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

Status:	Draft Report for Panel Review
Release Date:	August 18, 2023
Panel Meeting Date:	September 11-12, 2023

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

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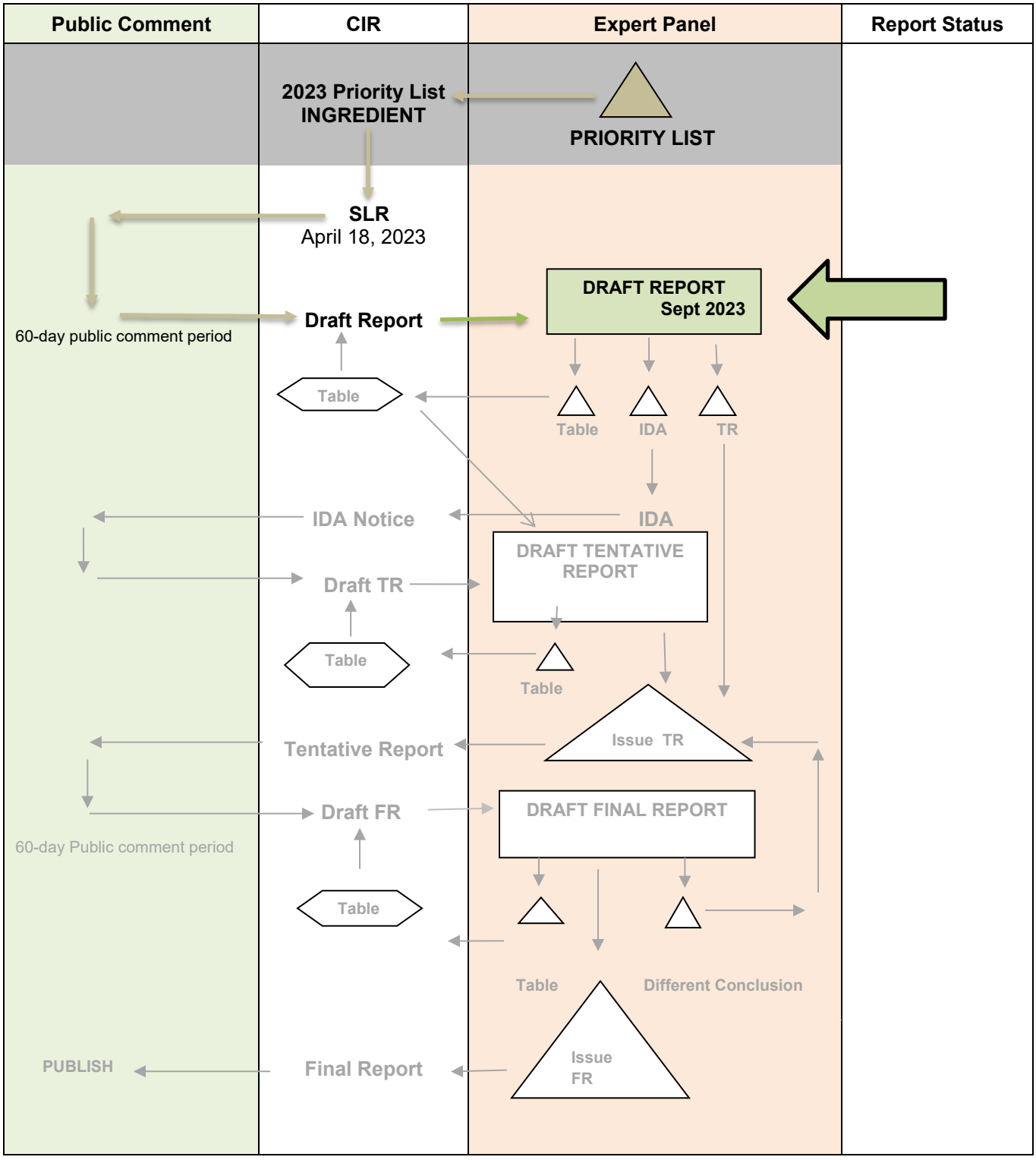
SAFETY ASSESSMENT FLOW CHART

INGREDIENT/FAMILY Pentapeptide Ingredients

MEETING September 2023



Cosmetic Ingredient Review

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Memorandum

To: Expert Panel for Cosmetic Ingredient Safety Members and Liaisons
From: Preethi S. Raj, M.Sc.
Senior Scientific Analyst/Writer, CIR
Date: August 18, 2023
Subject: Safety Assessment of Myristoyl Pentapeptide-4, Palmitoyl Pentapeptide-4, and Pentapeptide-4 as Used in Cosmetics

Enclosed is the Draft Report of the Safety Assessment of Myristoyl Pentapeptide-4, Palmitoyl Pentapeptide-4, and Pentapeptide-4 as Used in Cosmetics (identified as *report_Pentapeptides_092023* in the pdf). This is the first time the Expert Panel for Cosmetic Ingredient Safety (Panel) is seeing a safety assessment of this group of 3 cosmetic ingredients. A Scientific Literature Review (SLR) was announced on April 18, 2023. Of note, data for two amino acid sequences of Palmitoyl Pentapeptide-4 have been included, namely palmitoyl-lysine-threonine-threonine-lysine-serine (Pal-KTTKS) and palmitoyl-lysine-threonine-serine-lysine-serine (Pal-KTSKS). Test article sequences have been indicated throughout the report.

Although this is the first time the Panel is seeing a report on this group of 3 ingredients, Palmitoyl Pentapeptide-4 was initially included as part of a 2013 Draft Report on the safety of Palmitoyl Oligopeptides. Subsequently, the Palmitoyl Oligopeptides nomenclature was retired from the *Dictionary*, and the Panel decided to reorganize and regroup these ingredients into separate reports based on shared amino acid sequence; accordingly, this ingredient family was formed. Data on Palmitoyl Pentapeptide-4 that were submitted for use in that 2013 draft report (*data2_Pentapeptides_092023; data3_Pentapeptides_092023)* are now included in this package for your review.

The following is a complete listing of all unpublished data that have been submitted (currently and previously) and included in this draft report:

data1_Pentapeptides_092023

- 2022 Concentration of Use by FDA Product Category: Pentapeptide-4

data2_Pentapeptides_092023

- 2013 Concentration of Use by FDA Product Category for Palmitoyl Peptide Ingredients (Palmitoyl Pentapeptide-4) (included for complete transparency)

data3_Pentapeptides_092023

- Information on Palmitoyl Pentapeptide-4 (**all on the Pal-KTTKS sequence**; submitted November 13, 2012)
  - Sederma. 2012. Summary of information on Palmitoyl Pentapeptide-4 (previously named Pentapeptide-3).
  - Summary of acute dermal irritation of 0.01% Palmitoyl Pentapeptide-4 in rabbits. Laboratory study number 18839 TAL. CIT, 1999
  - Summary of acute eye irritation in rabbits tested with 0.01% Palmitoyl Pentapeptide-4. Laboratory study number 18840 TAL. CIT, 1999
  - Summary of acute oral toxicity of 0.01% Palmitoyl Pentapeptide-4 in rats. Laboratory study number 18838 TAR. CIT, 1999
  - Summary of local tolerance study after repeated topical application of 0.01% Palmitoyl Pentapeptide-4 for 2 weeks in guinea pigs. Laboratory study number 18842 TSG. CIT, 1999
  - Summary of skin sensitization test in guinea pigs using 0.01% Palmitoyl Pentapeptide-4. Laboratory study number 18841 TSG. CIT, 1999
  - Summary of a bacterial reverse mutation test of 0.5% Palmitoyl Pentapeptide-4. Laboratory study number 18796 MMJ. CIT, 1999

- Summary of a HET-CAM assay and human primary cutaneous tolerance of a trade name mixture containing 0.01% Palmitoyl Pentapeptide-4. Report No. 80503RD2. Institut D'Expertise Cliniqu, 1998
- Summary of repeated insult patch test of a trade name mixture containing 0.01% Palmitoyl Pentapeptide-4. Experiment Reference Number: C99-0567.02. Consumer Product Testing Co, 1999

data4_Pentapeptides_092023

- Summary Information on Palmitoyl Pentapeptide-4 (provides an overview of the individual data files listed below, **all on the Pal-KTSKS sequence**)

data5_Pentapeptides_092023

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

data6_Pentapeptides_092023

- In vitro mammalian cell micronucleus test on cultured human lymphocytes of Palmitoyl Pentapeptide-4 (81.6% pure). Report No. FSR-IPL 210103. Institut Pasteur de Lille, 2021

data7_Pentapeptides_092023

- XenoScreen YES/YAS endocrine disruptor testing of a formulation containing 0.12% Palmitoyl Pentapeptide-4. Study No. SEDE2010. Xenometrix, 2020

data8_Pentapeptides_092023

- XenoScreen XL YES endocrine disruptor testing of a formulation containing 0.12% Palmitoyl Pentapeptide-4. Study No. SEDE2105. Xenometrix, 2021

data9_Pentapeptides_092023

- Study performed on an EpiSKIN® reconstructed human epidermis model: Evaluation of the interaction of a formulation containing 0.12% Palmitoyl Pentapeptide-4 with MTT and staining power. Report No. 200840RD. IEC France, 2020

data10_Pentapeptides_092023

- Human patch test under dermatological control of a formulation containing 0.12% Palmitoyl Pentapeptide-4. Report No. ER 21/049 P21 0044. Eurofins EVIC Product Testing Romania SRL, 2021

data11_Pentapeptides_092023

- In chemico skin sensitization: Direct Peptide Reactivity Assay (DPRA) of Palmitoyl Pentapeptide-4 (81.6% pure), according to the OECD 442C guideline. Study No. 6.53-52287-ID-19/09966. IDEA Lab, 2019

data12_Pentapeptides_092023

- In vitro sensitization test: KeratinoSens™ keratinocytes test based on the signaling pathway Keap1-Nrf2-ARE coupled to the luciferase reporter gene, performed with Palmitoyl Pentapeptide-4 (81.6% pure). Study No. 6.52-52291-ID-19/09966. IDEA Lab, 2019

data13_Pentapeptides_092023

- Human repeated insult patch test with challenge of a formulation containing 0.12% Palmitoyl Pentapeptide-4. Report No. ER 21/048-14 P21 0045. Eurofins EVIC Product Testing Romania SRL, 2021

data14_Pentapeptides_092023

- Test report: UVA spectrum on 0.0015% Palmitoyl Pentapeptide-4. Sederma, 2019

data15_Pentapeptides_092023

- Evaluation of the ocular irritant potential of a formulation containing 0.12% Palmitoyl Pentapeptide-4 by application onto the hen egg chorio-allantoic membrane (HET-CAM). Study No. 6.02-54075-ID-20/00404. IDEA Lab, 2020

data16_Pentapeptides_092023

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

data17_Pentapeptides_092023

- Memorandum from the Council clarifying that the amount of Palmitoyl Pentapeptide-4 in the test article named in a published study does not exceed the maximum reported concentration of use of this ingredient in non-spray face and neck products that was reported to the Council in response to the use survey

Comments on the SLR (*PCPCcomments_Pentapeptides_092023*) that were received from the Council have been addressed and follow this memo. A comments response checklist is included (*response-PCPCcomments_Pentapeptides_092023*).

The following are also included for your review: a flow chart (*flow_Pentapeptides_092023*), literature search strategy (*search_Pentapeptides_092023*), data profile (*dataprofile_Pentapeptides_092023*), and ingredient history (*history_Pentapeptides_092023*). The meeting minutes associated with the previous review of Palmitoyl Pentapeptide-4 are also included herein (*originalminutes_Pentapeptides_092023*).

After reviewing these documents, if the available data are deemed sufficient to make a determination of safety, the Panel should issue a Tentative Report with a safe as used, safe with qualifications, split, or unsafe conclusion, and Discussion items should be identified. If the available data are insufficient, the Panel should issue an Insufficient Data Announcement (IDA), specifying the data needs therein.

Personal Care Products Council
Committed to Safety, Quality & Innovation

Memorandum

TO: Bart Heldreth, Ph.D.
Executive Director - Cosmetic Ingredient Review

FROM: Alexandra Kowcz, MS, MBA
Industry Liaison to the CIR Expert Panel

DATE: April 27, 2023

SUBJECT: Scientific Literature Review: Safety Assessment of Myristoyl Pentapeptide-4, Palmitoyl Pentapeptide-4 and Pentapeptide-4 as Used in Cosmetics (release date: April 18, 2023)

The Personal Care Products Council has no suppliers listed for Pentapeptide-4.

The Personal Care Products Council respectfully submits the following comments on the Scientific Literature Review Safety Assessment of Myristoyl Pentapeptide-4, Palmitoyl Pentapeptide-4 and Pentapeptide-4 as Used in Cosmetics.

Cosmetic Use – Please correct: “is used as up to” to “is used at up to”

Genotoxicity – It would be clearer to state the maximum dose (up to 5000 µg/plate) rather than the concentration in the second last sentence in the description of the Ames test.

Dermal Irritation and Sensitization; Ocular Irritation; Summary; Table 5 – For those studies in which the concentration of Palmitoyl Pentapeptide-4 is stated in ppm, it would be helpful to also indicate the concentration as a percentage, e.g., 100 ppm (0.01%).

Pentapeptides - September 11-12, 2023 Panel Meeting – Preethi Raj			
Comment Submitter: Personal Care Products Council **Date of Submission: April 27, 2023 (comments received on SLR posted April 18, 2023)**			
#	**Report section/Comment**	**Response/Action**	**Needs Panel Input**
1	Cosmetic Use – Please correct "is used as up to" to "is used at up to"	- revised	
2	Genotoxicity – It would be clearer to state the maximum dose (up to 5000 μg/plate) rather than the concentration in the second last sentence in the description of the Ames test.	- revised	
4	Dermal Irritation and Sensitization; Ocular Irritation; Summary; Table 5: For those studies in which the concentration of Palmitoyl Pentapeptide-4 is stated in ppm, it would be helpful to also indicate the concentration as a percentage, e.g., 100 ppm (0.01%)	- revised	

CIR History of:

Myristoyl Pentapeptide-4, Palmitoyl Pentapeptide-4, and Pentapeptide-4

March 2013

The Panel initially reviewed a large family of ingredients called Palmitoyl Oligopeptides. Since, this grouping was quite broad, the Panel decided to table this report to regroup the ingredients. Palmitoyl Petnapeptide-4 was one of said ingredients.

Previously received (unpublished) data for Palmitoyl Pentapeptide-4 includes:

- January 2013: concentration of use info

- November 2012: data from industry:

- Palmitoyl Pentapeptide-4 tested at 0.01% (vehicle and other contents not specified):
  -acute dermal irritation and acute eye irritation in rabbits, acute oral toxicity in rats, 2-wk dermal irritation in guinea pigs, HET-CAM assay, acute dermal irritation in 10 subjects, HRIPT in 51 subjects
- GPMT (0.0075% in saline and 0.01% during induction; 0.0025% in saline during challenge)
- Ames test (0.5% Palmitoyl Pentapeptide, in ethanol and water)

July 2022; February 2023

-Concentration of use data submitted by Council**;** Updated frequency of use data received from the VCRP program

April 2023

-SLR posted on CIR website; comments on SLR received from Council

- A memo was received from the Council stating that in a study summarized in the report (SLR ref 36), Palmitoyl Pentapeptide-4 did not exceed the concentration of this ingredient in face and neck products reported to the PCPC concentration of use survey

May - July 2023

In response to the SLR, the following data were received:

- Summary Information on Palmitoyl Pentapeptide-4 (provides an overview of the individual data files listed below)
- EpiSKIN® test with MTT assay (formulation containing 0.12% Palmitoyl Pentapeptide-4)
- Human patch test (formulation containing 0.12% Palmitoyl Pentapeptide-4)
- HET-CAM assay (formulation containing 0.12% Palmitoyl Pentapeptide-4)
- In vitro ocular irritation: SkinEthic™ model (formulation containing 0.12% Palmitoyl Pentapeptide-4)
- HRIPT (formulation containing 0.12% Palmitoyl Pentapeptide-4)
- XenoScreen YES/YAS endocrine disruptor testing (formulation containing 0.12% Palmitoyl Pentapeptide-4)
- XenoScreen XL YES endocrine disruptor testing (formulation containing 0.12% Palmitoyl Pentapeptide-4)
- DPRA (81.6% Palmitoyl Pentapeptide-4)
- In vitro sensitization test using KeratinoSens™ cell line (81.6% Palmitoyl Pentapeptide-4)
- Ames test (81.6% Palmitoyl Pentapeptide-4)
- In vitro mammalian cell micronucleus test (81.6% Palmitoyl Pentapeptide-4)
- Phototoxicity test (Palmitoyl Pentapeptide-4, tested at 0.0015%)

September 2023

A Draft Report is being presented to the Panel.

Pentapeptides Data Profile* – September 11-12, 2023 – Writer, Preethi Raj

				Toxicokinetics			Acute Tox			Repeated Dose Tox			DART		Genotox		Carci		Dermal Irritation			Dermal Sensitization				Ocular Irritation		Clinical Studies	
	Reported Use	Method of Mfg	Impurities	log P/log K_{ow}	Dermal Penetration	ADME	Dermal	Oral	Inhalation	Dermal	Oral	Inhalation	Dermal	Oral	In Vitro	In Vivo	Dermal	Oral	In Vitro	Animal	Human	In Vitro	Animal	Human	Phototoxicity	In Vitro	Animal	Retrospective/ Multicenter	Case Reports
Myristoyl Pentapeptide-4	X																												
Palmitoyl Pentapeptide-4	X	X	X	X	X			X		X					X				X	X	X	X	X	X	X	X	X		
Pentapeptide-4	X			X	X																								

* "X" indicates that data were available in a category for the ingredient

[Pentapeptides]

Ingredient	CAS #	PubMed	FDA	HPVIS	NIOSH	NTIS	NTP	FEMA	EU	ECHA	ECETOC	SIDS	SCCS	AICIS	FAO	WHO	Web
Palmitoyl Pentapeptide-4	521091-64-5 214047-00-4	✓	NR	NR	NR	NR	NR	NR	✓*	NR	NR	NR	NR	NR	NR	NR	✓
Pentapeptide-4	NA	✓	NR	NR	NR	NR	NR	NR	✓*	NR	NR	NR	NR	NR	NR	NR	✓
Myristoyl Pentapeptide-4	NA	NR	NR	NR	NR	NR	NR	NR	✓*	NR	NR	NR	NR	NR	NR	NR	✓

NR- not reported; ✓ - data available; ✓*- data available, but not relevant

Search Strategy

PubMed

[*total # of hits / # hits that were useful*] – search last performed: 7/27/2023

((((((((((((((myristoyl pentapeptide-4) OR (Myristoyl Pentapeptide-3)) OR (Collasyn 514KS)) OR (Palmitoyl Pentapeptide-3)) OR (Palmitoyl Pentapeptide-4)) OR (521091-64-5)) OR (214047-00-4)) OR (N2-(1-oxohexadecyl)-L-lysyl-L-threonyl-L-seryl-L-lysyl-L-serine)) OR (N2-(1-oxohexadecyl)-L-lysyl-L-threonyl-L-threonyl-L-lysyl-L-serine)) OR (Palmitoyl Pentapeptide-3)) OR (Lipopentapeptide 3)) OR (OriStar POPP)) OR (SpecPed SC-PP4)) OR (ApepPPP-5)) OR (BsPep-5)) OR (Matrixyl)
- 4,283/6 results

AND

DPRA (Direct Peptide Reactivity Assay) – 1 hit/0 useful
ADRA (Amino acid Derivative Reactivity Assay)- 0 hits
kDPRA (Kinetic DPRA) – 0 hits
IL-8-Luc (Interleukin8 Reporter Gene Assay) - 0 hits
GARD skin – 0 hits
SenCeeTox – 0 hits
VITOSens – 0 hits
PBMDC – 0 hits
SensiDerm – 0 hits
mMUSST – 0 hits

General Search

palmitoyl pentapeptide-4 cosmetic toxicity – 403,000/3

oligopeptide toxicity pentapeptide-4 – 264,000/4

LINKS

Search Engines

- Pubmed - http://www.ncbi.nlm.nih.gov/pubmed
  - appropriate qualifiers are used as necessary
  - search results are reviewed to identify relevant documents
- Connected Papers - https://www.connectedpapers.com/

Pertinent Websites

- wINCI - https://incipedia.personalcarecouncil.org/winci/ingredient-custom-search/
- FDA databases http://www.ecfr.gov/cgi-bin/ECFR?page=browse
- FDA search databases: http://www.fda.gov/ForIndustry/FDABasicsforIndustry/ucm234631.htm;,
- Substances Added to Food (formerly, EAFUS): https://www.fda.gov/food/food-additives-petitions/substances-added-food-formerly-eafus
- GRAS listing: http://www.fda.gov/food/ingredientspackaginglabeling/gras/default.htm
- SCOGS database: http://www.fda.gov/food/ingredientspackaginglabeling/gras/scogs/ucm2006852.htm
- Indirect Food Additives: http://www.accessdata.fda.gov/scripts/fdcc/?set=IndirectAdditives
- Drug Approvals and Database: http://www.fda.gov/Drugs/InformationOnDrugs/default.htm
- FDA Orange Book: https://www.fda.gov/Drugs/InformationOnDrugs/ucm129662.htm
- (inactive ingredients approved for drugs: http://www.accessdata.fda.gov/scripts/cder/iig/
- HPVIS (EPA High-Production Volume Info Systems) - https://iaspub.epa.gov/oppthpv/public_search.html_page
- NIOSH (National Institute for Occupational Safety and Health) - http://www.cdc.gov/niosh/
- NTIS (National Technical Information Service) - http://www.ntis.gov/
  - technical reports search page: https://ntrl.ntgis.gov/NTRL/
- NTP (National Toxicology Program) - http://ntp.niehs.nih.gov/
- Office of Dietary Supplements https://ods.od.nih.gov/
- FEMA (Flavor & Extract Manufacturers Association) GRAS: https://www.femaflavor.org/fema-gras
- EU CosIng database: http://ec.europa.eu/growth/tools-databases/cosing/
- ECHA (European Chemicals Agency – REACH dossiers) – http://echa.europa.eu/information-on-chemicals;jsessionid=A978100B4E4CC39C78C93A851EB3E3C7.live1
- ECETOC (European Centre for Ecotoxicology and Toxicology of Chemicals) - http://www.ecetoc.org
- European Medicines Agency (EMA) - http://www.ema.europa.eu/ema/
- OECD SIDS (Organisation for Economic Co-operation and Development Screening Info Data Sets)- http://webnet.oecd.org/hpv/ui/Search.aspx
- SCCS (Scientific Committee for Consumer Safety) opinions: http://ec.europa.eu/health/scientific_committees/consumer_safety/opinions/index_en.htm
- AICIS (Australian Industrial Chemicals Introduction Scheme)- https://www.industrialchemicals.gov.au/
- International Programme on Chemical Safety http://www.inchem.org/
- FAO (Food and Agriculture Organization of the United Nations) - http://www.fao.org/food/food-safety-quality/scientific-advice/jecfa/jecfa-additives/en/
- WHO (World Health Organization) technical reports - http://www.who.int/biologicals/technical_report_series/en/
- www.google.com - a general Google search should be performed for additional background information, to identify references that are available, and for other general information

Botanical Websites, if applicable

- Dr. Duke's - https://phytochem.nal.usda.gov/phytochem/search
- Taxonomy database - http://www.ncbi.nlm.nih.gov/taxonomy
- GRIN (U.S. National Plant Germplasm System) - https://npgsweb.ars-grin.gov/gringlobal/taxon/taxonomysimple.aspx
- Sigma Aldrich plant profiler- http://www.sigmaaldrich.com/life-science/nutrition-research/learning-center/plant-profiler.html
- American Herbal Products Association Botanical Safety Handbook (database) - http://www.ahpa.org/Resources/BotanicalSafetyHandbook.aspx
- National Agricultural Library NAL Catalog (AGRICOLA) https://agricola.nal.usda.gov/
- The Seasoning and Spice Association List of Culinary Herbs and Spices
- http://www.seasoningandspice.org.uk/ssa/background_culinary-herbs-spices.aspx

Fragrance Websites, if applicable

- IFRA (International Fragrance Association) – https://ifrafragrance.org/
- Research Institute for Fragrance Materials (RIFM) - https://www.rifm.org/#gsc.tab=0
  http://fragrancematerialsafetyresource.elsevier.com/

MARCH 2013 PANEL MEETING – INITIAL REVIEW/DRAFT REPORT

Belsito Team – March 18, 2013

DR. BELSITO: Well, I thought that -- I mean, those are case reports. I thought that the sensitization data was fine in terms of covering the range of use. So I really didn't have an issue with that.

I mean, you're occasionally going to get a case of a report of something causing a problem in somebody. I thought your data was sufficient enough to support the concentrations of use.

Anything else? Okay, palmitoyl oligopeptides. The day has come. I think that this is the first time we're looking at this. We do have some data -- safety test data on a few ingredients.

But the issue I had was that these peptide residues vary in chain length. We're told that the order of the amino acids within the residue can be very different despite having the same name. Clearly, some of these are biologically active, not only in terms of things that might not give me concern, like collagen synthesis in getting rid of wrinkles, but muting proinflammatory cytokines like IL6.

So one of the questions I would have is, what happens if you put this over a skin cancer, like a melanoma, and you reduce immune response, do you enhance the risk of metastases?

My safety issues with this group went on and on, and I really thought that sort of at the end of the day maybe -- let me see if I can see all the comments.

It's really difficult, and I almost felt that there were only two products -- the nanofiber gel and the biocide, nanofiber gel and the biopeptide CL -- that maybe had enough data, but even then you had to go set up with trade name because the other ones might have totally different amino acid sequences as far as my understanding.

So I had a real hard time wrapping myself around this family of ingredients and even thinking that we could do palmitoyl oligopeptides as a family.

So, with that as an overview, I'll turn it over to -- I had a lot of comments throughout the document, but I'll turn that over to Dan and Paul.

DR. LIEBLER: Okay. So, this one -- I had a lot of comments on it.

It seems more complicated than it really is, just from a chemistry perspective. But these products are all peptides, and the end terminus is modified by palmitic acid. So it's an N-palmitoyl version of each of these peptides.

The ingredients have a nomenclature that's confusing at first because a palmitoyl dipeptide, for example, could have a number of different ingredients. But the number after the dipeptide is apparently a code for which amino acids are in the dipeptide or in the tripeptide or in the tetrapeptide and so forth.

And I think table 1 provides some of that information, but it's confusing to me because when I read the method of manufacturer it indicates that most of these and almost all of these are produced by solid-phase peptide synthesis, which generally means you put on specifically one amino acid; then you put on another amino acid; then you put on another and another. And so, that will produce peptide sequences of high purity.

And of course, the last step -- you put on the palmitic acid on the end terminus, and you're done. That generally produces peptides of high purity in a very well defined composition sequence.

Now the table 1 is confusing because it has entries that indicate this tripeptide, for example, may contain lysine and glycine, for example, or two other amino acids. At least three amino acids with at least two of these -- I mean -- I'm sorry. A tripeptide with at least two of these amino acids in any order.

The in-any-order part, which appears in the table, seems to be inconsistent with the way solid-phase peptide synthesis works unless the synthesis actually took as the first amino acid a pool of the possible amino acids, added that, and then in the second step used a pool instead of a pure amino acid and added that.

And I can't tell -- I mean, I don't know how it's done. I could imagine that that could be done.

I'm not sure why you would do it except to have more diversity in the structures and only have to do one batch process to make the compound.

So I think we need to have more information about that because the method of synthesis suggests logically that these should be defined sequences whereas the table information suggests that these are semi-randomized sequences of a least defined composition in terms of the amino acids.

DR. BELSITO: Well, we're talking about table 1, Wilbur. There were three ingredients where you say monograph development in progress. Is this a REACH dossier? What is this monograph that's in process?

DR. ANDERSEN: Something new.

DR. BRESLAWEC: It's an INCI monograph.

DR. BELSITO: INCI monograph.

DR. BRESLAWEC: Nomenclature.

DR. BELSITO: Nomenclature, okay.

DR. ANDERSEN: Something new is being added to the dictionary.

DR. BELSITO: Okay.

DR. ANDERSEN: Dan, what's the counterpart methodology that would yield a gemisch of ordering?

DR. LIEBLER: Well, if you did pools, if you used the solid-phase method and you did pools instead of -- for example, let's say you were making -- I'm trying to find an example.

Here's palmitoyl peptide-4, which is page 33 of the PDF file. It's got lysine-threonine, lysine-serine as the four amino acids. You could start with a pool of all of serine, lysine, threonine, and then the first residue would be a mixture of those, proportional to what's in the pool. And then in the next you could use another pool. But then you wouldn't get a defined sequence that is portrayed here.

It seems to me that something is not correct in the way that this is described. It could be that these are actually randomized sequences that contain the named amino acids, but it might be that they're not really randomized. So I don't know.

That information should be available. It's just a matter of asking the precise of the manufacturers about how it's actually done and what these do contain.

DR. EISENMANN: I think they've had difficult naming these ingredients over the years as I think you've noticed. So they've started -- one of the first ones that was ever named is this palmitoyl oligopeptide.

DR. LIEBLER: Right.

DR. EISENMANN: And when they gave it a name, I think they gave a name to cover more than one peptide.

DR. LIEBLER: Yes, right. That sounds like a catch-all name.

DR. EISENMANN: Right, right. It's a catch-all name, and now -- right now -- one company is using that name for two sequences.

And then there are other names in there where they -- and then they've gone to this naming, you know, palmitoyl peptide, dipeptide-2. So the next peptide will get the next number.

DR. LIEBLER: Right.

DR. EISENMANN: And, unfortunately, the name is not sequence-specific, and I'm not sure why the INCI committee decided to do it this way. But it seems like the ingredients are sequence-specific based on the information that's been coming in.

DR. LIEBLER: Yes. So, I mean, if the decision has been taken not to make the name sequence-specific, that's out of our hands. We can still deal with these. It just makes it a little trickier.

But what we need is the table 1, essentially, to be a more accurate look-up representation. So, if we look at palmitoyl oligopeptide-6 and we look to table 1, we can see that that's, you know, valine-lysine, valine-histamine, or something like that. We can find it.

DR. EISENMANN: Right. They're just putting them in alphabetical order in the definition, but the information that's been coming in made them telling the sequence of what they actually are.

DR. LIEBLER: So, if these are defined sequence, table 1 currently suggests that they're semi-random.

DR. EISENMANN: Correct.

DR. LIEBLER: So that probably isn't correct. So we need to fix table 1 and then just add the defined sequence.

DR. EISENMANN: Unfortunately, that's the definition.

And I don't know if you've read the memo -- that we're suggesting that you cut back on these ingredients, not to do all the peptides together, that you should pick a peptide in addition to like palmitoyl.

DR. BELSITO: When did we get that memo?

DR. LIEBLER: I might not have gotten that memo.

DR. EISENMANN: I put it on your (inaudible) this morning.

DR. LIEBLER: Oh, I didn't look at that.

DR. EISENMANN: From CIR SSC.

DR. LIEBLER: So what do you want to do?

DR. BRESLAWEC: Well, our proposal is --

DR. EISENMANN: Do a peptide instead of basing it on palmitoyl because, unfortunately, the two peptides that are in palmitoyl oligopeptide also have other names. They're also tripeptide-1 and hexapeptide-12.

DR. LIEBLER: Okay. So we respectfully suggest that CIR Expert Panel table as before so that the panel and staff can consider the following suggestions and develop reasonable science-based strategy for grouping.

So I definitely agree with the table again at this point.

DR. EISENMANN: So then the focus would be for this report just to do tripeptide, which is this three sequence. It's lysine-lysine and the other one is valine- glysine, valine-alanine-lysine. That's the two --

DR. BELSITO: So do a specific amino acid sequence.

DR. EISENMANN: Correct.

DR. BELSITO: And say that specific sequence --

DR. EISENMANN: Correct.

DR. BELSITO: -- is okay.

DR. EISENMANN: Right. That sequence would be sold under these names, under more than one name, unfortunately, currently.

DR. LIEBLER: Well, that would the least of our problems.

DR. BELSITO: But would there be -- I guess I'm less concerned about the same thing having different names than different things having the same name.

DR. BRESLAWEC: But that's a situation that exists with the current naming.

DR. BELSITO: How is that going to be rectified?

DR. EISENMANN: You would have to say that your judgment is on this sequence, and if something else is being sold under this name --

DR. BELSITO: So, in other words, the report could not be titled The Safety of Ingredient X. It would have to be The Safety of Amino Acid ABCD Palmitoyl and Amino Acid.

I mean, how are you going to do that?

DR. EISENMANN: In some ways it's similar to when you do botanicals. For some botanicals, you say the safety you're assessing is of the extract that was tested.

So that also would be true here. It would be the safety of the sequence for which you have data.

DR. BRESLAWEC: What this does is it takes it out of the basic review on the palmitoyl component and focusing on the peptide.

DR. BELSITO: Yes, which is where we need to focus.

I mean, I don't have a problem with that. I don't have a problem looking over the data again. I think that would certainly make it much easier -- to look at a specific amino acid sequence and see what data are out there for it.

I guess I just want to go on record; if one of them is the one that down-regulates IL6, I'm very concerned about a cosmetic product that now is having a potentially significant biological effect in terms of immune responses.

That might be very beneficial for the rosacea patient who has erythema, but I'm concerned about the patient who has a skin cancer who's throwing something on that's going to down-regulate proinflammatory immunity. Just, my big concern with these.

DR. LIEBLER: Yes.

DR. BELSITO: I'm not concerned about sensitization. I'm not concerned about irritation.

DR. LIEBLER: I do think that those claims are being somewhat oversold when I look at the references that supposedly support these kinds of biospecific effects based on these peptides.

I mean, we'll have to come back to that when we have specific ingredients in the table and consider that. It's a potentially important consideration, but I do believe from what I saw that some of that stuff is hype. For example, in a couple of spots in the report, some of these sequences were referred to as being part of a certain collagen or a certain antibody, but with a di or tripeptide sequence that doesn't mean squat, you know, when it comes down to biological activity.

And those sequences are also parts of many other proteins, especially when you're down to a tri or tetrapeptide sequence. They're highly -- appear in many other proteins.

DR. BELSITO: Okay. I mean, I guess the only other comment -- I know this is probably meaningless given the fact that we're going to table this, and I certainly agree with it.

You know, since these are small peptides, when you look at the skin irritation and sensitization studies in table 3, page 41 of the Panel Book and you start looking, early on you see a lot of reports of slight erythema, slight erythema, slight erythema, erythema -- all of which were considered to be nonirritant. But it just makes me a little bit concerned were these urticarial reactions to these peptides.

So, I mean, just as we look at it again I would just like everyone to keep that in mind.

Those are my two major issues.

DR. LIEBLER: So I also was going to suggest removing the palmitoylated hydrolyzed plant and animal proteins as being a bridge too far, and that's, I guess, the last paragraph of this suggestion from the council.

And then these potentially other derivatives, like the acetyl tripeptide, azole tripeptide and the copper complexes and manganese complexes and so forth -- I think we do need to have some further discussion and consideration of how to make this grouping more rationale.

DR. SNYDER: So how easy is it going to be -- if we go for the peptide, how easy is it going to be to then know exactly what in the dictionary is encompassed under the peptide designation rather than the lead ingredient, palmitoyl?

So, I mean, how are we going to capture -- are we going to be able to capture all the ingredients?

DR. BRESLAWEC: First of all, I would be glad to bring the editor of the INCI dictionary to provide a briefing about the nomenclature and how it's applied to peptides. I'm not sure it's going to clear everything up, but I think it might provide a better framework for the discussion.

We're not suggesting that from now on every single protein, or peptide, be looked at separately. Our suggestion is based on the fact that this is a new type of ingredient for the panel and work through a more limited sort of report, identify the issues that are critical and then consider, or reconsider, different grouping mechanisms.

So we're not suggesting every peptide should be reviewed on its own from now on.

DR. BELSITO: I totally agree. My thought as I tried to wrap my arms around this family -- I just didn't see it as a family.

So I applaud PCPC for coming up with that approach and also having us look at defined amino acid groups rather than saying, well, this is four amino acids that can be arranged any way you want.

DR. BRESLAWEC: Then again, I don't know. Maybe at the end of your discussion you'll determine that, gee, that's okay.

DR. BELSITO: Right.

DR. BRESLAWEC: But I'm not sure that you can reach that conclusion now.

DR. BELSITO: Right.

DR. BRESLAWEC: And we certainly can't.

DR. BELSITO: Mm-hmm.

DR. LIEBLER: So the idea would be that we would do a focused report with a couple of ingredients and then once we've got our bearings either reopen the report or do another report with more ingredients?

DR. BRESLAWEC: I think that's an administrative question --

DR. LIEBLER: Okay.

DR. BRESLAWEC: -- and can be handled pretty much a lot of different ways.

DR. LIEBLER: Okay, but I agree with that strategy.

DR. BELSITO: But don't you think the order of the amino acid is going to have very significant effects on their biological activities, or you think these are so small and they're linked to this palmitoyl group that it really doesn't matter?

DR. LIEBLER: I favor the latter. I mean, in other words, I don't think that these -- I don't think that we're going to be running into magic sequences that have profound biological activities with these very small peptides.

My only concern is when we actually get into larger peptides from hydrolyzed proteins. That's when we might actually get into antigenic epitopes that would be likely to produce allergic responses. I'm not sure that we would be getting, you know, profound mimicry of biological signaling molecules with these peptides, for example, certainly not with dipeptides, tetrapeptides, those kinds of things here.

DR. BELSITO: Anything else? Okay, if not, tabled.

DR. ANDERSEN: Tabling it is easy. Thinking about what the right groupings should be is a step that I'm not comfortable that I understand what's being proposed by the council.

So I think we've got a lot of work ahead of us to figure out just what such a grouping would look like, strategically. I wish it was clear to me now, but it's not.

DR. BRESLAWEC: We also wish we could propose a clear path forward here, and I'm not sure that we can. We just think it deserves, you know, a little more time and consideration.

MR. JOHNSON: I just have one question regarding one of the comments provided, you know, stating that the substances in the report do not have INCI names and they should be deleted from the safety assessment.

DR. BELSITO: They don't have cosmetic uses. I don't know that they should be deleted. I think it's the purview of the panel, you know, as to whether we think it contributes to the understanding of the safety of the ingredients that are actually used as cosmetics.

DR. EISENMANN: Well, if the report now is going to be on a specific sequence, then data on other sequences are not necessary.

DR. BELSITO: Right. That would be --

DR. EISENMANN: So that's those other -- some sequences they're developing just to simulate the immune system, and those aren't relevant to --

DR. BELSITO: No, no, no. We're going to look at two or three specific amino acid sequences, and all of this other -- any data on other sequences should not be included in the report.

DR. BRESLAWEC: I guess my question is, did you decide then to go ahead with this study but limited to the one ingredient which has two different specific peptides in it and table the rest?

Are you planning on moving ahead --

DR. BELSITO: No, we're going to table the whole thing.

DR. BRESLAWEC: The whole thing, okay.

DR. BELSITO: Yes. I mean, I think it will be easier to look at the data again --

DR. LIEBLER: Right.

DR. BELSITO: -- when all the extraneous stuff has been removed and we know what we're looking at.

DR. BRESLAWEC: Okay.

DR. ANDERSEN: My inclination at this point -- until we better understand the proposal on the flipside here of keeping palmitoyl oligopeptide and adding a whole bunch of things that weren't previously in the report, it's hard to assess that without doing some homework. I could see just backing the report off to the oligopeptide with its two incarnations.

DR. EISENMANN: See, but those two incarnations have other names. That's the thing. The peptide part of it is tripeptide-1 or hexapeptide-12.

DR. ANDERSEN: Okay.

DR. EISENMANN: So that's why I suggested some of these other components.

DR. LIEBLER: Yes, I think having, you know, palmitoyl oligopeptide as sort of the blanket name and then there are other ingredients that are chemically defined, with different names, doesn't really pose a big problem for us for reviewing those.

There could be a problem more on the end of the definitions in the dictionary and how council deals with it. That's another issue really.

But I think particularly for this prototype, if we end up dealing with two or three chemically well-defined substances, then I think we're back in business.

DR. ANDERSEN: Okay. So let me feed it back to you, Carol, and see if I understand the rationale.

Palmitoyl oligopeptide is an old INCI name that is, in truth, two more specific INCY names -- palmitoyl tripeptide-1 and palmitoyl hexapeptide-12.

DR. EISENMANN: Correct.

DR. ANDERSEN: Okay. So you would add those two. And then taking off on that, any safety assessment of palmitoyl tripeptide-1 would legitimately address tripeptide-1 by itself. Any review of palmitoyl hexapeptide-12 would naturally address hexapeptide-12. And as long as you're going to focus on tripeptide-1, why not look at manganese tripeptide-1?

DR. EISENMANN: Right, and I don't know where I cut it, or we cut it -- you know, these other -- I didn't think these other ones in the bottom group were necessarily appropriate to look at, but I just wanted to be inclusive and include every tripeptide-1 that was in the dictionary at this point.

DR. ANDERSEN: I understand, but in a limited fashion --

DR. EISENMANN: Correct.

DR. ANDERSEN: -- the logic is oligopeptide is actually, again, an old definition that includes two specific newly identified names.

So, in a formulation, a company could use either palmitoyl tripeptide-1 or palmitoyl oligopeptide and be kosher as it now stands?

DR. EISENMANN: Probably both. If one company has the name palmitoyl oligopeptide and if you probably bought the ingredient from them, you would use -- so if you bought it from another company, you would probably use palmitoyl tripeptide-1.

DR. ANDERSEN: But for purposes of constructing a family, I think get the logic represented in this first grouping.

DR. LIEBLER: So, Alan, are you suggesting that this construction include the palmitoyl versions of these peptides as well as the non-palmitoyl versions of these peptides?

DR. BELSITO: That would be more reasonable, yes.

DR. ANDERSEN: I think so because I can't picture that the palmitoyl moiety is going to be the issue.

DR. LIEBLER: Well, it's going to change the properties of these a lot.

DR. EISENMANN: Frequently, the peptides -- as I understand it, the peptides themselves aren't necessarily used. They've all been added to the dictionary as part of the naming process. So, if somebody just comes in and wants the palmitoyl peptide, they will also name the peptide itself now, so whether or not the peptide itself is used.

DR. ANDERSEN: It's a building block.

DR. LIEBLER: So that's my concern. If the -- because I think the palmitoyl versions of these short peptides are going to have very different properties than the nonmodified versions and these short peptides are going to really fall into our short peptide analysis family. I'm wondering if they could be included in the other reports we're doing on short peptides or hydrolyzed proteins.

DR. BELSITO: Well, why don't we see what's there?

DR. LIEBLER: Yes.

DR. BELSITO: We don't even know what's in the dictionary. It may turn out that there's only palmitoyl for these, I mean.

DR. EISENMANN: No, they're listed --

DR. LIEBLER: They're listed at the bottom.

DR. BELSITO: So, I mean, let's look at them. We can always delete the ingredients, but I'm certainly more comfortable using the framework of the amino acid sequence and then look at what's added to it rather than palmitoyl with any old amino acid sequence.

DR. LIEBLER: Okay. I mean, we're going to go through another kind of --

DR. BELSITO: We're going to table it again anyway.

DR. LIEBLER: Yes, we're going to go through another round of thinking about this and another round of discussion, and we may end up backtracking a little bit on some things that we come up with today. That's fine.

DR. BELSITO: Okay. Anything else?

MR. JOHNSON: Dr. Belsito, I have one question.

DR. BELSITO: Yes.

MR. JOHNSON: Carol mentioned two palmitoyl oligopeptides. I know on Panel Book page 14 under Composition and Impurities there are two different CAS numbers listed for palmitoyl oligopeptide. And I was wondering, are those indicative of the two different names that you were referring to earlier?

Those two, okay.

DR. BELSITO: We will also see structures next time and have very specific molecules to work with.

DR. EISENMANN: They provided sequences.

DR. BELSITO: Yes.

DR. EISENMANN: All right.

DR. BELSITO: Okay, Christina, you're up -- 6- hydroxyindole.

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DR. MARKS: Are there any other comments? If not, we'll move on to the next ingredient. Wilbur, you're up again. This is the palmitoyl oligopeptides, and this is the first time we're looking at this group of ingredients. We received a memo dated March 18 on paper. This morning I found it on my desk here from the CIR Science and Support Committee of the Personal Care Products Council suggesting that this group of ingredients should be tabled. There are some issues in terms of nomenclature and also a suggestion as to what should be included. Do you want to address that memo? I think that's important. Have you seen this memo, Tom, Ron, Ron? Are you reading it now?

DR. JOHNSON: I think our primary objection is that the family is generated by the palmitoyl moiety when we don't think the palmitoyl moiety is going to drive the physiologic activity. We think it would be more appropriate from a toxicologic standpoint to generate the families based on the protein loyalty which is more likely to be the driver. We're not suggesting that the family is wholly inappropriate or that it needs to be done material by material, but we would slice and dice this group quite differently and not based on alphabetization but, rather, generated on chemistry and physiology and therefore are not really prepared to suggest how the grouping should be formed today but that it be tabled and reexamined.

DR. MARKS: I see Ron Hill shaking his head yes, nonverbally communicating he likes that idea. Ron Shank, Tom?

DR. SHANK: I have other concerns. There are several pages in the report on cellular activity. These compounds are capable of stimulating collagen synthesis, angiogenesis and others, and I wonder does that not make them drugs? And if the answer is, no, it does not, could the Council please address that for us? And if it does, should these be reviewed as a cosmetic ingredient? Also I think there is a strong data need because of this that we need reproductive and developmental toxicity data. In Wave 2 we got some information on penetration of these compounds and it shows that they penetrate into the dermis. But then the author concluded it would not therefore to into lymph and blood and I don't quite understand that. If it gets into the dermis I think it would get into the lymph and blood. I have several concerns about the safety not just the grouping.

DR. MARKS: Thank you, Ron. I was going to ask for a preview of where we would be going with these ingredients. You had also endorsed tabling it, but I raise these concerns at this point so that the next time we see these ingredients these issues may be addressed.

DR. SHANK: Yes. It's going to a scientific committee of the Council? If they could consider not only how to group these chemically but also are these drugs as well as cosmetic ingredients?

DR. ANSELL: I think the CIR Support Committee would be more than happy to reply suggesting a more appropriate grouping. We would be happy to address any questions. As it relates to the physiologic activity as it relates to its designation as a drug or cosmetic, I would suggest that would be directed to the FDA liaison as to its regulatory status.

DR. SLAGA: Do these have a type of effect in vivo if the doses are used in cosmetics? I agree with Ron that the cellular effects are quite pronounced in some cases, but what I'm suggesting is does this really occur in vivo as a cosmetic?

DR. SHANK: I could see advantages in cosmetics to stimulate collagen synthesis.

DR. SLAGA: But not angiogenesis.

DR. SHANK: Not angiogenesis, no, but certainly collagen synthesis for removing wrinkles.

DR. BERGFELD: That's very significant. Was there significance in the paper?

DR. SHANK: Yes.

MS. LORENTZ: May I comment that that gets to the whole grouping question too, again the driver not being the palmitoyl.

DR. SHANK: Using the driver is maybe not the right term because it's the palmitoyl that drives the peptide into the skin. Just the peptide itself would be absorbed I would think rather slowly, but when you add a fatty acid to it, that makes penetration much more likely.

DR. HILL: Not just into the skin, but into cells once it gets inside. The other thing I picked up on this was that it also increases the interest in impurities if you have for example a palmitoyl pentapeptide which perhaps in and of itself isn't inactive, you would want to know if there was a tripeptide or tetrapeptide impurity for example based on the ways that peptide synthesis is done. It's challenging to get 100 percent pure peptides. Then you need information about those impurity levels and some biological activity, so that would be another related issue to this in my mind. I was looking for ingredient-by-ingredient information if we were going to go forward with these as they were.

DR. ANSELL: We're not suggesting what the conclusion would be. We're certainly not suggesting ingredient-by-ingredient reviews. But we do think that if we pulled the family apart and reassemble it into smaller groups, perhaps they would lend themselves to building new and larger families but not based solely on the derivation but, rather, a look at both sides.

