant mixed with the test substance formulation (treated group) or the vehicle (control group) were performed in the interscapular region.

On day 7, the same region received a topical application of sodium lauryl sulfate in vaseline (10%, w/w) in order to induce local irritation.

On day 8, the test substance formulation (treated group) or the vehicle (control group) was applied to the same test site which was then covered by an occlusive dressing for 48 hours.

On day 22, after a rest period of 12 days, all animals of the treated and control groups were challenged by a cutaneous application of the test substance formulation to the right flank. The left flank served as control and received the vehicle only. Test substance formulation and vehicle were maintained under an occlusive dressing for 24 hours.
Skin reactions were evaluated approximately 24 and 48 hours after removal of the dressing.

Test substance concentrations were as follows:

Induction (treated group)
- intradermal injections: PALMITOYL-PENTAPEPTIDE 4 formulation at the concentration of 75% (w/w) in sterile isotonic saline solution (0.9% NaCl).
- topical application: PALMITOYL-PENTAPEPTIDE 3 formulation undiluted.

Challenge (all groups)
- topical application: PALMITOYL-PENTAPEPTIDE 4 formulation at the concentration of 25% (w/w) in sterile isotonic saline solution (0.9% NaCl).

At the end of the study, animals were killed without examination of internal organs.
No skin samples were taken from the challenge application sites.

The sensitivity of the guinea-pigs in CIT experimental conditions was checked with a positive sensitizer, MERCAPTOBENZOTHIAZOLE. During the induction period, the reference substance was applied at the concentrations of 1% (w/w) (day 1) and 20% (w/w) (day 8) in corn oil. For the challenge application, the reference substance was applied at the concentration of 20% (w/w) in corn oil.

Results

No clinical signs and no deaths were noted during the study.

After the challenge application, no cutaneous reactions were observed.

The species and strain which were used showed a satisfactory sensitization response in 100% animals treated with MERCAPTOBENZOTHIAZOLE.

Conclusion

Under our experimental conditions and according to the maximization method of Magnusson and Kligman, the formulation of the test substance PALMITOYL-PENTAPEPTIDE 3 does not induce delayed contact hypersensitivity in guinea-pigs. 4

SPONSOR
Société Séderma
29 rue du Chemin Vert
B.P. 33
78610 Le-Perray-en-Yvelines CEDEX
France

TEST SUBSTANCE
PALMITOYL-PENTAPEPTIDE 4

STUDY TITLE
BACTERIAL REVERSE MUTATION TEST

STUDY DIRECTOR
Hasnaà Haddouk

STUDY COMPLETION DATE
6 October 1999

PERFORMING LABORATORY
CIT
Centre International de Toxicologie
Miserey - 27005 Evreux - France

LABORATORY STUDY NUMBER
18796 MMJ

STATEMENT OF THE STUDY DIRECTOR AND CIT SCIENTIFIC MANAGEMENT

The study was performed in compliance with the following Principles of Good Laboratory Practice Regulations:

. OECD Principles on Good Laboratory Practice (as revised in 1997), ENV/MC/CHEM (98) 17.
. Council Directive 87/18/EEC of 18 December 1986 on the harmonization of laws, regulations or administrative provisions relating to the application of the Principles of Good Laboratory Practice and the verification of their applications for tests on chemical substances (OJ No. L 15 of 17.1.87).
. Décret N° 90-206 du 7 mars 1990 concernant les Bonnes Pratiques de Laboratoire (Journal Officiel du 9 mars 1990), Ministère de l'Industrie et de l'Aménagement du Territoire.
. Japanese Ministry of Health and Welfare, Good Laboratory Practice Standards, Pharmaceutical Affairs Bureau, YaKuHatsu No. 313 of, March 31, 1982 (and subsequent amendments).

I declare that this report constitutes a true and faithful record of the procedures undertaken and the results obtained during the performance of the study.

This study was performed at CIT (Centre International de Toxicologie), BP 563, 27005 Evreux, France.

Mutagenicity

H. Haddouk Date: 6 October 1999
Study Director
Doctor of Applied Biochemistry
Head of Genetic Toxicology

S. de Jouffrey Date: 6 October 1999
Doctor of Veterinary Medicine
Scientific Management

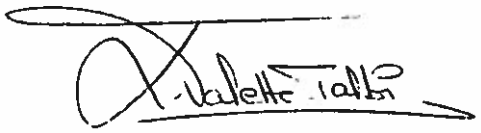
STATEMENT OF QUALITY ASSURANCE UNIT

Type of inspection	Dates		
	Inspection	Reported to Study Director (*)	Reported to Management (*)
Protocol	21 June 1999	22 June 1999	22 June 1999
Report	27 September 1999	27 September 1999	28 September 1999

In addition to the above-mentioned inspections, at about the same time as the study described in the present report, "process-based" and routine facility inspections of critical procedures relevant to this study type were also made by the Quality Assurance Unit.
The findings of these inspections were reported to the Study Director and to CIT Management.