MS. WEINTRAUB: I also noted the lack of reproductive and developmental toxicity data, also no carcinogenicity data and absorption, distribution, metabolism and excretion wasn't found either. So there seems to be a lot of data needed in addition to other concerns.

DR. MARKS: Tom, were you concerned about carcinogenicity of these compounds?

DR. SLAGA: No.

DR. MARKS: With the lack of data, the reason you weren't, again going forward? Or you thought there was enough in here in answering Rachel's concern?

DR. SLAGA: On carcinogenicity? I didn't have a concern about carcinogenicity. I do believe that we had some genotoxicity data and that was sufficient.

DR. SHANK: But there is a data need for reproductive and developmental.

DR. SLAGA: Yes.

DR. MARKS: That was clear. Rachel was anticipating my next question which was were there any other obvious data needs? Obviously since we're tabling it we're not going to sort through the compounds. That was one of the things I had printed out, the long list of compounds or ingredients.

DR. HELDRETH: I wanted to add that if we're going to look at these in a different grouping fashion based on the protein portion of the molecule, you'll note in the definition that there's a great amount of possible variability of what the amino acids are and what order they're in. So if we're going to base it on that part of the molecule, it would be nice if the support committee could provide us with which actual compounds are designated, say, palmitoyl tetrapeptides because that can be a number of different actual ingredients that if we're looking at the protein function or protein side of it are quite different.

DR. MARKS: Is there any comment about that, Jay?

DR. ANSELL: Yes. There was a change in nomenclature from the parent ingredient to nomenclature of other naming conventions later on in the INCI process that are perhaps better and would certainly try to point out the correct INCI nomenclature.

DR. HILL: One thing I wanted to follow-up on to Dr. Slaga's comment is while there is nothing indicative of carcinogenicity, I don't remember if it's in the first submission or in Wave 2, there is language to suggest that there might be changes in invasiveness of cells depending on the particular peptide so that's something that needs some attention. It's different than tumorigenicity and any of that, but it's changing the invasive character of the cells, then that's important.

DR. SLAGA: That relates to the angiogenesis that Ron has concern about.

DR. MARKS: Tom, going back to Rachel's question, it doesn't change your concern.

DR. SLAGA: No, it doesn't change anything about the genesis.

DR. MARKS: But the question is could it enhance the invasion if were metastatic?

DR. SLAGA: Angiogenesis inhibitors are used a lot in treatment of malignancy.

DR. HILL: Angiogenesis and cell invasion are related, but distinct phenomena as well.

DR. MARKS: We have some significant issues to resolve with these ingredients in terms of its biologic activity and back to originally is this a drug or a cosmetic. Perhaps we could dodge it if we say it's a drug, but I don't think we can do that. We have to address this. Rachel?

MS. WEINTRAUB: I have one question for Tom about genotoxicity that nanofiber gel CS was found to be genotoxic but not without metabolic activation in certain strains.

DR. SLAGA: What you have to do is take the total data for all of genotoxicity and in general it's more predominantly negative. That was the only positive and that happens now and again. I can't argue that that's not true, but generally since the majority are negative, I don't have a concern.

DR. MARKS: Anticipating when this is resubmitted, the different ingredients, was there any concern of several of these ingredients having hydrolyzed collagen? You were talking about the peptide variability. Is there any concern that these have collagen? You, Ron Shank, mentioned right in the beginning that they can increase collagen synthesis, but if you look at sodium palmitoyl hydrolyzed collagen, there is palmitoyl hydrolyzed collagen and then there are these proteins also. If we hadn't looked at this group I was going to say did you want to split any of these things out because of the difference there. But what's your sense with those? Did that raise any concerns or not, different than the peptide?

DR. SHANK: No concerns other than the other report which is on hydrolyzed amino acids and proteins.

DR. MARKS: Presumably tomorrow I'll be seconding a motion that these ingredients be tabled and I'll raise the concerns that were raised here, the need for repro and developmental toxicity. And I'll raise the thorny question whether this is a drug or a cosmetic or at least mention it because of the activity of increased collagen synthesis, the angiogenesis, and obviously the grouping is the main driver.

DR. BERGFELD: I want to add a comment that in many cosmetic products that have antioxidants in them there is increased collagen being formed and there is a biological activity of the epidermis and the upper portions of the dermis and that is still considered a cosmetic. It's dependent on concentration of the antioxidant and there are some that are released only to physicians which are higher concentrations and to my knowledge there is no prescription item in the antioxidant group.

DR. HILL: Are you talking about something like kojic acid?

DR. BERGFELD: We have a lot of antioxidants that fall into the fruit acid groups and the retinoids in a whole line of cosmetics now, but they all have biological activity and we can even see it on histology as well as clinical improvement of skin texture and wrinkles.

DR. HILL: I brought up sunscreens at the last meeting in a moment of lapse, but I had read just a few weeks before that the very extended version of what's a drug, a medical device and a cosmetic and there are clearly gray areas and maybe this is a case where we need some further clarification, and it may not be forthcoming anytime soon I guess.

DR. MARKS: I'll mention it tomorrow, but as Wilma you've said, we have other ingredients that have had biologic effects like alteration of the immune system and perhaps one of them that we're discussing today can have an impact. It should be raised and then we'll see where we go with it as we review the ingredient.

DR. BERGFELD: Ron, as to localized effects versus a systemic effect, would that make a difference for you between cosmetic and drug?

DR. SHANK: Certainly if it's systemic, yes. As to local, I'm not so much concerned.

DR. BERGFELD: I think these are mainly local.

DR. ANSELL: At very low-use concentrations with some products as well at ten-thousandth of a percent.

DR. MARKS: Are there any other comments about these palmitoyl oligopeptides?

DR. JOHNSON: I have one question, Dr. Marks, with respect to the Wave 2 data. Is it agreeable that all of those data will be incorporated into the safety assessment?

DR. MARKS: I guess if it's applicable to the ingredients in this new presentation. We'll see in the next draft report. Is there anything else about these ingredients?

DR. HILL: Wilbur, I assume you would like whatever commentary, thoughts or feedback on that, you would like that along with the actual report.

DR. JOHNSON: Yes.

DR. HILL: It will be forthcoming. I just wanted to be sure.

DR. MARKS: Thanks for bringing that, Ron. For the writers, is a better way as we work through going paperless to have a flash drive and at the end of the meeting tomorrow give you the flash drive with our changes? Some of us will have it in paper. Some of us will be on either a Word document or PDF. Which is the best way to give those back to you? In the past we got the books, and I assume what you did, Wilbur, was go page by page.

DR. JOHNSON: Yes.

DR. MARKS: That hasn't changed. We'll go page by page. It's just going to be on a screen. Lillian?

DR. GILL: I think we suggested in our memorandum to you that we would like to have the flash drive back and we can get it around to each of the writers and get your comments. Certainly you've had some team members that sent them by email. If that's better for you and you want to spend some more time, that's good as well. Either way, Jim, works for us as long as the reviewers or the writers get the comments.

DR. HILL: One thought I had on that subject was a full version of that group, that would allow lifting the pages which I don't have on this, but it would allow lifting out the pages from for example Wave 2 on the pertinent ingredient and you could tack them onto the end of the others. Maybe in the future we should aim for something along those lines.

DR. MARKS: I think we need to continue as we work though this certainly for the next few meetings as to which way to handle the data electronically and how that works and it may work one for one individual and a little differently for the other. I think sharing the way we do it is important. Jay just told me to download it from the flash drive and put it on my hard drive and showed me how to do that so that there is going to be a learning curve with varying steepness depending on the individual.

DR. ANSELL: May I also put in a request for the naming convention to use a little more of the title? I found it very difficult to find which file aligns with which report because you're only picking the first four letters. They're hard to find.

DR. BERGFELD: The memo that I reviewed said that we were to go in and rename it. I thought the responsibility was transferred to us to go in and fill in the whole name with our initials after it.

DR. GILL: I think the example allowed for renaming at the end with just the initials, but if you name it something and we can find it, Wilma, that will be fine.

DR. BERGFELD: Whatever we're supposed to do, I'd like to know that.

DR. GILL: The most critical issue was putting your initials so we would know from the comment.

DR. HILL: The only difficulty I had with that is the places where the alphabetization varied from the ingredient name and sometimes those change over time anyway, but when it's substantially different, so I marked up the agenda with what the names were so that I could quickly find them. That's a small, small thing in my opinion.

DR. BERGFELD: I would like to make another comment here. I think what was in the Buff Book before used to be in our transmission on this because we can go back and we can figure out the order of events and order up our ingredients in that way. With not having an agenda, I found that it was hard because it was free-flowing whatever compound came up.

DR. SHANKS: Wasn't that the admin Buff?

DR. BERGFELD: I didn't see it.

DR. HILL: It's there.

DR. SHANK: It's in the PDF for, but I don't think it's in the Word book.

DR. BERGFELD: I didn't see it.

DR. SHANK: The PDF files were much more complete than the Word.

DR. HILL: The first thing I did was go in and print the first 10 pages of the Buff Book that had the order of ingredients. If you were in the MS Word, you didn't see it. When I got it on the flash drive the name had changed from what was on the original website.

DR. MARKS: Obviously the data needs to be sent consistently. I gather there is among our members here a difference. You used Word and PDF, Ron?

DR. SHANK: Right. I appreciated having both forms available. I can do it in Word much faster than I can do it in PDF. I have Acrobat, but for me it's just faster in Word.

DR. MARKS: Do you use track change?

DR. SHANK: Yes, I do.

DR. MARKS: So that you can understand, Wilma, now why you were asking about the admin Buff because it is on the PDF. I guess that won't happen again, that if that were sent in Word there won't be inconsistency.

DR. ANSELL: If you use a tablet, you can't use the flash drive, you have to download it from the CIR site.

DR. HILL: The way you do that is you take the flash drive files, you put them on the computer, you upload them to Box.net and then you download them into the tablet. It's not hard, but it includes additional steps. It works slickly.

DR. ANSELL: I would suggest we recommend to staff that they pick a file name and stick to it because my file name is different that I got from the CIR from the file name for those people who use the flash drive to upload their documents.

DR. MARKS: I see this as part of growing pains, and when you make changes there are always going to be difficulties. It's going to be interesting to see how it works out over the next three or four meetings. We've obviously committed to that. I haven't heard anybody say I can't do this. I'm quitting. So that's good. Again I'll reiterate that it's a good thing we didn't have 2 hours of lectures this morning because we'll make up the difference in working through how to do this electronically. I appreciate everybody's willingness to share and in my case reveal my ignorance.

Next is 6-hydroxyindole. How many like using the down button, the little scroller or just dragging? My finger gets tired if I keep rolling the little whatever this thing is called.

Full Team – March 19, 2013

DR. BERGFELD: Thank you, unanimous. Okay, the next ingredient is Dr. Snyder, Palmitoyl Oligopeptides.

DR. SNYDER: Yes, the Palmitoyl Oligopeptide is a -- the document, the first time we've seen this, the document comprises of 45 ingredients based upon a scientific literature research that was conducted in August of 2014. We had quite a lengthy discussion about this ingredient, also, and our team came to the conclusion that we wanted to make a motion to table this ingredient to identify the correct groupings and have the groupings be based upon the peptide, not based upon what the peptide is bound to. We thought that would be a better way to look at these ingredients and maybe to bring other ingredients into the mix. And, so, we would make a motion to table this ingredient.

DR. MARKS: Second.

DR. BERGFELD: Motion's been made and a second. There's no comment on that. All those in favor at the table?

DR. MARKS: Well --

DR. BERGFELD: Well, you can comment afterwards.

(Hands raised)

DR. BERGFELD: Okay, it's approved. To table and comments now.

DR. MARKS: Yes, our team discussed some needs. Even though we tabled it, we wanted to alert interested parties that we were concerned about reproductive and developmental toxicity and we needed data on that. And then we actually had a fairly robust discussion about the potential drug effects of these particularly increased angiogenesis from these compounds. So, again, we wanted to delve into the cellular activity of these compounds and get some more --

DR. SLAGA: No, the cellular effects and cell culture, that's where this data came from being stimulating angiogenesis and really the critical thing is does this occur in vivo?

DR. BERGFELD: Ron Shank?

DR. SHANK: Well, and also it has many biological activities and I questioned whether these were actually drugs and should be reviewed as cosmetic ingredients at all. I think when there is a discussion, that has to be handled somehow. And that's why we felt a need for reproductive and developmental toxicity data.

DR. BERGFELD: Paul?

DR. SNYDER: We would agree with that assessment.

DR. BERGFELD: All right, any other discussion points that need to be put on the table for the minutes?

Dan?

DR. LIEBLER: One other point was just some clarification of the actual chemical composition of whichever of the Palmitoyl Oligopeptides are advanced for a consideration in the future. Most of these appear to be made by solid phased peptide synthesis, which should produce defined sequences, mixtures are pretty high purity, but the table one indicates that they're semi-random mixtures.

So, I mean, I suppose you could do a pooled approach to solid phase synthesis where you use a pool of amino acids for the first cycle, a pool for the second cycle. I'm not sure what's done, so, that needs to be clarified and the naming conventions, well, there isn't any, but it is completely unsatisfactory for these, so, it's really hard to tell what we're reviewing.

So, whether it's industry or whether it's the CIR staff or some interaction between those to come up with information to tell us exactly what it is that we're looking at. It's important and it's particularly going to be important if we're considering any biological activities of these compounds because if they are specific to sequences, then, obviously, we need to know the sequences involved.

DR. BERGFELD: Ron Hill?

DR. HILL: Yes, in follow-up to that, it's very well-known that when you do peptide synthesis of any kind, be it solid phase or liquid phase, certain steps are more problematic and sometimes they don't go to 100 completion. So, then what you have a fraction, however small, depending on how the analytical chemistry is done where there's a missing amino acid, and, so, then if it does on to the next step, suddenly, you've got a sequence that's different, it's shorter, and sometimes that can be 10 percent of the mixture and then that's typically handled at the end with purification.

So, that's the kind of information that would be needed particularly when we're looking at biological activity and I was trying to do a search here because they tried to use -- I didn't know whether this came from staff or from industry or who, but they tried to use DEREK to justify lack of hits and I think that's an entirely inappropriate use of that program in this particular case, and, so, I wouldn't really buy that without a lot more detail.

DR. SNYDER: I'll throw one more piece on the pile here.

DR. BERGFELD: Okay.

DR. SNYDER: The only acceptable method for characterizing compounds like this these days is mass spectrometry.

DR. BERGFELD: Thank you. All right, we'll move ahead then. This has been tabled.

Dr. Marks presenting on tromethamine.

Meeting Summary

Palmitoyl oligopeptides

The report was tabled pending reorganization of this document. These ingredients were preliminarily grouped together, as they are related structurally by an identical fatty, hydrophobic tail connected to a variable sequence of peptides.

The Panel noted, however, that the terminology used for these ingredients does not enable adequate evaluation.

Further information is sought to better understand the extent and manner in which solid-phase peptide synthesis is used to create the peptide portion of such fatty acid peptide ingredients. If additional information enables a better understanding of the amino acid sequences of the peptides of these ingredients than afforded by their definitions in the dictionary, then grouping them together in some fashion may be reasonable.

If there is a substantial degree of randomness associated with the peptides of these ingredients, then it would be important for the Panel to consider how that might influence the safety evaluation. For example, some small peptides are potent stimulators of angiogenesis. The potential for such an activity to promote tumor growth and metastasis in people with undiagnosed skin cancer might then be an issue. Given the present uncertainties, grouping a large number of these ingredients together might be inappropriate.

At the time the report was tabled, the following ingredients were included:

palmitoyl oligopeptide
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 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

MARCH 2014 PANEL MEETING – SECOND REVIEW/DRAFT TENTATIVE REPORT

Belsito Team – March 17, 2014

DR. SNYDER: You have palmitoyl oligopeptides?

DR. BELSITO: No, tripeptides.

MR. JOHNSON: Well, they have the whole name, palmitoyl oligopeptides. So that's the same report.

DR. BELSITO: Okay.

DR. KLAASSEN: You have to look under the palmitoyl.

MR. JOHNSON: Right.

DR. BELSITO: Okay, so we got these very convincing arguments from Lintner in Wave 2.

DR. EISENMANN: You know Dr. Lintner is here?

DR. BELSITO: Okay. Very convincing, Dr. Lintner, thank you.

DR. LINTNER: Thank you. I'm open to your questions. I shared some in the other room this morning, so I'll be happy to answer any further questions you have.

DR. BELSITO: Page 21; that was just my general comments. So we need to discuss with teams. Given the 1 percent report -- I'm not sure what that's --

DR. EISENMANN: Now, the 1 percent is definitely wrong.

DR. BELSITO: Okay.

DR. EISENMANN: I haven't provided new use data because I still have 0.5 and 0.25 percent, and those are probably also wrong because those are probably -- they're providing the concentration of the mixture rather than the ingredient itself.

DR. BELSITO: Okay. So assuming those are wrong and assuming -- you know, we were told the absorption with similar peptide was 3 percent. But even if you assume 100 percent, it's still within a safe limit --

DR. LINTNER: Absolutely.

DR. BELSITO: -- from your calculations. Then when I said well, 100 ppm seems to have some irritation and that would be an issue at 1 percent. But if 1 percent goes away, that's not an issue in terms of the irritation. So then if the 1 percent goes away and the 0.25 goes away, then I think we can go with a safe as used in those very low limits with the calculation even assuming 100 percent absorption.

DR. LINTNER: I can guarantee you that nobody uses these specific peptides any higher than 10 ppm, 0.0001 percent in a cosmetic cream. There is no reason to do it, and it's much too expensive. So the use levels of 0.1, 0.25, and 1 percent are absolutely wrong. They're based on, as Carol said, on the use level of the 100 ppm commercial solution, but not the true peptide content. Most people use it at 3, 5, maximum 10 ppm of peptide. The rest is just water glycerin and butylene glycol.

MR. JOHNSON: And that's for any peptide.

DR. LINTNER: I can only speak about the Sederma products, the ones under discussion here -- tripeptide-1, palmitoyl, hexapeptide-12. I don't speak for competitors' products. Most of them, indeed, are similarly formulated, but I don't have any data on those.

MR. JOHNSON: Well, let me ask you a question. In terms of --

DR. BELSITO: When you say similarly formulated, this is where we get back to my being upset with the INCI names and not being specific to a given chemical again. My assumption is that when we're saying tripeptide-1 and hexapeptide-12, we are now talking about a very specific amino acid sequence.

DR. LINTNER: Correct.

DR. BELSITO: And regardless of whether it's your specific amino acid sequence or another company's specific amino acid sequence, it would be the same, no?

DR. LINTNER: Well, well, unfortunately, that is more complicated historically. Twenty years ago our company introduced the first synthetic palmitoyl peptide to the cosmetic industry. We got the INCI name, CTFA name then, of palymitoyl oligopeptide. Why? I don't know. Strange, but that's the way it is. We supplied it. A couple of weeks later the second peptide, totally different chemical structure, it got the same INCI name, palmitoyl oligopeptide. Recently, this error was

corrected and the INCI Nomenclature Committee now gave the name tripeptide-1, palmitoyl tripeptide-1, to the tripeptide with glycine- histidine-lysine sequence, and palmitoyl and hexapeptide-12 to hexapeptide with six amino acids of a different sequence. That should have resolved the issue, to have one INCI name for one specific sequence. From what I've heard this morning in the other group, somebody -- I don't know, I've never heard of this and never run across it before -- some company seems to supply or propose a hexapeptide, palmitoyl hexapeptide, with the same amino acid composition with a different sequence. Now, this is, of course, a totally different chemical entity with totally different possible biological activity and/or, if ever, toxicity. But apparently -- Wilbur may correct or confirm -- this other competitor's product is also named palmitoyl hexapeptide-1, which just shouldn't be.

The Sederma Company also proposes and sells a palmitoyl tripeptide, but with a different number, which is composed of the same amino acids as the tripeptide-1, but again with a different sequence. Instead of glycine- histidine-lysine, it's glycine-lysine-histidine; two peptides in cell culture, but very different biological activities. So it is really up to the INCI Nomenclature Committee to clean up their act, if I may say so.

DR. BELSITO: Well, I agree, but we have a way around that. We can say that tripeptide-1 is defined as the trimer specifically composed of this sequence and that is safe as used, and the hexapeptide-12 is the hexapeptide specifically composed of this sequence, and any other resequencing of these would be insufficient.

DR. LINTNER: Correct. Yes, I agree with that.

DR. BELSITO: I mean that's easy for us to deal with.

DR. LINTNER: Good.

MR. JOHNSON: One question. In terms of the maximum use concentration, are we going to rely on industry or the Council for that information, or are we going to rely on Dr. Lintner's information?

DR. BELSITO: Well, I think you know, again -- I mean I didn't realize you were in the audience when I complimented you, but I'm glad you were here.

DR. LINTNER: Thank you for having me.

DR. BELSITO: I think that looking at what companies are trying to do with these products, looking at the activity -- I mean when I was looking at those very high levels, I reviewed this before we got Wave 2. On page 36 of the document, I said "not liking these and do we not really know what we're reviewing?" As I started seeing all of these rather potent biological effects on angiogenesis, et cetera, et cetera, and then getting your letter and putting this into perspective and the cost of manufacturing them and their solubility and the impossibility of getting them to be used at those concentrations, it sort of made sense. Companies are using these -- it's marketing tools.

DR. LINTNER: Of course.

DR. BELSITO: And they're using them because they have biological effects and they may or may not have some trivial biological effects in their cosmetic agents, but people are wanting to suck up and pay $100 for something that promises them a face lift without visiting a plastic surgeon, right?

DR. LIEBLER: But what to do about the concentration?

DR. EISENMANN: I plan to go to go back to the companies -- I've gone back to one -- and provide them with Dr. Lintner's information. They're likely to change by that simple fact and say oh, we made a mistake.

DR. BELSITO: Well, I think what we can do with it is go with Dr. Lintner's information about concentration and say that it is the assumption of the CIR -- we're making several assumptions here on the safety of these; that the ingredients are not used above the level that Dr. Lintner said and that what we call the tripeptide is this specific sequence. It's not any rearrangement of those various amino acids, and what we call the hexapeptide is this very sequence. It's not any rearrangement of those sequences. And anyone who -- and even if INCI calls it the same thing, if anyone wants to use a hexapeptide that falls under that nomenclature, but is a different sequence, the data is insufficient. And we can even put that in our conclusion. "The data are sufficient for the tripeptide of this specific sequence, but insufficient for other tripeptides that may be found under that INCI name. And it's sufficient for this sequence, but insufficient for any other hexapeptides that may fall under that INCI name." And I think we've covered our bases.

DR. LIEBLER: Well, I agree with all of that, and I also appreciate Dr. Lintner's comments. And I just think that we ought to be able to certify that industry has supplied us with the correct numbers, even if we have to prompt them with Dr. Lintner's information to tell them to check their calculations.

DR. SNYDER: So what would the title of this document be because it won't be linked to an INCI ingredient?

DR. BELSITO: It has to be linked to an INCI name, and I think that again it just points out that the dictionary has to get on mark and start giving specific names to chemicals that they group and are very different. It's very annoying.

DR. BRESLAWEC: Again, this was an attempt to do so. Obviously, some tweaks are needed in the system and Joanne Nikitakis, who is the new editor of the INCI Dictionary, will be here tomorrow and prepared to discuss this and answer any questions. That's certainly our intent.

DR. SNYDER: So, again, what are we going to title it?

DR. BELSITO: We're going to title it -- we can even put an asterisk to the title, or we can put in the title, which is going to be too long for the journal, a specific sequence that we're defining as the tripeptide and the hexapeptide.

DR. SNYDER: Excellent.

DR. LIEBLER: I think we could keep the title and add in the conclusion the clarification as to what specifically we're reviewing.

DR. BELSITO: And put it in the introduction that "there may be different hexapeptides and tripeptides with different sequences that would fall under this same INCI name; however, this review is strictly being limited to these specific sequences."

DR. SNYDER: But my only question is if you go to INCI, you're not going to find tripeptide-1, hexapeptide-12.

DR. BRESLAWEC: Yes, you will.

DR. BELSITO: Yes, they're existing INCI names, but the tripeptide may have a different sequence.

DR. SNYDER: Oh, okay. I'm sorry. I thought they were all under --

DR. EISENMANN: Actually, that name's been retired so they're working on transitioning everything over to the other name.

DR. SNYDER: Okay, I understand.

MR. STEINBERG: Yes, it's on Social Security now.

DR. BRESLAWEC: But you can't take a name and just throw it out of the dictionary because of legal and time to change the labeling in China and a lot of other things.

DR. LINTNER: If I may say, having read your preliminary draft for this meeting, it's a long list of many, many other acetyl and palmitoyl and biotinoyl and whatever peptides you have there. You're going to have a lot of work in investigating each and every one because of different chemical entities or just different substances. You can't group peptides together like you can maybe other ingredients. These peptides are each very different with their own data.

DR. LIEBLER: Well, it does raise the issue of whether those other peptide derivatives belong in this report or not.

DR. BELSITO: I think that that's --

DR. EISENMANN: Especially the ones where you haven't reviewed the other portion?

DR. LIEBLER: Right.

DR. HELDRETH: The ones that were recommended to us.

DR. SNYDER: So in this document, in the introduction, we'll have to have something that we really haven't had in specifying specifically -- so currently we don't -- I think we need to --

DR. BELSITO: Well, we're dealing with these small molecules that have biological activity and when you rearrange the amino acid sequences, you get very different activity. So that's the problem. The INCI name may not specify the sequence. So I mean I think that we are where we were at when we first started struggling with botanicals and decided to look at composition as a way of dealing with it. I think with these small peptide molecules in cosmetics, we're going to have to deal with specific sequences. And if there are several different chemicals that have the same INCI name with different sequences, we're going to have to create different reports on each of those chemicals. We're going to have to say that tripeptide-1 defined as this sequence is safe as used up to this concentration. Tripeptide-1 as defined by this different sequence is insufficient, is unsafe. I mean the conclusions may be very different.

DR. LINTNER: I think it's somewhat simpler. I think you have this situation only for the palmitoyl hexapeptide-12, which for historical reasons was only for peptide like the other tripeptides. I think today anybody who supplies or who asks for an INCI name for a hexapeptide, even if it's a palmitoyl hexapeptide, will have a new number, a chronological number. So this ambiguity in my understanding exists only on the term hexapeptide-12 because under the palmitoyl oligopeptide name, there was a tripeptide and we've taken care of that. But there were two hexapeptides, one supplied by Sederma with the leave-on, and somebody else's -- I don't know whose -- hexapeptide. They also swam in the pool under oligopeptides probably to get around the patent by Sederma and have a different sequence, but also was called oligo. And now with the change from oligo to hexapeptide-12, apparently also is called hexapeptide-12. But I don't think that you will have other instances where two

different sequences will have the same INCI name. So it shouldn't be too difficult in the future. But in your report, if you make sure that you declare the leave-on hexapeptide as safe and the other as insufficient, then that's fine.

DR. BRESLAWEC: I actually think it will be other such cases --

DR. LINTNER: Really?

DR. BRESLAWEC: Yeah. Yeah.

DR. LINTNER: Well that's a shame.

DR. BRESLAWEC: They have not been naming -- INCI has not been providing some of the names for another -- other peptide ingredients.

DR. LINTNER: Right.

DR. BRESLAWEC: So, in other categories there will be a similar sort of an issue.

DR. LINTNER: Absolutely, yeah. You should use the IUPAC Nomenclature after all, that's a group we have. Any chemist knows IUPAC, I don't know -- sorry.

DR. HELDRETH: Call it legal labeling.

DR. LINTNER: But anyway --

DR. BELSITO: Then the only other question that I had for this, looking at even -- with defining the tripeptide specific sequence is this Ursoloyl Tripeptide? Do you have any concern with that ursoloyl part of it? I mean, it looks so different from all the other animals that we looked at here?

DR. LIEBLER: I'm looking again.

DR. BELSITO: It's on page 45 of the document.

DR. LIEBLER: Ursoloyl? I guess I have the general peptide -- a general question. Are these peptides -- are we looking at the peptides or the other piece? I mean, we've got all these -- we even have it in the thioctoyl, the retinoyl ones, the quinoyl ones --

DR. BELSITO: It's my understanding that it's the -- I mean it's really the tripeptide sequence that is the driver here. Is it not? And then we are only concerned about what it's attached to, if we think that there could issues with it? But maybe I'm wrong.

DR. LIEBLER: Some of the -- some of the modifying groups could have at least as much biology as the peptides, so.

DR. BELSITO: So, then do we want to look at only the peptides with straight chains, and then look at the ones that are -- have separate chains. I mean, how do we want to split it? Because right now we are looking at -- so we are at the point where we said okay, the tripeptides with this specific amino acid sequence are fine. But now we have a whole list of tripeptides that may have that specific amino acid sequence. Are we saying those are all fine? I guess that's my question. When I looked at them, I just pulled out that last one that looked beastly as compared to the others.

DR. LIEBLER: Right. But you could make the same argument about the retinoyl one, for example.

DR. BELSITO: Okay. So then we need to -- we need --

DR. KLASSEN: I think that these -- these molecules that likely have very specific biological actions that we don't know of, we really can't put them together like -- you know, on Table 1 we've got a, you know, big steroid in here, and when that split off, what's that going to do? I mean, I think, you know we -- so I have great difficulty (inaudible) -- at least some of them, and you know, same with the retinol. I mean, you know, retinoic acid and its derivatives have tremendous biological effects just like, you know, these other more classical steroids. I think we have to be very careful here.

DR. BELSITO: So we are saying that -- we need to go back and look at our conclusion because we are going to say, safe as used with specific amino acid sequences within the limits that Dr. Lintner had told us these were being used at, and now I'm hearing from my experts that not all these tripeptides necessarily belong in this group. So are we prepared to carve these out? Or, do we want to say, insufficient, and we want industry to clarify the concentrations of use. And we want time to relook at the moieties that are attached to these tripeptides, to decide whether this is a correct grouping of the tri and hexapeptides, because I'm not the person to do this. This is not my area of expertise.

DR. SNYDER: We are talking about the single-digit parts per million for this range?

DR. BELSITO: Yeah.

SPEAKER: Right.

DR. SNYDER: I mean I don't think that's going to be (inaudible)

DR. BELSITO: I mean, that's fine, I'm just clarifying that --

DR. KLASSEN: But that just relies on argument that, well, nothing at that level could have any safety concerns. I understand the practical reaction to it, Paul, but I don't think it's a good rationale for us to be using. So, I'm trying to come up with --

DR. BELSITO: So you're prepared to slice and dice today? Or do you want to table it for a -- you know, let industry know, this is where we are going, we want concentration of use, because it doesn't seem to be what -- the reported usage seems exorbitantly high, from what we heard from industry experts. And in the meantime, give you and Curt, and other people who understand the chemistry, time to look through the list and get rid of ones that don't belong in this grouping, and create a new name of linear palmitoyl tripeptide ones?

DR. LIEBLER: If you were to ask me today, I would delete all the modified peptides with the exception of the fatty acyl derivatives, the palmitoyls.

DR. BELSITO: What's driving this report? What is the ingredient driving it?

DR. HELDRETH: It was originally palmitoyl and oligopeptide, which is now being retired so essentially, palmitoyl -- tripeptide-1, palmitoyl and hexapeptide-12, would be the driving wheel.

DR. BELSITO: Okay. So then why don't we -- so what's that family, what are we going to call that family?

DR. HELDRETH: The rest of the ingredients that the group is preparing were suggested by industry --

DR. LIEBLER: Yeah. Right. It doesn't look like there's any uses for almost all of them.

SPEAKER: Yeah. In that case it does --

DR. LIEBLER: Biotinoyl has a handful, and other than that, we've got the copper complex which I don't have as much problem with, and the palmitoyl, those are the only ones that really seem to have any uses.

DR. BELSITO: So, we'll call it palmitoyl and copper tripeptide-1?

DR. LIEBLER: I think we could call it oligopeptide -- or the tripeptide-1, and hexapeptide-12 --

DR. BELSITO: Hexapeptide-12 --

DR. LIEBLER: -- and relate it to amides, but just delete most of those other compounds.

DR. BELSITO: And related to amides. And the others aren't amides? They are, and that's --

DR. LIEBLER: They are. Yeah.

DR. BELSITO: So we can't call it --unless you can prove it.

DR. LIEBLER: But the copper complex isn't in amide.

DR. BELSITO: Then, so why don't we just --

DR. LIEBLER: But the others are all -- actually they are all attached as amides I believe.

DR. BELSITO: Right. So we can --

DR. LINTNER: No. No. No. No.

DR. LIEBLER: I'm scrolling to it.

DR. LINTNER: These are nothing but -- these are carboxyl groups --

SPEAKER: Carboxamide?

DR. LINTNER: No.

DR. HELDRETH: They are attached to the --

DR. LINTNER: COOH, they are acid --

DR. LIEBLER: They are attached to the N-terminus? That's what I was referring to.

DR. LINTNER: The palmitoyl is attached to N- terminal. Yeah.

DR. LIEBLER: Oh. The peptide -- the retinoyl is attached to the N-terminus of the peptide.

DR. LINTNER: Oh. Okay. Sorry.

DR. LIEBLER: That's what I was referring to. The quinoyl is attached to the N-terminus of the peptide, all of these are attached to the N-terminus?

DR. LINTNER: Right.

DR. BELSITO: Well, if we use a name that would include all these ingredients for excluding that doesn't make sense to me. If all you want to review is copper and palmitoyl tripeptide, then I would say, safety assessment of copper tripeptide-1, palmitoyl tripeptide-1, and then what hexapeptides do we want to review?

DR. HELDRETH: Just number 12.

DR. BELSITO: Just hexapeptide-12. So it is --

DR. HELDRETH: That's the essential (inaudible) --

DR. BELSITO: Safety assessment of copper tripeptide-1, palmitoyl tripeptide-1 and hexapeptide-12 is used in cosmetics.

DR. HELDRETH: That's just myristoyl hexapeptide- 12.

DR. BELSITO: That's fine. So then how do we --

DR. LIEBLER: And their fatty acyl amides.

DR. BELSITO: Okay. So, safety assessment of copper tripeptide-1, fatty acyl amide tripeptide-1 derivatives, related ingredients, and hexapeptide-12?

DR. LIEBLER: Safety assessment of tripeptide-1, hexapeptide-1, copper tripeptide-1, and their fatty acyl derivatives.

DR. BELSITO: Repeat that again. Relabel as?

DR. LIEBLER: Tripeptide-1, hexapeptide-12, copper tripeptide-1, and their fatty acyl derivatives.

DR. HELDRETH: Is the manganese tripeptide-1 an issue?

DR. KLASSEN: Tripeptide-1, hexapeptide-12, their metal salts and fatty acyl derivatives. How's that?

DR. BELSITO: Bart, how's that? Are we capturing everything?

DR. HELDRETH: Except they find another one.

DR. LIEBLER: And not the weird stuff.

DR. HELDRETH: As you think of -- No.

DR. BELSITO: Okay. So, it's going to be relabeled as tripeptide-1, hexapeptide-12, and their metal salts and fatty acid -- fatty acyl derivatives as used in cosmetics.

(Recess)

DR. SNYDER: So how much of Table 2 will go away?

DR. BELSITO: A lot.

DR. SNYDER: Yeah. And how much -- I mean --

DR. LIEBLER: Of Table 2 will go away? On Table 2 the only thing that gets deleted from Table 2 is biotinoyl tripeptide, Table 1.

DR. SNYDER: Well we are not -- we are referring to it as palmitoyl oligopeptide, GHK palmitoyl oligopeptide, VGVAPG levered.

MS. EISENMANN: You see, the problem is treasury survey was done before they decided to retire the name.

DR. SNYDER: So we have -- anyway, that has to be redone.

MS. EISENMANN: I have suggested that we will combine the GHK, and tripeptide-1 did in the palmitoyl VG, the other -- those two together, so you only have one person, but there's still a number of companies who could not tell me which sequence they were using under the name coumaroyl oligopeptide at that point.

DR. BELSITO: Okay. Well we are going to clarify it. We are going to clarify the sequence they can use. So, we don't really care. We are just going to clarify -- we want to go out to them Carol, and we don't want to say, "What concentration are you using in palmitoyl tripeptide- 1?" We want to say, "What concentration are you using palmitoyl tripeptide-1 as defined by this specific sequence in tripeptide?" And you need to let us know -- you need to know what sequence of tripeptide you're using, and if you are using another sequence of tripeptide, then your use is zero, and your concentration is zero.

Because basically what we are going to say is any other sequence is insufficient till we have biological data on that sequence.

MS. EISENMANN: So, was not planning on doing another complete concentration of ursoloyl, it was just planning on --

DR. BELSITO: No. just take the people who told you they are using it at --

MS. EISENMANN: High concentration.

DR. BELSITO: -- high concentration.

MS. EISENMANN: Right.

DR. BELSITO: You know, you don't need to reinvent the wheel. All the concentrations that are below what Dr. Lintner has told us are feasible to use. Don't go back and ask them.

MS. EISENMANN: Right.

DR. BELSITO: And, if they come back and say they are using it at that level, then our go-around to come to the safe as use concentration is to set the limits that Dr. Lintner told us are reasonably used in cosmetic products.

DR. LINTNER: If they use it at this level, I'm going to go after them for a patent infringement, because they can't do that. Sorry.

DR. BELSITO: Right -- well, I mean, no. If they use it at that level just as the reported levels for the cell tones, FDA should be going after them.

DR. LINTNER: Right.

DR. BELSITO: Right? And they can write all the nasty letters they want to us, in the end if they don't agree with our opinions, then let them formulate their own opinions because we are not doing it; and if they don't want to formulate their own opinions, take our opinions and enforce it, which they are also not doing, so.

MR. JOHNSON: Dr. Belsito, I'd like to call the Panel's attention to page 24.

DR. BELSITO: Yes.

MR. JOHNSON: Apparently on one of the trade names under which palmitoyl, oligopeptide is being marketed in Matrixyl 3000, and the Matrixyl 3000 consists of palmitoyl tripeptide-1 and palmitoyl tetrapeptide-7, and along with the -- and industry safety assessment are on Matrixyl 3000. Now, the amino acid sequence associated with the tetrapeptide-7, is included in the safety assessment, and we have a reference for that, but that sequence is not included in the dictionary. So, we actually have another sequence --

DR. BELSITO: Okay.

MR. JOHNSON: -- in the safety assessment.

DR. BELSITO: For tripeptide-1?

DR. SNYDER: No, for --

MR. JOHNSON: No. For palmitoyl tetrapeptide-7.

DR. SNYDER: Tetrapeptide-7 -- tripeptide, the new ones.

DR. BELSITO: But that's not in the safety assessment, we are just looking at tripeptide-1 --

DR. SNYDER: But that's he's saying, some of the data that is using that, a lot of the data we've got is on the Matrixyl 3000, which is containment --

DR. BELSITO: So they want to create tetrapeptide- in this report?

DR. LINTNER: That's right. I just sent by email to Dr. Gill, the safety information on this tetrapeptide -- on computer.

DR. BELSITO: Seven?

DR. LINTNER: So it's HRIPT, skin irritation, eye irritation, and Ames test.

DR. BELSITO: Okay. So then, I guess we are now changing what we are doing, and we are tabling it to include the tetrapeptide-7 --

DR. LINTNER: Palmitoyl and tetrapeptide-7.

DR. BELSITO: Palmitoyl -- No. It will be whatever the title --

SPEAKER: --

DR. BELSITO: -- we just created, metal, salts and fatty acyl derivatives of tripeptide-1, hexapeptide-6, and tetrapeptide-7.

DR. LINTNER: Right.

DR. BELSITO: And those peptides will be defined by your Company's specific sequences, and the data would be insufficient for biological activity on any other combination of those amino acid sequences.

DR. LINTNER: Right.

DR. BELSITO: So, I guess that's where we are at now.

MS. EISENMANN: Correct. So these tabled -- I think because --

DR. BELSITO: That's what I just said, we are changing what we are saying.

MS. EISENMANN: Because I --

DR. BELSITO: We are tabling it to include tetrapeptide-7 as defined by this specific amino acid sequence. The assumption is, we will get enough data from industry to support the safety, because we'll get the sensitization, irritation data. We already have the biological activity data. We'll clarify concentrations of use for that.

MS. EISENMANN: To actually do it while new --

DR. BELSITO: Yeah. I understand. I understand. But, you know, it gets to another thing off the place that we actually have data for, it gets a lot of stuff off the plate that we don't have data for. Good pick up, Wilbur. Thank you.

MR. JOHNSON: Okay.

DR. LINTNER: It says, the point of information to this -- this tetrapeptide-7 is sold as a solution, as such, the tetrapeptide -- under tetrapeptide-7, under the commercial name from rigin, R-I-G-I-N, and it's concentrated at 500 ppm in that solution. The same peptide is used at 50 ppm in the Matrixyl 3000 combination. So all the data that we have from Matrixyl 3000 include quite a combination; the safety of this combination, 100 ppm tripeptide-1, and 50 ppm of tetrapeptide-7. Right? So it's just in the -- 10 times concentrated version of this tetrapeptide-7 -- it is unique, you know, to Dr. Gill. Go ahead, Doc.

DR. LIEBLER: Just on one thing Dr. Belsito, I'd just like to be clear on the -- each ingredient that will be included in the revised safety assessment.

DR. BELSITO: Will be the metal salts and the fatty acyl derivatives of tripeptide-1, hexapeptide-2, and tetrapeptide-7. So this report will be labeled Safety Assessment of Tripeptide-1, Hexapeptide-12, Tetrapeptide-7 and their Metal Salts and Fatty Acyl Derivatives as Used in Cosmetics, will be the title of the new report.

And I don't think you have to do -- change much of the report, except get -- you know, get rid of the ingredients that we are not reviewing, that don't fall under that rubric. And I don't think we have data in the report on any of those other ingredients, so it's really just your tables and the list of ingredients we are reviewing that will change.

SPEAKER: Sure.

DR. BELSITO: Any other surprises that we didn't think of?

MR. JOHNSON: I think that's the last one.

DR. BELSITO: Okay. That was a good point Wilbur, we don't want to miss that. Thank you.

MR. JOHNSON: You're welcome.

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DR. MARKS: Yes. Actually we're removing sodium sulfate and the three per-sulfates that had been previously removed, or previously reviewed and concluded on. Okay. Any other comments about the inorganic sulfates? Thanks, Wilbur.

Next is the palmitoyl oligopeptides. So this is the second look at this group of -amides or -amides. It's a draft report. It was tabled to focus on knowing the peptide sequence at our last meeting. So we're back to Tom and Ron's -- are the ingredients okay? There were significant amount of comments about -- on Wave 2 from the Lintner letter. Ron Shank had raised concerns about the biologic activities, like angiogenesis, collagen census on repro development and I will open it up now. It's been retitled to the tripeptide-1, hexapeptide-12, and that was the focus on the Lintner letter. So Rons and Tom -- comments? And Hal, how do you want to proceed?

DR. SHANK: I still have the question, are these considered drugs? And I think we've had some response. Dr. Lintner says absolutely no. On the other hand I would like to hear what the FDA -- is there an official position? Oh -- okay, sorry --

DR. LINTNER: Dr. Lintner.

DR. SHANK: You are Dr. Lintner, all right. Good morning.

DR. ANSELL: Thank you for being here.

DR. SHANK: I very much appreciate --

DR. LINTNER: Thank you for letting me be here.

DR. SHANK: I very much appreciated all the material that you submitted, and I understand your position's saying no these are not drugs, but I would like to know what FDA says, so -- there is significant biological activity by these compounds, but what does FDA say? Are they drugs or are they not?

DR. GILL: We don't have any representatives from FDA present today, as far as I know.

DR. SHANK: And they didn't comment -- in the past?

DR. GILL: We got no comment from FDA on it.

DR. HILL: Yes, so I agree. I'm happy, since you're sitting three feet from me, to say I agree with everything you said. I still have the same question that Dr. Shank has, of, are these drugs or not, but it seems if FDA is not commenting, then we're reviewing them. But then you'll like me even less than with the metals report because for me, because we are seeing activity, it puts us, from where I sit, into a peptide review regime. One ingredient -- what do we know about the biology? Because if we are effecting things like keratinocyte proliferation, then that's going to be a very specific biological activity and not just something that is non-specific membrane effects, or like that, so -- I completely agreed. I was thrilled to see your letter. It pretty much put my thoughts nicely into writing, so that I didn't have to do it. So what he said is my response, and then that, to me, means we don't group. We put them in one ingredient -- one report. And nobody's going to like that -- that wants to have efficiency and contrasting and all of that, but, for me, mixing toxicology in this way, unless there's a specific read across, and then when you see a specific biological effect, with a dose response, then how do you read across? You can't, so that's how I felt about the whole thing.

DR. BERGFIELD: Could I respond on the collagen synthesis? We have a lot of cosmetic ingredients that do that. The angiogenesis is the new kind of statement. But with collagen synthesis you're going to have some angiogenesis in the skin. But we have the alpha-hydroxys, all of the fruit acids that are in that group -- all of them do that -- it's been well established.

DR. HILL: And those ought to be drugs, number one, and since they're not, I think I would contrast, in teaching -- I'm sorry, I know I shouldn't have come out and said that in open meeting, it will be captured on transcript -- but if not, fine. That's up for the FDA to decide and only the FDA to decide. But if not, then the mechanism matters. So when I began teaching medicinal chemistry to people who have never heard it before, I talk about specific versus non-specific actions. And we use the example of molecules that simply modify membrane characteristics in such a way that tadpoles stop swimming, just as an example. So the mechanism then matters, and mechanisms for compounds such as Wilma was talking about -- I think I have some sense of how those go. These I suspect aren't of that same kind of nature -- that there's some more specific mechanisms going on here. If we knew that that was not the case, then that changes things, but without knowing that that's the case, I don't know how to decide whether one can read across or not.

DR. SLAGA: Well I had a concern that there was many very strong biological activities. Most of these are done in cells in culture, which are very different from you applying on the stratum corneum of the whole skin. And first of all, I'd like to see if any of these angiogenesis really occurs when you put them on the skin. I really doubt it but we don't have that data to really do that. Our big concern last time was if you have some latent tumor cells related to either non-melanoma skin cancer or even melanoma, what these type of things may have on pushing those cells to a more active state. All of that's going to be very, very difficult to get at. So it's really, when it comes down to concentration, what effect they really have on the skin -- not what they do in vivo, or in cells in culture.

DR. MARKS: So I think it would be helpful. We keep referring to your letter, Dr. Lintner, and how good it is, but maybe you can summarize it, because what I took out of it was -- you really focused -- there were just two peptides being used -- the ones mentioned in this draft -- the tri-peptide, the hexapeptide-12 -- that it's in very small amounts -- like 10 parts per million I think was what you said.

DR. LINTNER: Even less, yes.

DR. MARKS: There's small size and low penetration, therefore these are -- these two are safe, but that -- I may have interpreted what you said in your letter incorrectly, so, why don't you embellish that, and I guess the Panel Members might say -- if you want to split out, maybe we should only do these two ingredients and only relate to them. But I don't know if that will answer the concerns about biological activity. But Dr. Lintner, please elucidate and correct what I may have misinterpreted.