The inspections were performed in compliance with CIT Quality Assurance Unit procedures and the Good Laboratory Practice.

The reported methods and procedures were found to describe those used and the results to constitute an accurate and complete reflection of the study raw data.



L. Valette-Talbi Date: 6 October 1999
Doctor of Biochemistry
Head of Quality Assurance Unit
and Scientific Archives

(*) The dates indicated correspond to the dates of signature of audit reports by Study Director and Management.

SUMMARY

The objective of this study was to evaluate the potential of the test substance PALMITOYL-PENTAPEPTIDE 3 to induce reverse mutation in *Salmonella typhimurium* and *Escherichia coli*.

Methods

A preliminary toxicity test was performed to define the dose-levels of PALMITOYL-PENTAPEPTIDE 3 to be used for the mutagenicity study. The test substance was then tested in two independent experiments, with and without a metabolic activation system, the S9 mix, prepared from a liver microsomal fraction (S9 fraction) of rats induced with Aroclor 1254.

Both experiments were performed according to the direct plate incorporation method except for the second test with S9 mix, which was performed according to the preincubation method (60 minutes, 37°C).

Four strains of bacteria *Salmonella typhimurium*: TA 1535, TA 1537, TA 98 and TA 100 and one strain of *Escherichia coli*: WP2 uvrA were used. Each strain was exposed to five dose-levels of the test substance (three plates/dose-level). After 48 to 72 hours of incubation at 37°C, the revertant colonies were scored.
The evaluation of the toxicity was performed on the basis of the observation of the decrease in the number of revertant colonies and/or a thinning of the bacterial lawn.

At the request of the Sponsor, the test substance PALMITOYL-PENTAPEPTIDE 3 was prepared as follows:

- a test substance solution at 0.5% was prepared in distilled water/ethanol (75/25) and homogenized during 15 minutes, this formulation was prepared once and stored at +4°C until use
- a preparation at 2% (from the test substance solution at 0.5%) was performed in distilled water

This preparation at 2% was considered as the final test substance to be tested in the present study.

The dose-levels of the positive controls were as follows:

without S9 mix:

- 1 µg/plate of sodium azide (NaN_3): TA 1535 and TA 100 strains,
- 50 µg/plate of 9-Aminoacridine (9AA): TA 1537 strain,
- 0.5 µg/plate of 2-Nitrofluorene (2NF): TA 98 strain,
- 2 µg/plate of 4-Nitroquinoline 1-oxide (4NQO): WP2 uvrA strain.

with S9 mix:

- 2 µg/plate of 2-Anthramine (2AM): *Salmonella typhimurium* strains,
- 10 µg/plate of 2-Anthramine (2AM): *Escherichia coli* WP2 uvrA strain.

Results

Since the test substance was freely soluble and non-toxic in the preliminary test, the highest dose-level for the main test was 5000 µg/plate, according to the criteria specified in the international guidelines.

The selected treatment-levels were: 312.5, 625, 1250, 2500 and 5000 µg/plate, for both mutagenicity experiments with and without S9 mix.

No emulsion was observed in the Petri plates when scoring the revertants at all dose-levels.

No toxicity was noted towards all the strains used, both with and without S9 mix.

The test substance did not induce any noteworthy increase in the number of revertants, both with and without S9 mix, in any of the five strains.

The number of revertants for the vehicle and positive controls was as specified in the acceptance criteria. The study was therefore considered valid.

Conclusion

Under our experimental conditions, the test substance PALMITOYL-PENTAPEPTIDE 4 does not show mutagenic activity in the bacterial reverse mutation test with *Salmonella typhimurium* and *Escherichia coli*.

IEC INSTITUT D'EXPERTISE CLINIQUE

REPORT

SPONSOR : **SEDERMA**

IN VITRO STUDY : **OCULAR TOLERANCE ASSESSMENT**

IN VITRO STUDY REALISED ON HEN'S EGG CHORION-ALLANTOIC MEMBRANE FOR ASSESSING OCULAR TOLERANCE (According to the HET CAM protocol published in the J.O.R.F., dated 26 December 1996)

CLINICAL STUDY : **EVALUATION OF THE PRIMARY CUTANEOUS TOLERANCE**

VERIFICATION OF THE GOOD EPICUTANEOUS LOCAL TOLERANCE, AFTER A SINGLE APPLICATION TO THE SKIN OF THE BACK AND UNDER OCCLUSIVE PATCH FOR 48 HOURS, IN 10 ADULT VOLUNTEERS (Single patch test)

TEST ARTICLE : MATRIXYL (batch n° MATRIX74E1)
contains 100 ppm Palmitoyl Pentapeptide-4

REPORT : N° 80503RD2, of 19 June 1998

For the attention of :
Mr. P. FERRANDON
SEDERMA
29, rue du chemin Vert - BP 33
78610 LE PERRAY EN YVELINES - France

Clinical Investigator :
Dr. G. RIGOT-MULLER
Dermatologist
I.E.C.
88, boulevard des Belges
69006 LYON - France

Study Director :
Mr. J.R. CAMPOS
Doctor in Cellular Biology and Microbiology
Graduate in Dermocosmetology
I.E.C.
87, rue de Sèze
69006 LYON - France

10 page document

RESULTS AND CONCLUSION

According to the experimental conditions used, the Study Assessing Ocular Tolerance by HET CAM test allowed to obtain the following results :

Positive Control : Sodium Dodecyl Sulfate (0.5% (W/V))

Mean Irritation Index = 12.0

Test article : MATRIXYL (batch n° MATRIX74E1), as supplied

Mean Irritation Index = 6.0

As a conclusion,

According to the classification published in the J.O.R.F. :

- The positive control (Sodium Dodecyl Sulfate at 0.5% (W/V)) is irritant at the ocular level.

- The test article "MATRIXYL (batch n° MATRIX74E1)", as supplied, is moderately irritant at the ocular level.

Lyon,
19 June 1998

J.P. GUILLOT
Senior Pharmacologist - Toxicologist
I.E.C. Manager

J.R. CAMPOS
Doctor in Cellular Biology
and Microbiology
Graduate in Dermocosmetology
Study Director

PROTOCOL

The test article was applied as supplied, once only, at the dose level of about 0.02 ml per panellist, on a surface of about 50 mm2 of skin on the back of 10 volunteers. The test article being under a liquid form, was put onto a disc of filter paper (7 mm in diameter) just before administration and kept in contact with the skin under an occlusive patch (Finn Chambers on Scanpor) for 48 consecutive hours. This application was performed in parallel and under the same conditions with a patch alone (without test article), as "negative" control.

Cutaneous clinical examinations were performed about 30 minutes after removal of the patches. Evaluation of the reactions was made according to a given numerical scale.

The values obtained allowed interpretation of the results according to the type of test article.

RESULTS AND CONCLUSION

No reaction of pathological irritation and significant of a cutaneous intolerance was noted. No subordinate effect was observed.

It was only noted a very slight erythema (hardly visible) in one out of the 10 panellists examined.

The index of Primary Cutaneous Irritation (P.C.I.) was equal to 0.10.

From the results obtained under the experimental conditions used, the single application of this test article to the skin of the back and under occlusive patch for 48 hours, in the adult volunteer, may be considered as : WELL TOLERATED.

Lyon,
19 June 1998

J.P. GUILLOT
Senior Pharmacologist - Toxicologist
I.E.C. Manager

Dr. G. RIGOT-MULLER, M.D.
Post graduate in Dermatology
Investigator
Study Director

CABINET DE CONSULTANT ET D'EXPERTISE

Jean-Pierre GUILLOT

Expert Toxicologue - Pharmacologue
Expert au Conseil Supérieur d'Hygiène Publique de France
Expert auprès de la D.G.C.C.R.F.
(Répression des Fraudes)
Expert national à l'O.C.D.E. et à la C.E.E.

ATTESTATION

On request of the Company **SEDERMA**, we have examined the dossier for the evaluation of the primary tolerance of the test article designated :

"MATRIXYL (batch n° MATRIX74E1)"

Examination of the information included in this dossier concerned principally :
- the normal conditions of use,
- the attestation of the manufacturer, stating that the formula to be studied was elaborated in conformity with the regulations in effect,
- the results of the cutaneous and ocular primary tolerance tests.

This examination allows us to ascertain that, to the best of our knowledge, this test article may be considered as **"RATHER WELL TOLERATED"**, as regards its ocular primary tolerance and **"WELL TOLERATED"**, as regards its cutaneous primary tolerance.