DR. LINTNER: I don't think you misinterpreted anything. Thank you for all your comments. There are a number of things which I have to answer. First the question of FDA -- I've had some informal discussion with the person from FDA. I think his name is John, but I don't remember, was it at the SEC meeting recently, where he says the FDA has a difficulty defining what is a drug and what is a cosmetic, because they realize that almost everything that you put on the skin has some biological activity. J&J has recently published a small item showing that glycerin modulates keratin proliferation so shall we call glycerin a drug? I don't think so. The question is -- and the FDA actually -- CFR 21 clearly says what makes it a drug is the intent presented to the consumer, not what the biological activity of the substance is. So these products, as long as they are used in cosmetic pilots, in cosmetic presentation, with claims that are acceptable -- they are not to be considered as drugs. If they have systemic activity, that would be something different. But as I've tried to show in my paper, the amount of peptides that could even get into blood is so tiny, that there is just no concern. Caroline Eisenmann suggested that I focus on these two peptides, especially because of the fact that there is 50 or 60 in your list. Most of them are from companies I don't -- I've never worked for -- I don't know their product so I cannot say anything about peptides from other people, and so she has suggested I talk with

just on this two, which I did. As you said, these two peptides, they are being used in cosmetic products worldwide, in general at use levels of between 1 and maybe 10 ppm -- very, very unlikely that they are used at higher concentrations because there is no need, because these are the recommended concentrations by the supplier, and because they are also very expensive. So as I said, in one small comment -- the FDA usage showing that people have used it at one percent, is absurd. That cannot be imagined -- 5 ppm, 10 ppm is the usual use concentration in any cosmetic cream or other Galenic form. Most of these peptides are used just in eye creams, skin care creams, and not in large volume products. As an estimate, over the last 20 years since they have been introduced, there is not more than 50 kilograms, 80 kilograms -- that the total maximum of these peptides worldwide. So the amounts are very small. Amount doesn't mean anything. We know that there are some substances that are highly toxic, but this is certainly not the case with these peptides. One thing I tried also to show in my paper, is that if you take into account penetration, or even if you don't and you assume 100 percent penetration, you will still be at an extremely low concentration of these peptides in the tissue -- in the living tissue. And especially these two peptides being fragments of natural proteins, such as the elastin and collagen, I've tried to do a calculation and estimation of the ratio of the peptide being applied to the skin at the ratio of these fragments being present constantly in our body, in our skin, due to skin renewal, which is appearing all the time. So, if these peptides have a negative affect being a thousand times more concentrated in our tissue constantly, than those that we put on the skin, then these peptides would do us damage rather than help us, which is their purpose in the so-called matrikine concept.

DR. HILL: Could I interrupt briefly, since I have the luxury of you sitting here. The one place where I did part from your logic is palmitoylation does several specific things. One is -- it would considerably increase the likelihood that we at least, to get down to the viable epidermis. Two -- it will greatly increase the ability of a peptide to get inside a cell if there is any action there. And three -- palmitoylation specifically protects peptides and small proteins from enzymatic degradation. So if we have free peptides generated as fragments from collagen, for example, they can be enzymatically handled. But for example, maybe this is a bad example, but the drug Liraglutide uses the strategy of palmitoylation to greatly extend the half-life of that protein in the blood stream and in tissues, and as far as I understand it, although the manufacturers are unclear and there are no publications -- that palmitoyl group does not have to be removed in vivo for it to exert its anti-diabetic action -- so in cretin type effect. So I think we can see that they are doing something at very low concentrations. That argues for a specific rather than a non-specific activity. I think with the palmitoyl group in place, it's highly likely that they would persist for a substantially longer period of time and so to right off that -- well, we have a lot more of that naturally present because we're always turning over collagen which is true, doesn't completely put to bed in my mind, the fact that these things are doing something biologically specific, and since FDA has washed their hands and I don't -- that's okay -- I think our science is at least as good as theirs in some cases -- we're going to just review what goes on with these things under the conditions of use. But the palmitoylation was the one place where I think I parted ways with you in terms of all of the overall logic.

DR. LINTNER: Thank you Ron. This is of course a very pertinent comment, and I agree partly with you. Palmitoylation is a natural process in our body, but that means the body has enzymes to cut the palmitoyl and to put it there. So the natural mechanisms to take off the palmitoyl are in the skin. It is indeed extremely difficult to study what happens to peptides that you use at 3 ppm in a cell culture and to see, does it get cut down into smaller pieces? Two comments, nevertheless. One is -- the study that we published in the International Journal of Cosmetic Science, 2000, which has cited where, with the radioactive label study on it -- even smaller peptides. The smaller reduced, the higher per possibility of the peptide getting through the stratum corneum, and we compared the Lipo- carnosine dipeptide with just the carnosine without the palmitoyl. And the purpose was, indeed, to show that skin diffusion into the epidermis is improved by the palmitoyl. But, with this diffusion cell -- France diffusion cell -- we also looked at the radioactivity in the receptor fluid. And not only did we get a 100th of a percent of the applied radioactivity to the skin, in the receptor fluid, over six hours of study, 100th -- 0.01 percent, only, and secondly -- the small amount of radioactivity that we found in the receptor fluid was almost following the same identical curve for both the carnosine and the Lipo-carnosine, which I interpret, because the radioactivity was not the whole peptide -- it was just on one amino acid, the histadine residue. I interpret this as showing that once you are below or in the dermis, where you get the contact with receptor fluid, the peptide has been cut short, otherwise we would have a much more significant difference in the trans- dermal diffusion, or penetration of the peptide -- into the receptor fluid. That is one aspect. The other is, as I mentioned also, on a different, slightly longer but highly charge anhydrophilic peptide, KTPKS -- also palmitoyl -- we did a penetration study, again, with the radioactive label, and we found only three percent of the applied radioactivity in the dermis. And again, I don't know if the peptide or just the lysine residue is what we found. But only three percent was found in the dermis. So I think the two assume that negligible amount of entire or still active peptides are found in the blood stream, if it gets there.

DR. HILL: I want to be very clear. I wasn't the least bit concerned about any systemic toxicology -- only things going on in the skin.

DR. LINTNER: Yes, but in --

DR. HILL: In all the statements I said before, I wasn't concerned about anything systemic -- only things happening in the skin.

DR. LINTNER: Okay, but in the skin, it's perfectly fine if we stimulate collagen, like retinol does, or alpha-hydroxy acids and other things. These two peptides have never claimed angiogenesis, neither inhibition nor stimulation. They are simple collagen and tissue ENC extra cell ACM tissue modulating activities.

DR. MARKS: So let's get back to the ingredients. Because I, in my own mind, and not clear if we go on, however you want to -- which page you want to go to. We go on page 6 of this report. Wilbur has listed all the ingredients and right at the top is the palmitoyl oligopeptide which I understand now as basically a synonym or the INCI is for the tripeptid-1 and the -- or I mean for the palmitoyl tripeptide-1 and palmitoyl hexapeptide-12. So I kind of in my mind group those three different things together, or ingredients together, and then everything else is -- what do the Team Members want to do? Ron Shank, do you want to limit this report to just the tripeptide-1 and palmitoyl hexapeptide-12, since we seem to know the most about that? And again, that being the equivalent of the palmitoyl oligopeptide, is what I understand.

DR. LINTNER: This is a historical mistake by the nomenclature committed of the then CTFA, who, for the first time, were confronted with a synthetic peptide with a -- as a cosmetic ingredient. They named the first, and the second, and bunched it together as palmitoyl, which I thought, well -- strange. But recently this was corrected and now term INCI name palmitoyl oligopeptide disappears and is replaced by a specific name for each of the two peptides.

DR. HILL: Right.

DR. LINTNER: So you can -- I don't want to teach you but --

DR. HILL: You can teach us.

DR. LINTNER: You can write your report on tripeptide-1 and hexapeptide -- palmitoyl hexapeptide-12 -- and to keep the palmitoyl hexapeptide without the palmitoyl, is a different story, like the copper peptides and so on.

DR. MARKS: So team, what do you want to do on that, because the title is enro -- interrelated -- whatever chemicals -- I have to go back and look at it. Do you want to limit it to these two? Can you read across to all of the rest? I can see Ron Shank shaking his head yes, limit. So -- oh, he did it non-verbally. But we'll get his verbal comment for the recording, too. So we would limit to the tripeptide-1 and the hexapeptide-12 and not -- and eliminate the related -amides. Which is the correct name, -amides or - amides?

DR. LINTNER: Yes.

DR. MARKS: Or is it like either?

DR. LINTNER Yes.

DR. HILL: I was going to say potayto, potahto, but it was recently brought to my attention that nobody says potahto.

DR. MARKS: Yes, anymore. So let's --

DR. HILL: However, there's a remaining unresolved issue which is that hexapeptide-12 can apparently be one of two different molecules and we have a lot of biological data about one and zip on the other.

DR. MARKS: Well let's get back to -- well, we'll answer that question in a minute. Do you want to limit it to those two, or can you read across to the other ingredients that --

DR. HILL: I would very much -- I think you heard me earlier -- like to limit it to no more than those two.

DR. MARKS: Okay. Ron Shank?

DR. SHANK: Just the two, yes.

DR. MARKS: Okay, so we're limited to those two. And then, Ron Hill, you had a concern about the hexapeptide-12.

DR. HILL: I think Dr. Lintner was going to make a comment and the one he was -- there was one of the two that he was familiar with, but it appears that there's another vendor or another company out there who decided to do a sequence scrambling, I assume, so they could get around the patent, and it still fits within the INCI description. So if it is doing something specific, which the kinds of concentrations that are being applied suggest that there is -- there are biochemically specific effects in the cells and in the skin, then that would be presumably fairly highly dependent on the sequence, and we don't have any biology on that other one. And so if I were to draft a conclusion on my own, with no other input, I would say, evidence is sufficient for those two, and insufficient for the third.

DR. MARKS: Ron Shank? Or Dr. Lintner.

DR. LINTNER: Just a comment. I was not aware of this other hexapeptide until I read your draft report -- never heard of this before, and I have absolutely no idea who or what and why.

DR. MARKS: Wilbur?

MR. JOHNSON: So the safety assessment will just be on -- based upon your recommendation, tripeptide-1 and hexapeptide-12?

DR. MARKS: Correct.

MR. JOHNSON: What about the palmitoyl peptide-1 and palmitoyl hexapeptide-12?

DR. LINTNER: That's what I meant. The palmitoyl --

DR. MARKS: Yes.

MR. JOHNSON: So all four?

DR. LINTNER: No, no, no, no, no.

DR. MARKS: Two -- palmitoyl.

MR. JOHNSON: There are two palmitoyls.

DR. MARKS: Right.

MR. JOHNSON: But the tri-peptide-1 and hexapeptide-12 will not be included?

DR. MARKS: Correct. That's what Lintner referred to in his letter. So -- but, let's get back to this supposed other hexapeptide-12. Wilbur, do you want to comment on that, since Dr. Lintner wasn't aware of its existence? Where did that come from Ron Hill? You focused on that.

DR. HILL: So this derma molecule is apparently APGVGV -- which is alanine, proline, glycine, valine, glycine -- the other one -- it's the other one.

DR. LINTNER: It's the other one --

DR. HILL: Okay, no, you said the APGVGV sequence was never proposed by anyone in the capes but it appears pretty clearly that it is being made. We had some specific information to say that yes, this other sequence is out there in the market.

DR. LINTNER: I don't know everything.

DR. HILL: I know -- I know, I wasn't suggesting you should or did, but we have means of getting some of that information and the report suggests that other peptide is out there, and I can only presume that gets them around the patent, but we don't have any biology.

DR. LINTNER: Right.

DR. MARKS: Wilbur?

MR. JOHNSON: One other concern that I have is the fact that data on Matrixyl 3000 are included in the safety assessment but that trade name material consists of palmitoyl tripeptide-1 and palmitoyl tetrapeptide-7. So with that in mind, should data on Matrixyl 3000 be used in this safety assessment?

DR. HILL: Do we know if that hexapeptide's -- no, the peptide-7 -- is it hexapeptide-7?

MR. JOHNSON: A palmitoyl tetrapeptide-7.

DR. HILL: Tetrapeptide-7 -- do we know if that's being used separately and individually? Is that one of these ingredients? I'm not looking back. I should.

MR. JOHNSON: It is.

DR. HILL: Okay, so then we probably need to include that in the report and go ahead and use the data on that mixture. What do you think?

DR. SLAGA: Well, maybe, maybe not. That's most of the data, isn't it?

MR. JOHNSON: Yes, that's --

DR. SLAGA: If you eliminate that, you eliminate the report.

DR. MARKS: Ron Shank, comment?

DR. SHANK: So let's go back to your -- I want to get -- that's another issue. Let's resolve Ron Hill's issue of this other peptide sequence for the hexapeptide-12. Is that correct?

DR. HILL: Yes, I don't think we need to resolve it. I'm just saying, if I were to draft a conclusion, based on what's in there now, it's sufficient for -- but we have the issue that we have a dictionary name that's ambiguous. That's the catch.

DR. MARKS: Okay. So, and then, now Wilbur, so you're not concerned it can still say is the tripeptide-1, hexapeptide-12, and the Wilbur, you bring up the issue that the material that's being tested contains, besides the 12, it contains --

MR. JOHNSON: Palmitoyl tetrapeptide-7.

DR. HILL: So we ought to leave that ingredient in, as part of the review, and see if we get any more data on that one specifically. I'm guessing we already beat on industry, and this is what we're getting.

DR. MARKS: So actually, the ingredients will be three now. It will be the tetra -- the tripeptide-1, the hexapeptide-12 and the third ingredient will be the --

DR. LINTNER: The tetrapeptide-7. But I'm sure that Sedema will supply information on that one too. Because again, it's a Sedema product.

DR. MARKS: If not, it sounds like we might can get it -- tomorrow.

MR. JOHNSON: I think the data on that ingredient are included in the initial safety assessment on palmitoyl oligopeptide, so if that is the case, those data can be incorporated here.

DR. MARKS: That would be wonderful. So tomorrow, how do you want to proceed? Are we going to do a tentative report with safe? Are we reassured now with the biologic effects for these three ingredients? If not safe, then is there an insufficient data notice?

DR. HILL: I would like it to be insufficient with respect to that alternative peptide - palmitoyl peptide sequence -- the impersonator hexapeptide -- hexapeptide-

-- palmitoyl hexapeptide-12. For me, that's still insufficient. So I don't how we make a conclusion where we have one ingredient that's schizophrenic, but that's -- that's where it lands in my mind.

DR. MARKS: Ron Shank? Tom?

DR. SHANK: Do we have clear use concentration data? Because the report seems to imply that some of the products use too much. It's not realistic that they would use so much. The reason I ask that is because the skin sensitization data that we have is well below what we have use concentration. But certainly agrees with what you have said. The use concentrations would be much lower, and therefore, we have sensitization data. So I'm just confused as what data are we using for concentration of use?

DR. HILL: For me that's easy. We add to the conclusion and say, no more than x percent. And then if somebody's out there actually using it higher, leave them to support they can prove it's okay. That's how I would look at it.

DR. MARKS: Yes, I thought the sensitization was fine, but I was going on what Dr. Lintner's concentrations of the 0.001 percent, and we have HRIPT on .1 and .01 percent.

DR. ANSELL: Yes, Carol is chasing after this, but it's almost certain that they're using one percent of a solution that they bought.

DR. MARKS: Right.

DR. ANSELL: Which itself contains a few ppm of the material.

DR. LINTNER: So one hundredth --

DR. ANSELL: Yes, yes, and so that would be much more typical in reports like this, consistent with the entire marketplace. It's inconceivable that anyone would actually use it at these percentages. They'd essentially have to buy the worldwide supply of it for their one product.

DR. HILL: And if they were, then I retract what I said about systemic toxicology, because that, I think would put in a regime where we might be concerned.

DR. MARKS: Yes, I actually -- go to page 46 Ron Shank.

DR. SHANK: Forty-six?

DR. MARKS: Yes. So, if we look at the 1 -- the use concentration's.001, and if we look at the 12, it's.002, so in that use concentration, it's very low. It's consistent with what we heard. Where was the one that -- and then the other one that we're going to include is the tetra -- is that right? Is that even being used? Yes. What was the third ingredient? I have to go back and --

DR. LINTNER: Palmitoyl tetrapeptide-7.

DR. MARKS: Seven. Is that -- is that on the --

DR. HILL: It's not showing up on the creative use table though.

DR. MARKS: Yes, exactly. So, that's where I got confused, I think, Wilbur, when you brought that up. If it's in the material, why isn't it in the table?

DR. LINTNER: Maybe it's not much sold in the U.S.

MR. JOHNSON: Yes, with respect to the memo-

DR. MARKS: Yes.

MR. JOHNSON: We received from industry, that wasn't one of the ingredients that was recommended for inclusion in the revised safety assessment. So that's why it doesn't appear here.

DR. HILL: Well if we can find out that that's because it's not being sold as a separate ingredient in the United States, then that -- we just have to figure out how to put that in the report.

DR. LINTNER: We can't guarantee that, but perhaps so small amount that it doesn't show up in the FDA. I don't know.

DR. HILL: That's just a direct -- somebody can easily get that answer. Is it being sold separately in the United States, or only as a combination?

MR. JOHNSON: And one other thing -- the amino acid sequence was not included -- the specific amino acid sequence was not included in the dictionary. But I think that according to one of the publications included in the text, that amino acid sequence is stated.

DR. HILL: Well the point is with the hexapeptide, there are two alternative ingredients -- two different molecules that meet the criteria to be called that particular ingredient name, by INCI's name. The point is that --

DR. ANSELL: The point is, as Dr. Lintner pointed out, that's an historical artifact, it has been corrected and that name is retired.

DR. HILL: So hexapeptide --

DR. ANSELL: Yes. The palmitoyl peptide.

DR. HILL: I thought it was the palmitoyl oligopeptide that's been retired. So now we have hexapeptide, but the point is that there are two different sequences that both meet the hexapeptide name. So there are two different molecules, which, if they're not doing anything specific in the tissue, and we know that, fine. But all the evidence suggests to me that that's not the case, and that these two different sequences would be biologically disparate, and so, that's why I say, a conclusion for me would be -- that ingredient with this specific hexapeptide sequence -- safe as used, capped at.1 percent. The other one -- insufficient data.

MR. JOHNSON: On page 24 --

DR. HILL: I'm there, yes.

MR. JOHNSON: Yes. The amino acid sequence for the palmitoyl tetrapeptide-7 is included. And that paragraph immediately above -- physical and chemical properties --

DR. HILL: Yes, I wasn't talking about 7 right then, though. I was talking about the two different flavors of palmitoyl hexapeptide.

DR. MARKS: Which one is it?

DR. HILL: 12 -- hexapeptide-12. There are two different versions of palmitoyl -- two different molecules that are both called palmitoyl hexapeptide-12.

MR. JOHNSON: Right. Yes.

DR. HILL: All right, one -- I think we have enough information to conclude -- safe as used if, in reality the use is.1 percent. The other one, we have no data, for me, that one's insufficient. So if we say it's sufficient, meeting -- let's see -- which is the current -- the sequence we know a lot about --

DR. LINTNER: VGB.

DR. HILL: Yes, VGB. That one we know. The one that is VGV -- no, let me see, wait a minute, where is it --

DR. LINTNER: On the (inaudible), VGVAPG.

DR. GILL: I have it on page 23, Ron. I think it's -- Wilbur has it as also known as tripeptide-1. It's that second sequence for the hexapeptide-12.

DR. HILL: Yes. Okay. So tripeptide-1 is a different ingredient.

DR. LINTNER: Yes.

DR. HILL: And so -- so how do we deal with this? So if it's being sold under both names, that's a problem. I should say, if it's being labeled under both names -- the hexapeptide-12 is maybe actually two different molecules. That's a problem. Because

one of them we have data for and the other one we don't. I need to write down so I can use a cheat sheet and reference it, but -- am I losing you totally? There's two different molecules --

MR. JOHNSON: I know -- I know what you're saying.

DR. HILL: All right. There's two different molecules called hexapeptide -- palmitoyl hexapeptide-12. One of them we have plenty of data on. The other one, we have zero data on. So in my mind, we're sufficient -- safe,.1 percent or below and the other one --

DR. MARKS: I don't think we need the 1 percent limit, do we? Because it's not being used there.

DR. HILL: If we know for very certain that everybody is already in use at.1 percent --

DR. MARKS: Well, it's much less than 1 percent.

DR. HILL: Then we should make sure we state that in the discussion --

DR. LINTNER: .1 is fine.

DR. MARKS: But we normally have a conclusion, the present use and concentration, the present use of that, or the present concentration in the table was much lower than.1 percent.

DR. HILL: But we have tabulated data up to 10 percent, which we think is fictional, but we don't have information to suggest it's fictional.

DR. LINTNER: No, one percent is --

DR. HILL: Or one percent rather.

DR. LINTNER: But that is impossible.

DR. HILL: And he says it's impossible, but --

DR. GILL: And Carol is checking on that, correct?

DR. HILL: So if we know that that's impossible, I agree, we can take that out. And we still have insufficient for the second version of palmitoyl hexapeptide-12. If it has an alternative name, then the problem is just that it's being sold under the wrong name. Labeled under the wrong name -- I don't know.

DR. LINTNER: Probably, it may pop up with other peptides, because the INCI Nomenclature Committee has decided not to reveal the sequences -- just amino acid composition. And if different sequences may be even supplied under CDA -- if there's a supplier doesn't want to give the sequence, which I find absurd, but if that's the case, then that's okay, but at least different chemical entities should have different INCI name.

DR. HILL: Well, if, in terms of the safety review, from where I sit, if we have some sense that something specific's going on, and it's just nonspecific effects, then they need to -- I can't reach a conclusion if they don't reveal the sequence.

DR. MARKS: So which is the alt -- the other one you said -- what was that sequence, just so I have it noted here, Ron Hill?

DR. HILL: VGVAPG is the --

DR. LINTNER: The correct one, the safe one.

DR. HILL: VPG, or VGVAPG, which is valine, lysine, valine, alamine, proline, glycine. I'm going to put --

DR. MARKS: All right, I'm going to call on you tomorrow, Ron --

DR. HILL: Okay, I'll make sure I make myself cheat notes so that I don't have this problem referring to --

DR. MARKS: Ron Shank and Tom Slaga -- do you have the same concerns, with this different amino acid sequence, that that's going to make a big difference?

DR. SLAGA: Well, the amino acids will make a difference and I think we have to be specific to the sequence.

DR. MARKS: Okay.

MR. JOHNSON: One point -- the reason why I mentioned the amino acids sequence for palmitoyl tetrapeptide-7 is because, the reason for this particular grouping of ingredients in this report was based upon the known amino acid sequence. The known amino acid -- there was no known amino acid sequence in the dictionary with respect to palmitoyl tetrapeptide-7, so this if from --

DR. HILL: It's given in the report.

MR. JOHNSON: Yes, but this is from another reference. It's not from the dictionary.

DR. HILL: Ah. Well, then the deal would be the same. If it meets the sequence, we have data, and it supports the safety and we're find, and if it does not meet the sequence -- insufficient.

MR. JOHNSON: Um-hm.

DR. MARKS: And where, Wilbur, on this report, do you refer to the tetrapeptide-7 so --

MR. JOHNSON: It's on --

DR. MARKS: What page or pages?

MR. JOHNSON: Page 24, in the section immediately above physical and chemical properties.

DR. HILL: The sequence it's giving in the text is PAL, which is palmitoyl GQPR -- Gentleman's Quarterly Public Relations.

DR. MARKS: Wilbur, where is the testing where you say it contained the tetrapeptide-7? So which, and then the comment was made -- well, that's in everything that's being tested. Which product was that again, that you were talking --

MR. JOHNSON: That's Matrixyl 3000. That's a trade name.

DR. MARKS: And what page is that?

MR. JOHNSON: Well, it's on page 24 and I think I have it in the introduction.

DR. MARKS: Page 24.

DR. HILL: Well, it's in that same section where you just referred us to. It says, according to another source, let's see -- data on Matrixyl 3000 are included in the safe palmitoyl -- okay, somewhere you say, right there -- is one of two active ingredients in that -- right at the beginning of that paragraph.

DR. MARKS: Introduction, okay. Let me go back and -- I'm missing it. Where in the introduction is it?

DR. HILL: No, it's that same paragraph right above physical and chemical properties. I don't know if it's written again in the introduction or not. But that same paragraph, he just referred us to, where we had the GQPR -- on page 24.

DR. MARKS: So it's right above -- yes, okay. So that's the Matrixyl.

DR. HILL: And then it says the other active ingredient is tetrapept -- palmitoyl tetrapeptide-7.

MR. JOHNSON: Yes, that's the first occurrence of it, on page 24.

DR. MARKS: Okay.

MR. JOHNSON: Oh no, it is -- it is mentioned in the introduction, I'm sorry, it is. It is there, yes.

DR. SHANK: Yes, it is.

MR. JOHNSON: So would we need to -- would the Council need to confirm that that is the amino acid sequence for palmitoyl tetrapeptide-7, or is the Panel accepting this reference?

DR. HILL: Is there any reason to believe that reference is incorrect?

MR. JOHNSON: You'd have to ask the Council.

DR. HILL: Me? That's a rhetorical question at this point.

DR. LINTNER: Tetrapeptide -- palmitoyl tetrapeptide-7 is the sequence that you have announced. It is the main ingredient that is mixed with the palmitoyl tripeptide-1 in the commercially called blend Matrixyl 3000, but you have data on biopeptide CL which contains the 100 ppm of tripeptide -- palmitoyl tripeptide number 1, and then this Matrixyl -- it's just added another peptide. But it's still safe. So addition of the peptide, tetrapeptide-7 to the tripeptide-1, does not change the safety. So it does not --

DR. MARKS: The only problem I have when I'm looking at the Matrxyl under sensitization is, I don't know what concentration -- I don't know what amount of the seven is in there, to say this is a safe limit. Did I miss that?

So I would probably not include seven in the report, just because it's not under the use concentration, and I'm not sure it's --

DR. LINTNER: The amount is half of -- it's 50 ppm.

DR. MARKS: Fifty.

DR. LINTNER: Fifty. It's half of the tripeptide.

DR. MARKS: Okay.

DR. HILL: Well, then that poses the difficulty. If it was sold individually and separately, would they put it in there up to a hundred? In which case we don't have data to support it which I think is what you were trying to say.

DR. LINTNER: But we can send data -- specific data on the palmitoyl tetrapeptide-7. That is another product from Sederma. That is sold individually. Maybe not much in the U.S., but there are some sales, so, we have data on this like for the other peptide.

DR. MARKS: Okay.

DR. HILL: So if we could get that, that would be nice to keep that in, even if it's not sold in the U.S., because it rounds out this -- I think it nicely rounds out the evaluation.

DR. MARKS: So one of the things we could move forward, again an insufficient data notice to clarify the alternative -- hexapeptide-12 -- Ron Hill, you were asking for that -- that amino acid sequence and the other would be further data on the tetrapeptide-7, in terms of sensitization you said. So there could be an insufficient data notice or we could go forward with a safe. Which way would you prefer?

DR. SLAGA: Well it's early in the game, so insufficient --

DR. HILL: Yes, I think even if you just include that one insufficiency, that right now for me that's enough, and then we just ask for the other, and if we have it great, and if not, we limit to fifty -- I don't know. Matrixyl is okay and we don't know about the individual ingredient.

DR. MARKS: Yes, so the other would be an HRIPT of the tetrapeptide-7 -- even at use concentration, since we don't even have a use in the U.S., at least in the table. Yes, Rachel?

DR. WEINTRAUB: In my notes, I had there's still no reproductive or development toxicity and carcinogenicity data, so would that be a need, or is the Panel comfortable with not having that data based on other information?

DR. SHANK: We felt we didn't need because of the very low use concentration applied to the skin -- there wouldn't be sufficient systemic exposure.

DR. SLAGA: I agree with Ron Shank. It's so low -- genotoxicity and carcinogenicity is not an issue.

DR. WEINTRAUB: Thank you.

DR. HILL: However, I think it would be beneficial in the discussion to capture salient points made in Dr. Lintner's letter. It's not a published reference, but he provides references where we can capture that and reference it.

DR. GILL: You can reference a letter.

DR. HILL: Okay, personal communication.

DR. GILL: Right.

DR. MARKS: Okay, any other comments, Ron Hill, again, I'm looking at the paragraph down here on the 12 -- is it the one that is the PAL-alanine or the one that begins with PAL-valine, as the one that you need?

DR. HILL: PAL-alanine.

DR. MARKS: Okay.

MR. JOHNSON: What (inaudible)?

DR. HILL: Anything. We have nothing. Or it goes insufficient on that version of it, which is cumbersome, I know, because we have two different molecules, one ingredient but that's the deal, from where I sit. Dr. Liebler may have another take, but --

DR. MARKS: PAL-alanine was the one that you needed, right?

DR. HILL: Yes sir.

DR. MARKS: Okay. Anything else? So tomorrow I'll move that we issue an insufficient data notice. That's not uncommon. That doesn't mean that we're coming --

DR. LINTNER: That's just for the PAL-alanine?

DR. MARKS: Yes, for the PAL-alanine and then also an HRIPT on the tetrapeptide-7 or other information, but I particularly would like to see that.

DR. LINTNER: Oh sure. I'll send it tomorrow. I'll send it to you.

DR. MARKS: Yes, and Ron Shank, or Tom Slaga or Ron Hill, would you like to see anything more on the tetrapeptide-7? Are you concerned about the other toxicities with this, or not?

DR. HILL: The only other piece of missing information for me was in the summary of the manufacturing process, we -- not that I'm worried that much about it from a safety point of view, but we were not given any information about the palmitoylation

process. That's still a black box, in terms of what I see in the report. I can surmise how it would likely be done, but we don't have information.

DR. LINTNER: It is done exactly the same way as the other amino acids -- it is acid that is coupled to --

DR. HILL: Yes, but how is it coupled? We don't get that. So is it mixed anhydride, acid chloride? That's the point. And like I say, I don't really have any overwhelming concerns, because those processes are going to be proprietary.

DR. LINTNER: It is phase synthesis in --

DR. HILL: It's all with phase synthesis using what? Palmitoyl? How is it activated, I guess?

DR. MARKS: Okay, well that was a robust discussion. We're going to limit it to the one, twelve and seven ingredients -- seven meaning the number seven, not that there are seven ingredients. So the tetrapeptide-1, hexapeptide-12, and the tetrapeptide-7, so we're only going to have three ingredients in this report, and I'm going to suggest that we are moved that we have an insufficient data notice for what I said before, in terms of the hexapeptide- 12, the PAL-alanine, etcetera, amino acid sequence -- we need more information on that, and then the HRIPT and other information, if available on the tetrapeptide-7 -- use concentration.

DR. LINTNER: I'll send that tomorrow.

DR. MARKS: Ron, Ron, Tom -- does that summarize where we're at?

DR. SLAGA: Yes.

DR. MARKS: Wilbur?

DR. SLAGA: I think it's safe. But we'll get that next time.

DR. MARKS: Yes, okay. Yes, it's probably going to get there, but let's be sure. If we're going to err, we're going to err on being on the safe side. Any other comments? Okay. It's after twelve.

DR. LINTNER: Thank you ladies and gentlemen.

DR. SLAGA: Thank you.

DR. HILL: Thank you.

DR. MARKS: Thank you. That's okay. I would have kept on going, but I looked at the clock. I think we will take a break then for lunch.

Full Panel – March 18, 2014

DR. BERGFELD: And then the last item in our total list here is Dr. Marks. Palmitoyl oligopeptides.

DR. MARKS: So there was a draft report on these oligopeptides in March of last year, which was tabled. There was discussion as to what ingredients really we should be reviewing and there was a robust discussion in our team meeting on that. We felt that we would limit to three ingredients -- tripeptide 1 as in the draft report titles from Wilbur dated February 21st; the hexapeptide 12; and the only other amide would be the tetrapeptide 7. And so we would recommend limiting it to those three ingredients, that there be an insufficient data notice. And what we wanted to confirm is the hexapeptide. Ron Hill would clarify that. There are different amino acid sequences -- and if I remember of the hexapeptide 12 -- and the one I believe Ron Hill, you were concerned about, was the palmitoyl alanine. And then we need an HRIPT for the tetrapeptide 7 use concentration.

So the motion would be insufficient data notice for those three ingredients and those were the needs.

DR. BERGFELD: Ron Hill, did you want to comment before we ask the Belsito team?

DR. HILL: I just wanted to be clear that we were talking about the palmitoyliated species.

DR. MARKS: Correct.

DR. HILL: Which we have.

DR. MARKS: Thank you.

DR. HILL: And I don't know -- and further reflecting overnight, I'm not sure what we want to do with the unpalmitoyliated peptides that are the same peptides. So whether we want to keep that in this same report or not. So, I apologize. That was further reflection last night and this morning. Hopefully, the other team will have comment on that.

In terms of the hexapeptide 12, it's because there are two different versions on the market and all the biology we have is for the VGV APG and not the APG VGV. But I'm also told that we will probably be able to get the data on that alternative hexapeptide 12 and then the big question is what about the INCI nomenclature that's ambiguous in this case.

DR. BERGFELD: Dr. Belsito?

DR. BELSITO: Well, we took a similar but different approach.

So, first of all, we thought we needed to relabel this as tripeptide 1, hexapeptide 12. There are metal salts and there are fatty acyl derivatives, to address Ron's point that we are cutting out, other than the metal salts from the fatty acyl derivatives and tetrapeptide 7 as used in cosmetics.

We agree that we remain concerned that an INCI name can refer to different chemicals, and in this case it really does make a point because the amino acid sequence can have very different effects. In other discussion, we point out the specific amino acid sequences that we're referring to when we talk about the hexapeptide and say that only that specific amino acid sequence, hexapeptide, is safe as used and that any other hexapeptide under the INCI name that didn't have that exact sequence was not safe. Furthermore, we appreciated Dr. Lintner's comments that these ranges of ingredients that were used in products made absolutely no sense. We wanted some further clarification on that. Otherwise, we would restrict to the range that we're being told is the range that could be used, and therefore, we would not need the HRIPT that you're asking for.

So our group was recommending that with a relabel as tripeptide 1, hexapeptide 12 -- hexapeptide 6, right? Not 12 -- 12, and their metal salts and fatty acyl derivatives and tetrapeptide 7 as used in cosmetics is safe as used. And the as used will be the new defined range of limits that Dr. Lintner told us were used. And again, the discussion would clearly say the safe as used implies that the hexapeptide 12 has the exact same amino acid sequence as the one we reviewed, and any other hexapeptide 12 that had a different sequence would be insufficient.

DR. BERGFELD: Dr. Marks?

DR. MARKS: I'm going to ask Ron Hill and Ron Shank.

Okay.

DR. BERGFELD: So are you withdrawing your motion?

DR. MARKS: Yes, I'll withdraw.

DR. BERGFELD: And your motion?

DR. MARKS: My motion.

DR. BERGFELD: Do you want to make a motion?

DR. BELSITO: So my motion is that we go out and resurvey Industry because we're told the concentration of uses we've gotten are impossible. If we get them back, we will go back and say we found these ranges of concentration of use; however, we've been informed that the usual concentration of use is these extremely low uses and that that's the ones that we're reporting on safety where the manufacturer tells us is the concentrations that they recommend these be used as. And in the discussion, again point out that it's not any hexapeptide 12; it's the hexapeptide 12 with that specific amino acid sequence. With all of those aspects in the discussion and with the relabel of the report as tripeptide 1, hexapeptide 12, their metal salts and the fatty acyl derivatives, getting rid of all of the other ingredients that aren't metal salts or fatty acyl derivatives, we felt we could go with a safe as used conclusion.

DR. BERGFELD: Now, I have to ask you a question. You're asking for a search on the actual concentrations prior to this going out?

DR. BELSITO: I'm asking -- I'm asking that Carol go with the ingredients we are now including, which is a much smaller universe than the ingredients we looked at.

DR. BERGFELD: Okay.

DR. BELSITO: Whether any of those that fall outside of the bound that Dr. Lintner told us yesterday is the usual and customary use that she go back to those companies and say, "Are you really using it at that concentration?" If they say yes, you know, it will be reported. But then in the Cosmetic Use section I think there would be a paragraph that, you know, information that we've received from the manufacturer is that these are -- their recommendations are for concentrations of use within a given range.

Is that not correct, Dr. Lintner, that you have that?

DR. LINTNER: Yes.

DR. BELSITO: And that we say these are the ranges that we would consider safe as used.

DR. BERGFELD: Ron Hill and then --

DR. HILL: Yeah. Because out discussion yesterday was only the palmitoyl. So I want to be clear. When you say fatty acyl, are you restricting to myristoyl and palmitoyl?

DR. BELSITO: Yes.

DR. HILL: Okay. I'm okay with that.

DR. BELSITO: I think those were the only other two fatty acids.

DR. HILL: I think so. I just want to be clear.

DR. BELSITO: So we're eliminating all of the others. It's a very small group. And those are the ones that are used.

DR. JOHNSON: Dr. Lintner, just for the record, what is that use concentration range?

DR. LINTNER: It is in what is called a PPM range between one and let's say 20, 30 PPM, parts per million of peptide in a cosmetic product.

DR. JOHNSON: Between one and --

DR. LINTNER: One and 30.

DR. JOHNSON: But what is the customary concentration?

DR. LINTNER: It is below 10.

DR. JOHNSON: Below 10. Okay, thank you.

DR. MARKS: So Don, do you mind going on to page six and saying which specific ingredients going from that list? I know you grouped it.

DR. BELSITO: Basically -- I was moving on to the next one, so let me go back.

DR. MARKS: Oh, that can be clarified later. You can clarify that.

DR. BELSITO: I mean, basically, where the metal salts and palmitoyl and myristoyl.

DR. BERGFELD: Okay.

DR. MARKS: Okay.

DR. BERGFELD: All right. We understand what we're voting on now?

All right. No other discussion? I'm going to call the question then. All those in favor of this motion please indicate by raising your hand. Thank you. It is unanimous.

(Motion passed)

Meeting Summary

Tripeptide-1, Hexapeptide-12, their Metal Salts and Fatty Acyl Derivatives, and Palmitoyl Tetrapeptide-7

The Panel issued a tentative safety assessment for public comment with the conclusion that the following 10 ingredients identified as tripeptide-1, hexapapeptide-12, their metal salts and fatty acyl derivatives, and palmitoyl tetrapeptide-7, are safe in the present practices of use and concentration in cosmetics. This conclusion is applicable only to ingredients with peptide sequences that are defined as follows: tripeptide-1 (glycine-histidine-lysine), hexapeptide-12 (valine-glycine-valine-alanine-proline-glycine only), and tetrapeptide-7 (glycine-glutamine-proline-arginine).

tripeptide-1

palmitoyl tripeptide-1

myristoyl tripeptide-1*

hexapeptide-12*

palmitoyl hexapeptide-12

myristoyl hexapeptide-12*

copper tripeptide-1

bis(tripeptide-1) copper acetate*

manganese tripeptide-1*

palmitoyl tetrapeptide -7

*Not reported to be in current use. Were ingredients in this group not in current use to be used in the future, the expectation is that they would be used in product categories and at concentrations comparable to others in this group.

Palmitoyl hexapeptide-12 is reported to function as an antioxidant in cosmetic products; the remaining 9 ingredients reportedly function as skin conditioning agents.

These ingredients were initially included in the CIR safety assessment titled Palmitoyl Oligopeptides. This group was subsequently revised to include only ingredients with a defined peptide sequence (i.e., tripeptide-1[glycine-histidine-lysine] and hexapeptide-12 [valine-glycine-valine-alanine-proline-glycine]) bonded to a palmityl group or one of various other groups. The Panel specifically pointed out that this assessment does not apply to the other sequence listed in the INCI dictionary for hexapeptide-12 (i.e., ala-pro-gly-val-gly-val). Because of major differences in chemistry/biological activity between some of the more complex groups attached to the peptide, the Panel determined that the current safety assessment should include only tripeptide-1, hexapeptide-12 (valine-glycine-valine-alanine-proline-glycine only), their metal salts and fatty acyl derivatives, and palmitoyl tetrapeptide-7 (Pal-glycine-glutamine-proline-arginine). The latter ingredient was added because it is a component of one of the trade name mixtures containing palmitoyl tripeptide-1, for which safety test data are available.

During the Panel discussion, an expert research scientist in the field of cosmetic peptide chemistry commented that peptide ingredients are used in cosmetic products at concentrations between 1 ppm and 30 ppm, but concentrations < 10 ppm are customary. He also provided genotoxicity, ocular irritation, and human repeated insult patch test data on palmitoyl tetrapeptide-7. The Panel determined that, overall, the data in the safety assessment, were sufficient to support the safety of these ingredients in present practices of use and concentration in cosmetics.

JUNE 2014 PANEL MEETING – THIRD REVIEW/DRAFT FINAL REPORT

Belsito Team - June 9, 2014

DR. BELSITO: Good, so that takes care of that. Moving along. Okay, so tripeptide 1, hexapeptide 12, and then metal salts. At the March meeting we concluded that they -- tripeptide 1, hexapeptide 12, their metal salts and fatty acid derivatives and palmitoyl tetrapeptide 7 were safe in the present practice of use and concentration in cosmetics, and went on to further specify that the safe conclusion is applicable only to the named ingredients that have the following peptide sequences, and we defined them because as we learned, the INCI dictionary is not inadequate for ceramides, it's inadequate for these as well, so we felt the need to define the chemical sequence, and I thought it looked good. I don't know if I have any comments. Let me open the document after I save this. That's under peptides, right?

DR. BERGFELD: Right. Peptides.

DR. BELSITO: Page 36. Oh, I guess we don't do the inhalation boilerplate until the discussion. Is that correct? So, we don't need that there, so that I can delete. And then --

MR. JOHNSON: Excuse me, Dr. Belsito, generally we do include that information in the use section because we include the references --

DR. BERGFELD: Right.

MR. JOHNSON: -- in the use section. But I guess during the last review it wasn't requested that information be included in the use section. You just mentioned the discussion.

DR. BELSITO: Okay, so we normally do put it in?

MR. JOHNSON: We normally do put it in there.

DR. BELSITO: So, then it should be in.

MR. JOHNSON: Okay, so that will be added.

DR. BELSITO: And then just a comment here on page 54 on palmitoyl tetrapeptide 7, I'm just shocked that it has 249 uses and no reported concentration.

DR. EISENMANN: That's because you put it in last meeting there and they didn't want to delay it, so there wasn't time to do a concentration of use survey, so I'm assuming you're limiting it to --

DR. BELSITO: The same low levels --

DR. EISENMANN: -- same -- 30 ppm with a typical of 10 ppm. That's --

DR. BELSITO: Is there a request out to get ranges?

DR. EISENMANN: No, I have not sent out a request to include that in the -- because I knew you wanted to finalize it at this meeting, and there just wasn't going to be time between March and now, so if you want to delay it -- to ask, I—

DR. BELSITO: You know, from Dr. Lintner's presentation, it's quite clear that from a financial and chemical standpoint these molecules are going to be used in very low levels, so I presume that the palmitoyl tetrapeptide-7 will be in that same range? If I were wanting to be in a group that was negative of what the CIR does and our thought processes, I would look at that and go, "There are

250 uses of this product, and they don't know how much is in these 250 products." I know you don't like to delay things. I'd be fine going out as a final, but I mean that would be purely editorial to add that information unless it ended up changing our discussion and then we could go "oops," so I would like to see you get that and put it in before this paper gets published.

DR. LIEBLER: I agree. And it's one thing to sort of proceed provisionally with the expectation that the use levels would be very low so we can finalize our discussion and all of that. That's what we've done, but to not have it there when that's sort of a basic due diligence.

DR. BELSITO: Yeah, there are no reported uses for it if it's used in one product and we're not getting any information. It doesn't bother me, but when there are 250 reported uses and we don't have a single concentration range, I don't like my name being on a document like that.

DR. LIEBLER: (inaudible) some other changes.

DR. BELSITO: Yes, go ahead.

DR. LIEBLER: So, I think in terms of readability, with all these peptide names and then the three-letter abbreviations for the peptides which are really not very much used in the literature on proteins and peptides because they're not that much easier to read than reading the whole names, so I suggest that after you introduce these either full spelled out names, you abbreviate them with their single letter abbreviations and use those thereafter, and so I've indicated where you can do that. The other thing is that I think in the conclusion there's a nice layout of the ingredients and the sequences of how they relate to each other which is the two-column display in the conclusion. That would be so useful up front in the introduction for the reader to see, okay, this is what we're dealing with rather than the sort of gobbledygook of names in a paragraph. So, I suggest that that also be put into the introduction as a way to introduce the reader to what we're dealing with here because then they can see, oh, we've got essentially a group of peptides that are GHK and derivatives of GHK. And then we've got another group that are VGVAPG and derivatives of those, and then we've got the GQPR-oddball by itself, the palmitoyl tetrapeptide. And it just makes the report easier to understand just in terms of the ingredients just from the get-go.

DR. BELSITO: So you want this put into the introduction as well?

DR. LIEBLER: Yes, it's a two-column display, and in fact, put all the GHKs in the left-hand column, and then put all the VGVs in the right-hand column, and you'll have a nice lineup.

DR. KLAASEN: And GQ by itself.

DR. LIEBLER: At the bottom of the right.

DR. KLAASEN: Right, less space in-between.

DR. BELSITO: Mr. GQ.

DR. LIEBLER: Now, I think in several places and even in one of the figures in referring to hexapeptide 12 there's this bad history of hexapeptide 12 representing two distinct sequences, and then you were saying not alaprolivale that one. Instead of saying that not in italics everywhere in the report, just take that out and instead you have the sentence right up at the beginning that --

DR. BELSITO: Page?

DR. LIEBLER: Well, this is what I'm adding, but this would be in the introduction. It would be --

DR. BELSITO: Page 32?

DR. LIEBLER: Yeah, page 32.

DR. BELSITO: So, you would put your table after the first sentence, the safety of --

DR. LIEBLER: So, you put the table somewhere in that, either right after that introductory paragraph, right there, okay. And

then I think I suggest you have that first introductory paragraph is fine as it is. You can perhaps go -- let's see. The first one, two, three sentences and then splice in that two-column display; so, three sentences, then put in the two-column display.

DR. BELSITO: So, you're putting it where? After --

DR. LIEBLER: Right after the word "dictionary," halfway through that paragraph.

DR. BELSITO: Okay, so in the dictionary (inaudible) there.

DR. LIEBLER: Yeah, carriage return, couple carriage returns, put in those two columns, and then begin a new paragraph still under introduction with this safety assessment also includes data on trade name material, blah-blah. And all the way to the end of that sentence that ends with an oligo peptide component." Now at that point I suggest you splice in a sentence that says

"This safety assessment addresses only these specified sequences: The data or conclusions are not applicable to other peptide sequences."

MR. JOHNSON: Do you have that?

DR. LIEBLER: I have it right here.

MR. JOHNSON: Okay.

DR. LIEBLER: I'm just reading you what my edit is.

MR. JOHNSON: Okay.

DR. BELSITO: This safety assessment addresses --

DR. LIEBLER: -- only these specified sequences, and they're already laid out for you.

DR. BELSITO: Peptides with the specified sequences?

DR. LIEBLER: "This safety assessment addresses only peptides with these specified sequences: The data or conclusions are not applicable to other peptide sequences." And having said that you can scratch out everywhere where we have an italics not that other sequence so that saves you from having to throw those in all over the place. And then after the example structures, you have another paragraph on page 33 that starts the ingredient name palmitoyl oligopeptide in the INCI dictionary's been retired. You've actually just said that up above, so it's not necessary to have this entire paragraph, and I suggest leaving that paragraph (inaudible).

DR. BELSITO: Where are you?

DR. LIEBLER: It's on page 33 of the PDF right under Figure 1, Example Structures. That entire paragraph can be deleted, and then you resume with this short sentence: "The definition structures and functions of the ingredients in this report are included in Table 1." I think it will just make this report much more digestible for the reader.

DR. BELSITO: But this isn't a GRAS substance.

DR. LIEBLER: I have a couple other comments. On page 42, this is under the cell --it's on the cellular effects study. Page 42

there's a study on cell proliferation and another one, "Effect of Growth Factor Production." I

didn't look at those references, but I would delete -- those are probably of marginal relevance. I would delete them unless there is really strong evidence for a peptide sequence-specific effect on the biological endpoints described. In other words, do they have adequate controls?

DR. BELSITO: Which are you talking about now?

DR. LIEBLER: Cell proliferation and effect on growth factor production.

DR. BELSITO: And the reason for deleting them is?

DR. LIEBLER: Because the way it reads right now it doesn't look like this is going to be any evidence specific for that particular peptide, or in other words they might have thrown in that peptide, but how do they know that it's anything specific to that sequence? They have adequate controls to show that it's not a non- specific effect. I doubt that these are going to be relevant to our consideration anyway. And then on page 43 there's a section called, "Enzyme of Regulation Release Metallic Proteases," and that's all at very high concentrations that I think are irrelevant, relatively high concentrations that are irrelevant.

MR. JOHNSON: So, delete that entire --

DR. LIEBLER: Delete that section, and then there's a section on page 44 called, "Effect on Cell Adhesion." Same thing for the very high concentrations. Delete the "Effect on Cell Adhesion," and then under other –

DR. BELSITO: You're getting -- I'm not a typist, so you got rid of the Growth and then the next one before the Cell Adhesion was --

DR. LIEBLER: Enzyme of Regulation Release.

DR. BELSITO: That's page 44?

DR. LIEBLER: Correct. That's 43 actually.

DR. BELSITO: Forty-three, and then Cell Adhesion is 44.

DR. LIEBLER: Correct.

DR. BELSITO: And you're deleting all of those because of dose?

DR. LIEBLER: Right, and I'm not finished.

DR. BELSITO: Okay, but I mean that doesn't give you information that you want to consider and then the discussion saying it's not relevant because of dose, because otherwise doesn't it look like we're not looking at this data?

DR. LIEBLER: I thought this stuff was really of marginal relevance regardless of whether we looked at it. I mean it's -- the same thing as the effect of (inaudible).

DR. BELSITO: I mean I guess my only concern is that our approach has always been to be as transparent as possible and to look at all of the data that is out there. I mean, yeah, I don't have a problem with deleting the paragraph that some boy stuffed starring down his ear canal, but I guess these paragraphs do describe activity of the compounds that we're looking at, albeit not at levels that we're concerned about, and to delete them would be to the individual reading the report would (inaudible) weren't these guys and gals aware that there's data on cell adhesion and cell proliferation for these molecules? Did they not take that into account? And it sounds like you're saying, "Yeah, I took it into account, but it's not relevant to the concentrations that are used in cosmetics", and that would be something we would say in the discussion.

DR. LIEBLER: So, this takes up about two pages.

DR. BELSITO: I understand, but they're small little summaries. It's not like it's two pages on cell adhesion, two pages on growth promotion. They're saying there's a study. Here are the details, and then in the discussion we're saying we're aware that there are these biological effects of these compounds, but at concentrations used in cosmetics it's not relevant.

DR. LIEBLER: So, I think you could shrink this all down to about a paragraph.

DR. BELSITO: I don't have a problem with shrinking it as much as you want to shrink it. I mean you could even shrink it into a table and say there are reports that these peptide sequences can have biological effects, table, whatever, and you put in the reference. You put in the dose range. You put in the effects, and add the discussion –

DR. LIEBLER: (inaudible) way to deal with it is to use a table at the end and then have a brief section on cellular effects in various cellular models where you essentially indicate that these peptides have been studied and shown to have effects on A, B, C, D & E and various cellular models, see table 12. And then we can talk about that at the discussion that we noted this, that these were at levels of well above the use concentrations and probably not relevant to safety.

DR. BELSITO: And not relevant, not probably not relevant. Not relevant.

DR. LIEBLER: I say "probably" in discussion, but not in writing.

DR. BELSITO: Okay.

DR. LIEBLER: I've said my piece. I'm trying to save a tree or a byte, kilobyte.

DR. BELSITO: So instead of deleting everything we just talked about you were going to tabulate it, and then we'll just in the discussion point out that these various biological effects of these peptides are seen at concentrations that are well above what would be in a cosmetic product. Anything else? Okay. Well, let's save this puppy -- and styrene.

Marks Team – June 9, 2014

DR. MARKS: So, next are the peptides and we have before us -- Wilbur, you're really up here in the beginning.

MR. JOHNSON: Yeah.

DR. MARKS: We have Wilbur before us again. We have the draft final report on -- it's important that we notice that it's tripeptide-1, hexapeptide-12, their metal salts, fatty acyl derivatives, and palmitoyl tetrapeptide-7. The conclusion was safe. And are there any discussions to that? Let me see. That's the motion I'm going to make that we issue a final report where these ingredients are safe.

DR. SLAGA: Agreed.

DR. SHANK: I agree with the conclusion.

DR. MARKS: I guess -- and this is really minor, Wilbur, but when you look at the conclusion, my first look at that was I looked at the initials after and then I had to look up in the discussion to see what the initials stood for. I don't know whether we want to keep the initials the way they are in the conclusion. That's page 49. Very minor, but if somebody looked at the conclusion tripeptide-1 and then has GHK. Would you know what the GHK stands for under the, say, palmitoyl tetrapeptide-12 as VGVAPG? And that's minor. I mean, you could do it with an asterisk, as you've done with the others, or in this case maybe two asterisks, but you go right above in the same page, you have what they are.

MR. JOHNSON: Yes, that's what was mentioned. Yes.

DR. MARKS: So, I don't -- to me, I don't know whether Ron -- obviously, Ron Shank, Tom and Ron Hill, you didn't pick that out, but for me, when I first looked at the conclusion I said, what do these initials mean? Maybe not being a peptide chemist --

DR. HILL: There was a place where it became GHL instead of GHK, so I did flag that.

DR. MARKS: Okay, so --

DR. HILL: I mean, we --

DR. MARKS: Are you fine with the conclusion, with the initials in the way they are, guys? If you are, then --

DR. SHANK: I am.

DR. MARKS: You are? Okay, fine. So, ignore that comment.

MR. JOHNSON: Okay.

DR. HILL: I did have a couple of questions on page 38. These might be slightly rhetorical, but -- hang on a second. The comment I had in the document was, it's at PDF page 38, I need to be enlightened as to how 38 percent cell loss is declared not cytotoxic. They didn't do a dose response, so I was bugged by that, not in terms of changing the conclusion, and the other thing is --

MR. JOHNSON: Oh, excuse me, Dr. Hill, you mean the negligible cytotoxicity?

DR. HILL: Yeah, it said negligible cytotoxicity, I think, but then they said 38 percent cell loss, so I was puzzled how that could be declared not cytotoxic and maybe it relates to how they judge the (inaudible) controls. I'm not sure. But the way it's written sounds funny, so we might need to go back and look at the original reference and see.

MR. JOHNSON: Now, you said 38 percent cell loss, is that --

DR. HILL: That's why I'm trying to find out if I'm on the right page because --

MR. JOHNSON: Because I see 37.

DR. HILL: All right, well, 37 percent, maybe 38 -- page 38 and 37 percent? But the question is, how is that not cytotoxic? So, that was one question and then the other one was --

MR. JOHNSON: Because it said negligible cytotoxicity, is that --

DR. HILL: Yeah, how do you call 37 percent cell loss negligible cytotoxicity? I'm guessing it's because the controls also exhibit cell loss or something along those lines, but it just sounds really odd.

The other question I had in this was maybe more biologically significant. It talks about a beige color and I just wondered, from the dermatologists, was there any chance that that was indicative of increased melanin secretion.

MR. JOHNSON: What page are you on, doctor?

DR. HILL: It's also on page 38 and it is the second to last paragraph. So, it's the biopeptide CL study. It says, "A very slight beige coloration of the skin was observed in each animal." Which would not be irritation, of course, but my question was, is that indicative of an effect on melanin secretion? That's the only place I ran across that kind of effect in all of this.

DR. MARKS: I thought that was insignificant.

DR. HILL: Okay, well, I think everybody probably did, but I thought while I had a panel of dermatologists sitting here, I would ask.

DR. MARKS: Anything else?

MS. LORETZ: I have a question about the hexapeptide-12, so there's difference sequences but only the one was reviewed, and in the future, other sequences could be added that would fall under that name. Should that just be made really clear what CIR --

DR. HILL: I thought it was, isn't it?

DR. MARKS: I also thought it was clear, that's why the peptide sequence was identified.

DR. HILL: Somewhere there's language, but if it --

MS. LORETZ: I just wondered about, like, the naming, how they're named, because there is the potential for the future for more to be added.

DR. HILL: I think somewhere we said exactly this sequence and only this sequence the safety review pertains to and I think it was written in a way that -- did I get that somewhere?

DR. MARKS: If you look on page 49 --

DR. HILL: That's where I'm at.

DR. MARKS: -- at the conclusion in that paragraph at the top it says, "Noted, the safe conclusion applicable only to ingredient names associated with the following known peptide sequences." So, that's where it's being very specific. So, I guess if you came back with a different peptide sequence you would -- there better be safety to support that.

DR. HILL: And we know those are out there because we saw that in the last version of the document, but we didn't get safety data and it was asserted that there might be safety data, but we haven't seen it.

So, then we would have to reopen. Yes?

DR. MARKS: Yes. Absolutely. Does that answer your question?

MS. LORETZ: Yeah, I guess I was thinking more in the introduction it would be kind of nice to set that up rather than putting it at the end.

DR. MARKS: Again, that would be editorial, so, Wilbur, if you think that -- I mean, I have no problems with that. It's easy to repeat it again in the introduction, just it's, again, the sort of -- my -- in the conclusion, having the GHK and the VGBAPG -- it's, again, pretty obvious with that in there once you look at what those mean were very specific in terms of the sequencing.

DR. HELDRETH: It also is mentioned in the first sentence of the chemistry section. Specifically your hexapeptide-12 that we mean the sequence that's in the definition and not the other one.

DR. HILL: However, I mean, it would be editorial and almost inconsequential to add to that second sentence in the conclusion -- in the footnote. So, were ingredients in this group not -- seemed like what somebody said, add another footnote or something, I don't know.

It didn't jump out at her, it might not jump out at everybody that we're talking about exactly these sequences.

DR. MARKS: Okay. And then maybe add that in the introduction. Tom, Ron, Ron, do you feel strongly? Or do you think it's a good idea --

DR. HILL: I'll let everybody else hash it out at CIR staff.

DR. MARKS: Well, we need to give guidance. To me, repeating it causes no harm. It's like one more sentence.

MS. LORETZ: It's just an unusual naming convention, that's a little a different in that respect.

DR. SLAGA: That would be fine.

DR. MARKS: Yes. Okay, so, Wilbur, if you'd put it in the introduction and also to draw emphasis to this nuance.

Okay, so --

DR. SHANK: I have one comment -- comment on the discussion. Since the body of the report has some in vitro studies showing that some of the peptides induced angiogenesis, this is important in carcinogenesis, but we don't mention it in the discussion and I think it might be helpful if we add a sentence or two to the discussion saying that these peptides can induce angiogenesis in in vitro studies, but because these are low concentrations and are rapidly hydrolyzed in the plasma, there's little concern about promotion of skin tumors through cosmetic use.

DR. MARKS: That's sort of --

DR. SHANK: Just so it shows (inaudible) did consider it. I have the words (inaudible).

DR. MARKS: I think tomorrow, Ron, I'm going to ask you to make that comment.

DR. SHANK: Okay.

DR. MARKS: I think that's an important editorial comment. It doesn't change the conclusion, obviously, but --

DR. SHANK: It just shows we recognize that --

DR. SLAGA: Yeah, we had a lot of discussion about that.

DR. SHANK: Yes, we did.

DR. MARKS: Is that -- which do you prefer, Tom? Do you want to comment or Ron Shank, since Ron --

DR. SHANK: This is your --

DR. SLAGA: No, no, no.

DR. MARKS: Yeah, I know. That's why --

DR. SLAGA: We had extensive discussion last time about that.

DR. HILL: Well, and yeah, because I mean it's applicable in the skin, so your proviso about rapidly hydrolyzing the plasma, I don't know that that necessarily applies to things happening dermally, but then the flip side --

DR. SHANK: But the fact that it's used in such low concentration, we really didn't have any issues with the --

DR. HILL: That and also the fact that there are basically biochemical systems in place in the skin to keep that from becoming overactive. I mean, I think that is there in the report. So, I wasn't worried about it. I'm just saying, you raise it -- if you bring it up in the discussion, somebody could ask that question.

DR. SLAGA: It's already in the --

DR. HILL: I can give this to you.

DR. SLAGA: -- document, so -- I could go either way. It doesn't matter to me.

DR. SHANK: Okay. Well, if you don't need to add it.

DR. MARKS: What do you feel, Tom?

DR. SLAGA: I don't think we need it.

DR. MARKS: Okay.

DR. SLAGA: I mean, if we dismissed it because it's low concentration --

DR. MARKS: Okay. So, you don't feel we need it in the discussion and comment to that effect on angiogenesis. And that's fine. We haven't had it up to this point. I think your comments are straight on. The question is, does it need to be repeated or not?

DR. SHANK: I guess not. No.

DR. HILL: Well, you could bring it up tomorrow and see --

DR. MARKS: Well, no. If Tom and our team doesn't feel --

DR. SLAGA: Let me see -- presented?

DR. MARKS: I'm presenting. So, I'll be moving the final safe and then we'll ask for editorial comments. Tom and Ron, you'll have time to think about it more between now and tomorrow and Tom, if you feel the same, I'm not going to bring it up, Tom, since it sounds like now the way we've come to the conclusion it's adequately addressed in the document already, but we want to clarify it more tomorrow, Ron, we can get the reaction of the other team.

DR. SHANK: Fine.

DR. MARKS: Okay, any other comments? So, I will move tomorrow that a final report be issued with a safe conclusion for these peptides. So, we're down -- next ingredient is styrene. And this is the first time we've seen these ingredients.

Full Panel – June 10, 2014

DR. MARKS: So, we have the draft final report on these Peptides, Tripeptide-1, Heptapeptide-12, they are metal salts and fatty acid salt derivatives, and Palmitol Tetrapeptide-7, and we move a final report could be issued with these Peptides having a conclusion of safe in the present practice of use and concentration.

DR. BELSITO: Second.

DR. BERGFIELD: Any discussion or comment regarding this ingredient?

DR. BELSITO: We made a number of changes within the document, particularly Dan liked that table in the conclusion and recommended that it be brought up into the introduction so it was quite clear immediately that we're dealing with only specific Peptide sequences, but it was all editorial through the document and nothing major.

DR. BERGFIELD: Any other comments that you want to make.

DR. MARKS: Similar. We had editorial comments, but that's --

DR. BERGFIELD: Nothing changing anything.

DR. MARKS: No, nothing changing the conclusion.

DR. BERGFIELD: Dan, did you have a comment, or you just raised your hand out of fun? Okay. All right.

DR. MARKS: He's already voting.

DR. BERGFIELD: I'll call the question, all those in favor. Please indicate by raising your hands. Thank you, unanimous. Then moving on to the next ingredient, Dr. Belsito, the Pentaerythrityl.

Meeting Summary

Tripeptide-1, Hexapeptide-12, their Metal Salts and Fatty Acyl Derivatives, and Palmitoyl Tetrapeptide-7

The Panel issued a final safety assessment with the conclusion that the following 10 ingredients, identified as tripeptide-1, hexapapeptide-12, their metal salts and fatty acyl derivatives, and palmitoyl tetrapeptide-7, are safe in the present practices of use and concentration in cosmetics.

tripeptide-1
palmitoyl tripeptide-1
myristoyl tripeptide-1*
hexapeptide-12*
palmitoyl hexapeptide-12
myristoyl hexapeptide-12*
copper tripeptide-1
bis(tripeptide-1) copper acetate*
manganese tripeptide-1*
palmitoyl tetrapeptide-7

*Not reported to be in current use. Were ingredients in this group not in current use to be used in the future, the expectation is that they would be used in product categories and at concentrations comparable to others in this group.

This conclusion is applicable only to ingredients with peptide sequences that are defined as follows: tripeptide-1 (glycine-histidine-lysine), hexapeptide-12 (valine-glycine-valine-alanine-proline-glycine only), and tetrapeptide-7 (glycine-glutamine-proline-arginine). This assessment does not apply to the hexapeptide-12 (i.e., ala-pro-gly-val-gly-val) sequence listed in the INCI dictionary, because of the potential for major differences in chemistry and biological activity of some of the more complex groups attached to the peptide compared to those of the ingredients included in this safety assessment.

The peptides are used in cosmetic products at concentrations between 1 ppm and 30 ppm, and use at concentrations < 10 ppm is customary. However, data on the use concentrations of palmitoyl tetrapeptide-7 were not provided for this safety assessment. Given the high use frequency of use of palmitoyl tetrapeptide-7 reported to FDA, industry was urged to complete a use concentration survey for this ingredient.
Palmitoyl hexapeptide-12 is reported to function as an antioxidant in cosmetic products; the remaining 9 ingredients reportedly function as skin conditioning agents.

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

Status: Draft Report for Panel Review
Release Date: August 18, 2023
Panel Meeting Date: September 11-12, 2023

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

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ABBREVIATIONS

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

INTRODUCTION

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

The 3 ingredients included in this safety assessment are synthetic peptides which comprise a 5-amino-acid-sequence (pentapeptide) containing lysine, serine, and threonine. One such sequence is lysine-threonine-threonine-lysine-serine, also represented as Lys-Thr-Thr-Lys-Ser, or, KTTKS.[2] Myristoyl Pentapeptide-4 and Palmitoyl Pentapeptide-4 have an additional saturated fatty acid group attached to the peptide structure, namely myristic acid and palmitic acid, respectively. The amino acid sequence of the pentapeptide portion of these ingredients can vary; thus, data for two variations of Palmitoyl Pentapeptide-4, namely, Pal-KTTKS and Pal-KTSKS (palmitoyl-Lys-Thr-Ser-Lys-Ser), are included in this report.

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

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

CHEMISTRY

Definition and Structure

Pentapeptide-4 is a synthetic peptide comprised of the amino acids, lysine, serine, and threonine, which are linked in varied 5-amino-acid sequences (forming a pentapeptide), two of which are lysine-threonine-threonine-lysine-serine (also represented as Lys-Thr-Thr-Lys-Ser or KTTKS) and, lysine-threonine-serine-lysine-serine (also represented as Lys-Thr-Ser-Lys-Ser or KTSKS) (Figure 1).[1,2] Myristoyl Pentapeptide-4 (no CAS No. but has been assigned the UNII identifier, PMA59A699X) and Palmitoyl Pentapeptide-4 (CAS No. 521091-64-5; 214047-00-4) each have a myristic acid or palmitic acid group, respectively, attached to the *N*-capped end of this sequence. The definitions and structures of the ingredients included in this review are provided in Table 1.

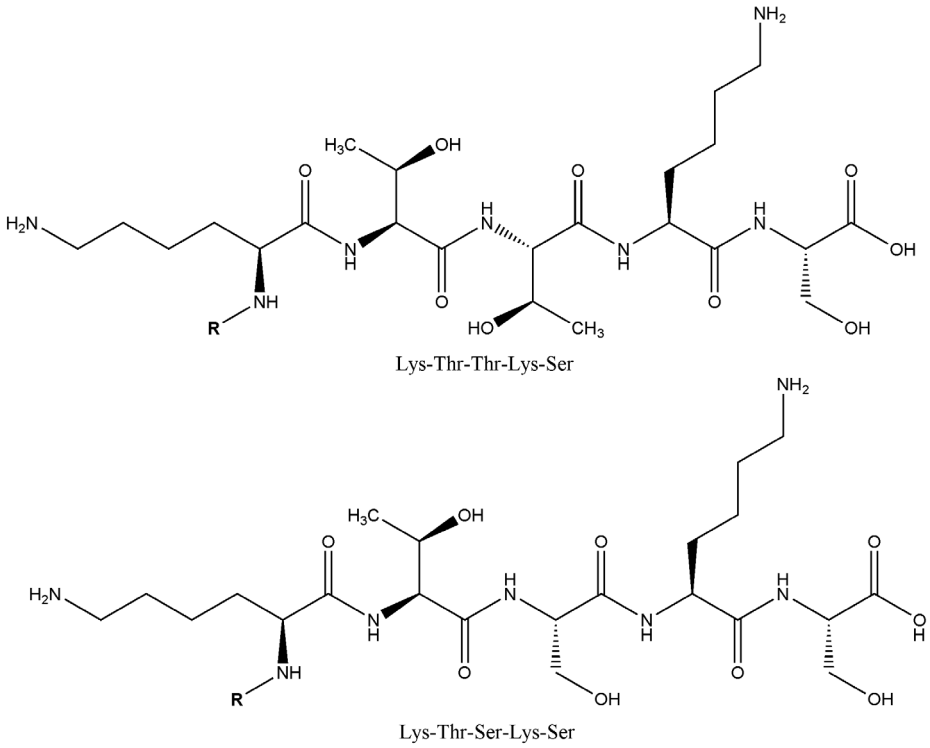


Figure 1. Pentapeptide-4 (when R is hydrogen) and *N*-capped derivatives (when R is the residue of myristic or palmitic acid)

Pentapeptide-4 is a subfragment of type I collagen propeptide, and is regarded as a signal peptide and a matrikine, which possesses the ability to enhance dermal remodeling by triggering cellular processes, such as inhibiting collagenase activity and increasing extracellular matrix production.[2,8-11] The hydrophilic and charged nature of Pentapeptide-4 makes it difficult for it to pass through the intact stratum corneum.[12] However, through the attachment of a fatty acid, such as palmitic acid, which has a 16-carbon chain, the peptide is rendered more lipophilic and is more easily able to penetrate into the skin.[13]

Chemical Properties

Myristoyl Pentapeptide-4, Palmitoyl Pentapeptide-4, and Pentapeptide-4 have molecular weights of 774 g/mol,[14] 802.1 g/mol,[15,16] and 563.6 g/mol,[17] respectively. Additionally, Myristoyl Pentapeptide-4 has an estimated log p value of -0.3,[14] while Palmitoyl Pentapeptide-4 and Pentapeptide have estimated log p values of 3.32 and -3.27, respectively.[2] Chemical properties for ingredients in this report are further outlined in Table 2.

Method of Manufacture

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

Palmitoyl Pentapeptide-4

Two samples of Palmitoyl Pentapeptide-4 (Pal-KTTKS and Pal-KTSKS) are described by a supplier as being obtained via solid phase synthesis at room temperature using Fmoc-amino acid derivatives.[20] An N_α-fluorenylmethoxycarbonyl-N_ε-(*t*-butoxycarbonyl)-lysine (Fmoc-Lys(Boc)-OH) complex is first activated with a coupling agent and reacted on serine-protected resin. Deprotection of the Fmoc residue with a base produces a dipeptide on the resin. For the Pal-KTTKS sequence, both activation and coupling are achieved using the N_α-fluorenylmethoxycarbonyl-*O*-(*t*-butyl)-L-threonine (Fmoc-Thr(tBu)-OH) complex, and deprotection is achieved with the Fmoc-Lys(Boc)-OH group. For the Pal-KTSKS sequence, the N_α-fluorenyl-methoxycarbonyl-*O*-(*t*-butyl)-L-serine (Fmoc-Ser(tBu)-OH), Fmoc-Thr(tBu)-OH, and Fmoc-Lys(Boc)-OH groups are utilized for activation, coupling, and deprotection, respectively. After the last Fmoc-deprotection step, palmitic acid is

reacted in the same manner in each process and the resulting products are fully deprotected and purified to yield the final amino acid sequences (Pal-Lys-Thr-Thr-Lys-Ser-OH and Pal-Lys-Thr-Ser-Lys-Ser-OH).

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

Impurities

Palmitoyl Pentapeptide-4

The impurities found in a sample of Palmitoyl Pentapeptide-4 (Pal-KTTKS), as described by a supplier, were: acetate (< 10%), palmitic acid (< 5%), water (< 5%), and residual solvents (in accordance with the International Council for Harmonisation Of Technical Requirements for Pharmaceuticals for Human Use Guideline for Residual Solvents (ICH Q3C)).[15] Two distinct samples of Palmitoyl Pentapeptide-4, each comprising the Pal-KTTKS or Pal-KTSKS sequence, were described by a supplier as having ≥ 90% purity at 210 nm.[20] The supplier described the impurities in the first sample of Palmitoyl Pentapeptide-4 (Pal-KTTKS) as stereoisomers of Pal-KTTKS-OH, myristine-lysine-threonine-threonine-lysine-serine-OH, and stearyl-lysine-threonine-threonine-lysine-serine-OH. The impurities in the second Palmitoyl Pentapeptide-4 sample (Pal-KTSKS) were described by the supplier as stereoisomers of Pal-KTSKS-OH, myristine-lysine-threonine-serine-lysine-serine-OH, and stearyl-lysine-threonine-serine-lysine-serine-OH.

USE

Cosmetic

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

According to 2023 VCRP survey data, Palmitoyl Pentapeptide-4 has the greatest reported frequency of use; it is reported to be used in 239 formulations, 223 of which are leave-on products (Table 3).[21] Myristoyl Pentapeptide-4 is reported to have 4 uses, while Pentapeptide-4 has 1 reported use. The results of the concentration of use survey conducted by the Council in 2022 indicate Myristoyl Pentapeptide-4 has the highest maximum reported concentration of use, at up to 0.05% in other eye makeup preparations.[22]

Historical frequency and concentration of use data are available for Palmitoyl Pentapeptide-4. The frequency of use of Palmitoyl Pentapeptide-4 has increased notably since 2012, at which time it was reported to the VCRP to be used in 51 formulations.[23] The highest reported maximum concentration of use for Palmitoyl Pentapeptide-4 in 2013 was 0.00061% in eye lotions and face and neck preparations.[24]

Some of these ingredients are reported to be used in products that are applied near the eye; as stated above, Palmitoyl Pentapeptide-4 is used at up to 0.05% in eye makeup preparations. Palmitoyl Pentapeptide-4 is reported to be used in a face powder (concentration not provided) and could possibly be inhaled. In practice, as stated in the Panel's respiratory exposure resource document (https://www.cir-safety.org/cir-findings), most droplets/particles incidentally inhaled from cosmetics would be deposited in the nasopharyngeal and tracheobronchial regions and would not be respirable (i.e., they would not enter the lungs) to any appreciable amount. Conservative estimates of inhalation exposures to respirable particles during the use of loose powder cosmetic products are 400-fold to 1000-fold less than protective regulatory and guidance limits for inert airborne respirable particles in the workplace.

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

The Pentapeptide-4 ingredients named in the report are not restricted from use in any way under the rules governing cosmetic products in the European Union.[25]

Non-Cosmetic

Palmitoyl Pentapeptide-4 (Pal-KTTKS) has been tested for its wound-healing effects.[26] Palmitoyl Pentapeptide-4 applied in a patch (0.1 and 1 mg) and cream (1 mg) form had a larger impact on wound healing in animals, compared to negative controls (untreated) and positive controls (ready-to-wear dressing; $p < 0.05$).

TOXICOKINETIC STUDIES

Dermal Permeation

In Vitro

Palmitoyl Pentapeptide-4; Pentapeptide-4

The permeability of Palmitoyl Pentapeptide-4 (Pal-KTTKS) and Pentapeptide-4 (KTTKS) was evaluated in an in vitro study using 3 replicate skin samples of CrlOri: SKH1-hr strain hairless mice.[27] Intact hairless mouse skin was mounted on Franz diffusion cells with the epidermal side facing the donor compartment. In the receptor compartment, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) buffer was mixed with 15% ethanol containing phenylmethane-sulfonylfluoride and 1,10-phenanthroline at final concentrations of 5 and 1 mM, respectively, as proteolytic enzyme inhibitors. The donor compartment was loaded with a 1 ml of Palmitoyl Pentapeptide-4 or Pentapeptide-4 (100 µg/ml in 15% ethanol) solution. After 24-h incubation, the skin was removed from the diffusion cell and the remaining donor solution on the skin surface was washed 4 times with 1 ml of distilled water. Upon drying, separation, and mincing of the skin layers (stratum corneum, epidermis, and dermis), the amount of Palmitoyl Pentapeptide-4 or Pentapeptide-4 distributed in each skin layer was extracted using 1 ml of methanol for 24 h with continuous shaking. The extracted samples were centrifuged and the supernatants were analyzed using liquid chromatography with tandem mass spectrometry (LC-MS/MS). No detectable level of Pentapeptide-4 was observed in the receptor solution over an observation period of 48 h. A trace amount of Palmitoyl Pentapeptide-4 was detected in the receptor solution after 24 h by LC-MS/MS; however, it was below the limit of quantification (LOQ; < 0.5 µg/ml). No amount of Pentapeptide-4 was detected in any of the skin layers over a period of 24 h. Palmitoyl Pentapeptide-4 was observed in every skin layer: 4.2 ± 0.7 µg/cm^2 in the stratum corneum, 2.8 ± 0.5 µg/cm^2 in the epidermis, and 0.3 ± 0.1 µg/cm^2 in the dermis. Overall, 14.6% of the applied Palmitoyl Pentapeptide-4 was retained in the skin: 8.3% in the stratum corneum, 5.6% in the epidermis, and 0.6% in the dermis. Therefore, the researchers concluded that neither Palmitoyl Pentapeptide-4 nor Pentapeptide-4 could permeate through full-thickness hairless mouse skin over the time period used in these experiments.

The dermal stability of Palmitoyl-Pentapeptide-4 (Pal-KTTKS) and Pentapeptide-4 (KTTKS) was evaluated in vitro in epidermal and dermal skin extracts and whole skin homogenate prepared from hairless mouse skin.[27] Pentapeptide-4 (200 µl) or Palmitoyl Pentapeptide-4 (40 µg/ml in 10 mM HEPES buffer, pH 7.4, as peptide concentration) was incubated with 200 µl of the epidermal skin extract, dermal skin extract, or whole skin homogenates at 37 °C for 120 min. At predetermined times, the amount of Palmitoyl Pentapeptide-4 and Pentapeptide-4 present in the incubated mixtures was sampled and analyzed by LC-MS/MS. Pentapeptide-4 was almost fully degraded in the dermal skin extract and whole skin homogenate, with 3.2% remaining in the dermal skin extract at 30 min and 1.5% remaining in the whole skin homogenate at 60 min. The degradation of Pentapeptide-4 in the epidermal skin extract was slower than that seen in the dermal skin extract and whole skin homogenate, which was potentially attributed to lower amounts of proteolytic enzymes. Palmitoyl Pentapeptide-4 was more stable in the skin extracts over time, compared to Pentapeptide-4. The concentration of Palmitoyl Pentapeptide-4 detected in the epidermal skin extract after 120 min was similar to the initial concentration. After 60 min, 11.2% Palmitoyl Pentapeptide-4 remained in the whole skin homogenate, and, after 120 min, 9.7% Palmitoyl Pentapeptide-4 remained in the dermal extract.

TOXICOLOGICAL STUDIES

Acute Toxicity Studies

Oral

Palmitoyl Pentapeptide-4

The acute oral toxicity of Palmitoyl Pentapeptide-4 (Pal-KTTKS), tested at 0.01% (vehicle not specified), was evaluated in Sprague-Dawley rats (5/sex), in accordance with Organisation for Economic Cooperation and Development (OECD) test guideline (TG) 401.[15,28] A single dose of the test substance (20 ml/kg) was administered via gavage. Mortality, clinical abnormalities, and body weight gain were monitored for a period of up to 14 d; all animals were killed at the end of the study. No deaths occurred during the study and no apparent changes or abnormalities were observed in general behavior, body weight gain, or upon necropsy.

Short-Term Toxicity Studies

Dermal

Palmitoyl Pentapeptide-4

Groups of guinea pigs (5/sex; strain not specified) were treated with 0.01% Palmitoyl Pentapeptide (0.05 ml; vehicle not specified; Pal-KTTKS) in a 2-wk dermal irritation study.[15,29] No deaths or clinical signs related to treatment were noted during the study; internal organs were not examined. No further details were provided.

Subchronic, and Chronic Toxicity Studies

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

DEVELOPMENTAL AND REPRODUCTIVE TOXICITY STUDIES

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

GENOTOXICITY STUDIES

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

A solution of 0.5% Palmitoyl Pentapeptide-4 (Pal-KTTKS) in distilled water and ethanol (75/25), tested at 2% in distilled water, was not mutagenic in an Ames test at concentrations up to 5000 µg/plate using *Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537 and *Escherichia coli* WP2*uvr*A.[15,30] In another Ames test, performed in accordance with OECD TG 471, Palmitoyl Pentapeptide-4 (81.6% pure, Pal-KTSKS) in dimethyl sulfoxide (DMSO) was not mutagenic when tested at concentrations up to 5000 µg/plate using *S. typhimurium* strains TA98, TA100, TA102, TA1535, and TA1537, with or without metabolic activation; signs of cytotoxic activity were observed under test conditions.[20,31] The genotoxic potential of Palmitoyl Pentapeptide-4 (> 96% pure, Pal-KTSKS) in water was evaluated in an in vitro mammalian cell micronucleus test in accordance with OECD TG 487 using cultured human lymphocytes.[20,32] Cells were treated with 250, 500, or 1000 µg/ml of the test article in the presence of metabolic activation for 4-h, followed by a 24-h recovery period; cells were also treated with 375, 500, or 750 µg/ml of the test article in the absence of metabolic activation for 4 h, followed by a 24-h recovery period (short treatment). In an additional assay, cells were treated with concentrations of 250, 320, or 400 µg/ml Palmitoyl Pentapeptide-4 for 24 h without a recovery period (continuous treatment). Neither statistically nor biologically significant increases in the number of micronucleated cells were observed with either treatment period; the test article was deemed not genotoxic.

CARCINOGENICITY STUDIES

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

OTHER RELEVANT STUDIES

Endocrine Activity

Palmitoyl Pentapeptide-4

The estrogenic and androgenic activity of a formulation containing 0.12% Palmitoyl Pentapeptide-4 (other contents not specified; Pal-KTSKS) was evaluated in transformed yeast cells using the XenoScreen Yeast Estrogen Screen (YES) and Yeast Androgen Screen (YAS) assays.[20,33] *Saccharomyces cerevisiae* cells were genetically transformed with human estrogen receptor α (hERα) and human androgen receptors (hAR) and, additionally, had an expression plasmid carrying the reporter gene lacZ inserted. Binding of the test article with hERα or hAR receptors resulted in the interaction of these receptors with the corresponding response elements on the expression plasmid, in turn affecting β-galactosidase gene expression. Thus, the amount of secreted β-galactosidase, which was correlated with colorimetric quantification of the conversion of the yellow substrate, chlorophenol red-β-D-galactopyranoside, into a red product at 570 nm (corrected for unspecific absorption and light scattering at 690 nm), indicated the estrogenic or androgenic activity of the test article. The difference between these optical density (OD) absorbance values (OD_{690} - OD_{570}) was used to calculate growth factor values and induction ratios. Eight serial dilutions of the test article (half-log steps) in DMSO, resulting in final concentrations of 1 x 10^{-2} – 3.16 x 10^{-6} M, were added to yeast cells in the agonist assays. For the agonist YES assay, 17-β estradiol (E2) was used as the positive control at 7 final concentrations between 1 x 10^{-11} – 1 x 10^{-8} M; 5α-dihydrotestosterone (DHT) was used as the positive control for the agonist YAS assay at 7 final concentrations between 1 x 10^{-9} – 1 x 10^{-6} M, using half-log dilution steps. DMSO (1%) was used as the solvent control. The inhibitory activity of the test article dilutions were evaluated in the presence of E2 (1.3 x 10^{-9} M) in an antagonistic YES assay and in the presence of DHT (3 x 10^{-8} M) in an antagonistic YAS assay. Serial dilutions of 4-hydroxytamoxifen and flutamide were used as antagonist positive controls. The test article exhibited cellular toxicity (growth factors ≤ 0.5) at the two highest tested concentrations and estrogenic activity with a 10% effect concentration (EC_{10}) value of 6.9 x 10^{-3} in the YES agonist assay. No estrogenic antagonist or androgenic agonist/antagonist activities were observed.

Similarly, the estrogen agonist effects of a formulation containing 0.12% Palmitoyl Pentapeptide-4 (other contents not specified; Pal-KTSKS), were assessed in a XenoScreen XL YES assay.[34] Lyticase and a detergent were used to facilitate the secretion of the intracellularly synthesized β-galactosidase. Test article samples were serially diluted in 8 steps (half-log steps) in water with 1% DMSO, with concentrations ranging from 5.21 x 10^{-5} – 6.7 x 10^{-3} M. E2 was used as the positive control in 8 final concentrations between 2.1 x 10^{-12} – 6.7 x 10^{-9} M, using half-log dilution steps; 1% DMSO served as the solvent control. The limit of detection (LoD) for estrogenic activity was 1.49 x 10^{-11} M E2. No inhibition of cellular growth or estrogenic agonist activity was observed at any concentration tested.

DERMAL IRRITATION AND SENSITIZATION STUDIES

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

A formulation containing 0.12% Palmitoyl Pentapeptide-4 (tested as supplied; Pal-KTSKS) did not cause irritation when applied to a reconstructed human epidermis model (EpiSkin®) in a cutaneous primary irritation test performed in accordance with OECD TG 439.[20,35] Palmitoyl Pentapeptide-4, tested at 0.01% (vehicle not specified; Pal-KTTKS), was not irritating in an acute dermal irritation test performed in accordance with OECD TG 404 using New Zealand white rabbits nor in a 2-wk dermal irritation study performed in accordance with OECD TG 404 using guinea pigs.[15,29,36] A trade name mixture containing 0.01% Palmitoyl Pentapeptide-4 (applied neat; Pal-KTTKS) was tested for acute skin irritation using 10 subjects.[15,37] Very slight erythema was observed in 1 of the subjects and the primary cutaneous irritation (PCI) score was determined to be 0.10. The test substance was considered to be well-tolerated. A formulation containing 0.12% Palmitoyl Pentapeptide-4 (tested at 15% in distilled water; Pal-KTSKS) was not irritating when applied for 48 h, under semi-occlusive conditions in a patch test using 11 subjects.[20,38]

Palmitoyl Pentapeptide-4 (81.6% pure, Pal-KTSKS) was predicted to be non-sensitizing when tested at 5 mM (5 µl) and 25 mM (250 µl) in water in a direct peptide reactivity assay (DPRA) performed in accordance with OECD TG 442C.[20,39] Palmitoyl Pentapeptide-4 (81.6% pure; Pal-KTSKS) was tested at up to 200 µM (0.05 ml) in DMSO using the KeratinoSens™ cell line in an antioxidant/electrophile response element (ARE)-Nrf2 luciferase assay, performed in accordance with OECD TG 442D.[20,40] The test article yielded a maximal response value (I_{max}) of 1.35 compared to an I_{max} of 5.12 for the positive control, cinnamaldehyde; the test article was predicted to be non-sensitizing. A guinea pig maximization test was performed in accordance with OECD TG 406, to evaluate the sensitization potential of Palmitoyl Pentapeptide-4 (0.01%; Pal-KTTKS).[15,41] Thirty guinea pigs (test animals: 10/sex; controls: 5/sex), received the test substance at an effective concentration of 0.0075% (w/w; in saline) followed by an undiluted epicutaneous application during induction, and a dermal application of the test substance at an effective concentration of 0.0025%, in saline, during challenge. No skin reactions were observed during evaluation of the test sites 24 and 48 h after patch removal; the test substance was deemed non-sensitizing. A formulation containing 0.12% Palmitoyl Pentapeptide-4 (tested at 15% in distilled water; Pal-KTSKS) was not irritating or sensitizing when applied under semi-occlusive conditions in a human repeated insult patch test (HRIPT) using 106 subjects.[20,42] A trade name mixture containing 0.01% Palmitoyl Pentapeptide-4 (Pal-KTTKS) did not cause irritation or sensitization in a HRIPT using 51 subjects.[15,43] No further details were provided.

Phototoxicity Studies

Palmitoyl Pentapeptide-4

The potential for a sample of Palmitoyl Pentapeptide-4 (tested at 0.0015%; Pal-KTSKS), in water, to absorb ultraviolet light A (UVA) and ultraviolet light B (UVB) was evaluated, in accordance with OECD TG 101.[20,44] The diluted article (1 ml) was placed in a calibrated spectrophotometer in order to read UVA/UVB absorption. No absorbance peak was observed between 290 and 400 nm, which was suggestive of a molar extinction coefficient (ε; a measure of how strongly a chemical species or substance absorbs light at a particular wavelength; is an intrinsic property of chemical species that is dependent on structure) < 1000 M^{-1} cm^{-1}. The test article was predicted to be non-phototoxic.

OCULAR IRRITATION STUDIES

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

A formulation containing 0.12% Palmitoyl Pentapeptide-4 (300 µl dose; Pal-KTSKS) was tested in an in vitro hens egg-chorioallantoic membrane (HET-CAM) assay, following the 1996 HET CAM protocol published in the *Journal Officiel Republique Francaise*.[20,45] The mean score calculated for hyperemia, hemorrhage, and coagulation, opacity, and/or thrombosis was 4.25; the test article was classified as slightly irritating. In another HET-CAM assay, a trade name mixture containing 0.01% Palmitoyl Pentapeptide-4 (Pal-KTTKS), which was tested as supplied, produced a mean irritation index of 6.0; the mean irritation index of the positive control, sodium dodecyl sulfate, was 12.0.[15,37] The test article was classified as moderately irritating. The ocular irritation potential of a formulation containing 0.12% Palmitoyl Pentapeptide-4 (tested at 30% in glycerin and water; Pal-KTSKS) was tested in a SkinEthic™ human corneal epithelial model, in accordance with OECD TG 492.[20,46] Mean cell viability when tested with the test article was 104.3%; the test article was considered not irritating. Palmitoyl Pentapeptide-4 tested at 0.01% (vehicle not specified; Pal-KTTKS) was assessed for ocular irritation in 3 male New Zealand white rabbits, in accordance with OECD TG 405.[15,47] A single dose of 0.1 ml was instilled into the conjunctival sac of the left eye, and the eye was not rinsed. All mean values for chemosis, redness of the conjunctiva, iris lesions, and corneal opacity were 0 at each tested time interval. The test substance was deemed non-irritating to rabbit eyes under the conditions of this study.

CLINICAL STUDIES

Use Studies

Palmitoyl Pentapeptide-4 has been tested in several clinical studies for its use as an anti-wrinkle agent. A moisturizer containing 3 ppm Palmitoyl Pentapeptide-4 was well tolerated in a 12-wk, double-blind, placebo-controlled, split face, left-right randomized clinical study performed in 93 female subjects.[48] In an 8-wk, randomized parallel-group study conducted in

196 women, a cosmetic product regimen containing niacinamide, Palmitoyl Pentapeptide-4, palmitoyl-lysine-threonine, retinyl propionate, and carnosine in a moisturizing base was well tolerated compared to a moisturizer containing 0.02% tretinoin;[49] the concentration of Palmitoyl Pentapeptide-4 in the moisturizing base does not exceed the maximum reported concentration of use of this ingredient in non-spray face and neck products that was reported to the Council in response to the use survey (i.e., 0.0012%).[50] Palmitoyl Pentapeptide-4 was also well tolerated in another 8-wk, double-blind randomized trial evaluating the effectiveness of 3 cream formulations containing either acetylhexapeptide-3, Pentapeptide-4, or placebo (concentrations not provided).[51]

SUMMARY

This assessment reviews the safety of Myristoyl Pentapeptide-4, Palmitoyl Pentapeptide-4, and Pentapeptide-4 as used in cosmetic formulations. These 3 synthetic peptides are comprised of a varied 5-amino-acid-sequence containing lysine, threonine, and serine; this report reviews the safety of two sequences, namely Pal-KTTKS and Pal-KTSKS. According to the *Dictionary*, these ingredients are reported to function in cosmetics as skin-conditioning agents. As reported in 2023 VCRP data, Palmitoyl Pentapeptide-4 is used in 239 formulations. Myristoyl Pentapeptide-4 had the highest concentration of use reported in response to a 2022 concentration of use survey; it is used at up to 0.05% in other eye makeup preparations.

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

The in vitro dermal stability of Palmitoyl Pentapeptide-4 and Pentapeptide-4 was evaluated in several mouse skin extracts. Either 200 µl Pentapeptide-4 or 40 µg/ml Palmitoyl Pentapeptide-4 (in 10 mM HEPES buffer) was incubated with 200 µl of the epidermal skin extract, dermal skin extract, or whole skin homogenates at 37 °C for 120 min. The amounts of each substance present in the incubated mixtures were sampled and analyzed by LC-MS/MS. Pentapeptide-4 was almost fully degraded in the dermal skin extract and whole skin homogenate, with 3.2% remaining in the dermal skin extract at 30 min and 1.5% remaining in the whole skin homogenate at 60 min. Pentapeptide-4 degradation was slower in the epidermal skin extract which was attributed to lower amounts of proteolytic enzymes. Palmitoyl Pentapeptide-4 was more stable in the skin extracts over time; the amount detected in the epidermal skin extract after 120 min was similar to the initial concentration. After 60 min, 11.2% Palmitoyl Pentapeptide-4 remained in the whole skin homogenate and after 120 min, 9.7% Palmitoyl Pentapeptide-4 remained in the dermal extract.

In an acute oral toxicity study, performed in accordance with OECD TG 401, groups of Sprague-Dawley rats (5/sex) received a single dose of Palmitoyl Pentapeptide-4 (20 ml/kg; Pal-KTTKS), tested at 0.01%, via gavage. No deaths occurred during the study and no abnormalities were observed in the general behavior, body weight gain, or upon necropsy. No deaths or clinical signs related to treatment were noted in groups of guinea pigs (5/sex) treated with 0.01% Palmitoyl Pentapeptide (0.05 ml) in a 2-wk dermal irritation study.

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

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

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

Palmitoyl Pentapeptide-4 (81.6% pure; Pal-KTSKS) was predicted to be non-sensitizing when tested in a DPRA (OECD TG 442C) and a ARE-Nrf2 luciferase assay (OECD 442D). In a guinea pig maximization test, Palmitoyl Pentapeptide-4 (0.01%; Pal-KTTKS) was not sensitizing when injected at effective test concentrations of 0.0075% in saline during intradermal induction, applied at 0.01% during epicutaneous induction, and applied at 0.0025% in saline during challenge. A formulation containing 0.12% Palmitoyl Pentapeptide-4 (tested at 15% in distilled water; Pal-KTSKS) was not irritating or sensitizing when tested under semi-occlusive conditions in an HRIPT using 106 subjects. No irritation or sensitization was observed in an HRIPT in which 51 subjects were treated with a trade name mixture containing 0.01% Palmitoyl Pentapeptide-4.

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

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

Clinically, a moisturizer containing 3 ppm Palmitoyl Pentapeptide-4 was well-tolerated in a 12-wk, double blind placebo-controlled, split face, left-right randomized clinical study performed in 93 female subjects. Palmitoyl Pentapeptide-4 has also been shown to be well tolerated in other randomized trials where it was tested in cosmetic formulations (concentration did not exceed the maximum reported concentration of use in face and neck products).