Bessenay, 19 June 1998

J.P. GUILLOT
Senior Pharmacologist - Toxicologist

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e-mail : info@...

Consumer Product Testing Co.

EST. 1975

FINAL REPORT

CLIENT: SEDERMA
29, rue du Chemin Vert – BP 33
78610 Le PERRAY-en-Yvelines
CEDEX - FRANCE

ATTENTION: Dr. Pierre Ferrandon, Ph.D.
Scientific Coordination

TEST: Repeated Insult Patch Test
Protocol No.: 1.01

TEST MATERIAL: MATRIXYL Lot/Batch MATRIXV1/001
contains 100 ppm Palmitoyl Pentapeptide-4

EXPERIMENT REFERENCE NUMBER: C99-0567.02

Richard R. Eisenberg, M.D.
Board Certified Dermatologist

Kathleen Alworth, B.A.
Director of Quality Assurance

Robert W. Shanahan, Ph.D.
Principal Investigator

Joy Frank, R.N.
Study Director

70 New Dutch Lane • Fairfield, New Jersey 07004-2514 • (973) 808-7111 • Fax (973) 808-7234

Consumer Product Testing Co.
EST. 1975

QUALITY ASSURANCE UNIT STATEMENT

Study No.: C99-0567.02

The objective of the Quality Assurance Unit (QAU) is to monitor the conduct and reporting of clinical laboratory studies. The QAU maintains copies of study protocols and standard operating procedures and has inspected this study on the date(s) listed below. Studies lasting six months or more are inspected at time intervals to assure the integrity of the study. The findings of such inspections are reported to management and the Study Director. All materials and data pertinent to this study will be stored or disposed of in accordance with current Standard Operating Procedures.

Date(s) of inspection:
June 22, 1999
June 30, 1999
July 8, 1999
August 9, 1999
August 10, 1999

Senior personnel involved:

Joy Frank, R.N.	-	Executive Vice President Clinical Evaluations
Robert W. Shanahan, Ph.D.	-	Vice President, Technology
Johanna Erdmann	-	Clinical Laboratory Supervisor
OnChi Cheung, B.S.	-	Quality Assurance Associate

The representative signature of the Quality Assurance Unit on the front page signifies that this study has been performed in accordance with standard operating procedures and study protocol as well as government regulations regarding such procedures and protocols as outlined in the Federal Register (Vol. 46, No. 17 of Tuesday, January 27, 1981).

70 New Dutch Lane • Fairfield, New Jersey 07004-2514 • (973) 808-7111 • Fax (973) 808-7234
Clinical • Toxicology • Analytical Chemistry • Microbiology

Results: The results of each participant are appended (Table 1). Subject demographics are presented in Table 2.

Observations remained negative throughout the test interval.

Summary: Under the conditions of this study, test material, MATRIXYL Lot/Batch MATRIXV1/001, did not indicate a potential for dermal irritation or allergic contact sensitization.

For Matrixyl:
Fifty-nine (59) qualified subjects, male and female, ranging in age from 19 to 78 years, were selected for this evaluation. Fifty-one (51) subjects completed this study. The remaining subjects discontinued their participation for various reasons, none of which were related to the application of the test material.

Personal Care Products Council
Committed to Safety, Quality & Innovation

Memorandum

TO: F. Alan Andersen, Ph.D.
Director - COSMETIC INGREDIENT REVIEW (CIR)

FROM: Halyna Breslawec, Ph.D.
Industry Liaison to the CIR Expert Panel

DATE: January 23, 2013

SUBJECT: Concentration of Use by FDA Product Category: Palmitoyl Peptide Ingredients

1101 17th Street, N.W., Suite 300 | Washington, D.C. 20036-4702 | 202.331.1770 | 202.331.1969 (fax) | www.personalcarecouncil.org

Concentration of Use by FDA Product Category*

Palmitoyl Dipeptide-7
Palmitoyl Dipeptide-10
Palmitoyl Dipeptide-13
Palmitoyl Dipeptide-17
Palmitoyl Dipeptide-18
Palmitoyl Tripeptide-1
Palmitoyl Tripeptide-4
Palmitoyl Tripeptide-5
Palmitoyl Tripeptide-8
Palmitoyl Tripeptide-28
Palmitoyl Tripeptide-29
Palmitoyl Tripeptide-31
Palmitoyl Tripeptide-36
Palmitoyl Tripeptide-37
Palmitoyl Tripeptide-38
Palmitoyl Tripeptide-40
Palmitoyl Tripeptide-42
Palmitoyl Tetrapeptide-7
Palmitoyl Tetrapeptide-10
Palmitoyl Tetrapeptide-20
Palmitoyl Pentapeptide-4
Palmitoyl Pentapeptide-5
Palmitoyl Hexapeptide-12
Palmitoyl Hexapeptide-14
Palmitoyl Hexapeptide-15
Palmitoyl Hexapeptide-19
Palmitoyl Hexapeptide-26
Palmitoyl Hexapeptide-32
Palmitoyl Hexapeptide-36
Palmitoyl Hexapeptide-27 Acetate
Palmitoyl Heptapeptide-5
Palmitoyl Nonapeptide-6
Palmitoyl Decapeptide-21
Palmitoyl Hydrolyzed Collagen
Palmitoyl Hydrolyzed Milk Protein
Palmitoyl Hydrolyzed Wheat
Protein
Potassium Palmitoyl Hydrolyzed
Corn Protein
Potassium Palmitoyl Hydrolyzed
Oat Protein
Potassium Palmitoyl Hydrolyzed
Rice Protein
Potassium Palmitoyl Hydrolyzed
Sweet Almond Protein
Potassium Palmitoyl Hydrolyzed
Wheat Protein
Sodium Palmitoyl Hydrolyzed Collagen
Sodium Palmitoyl Hydrolyzed Wheat Protein

Ingredient	FDA Code †	Product Category	Maximum Concentration of Use
Palmitoyl Dipeptide-7	03D	Eye lotion	0.002-0.5%
Palmitoyl Tripeptide-5	03D	Eye lotion	0.001-0.013%
Palmitoyl Tripeptide-5	12C	Face and neck products not spray	0.001-0.0013%
Palmitoyl Tripeptide-5	12J	Other skin care preparations	0.002%
Palmitoyl Tripeptide-8	12C	Face and neck products not spray	0.0005-0.05%
Palmitoyl Tripeptide-8	12F	Moisturizing products not spray	0.0001%
Palmitoyl Tripeptide-8	12G	Night products not spray	0.0005%

Palmitoyl Tripeptide-28	12C	Face and neck products not spray	0.0015%
Palmitoyl Tripeptide-38	07E	Lipstick	0.00001-0.001%
Palmitoyl Tripeptide-38	12C	Face and neck products not spray	0.0005%
Palmitoyl Tetrapeptide-7	03C	Eye shadow	0.00015%
Palmitoyl Tetrapeptide-7	03D	Eye lotion	0.00005-0.02%
Palmitoyl Tetrapeptide-7	03G	Other eye makeup preparations	0.0001%
Palmitoyl Tetrapeptide-7	04B	Perfumes	0.001%
Palmitoyl Tetrapeptide-7	07C	Foundations	0.0003-0.2%
Palmitoyl Tetrapeptide-7	07I	Other makeup preparations	0.0001-0.003%
Palmitoyl Tetrapeptide-7	12A	Skin cleansing (cold creams, cleansing lotions, liquids and pads)	0.000005-0.0009%
Palmitoyl Tetrapeptide-7	12C	Face and neck products not spray	0.000025-0.0005%
Palmitoyl Tetrapeptide-7	12D	Body and hand products not spray	0.0002%
Palmitoyl Tetrapeptide-7	12F	Moisturizing products not spray	0.0009%
Palmitoyl Tetrapeptide-7	12G	Night products not spray	0.00045-0.0015%
Palmitoyl Tetrapeptide-7	12J	Other skin care products	0.001-0.0009%
Palmitoyl Pentapeptide-4	03D	Eye lotion	0.00001-0.00061%
Palmitoyl Pentapeptide-4	07C	Foundations	0.00005-0.00011%
Palmitoyl Pentapeptide-4	12A	Skin cleansing (cold creams, cleansing lotions, liquids and pads)	0.000085%
Palmitoyl Pentapeptide-4	12C	Face and neck products not spray	0.00001-0.00061%
Palmitoyl Pentapeptide-4	12D	Body and hand products not spray	0.00003-0.00011%
Palmitoyl Pentapeptide-4	12G	Night products not spray	0.00001-0.00031%
Palmitoyl Pentapeptide-4	12J	Other skin care preparations	0.00031%