TABLES

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

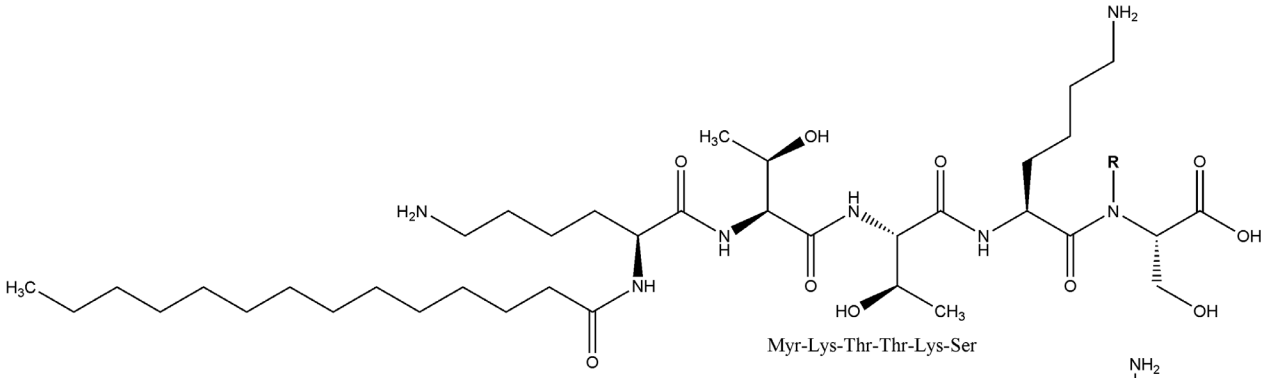
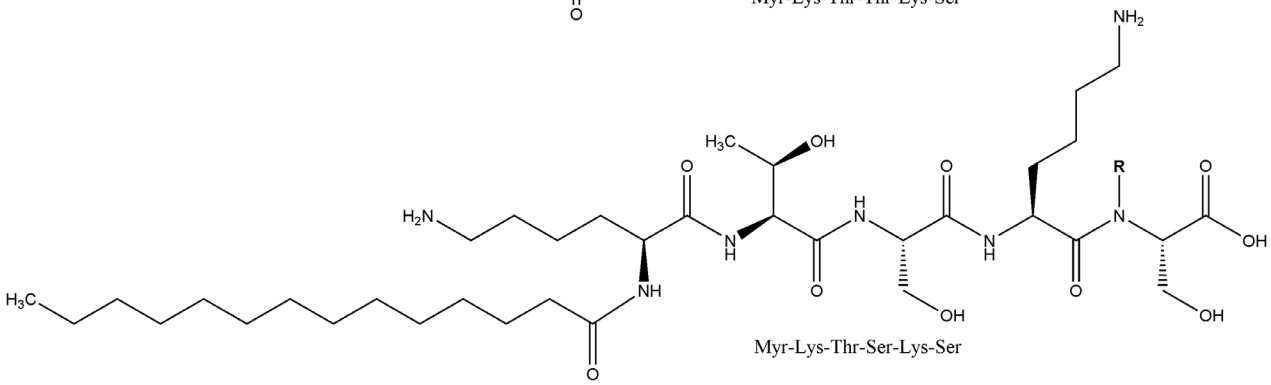
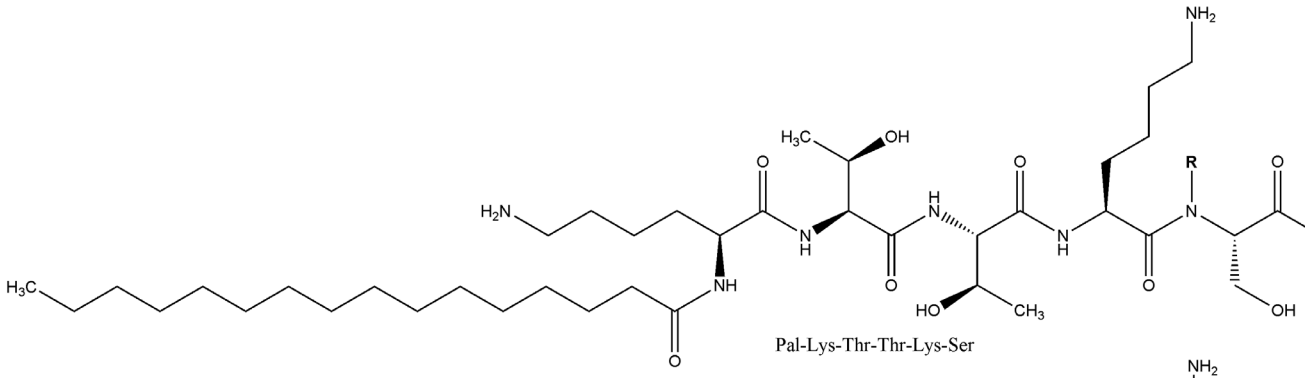
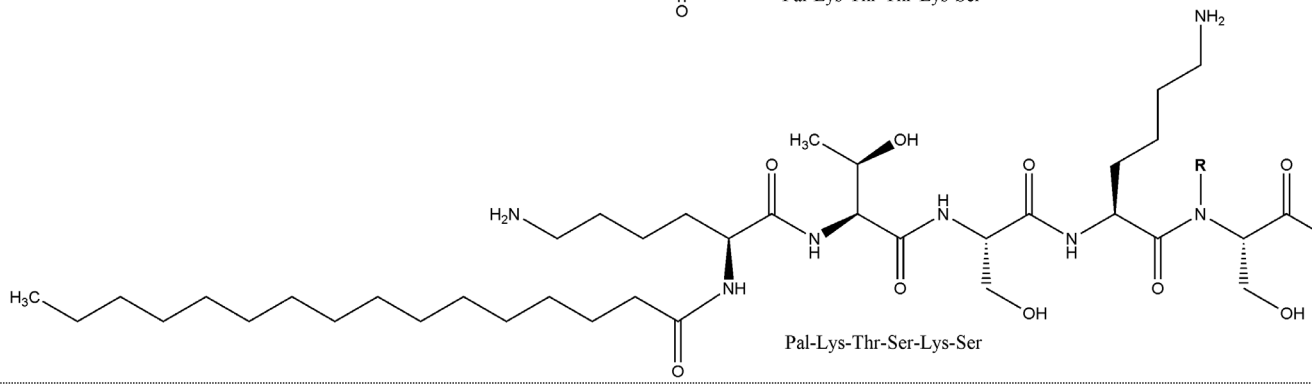
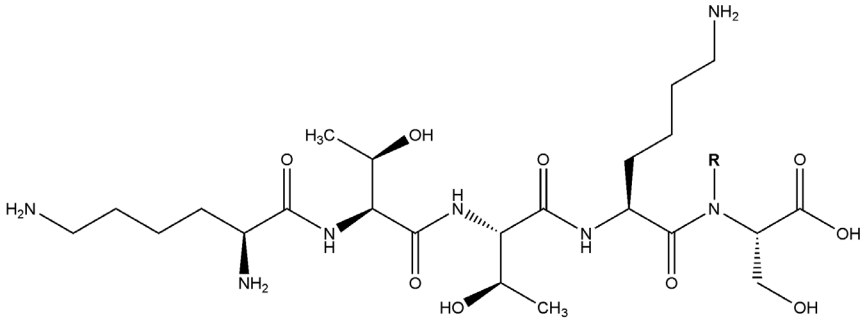
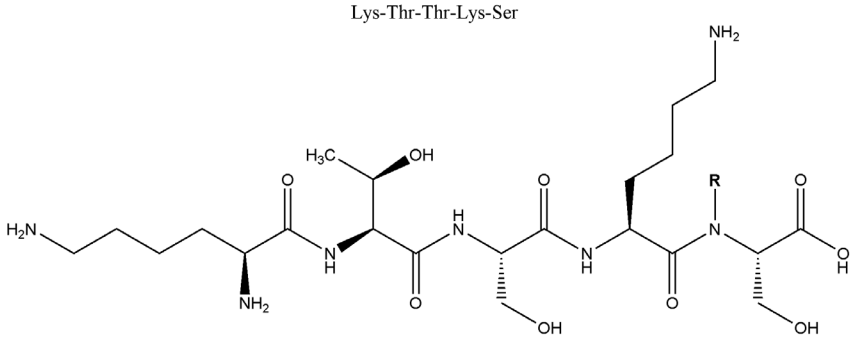
Ingredient	Definition	Function
Myristoyl Pentapeptide-4	Myristoyl Pentapeptide-4 is the reaction product of myristic acid and Pentapeptide-4.	Skin-conditioning agent - miscellaneous
	 Myr-Lys-Thr-Thr-Lys-Ser	
	 Myr-Lys-Thr-Ser-Lys-Ser	
Palmitoyl Pentapeptide-4 521091-64-5 214047-00-4	Palmitoyl Pentapeptide-4 is the reaction product of palmitic acid and Pentapeptide-4.	Skin-conditioning agent - miscellaneous
	 Pal-Lys-Thr-Thr-Lys-Ser	
	 Pal-Lys-Thr-Ser-Lys-Ser	

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

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



Lys-Thr-Thr-Lys-Ser



Lys-Thr-Ser-Lys-Ser

Table 2. Chemical properties

Property	Value	Reference
	Myristoyl Pentapeptide-4	
Molecular Weight (g/mol)	774 (Myr-Lys-Thr-Thr-Lys-Ser); 759.99 (Myr-Lys-Thr-Ser-Lys-Ser)	14
Topological Polar Surface Area (Å^2)	296 (estimated; Myr-Lys-Thr-Thr-Lys-Ser)	14
log p	-0.3 (estimated; Myr-Lys-Thr-Thr-Lys-Ser)	14
	Palmitoyl Pentapeptide-4	
Physical Form	Powder	15
Color	White	15
Molecular Weight (g/mol)	802.1 (Pal-Lys-Thr-Thr-Lys-Ser); 788.04 (Pal-Lys-Thr-Ser-Lys-Ser)	15,16
Topological Surface Area (Å^2)	296 (estimated; Pal-Lys-Thr-Thr-Lys-Ser)	16
log p	3.32; 3.48 (estimated; Pal-Lys-Thr-Thr-Lys-Ser)	2,15
	Pentapeptide-4	
Molecular Weight (g/mol)	563.65 (Lys-Thr-Thr-Lys-Ser); 549.63 (Lys-Thr-Ser-Lys-Ser)	17
Topological Polar Surface Area (Å^2)	292 (estimated; Lys-Thr-Thr-Lys-Ser)	17
log p	-3.27; -6.8 (estimated; Lys-Thr-Thr-Lys-Ser)	2,17

Table 3. Frequency (2023)[21] and concentration (2022)[22] of use according to likely duration and exposure by product category

	Myristoyl Pentapeptide-4		Palmitoyl Pentapeptide-4		Pentapeptide-4	
	# of Uses	Max Conc of Use (%)	# of Uses	Max Conc of Use (%)	# of Uses	Max Conc of Use (%)
Totals*	**4**	**0.05**	**239**	**0.000005-0.0035**	**1**	**NR**
summarized by likely duration and exposure**						
Duration of Use						
Leave-On	*4*	*0.05*	*223*	*0.00036 – 0.0012*	*1*	*NR*
Rinse-Off	*NR*	*NR*	*16*	*0.000005 – 0.0035*	*NR*	*NR*
Diluted for (Bath) Use	*NR*	*NR*	*NR*	*NR*	*NR*	*NR*
Exposure Type						
Eye Area	4	0.05	31	0.0012	NR	NR
Incidental Ingestion	NR	NR	NR	NR	NR	NR
Incidental Inhalation-Spray	NR	NR	117[a]; 64[b]	NR	1[a]	NR
Incidental Inhalation-Powder	NR	NR	1; 64[b]	0.00036 – 0.0012[c]	NR	NR
Dermal Contact	4	0.05	236	0.000005 – 0.0012	1	NR
Deodorant (underarm)	NR	NR	NR	NR	NR	NR
Hair - Non-Coloring	NR	NR	3	0.00035 – 0.0035	NR	NR
Hair-Coloring	NR	NR	NR	NR	NR	NR
Nail	NR	NR	NR	NR	NR	NR
Mucous Membrane	NR	NR	2	0.000005	NR	NR
Baby Products	NR	NR	NR	NR	NR	NR
as reported by product category						
Eye Makeup Preparations						
Eye Lotion			21	0.0012		
Other Eye Makeup Preparations	4	0.05	10	NR		
Hair Preparations (non-coloring)						
Hair Conditioner			1	0.0035		
Rinses (non-coloring)			1	NR		
Shampoos (non-coloring)			1	0.00035		
Makeup Preparations						
Face Powders			1	NR		
Foundations			4	NR		
Personal Cleanliness Products						
Bath Soaps and Detergents			1	0.000005		
Other Personal Cleanliness Products			1	NR		
Skin Care Preparations						
Cleansing			10	0.000005		
Face and Neck (exc shave)			59	0.0012 (not spray)		
Body and Hand (exc shave)			5	0.00036 (not spray)		
Moisturizing			101	0.00059 (not spray)	1	NR
Night			8	NR		
Paste Masks (mud packs)			1	NR		
Skin Fresheners			8	NR		
Other Skin Care Preparations			6	NR		

NR – not reported

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

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

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

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

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

Table 4. Genotoxicity studies

Test Article	Vehicle	Concentration/Dose	Test System	Procedure	Results	Reference
IN VITRO						
0.5% Palmitoyl Pentapeptide-4 in distilled water/ethanol (75/25) Pal-KTTKS	distilled water	tested at 2% 312.5, 625, 1250, 2500, and 5000 µg/plate, with or without metabolic activation	*S. typhimurium* TA98, TA100, TA1535, TA1537, and *E. coli* WP2*uvr*A	Ames test. For positive controls, sodium azide, 9-aminoacridine, 2-nitrofluorene, and 4-nitroquinoline were tested in the absence of metabolic activation, while 2-anthramine was tested in the presence of metabolic activation. Revertant colonies were scored after 48 to 72 h of incubation at 37 °C.	Not mutagenic. Results for the vehicle and positive controls were as expected.	[15,30]
Palmitoyl Pentapeptide-4, 81.6% pure Pal-KTSKS	DMSO	1.6, 5, 16, 50, 160, 500, 1600, and 5000 µg/plate, with or without metabolic activation	*S. typhimurium* TA98, TA100, TA102, TA1535, and TA1537	Ames test. OECD TG471. In the absence of metabolic activation, sodium azide and mitomycin were tested in water, and 2-nitroflourene and 9-aminoacridine were tested in DMSO, for positive controls. In the presence of metabolic activation, 2-aminoanthracine was tested in DMSO as a positive control.	Not mutagenic. Signs of cytotoxic activity were observed under test conditions for the test article; controls produced expected results.	[20,31]
Palmitoyl Pentapeptide-4, > 96% pure Pal-KTSKS	sterile water	as supplied with metabolic activation: 4-h treatment, 24-h recovery: 250, 500, or 1000 µg/ml without metabolic activation: 4-h treatment, 24-h recovery: 375, 500, or 750 µg/ml 24-h, continuous treatment: 250, 320, or 400 µg/ml	Cultured human peripheral blood lymphocytes	Micronucleus test. OECD TG 487. Cells were treated for 4 h, with a 24-h recovery period, with and without metabolic activation (short treatment). In an additional assay, cells were treated for 24 h without a recovery period (continuous treatment). Cells treated were treated for 4 h followed by a 24-h recovery period, with cyclophosphamide in the presence of metabolic activation and with mitomycin in the absence of metabolic activation. Mitomycin and griseofulvin were used as positive controls in the 24-h, continuous assay.	Not genotoxic. Neither statistically or biologically significant increases in the number of micronucleated cells were observed with the short-term or continuous treatments.	[20,32]

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

Table 5. Dermal irritation and sensitization studies

Test Article	Vehicle	Test Concentration/Dose	Test Population/ System	Procedure	Results	Reference
IRRITATION						
IN VITRO						
Formulation containing 0.12% Palmitoyl Pentapeptide-4, glycerin, and water Pal-KTSKS	tested as supplied	10 µl; 100% (effective test concentration: 0.12% Palmitoyl Pentapeptide-4)	EpiSkin® reconstructed human epidermis model	Cutaneous primary irritation test. OECD TG 439. The test article, positive control (10 µl SDS), and negative control (10 µl PBS) were in contact with the epidermis model for 15 min, followed by a 42-h incubation period. Cell viability was evaluated via an MTT assay.	Predicted to be not irritating. The test article, as supplied did not stain the cells or interact with MTT.	[20,35]
ANIMAL						
Palmitoyl Pentapeptide-4 Pal-KTTKS	not specified	0.01%; 0.5 ml	3 male New Zealand white rabbits	Acute dermal irritation study. OECD TG 404. Semi-occlusive application of the test substance was made to shaved skin for 4 h. Skin reactions were observed 1, 24, 48, and 72 h after patch removal. Mean values for erythema and edema were calculated for each animal.	Not irritating. Very slight erythema was observed in 1 animal, only on day 1. All erythema and edema mean scores over 24, 48, and 72 h were 0.	[15,36]

Table 5. Dermal irritation and sensitization studies

Test Article	Vehicle	Test Concentration/Dose	Test Population/ System	Procedure	Results	Reference
Palmitoyl Pentapeptide-4 Pal-KTTKS	not specified	0.01%; 0.05 ml	Guinea pigs (5/sex; strain not specified)	2-wk dermal irritation study. Open application to a shaved, 2 cm^2 area of the left flank daily for 14 d; the site was not rinsed. Purified water applied to the right flank served as the control. Skin reactions were evaluated before and approximately 24 h after each application; these values were used to calculate daily irritation and weekly mean irritation indices.	Non-irritating. Very slight erythema was noted in 1 animal on days 12 and 13. According to the researchers, these reaction were not attributed to an irritant effect of the test substance because they were very slight and only occurred in 1 animal.	15,29
HUMAN						
Trade name mixture containing 0.01% Palmitoyl Pentapeptide-4 Pal-KTTKS	tested as supplied	0.02 ml (effective test concentration: 0.01% Palmitoyl Pentapeptide-4)	10 subjects	Acute skin irritation study. A single occlusive, neat application of the test substance was made to a 50 mm^2 area of the back for 48 h using Finn chambers. Untreated sites covered with an occlusive patch served as negative controls. Skin reactions were scored 30 min after patch removal.	Well-tolerated. Very slight erythema (hardly visible) in 1 of the subjects. PCI = 0.10.	15,37
Formulation containing 0.12% Palmitoyl Pentapeptide-4 Pal-KTSKS	distilled water	160 µl; 15% (effective test concentration: 0.018% Palmitoyl Pentapeptide-4)	11 subjects; phototype II - IV	Patch test; semi-occlusive application to 400 mm^2 for 48 h; test sites were scored before patching and 15 – 30 min after patch removal	Not irritating. No reactions were observed in either the test or control subjects.	20,38
SENSITIZATION						
IN CHEMICO/ IN VITRO						
Palmitoyl Pentapeptide-4, 81.6% pure Pal-KTSKS	water	5 (50 µl) and 25 mM (250 µl)	cysteine and leucine	DPRA; OECD TG 442C and ECVAM DB-ALM Protocol No 154; 24-h incubation period; each concentration was tested 3 times; mean percent depletion of cysteine and lysine was evaluated; positive control: cinnamaldehyde in acetonitrile; negative control: peptide in buffer	Prediction of non-sensitizing. Mean percent depletion of cysteine and lysine was 4.58%, reflecting no or minimal reactivity.	20,39
Palmitoyl Pentapeptide-4, 81.6% pure Pal-KTSKS	DMSO	0.98 – 2000 µM; 0.05 ml	KeratinoSens™ cell line	ARE-Nrf2 Luciferase test method; OECD TG 442D and ECVAM DB-ALM protocol 155; performed 2 times; positive control: cinnamaldehyde; negative controls: 1% DMSO in treatment medium	Prediction of non-sensitizing. I_{max} of 1.35, compared to 5.12 for positive control.	20,40
ANIMAL						
Palmitoyl Pentapeptide-4, 0.01% Pal-KTTKS	saline	Induction: 75% (effective concentration 0.0075%); topical induction: applied neat (effective concentration 0.01%) Challenge: 25% (effective concentration: 0.0025%)	Guinea pigs (strain not specified) test animals: 10/sex controls: 5/sex	OECD TG 406. Guinea pig maximization test. Saline solution and mercaptobenzothiazole in corn oil served as negative and positive controls, respectively. On day 1, the test substance was mixed with FCA and injected intradermally in the back. After pretreatment of the test site with 10% SLS (pet) on day 7, the test substance was applied on day 8 under occlusion to the same region for 48 h. After a non-treatment period of 12 d, both test and control animals received an occlusive challenge application of the test substance to the right flank, as well as an occlusive application of the vehicle control to the left flank, both for 24 h. Skin reactions were evaluated 24 and 48 h after patch removal.	Not sensitizing. Controls yielded expected results.	15,41

Table 5. Dermal irritation and sensitization studies

Test Article	Vehicle	Test Concentration/Dose	Test Population/ System	Procedure	Results	Reference
			HUMAN			
Formulation containing 0.12% Palmitoyl Pentapeptide-4 Pal-KTSKS	distilled water	160 µl; 15% (effective concentration: 0.018% Palmitoyl Pentapeptide-4)	106 subjects; phototype II - III	HRIPT; semi-occlusive conditions (400 mm^2); induction: 9 applications (48 – 72 h) were made to the upper back over a 3-wk period. Concurrent applications of distilled water under the same conditions served as control sites. Challenge: after a non-treatment period of 2 wk, a 48-h application was made to an induction site and an untreated site. Treated sites were scored before patching, 15 – 30 min after patch removal, and, additionally, 48 h after patch removal during the challenge phase	Not irritating or sensitizing. No reactions were induced during the induction or challenge phases.	[20,42]
Trade name mixture containing 0.01% Palmitoyl Pentapeptide-4 Pal-KTTKS	not specified	not specified	51 subjects	HRIPT	Non-irritating and non-sensitizing	[15,43]

ARE – antioxidant/electrophile response element; DPRA – direct peptide reactivity assay; ECVAM DB-ALM - European Centre for Validation of Alternative Methods Database on Alternative Methods; FCA – Freund's Complete Adjuvant; HRIPT – human repeated insult patch test; MTT - 3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide; OECD – Organisation for Economic Cooperation and Development; PBS – phosphate-buffered solution; PCI – primary cutaneous irritation; SDS – sodium dodecyl sulfate; SLS – sodium lauryl sulfate; TG – test guideline

Table 6. Ocular irritation studies

Test Article	Vehicle	Test Concentration/Dose	Test Population	Procedure	Results	Reference
IN VITRO						
Formulation containing 0.12% Palmitoyl Pentapeptide-4 Pal-KTSKS	water	300 µl; 10% (effective test concentration: 0.012% Palmitoyl Pentapeptide-4)	4 eggs (test article); 2 eggs (reference controls)	In vitro HET-CAM assay; 1996 HET CAM protocol published in the *Journal Officiel Republique Francaise*; positive control: 0.4 and 3.2% lauryl sulfobetaine in saline solution; negative control: 0.05% lauryl sulfobetaine in saline solution	Classified as slightly irritating. The mean score calculated for hyperemia, hemorrhage, and coagulation, opacity, and/or thrombosis was 4.25.	[20,45]
Trade name mixture containing 0.01% Palmitoyl Pentapeptide-4 Pal-KTTKS	tested as supplied	dose not specified (effective test concentration: 0.01% Palmitoyl Pentapeptide-4)	HET-CAM	In vitro HET-CAM assay; 1996 HET CAM protocol published in the *Journal Officiel Republique Francaise*; positive control: SDS (0.05% (w/v))	Classified as moderately irritating. The mean irritation index for the SDS was 12, while the mean irritation index for the test substance was 6	[15,37]
Formulation containing 0.12% Palmitoyl Pentapeptide-4; glycerin, and water Pal-KTSKS	water	30 µl; 30% (effective test concentration: 0.036% Palmitoyl Pentapeptide-4)	human immortalized comeal epithelial cells	SkinEthic™ human corneal epithelial model. OECD TG 492, in agreement with French GLP, European Directive 2004/10/CE, and 2004 decree published in the *Journal Officiel Republique Francaise*. 2 epithelia were used as replicates; 30 min incubation period; positive control: methyl acetate; negative control: DPBS; cell viability evaluated via MTT assay	Not irritating. Mean cell viability for the test article was 104.3%. Positive controls yielded expected results.	[20,46]
ANIMAL						
Palmitoyl Pentapeptide-4 Pal-KTTKS	not specified	0.01%; 0.1 ml	3 male New Zealand white rabbits	OECD TG 405. A single dose was instilled into the conjunctival sac of the left eye. Treated eyes were not rinsed; right eyes served as control Ocular reactions were evaluated 1, 24, 48 and 72 h. Mean values for chemosis, redness of the conjunctiva, iris lesions, and corneal opacity were calculated for each animal	Classified as non-irritant. All mean values were 0 at each time interval.	[15,47]

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

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Concentration of Use by FDA Product Category – Pentapeptide-4*

Palmitoyl Pentapeptide-4
Pentapeptide-4
Myristoyl Pentapeptide-4

Ingredient	FDA Product Category	Maximum Concentration of Use
Palmitoyl Pentapeptide-4	Eye lotions	0.0012%
Palmitoyl Pentapeptide-4	Hair conditioners	0.0035%
Palmitoyl Pentapeptide-4	Shampoos (noncoloring)	0.00035%
Palmitoyl Pentapeptide-4	Bath soaps and detergents	0.000005%
Palmitoyl Pentapeptide-4	Skin cleansing (cold creams, cleansing lotions, liquids, and pads)	0.000005%
Palmitoyl Pentapeptide-4	Face and neck products Not spray	0.0012%
Palmitoyl Pentapeptide-4	Body and hand products Not spray	0.00036%
Palmitoyl Pentapeptide-4	Moisturizing products Not spray	0.00059%
Myristoyl Pentapeptide-4	Other eye makeup preparations	0.05%

*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.

Information collected in 2022
Table prepared: July 6, 2022

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

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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: 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.

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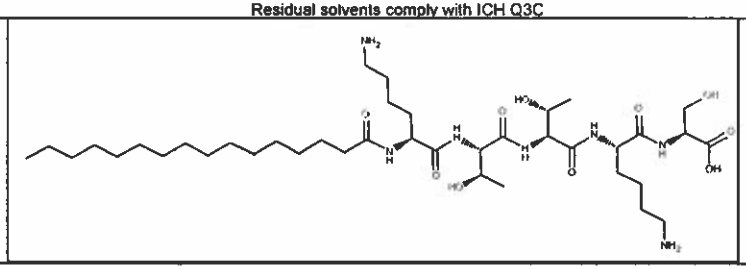
INCI name	Palmitoyl Pentapeptide-4
INCI Monograph ID	12108
Trade names of SEDERMA mixtures from PcPc website	MATRIXYL
Technical name from PcPc website	Palmitoyl Pentapeptide-3 L-Serine, N2-(1-oxohexadecyl)-L-lysyl-L-threonyl-L-seryl-L-lysyl- L-Serine, N2-(1-oxohexadecyl)-L-lysyl-L-threonyl-L-threonyl-L-lysy
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

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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

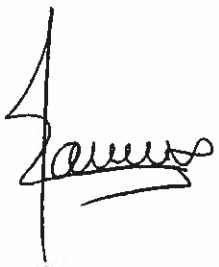
STATEMENT OF THE STUDY DIRECTOR

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

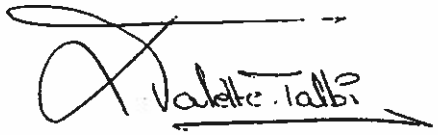
STATEMENT OF QUALITY ASSURANCE UNIT

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
Société Séderma
29 rue du Chemin Vert
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TEST SUBSTANCE
PALMITOYL-PENTAPEPTIDE 3 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

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

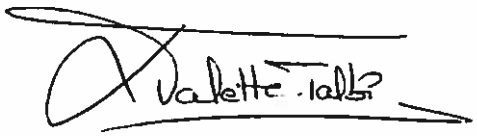
STATEMENT OF QUALITY ASSURANCE UNIT

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.

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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
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Centre International de Toxicologie
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LABORATORY STUDY NUMBER
18838 TAR

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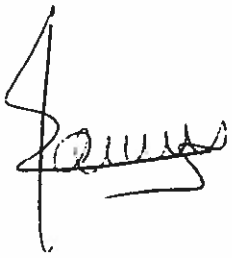
STATEMENT OF THE STUDY DIRECTOR

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: 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

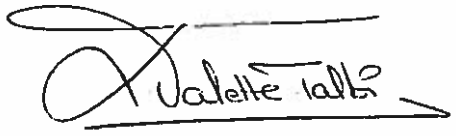
STATEMENT OF QUALITY ASSURANCE UNIT

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 4 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 4 at the concentration of 0.01% does not induce any signs of toxicity in rats.

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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.

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Société Séderma
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
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France

TEST SUBSTANCE
PALMITOYL-PENTAPEPTIDE 4

STUDY TITLE
BACTERIAL REVERSE MUTATION TEST

STUDY DIRECTOR
Hasnaà Haddouk

STUDY COMPLETION DATE
6 October 1999

PERFORMING LABORATORY
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Centre International de Toxicologie
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LABORATORY STUDY NUMBER
18796 MMJ

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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

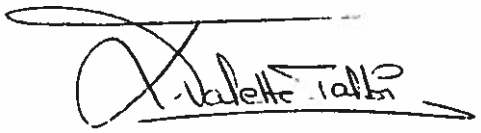
STATEMENT OF QUALITY ASSURANCE UNIT

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

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 74 70 94 98
e-mail : info@iec.fr - Internet : http : //www.iec.fr

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

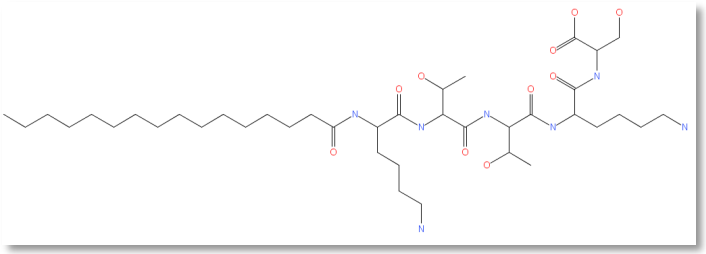
Results: The results of each participant are appended (Table 1). Subject demographics are presented in Table 2.

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.

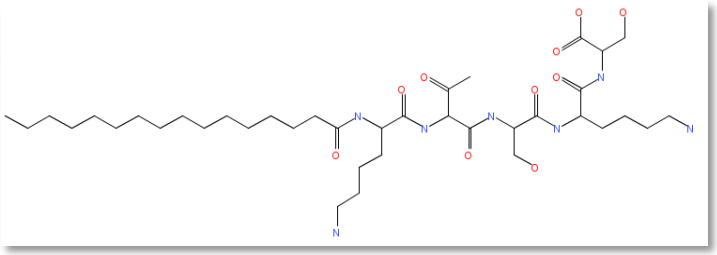
For Matrixyl:
Fifty-nine (59) qualified subjects, male and female, ranging in age from 19 to 78 years, were selected for this evaluation. Fifty-one (51) subjects completed this study. The remaining subjects discontinued their participation for various reasons, none of which were related to the application of the test material.

Summary Information on Palmitoyl Pentapeptide-4

INCI name	**Palmitoyl Pentapeptide-4 (Pal KTTKS)**
INCI Monograph ID	12108
Manufacturing process	Pal-Lys-Thr-Thr-Lys-Ser-OH is obtained by solid phase synthesis at room temperature using Fmoc-aminoacid derivatives: Fmoc-Lys(Boc)-OH is activated, with a coupling reagent, and reacted on Serine protected resin. Deprotection of the Fmoc residue, with a base, gives the dipeptide on resin. The same cycle of activation, coupling and deprotection is repeated with respectively Fmoc-Thr(tBu)-OH (2times) and Fmoc-Lys(Boc)-OH. After last Fmoc deprotection, palmitic acid is reacted in the same way and the palmitoyl-pentapeptide is fully deprotected and purified to give Pal-Lys-Thr-Thr-Lys-Ser-OH
Composition/ Impurities	**Pal KTTKS**: Pal-Lys-Thr-Thr-Lys-Ser L-Serine, N2-(1-oxohexadecyl)-L-lysyl-L-threonyl-L-threonyl-L-lysyl-  The chromatographic purity at 210 nm is ≥ 90% The impurities are listed below: - stereoisomers of pal-KTTKS-OH - Myr-Lys-Thr-Thr-Lys-Ser-OH - Stearyl- Lys-Thr-Thr-Lys-Ser-OH
Safety data	**Data already on the CIR Scientific Literature Review from unpublished reports from PcPc**

Patch Test in humans	June 19th, 1998	Well tolerated
In vitro Ocular tolerance assessment	June 19th, 1998	Rather well tolerated
Reverse Mutation Study	June 30th, 1999	Non mutagenic
HRIPT	August 10th, 1999	Non sensitizing

Other safety information	Pal KTTKS is used at approx. 100 ppm in our products, cosmetic ingredients recommended between 3 and 8% in finished cosmetic product (up to 8 ppm Palmitoyl Pentapeptide-4 in finished cosmetics). These products are widely supplied since 1999 in the EU, the US, Canada, China, Korea, Japan, Australia without any complaint concerning their innocuity.

<table>
<tr><td>INCI name</td><td>Palmitoyl Pentapeptide-4 (Pal KTSKS)</td></tr>
<tr><td>INCI Monograph ID</td><td>12108</td></tr>
<tr><td>Manufacturing process</td><td>Pal-Lys-Thr-Ser-Lys-Ser-OH is obtained by solid phase synthesis at room temperature using Fmoc-aminoacid derivatives:
Fmoc-Lys(Boc)-OH is activated, with a coupling reagent, and reacted on Serine protected resin. Deprotection of the Fmoc residue, with a base, gives the dipeptide on resin. The same cycle of activation, coupling and deprotection is repeated with respectively Fmoc-Ser(tBu)-OH, Fmoc-Thr(tBu)-OH and Fmoc-Lys(Boc)-OH.
After last Fmoc deprotection, palmitic acid is reacted in the same way and the palmitoyl-pentapeptide is fully deprotected and purified to give Pal-Lys-Thr-Ser-Lys-Ser-OH.</td></tr>
<tr><td>Composition Impurities</td><td>Pal KTSKS: Pal-Lys-Thr-Ser-Lys-Ser
L-Serine, N2-(1-oxohexadecyl)-L-lysyl-L-threonyl-L-seryl-L-lysyl
 
The chromatographic purity at 210 nm is≥ 90%

The impurities are listed below:
- stereoisomers of pal-KTSKS-OH
- Myr-Lys-Thr-Ser-Lys-Ser-OH
- Stearyl- Lys-Thr-Ser-Lys-Ser-OH</td></tr>
<tr><td>Safety data</td><td><u>Tests on the formula of the commercial product</u> (code TX 19011, containing 0.12% of Palmitoyl Pentapeptide-4):

<table>
<tr><td colspan="3">Local toxicity</td></tr>
<tr><td rowspan="2">Cutaneous primary tolerance</td><td><u>Cutaneous primary irritation test according to the OECD guideline n°439</u>
IEC, report n°200840RD
23 July 2020, (on TX 19011 at 100%)
Test at approx. 1200 ppm Palmitoyl Pentapeptide-4</td><td>Non irritant</td></tr>
<tr><td><u>Human Patch test</u>
Eurofins Romania, report n°ER 21/049 P21 0044
7 April 2021, (on TX 19011 at 15%)
Test at approx. 180 ppm Palmitoyl Pentapeptide-4</td><td>Non irritant</td></tr>
<tr><td rowspan="2">Ocular irritation</td><td><u>HET-CAM test</u>
IDEA Lab, report n°6.02-54075-ID-20/00404
14 February 2020, (on TX 19011 at 10%)
Test at approx. 120 ppm Palmitoyl Pentapeptide-4</td><td>Slightly irritant</td></tr>
<tr><td><u>EpiOcular test according to the OECD guideline n°492</u>
IDEA Lab, report n° 6.49_S-54682-ID-20/00404
2 November 2020, (on TX 19011 at 30%)
Test at approx. 360 ppm Palmitoyl Pentapeptide-4</td><td>Non irritant</td></tr>
<tr><td colspan="3">Allergenicity</td></tr>
<tr><td>In vivo sensitization</td><td><u>HRIPT</u>
Eurofins Romania, report n°ER 21/048-14 P21 0045
27 May 2021, (on TX 19011 at 15%)
Test at approx. 180 ppm Palmitoyl Pentapeptide-4</td><td>Non irritant nor sensitizing</td></tr>
</table>
</td></tr>
</table>

	Systemic toxicity
	Endocrine disruptor — **YES/YAS test** Xenometrix, report n°SEDE2010 / SEDE2015 10 December 2020 / 2 June 2021, (both on TX 19011 at 100%) Test at approx. 1200 ppm Palmitoyl Pentapeptide-4 — Non androgenic nor estrogenic activity
	Tests on the peptide Palmitoyl Pentapeptide-4 (Pal-KTSKS) (code TX 19006):
	Allergenicity
	***In vitro* sensitization** — **DPRA test according to the OECD guideline n°442C** IDEA Lab, report n°6.53-52287-ID-19/09966 25 November 2019, (on TX 19006 at 100%) — Negative prediction
	***In vitro* sensitization** — **KeratinoSens test according to the OECD guideline n°442D** IDEA Lab, report n° 6.52-52291-ID-19/09966 25 November 2019, (on TX 19006 at 100%) — Negative prediction
	Systemic toxicity
	Mutagenesis — **Ames test according to the OECD guideline n°471** IDEA Lab, report n°6.46_5S-53451-ID-19/09966 4 December 2019, (on TX 19006 at 100%) — Non mutagenic
	Clastogenicity — **Micronucleus test according to the OECD guideline n°473** IPL, report n°FSR-IPL 210103 12 April 2021, (on TX 19006 at 100%) — Non clastogenic
	Photoreactivity
	***In vitro* phototoxicity** — **UVA Spectrum according to the OECD guideline n°101** SEDERMA report 1 August 2019, (on TX 19006 at 100% with 15 ppm) — No significant absorption
Other safety information	Pal KTSKS is used at approx. 0.12% in our product, cosmetic ingredients recommended at 1% in finished cosmetic product (up to 12 ppm Palmitoyl Pentapeptide-4 in finished cosmetics). Used by customer since 2022 in all country without any complaint concerning their innocuity.

Test item
PENTA 18 479 - REF : TX 19006

Bacterial reverse mutation assay: determination of the mutagenic activity of a test item on *Salmonella typhimurium* (Ames test) according to the OECD #471

FINAL REPORT

Study Director : Francoise QUEFFURUS

Study # : 6.46_5S-53451-ID-19/09966

Sponsor	**Test Facility**
SEDERMA	IDEA Lab
29, rue du Chemin-Vert BP 33 78612 LE PERRAY EN YVELINES CEDEX FRANCE	Technopôle Brest-Iroise 90 rue René Descartes 29280 PLOUZANE FRANCE

Groupe IDEA TESTS - IDEA Lab
www.groupeideatests.com • idea@groupeideatests.com
SARL au capital de 19 600 € • RCS BORDEAUX 353 989 684 • APE 7120B • TVA FR 66 353 989 684
Site Martillac : Technopôle Montesquieu, 5 rue Jacques Monod CS 60077 33652 MARTILLAC CEDEX • Tél. 05 56 64 82 33 • Fax. 05 56 64 82 11
Site Brest : Technopôle Brest-Iroise 90, rue René Descartes 29280 PLOUZANE •Tél. 02 98 05 19 70 • Fax. 02 98 05 09 73

Table of content

GLP conformity statement

According to the Good Laboratory Practice (GLP) principles of France, the European Directive 2004/10/CE, the decree dated August 10th, 2004 from the JOFR, I state that:

- the study 6.46_5S-53451-ID-19/09966 was performed according to the GLP principles in IDEA Lab company laboratory, Brest location,
- the Study Plan and its modifications have been performed under my responsibility,
- all relevant SOPs have been followed,
- for confidentiality concerns, some characterisation data related to the test item composition may not be shown in this report. In this case, it is a deviation to GLP. However, this characterization had been provided by the Sponsor, brought to my attention, and then stored in a secure environment in accordance with the company procedures.
- raw data have been registered accurately,
- the study 6.46_5S-53451-ID-19/09966 is in conformity with the GLP principles despite of the following point which does not affect the reliability of results generated:
  the test item concentrations control in the different dilutions was not performed for the following reasons:
  - control of the test item preparation in its vehicle, particularly with micropipette and precision scales regularly controlled, calibrated and traceable with national or international standards of measurement,
  - the control of the homogeneity of the test item dilutions in the vehicle is performed using organoleptic criteria and is documented in the study log book,
  - test items dilutions are prepared extemporaneously,

This report accurately reflects the study carried out and the results obtained.
For these reasons, the Study Director acknowledges responsibility for the data validity of the study.

Date: 06 DEC. 2019

Francoise QUEFFURUS
Study Director
Microbiology Engineer

Quality Assurance statement

According to the Good Laboratory Practice, I state that:

- The General Study Plan was audited by the Quality Assurance and that the Specific Study Plan was verified before the beginning of the study.
- The different technical phases of the study 6.46_5S are regularly audited by the Quality Assurance. Facility audits are also carried out. The audit frequency is defined in the corresponding procedure.

  At the last technical audit (A-18/06), the following activities have been examined:
  Technical Phase preliminary cytotoxicity study
  - test item and controls preparation
  - S9-Mix preparation
  - test item and controls contact with the test system
  - plates incubation.
- The final report was audited by the Quality Assurance of IDEA Lab. It accurately reflects the raw data from the study and the application of the Standard Operating Procedures and the protocol.

Audit nature	Audits dates	Transmission dates of the audit report to the Study Director and the General Management
Technical phases of the study	From 25/06/2018 To 27/06/2018	03/07/2018
General Study Plan	21/12/2018	21/12/2018
Draft Report	28 OCT. 2019	28 OCT. 2019
Final Report	04 DEC. 2019	04 DEC. 2019

Date: 04 DEC. 2019

Quality Assurance
Delphine LEGEAIS

Study summary

The ability of the test item **PENTA 18 479 - REF : TX 19006**, supplied by **SEDERMA**, to induce mutation was assessed using the bacterial reverse mutation test (Ames test). The test was performed on five *Salmonella typhimurium* strains.

The test item dilutions were prepared in DMSO.
A preliminary cytotoxicity test was performed on *S. typhimurium* TA100 strain.
The test has been performed at the concentrations 5000, 1600, 500, 160 and 50 µg/plate, with and without S9-Mix.

As the preliminary experiment revealed cytotoxicity of the test item, the Study Director decided to define the first point of the range of concentrations at 1600 µg/plate and added two extra points of concentration. Therefore, the range of concentrations was the following: 1600, 500, 160, 50, 16, 5 and 1.6 µg/plate. The Test 1 also includes the test on the strain TA100.

According to the results obtained in the Test 1, the Study Director decided to maintain range of concentrations for Test 2.

The revertant analysis shows that:

- A cytotoxic effect has been observed in the following conditions:
  - Without metabolic activation:
    - With direct incorporation (Test 1): until 50 µg/plate for strain TA98, until 160 µg/plate for strains TA100, TA102 and TA1535, until 500 µg/plate for strain TA1537.
    - This cytotoxic effect has been confirmed with the pre-incubation assay (Test 2) until 50 µg/plate for strain TA100 and TA102, until 160 µg/plate for strains TA98 and TA1535, until 500 µg/plate for strain TA1537.
  - With metabolic activation:
    - With direct incorporation (Test 1): until 160 µg/plate for strain TA100, until 500 µg/plate for strains TA98, TA102, TA1535 and TA1537.
    - This cytotoxic effect has been confirmed with the pre-incubation assay (Test 2) until 160 µg/plate for strain TA102, until 500 µg/plate for strains TA98, TA100, TA1535 and TA1537
- No concentration of the test item showed ratio R higher or equal at least to the double of the spontaneous rate of reversion for TA98, TA100 and TA102 strains and to the triple of the spontaneous rate of reversion for TA1535 and TA1537 strains, with and without metabolic activation.
- No dose response was observed, whatever the test system or conditions of the test.
- In addition, signs of precipitate have been observed on plates and / or on mix reagent until 500 µg/plate, with and without metabolic activation.

Based on the result of this study, the test item PENTA 18 479 - REF : TX 19006, ID-19/09966 was found to be non mutagenic and non pro-mutagenic, but shows a cytotoxic activity under the test conditions.

Study presentation

1 Study objective

We have evaluated, using an *in vitro* test, the genotoxicity potential of a test item **PENTA 18 479 - REF : TX 19006, ID-19/09966,** according to the general study plan 6.46_5S.
This study has been performed according to the OECD #471 Guideline (July 21st, 1997) and the Directive 2000/32/CE, method B13/14, dated June 8th 2000.

2 Test item

Reception of test item (including recording and verification) has been managed by **IDEA Lab, – site de Martillac - Technopole Montesquieu, 33652 MARTILLAC Cedex,** which in this case resend the sample to IDEA Lab, Brest Test Location for the study.

This activity is under Study Director's responsibility and performed according procedures in place within IDEA Lab, and is within the scope of installation audits performed by the Quality Assurance of the test facility.

Information linked to the identification, purity and stability of the test item are under responsibility of the Sponsor of the study. The technical data sheet of the test item was provided by the Sponsor of the study. In case of missing or incomplete data, results obtained during solubility and stability study performed during preliminary experiment will serve as evidences and are available in study book.

Name	: PENTA 18 479 - REF : TX 19006
Internal code	: ID-19/09966
Batch number	: B1
Storage conditions	: Room temperature (20°C ± 5°C)
Test item nature	: Cosmetic ingredient
Retest date	: 27/02/2020
CAS number	: NA

Physico-chemistry properties

Physical state at 20 °C	: Solid
Color	: White
pH	: NA
Density (for liquid)	: NA
Homogeneity	: Yes
Test item purity	: 81.6%

Solubility and stability

Solvent	: Ethanol
Maximal concentration in the solvent	: 2.5 g/l
Stability in the solvent	: Not defined

The certificate of analysis of the test item is shown at the end of the report.

3 Study principle

- Contact of the five mutant strains with several concentrations of the test item, with and without metabolic activation.
- Counting of revertant colonies with several concentrations of test item and comparison with the spontaneous revertant colonies.
- Validation of the test by positive controls (mutagenic substances) and negative controls.

4 Study course

4.1 Experimentation phases

Start of the experimental part of the study (cytotoxicity test): 26/09/2019.

End of the experimental part of the study (end of Test 2): 11/10/2019.

Study occurred in 3 main phases:

- A preliminary experiment performed in order to evaluate the cytotoxicity of the test item and to select the range of dose levels for the further experiments,
- A first experiment of genotoxicity (Test 1), with and without metabolic activation, with the direct plate incorporation method, on the range of concentrations defined by the preliminary study.
- A second experiment (Test 2), using the pre-incubation method, with and without metabolic activation, with dose levels defined by Study Director after analysis of results obtained from the first experiment. This second experiment has been performed in order to confirm or for complement results of the first one.

4.2 Material and reagents

4.2.1 Material

- Petri plates
- Plastic vials with screw cap for strains conservation (cryotubes)
- Automatic pipettes 1-2000 µl
- Test tubes and vials with appropriate volumes
- Autoclave
- Vortex
- pH-meter
- Analytical weights
- Spectrophotometer
- Bacteriological incubators
- Thermostatic agitators
- Laminar flow hood (PSM) with extraction system
- Freezer at -80°C (± 5°C)
- Freezer at -20°C (± 5°C)
- Refrigerators at 6°C (± 3°C)
- Colony counter and data processing system
  - Plates reader : Sorcerer, version 2.2 (Perceptive Instrument)
  - Transfer and raw data storage : Ames Study Manager, version 1.22 (Perceptive Instrument)
  - Result tables edition : Ames Report Generator, version 1 (Perceptive Instrument)

4.2.2 Reagents

- Nutrient broth for the strains culture
- Bottom agar
  - Vogel-Bonner medium E (concentrated 50 times)
  - Glucose solution at 400 g/l
- Top agar for *Salmonella typhimurium* TA
- Phosphate buffer 0.2 M, pH 7.4
- Reagents for preparation of S9-Mix
- Microsomal fraction of rat liver (S9)

4.3 Test system

4.3.1 Strains

The choice of strains was made according to OECD Guideline. It is five strains of *Salmonella typhimurium* LT2 for which the properties are summarised in the following table.

	Target mutation	Excision repear	Plasmid	Cell wall	Type of mutation
TA98	His D 3052	uvrB	pkM101	rfa	Frameshift
TA100	His G 46	uvrB	pkM101	rfa	Base-pair substitution
TA102	His G 428	intact excision	pkM101	rfa	Base-pair substitution
TA1535	His G 46	uvrB	/	rfa	Base-pair substitution
TA1537	His C 3076	uvrB	/	rfa	Frameshift

Table 1

Besides their histidin mutation, each strain has 1 or 2 further mutations which increase really their sensibility to mutagens:

- uvrB: excision of reparation system,
- rfa: the lipo-polysaccharidic wall is made permeable to big molecules.

Control of essential characteristics of strains is periodically performed according to OECD #471 Guidelines and instruction IL 11:

- Growing in presence of histidin
- Permeability of bacterial wall (rfa mutation)
- Ampicillin resistance
- Deletion of the DNA repair potential
- Spontaneous revertant rate

4.3.2 Media and growth conditions

The composition of media is summarized in annex to this document.

For each experiment, the test strains cultures were prepared in nutrient broth from frozen stocks and incubated at 37°C ± 2 °C on shaken platter to allow the culture to grow up to the late exponential or early stationary phase of growth (approximately 10^8-10^9 cells/ml). The optical density of each culture has been used to check the cell density.

Microbial suspension was put in contact with the test item or reference items, mixed with top agar and poured over minimal agar medium plate. After solidification, plates were incubated at 37°C ± 2°C during 48 to 72 hours. Positive and negative controls were included in the experiment.

4.3.3 Metabolic activation

Bacteria were exposed to the test item with and without a metabolic activation system. The system used is a cofactor enhanced post-mitochondrial fraction (S9), prepared from rat livers treated with an enzymatic inducer. The post-mitochondrial fraction (certificate given in annex) is used at 10% (v/v). The composition of the S9-Mix is described in annex. The acceptation criteria for the post-mitochondrial fraction are described in the working instruction IL REAC 01.