Palmitoyl Hexapeptide-12	12C	Face and neck products not spray	0.002%
Palmitoyl Hexapeptide-14	07A	Blushers (all types)	0.0085%
Palmitoyl Hexapeptide-14	07B	Face powders	0.06%
Palmitoyl Hexapeptide-14	12J	Other skin care preparations	0.0018%
Palmitoyl Hexapeptide-19	12J	Other skin care preparations	0.00025%
Palmitoyl Hydrolyzed Wheat Protein	12C	Face and neck products not spray	0.37-0.42%
Potassium Palmitoyl Hydrolyzed Oat Protein	12A	Skin cleansing (cold creams, cleansing lotions, liquids and pads)	0.06%
Potassium Palmitoyl Hydrolyzed Wheat Protein	07C	Foundations	0.05%
Potassium Palmitoyl Hydrolyzed Wheat Protein	12C	Face and neck products not spray	0.6%
Potassium Palmitoyl Hydrolyzed Wheat Protein	12D	Body and hand products not spray	0.9%

*Ingredients included in the title of the table but not found in the table were included in the concentration of use survey, but no uses were reported.
†Product category codes used by FDA

Information collected in 2012
Table prepared January 23, 2013

Personal Care Products Council
Committed to Safety, Quality & Innovation

Memorandum

TO: F. Alan Andersen, Ph.D.
Director - COSMETIC INGREDIENT REVIEW (CIR)

FROM: Halyna Breslawec, Ph.D.
Industry Liaison to the CIR Expert Panel

DATE: September 26, 2012

SUBJECT: Comments on the Scientific Literature Review on Palmitoyl Peptide Ingredients

Key Issues

In the Chemistry section, please explain the INCI Committee nomenclature conventions for peptide ingredients. As these ingredients are named without reference to the position of the amino acids, all the additional [CAS numbers] identified in Table 1 have been added to the Dictionary database.

Please add a rational for grouping these ingredients to the Chemistry section.

p.3 - Please delete the following from the Use section as the CIR Expert Panel agreed that a discussion of potential toxicity is not appropriate for inclusion in the Use section. "However, the potential for inhalation toxicity is not limited to respirable droplets/particles deposited in the lungs. Inhaled droplets/particles deposited in the nasopharyngeal and thoracic regions of the respiratory tract may cause toxic effects, depending on their chemical and other properties."

Additional Comments

p.3 - Until the SLR was received, the Council was expecting that this report would contain 2 ingredients. How can use information on all of the ingredients be "anticipated" when the Council did not know the additional 43 ingredients were in the report?

p.3, 8 - Please use the INCI name (Palmitoyl Dipeptide-10) for palmitoyl alanine-histidine (also called palmitoyl carnosine).

p.4 - Please include the frequency of application, e.g., daily, used in the study of Palmitoyl Tripeptide-1 (reference 11).

p.4-5 - In the description of reference 5, it is not clear what is meant by "excipient". If this is the same as "placebo" the same word should be used.

p.5, 9 - Please use the INCI name (Palmitoyl Pentapeptide-4) for Palmitoyl-KTTKS.

p.7 - The tetrapeptide serine-serine-asparagine-alanine has not been given an INCI name. The description of reference 12 indicates that the mitogenic activity is in the tetrapeptide moiety.

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This moiety is not part of this report. Therefore, this reference 12 should be deleted from the report. If this reference is not deleted from the report, the report should clearly state that the ingredient used in reference 12 is not included in the report.

p.7 - Tripalmitoyl pentapeptide is not an ingredient in this report, nor is the pentapeptide cysteinyl-seryl-seryl-asparginyl-alanine included in any ingredient in this report. Therefore, reference 24 should be deleted from the report, or it should be made clear that tripalmitoyl pentapeptide is not an ingredient included in this report.

p.7 - As genotoxicity and gene activation are not related, they should not be in the same section heading.

p.8 - Please delete the following as the materials tested are not cosmetic ingredients: "Study results have established palmitoyl tetrapeptide as a novel B-lymphocyte mitogen and tripalmitoyl pentapeptide as a potent immune adjuvant." If this information is left in the report, it should be stated that these compounds are not cosmetic ingredients and the amino acid composition of the peptides needs to be stated.

p.16, Table 3 - Please include the INCI names in this table. Palmitoyl-lysine-threonine-threonine-lysine-serine is Palmitoyl Pentapeptide-4.

p.18, Table 4 - All of the studies on palmitoyl-seryl-seryl-asparaginyl-alanine and tripalmitoyl-cysteinyl-seryl-seryl-asparaginyl-alanine should be deleted from Table 4 as these compounds are not cosmetic ingredients.