4.4 Reference items

Negative controls: the spontaneous revertant count with the solvent, with and without metabolic activation, was included in each experiment.

Negative control without treatment: the spontaneous revertant count without the solvent, with and without metabolic activation was included in each experiment for the control of absence of mutagen activity of vehicle.

Positive controls: known mutagens as defined in table 2.

Strain	Reference items used							
	-S9-Mix	Solvent	Dose (µg/plate)	Dose (µl)	+S9-Mix	Solvent	Dose (µg/plate)	Dose (µl)
TA98	2-NITROFLUORENE 0.1 mg/ml CAS No. (607-57-8)	DMSO	5	50	2-AMINOANTHRACENE 0.1 mg/ml CAS No. (613-13-8)	DMSO	5	50
TA100	SODIUM AZIDE 0.2 mg/ml CAS No. (26628-22-8)	Water	10	50	2-AMINOANTHRACENE 0.1 mg/ml CAS No. (613-13-8)		5	50
TA102	MITOMYCIN 0.01 mg/ml CAS No. (50-07-7)	Water	0.5	50	2-AMINOANTHRACENE 0.5 mg/ml CAS No. (613-13-8)		25	50
TA1535	SODIUM AZIDE 0.2 mg/ml CAS No. (26628-22-8)	Water	10	50	2-AMINOANTHRACENE 0.1 mg/ml CAS No. (613-13-8)		5	50
TA1537	9-AMINOACRIDINE 0.6 mg/ml CAS No. (90-45-9)	DMSO	30	50	2-AMINOANTHRACENE 0.1 mg/ml CAS No. (613-13-8)		5	50

Table 2

4.5 Solvent choice and test item preparation

The most commonly used solvents are deionized water for the analysis and dimethylsulfoxide (DMSO), or any appropriate solvent compatible with the test system and the test item or other solvent can be used at the request of the Sponsor if they are known or if it has been demonstrated that they are not cytotoxic nor genotoxic. The compatibility with the test item is therefore under the Sponsor responsibility.

A preliminary dissolution test was performed in order to define the most appropriate solvent as well as the maximal concentration tested.

A stock solution of the test item PENTA 18 479 - REF : TX 19006 was prepared DMSO (DMSO final concentration 1.89%) as solubility rate of the solvent proposed by the Sponsor (ethanol) was not compatible with the study.

The other tested solutions have been obtained by serial dilution from this stock solution in the same solvent. These solutions were prepared extemporaneously each day of manipulation.

4.6 Series definition

The solubility test showed no insolubility of the test item. Therefore, the maximal concentration retained was 5000 µg/plate.

As purity of test item defined by sponsor was 81.6%, the percentage of purity is taken into account for the study (concentration of stock solution at 122.55 mg/ml for the maximal concentration of test item at 100 mg/ml).

According to OECD Guideline, 5 concentrations of test item have been studied with approximately half log (i.e. approximately √10) interval. These doses (rounded to the higher value) used for the preliminary cytotoxicity test were therefore the following: 5000, 1600, 500, 160 and 50 µg/plate.

As the preliminary experiment revealed cytotoxicity of the test item (cf. paragraph 7.1), the Study Director decided to define the first point of the range of concentrations at 1600 µg/plate and added two extra points of concentration. Therefore, the range of concentrations was the following: 1600, 500, 160, 50, 16, 5 and 1.6 µg/plate. The Test 1 also includes the test on the strain TA100.

According to the results obtained in the Test 1, the Study Director decided to maintain range of concentrations for Test 2.

Each test item dilution and each reference item are tested on 3 Petri plates.

4.7 Test performance

4.7.1 Preliminary cytotoxicity study

The preliminary cytotoxicity study of the test item has been performed on the strain *S. typhimurium* TA100, in the same conditions as the Test 1 (cf. paragraph 4.7.3).

Results obtained are part of the Test 1 results if no cytotoxicity is observed. The test item has been dissolved in the suitable solvent.

The applied protocol was the following one:

- In 3 hemolysis tubes, introduce:
  - assay without metabolic activation:
    - 0.05 ml of the different test item concentrations,
    - 0.5 ml sterile phosphate buffer 0.2 M, pH 7.4,
    - 2 ml of top agar for *S. typhimurium*,
    - 0.1 ml of bacterial inoculum (TA100).
  - assay with metabolic activation:
    - 0.05 ml of the different test item concentrations,
    - 2 ml of top agar for *S. typhimurium*,
    - 0.1 ml of bacterial inoculum (TA100),
    - 0.5 ml of S9-Mix.
- Mix and pour on the surface of the bottom agar previously distributed in Petri dishes.
- Incubate at 37°C ± 2 °C for 48 to 72 hours.
- Count colonies. Results are interpreted according to the paragraph 5.

4.7.2 Control tests

These assays were performed for each test: preliminary cytotoxicity test, Test 1 and Test 2.
Non treated control, negative controls and positive controls made during pre-incubation method were incubated during 20-30 minutes at 37°C ± 2°C before pouring top agar, according described method paragraph 4.7.3.2.

4.7.2.1 Sterility control of the solvent used, the test item, the S9-Mix and the top agar

The applied protocol was the following one:

- In 4 fractions of 2 ml top agar for *S. typhimurium*, introduce:
  - 0.1 ml of phosphate buffer 0.2 M, pH 7.4,
  - 0.1 ml of solvent,
  - 0.1 ml of S9-Mix.
  - 0.1 ml of the test item preparation at the higher concentration,
- One fraction of 2 ml top agar for *S. typhimurium* is used to control its sterility.
- Mix and pour on the surface of the bottom agar previously distributed in Petri dishes.
- Incubate at 37°C ± 2°C for 48 to 72 hours.
- The test is performed in triplicate.
- No bacterial growth must be observed.

4.7.2.2 Non treated control and negative control (control of non mutagenic activity of the solvent used)

The applied protocol was the following one:

- For each strain, in 3 hemolysis tubes, introduce:
  - assay without metabolic activation:
    - 0.5 ml sterile phosphate buffer 0.2 M, pH 7.4,
    - 2 ml of top agar,
    - 0.1 ml of bacterial inoculum.
  - assay with metabolic activation:
    - 2 ml of top agar,

  - 0.1 ml of bacterial inoculum,
  - 0.5 ml of S9-Mix.
- For the negative control add 0.05 ml of the solvent used.
- Mix and pour on the surface of the bottom agar previously distributed in Petri dishes.
- Incubate at 37°C ± 2°C for 48 to 72 hours.
- Count colonies. Results are interpreted according to the paragraph 5.

4.7.2.3 Positive control

Each mutagenicity assay of a test item must include well-known mutagenic products, specific for each strain. Positive controls used are described in table 2.

The applied protocol was the following one:

- For each strain, in 3 hemolysis tubes, introduce:
  - assay without metabolic activation:
    - 0.5 ml sterile phosphate buffer 0.2 M, pH 7.4,
    - The quantity of positive control defined in table 2,
    - 2 ml of top agar,
    - 0.1 ml of bacterial inoculum.
  - assay with metabolic activation:
    - The quantity of positive control defined in table 2,
    - 2 ml of top agar,
    - 0.1 ml of bacterial inoculum,
    - 0.5 ml of S9-Mix.
- Mix and pour on the surface of the bottom agar previously distributed in Petri dishes.
- Incubate at 37°C ± 2°C for 48 to 72 hours.
- Count colonies. Results are interpreted according to the paragraph 5.

4.7.3 Test itself: Research of mutagenic activity

For at least 5 concentrations of the test item, a test without metabolic activation and a test with metabolic activation have been performed simultaneously as follow:

4.7.3.1 Test 1: direct method

- For each strain, in 3 hemolysis tubes, introduce:
  - assay without metabolic activation:
    - 0.05 ml of the different test item concentrations,
    - 0.5 ml sterile phosphate buffer 0.2 M, pH 7.4,
    - 2 ml of top agar,
    - 0.1 ml of bacterial inoculum.
  - assay with metabolic activation:
    - 0.05 ml of the different test item concentrations,
    - 2 ml of top agar,
    - 0.1 ml of bacterial inoculum,
    - 0.5 ml of S9-Mix.
- Mix and pour on the surface of the bottom agar previously distributed in Petri dishes.
- Incubate at 37°C ± 2°C for 48 to 72 hours.
- Count colonies. Results are interpreted according to the paragraph 5.

4.7.3.2 Test 2: method with pre-incubation

Pre-incubation method allows revealing more effectively mutagen activity of some compounds like aliphatic nitrosamines, bivalent metals, aldehydes, azoic coloring agent.

The applied protocol was the following one:

- For each strain, in 3 hemolysis tubes, introduce:
  - assay without metabolic activation:
    - 0.05 ml of the different test item concentrations,
    - 0.5 ml sterile phosphate buffer 0.2 M, pH 7.4,
    - 0.1 ml of bacterial inoculum.
  - assay with metabolic activation:
    - 0.05 ml of the different test item concentrations,
    - 0.1 ml of bacterial inoculum,
    - 0.5 ml of S9-Mix.
- Incubate at 37°C ± 2°C for 20 to 30 min.
- Add the 2 ml of top agar.
- Mix and pour on the surface of the bottom agar previously distributed in Petri dishes.
- Incubate at 37°C ± 2°C for 48 to 72 hours.
- Count colonies. Results are interpreted according to the paragraph 5.

4.8 Data evaluation

For each assay the following observations were performed and reported:

- Observation of the reagent mix before Petri plates pouring: reporting of any abnormal sign (precipitate, trouble, etc.),
- Petri plates observation and reporting of any cytotoxicity sign (bottom bacterial layer reduction). The cytotoxicity intensity on the bottom bacterial layer is evaluated qualitatively on each plate by naked eyes:
  - total destruction of the bottom bacterial layer (the revertants development does not occur in this case), this one is noted in tables of results as “A”.
  - moderated destruction of the bottom bacterial layer. This one is noted in tables of results as “S”.

Acquisition and storage of raw data were managed by the following electronic system:

- Reading of plates: Sorcerer, version 2.2.
- Transfer and storage of raw data: Ames Study Manager, version 1.22.

The result tables edition was managed by the Ames Report Generator, version 1.

5 Results expression and interpretation

5.1 Processing and presentation of the results

After 48 to 72 hours incubation at 37°C ± 2°C, revertants were counted on each plate. (If counting has not been performed at the end of incubation period, plates have been stored in fridge (6°C ± 3°C) and read within 72 hours, without affecting results of test).

Results are expressed in number of revertants (mean ± sd) per plate for each concentration of the test item.

The following ratio can be established:

$$R = \frac{\text{Number of revertants with test item}}{\text{Number of revertants with solvent without test item}}$$

Tables were set up where we can see all individual results obtained with the test item and the positive and negative controls.

Mean and standard deviation were calculated for each concentration of the test item.

The preliminary cytotoxicity test results are shown with the Test 1 results (cf. paragraph 7.2) if there is no cytotoxicity or if this one can be shown only at the maximum concentration. In case of the opposite, they are presented in paragraph 7.1 in a table in which bacterial layer aspect and R ratio values are shown for each concentration tested.

5.2 Acceptance criteria of data

The test is considered valid if the following criteria are fulfilled:

- The sterility tests are conform,
- The mean negative controls are within the historical data,
- The solvent used (negative control) must not show genotoxic nor cytotoxic activity,
- The revertants rate obtained for the positive controls must be in agreement with the historical data,
- The positive controls must show a revertants number equal at least to the double of the spontaneous rate of reversion for TA98, TA100 and TA102 ($R \geq 2$) and the triple of the spontaneous rate of reversion for TA1535 and TA1537 ($R \geq 3$),
- No more than 5% of the plates of the test are lost through contamination or any other unforeseen event,
- At least 3 concentrations are available for mutagenicity assessment.

5.3 Results interpretation

5.3.1 Preliminary test of cytotoxicity

We consider the test item as cytotoxic if the spontaneous revertant rate is lower than 0.7 ($R < 0.7$). The possible destruction of the bottom bacterial layer is also taken into account.

5.3.2 Mutagenicity test

- **The test item is considered as mutagenic** if at the end of the verifications steps, it has been obtained, in a reproducible way, a relation dose-effect on one or some of 5 strains with and/or without metabolic activation. The mutagenicity is taken into account for a given concentration only when the number of revertants is equal at least to the double of the spontaneous rate of reversion for TA98, TA100 and TA102 strains ($R \geq 2$) and the triple of the spontaneous rate of reversion for TA1535 and TA1537 strains ($R \geq 3$).
- **The test item is considered as non-mutagenic** if, in the outcome of the Test 1 and the Test 2, the rate of revertants always remained lower than the double of the rate of spontaneous reversion for all the concentrations of tested product, for TA98, TA100 and TA102 strains ($R < 2$) and lower than the triple of the spontaneous rate of reversion for TA1535 and TA1537 strains ($R < 3$), with and without metabolic activation, and on the condition of having made sure that the absence of mutagen effect is not bound to the toxicity of the tested concentrations.

The result validation was performed by the Study Director in agreement with the working instruction IL 04.

6 Study plan deviations and amendments

No deviation or amendment to the study plan has been observed during this study.

7 Results

7.1 Preliminary cytotoxicity

Preliminary cytotoxicity test performed on the TA 100 strain showed a cytotoxicity of the test item. The results are shown in the following table:

Concentration (µg/plate)	5000	1600	500	160	50
Total destruction of bacterial layer	-S9 : Yes +S9 : Yes	-S9 : Yes +S9 : No	-S9 : No +S9 : No	-S9 : No +S9 : No	-S9 : No +S9 : No
Moderate destruction of bacterial layer	-S9 : No +S9 : No	-S9 : No +S9 : Yes	-S9 : Yes +S9 : Yes	-S9 : Yes +S9 : No	-S9 : No +S9 : No
R ratio value without S9	0	0	0	0,1	0,7
R ratio value with S9	0	0	0	0,2	1

According to the results of cytotoxicity test, the Study Director decided to define the first point of the range of concentrations at 1600 µg/plate and added two extra points of concentration. Therefore, the range of concentrations was the following: 1600, 500, 160, 50, 16, 5 and 1.6 µg/plate. The Test 1 also includes the test on the strain TA100.

According to the results obtained in the Test 1, the Study Director decided to maintain range of concentrations for Test 2.

7.2 Revertants analysis

Revertant analysis was performed on the 7 concentrations chosen following the cytotoxicity study. The results are shown in table 3, 4, 5 and 6.

- Test 1, without S9-Mix, direct assay: table 3
- Test 1, with S9-Mix, direct assay: table 4
- Test 2, without S9-Mix, with pre-incubation: table 5
- Test 2, with S9-Mix, with pre-incubation: table 6

Revertant analysis tables

Table 3: Test 1, without S9-Mix, direct assay

Study Name: 6.46-5S-53451-ID-19/09966
Experiment: Test 1. ID-19/09966
Assay Conditions: Plate incorporation assay

Study Code: ID-19/09966
Date Plated: 01/10/2019
Date Counted: 04/10/2019

Without metabolic activation

Strain	Compound	Dose level per plate	Mean revertants per plate	Standard Deviation	Ratio treated / solvent	Individual revertant colony counts
TA100	**ID-19/09966**	1600 µg	*0,0*	*0,0*	*0,0*	0 P A, 0 P A, 0 P A
		500 µg	*9,7*	*2,1*	*0,1*	9 S, 8 S, 12 S
		160 µg	*36,3*	*2,1*	*0,2*	34 S, 38 S, 37 S
		50 µg	*139,3*	*11,7*	*0,8*	129, 137, 152
		16 µg	*193,7*	*37,1*	*1,1*	158, 191, 232
		5 µg	*206,0*	*65,8*	*1,1*	158, 179, 281
		1.6 µg	*163,7*	*7,0*	*0,9*	171, 157, 163
	DMSO		*182,7*	*36,5*		173, 152, 223
	Untreated Control		*189,0*	*29,4*		172, 172, 223
TA102	**ID-19/09966**	1600 µg	*0,0*	*0,0*	*0,0*	0 P A, 0 P A, 0 P A
		500 µg	*0,0*	*0,0*	*0,0*	0 S, 0 S, 0 S
		160 µg	*121,7*	*11,1*	*0,3*	132 S, 110 S, 123 S
		50 µg	*345,3*	*47,9*	*0,9*	356, 293, 387
		16 µg	*443,0*	*73,1*	*1,1*	388, 415, 526
		5 µg	*464,3*	*44,2*	*1,2*	444, 434, 515
		1.6 µg	*353,0*	*31,0*	*1,0*	348, 394, 407
	DMSO		*399,3*	*19,6*		379, 418, 401
	Untreated Control		*406,0*	*12,0*		418, 394, 406
TA1535	**ID-19/09966**	1600 µg	*0,0*	*0,0*	*0,0*	0 P A, 0 P A, 0 P A
		500 µg	*2,0*	*1,0*	*0,1*	2 S, 1 S, 3 S
		160 µg	*5,7*	*6,4*	*0,4*	2, 2, 13
		50 µg	*10,0*	*1,7*	*0,7*	9, 12, 9
		16 µg	*15,3*	*1,5*	*1,1*	15, 17, 14
		5 µg	*13,3*	*1,2*	*0,9*	14, 12, 14
		1.6 µg	*17,3*	*2,9*	*1,2*	19, 14, 19
	DMSO		*14,3*	*3,2*		13, 12, 18
	Untreated Control		*16,7*	*2,5*		17, 14, 19
TA1537	**ID-19/09966**	1600 µg	*0,0*	*0,0*	*0,0*	0 P S, 0 P S, 0 P S
		500 µg	*7,7*	*1,2*	*0,5*	7 S, 9 S, 7 S
		160 µg	*12,7*	*5,7*	*0,9*	8, 19, 11
		50 µg	*17,0*	*2,0*	*1,2*	15, 17, 19
		16 µg	*13,0*	*5,6*	*0,9*	7, 14, 18
		5 µg	*14,0*	*3,0*	*1,0*	17, 14, 11
		1.6 µg	*16,7*	*2,5*	*1,1*	17, 14, 19
	DMSO		*14,7*	*3,8*		12, 13, 19
	Untreated Control		*19,0*	*5,0*		24, 14, 19

Key to Plate Postfix Codes

P	Precipitate
A	Lawn absent
S	Sparse lawn

Study Name: 6.46-5S-53451-ID-19/09966
Experiment: Test 1. ID-19/09966
Assay Conditions: Plate incorporation assay

Study Code: ID-19/09966
Date Plated: 01/10/2019
Date Counted: 04/10/2019

Without metabolic activation

Strain	Compound	Dose level per plate	Mean revertants per plate	Standard Deviation	Ratio treated / solvent	Individual revertant colony counts
TA98	**ID-19/09966**	1600 µg	*0,0*	*0,0*	*0,0*	0 P S, 0 P S, 0 P S
		500 µg	*10,7*	*2,1*	*0,4*	9 S, 13 S, 10 S
		160 µg	*14,0*	*7,0*	*0,6*	22 S, 9 S, 11 S
		50 µg	*26,0*	*4,0*	*1,0*	30 S, 22 S, 26 S
		16 µg	*30,0*	*2,6*	*1,2*	27, 32, 31
		5 µg	*27,0*	*4,6*	*1,1*	22, 28, 31
		1.6 µg	*24,3*	*2,1*	*1,0*	22, 25, 26
	DMSO		*25,0*	*6,2*		18, 30, 27
	Untreated Control		*25,3*	*0,6*		25, 25, 26
TA100	**AZI**	10 µg	*1712,0*	*136,0*	*9,4*	1638, 1629, 1869
TA102	**MIT**	0.5 µg	*2310,0*	*91,8*	*5,8*	2319, 2397, 2214
TA1535	**AZI**	10 µg	*2150,0*	*133,0*	*150,0*	2062, 2303, 2085
TA1537	**9AA**	50 µg	*211,3*	*67,1*	*14,4*	142, 276, 216
TA98	**2NIT**	5 µg	*1179,7*	*81,0*	*47,2*	1159, 1111, 1269

Key to Positive Controls

AZI	SODIUM AZIDE
MIT	MITOMYCIN
9AA	9-AMINOACRIDINE
2NIT	2-NITROFLUORENE

Key to Plate Postfix Codes

P	Precipitate
A	Lawn absent
S	Sparse lawn

Table 4: Test 1, with S9-Mix, direct assay

Study Name: 6.46-5S-53451-ID-19/09966
Experiment: Test 1. ID-19/09966
Assay Conditions: Plate incorporation assay

Study Code: ID-19/09966
Date Plated: 01/10/2019
Date Counted: 04/10/2019

With metabolic activation

Strain	Compound	Dose level per plate	Mean revertants per plate	Standard Deviation	Ratio treated / solvent	Individual revertant colony counts
TA100	**ID-19/09966**	1600 µg	*0,0*	*0,0*	*0,0*	0 P A, 0 P A, 0 P A
		500 µg	*0,0*	*0,0*	*0,0*	0 S, 0 S, 0 S
		160 µg	*58,0*	*14,0*	*0,3*	48, 52, 74
		50 µg	*192,0*	*11,5*	*1,0*	188, 205, 183
		16 µg	*171,0*	*2,6*	*0,9*	168, 173, 172
		5 µg	*196,7*	*17,0*	*1,0*	184, 190, 216
		1.6 µg	*156,0*	*7,0*	*0,8*	151, 164, 153
	DMSO		*196,7*	*20,6*		181, 189, 220
	Untreated Control		*215,0*	*18,5*		197, 214, 234
TA102	**ID-19/09966**	1600 µg	*0,0*	*0,0*	*0,0*	0 P A, 0 P A, 0 P A
		500 µg	*0,0*	*0,0*	*0,0*	0 S, 0 S, 0 S
		160 µg	*337,7*	*27,5*	*0,8*	360, 346, 307
		50 µg	*531,3*	*69,3*	*1,2*	485, 611, 498
		16 µg	*450,0*	*35,7*	*1,0*	426, 491, 433
		5 µg	*482,3*	*20,3*	*1,1*	466, 476, 505
		1.6 µg	*402,3*	*25,5*	*0,9*	382, 394, 431
	DMSO		*450,0*	*27,8*		425, 445, 480
	Untreated Control		*495,7*	*30,0*		463, 502, 522
TA1535	**ID-19/09966**	1600 µg	*0,0*	*0,0*	*0,0*	0 P A, 0 P A, 0 P A
		500 µg	*3,7*	*1,5*	*0,2*	4 S, 5 S, 2 S
		160 µg	*11,7*	*3,2*	*0,7*	8, 14, 13
		50 µg	*10,0*	*3,6*	*0,6*	9, 7, 14
		16 µg	*19,0*	*4,6*	*1,2*	15, 18, 24
		5 µg	*14,3*	*5,5*	*0,9*	8, 17, 18
		1.6 µg	*16,0*	*6,6*	*1,0*	9, 22, 17
	DMSO		*15,7*	*2,1*		18, 15, 14
	Untreated Control		*18,0*	*4,0*		14, 22, 18
TA1537	**ID-19/09966**	1600 µg	*0,0*	*0,0*	*0,0*	0 P S, 0 P S, 0 P S
		500 µg	*14,0*	*6,6*	*0,9*	8 S, 21 S, 13 S
		160 µg	*17,3*	*4,5*	*1,1*	22, 13, 17
		50 µg	*20,7*	*7,1*	*1,3*	22, 27, 13
		16 µg	*16,3*	*2,1*	*1,0*	17, 14, 18
		5 µg	*19,7*	*4,6*	*1,3*	17, 25, 17
		1.6 µg	*16,3*	*3,1*	*1,0*	19, 17, 13
	DMSO		*15,7*	*2,9*		14, 19, 14
	Untreated Control		*18,0*	*3,6*		17, 22, 15

Key to Plate Postfix Codes

P	Precipitate
A	Lawn absent
S	Sparse lawn

Study Name: 6.46-5S-53451-ID-19/09966
Experiment: Test 1. ID-19/09966
Assay Conditions: Plate incorporation assay

Study Code: ID-19/09966
Date Plated: 01/10/2019
Date Counted: 04/10/2019

With metabolic activation

Strain	Compound	Dose level per plate	Mean revertants per plate	Standard Deviation	Ratio treated / solvent	Individual revertant colony counts
TA98	**ID-19/09966**	1600 µg	*0,0*	*0,0*	*0,0*	0 P S, 0 P S, 0 P S
		500 µg	*19,3*	*2,5*	*0,6*	17 S, 22 S, 19 S
		160 µg	*29,7*	*3,2*	*0,9*	31, 32, 26
		50 µg	*30,7*	*4,2*	*0,9*	32, 26, 34
		16 µg	*30,3*	*5,7*	*0,9*	24, 32, 35
		5 µg	*33,3*	*7,6*	*1,0*	40, 35, 25
		1.6 µg	*29,7*	*14,0*	*0,9*	41, 34, 14
	DMSO		*34,0*	*3,0*		31, 34, 37
	Untreated Control		*35,0*	*2,6*		38, 33, 34
TA100	**A2A**	5 µg	*4225,3*	*66,7*	*21,5*	4211, 4298, 4167
TA102	**A2A**	25 µg	*3943,0*	*289,9*	*8,8*	4175, 4036, 3618
TA1535	**A2A**	5 µg	*296,7*	*22,4*	*18,9*	292, 277, 321
TA1537	**A2A**	5 µg	*662,3*	*59,2*	*42,3*	697, 696, 594
TA98	**A2A**	5 µg	*4698,0*	*163,1*	*138,2*	4871, 4676, 4547

Key to Positive Controls

A2A	2-AMINOANTHRACENE

Key to Plate Postfix Codes

P	Precipitate
A	Lawn absent
S	Sparse lawn

Table 5: Test 2, without S9-Mix, with pre-incubation

Study Name: 6.46-5S-53451-ID-19/09966
Experiment: Test 2. ID-19/09966
Assay Conditions: Pre-incubation assay

Study Code: ID-19/09966
Date Plated: 08/10/2019
Date Counted: 11/10/2019

Without metabolic activation

Strain	Compound	Dose level per plate	Mean revertants per plate	Standard Deviation	Ratio treated / solvent	Individual revertant colony counts
TA100	**ID-19/09966**	1600 µg	*0,0*	*0,0*	*0,0*	0 P S, 0 P S, 0 P S
		500 µg	*4,0*	*1,0*	*0,0*	3 S, 5 S, 4 S
		160 µg	*20,0*	*3,6*	*0,1*	23 S, 16 S, 21 S
		50 µg	*96,7*	*9,3*	*0,6*	101, 103, 86
		16 µg	*153,7*	*49,7*	*1,0*	100, 163, 198
		5 µg	*169,0*	*45,0*	*1,1*	135, 152, 220
		1.6 µg	*153,7*	*14,6*	*1,0*	152, 169, 140
	DMSO		*157,7*	*11,6*		150, 152, 171
	Untreated Control		*175,0*	*19,5*		153, 182, 190
TA102	**ID-19/09966**	1600 µg	*0,0*	*0,0*	*0,0*	0 P S, 0 P S, 0 P S
		500 µg	*0,0*	*0,0*	*0,0*	0 S, 0 S, 0 S
		160 µg	*85,7*	*21,8*	*0,2*	105 S, 90 S, 62 S
		50 µg	*207,7*	*33,7*	*0,5*	243, 204, 176
		16 µg	*298,0*	*28,2*	*0,7*	309, 266, 319
		5 µg	*411,7*	*6,7*	*1,0*	410, 406, 419
		1.6 µg	*432,7*	*28,1*	*1,1*	465, 419, 414
	DMSO		*405,3*	*11,6*		392, 413, 411
	Untreated Control		*413,3*	*30,0*		395, 397, 448
TA1535	**ID-19/09966**	1600 µg	*0,0*	*0,0*	*0,0*	0 P S, 0 P S, 0 P S
		500 µg	*1,3*	*0,6*	*0,1*	2 S, 1 S, 1 S
		160 µg	*2,0*	*0,0*	*0,1*	2, 2, 2
		50 µg	*12,7*	*4,0*	*0,8*	12, 9, 17
		16 µg	*11,0*	*0,0*	*0,7*	11, 11, 11
		5 µg	*12,0*	*4,4*	*0,7*	14, 15, 7
		1.6 µg	*13,7*	*2,9*	*0,8*	12, 12, 17
	DMSO		*16,3*	*2,1*		18, 14, 17
	Untreated Control		*10,0*	*5,3*		14, 12, 4
TA1537	**ID-19/09966**	1600 µg	*0,0*	*0,0*	*0,0*	0 P S, 0 P S, 0 P S
		500 µg	*9,7*	*2,5*	*0,6*	12 S, 10 S, 7 S
		160 µg	*11,3*	*1,5*	*0,7*	10, 13, 11
		50 µg	*7,3*	*1,2*	*0,5*	6, 8, 8
		16 µg	*11,7*	*0,6*	*0,7*	12, 12, 11
		5 µg	*16,3*	*3,2*	*1,0*	20, 14, 15
		1.6 µg	*16,3*	*4,0*	*1,0*	17, 20, 12
	DMSO		*15,7*	*6,1*		21, 17, 9
	Untreated Control		*21,0*	*2,6*		19, 24, 20
TA98	**ID-19/09966**	1600 µg	*0,0*	*0,0*	*0,0*	0 P S, 0 P S, 0 P S
		500 µg	*6,3*	*2,1*	*0,3*	4 S, 8 S, 7 S
		160 µg	*11,7*	*2,1*	*0,5*	11 S, 10 S, 14 S
		50 µg	*25,7*	*6,0*	*1,1*	20, 32, 25
		16 µg	*29,7*	*3,8*	*1,3*	27, 28, 34
		5 µg	*26,7*	*12,5*	*1,2*	18, 21, 41
		1.6 µg	*20,0*	*0,0*	*0,9*	20, 20, 20
	DMSO		*22,3*	*4,2*		27, 19, 21
	Untreated Control		*19,0*	*5,3*		17, 25, 15

Key to Plate Postfix Codes

P	Precipitate
S	Sparse lawn

Study Name: 6.46-5S-53451-ID-19/09966
Experiment: Test 2. ID-19/09966
Assay Conditions: Pre-incubation assay

Study Code: ID-19/09966
Date Plated: 08/10/2019
Date Counted: 11/10/2019

Without metabolic activation

Strain	Compound	Dose level per plate	Mean revertants per plate	Standard Deviation	Ratio treated / solvent	Individual revertant colony counts
TA100	**AZI**	10 µg	*2009,3*	*121,8*	*12,7*	2144, 1977, 1907
TA102	**MIT**	0.5 µg	*2981,7*	*497,6*	*7,4*	2489, 2972, 3484
TA1535	**AZI**	10 µg	*2176,7*	*64,1*	*133,3*	2220, 2103, 2207
TA1537	**9AA**	30 µg	*240,7*	*92,2*	*15,4*	261, 321, 140
TA98	**2NIT**	5 µg	*1252,0*	*107,4*	*56,1*	1312, 1128, 1316

Key to Positive Controls

AZI	SODIUM AZIDE
MIT	MITOMYCIN
9AA	9-AMINOACRIDINE
2NIT	2-NITROFLUORENE

Key to Plate Postfix Codes

P	Precipitate
S	Sparse lawn

Table 6: Test 2, with S9-Mix, with pre-incubation

Study Name: 6.46-5S-53451-ID-19/09966
Experiment: Test 2. ID-19/09966
Assay Conditions: Pre-incubation assay

Study Code: ID-19/09966
Date Plated: 08/10/2019
Date Counted: 11/10/2019

With metabolic activation

Strain	Compound	Dose level per plate	Mean revertants per plate	Standard Deviation	Ratio treated / solvent	Individual revertant colony counts
TA100	**ID-19/09966**	1600 µg	*0,0*	*0,0*	*0,0*	0 P S, 0 P S, 0 P S
		500 µg	*8,0*	*4,4*	*0,0*	13 S, 6 S, 5 S
		160 µg	*108,0*	*13,5*	*0,7*	97, 104, 123
		50 µg	*162,3*	*8,1*	*1,0*	155, 171, 161
		16 µg	*149,7*	*10,1*	*0,9*	138, 155, 156
		5 µg	*176,0*	*14,1*	*1,1*	161, 178, 189
		1.6 µg	*145,7*	*17,4*	*0,9*	126, 152, 159
	DMSO		*166,0*	*6,1*		159, 170, 169
	Untreated Control		*195,7*	*10,7*		205, 198, 184
TA102	**ID-19/09966**	1600 µg	*0,0*	*0,0*	*0,0*	0 P S, 0 P S, 0 P S
		500 µg	*0,0*	*0,0*	*0,0*	0 S, 0 S, 0 S
		160 µg	*305,7*	*17,6*	*0,6*	295, 296, 326
		50 µg	*472,7*	*10,2*	*0,9*	477, 461, 480
		16 µg	*444,7*	*12,4*	*0,9*	437, 459, 438
		5 µg	*482,7*	*8,1*	*0,9*	490, 484, 474
		1.6 µg	*448,7*	*50,5*	*0,9*	420, 419, 507
	DMSO		*516,0*	*33,0*		494, 500, 554
	Untreated Control		*481,7*	*17,6*		465, 480, 500
TA1535	**ID-19/09966**	1600 µg	*0,0*	*0,0*	*0,0*	0 P S, 0 P S, 0 P S
		500 µg	*2,3*	*2,1*	*0,2*	4 S, 0 S, 3 S
		160 µg	*8,7*	*0,6*	*0,7*	9, 9, 8
		50 µg	*15,7*	*3,8*	*1,2*	13, 20, 14
		16 µg	*12,7*	*0,6*	*1,0*	13, 12, 13
		5 µg	*16,0*	*2,6*	*1,2*	14, 19, 15
		1.6 µg	*18,3*	*1,2*	*1,4*	17, 19, 19
	DMSO		*13,0*	*1,7*		14, 11, 14
	Untreated Control		*16,3*	*2,3*		15, 15, 19
TA1537	**ID-19/09966**	1600 µg	*0,0*	*0,0*	*0,0*	0 P S, 0 P S, 0 P S
		500 µg	*13,7*	*2,1*	*0,7*	13 S, 12 S, 16 S
		160 µg	*19,0*	*1,7*	*1,0*	18, 21, 18
		50 µg	*17,3*	*3,5*	*0,9*	17, 14, 21
		16 µg	*21,3*	*6,4*	*1,1*	14, 24, 26
		5 µg	*18,3*	*5,5*	*1,0*	21, 12, 22
		1.6 µg	*16,7*	*3,8*	*0,9*	15, 21, 14
	DMSO		*19,0*	*7,2*		21, 25, 11
	Untreated Control		*25,7*	*0,6*		26, 25, 26
TA98	**ID-19/09966**	1600 µg	*0,0*	*0,0*	*0,0*	0 P S, 0 P S, 0 P S
		500 µg	*17,3*	*3,8*	*0,6*	19 S, 13 S, 20 S
		160 µg	*27,7*	*3,5*	*0,9*	24, 28, 31
		50 µg	*28,3*	*8,1*	*1,0*	37, 21, 27
		16 µg	*37,7*	*6,4*	*1,3*	33, 45, 35
		5 µg	*34,0*	*6,1*	*1,2*	30, 31, 41
		1.6 µg	*31,3*	*3,1*	*1,1*	28, 34, 32
	DMSO		*29,3*	*3,8*		32, 25, 31
	Untreated Control		*31,3*	*6,5*		25, 31, 38

Key to Plate Postfix Codes

P Precipitate
S Sparse lawn

Study Name: 6.46-5S-53451-ID-19/09966
Experiment: Test 2. ID-19/09966
Assay Conditions: Pre-incubation assay

Study Code: ID-19/09966
Date Plated: 08/10/2019
Date Counted: 11/10/2019

With metabolic activation

Strain	Compound	Dose level per plate	Mean revertants per plate	Standard Deviation	Ratio treated / solvent	Individual revertant colony counts
TA100	**A2A**	5 µg	*2791,7*	*79,3*	*16,5*	2709, 2799, 2867
TA102	**A2A**	25 µg	*4196,7*	*502,2*	*8,1*	3676, 4236, 4678
TA1535	**A2A**	5 µg	*176,3*	*5,5*	*13,6*	176, 171, 182
TA1537	**A2A**	5 µg	*452,7*	*142,5*	*23,8*	330, 419, 609
TA98	**A2A**	5 µg	*2899,0*	*32,7*	*98,8*	2887, 2874, 2936

Key to Positive Controls

A2A 2-AMINOANTHRACENE

Key to Plate Postfix Codes

P Precipitate
S Sparse lawn

8 Discussion

Test validation

All the criteria defined in paragraph 5.2 are met. This allows to validate the test.

Test item

Revertant analysis tables show that:

- A cytotoxic effect has been observed in the following conditions:
  - Without metabolic activation:
    - With direct incorporation (Test 1): until 50 µg/plate for strain TA98, until 160 µg/plate for strains TA100, TA102 and TA1535, until 500 µg/plate for strain TA1537.
    - This cytotoxic effect has been confirmed with the pre-incubation assay (Test 2) until 50 µg/plate for strain TA100 and TA102, until 160 µg/plate for strains TA98 and TA1535, until 500 µg/plate for strain TA1537.
  - With metabolic activation:
    - With direct incorporation (Test 1): until 160 µg/plate for strain TA100, until 500 µg/plate for strains TA98, TA102, TA1535 and TA1537.
    - This cytotoxic effect has been confirmed with the pre-incubation assay (Test 2) until 160 µg/plate for strain TA102, until 500 µg/plate for strains TA98, TA100, TA1535 and TA1537
- No concentration of the test item showed ratio R higher or equal at least to the double of the spontaneous rate of reversion for TA98, TA100 and TA102 strains and to the triple of the spontaneous rate of reversion for TA1535 and TA1537 strains, with and without metabolic activation.
- No dose response was observed, whatever the test system or conditions of the test.
- In addition, signs of precipitate have been observed on plates and / or on mix reagent until 500 µg/plate, with and without metabolic activation.

9 Conclusion

Based on the result of this study, the test item **PENTA 18 479 - REF : TX 19006, ID-19/09966 was found to be non mutagenic and non pro-mutagenic, but shows a cytotoxic activity** under the test conditions.

10 Archive

The study folder (study plan and any amendment, report, raw data) will be stored in the IDEA Lab archive room for 10 years.

According to the GLP principles for the short term studies, the test item won't be archived, but will be stored 2 months after the end of study, or until its expiry date, in the Ames study room. After this period of time, it will be destroyed or sent back to the Sponsor according to his choice.

The reference item samples will be stored 10 years, or until their expiry date, in the Ames study room, in the storage condition described in the quality form FL REAC 04.

Annex

Historical data

Positive and negative control follow-up:

HISTORIQUE MANIPULATIONS : NOMBRE DE REVERTANTS SPONTANES SANS S9 Mix / *Spontaneous revertants without S9 Mix*

TA98		TA100		TA102		TA1535		TA1537	
Nb de valeurs / *Nb of values*	541,0	Nb de valeurs / *Nb of values*	566,0	Nb de valeurs / *Nb of values*	530,0	Nb de valeurs / *Nb of values*	534,0	Nb de valeurs / *Nb of values*	541,0
Moyenne / *Mean*	25,7	Moyenne / *Mean*	155,1	Moyenne / *Mean*	373,1	Moyenne / *Mean*	17,2	Moyenne / *Mean*	16,6
Ecart-type / *Std deviation*	3,6	Ecart-type / *Std deviation*	33,7	Ecart-type / *Std deviation*	34,1	Ecart-type / *Std deviation*	4,8	Ecart-type / *Std deviation*	3,6
Nb mini / *Minimal value*	16,0	Nb mini / *Minimal value*	49,7	Nb mini / *Minimal value*	275,3	Nb mini / *Minimal value*	9,0	Nb mini / *Minimal value*	6,7
Nb maxi / *Maximal value*	42,0	Nb maxi / *Maximal value*	243,3	Nb maxi / *Maximal value*	587,7	Nb maxi / *Maximal value*	38,0	Nb maxi / *Maximal value*	30,3

HISTORIQUE MANIPULATIONS : NOMBRE DE REVERTANTS SPONTANES + S9 Mix/ *Spontaneous revertants with S9 Mix*

TA98		TA100		TA102		TA1535		TA1537	
Nb de valeurs / *Nb of values*	543,0	Nb de valeurs / *Nb of values*	553,0	Nb de valeurs / *Nb of values*	518,0	Nb de valeurs / *Nb of values*	527,0	Nb de valeurs / *Nb of values*	531,0
Moyenne / *Mean*	33,8	Moyenne / *Mean*	171,7	Moyenne / *Mean*	425,8	Moyenne / *Mean*	17,6	Moyenne / *Mean*	20,3
Ecart-type / *Std deviation*	4,5	Ecart-type / *Std deviation*	35,1	Ecart-type / *Std deviation*	38,4	Ecart-type / *Std deviation*	4,9	Ecart-type / *Std deviation*	4,8
Nb mini / *Minimal value*	23,3	Nb mini / *Minimal value*	58,3	Nb mini / *Minimal value*	310,0	Nb mini / *Minimal value*	10,0	Nb mini / *Minimal value*	10,0
Nb maxi / *Maximal value*	56,7	Nb maxi / *Maximal value*	276,3	Nb maxi / *Maximal value*	685,3	Nb maxi / *Maximal value*	38,3	Nb maxi / *Maximal value*	39,0

HISTORIQUE MANIPULATIONS : CONTROLES POSITIFS - S9 Mix/ Positive controls without S9 Mix

TA98		TA100		TA102		TA1535		TA1537	
Nb de valeurs / *Nb of values*	211,0	Nb de valeurs / *Nb of values*	221,0	Nb de valeurs / *Nb of values*	206,0	Nb de valeurs / *Nb of values*	208,0	Nb de valeurs / *Nb of values*	211,0
Moyenne / *Mean*	1203,6	Moyenne / *Mean*	2030,0	Moyenne / *Mean*	2699,8	Moyenne / *Mean*	2124,0	Moyenne / *Mean*	388,4
Ecart-type / *Std deviation*	218,0	Ecart-type / *Std deviation*	241,7	Ecart-type / *Std deviation*	542,9	Ecart-type / *Std deviation*	203,6	Ecart-type / *Std deviation*	221,8
Nb mini / *Minimal value*	746,3	Nb mini / *Minimal value*	1172,3	Nb mini / *Minimal value*	1737,7	Nb mini / *Minimal value*	1551,7	Nb mini / *Minimal value*	117,7
Nb maxi / *Maximal value*	2125,0	Nb maxi / *Maximal value*	3329,7	Nb maxi / *Maximal value*	4020,0	Nb maxi / *Maximal value*	2803,3	Nb maxi / *Maximal value*	1451,7

HISTORIQUE MANIPULATIONS : CONTROLES POSITIFS + S9 Mix/ Positive controls with S9 Mix

TA98		TA100		TA102		TA1535		TA1537	
Nb de valeurs / *Nb of values*	210,0	Nb de valeurs / *Nb of values*	212,0	Nb de valeurs / *Nb of values*	200,0	Nb de valeurs / *Nb of values*	203,0	Nb de valeurs / *Nb of values*	205,0
Moyenne / *Mean*	2683,0	Moyenne / *Mean*	3046,4	Moyenne / *Mean*	3699,5	Moyenne / *Mean*	236,8	Moyenne / *Mean*	351,6
Ecart-type / *Std deviation*	966,0	Ecart-type / *Std deviation*	1020,0	Ecart-type / *Std deviation*	769,2	Ecart-type / *Std deviation*	71,0	Ecart-type / *Std deviation*	135,6
Nb mini / *Minimal value*	931,0	Nb mini / *Minimal value*	923,3	Nb mini / *Minimal value*	1955,7	Nb mini / *Minimal value*	115,0	Nb mini / *Minimal value*	61,7
Nb maxi / *Maximal value*	4855,3	Nb maxi / *Maximal value*	5424,0	Nb maxi / *Maximal value*	6035,3	Nb maxi / *Maximal value*	433,7	Nb maxi / *Maximal value*	725,3

Mise à jour du : 11/10/2019

Annex

Culture media

- Nutrient broth for the strain culture

Beef extract	10 g
Peptone	10 g
Sodium chloride	5 g
Water for analysis	1 000 ml

- Bottom agar
    - Vogel-Bonner medium (concentrated 50 times)

Magnesium sulfate heptahydrated ($MgSO_4.7H_2O$)	10 g
Citric acid monohydrated ($C_6H_8O_7.H_2O$)	100 g
Potassium hydrogeno phosphate (K_2HPO_4)	500 g
Sodium and ammonium hydrogeno phosphate tetrahydrated ($NaNH_4HPO_4.4H_2O$)	175 g
Water for analysis	670 ml

    - Glucose solution 400 g/l
    - Completed medium

Powder agar	15 g
Water for analysis	930 ml
Vogel-Bonner medium (concentrated 50 times)	20 ml
Glucose solution 400 g/l	50 ml

- Top agar for *Salmonella typhimurium* TA

Powder agar	6 g
Sodium chloride	5 g
Water for analysis	1 000 ml
L-histidine and D-biotin mix solution at 0.5 mmol/l	10%

Reagents

- Metabolic activation S9-Mix

Reagents used for preparation of S9-Mix are prepared according to the working instruction IL REAC 04.

	Final concentration
$MgCl_2$ (0.4 M) + KCl (1.65 M)	8 mM + 33 mM
Glucose 6 Phosphate (0.2 M)	5 mM
NADP (0.1 M),	4 mM
Phosphate buffer for S9-Mix (pH 7.4 – 0.2 M),	0.1 M
S9 fraction	10%

Annex

Certificate of analysis

ID-19/09966

sederma	**ENREGISTREMENT QSHE**	Codification **EE1/33** Date : 02/04/15 Révision : c
	BULLETIN D'ANALYSES *CERTIFICATE OF ANALYSIS*	Page 1 sur 1

PRODUIT / *PRODUCT* : TX 19006

N° de LOT / *BATCH Nr* : B1

Stockage / *Storage* : **Stockage entre +15°C et +25°C**
Storage between +15°C and +25°C

Date de fabrication : 27 mai 2019
Date of manufacturing

Date de retest : 27 février 2020
Retest date

Les analyses effectuées sont garanties quand le produit est stocké dans son emballage d'origine et à la température recommandée
The performed analysis are guaranteed when product is stored in original packaging and at recommended temperature

	Résultats *Results*
Aspect *Appearance*	Poudre blanche *White powder*
Teneur en Pal KTSKS (HPLC) *Pal KTSKS content*	81.6%

Liliane IACUZZI
Responsable Assurance Qualité Produit / Product Quality Assurance Manager

Ce document est une copie informatique et de ce fait ne porte pas de signature / This certificate is a computer printout and therefore has no signature.

SEDERMA 29 rue du Chemin Vert – 78610 LE PERRAY EN YVELINES – France
Tél. : 01.34.84.10.10 – Fax : 01.34.84.11.30

Annex

S9 certificate of analysis

B 4004

TRINOVABIOCHEM

MOLTOX®
Molecular Toxicology, Inc.

POST MITOCHONDRIAL SUPERNATANT (S9)
QUALITY CONTROL & PRODUCTION CERTIFICATE

Animal Information
SPECIES: Rat
STRAIN: Sprague Dawley
SEX: Male
AGE: 5 – 6 weeks
WEIGHT: 175 – 199 g
TISSUE: Liver

Part Number Information
LOT NO.: 4052
PART NO.: 11-101
VOLUME: 5 mL
BUFFER: 0.15 M KCl
STORAGE: At or below -70°C

PREP: January 23, 2019
EXPIRY: January 23, 2021
INDUCING AGENT: Aroclor 1254, (Monsanto KL615), 500 mg/kg i.p.

REFERENCE: Maron, D & Ames, B., *Mutat Res*, **113**: 173, 1983. **For Research Purposes Only**

BIOCHEMISTRY:

- PROTEIN: 36.6 mg/ml — Assayed according to the method of Lowry et al., *JBC* **193**:265, 1951 using bovine serum albumin as the standard.

- ALKOXYRESORUFIN-0-DEALKYLASE ACTIVITIES

Activity	P450	Fold - Induction
BROD	2B1, 2B2	57.1
EROD	1A1, 1A2	98
MROD	1A1, 1A2	72.8
PROD	2B1, 2B2	37.7

Assays for ethoxyresorufin-0-deethylase (EROD), pentoxy-, benzyl- and methoxyresorufin-0-dealkylases (PROD, BROD, & MROD) were conducted using a modification of the methods of Burke, et al., *Biochem Pharm* **34**:3337, 1985. Fold-inductions were calculated as the ratio of the sample vs. uninduced specific activities (SA's). Control SA's (pmoles/min/mg protein) were 120.7, 105.5, 48.9, & 48.7 for BROD, EROD, MROD and PROD, respectively.

BIOASSAY:

- TEST FOR THE PRESENCE OF ADVENTITIOUS AGENTS
  Samples of S-9 were assayed for the presence of contaminating microorganisms by plating 1.0 ml volumes on Nutrient Agar and Minimal Glucose (Vogel-Bonner E, supplemented with 0.05 mM L-histidine and D-biotin) media. Duplicate plates were read after 40 - 48 h incubation at 35 ± 2°C. The tested samples met acceptance criteria.

- PROMUTAGEN ACTIVATION

No. His+ Revertants	
TA98	TA1535
116.8	1312

The ability of the sample to activate ethidium bromide (EtBr) and cyclophosphamide (CPA) to intermediates mutagenic to TA98 and TA1535, respectively, was determined according to Lesca, et al., *Mutation Res* **129**: 299, 1984. Data were expressed as revertants per µg EtBr or per mg CPA.

Dilutions of the sample S9, ranging from 0.2 – 10% in S9 mix, were tested for their ability to activate benzo(a)pyrene (BP) and 2-aminoanthracene (2-AA) to metabolites mutagenic to TA100. Assays were conducted as described by Maron & Ames, (*Mutat Res* **113**: 173, 1983).

	µl S9 per plate/number *his*+ revertants per plate					
Promutagen	0	1	5	10	20	50
BP (5 µg)	99	211	298	662	723	1036
2-AA (2.5 µg)	105	434	816	1511	1720	1481

Approved: Kathy Pearson 01/25/19

MOLECULAR TOXICOLOGY, INC. www.moltox.com (828) 264-9099

06 AOUT 2019

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TRINOVABIOCHEM

MOLTOX®
Molecular Toxicology, Inc.

POST MITOCHONDRIAL SUPERNATANT (S9)
QUALITY CONTROL & PRODUCTION CERTIFICATE

Animal Information
SPECIES: Rat
STRAIN: Sprague Dawley
SEX: Male
AGE: 5 – 6 weeks
WEIGHT: 175 – 199 g
TISSUE: Liver

Part Number Information
LOT NO.: 3974
PART NO.: 11-101
VOLUME: 1 & 2 mL
BUFFER: 0.15 M KCl
STORAGE: At or below -70°C

PREP: June 13, 2018
EXPIRY: June 13, 2020
INDUCING AGENT: Aroclor 1254, (Monsanto KI 615), 500 mg/kg i.p.

REFERENCE: Maron, D & Ames, B., *Mutat Res*, **113**: 173, 1983. **For Research Purposes Only**

BIOCHEMISTRY:
- PROTEIN: 35.1 mg/ml — Assayed according to the method of Lowry et al., *JBC* **193**:265, 1951 using bovine serum albumin as the standard

- ALKOXYRESORUFIN-O-DEALKYLASE ACTIVITIES

Activity	P450	Fold - Induction
BROD	2B1, 2B2	186.9
EROD	1A1, 1A2	386.9
MROD	1A1, 1A2	109.3
PROD	2B1, 2B2	116

Assays for ethoxyresorufin-O-deethylase (EROD), pentoxy-, benzyl- and methoxyresorufin-O-dealkylases (PROD, BROD, & MROD) were conducted using a modification of the methods of Burke, et al., *Biochem Pharm* **34**:3337, 1985. Fold-inductions were calculated as the ratio of the sample vs. uninduced specific activities (SA's). Control SA's (pmoles/min/mg protein) were 51.9, 30.4, 24.3, & 15.2 for BROD, EROD, MROD and PROD, respectively.

BIOASSAY:
- TEST FOR THE PRESENCE OF ADVENTITIOUS AGENTS
Samples of S-9 were assayed for the presence of contaminating microorganisms by plating 1.0 ml volumes on Nutrient Agar and Minimal Glucose (Vogel-Bonner E. supplemented with 0.05 mM L-histidine and D-biotin) media. Duplicate plates were read after 40 - 48 h incubation at 35 ± 2°C. The tested samples met acceptance criteria.

- PROMUTAGEN ACTIVATION

No. His+ Revertants	
TA98	TA1535
388	864

The ability of the sample to activate ethidium bromide (EtBr) and cyclophosphamide (CPA) to intermediates mutagenic to TA98 and TA1535, respectively, was determined according to Lesca, et al., *Mutation Res* **129**: 299, 1984. Data were expressed as revertants per µg EtBr or per mg CPA.

Dilutions of the sample S9, ranging from 0.2 – 10% in S9 mix, were tested for their ability to activate benzo(α)pyrene (BP) and 2-aminoanthracene (2-AA) to metabolites mutagenic to TA100. Assays were conducted as described by Maron & Ames, (*Mutat Res* **113**: 173, 1983).

	µl S9 per plate/number *his*⁺ revertants per plate					
Promutagen	0	1	5	10	20	50
BP (5 µg)	78	158	268	381	530	833
2-AA (2.5 µg)	94	383	1369	1781	1939	1674

Approved: 06/19/18

MOLECULAR TOXICOLOGY, INC. www.moltox.com (828) 264-9099

06 AOUT 2019

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Final Study Report

***In vitro* MAMMALIAN CELL MICRONUCLEUS TEST**
On Cultured Human Lymphocytes

Study Number
FSR-IPL 210103

Study Completion
12/04/21

Test Item
PENTA 18 479 – TX 19006

Study Director
Dr. Sophie SIMAR

Sponsor
Sederma

TEST FACILITY
INSTITUT PASTEUR DE LILLE
Genetic Toxicology Laboratory
1, rue du Professeur Calmette - BP. 245
59019 LILLE CEDEX

SPONSOR
Sederma
29, rue du Chemin Vert
BP 33
78612 LE PERRAY EN YVELINES

SPONSOR REPRESENTATIVE
Mr. Vincent VICEDO

CONTENTS

STUDY INFORMATION

Study	***In vitro* MAMMALIAN CELL MICRONUCLEUS TEST On Cultured Human Lymphocytes**
Test Item	**PENTA 18 479 – TX 19006**

SPONSOR INFORMATION

Sponsor	**Sederma**
Address	29, rue du Chemin Vert BP 33 78612 LE PERRAY EN YVELINES
Sponsor Representative	**Mr. Vincent VICEDO**

TEST FACILITY INFORMATION

Test Facility	**INSTITUT PASTEUR DE LILLE** Genetic Toxicology Laboratory 1, rue du Professeur Calmette - BP. 245 59019 LILLE CEDEX
Study Director	**Dr. Sophie SIMAR**
Quality Assurance	**Mrs. Aurélie RIZZA**
Test Facility Management	**Dr. Fabrice NESSLANY**

TEST SITE INFORMATION FOR CONTROL OF CONCENTRATIONS IN TREATMENT PREPARATIONS

Control of concentrations in treatment preparations was not scheduled

GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT AND REPORT AUTHENTICATION

I consider the data generated and reported to be valid and I declare that this report is a true and accurate record of the results obtained.

As described in the Study Plan, the Sponsor certifies that the test item provided by **Sederma** was identical to the test item described in the Final Study Plan and in the Certificate of Analysis.

Note: The Certificate of Analysis was provided by a laboratory that is compliant with ISO 9001 regulations.

The study was performed at the Toxicology Department of Institut Pasteur de Lille for genotoxicity assays.

The computer application used to acquire and derive data for this study included Microsoft® Excel. This application has been validated in the laboratory (Conformity certificate F-TOX-INF-052).

The work described in this report was performed according to the agreed study plan and with the Standard Operating Procedures of the test facility, unless otherwise stated, and was conducted in accordance with:

- OECD Principles of Good Laboratory Practice (as revised in 1997), ENV/MC/CHEM(98)17;
- GLP departmental order 10/8/2004 (Official Journal of 18th September 2004);
- EC Commission Directive 2004/10/EC of 11th February 2004 (Official Journal No. L050);

except that:

- No control of concentration in treatment preparations was performed. This should constitute a deviation to the recommendations of the Good Laboratory Practice (OECD, 1997: §6.2, Characterisation). Nevertheless, according to the BO Santé - Protection sociale - Solidarité n°2010/5 du 15 juin 2010, as the test item is a raw cosmetic material, the control of concentration in treatment preparations is not mandatory. Moreover, weighing tickets are preserved in the archives of the study, calculations for treatment preparations and successive dilutions are checked by a second operator and preparations were done with verified material,
- No data about stability of the test item in solution was provided. Nevertheless, regarding the lack of data about the stability of the test item in solution, preparations were done extemporaneously.

Overall, these GLP exceptions were considered as being minor with no impact on the outcome, the integrity and the achievement of the objectives of the study.

Submitted by:

Study Director **Dr. Sophie SIMAR**

12.4.21
Date Signature

Study	***In vitro* MAMMALIAN CELL MICRONUCLEUS TEST On Cultured Human Lymphocytes**
Test Item	**PENTA 18 479 – TX 19006**
Sponsor	**Sederma**

This report was reviewed and approved by:

Test Facility Management	**Dr. Fabrice NESSLANY** Head of Toxicology Department	12/04/2021 Date	 Signature

QUALITY ASSURANCE STATEMENT

Study — ***In vitro* MAMMALIAN CELL MICRONUCLEUS TEST On Cultured Human Lymphocytes**

Test Item — **PENTA 18 479 – TX 19006**

Sponsor — **Sederma**

Test Facility — **INSTITUT PASTEUR DE LILLE**
Genetic Toxicology Laboratory
1, rue du Professeur Calmette
B.P. 245
59019 LILLE CEDEX FRANCE

This study was audited by the Quality Assurance Unit, employing methods detailed in Standard Operating Procedures used at the Toxicology Department of Institut Pasteur de Lille:

STUDY PLAN AUDIT

Audit	Date of audit	Approved by the Study Director on	Approved by the Test Facility Management on
Study Plan	28/01/21	28/01/21	28/01/21

IN STUDY AUDITS

	Inspection/audit on critical phases of this study			Inspection/audit on critical phases associated with this type of study		
Phases audited	**Dates of Inspection/ Audit**	**Approved by the Study Director on**	**Approved by the Test Facility Management on**	**Dates of Inspection/ Au/dit**	**Approved by the Study Director on**	**Approved by the Test Facility Management on**
Preparation of treatment preparations				08/03/21	10/03/21	10/03/21
Culturing of human lymphocytes				05/01/21	06/01/21	06/01/21
Treatment				28/01/21	28/01/21	29/01/21
Sampling	12/02/21	12/02/21	12/02/21			
Reading of slides				29/10/20	30/10/20	30/10/20

In addition, process and facility-based inspections are carried out according to the annual quality assurance program.

REPORT AUDITS

Audit	Date of audit	Approved by the Study Director on	Approved by the Test Facility Management on
Draft report	29/03/21	30/03/21	30/03/21
Final report	07/04/21	07/04/21	07/04/21

CONCLUSION

Based on these audits, I declare that the data presented in this report accurately reflect the raw data collected during the current study.

Quality Assurance **Mrs. Aurélie RIZZA**

12.04.21 

Date Signature

ARCHIVE STATEMENT

The following documents will be retained in the archives of the Toxicology Laboratory of the Institut Pasteur de Lille (1, rue du Professeur Calmette, BP 245, 59019 Lille Cedex - FRANCE):

- The study plan,
- All raw data,
- Slides,
- Correspondence and documentation,
- Final report and possible amendment(s),
- The Quality Assurance Unit inspection report.

Archives are conserved at the Institut Pasteur de Lille during at least 10 years after the end of the study. After the end of this period, they will be returned to the sponsor or destroyed at sponsor's written request.

In addition, raw data not specific to the study, including but not limited to equipment calibration, are also archived at Institut Pasteur de Lille for at least 20 years.

According to OECD Guideline Number 7 (as revised in 1997) point II 6.2.6 relative to the application of the Good Laboratory Practice Principles to short-term studies, **the test item does not need to be preserved**.

After the archiving of the study, according to the Sponsor Representative's request and in accordance with the Final Study Plan, Institut Pasteur de Lille should destroy the remaining test item.

SUMMARY

Study	***In vitro* MAMMALIAN CELL MICRONUCLEUS TEST On Cultured Human Lymphocytes**
Sponsor	**Sederma**
Test Item	**PENTA 18 479 – TX 19006**
Batch number	**0001684789**

THIS STUDY WAS CARRIED OUT IN COMPLIANCE WITH GOOD LABORATORY PRACTICE REGULATIONS

Study initiation date (date Study Director signed Study Plan)	28/01/21
Experimental start date	01/02/21
Experimental completion date	29/03/21
Study completion	12/04/21

PURPOSE

The investigation of a genotoxic activity of the test item **PENTA 18 479 – TX 19006** (batch **0001684789**) sponsored by **Sederma** has been carried out in compliance with the OECD Guideline 487 (2016), using the *in vitro* mammalian cell micronucleus test on cultured human lymphocytes with the highest dose compatible with the cytotoxic activity of the test item.

All the concentrations tested in this study are expressed as **µg/mL** of **PENTA 18 479 – TX 19006** as supplied**.**

METHODS

Study carried out both without and with metabolic activation using Aroclor1254-induced S9 from rat livers.

Cell strain	Human peripheral blood lymphocytes
Culture medium	RPMI 1640
Cytochalasin B concentration	6 µg/mL
Solvent used	sterile water
Stability in solvent	unknown (dilutions were prepared extemporaneously)
Purity	>96%
Correction factor	none at the Sponsor's request
Expression of the concentrations	**µg/mL** of **PENTA 18 479 – TX 19006** as supplied
Treatment durations	
Without S9-mix	4 h + 24 h recovery period (short treatment) 24 h without recovery period (continuous treatment)
With S9-mix	4 h + 24 h recovery period, with 5% S9-mix

PRELIMINARY CYTOTOXICITY ASSAY

Number of cultures/concentration 1

Factor limiting the maximum concentration analyzed: maximum concentration according to OECD guideline, *i.e.* 2000 µg/mL

Concentrations tested in **µg/mL** of **PENTA 18 479 – TX 19006** as supplied

- Assay with 4 h treatment and 24 h recovery period without S9-mix
- Assay with 4 h treatment and 24 h recovery period with S9-mix
- Assay with 24 h treatment and no recovery period

2000 – 1000 – 500 – 250 – 125 – 62.5 – 31.25

Number of analyzed cells: 500 cells / concentration of **PENTA 18 479 – TX 19006**

Results

	Assay S9- 4h/+24h				Assay S9+ 4h/+24h				Assay S9- 24h/+0h			
Test item	**Conc. in µg/mL**	**CBPI**	**% Replication Index**	**% Cytostasis**	**Conc. in µg/mL**	**CBPI**	**% Replication Index**	**% Cytostasis**	**Conc. in µg/mL**	**CBPI**	**% Replication Index**	**% Cytostasis**
Solvent	**0**	1.7	100	0	**0**	1.7	100	0	**0**	1.8	100	0
PENTA 18 479 – TX 19006	**2000***	*	*	*	**2000***	*	*	*	**2000***	*	*	*
	1000**	1.3	43.9	56.1	**1000****	1.2	27.3	72.7	**1000***	*	*	*
	500**	1.3	49.7	50.3	**500****	1.3	43.9	56.1	**500*****	1.2	25.3	74.7
	250**	1.7	103.2	-3.2	**250****	1.7	87.2	12.8	**250*****	1.7	91.6	8.4
	125	1.6	92.1	7.9	**125**	1.6	78.1	21.9	**125**	1.7	85.2	14.8
	62.5	1.7	106.7	-6.7	**62.5**	1.7	93.9	6.1	**62.5**	1.8	103.3	-3.3
	31.25	1.7	106.4	-6.4	**31.25**	1.7	96.0	4.0	**31.25**	1.8	97.7	2.3

**: No cell growth/hemolysis; calculations could not be performed*

***: Low cell density at 1000 and 500 µg/mL and hemolysis at 1000, 500 and 250 µg/mL*

****: Low cell density at 500 and 250 µg/mL and hemolysis*

In the preliminary cytotoxicity assays using a 4-hour treatment without and with metabolic activation followed by a recovery period, a very important cytotoxicity was observed at the highest concentration tested of 2000 µg/mL with no cell harvested. The immediately lower concentration of 1000 µg/mL induced an important cytotoxicity while the concentration of 500 µg/mL induced an acceptable cytotoxic activity with 50.3 and 56.1% of cytostasis without and with metabolic activation, respectively. The concentration of 1000 µg/mL was hence retained as the top concentration to be tested in the main genotoxicity assays. A narrowed range of concentration was chosen to reach a cytotoxicity comprised between 50 and 60%.

In the preliminary cytotoxicity assays using a 24-hour continuous treatment without metabolic activation, a very important cytotoxicity was observed at the 2 highest concentrations tested of 2000 and 1000 µg/mL with no cell harvested. The immediately lower concentration of 500 µg/mL induced an important cytotoxicity while the concentration of 250 µg/mL induced no real cytotoxic activity with 8.4% of cytostasis. The concentration of 500 µg/mL was hence retained as the top concentration to be tested in the main genotoxicity assays. A narrowed range of concentration was chosen to reach a cytotoxicity comprised between 50 and 60%.

GENOTOXICITY ASSAY

Number of experimental conditions	3
Number of cultures/concentration	2
Factor limiting the maximum concentration analyzed	cytotoxicity
Concentrations tested in	**µg/mL** of **PENTA 18 479 – TX 19006** as supplied

- Assay with 4 h treatment and 24 h recovery period without S9-mix
  1000 – **750 – 500 – 375** – 250 - 125
- Assay with 4 h treatment and 24 h recovery period with S9-mix
  1000 – 750 – **500** – 375 – **250** – 125
- Assay with 24 h treatment and no recovery period
  500 – **400 – 320 – 250** – 125 – 62.5

In bold, concentrations actually assessed

Positive controls

- Assay with 4 h treatment and 24 h recovery period without S9-mix
  mitomycin C 0.15 µg/mL
- Assay with 4 h treatment and 24 h recovery period with S9-mix
  cyclophosphamide 10 µg/mL
- Assay with 24 h treatment and no recovery period
  mitomycin C 0.075 µg/mL
  griseofulvin 10 µg/mL

Number of analyzed cells	2000 binucleated cells / concentration

Results:

			BINUCLEATED CELLS		
Test item	**Conc. in µg/mL**	**% cytostasis**	**Nb of MBNC / 2000 BNC**	***p***	**ANOVA**
Assay S9- 4h/+24h					
Solvent	**0**	*0.0*	**20**	**-**	**-**
Mitomycin C	**0.15 µg/mL**	*16.9*	**41**	***<0.01***	**-**
PENTA 18 479 – TX 19006	**750**	*27.4*	**8**	***<0.05***	***N.S.***
	500	*18.1*	**12**	***N.S.***	
	375	*1.1*	**11**	***N.S.***	
Assay S9+ 4h/+24h					
Solvent	**0**	*0.0*	**9**	**-**	**-**
Cyclo-phosphamide	**10 µg/mL**	*35.8*	**45**	***<0.001***	**-**
PENTA 18 479 – TX 19006	**1000**	*34.5*	**7**	***N.S.***	***N.S.***
	500	*30.8*	**13**	***N.S.***	
	250	*-1.5*	**6**	***N.S.***	
Assay S9- 24h/+0h					
Solvent	**0**	*0.0*	**14**	**-**	**-**
Mitomycin C	**0.075 µg/mL**	*33.6*	**34**	***<0.01***	**-**
Griseofulvin	**10 µg/mL**	*24.9*	**100**	***<0.001***	
PENTA 18 479 – TX 19006	**400**	*38.2*	**9**	***N.S.***	***N.S.***
	320	*18.9*	**14**	***N.S.***	
	250	*14.2*	**10**	***N.S.***	

MBNC: MicroBiNucleated Cells; BNC: BiNucleated Cells

% cytostasis *= 100 - 100 [(CBPI $_{treated}$ -1) / (CBPI $_{control}$ -1)]*

With CBPI *(Cytokinesis-Block Proliferation Index) =*

(Nb of mononucleated cells + 2 x Nb of binucleated cells + 3 x Nb multinucleated cells) / Total Nb of cells

Chi² *test for number of micronucleates*

N.S. *=not statistically significant at the threshold of p< 0.05*

The test item **PENTA 18 479 – TX 19006** induced neither statistically nor biologically significant increases in the number of binucleated micronucleated cells, either in the 24-hour treatment without metabolic activation without recovery period or in the short-term treatments with or without metabolic activation.

The test item **PENTA 18 479 – TX 19006** is thus **not genotoxic** in this test system.

CONCLUSION:

The search for genotoxic potential of the test item PENTA 18 479 – TX 19006 (batch 0001684789) sponsored by Sederma was done by means of the *in vitro* micronucleus test in human lymphocytes treated in presence and in absence of metabolic activation, either with a short or a long-term treatment according to OECD guideline (OECD 487, 2016), using the highest concentration compatible with the cytototoxic activity of the test item.

The acceptance criteria for the assay were considered as fulfilled. The current study was valid.

Under these experimental conditions, no genotoxic activity was revealed in presence and in absence of metabolic activation with a short-term treatment or in absence of metabolic activation, with a continuous treatment.

In vitro MAMMALIAN CELL MICRONUCLEUS TEST
On Cultured Human Lymphocytes

1. PURPOSE OF THE STUDY

The *in vitro* micronucleus assay is a genotoxicity test system for the detection of chemicals or physical mutagens which induce the formation of micronuclei in the cytoplasm of interphase cells. These micronuclei may originate from acentric fragments (chromosome fragments lacking a centromere) or whole chromosomes, which are unable to migrate with the other chromosomes during the anaphase of cell division (Fenech and Morley, 1985).

The purpose of the *in vitro* micronucleus assay is to detect those agents, which modify chromosome structure and/or segregation in such a way as to lead to induction of micronuclei in interphase cells.

Analysis of the induction of micronuclei in primary cell cultures has shown that the most convenient stage to score micronuclei in this cell system is the binucleate interphase stage (Fenech *et al*., 2003; Fenech, 2000). Such cells have completed one cell division after mutagen treatment and are therefore capable of expressing micronuclei.

Treatment of cultures with the inhibitor of actin polymerisation cytochalasin B results in the "trapping" of cells at the binucleate stage where they can be easily identified (Fenech, 2000; Fenech *et al*., 2003). The measurement of the relative frequencies of binucleated to mononucleated cells within a culture also provides a simple method of measuring the proliferation rate and indirectly the cytotoxicity of a treatment (Fenech and Morley, 1985).

The current study was performed in accordance with the Final Study Plan FSP-IPL 210103 (see Appendix No. 8) and Study Plan Adherence (see § 14).

Experimental phase:

Initiated – completed : 01/02/21 – 29/03/21

2. PRINCIPLE

Cell cultures are exposed to the test item both with and without metabolic activation. After exposure to a test item, cell cultures are grown for a period sufficient to undergo one mitosis and allow chromosome damage or impairment of chromosome segregation to lead to the formation of micronuclei in interphase cells. Harvested and stained interphase cells are then analysed microscopically for the presence of micronuclei among binucleates.

3. MATERIALS

3.1. Reason for the choice of the reactive system

Human lymphocytes are used for three different reasons:

- Lymphocyte cell division can be stimulated in culture by treatment with a mitogen such as phytohaemagglutinin (PHA) and the cytokinesis-block method is easy to perform using Cytochalasin B, extract from filtrate of cultured fungus.
- Test results on cultured human lymphocytes are more pertinent in the assessment of possible human hazard.
- Human lymphocytes are recommended by OECD Guideline for assessing clastogenic activity and by IWGTP meeting (Kirsch-Volders *et al*., 2000 and 2003).

3.2. Cells

Human lymphocytes were taken from young healthy non-smoker subjects receiving no medication and who have not suffered any recent viral infection. The blood was drawn onto lithium-heparin in a sterile Venoject tube.

The cytotoxicity assay was carried out using cells taken from one woman donor 45 years old.

The genotoxicity test was carried out using cells taken from a man and a woman donor of 35 and 34 years old. The cells were pooled.

3.3. Culture medium

The culture medium was RPMI 1640 containing inactivated fetal calf serum, glutamine solution, antibiotics (penicillin, streptomycin), heparin and phytohaemagglutinin A solution.

3.4. Metabolic activation system

3.4.1. Preparation of the S9 fraction

The S9 fraction was prepared at Institut Pasteur de Lille (IPL).

This preparation is carried out using the method described by Ames *et al.* (1975) in male OFA Sprague Dawley rats induced by Aroclor 1254 (origin - Monsanto, Saint Louis, U.S.A) according to the standard operating procedures of the Institut Pasteur de Lille.

The S9 batch numbers used in this study were IPL 19-E.

For controls of sterility, proteins content and activity, see Appendix No. 7.

3.4.2. Preparation of S9 mix (Kirkland *et al.*, 1989)

The S9 mix was composed as follows. For example, for 5 mL:

- S9 fraction	2	mL
- 150 mM KCl	1	mL
- 25 mg/mL NADP	1	mL
- 180 mg/mL Glucose-6-phosphate	1	mL

All the solutions (except S9 fraction) were mixed the day of each assay, filtered through a sterilizing membrane and preserved in a refrigerated place pending use. The S9 fraction was added extemporaneously.

4. TEST ITEM INFORMATION

TEST ITEM	**PENTA 18 479 – TX 19006**
OTHER NAME / CODE	/
NAME ON IDENTIFICATION TAG	**PENTA 18 479** **Ref: TX 19006**
IPL REGISTRATION NUMBER	210111
BATCH NUMBER	**0001684789**
QUANTITY SUPPLIED	1.5 g
APPEARANCE	white powder
WATER CONTENT	2.5%
PURITY / COMPOSITION	>96%
SALT / BASE RATIO	unknown
CORRECTION FACTOR	None (at the Sponsor's request)
MOLECULAR WEIGHT	base form: 788.04 g/mol
DENSITY	unknown

STORAGE CONDITIONS**	room temperature (+15 to +28°C)
MANUFACTURING DATE	30/03/20
☐EXPIRY DATE	
☒RETEST DATE	30/03/22
ANALYSIS DATE	unknown
STABILITY UNDER STORAGE CONDITIONS	at least 2 years, up to 30/03/22 for the batch **0001684789**

***: Immediately upon receipt, the test item was registered, then stored at room temperature, in accordance with the Sponsor's instructions. The complete description of the chemical and physical properties of the test item including stability is the responsibility of the Sponsor.*

This test item, the characteristics of which are given in Appendix No. 5, was tested in accordance with the Final Study Plan FSP-IPL 210103 (see Appendix No. 8) and Study Plan Adherence (see § 14).

5. SOLUBILITY TRIALS

Prior to the implementation of the current study, trials for solubility were performed and demonstrated that **PENTA 18 479 – TX 19006** could be dissolved in sterile water at a maximum initial concentration of 20 mg/mL leading to a maximum final concentration of 2000 µg/mL when used at 10% in the culture medium.

The stability of the test item in the solvent was unknown but preparations for treatment were performed just before use.

6. pH MEASURMENT AND ASSESSMENT OF OSMOLALITY

The test item was dissolved in sterile water at a maximum initial concentration of 20 mg/mL and used at 10% in culture medium, giving a final concentration of 2000 µg/mL.

Two successive dilutions were also performed in sterile water and used at 10 % and pH and osmolality were measured.

Compound	Final concentration in µg/mL	pH	Osmolality (mOsmol/kg)	Osmolality variation (mOsmol/kg) Compared to solvent control
Culture medium (RPMI)	0	7.46	275	+27
Solvent Control (culture medium + 10% sterile water)	0	7.60	248	-
PENTA 18 479 – TX 19006	2000	7.18	253	+5
	1000	7.36	251	+3
	500	7.43	250	+2

These concentrations induced no variation in osmolarity higher than 50 mOsmol/kg when compared to the solvent control. Furthermore, the pH was superior to 6, and did not vary for more than one unit from the one of the solvent control at the 3 highest concentrations tested from 2000 to 500 µg/mL.

The concentration of 2000 µg/mL was thus retained as the highest concentration to be assessed in the cytotoxicity assay.

7. CYTOTOXICITY ASSESSMENT

7.1. Formulation of the test item

The test item was dissolved in sterile water (Fresenius, Batch 13PLP253) at a maximum initial concentration of 20 mg/mL and used at 10% in culture medium, giving a final concentration of 2000 µg/mL.

Successive dilutions were also performed in sterile water and used at 10 %

The stability of the test item in the solvent was unknown but preparations for treatments were performed just before use.

7.2. Determination of the cytotoxicity of the test item

The cytotoxic effect on human lymphocytes was determined in:

- the preliminary assay which was performed using the protocol used for the main assay (see relative paragraph). Three treatment schedules were performed as described in the relative paragraph, except that a single culture was performed instead of 2. The cytotoxicity assay was carried out with a wide range of concentrations according to a half progression.
- the determination of the cytotoxicity was also combined to the main assays.

A minimum of 500 lymphocytes was scored for determining the percentage of cells with 1, 2 or ≥ 3 nuclei.

Cytostasis/cytotoxicity can be quantified from the Cytokinesis-Block Proliferation Index (CBPI) or may be derived from the Replication Index (RI). The CBPI indicates the average number of cell cycles per cell during the period of exposure to cytoB, and may be used to calculate cell proliferation. The RI indicates the relative number of nuclei in treated cultures compared to control cultures and can be used to calculate the % cytostasis. These measurements can be used to estimate cytotoxicity by comparing values in the treated and control cultures.

$$\% \text{ Cytostasis} = 100\text{-}100\{(CBPI_T - 1) \div (CBPI_C - 1)\}$$

Where:

$$CBPI = \frac{((\text{No. mononucleated cells}) + (2 \times \text{No. binucleated cells}) + (3 \times \text{No. multinucleated cells}))}{(\text{Total number of cells})}$$

and:

T = test item treatment culture

C = solvent control culture

Thus, a CBPI of 1 (all cells are mononucleated) is equivalent to 100% cytostasis.

$$\text{Cytostasis} = 100\text{-RI}$$

$$RI = \frac{((\text{No. binucleated cells})+(2\times\text{No.multinucleated cells}))\div(\text{Total number of cells treated cultures})}{((\text{No. binucleated cells})+(2\times\text{No.multinucleated cells}))\div(\text{Total number of cells control cultures})} \times 100$$

8. TEST FOR GENOTOXIC ACTIVITY

(See Figure 1)

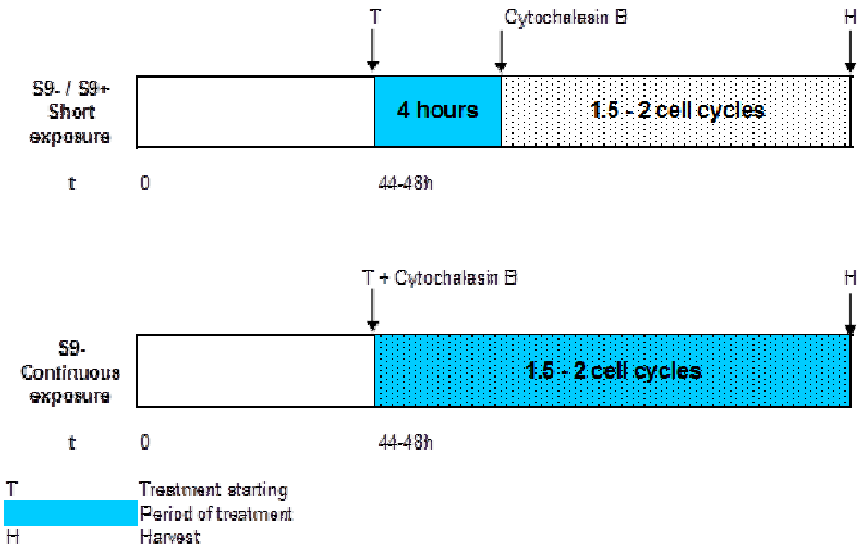
8.1. Formulation of the test item

The test item was dissolved in sterile water (Fresenius, Batch 13PLP253) at a maximum initial concentration of 20 mg/mL then half diluted and used at 10% in culture medium, giving a final concentration of 1000 µg/mL.

8.2. Treatments

The protocol described below is in compliance with the current OECD guideline (OECD 487, 2016).

Figure 1: In vitro MAMMALIAN CELL MICRONUCLEUS TEST On Cultured Human Lymphocytes – STUDY DESIGN



8.2.1. Without metabolic activation: short-term exposure

A volume of 0.5 mL of whole blood taken under the conditions mentioned above was added to an appropriate volume of complete RPMI 1640 medium with phytohaemagglutinin A in 15 mL tubes.

Approximately 44-48 hours after starting cell culture, the preparations of the test item at different concentrations chosen according to the preliminary cytotoxicity assay were added. The tubes were closed, gently stirred using a vortex and then incubated at *ca.* 37 °C in a tilted position without shaking.

After a period of 4-hour treatment, the culture medium was discarded and the cells washed twice with culture medium containing 10 % inactivated fetal calf serum. Fresh complete medium containing cytochalasin B (Sigma, batch 087M4005V) at a final concentration of 6 µg/mL was then added and the lymphocytes were incubated again at *ca.* 37 °C and harvest 1.5 – 2.0 normal cell cycles after the beginning of treatment (*i.e.* around 24 hours after the end of treatment).

For each culture, the cells were collected by centrifugation for 5 min at 1000 rpm and washed twice with the culture medium containing 2% of inactivated fetal calf serum. Thereafter, the cells were subjected to hypotonic shock for 10 min using RPMI 1640 containing 2% of inactivated fetal calf serum and sterile water: RPMI 1640 medium – sterile water (1 vol / 4 vol). A step of pre-fixation was realized by adding 2 mL of Carnoy ethanol mix: ethanol-acetic acid (3:1). After centrifugation, as much as possible of the supernatant was eliminated and the cells were fixed for 10 min using 10 mL of Carnoy-ethanol mix.

The cells were centrifuged, spread on slides, exposed to air for 24 hours and then stained with a dilution of Giemsa reagent in mineral water.

After coding the slides by a person not involved in the reading, the cells were examined under the immersion microscope and screened for micronuclei in binucleated cells.

The incidence of micronucleated cells out of 1000 binucleated cells per culture were counted (2000 binucleated cells/concentration).

8.2.2. With metabolic activation
The assay was performed as described above but S9 was added at 5 % to the culture medium during treatment, *i.e.* 2% of S9 in final concentration in culture medium.

After 44 to 48-hour induction of cell division, the test item and 5% S9-mix were added to the culture medium for 4-hour treatment with shaking. After that, the culture medium was discarded and the cells washed twice with the culture medium containing 10% of inactivated calf fetal serum. A volume of 5 mL of fresh complete culture medium containing cytochalasin B (Sigma) at a final concentration of 6 µg/mL was then added and the lymphocytes were incubated again at 37 °C for 1.5 – 2.0 normal cell cycles after the beginning of treatment (*i.e.* around 24 hours after the end of treatment).

For each culture, the cells were collected and slides were prepared as mentioned above.

8.2.3. Without metabolic activation: extended exposure
For this assay, the same protocol above mentioned without metabolic activation was used, but a continuous treatment was performed for 1.5 – 2.0 normal cell cycles (*i.e.* around 24 hours) in the presence of cytochalasin B.

At the end of the treatment period, the cells are harvested and slides are prepared.

8.2.4. Duplicate cultures
Duplicate cultures were conducted since the Guidelines of OECD (2016) have strongly recommended that for each treatment, treated and control cultures should be duplicated throughout the entire experiment.

8.3. Reference products used for the controls

Solvent controls were studied in parallel under the same conditions. Moreover, concurrently to the main assays, tests were also carried out with reference mutagenic compounds (mitomycin C in the absence of metabolic activation and cyclophosphamide in the presence of metabolic activation via S9-mix) and with a reference aneugenic compound (griseofulvin in the continuous treatment in absence of metabolic activation), in order to demonstrate the sensitivity of the cells and the effectiveness of the metabolic activation system.

The positive controls are depicted hereafter:

- Short treatment
  - Without S9-mix : mitomycin C 0.15 µg/mL (Sigma, batch SLBN5647V)
  - With S9-mix : cyclophosphamide 10 µg/mL (Sigma, batch MKCF1756)
- Continuous treatment : mitomycin C 0.075 µg/mL (Sigma, batch SLBN5647V)
  griseofulvin 10 µg/mL (Sigma, batch MKCD6584)

9. DETERMINATION OF TEST ITEM CONCENTRATION IN TREATMENT PREPARATIONS

The Sponsor chose not to perform the determination of the concentration of active substance in treatment preparations.

10. EXPRESSION OF THE RESULTS AND STATISTICAL ANALYSIS

10.1. Identification of micronuclei

The frequency of the number of micronuclei was assessed in binucleated cells. Micronuclei were identified according to the criteria of Fenech *et al.* (2000, 2003).

Micronuclei are morphologically identical to, but smaller, than nuclei. They also have the following characteristics:

- The diameter of micronuclei in human lymphocytes usually varies between $1/16^{th}$ and $1/3^{rd}$ of the mean diameter of the main nuclei, which corresponds to $1/256^{th}$, and $1/9^{th}$ of the area of one of the main nuclei in a binucleated cell, respectively.
- Micronuclei are non-refractile and they can therefore be readily distinguished from artefact such as staining particles;
- Micronuclei are not linked or connected to the main nuclei;
- Micronuclei may touch but not overlap the main nuclei and the micronuclear boundary should be distinguishable from the nuclear boundary;
- Micronuclei usually have the same staining intensity as the main nuclei but occasionally staining may be more intense.

10.2. Expression of the results

The results obtained in the different treatment cultures are presented in tabular form giving the mean number of micronuclei for 2000 binucleated cells per duplicate cell culture and the relative CBPI per concentration, the percentage of cytostasis and the replication index.

10.3. Statistical Analysis

Statistical analysis of the results obtained in the cells treated at each concentration level was performed using the χ^2 test in comparison with those in control groups. ANOVA trend test was also applied, using the statistical software Stat view®, version 5.

11. ACCEPTANCE CRITERIA FOR THE RESULTS

Historical data are presented in Appendix No. 4.

In the solvent control group, the number of micronucleated cells per binucleated cells were within or very close to the control limits of the distribution of the laboratory's historical solvent control database, and were lower than 16 per 1000 (Van Hummelen and Kirsch-Volders, 1992), with one exception (see § 14).

Regarding the results obtained with the positive reference substance, as expected, statistically significant increases in the number of micronucleated cells in the binucleated cells were observed in the presence of mitomycin C, cyclophosphamide and griseofulvin.

The responses were not compromised by cytotoxicity exceeding the limits specified.

The test item was cytotoxic, but despite the ranges of concentrations were narrowed in each treatment program, the highest concentration analysed induced cytotoxicity with a mean of percentage of cytostasis of below the interval of 55±5 %, as recommended (see also § 14).

The validity criteria for the test were considered as fulfilled. The study is thus valid.

12. INTERPRETATION OF THE RESULTS

Providing that all acceptability criteria are fulfilled, a test item is considered to be clearly positive if, in any of the experimental conditions examined

- at least one of the test concentrations exhibits a statistically significant increase (χ^2) compared with the concurrent solvent control and,
- the increase is dose-related in at least one experimental condition when evaluated with an appropriate trend test and,
- any of the results are outside the distribution of the historical solvent control data.

When all of these criteria are met, the test item is then considered able to induce chromosome breaks and/or gain or loss in this test system.

Providing that all acceptability criteria are fulfilled, a test item is considered clearly negative if, in all experimental conditions examined:

- none of the test concentrations exhibits a statistically significant increase compared with the concurrent solvent control and,
- there is no concentration-related increase when evaluated with an appropriate trend test and
- all results are inside the distribution of the historical solvent control data (95% control limits).

The test item is then considered unable to induce chromosome breaks and/or gain or loss in this test system.

13. RESULTS

13.1. Results for cytotoxic activity

The recapitulative results of preliminary cytotoxicity assays are depicted in Table 1, Appendix 1.

Individual results of preliminary cytotoxicity assays for the choice of the concentrations in the main genotoxicity assays are shown in Tables 3 to 5, Appendix 2.

Results of cytotoxicity during the definitive genotoxicity assays for the choice of the concentrations to be analyzed are presented in Tables 6 to 8, Appendix 2.

In the preliminary cytotoxicity assay using a 4-hour treatment without metabolic activation followed by a recovery period (Table 3), a very important cytotoxicity was observed at the highest concentration tested of 2000 µg/mL with no cell harvested and hemolysis. The immediately lower concentration of 1000 µg/mL induced an important cytotoxicity (56.1%) while the concentration of 500 µg/mL induced a strong but acceptable cytotoxic activity with 50.3 % of cytostasis corresponding to a replication index of 49.7%. Noteworthy, the concentrations ranging from 1000 to 250 µg/mL induced hemolysis, and a decrease in cell density at 1000 and 500 µg/mL.

The concentration of 1000 µg/mL was hence retained as the top concentration to be tested in the main genotoxicity assays. A narrowed range of concentration was chosen to reach a cytotoxicity comprised between 50 and 60%.

In the corresponding main experiment (Table 6), the highest concentration of 1000 µg/mL induced a strong cytotoxicity with so few cells on the slides that mitotic index could not be evaluated. The concentration of 750 µg/mL induced a slight cytotoxicity with a percentage of cytostasis of 27.4%, corresponding to a replication index of 72.6%, when compared to the respective solvent control. Noteworthy, a low cell density was noted at this concentration.

Under these conditions, the concentration of 750 µg/mL was retained as the maximum concentration to be analysed. Two lower concentrations (500 and 375 µg/mL) were also assessed for genotoxicity.

Interestingly, and important hemolysis was noted at 1000, 750 and 500 µg/mL and a moderate or slight hemolysis was observed at 375 µg/mL and 250 µg/mL, respectively.

In the preliminary cytotoxicity assay using a 4-hour treatment with metabolic activation followed by a recovery period (Table 4), a very important cytotoxicity was observed at the highest concentration tested of 2000 µg/mL with no cell harvested and hemolysis. The immediately lower concentration of 1000 µg/mL induced an important cytotoxicity (72.7%) while the concentration of 500 µg/mL induced an acceptable cytotoxic activity with 56.1% of cytostasis corresponding to a replication index of 43.9%. Interestingly, the concentrations ranging from 1000 to 250 µg/mL induced hemolysis, and a decrease in cell density at 1000 and 500 µg/mL.

The concentration of 1000 µg/mL was hence retained as the top concentration to be tested in the main genotoxicity assays. A narrowed range of concentration was chosen to reach a cytotoxicity comprised between 50 and 60%.

In the corresponding main experiment (Table 7), the highest concentration of 1000 µg/mL induced a moderate cytotoxicity with a percentage of cytostasis of 34.5%, corresponding to a replication index of 65.5%. Noteworthy, a low cell density was noted at this concentration.

Under these conditions, the concentration of 1000 µg/mL was retained as the maximum concentration to be analysed. Two lower concentrations (500 and 250 µg/mL) were also assessed for genotoxicity.

Interestingly, and important hemolysis was noted at 1000, 750 and 500 µg/mL and a moderate or slight hemolysis was observed at 375 µg/mL and 250 µg/mL, respectively.

In the preliminary cytotoxicity assay using a 24-hour continuous treatment without metabolic activation (Table 5), a very important cytotoxicity was observed at the 2 highest concentrations tested of 2000 and 1000 µg/mL with no cell harvested and hemolysis. The immediately lower concentration of 500 µg/mL induced an important cytotoxicity (74.7%) while the concentration of 250 µg/mL induced no real cytotoxic activity with 8.4% of cytostasis corresponding to a replication index of 91.6%. Noteworthy, the concentrations of 500 and 250 µg/mL induced hemolysis, and a decrease in cell density.

The concentration of 500 µg/mL was hence retained as the top concentration to be tested in the main genotoxicity assays. A narrowed range of concentration was chosen to reach a cytotoxicity comprised between 50 and 60%.

In the corresponding main experiment (Table 8), the highest concentration of 500 µg/mL induced a strong cytotoxicity with so few cells on the slides that mitotic index could not be evaluated. The concentration of 400 µg/mL induced a moderate cytotoxicity with a percentage of cytostasis of 38.2%, corresponding to a replication index of 61.8%, when compared to the respective solvent control. Noteworthy, a low cell density was noted at this concentration.

Under these conditions, the concentration of 400 µg/mL was retained as the maximum concentration to be analysed. Two lower concentrations (320 and 250 µg/mL) were also assessed for genotoxicity.

Interestingly, and important hemolysis was noted at 500, 400 and 320 µg/mL and a slight hemolysis was observed at 250 µg/mL.

13.2. Results for genotoxic activity

The investigation of a genotoxic activity of the test item **PENTA 18 479 – TX 19006** (batch **0001684789**) sponsored by **Sederma** has been carried out in compliance with the OECD Guideline 487 (2016), using the *in vitro* mammalian cell micronucleus test on cultured human lymphocytes with the m highest dose compatible with the cytotoxic activity of the test item.

All the concentrations tested in this study are expressed as **µg/mL** of **PENTA 18 479 – TX 19006** as supplied**.**

The summary of the test results is given in Appendix No. 1 (Table 2). The individual results for genotoxicity assays are shown in Appendix No. 2 (Tables 9 to 11). ANOVA trend tests are presented in Appendix No. 3.

In the short-term treatment without metabolic activation followed by a 24-hour recovery period (assay S9- 4h/+ 24h), the test item **PENTA 18 479 – TX 19006** induced neither statistically nor biologically significant increase in the number of micronucleated cells whatever the concentration analyzed from 375 to 750 µg/mL. Indeed, 8 to 12 micronucleated binucleated cells were observed per 2000 cells, *vs.* 20 in the solvent control (Table 9). A statistically significant decrease, rather due to the high spontaneous micronucleation (see § 14) was noted at 750 µg/mL without however any significance in terms of genotoxicity.

The test item **PENTA 18 479 – TX 19006** was thus **not genotoxic** under this condition.

In the short treatment with metabolic activation followed by a 24-hour recovery period (assay S9+ 4h/24h), the test item **PENTA 18 479 – TX 19006** induced neither statistically nor biologically significant increase in the number of micronucleated cells at all the concentrations analyzed from 250 to 1000 µg/mL. Indeed, 7 to 13 micronucleated binucleated cells were observed per 2000 cells, *vs.* 9 in the solvent control (Table 10).

The test item **PENTA 18 479 – TX 19006** was thus **not genotoxic** under this condition.

In the continuous treatment without metabolic activation without recovery period (assays S9- 24h/+0h), the test item **PENTA 18 479 – TX 19006** induced neither statistically nor biologically significant increase in the number of micronucleated cells at all the concentrations analyzed from 250 to 400 µg/mL. Indeed, 9 to 14 micronucleated binucleated cells were observed per 2000 cells, *vs.* 14 in the solvent control (Table 11).

The test item **PENTA 18 479 – TX 19006** was thus **genotoxic** under this condition.

14. STUDY PLAN ADHERENCE

14.1. Deviations to the Final Study Plan

Regarding the acceptance criteria for the results

- The test item was cytotoxic, but despite the ranges of concentrations were narrowed in each treatment program, the highest concentration analysed induced cytotoxicity with a mean of percentage of cytostasis of below the interval of 55±5 %, as recommended. Nevertheless, the top concentrations chosen demonstrated also decreases in cell density, meaning that cytotoxicity was underestimated by cytostasis calculation. The deviation was thus considered as minor with no impact on the global conclusion.
- in the solvent control group of the short-term treatment without metabolic activation, the number of micronucleated cells per binucleated cells was out of the limits of the 95% distribution of the laboratory's historical solvent control database with a value of 20 *vs*. 9.15–11.89. The value was also above the intervals of extreme values already observed (*i.e.* 6-16). The increase was mainly due to an out of range data (*i.e.* 10 for the culture A/reader 1 *vs*. 4 or 2 for the other results; Table 9). As no trend for genotoxicity was noted (a statistically significant decrease was even noted at the top concentration analysed), deviation was considered as minor without any impact on the current study. The conclusion remains the same.

Otherwise, this study was performed in accordance with the Final Study Plan FSP-IPL 210103. There were no other deviations from the Final Study Plan.

14.2. Notes

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15. CONCLUSION

The search for genotoxic potential of the test item PENTA 18 479 – TX 19006 (batch 0001684789) sponsored by Sederma was done by means of the *in vitro* micronucleus test in human lymphocytes treated in presence and in absence of metabolic activation, either with a short or a long-term treatment according to OECD guideline (OECD 487, 2016), using the highest concentration compatible with the cytototoxic activity of the test item.

The acceptance criteria for the assay were considered as fulfilled. The current study was valid.

Under these experimental conditions, no genotoxic activity was revealed in presence and in absence of metabolic activation with a short-term treatment or in absence of metabolic activation, with a continuous treatment.

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Appendix No. 1: Recapitulative results

TABLE 1

***In Vitro* MAMMALIAN CELL MICRONUCLEUS TEST**
On Cultured Human Lymphocytes

PRELIMINARY CYTOTOXICITY
SUMMARY OF THE PRELIMINARY TOXICITY ASSAYS

Sponsor: **Sederma** **Solvent: Sterile water**

Test item: **PENTA 18 479 – TX 19006**

		Assay S9- 4h/+24h				Assay S9+ 4h/+24h				Assay S9- 24h/+0h		
Test item	**Conc. in µg/mL**	**CBPI[(a)]**	**% Replication Index[(c)]**	**% Cytostasis[(b)]**	**Conc. in µg/mL**	**CBPI[(a)]**	**% Replication Index[(c)]**	**% Cytostasis[(b)]**	**Conc. in µg/mL**	**CBPI[(a)]**	**% Replication Index[(c)]**	**% Cytostasis[(b)]**
Solvent	**0**	1.7	100	0	**0**	1.7	100	0	**0**	1.8	100	0
PENTA 18 479 – TX 19006	**2000***	*	*	*	**2000***	*	*	*	**2000***	*	*	*
	1000**	1.3	43.9	56.1	**1000****	1.2	27.3	72.7	**1000***	*	*	*
	500**	1.3	49.7	50.3	**500****	1.3	43.9	56.1	**500*****	1.2	25.3	74.7
	250**	1.7	103.2	-3.2	**250****	1.7	87.2	12.8	**250*****	1.7	91.6	8.4
	125	1.6	92.1	7.9	**125**	1.6	78.1	21.9	**125**	1.7	85.2	14.8
	62.5	1.7	106.7	-6.7	**62.5**	1.7	93.9	6.1	**62.5**	1.8	103.3	-3.3
	31.25	1.7	106.4	-6.4	**31.25**	1.7	96.0	4.0	**31.25**	1.8	97.7	2.3

**: No cell growth/hemolysis; calculations could not be performed*
***: Low cell density at 1000 and 500 µg/mL and hemolysis at 1000, 500 and 250 µg/mL*
****: Low cell density at 500 and 250 µg/mL and hemolysis*

(a) *: **CBPI** = Cytokinesis-Block Proliferation Index*

CBPI = (Nb of mononucleated cells + 2 x Nb of binucleated cells + 3 x Nb multinucleated cells) / Total Nb of cells

(b): % cytostasis *= 100 - 100 [(CBPI $_{treated}$ -1) / (CBPI $_{control}$ -1)]*

(c): % Replication Index *= (RI $_{treated}$ / RI $_{control}$) x 100*

RI = (Nb of binucleated cells + 2 x Nb multinucleated cells) / Total number of cells

TABLE 2

***In Vitro* MAMMALIAN CELL MICRONUCLEUS TEST**
On Cultured Human Lymphocytes

SUMMARY OF GENOTOXICITY ASSAYS

Sponsor: **Sederma**

Test item: **PENTA 18 479 – TX 19006** **Solvent: Sterile water**

		BINUCLEATED CELLS			
		Assay S9- 4h/+24h			
Test item	**Conc. in µg/mL**	**% cytostasis**	**Number of MBNC / 2000 BNC**	***p***	**ANOVA**
Solvent	**0**	*0.0*	**20**	**-**	**-**
Mitomycin C	**0.15 µg/mL**	*16.9*	**41**	***<0.01***	**-**
PENTA 18 479 – TX 19006	**750**	*27.4*	**8**	***<0.05***	***N.S.***
	500	*18.1*	**12**	***N.S.***	
	375	*1.1*	**11**	***N.S.***	

		BINUCLEATED CELLS			
		Assay S9+ 4h/+24h			
Test item	**Conc. in µg/mL**	**% cytostasis**	**Number of MBNC / 2000 BNC**	***p***	**ANOVA**
Solvent	**0**	*0.0*	**9**	**-**	**-**
Cyclo-phosphamide	**10 µg/mL**	*35.8*	**45**	***<0.001***	**-**
PENTA 18 479 – TX 19006	**1000**	*34.5*	**7**	***N.S.***	***N.S.***
	500	*30.8*	**13**	***N.S.***	
	250	*-1.5*	**6**	***N.S.***	

		BINUCLEATED CELLS			
		Assay S9- 24h/+0h			
Test item	**Conc. in µg/mL**	**% cytostasis**	**Number of MBNC / 2000 BNC**	***p***	**ANOVA**
Solvent	**0**	*0.0*	**14**	**-**	**-**
Mitomycin C	**0.075 µg/mL**	*33.6*	**34**	***<0.01***	**-**
Griseofulvin	**10 µg/mL**	*24.9*	**100**	***<0.001***	
PENTA 18 479 – TX 19006	**400**	*38.2*	**9**	***N.S.***	***N.S.***
	320	*18.9*	**14**	***N.S.***	
	250	*14.2*	**10**	***N.S.***	

MBNC: MicroBiNucleated Cells; BNC: BiNucleated Cells

% cytostasis *= 100 - 100 [(CBPI $_{treated}$ -1) / (CBPI $_{control}$ -1)]*

With CBPI *(Cytokinesis-Block Proliferation Index) =*

(Nb of mononucleated cells + 2 x Nb of binucleated cells + 3 x Nb multinucleated cells) / Total Nb of cells

Chi^2 *test for number of micronucleates*

N.S. *=not statistically significant at the threshold of p< 0.05*

Appendix No. 2: Individual results

TABLE 3

***In Vitro* MAMMALIAN CELL MICRONUCLEUS TEST**
On Cultured Human Lymphocytes

PRELIMINARY CYTOTOXICITY ASSAY WITHOUT METABOLIC ACTIVATION
4-HOUR TREATMENT WITH 24-HOUR RECOVERY PERIOD

Sponsor: **Sederma** **Solvent: Sterile water**

Test item: **PENTA 18 479 – TX 19006** Beginning of the study: 03/02/2021

Test item	Conc. in µg/mL	Slide number M 21-	Number of cells with n nuclei for 500 cells/culture			CBPI[(a)]	% Replication Index[(c)]	% Cytostasis[(b)]
			n=1	n=2	n>2			
Solvent	**0**	***222***	199	260	41	1.7	*100*	**0**
PENTA 18 479 – TX 19006	**2000***	***223***	*	*	*	*	*	*
	1000**	***224***	361	128	11	1.3	*43.9*	**56.1**
	500**	***225***	334	162	4	1.3	*49.7*	**50.3**
	250**	***226***	188	271	41	1.7	*103.2*	**-3.2**
	125	***227***	212	261	27	1.6	*92.1*	**7.9**
	62.5	***228***	190	255	55	1.7	*106.7*	**-6.7**
	31.25	***229***	195	246	59	1.7	*106.4*	**-6.4**

**: No cell growth/hemolysis; calculations could not be performed*
***: Low cell density at 1000 and 500 µg/mL and hemolysis at 1000, 500 and 250 µg/mL*

(a) *: **CBPI** = Cytokinesis-Block Proliferation Index*

CBPI = (Nb of mononucleated cells + 2 x Nb of binucleated cells + 3 x Nb multinucleated cells) / Total Nb of cells

(b): % cytostasis *= 100 - 100 [(CBPI $_{treated}$ -1) / (CBPI $_{control}$ -1)]*

(c): % Replication Index *= (RI $_{treated}$ / RI $_{control}$) x 100*

RI = (Nb of binucleated cells + 2 x Nb multinucleated cells) / Total number of cells

TABLE 4

***In Vitro* MAMMALIAN CELL MICRONUCLEUS TEST**
On Cultured Human Lymphocytes

PRELIMINARY CYTOTOXICITY ASSAY WITH METABOLIC ACTIVATION
4-HOUR TREATMENT WITH 24-HOUR RECOVERY PERIOD

Sponsor: **Sederma** **Solvent: Sterile water**

Test item: **PENTA 18 479 – TX 19006** Beginning of the study: 03/02/2021

Test item	Conc. in µg/mL	Slide number M 21-	Number of cells with n nuclei for 500 cells/culture			CBPI[(a)]	% Replication Index[(c)]	% Cytostasis[(b)]
			n=1	n=2	n>2			
Solvent	**0**	***230***	176	274	50	1.7	*100*	**0**
PENTA 18 479 – TX 19006	**2000***	***231***	*	*	*	*	*	*
	1000**	***232***	398	102	0	1.2	*27.3*	**72.7**
	500**	***233***	345	146	9	1.3	*43.9*	**56.1**
	250**	***234***	202	270	28	1.7	*87.2*	**12.8**
	125	***235***	233	242	25	1.6	*78.1*	**21.9**
	62.5	***236***	183	283	34	1.7	*93.9*	**6.1**
	31.25	***237***	170	301	29	1.7	*96.0*	**4.0**

**: No cell growth/hemolysis; calculations could not be performed*
***: Low cell density at 1000 and 500 µg/mL and hemolysis at 1000, 500 and 250 µg/mL*

(a) *: **CBPI** = Cytokinesis-Block Proliferation Index*

CBPI = (Nb of mononucleated cells + 2 x Nb of binucleated cells + 3 x Nb multinucleated cells) / Total Nb of cells

(b): % cytostasis *= 100 - 100 [(CBPI $_{treated}$ -1) / (CBPI $_{control}$ -1)]*

(c): % Replication Index *= (RI $_{treated}$ / RI $_{control}$) x 100*

RI = (Nb of binucleated cells + 2 x Nb multinucleated cells) / Total number of cells

TABLE 5

***In Vitro* MAMMALIAN CELL MICRONUCLEUS TEST**
On Cultured Human Lymphocytes

PRELIMINARY CYTOTOXICITY ASSAY WITHOUT METABOLIC ACTIVATION
24-HOUR TREATMENT WITHOUT RECOVERY PERIOD

Sponsor: **Sederma** **Solvent: Sterile water**

Test item: **PENTA 18 479 – TX 19006** Beginning of the study: 03/02/2021

Test item	Conc. in µg/mL	Slide number M 21-	Number of cells with n nuclei for 500 cells/culture			CBPI[(a)]	% Replication Index[(c)]	% Cytostasis[(b)]
			n=1	n=2	n>2			
Solvent	**0**	***214***	157	294	49	1.8	*100*	**0**
PENTA 18 479 – TX 19006	**2000***	***215***	*	*	*	*	*	*
	1000*	***216***	*	*	*	*	*	*
	500**	***217***	402	97	1	1.2	*25.3*	**74.7**
	250**	***218***	168	305	27	1.7	*91.6*	**8.4**
	125	***219***	199	268	33	1.7	*85.2*	**14.8**
	62.50	***220***	164	267	69	1.8	*103.3*	**-3.3**
	31.25	***221***	172	273	55	1.8	*97.7*	**2.3**

**: No cell growth/Hemolysis; calculations could not be performed*
***: Low cell density at 500 and 250 µg/mL and hemolysis*

(a) *: **CBPI** = Cytokinesis-Block Proliferation Index*

CBPI = (Nb of mononucleated cells + 2 x Nb of binucleated cells + 3 x Nb multinucleated cells) / Total Nb of cells

(b): % cytostasis *= 100 - 100 [(CBPI $_{treated}$ -1) / (CBPI $_{control}$ -1)]*

(c): % Replication Index *= (RI $_{treated}$ / RI $_{control}$) x 100*

RI = (Nb of binucleated cells + 2 x Nb multinucleated cells) / Total number of cells

TABLE 6

***In Vitro* MAMMALIAN CELL MICRONUCLEUS TEST**
On Cultured Human Lymphocytes

CYTOTOXICITY ASSAY WITHOUT METABOLIC ACTIVATION
4-HOUR TREATMENT WITH 24-HOUR RECOVERY PERIOD

Sponsor: **Sederma** **Solvent: Sterile water**

Test item: **PENTA 18 479 – TX 19006** Beginning of the study: 11/02/2021

Test item	Conc. in µg/mL	Culture	Slide number M 21-	Number of cells with n nuclei for 500 cells/culture			CBPI[(a)]		Relative % Replication Index[(c)]		% Cytostasis[(b)]	
				n=1	n=2	n>2	per cult.	per conc.	per cult.	per conc.	per cult.	per conc.
Solvent	**0**	A	***285***	181	271	48	1.7	1.7	100	100	**0**	**0**
		B	***293***	171	285	44	1.7		100		**0**	
Mitomycin C	**0.15 µg/mL**	A	***286***	219	272	9	1.6	1.6	79.0	83.1	**21.0**	**16.9**
		B	***294***	189	297	14	1.7		87.1		**12.9**	
PENTA 18 479 – TX 19006	**1000**	A	***287***	*	*	*	*	*	*	*	*	*
		B	***295***	*	*	*	*		*		*	
	750**	A	***288***	240	255	5	1.5	1.5	72.2	72.6	**27.8**	**27.4**
		B	***296***	231	266	3	1.5		72.9		**27.1**	
	500	A	***289***	207	281	12	1.6	1.6	83.1	81.9	**16.9**	**18.1**
		B	***297***	210	279	11	1.6		80.7		**19.3**	
	375	A	***290***	160	314	26	1.7	1.7	99.7	98.9	**0.3**	**1.1**
		B	***298***	151	332	17	1.7		98.1		**1.9**	
	250	A	***291***	112	340	48	1.9	1.8	118.8	114.1	**-18.8**	**-14.1**
		B	***299***	139	314	47	1.8		109.4		**-9.4**	
	125	A	***292***	109	337	54	1.9	1.9	121.3	118.0	**-21.3**	**-18.0**
		B	***300***	120	332	48	1.9		114.7		**-14.7**	

Important hemolysis at 1000, 750 and 500 µg/mL moderate hemolysis at 375 µg/mL and slight hemolysis at 250 µg/mL
**: Important cytotoxicity; **: Low cell density*

(a) *: **CBPI** = Cytokinesis-Block Proliferation Index*
CBPI = (Nb of mononucleated cells + 2 x Nb of binucleated cells + 3 x Nb multinucleated cells) / Total Nb of cells
(b): % cytostasis *= 100 - 100 [(CBPI $_{treated}$ -1) / (CBPI $_{control}$ -1)]*
(c): % Replication Index *= (RI $_{treated}$ / RI $_{control}$) x 100*
RI = (Nb of binucleated cells + 2 x Nb multinucleated cells) / Total number of cells

TABLE 7

***In Vitro* MAMMALIAN CELL MICRONUCLEUS TEST**
On Cultured Human Lymphocytes

CYTOTOXICITY ASSAY WITH METABOLIC ACTIVATION
4-HOUR TREATMENT WITH 24-HOUR RECOVERY PERIOD

Sponsor: **Sederma** **Solvent: Sterile water**

Test item: **PENTA 18 479 – TX 19006** Beginning of the study: 11/02/2021

Test item	Conc. in µg/mL	Culture	Slide number M 21-	Number of cells with n nuclei for 500 cells/culture			CBPI[(a)]		Relative % Replication Index[(c)]		% Cytostasis[(b)]	
				n=1	n=2	n>2	per cult.	per conc.	per cult.	per conc.	per cult.	per conc.
Solvent	**0**	A	***301***	172	290	38	1.7	1.8	100	100	**0**	**0**
		B	***309***	142	328	30	1.8		100		**0**	
Cyclo-phosphamide	**10 µg/mL**	A	***302***	269	224	7	1.5	1.5	65.0	64.2	**35.0**	**35.8**
		B	***310***	260	234	6	1.5		63.4		**36.6**	
PENTA 18 479 – TX 19006	**1000***	A	***303***	291	208	1	1.4	1.5	57.4	65.5	**42.6**	**34.5**
		B	***311***	223	270	7	1.6		73.2		**26.8**	
	750	A	***304***	168	320	12	1.7	1.7	94.0	88.3	**6.0**	**11.7**
		B	***312***	201	276	23	1.6		83.0		**17.0**	
	500	A	***305***	237	247	16	1.6	1.5	76.2	69.2	**23.8**	**30.8**
		B	***313***	269	219	12	1.5		62.6		**37.4**	
	375	A	***306***	219	273	8	1.6	1.6	79.0	77.3	**21.0**	**22.7**
		B	***314***	214	278	8	1.6		75.8		**24.2**	
	250	A	***307***	164	303	33	1.7	1.8	100.8	101.5	**-0.8**	**-1.5**
		B	***315***	144	316	40	1.8		102.1		**-2.1**	
	125	A	***308***	169	308	23	1.7	1.7	96.7	97.6	**3.3**	**2.4**
		B	***316***	158	302	40	1.8		98.5		**1.5**	

Important hemolysis at 1000, 750 and 500 µg/mL moderate hemolysis at 375 µg/mL and slight hemolysis at 250 µg/mL
**: Low cell density*

(a) *: **CBPI** = Cytokinesis-Block Proliferation Index*
CBPI = (Nb of mononucleated cells + 2 x Nb of binucleated cells + 3 x Nb multinucleated cells) / Total Nb of cells
(b): % cytostasis *= 100 - 100 [(CBPI $_{treated}$ -1) / (CBPI $_{control}$ -1)]*
(c): % Replication Index *= (RI $_{treated}$ / RI $_{control}$) x 100*
RI = (Nb of binucleated cells + 2 x Nb multinucleated cells) / Total number of cells

TABLE 8

***In Vitro* MAMMALIAN CELL MICRONUCLEUS TEST**
On Cultured Human Lymphocytes

CYTOTOXICITY ASSAY WITHOUT METABOLIC ACTIVATION
24-HOUR TREATMENT WITHOUT RECOVERY PERIOD

Sponsor: **Sederma** **Solvent: Sterile water**

Test item: **PENTA 18 479 – TX 19006** Beginning of the study: 11/02/2021

Test item	Conc. in µg/mL	Culture	Slide number M 21-	Number of cells with n nuclei for 500 cells/culture			CBPI[(a)]		Relative % Replication Index[(c)]		% Cytostasis[(b)]	
				n=1	n=2	n>2	per cult.	per conc.	per cult.	per conc.	per cult.	per conc.
Solvent	**0**	**A**	***267***	82	339	79	2.0	1.9	100	100	**0**	**0**
		B	***276***	134	326	40	1.8		100		**0**	
Mitomycin C	**0.075 µg/mL**	**A**	***268***	213	284	3	1.6	1.6	58.4	66.4	**41.6**	**33.6**
		B	***277***	195	300	5	1.6		76.4		**23.6**	
Griseofulvin	**10 µg/mL**	**A**	***269***	179	301	20	1.7	1.7	68.6	75.1	**31.4**	**24.9**
		B	***278***	175	313	12	1.7		83.0		**17.0**	
PENTA 18 479 – TX 19006	**500**	**A**	***270***	*	*	*	*	*	*	*	*	*
		B	***279***	*	*	*	*		*		*	
	400*	**A**	***271***	231	267	2	1.5	1.6	54.5	61.8	**45.5**	**38.2**
		B	***280***	213	287	0	1.6		70.7		**29.3**	
	320	**A**	***272***	135	349	16	1.8	1.7	76.7	81.1	**23.3**	**18.9**
		B	***281***	160	329	11	1.7		86.5		**13.5**	
	250	**A**	***273***	140	345	15	1.8	1.8	75.5	85.8	**24.5**	**14.2**
		B	***282***	121	358	21	1.8		98.5		**1.5**	
	125	**A**	***274***	118	336	46	1.9	1.9	86.1	94.2	**13.9**	**5.8**
		B	***283***	122	333	45	1.8		104.2		**-4.2**	
	62.5	**A**	***275***	115	347	38	1.8	1.9	85.1	94.4	**14.9**	**5.6**
		B	***284***	105	361	34	1.9		105.7		**-5.7**	

Important hemolysis at 500, 400 and 320 µg/mL and slight hemolysis at 250 µg/mL
**: Important cytotoxicity*

(a) *: **CBPI** = Cytokinesis-Block Proliferation Index*
CBPI = (Nb of mononucleated cells + 2 x Nb of binucleated cells + 3 x Nb multinucleated cells) / Total Nb of cells
(b): % cytostasis *= 100 - 100 [(CBPI $_{treated}$ -1) / (CBPI $_{control}$ -1)]*
(c): % Replication Index *= (RI $_{treated}$ / RI $_{control}$) x 100*
RI = (Nb of binucleated cells + 2 x Nb multinucleated cells) / Total number of cells

TABLE 9

***In Vitro* MAMMALIAN CELL MICRONUCLEUS TEST**
On Cultured Human Lymphocytes

GENOTOXICITY ASSAY WITHOUT METABOLIC ACTIVATION
4-HOUR TREATMENT WITH 24-HOUR RECOVERY PERIOD

Sponsor: **Sederma** **Solvent:** **Sterile water**

Test item: **PENTA 18 479 – TX 19006** Beginning of the study : 11/02/2021

BINUCLEATED CELLS											
Test item	**Conc. in µg/mL**	**Cult.**	**Slide number M 21-**	**% cytostasis**		**Nb of cells with micronucleus / 500 cells observed**		**Number of MBNC / 1000 BNC**	**Number of MBNC / 2000 BNC**	***χ2***	***p***
				/cult.	**/conc.**	**reader 1**	**reader 2**				
Solvent	**0**	A	285	*0*	*0*	10	4	***14***	**20**	-	**-**
		B	293	*0*		4	2	***6***			
Mitomycin C	**0.15 µg/mL**	A	286	*21.0*	*16.9*	8	15	***23***	**41**	*7.341*	***<0.01***
		B	294	*12.9*		10	8	***18***			
PENTA 18 479 – TX 19006	**750**	A	288	*27.8*	*27.4*	2	1	***3***	**8**	*5.179*	***<0.05***
		B	296	*27.1*		3	2	***5***			
	500	A	289	*16.9*	*18.1*	5	2	***7***	**12**	*2.016*	***N.S.***
		B	297	*19.3*		4	1	***5***			
	375	A	290	*0.3*	*1.1*	5	2	***7***	**11**	*2.633*	***N.S.***
		B	298	*1.9*		2	2	***4***			

MBNC: MicroBiNucleated Cells; BNC: BiNucleated Cells

Chi² *test for number of micronucleates*

N.S. *=not statistically significant at the threshold of p< 0.05*

TABLE 10

***In Vitro* MAMMALIAN CELL MICRONUCLEUS TEST**
On Cultured Human Lymphocytes

GENOTOXICITY ASSAY WITH METABOLIC ACTIVATION
4-HOUR TREATMENT WITH 24-HOUR RECOVERY PERIOD

Sponsor: **Sederma** **Solvent:** **Sterile water**

Test item: **PENTA 18 479 – TX 19006** Beginning of the study : 11/02/2021

BINUCLEATED CELLS											
Test item	**Conc. in µg/mL**	**Cult.**	**Slide number M 21-**	**% cytostasis**		**Nb of cells with micronucleus / 500 cells observed**		**Number of MBNC / 1000 BNC**	**Number of MBNC / 2000 BNC**	***χ2***	***p***
				/cult.	**/conc.**	**reader 1**	**reader 2**				
Solvent	**0**	A	301	*0*	*0*	3	3	***6***	**9**	-	**-**
		B	309	*0*		2	1	***3***			
Cyclo-phosphamide	**10 µg/mL**	A	302	*35.0*	*35.8*	9	11	***20***	**45**	*24.328*	***<0.001***
		B	310	*36.6*		16	9	***25***			
PENTA 18 479 – TX 19006	**1000**	A	303	*42.6*	*34.5*	1	3	***4***	**7**	*0.251*	***N.S.***
		B	311	*26.8*		2	1	***3***			
	500	A	305	*23.8*	*30.8*	1	2	***3***	**13**	*0.731*	***N.S.***
		B	313	*37.4*		7	3	***10***			
	250	A	307	*-0.8*	*-1.5*	3	1	***4***	**6**	*0.602*	***N.S.***
		B	315	*-2.1*		2	0	***2***			

MBNC: MicroBiNucleated Cells; BNC: BiNucleated Cells

Chi² *test for number of micronucleates*

N.S. *=not statistically significant at the threshold of p< 0.05*

TABLE 11

***In Vitro* MAMMALIAN CELL MICRONUCLEUS TEST**
On Cultured Human Lymphocytes

GENOTOXICITY ASSAY WITHOUT METABOLIC ACTIVATION
24-HOUR TREATMENT WITHOUT RECOVERY PERIOD

Sponsor: **Sederma** **Solvent:** **Sterile water**

Test item: **PENTA 18 479 – TX 19006** Beginning of the study : 11/02/2021

BINUCLEATED CELLS											
Test item	**Conc. in µg/mL**	**Cult.**	**Slide number M 21-**	**% cytostasis**		**Nb of cells with micronucleus / 500 cells observed**		**Number of MBNC / 1000 BNC**	**Number of MBNC / 2000 BNC**	***χ2***	***p***
				/cult.	**/conc.**	**reader 1**	**reader 2**				
Solvent	**0**	A	267	*0*	*0*	6	1	***7***	**14**	-	**-**
		B	276	*0*		4	3	***7***			
Mitomycin C	**0.075 µg/mL**	A	268	*41.6*	*33.6*	9	9	***18***	**34**	*8.435*	***<0.01***
		B	277	*23.6*		3	13	***16***			
Griseofulvin	**10 µg/mL**	A	269	*31.4*	*24.9*	31	21	***52***	**100**	*66.780*	***<0.001***
		B	278	*17.0*		29	19	***48***			
PENTA 18 479 – TX 19006	**400**	A	271	*45.5*	*38.2*	3	3	***6***	**9**	*1.093*	***N.S.***
		B	280	*29.3*		1	2	***3***			
	320	A	272	*23.3*	*18.9*	1	5	***6***	**14**	*0.000*	***N.S.***
		B	281	*13.5*		6	2	***8***			
	250	A	273	*24.5*	*14.2*	3	0	***3***	**10**	*0.671*	***N.S.***
		B	282	*1.5*		3	4	***7***			

MBNC: MicroBiNucleated Cells; BNC: BiNucleated Cells

***Chi*^{2}** *test for number of micronucleates*

N.S. *=not statistically significant at the threshold of p< 0.05*

Appendix No. 3: ANOVA statistical analysis

GENOTOXICITY ASSAY WITHOUT METABOLIC ACTIVATION 4-HOUR TREATMENT WITH 24-HOUR RECOVERY PERIOD

ANOVA statistical analysis on MicroBiNucleated Cells:

Tableau ANOVA pour Nb. MBNC S9- 4h
Exclusion de lignes : Data S9- 4h.svd

	ddl	Somme des carrés	Carré moyen	Valeur de F	Valeur de p	Lambda	Puissance
Concentrations	3	39.375	13.125	1.296	.3907	3.889	.165
Résidu	4	40.500	10.125				

GENOTOXICITY ASSAY WITH METABOLIC ACTIVATION 4-HOUR TREATMENT WITH 24-HOUR RECOVERY PERIOD

ANOVA statistical analysis on MicroBiNucleated Cells:

Tableau ANOVA pour Nb. MBNC S9+
Exclusion de lignes : Data S9+.svd

	ddl	Somme des carrés	Carré moyen	Valeur de F	Valeur de p	Lambda	Puissance
Concentrations	3	14.375	4.792	.608	.6439	1.825	.102
Résidu	4	31.500	7.875				

GENOTOXICITY ASSAY WITHOUT METABOLIC ACTIVATION 24-HOUR TREATMENT WITHOUT RECOVERY PERIOD

ANOVA statistical analysis on MicroBiNucleated Cells:

Tableau ANOVA pour Nb. MBNC S9- 24h
Exclusion de lignes : Data S9- 24h.svd

	ddl	Somme des carrés	Carré moyen	Valeur de F	Valeur de p	Lambda	Puissance
Concentrations	3	10.375	3.458	.954	.4951	2.862	.133
Résidu	4	14.500	3.625				

Appendix No. 4: Historical data

MICRONUCLEUS HUMAN LYMPHOCYTES
04/19 - 10/20
20 Studies

	Number of micronuclei for 2000 BINUCLEATED cells	
	NEGATIVE CONTROL	**POSITIVE CONTROL**
	m ± *sd* [Confidence interval 95%]* *(Extreme deviations)*	m ± *sd* [Confidence interval 95%]* *(Extreme deviations)*
SHORT TREATMENT S9-		**Mitomycin C 0.15 µg/mL**
	10.52 ± *3.20*	**55.08** ± *10.51*
	9.15 - 11.89	50.35 - 59.80
	6.00 - 16.00	*39.00 - 72.00*
SHORT TREATMENT S9+		**Cyclophosphamide 10 µg/mL**
	10.59 ± *5.43*	**48.50** ± *10.93*
	8.32 - 12.86	43.71 53.29
	5.00 - 26.00	*33.00 - 71.00*
CONTINUOUS TREATMENT S9-		**Mitomycin C 0.075 µg/mL**
	11.89 ± *3.69*	**56.07** ± *13.86*
	10.38 - 13.40	50.14 - 62.00
	7.00 - 22.00	*36.00 - 98.00*
		Griseofulvin 10 µg/mL
		54.00 ± *15.13*
		47.53 - 60.47
		36.00 - 90.00

m: mean; sd: standard deviation
*=m ±(1.96xsd)/√n

Appendix No. 5: Analytical certificate

BULLETIN D'ANALYSES
CERTIFICATE OF ANALYSIS

08-06-E002 a

Date : 29/04/2020

Manufacturing site is certified according to ISO 9001, ISO 14001, ISO 45001 and EFfCi

PRODUIT / *PRODUCT* : TX 19006

N° de LOT / *BATCH Nr* : 1684789

Stockage / *Storage* :

Date de fabrication : 30/03/2020
Date of manufacturing

Date de retest : 30/03/2022
Retest date

Les analyses effectuées sont garanties quand le produit est stocké dans son emballage d'origine et à la température recommandée.
The performed analysis are guaranteed when product is stored in original packaging and at recommended temperature.

	Résultats *Results*
Poids moléculaire	788.04 g/mol
Aspect *Appearance*	Poudre fine banche *White fine powder*
Teneur en eau (Karl Fischer) (%) *Water content*	2.5
Teneur en peptide base (%) *Peptide base content*	85.2
Pureté DEDL (%) *DEDL purity*	99.2
Pureté 210 nm (%) *210nm purity*	96.1

Liliane IACUZZI

Assurance Qualité / Quality Assurance

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sederma
29 rue du Chemin Vert – 78610 LE PERRAY EN YVELINES – France
Tél. : 01.34.84.10.10 – Fax : 01.34.84.11.30

1 sur 1

Appendix No. 6: Slide coding

TEST ITEM: **PENTA 18 479 – TX 19006**
SPONSOR: **Sederma**

Assay S9- 4h/+24h				Assay S9+ 4h/+24h				Assay S9- 24h/+0h			
Conc. in µg/mL	Culture	M 21-	Code number **11**	Conc. in µg/mL	Culture	M 21-	Code number **12**	Conc. in µg/mL	Culture	M 21-	Code number **13**
0	*A*	285	AP/BI	**0**	*A*	301	AD/BN	**0**	*A*	267	AC/BQ
	B	293	AF/BO		*B*	309	AG/BK		*B*	276	AO/BG
Mito C 0.15 µg/mL	*A*	286	AL/BT	**CPA 10 µg/mL**	*A*	302	AP/BS	**Mito C 0.075 µg/mL**	*A*	268	AH/BX
	B	294	AC/BJ		*B*	310	AA/BF		*B*	277	AP/BT
								Griseo 10 µg/mL	*A*	269	AL/BR
									B	278	AB/BJ
750	*A*	288	AH/BS	**1000**	*A*	303	AO/BC	**400**	*A*	271	AD/BA
	B	296	AQ/BG		*B*	311	AM/BE		*B*	280	AU/BI
500	*A*	289	AD/BB	**500**	*A*	305	AH/BI	**320**	*A*	272	AV/BS
	B	297	AK/BM		*B*	313	AJ/BR		*B*	281	AF/BM
375	*A*	290	AN/BA	**250**	*A*	307	AT/BB	**250**	*A*	273	AW/BK
	B	298	AE/BR		*B*	315	AL/BQ		*B*	282	AN/BE

Mito C: Mitomycin C; CPA: Cyclophosphamide; Griseo: Griseofulvin

Appendix No. 7: Control of S9

BIOASSAY - S9 FRACTION

Batch number: **IPL 19- E**

Prepared at Institut Pasteur de Lille (IPL) on: **19/11/2019**

Animal species:	**Rat**	Strain:	**OFA Sprague Dawley**
Supplier:	**Charles River France**	Sex:	**Male**
Tissue:	**Liver**		
Inducting agent:	**Aroclor 1254**	Number of dose(s):	**1**
Route:	**intraperitoneal**	Dose(s):	**500 mg/kg**
Delay:	**5 days**		
Shelf life:	**18/11/2021**		
Storage:	**in liquid nitrogen**		

Sterility test **0** colonie(s) of contaminating microflora observed per 50 µL of S9 after a *ca.* 48-72 h-incubation on Vogel-Bonner medium at *ca.* 37°C

Controled at Institut Pasteur de Lille (IPL) on: ***26/11/2019***

Proteins

value in mg/mL[1]	*standard deviation*	***Minimum limit***
32.0	*1.3*	***20***

[1] *Mean of 3 determinations*

Biological activity: Assessment by the Ames test

Strain:	TA100	Volume of S9-mix:	0.5 mL/plate
Incubation:	48-72 h at *ca.* 37°C	Volume of S9 in S9-mix:	100 µL/mL

Reference compounds	Doses in µg/plate	Revertants/plate[2]	*Standard deviation*	Induced revertants/plate	***Minimum limit***
2 aminoanthracene[1]	0	64	*8.6*		
	0.5	859	*24.4*	795	***200***
	2	2293	*517.3*	2230	***1000***
benzo[a]pyrene[1]	0	64	*8.6*		
	1	1115	*260.1*	1051	***300***
	2	880	*127.0*	817	***800***
2-acetamidofluorene[1]	0	64	*8.6*		
	20	329	*61.3*	266	***150***
	40	537	*113.2*	474	***400***

[1] *Solvent: DMSO* [2] *Mean of 6 plates for negative controls. Mean of 3 plates for positive controls.*

Controled at Institut Pasteur de Lille (IPL) on: ***08/01/2020***

Biological activity: Assessment by the *in vitro* micronucleus test

Cell type:	L5178Y mouse lymphoma cells
Treatment time:	4h
Recovery time:	+ 24h
Final concentration of S9:	2%

Reference compound	Concentration in µg/mL	Number of **micronucleated** cells on 2000 mononucleated cells	Number of **induced micronucleated** cells on 2000 mononucleated cells	***Minimum limit***
Negative control: Aqueous solvent	0	4	-	-
Cyclophosphamide	10	241	237	***100***

Controled at Institut Pasteur de Lille (IPL) on: ***08/01/2020***

ACCEPTED

Appendix No. 8: Final Study Plan FSP-IPL 210103

Institut Pasteur de Lille
Vivre mieux, plus longtemps

Docteur Fabrice NESSLANY
Laboratoire de Toxicologie
Tél. : 33 (0)3 20 87 72 72
e-mail : fabrice.nesslany@pasteur-lille.fr

Final Study Plan

Confidential

***In Vitro* MAMMALIAN CELL MICRONUCLEUS TEST**
On Cultured Human Lymphocytes

Study Number
FSP-IPL 210103

Test Item
PENTA 18 479 – TX 19006

Study Director
Dr. Sophie SIMAR

Sponsor
Sederma

TEST FACILITY
INSTITUT PASTEUR DE LILLE
Genetic Toxicology Laboratory
1, rue du Professeur Calmette - BP. 245
59019 LILLE CEDEX

SPONSOR
Sederma
29, rue du Chemin Vert
BP 33
78612 LE PERRAY EN YVELINES

SPONSOR REPRESENTATIVE
Mr. Vincent VICEDO

FSP-IPL 210103 / PENTA 18 479 – TX 19006 / Sederma

CONTENTS

FSP-IPL 210103 / PENTA 18 479 – TX 19006 / Sederma

STUDY INFORMATION

Study	**In Vitro MAMMALIAN CELL MICRONUCLEUS TEST On Cultured Human Lymphocytes**
Test Item	**PENTA 18 479 – TX 19006**
Sponsor	**Sederma**
Address	29, rue du Chemin Vert BP 33 78612 LE PERRAY EN YVELINES
Sponsor Representative	**Mr. Vincent VICEDO** Email: vincent.vicedo@sederma.fr
Test Facility	**INSTITUT PASTEUR DE LILLE** Genetic Toxicology Laboratory 1, rue du Professeur Calmette - BP. 245 59019 LILLE CEDEX
Study Director[1]	**Dr. Sophie SIMAR** **INSTITUT PASTEUR DE LILLE** Genetic Toxicology Laboratory 1, rue du Professeur Calmette - BP. 245 59019 LILLE CEDEX TEL: 33 (0)3 20 87 77 25 Email: sophie.simar@pasteur-lille.fr
Quality Assurance[2]	**Mrs. Aurélie RIZZA** TEL: 33 (0)3 20 87 73 81 Email: aurelie.rizza@pasteur-lille.fr
Test Facility Management[2]	**Dr. Fabrice NESSLANY** Head of Toxicology Department TEL: 33 (0)3 20 87 72 72 Email: fabrice.nesslany@pasteur-lille.fr

[1] Any change will form the subject of an amendment of the study plan
[2] Any change will be indicated in the final report

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STUDY PLAN APPROVALS

ON BEHALF OF INSTITUT PASTEUR DE LILLE

		Dates	Signatures
Study Director	**Dr. Sophie SIMAR**	28.1.21	
Quality Assurance	**Mrs. Aurélie RIZZA**	28.01.21	
Test Facility Management	**Dr. Fabrice NESSLANY**	28/01/2021	

The signature shall constitute approval of the entire contents of this study plan

FSP-IPL 210103 / PENTA 18 479 – TX 19006 / Sederma

ON BEHALF OF Sederma

Sponsor Representative **Mr. Vincent VICEDO**

Vicedo, Vincent

Digitally signed by Vicedo, Vincent
Date: 2021.02.01 14:12:02 +01'00'

Date **Signature**

The signature shall constitute approval of the entire contents of this study plan

FSP-IPL 210103 / PENTA 18 479 – TX 19006 / Sederma

PROPOSED STUDY DATES

EXPERIMENTAL STARTING DATE	:	**week 5, 2021**
IF AGREEMENT REACHED BEFORE	:	**week 4, 2021**
EXPERIMENTAL COMPLETION DATE	:	**week 9, 2021**
DRAFT REPORT AVAILABLE	:	**week 12, 2021**

The starting and the completion dates of the study will be indicated in the final report.

Following completion of the study, an audited Draft Study Report will be issued. Sponsor comments are requested within **4 weeks** of receipt. If no comments are received by this time, the Sponsor will periodically be requested to provide comments and/or approve the report for finalization.

If no Sponsor comments are received within **4 months** of issue of the Audited Draft Report, it will be assumed that there are none, and the report will be archived (see "Report" section).

A Final Report will be issued to the Sponsor within 4 weeks of approval of the Audited Draft Study Report and the request to finalize, unless the entire documentation (*e.g.* analytical certificate, Final Phase Report if any…) is not provided in a timely manner.

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In Vitro MAMMALIAN CELL MICRONUCLEUS TEST
On Cultured Human Lymphocytes

A. GENERAL PROTOCOL

1. PURPOSE OF THE STUDY

The *in vitro* micronucleus assay is a genotoxicity test system for the detection of chemicals or physical mutagens which induce the formation of micronuclei in the cytoplasm of interphase cells. These micronuclei may originate from acentric fragments (chromosome fragments lacking a centromere) or whole chromosomes, which are unable to migrate with the other chromosomes during the anaphase of cell division (Fenech and Morley, 1985).

The purpose of the *in vitro* micronucleus assay is to detect those agents, which modify chromosome structure and/or segregation in such a way as to lead to induction of micronuclei in interphase cells.

Analysis of the induction of micronuclei in primary cell cultures has shown that the most convenient stage to score micronuclei in this cell system is the binucleate interphase stage (Fenech *et al.*, 2003; Fenech, 2000). Such cells have completed one cell division after mutagen treatment and are therefore capable of expressing micronuclei.

Treatment of cultures with the inhibitor of actin polymerisation cytochalasin B results in the "trapping" of cells at the binucleate stage where they can be easily identified (Fenech, 2000; Fenech *et al.*, 2003). The measurement of the relative frequencies of binucleated to mononucleated cells within a culture also provides a simple method of measuring the proliferation rate and indirectly the cytotoxicity of a treatment (Fenech and Morley, 1985).

2. PRINCIPLE

Cell cultures are exposed to the test item both with and without metabolic activation. After exposure to a test item, cell cultures are grown for a period sufficient to undergo one mitosis and allow chromosome damage or impairment of chromosome segregation to lead to the formation of micronuclei in interphase cells. Harvested and stained interphase cells are then analysed microscopically for the presence of micronuclei among binucleates.

3. MATERIALS AND METHODS

3.1 Reason for the choice of the reactive system

Human lymphocytes are used for three different reasons:

- Lymphocyte cell division can be stimulated in culture by treatment with a mitogen such as phytohaemagglutinin (PHA) and the cytokinesis-block method is easy to perform using Cytochalasin B, extract from filtrate of cultured fungus.
- Test results on Cultured Human Lymphocytes are more pertinent in the assessment of possible human hazard.
- Human lymphocytes are recommended by OECD Guideline for assessing clastogenic activity and by IWGTP meeting (Kirsch-Volders *et al.*, 2000 and 2003).

3.2 Cells

Human lymphocytes will be taken from young (around 18-35 years old; exception is allowed for the preliminary toxicity assay) healthy non-smoker subjects receiving no medication or radiation and who have not suffered any recent viral infection. The blood will be drawn onto lithium-heparin in a sterile Venoject tube. The toxicity assay will be carried out using cells taken from at least one donor. The genotoxicity test will be carried out using cells taken from either a man and a woman donor and/or at least two different donors. The number and the sex of the donors will be specified in the Final Study Report. The cells from more than 1 donor can be pooled; in this case, it will be specified in the Final Study Report.

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3.3 Culture medium

The culture medium is RPMI 1640 containing inactivated fetal calf serum, glutamine solution, antibiotics (penicillin, streptomycin), heparin and phytohaemagglutinin A solution.

3.4 Metabolic activation system

3.4.1 Preparation of the S9 fraction

The S9 fraction is prepared at Institut Pasteur de Lille (IPL).

This preparation is carried out using the method described by Ames *et al.* (1975) in male rat OFA Sprague Dawley induced by Aroclor 1254 (origin - Monsanto, Saint Louis, U.S.A) according to the standard operating procedures of the Institut Pasteur de Lille.

Sterility, proteins content and activity are tested on each batch of S9.

Alternatively, commercial S9 fraction may be purchased from external supplier.

In any case, a control sheet in which are presented data for sterility, protein content, activity on reference mutagenic products, will be included in the final report.

3.4.2 Preparation of S9 mix (Kirkland *et al.*, 1989)

The S9 mix is composed as follows. For example, for 5 mL:

- S9	2	mL
- 150 mM KCl	1	mL
- 25 mg/mL NADP	1	mL
- 180 mg/mL Glucose-6-phosphate	1	mL

All the solutions (except S9) will be mixed the day of each assay, filtered through a sterilizing membrane and preserved in a refrigerated place pending use. The S9 fraction will be added extemporaneously.

3.5 Choice of concentrations

For a freely soluble test item, the maximum concentration retained should be 2 mg/mL, 10 mM or 2 µL/mL whichever is the lowest (OECD, 2016).

When the test item is not of defined composition *e.g.* substance of unknown or variable composition, complex reaction products or biological materials (*i.e.* UVCBs), environmental extracts etc., the top concentration may need to be higher (*e.g.* 5 mg/ml), in the absence of sufficient cytotoxicity, to increase the concentration of each of the components.

However, the top concentration should be limited by either toxicity or solubility in culture medium:

- After the cytostasis has been determined, at least 3 concentrations are selected for cell analysis. When a test item presents toxicity, the maximum concentration should produce 55 ± 5% cytotoxicity compared with the control (*i.e.* the percentage of cytostasis should be ranged from 50 to 60% compared with the control) or is the concentration which, when added to the culture medium, produces slight precipitation. The intermediate concentration is chosen with a little toxic effect and the low concentration should present no significant toxic effect.
- For a relatively insoluble test item, and in the absence of cytotoxicity, it should be tested up to the limit of solubility or to a concentration, which provoke slight precipitates when added to the culture medium.

Furthermore, the highest concentration should present a variation in osmolality lower than 50 mOsm/kg when compared with the solvent control in order to avoid effects related to the variations of osmolality. Moreover the pH should vary no more than 1 unit when compared with the solvent control and be superior to 6 (Marzin et al., 1986; Nestmann et al., 1991; Scott et al., 1991) without reaching high pH, as it is known that high pH also induce DNA damage (Brusick, 1986).

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3.6 Determination of the cytotoxicity of the test item

The cytotoxic effect on human lymphocytes is determined in:

- a preliminary assay which is performed using the protocol used for the main assay (see relative paragraph). Three treatment schedules are performed as described in the relative paragraph, except that it is not performed in duplicate cultures. Moreover, no positive control is used. The cytotoxicity assay is carried out with a wide range of concentrations generally according to a half progression. The concentrations should be separated by no more than a factor of $\sqrt{10}$.
- a determination of the cytotoxicity is also combined to the main assay.

A minimum of 500 lymphocytes will be scored for determining the percentage of cells with 1, 2 or ≥ 3 nuclei.

Cytostasis/cytotoxicity can be quantified from the Cytokinesis-Block Proliferation Index (CBPI) or may be derived from the Replication Index (RI). The CBPI indicates the average number of cell cycles per cell during the period of exposure to cytoB, and may be used to calculate cell proliferation. The RI indicates the relative number of nuclei in treated cultures compared to control cultures and can be used to calculate the % cytostasis. These measurements can be used to estimate cytotoxicity by comparing values in the treated and control cultures.

$$\% \text{ Cytostasis} = 100\text{-}100\{(CBPI_T - 1) \div (CBPI_C - 1)\}$$

Where:

$$CBPI = \frac{((\text{No. mononucleated cells}) + (2 \times \text{No. binucleated cells}) + (3 \times \text{No. multinucleated cells}))}{(\text{Total number of cells})}$$

and:

T = test item treatment culture

C = solvent control culture

Thus, a CBPI of 1 (all cells are mononucleated) is equivalent to 100% cytostasis.

$$\text{Cytostasis} = 100\text{-RI}$$

$$RI = \frac{((\text{No. binucleated cells})+(2\times\text{No.multinucleated cells}))\div(\text{Total number of cells treated cultures})}{((\text{No. binucleated cells})+(2\times\text{No.multinucleated cells}))\div(\text{Total number of cells control cultures})} \times 100$$

After determining this index, at least 3 concentrations should be analysable in the genotoxicity study.

The concentrations should be separated by no more than a factor of $\sqrt{10}$. For test substances that exhibit a steep concentration-response curve, it may be necessary to more closely space the test substance concentrations so that cultures in the moderate and low toxicity ranges also will be scored.

3.7 Methods

The protocol described below is in compliance with the current OECD guideline (OECD 487, 2016) (See Figure 1).

3.7.1 Without metabolic activation: short exposure

A volume of 0.5 mL of whole blood taken under the conditions mentioned above is added to an appropriate volume of complete RPMI 1640 medium with phytohaemagglutinin A in 15 mL tubes.

Approximately 44-48 hours after starting cell culture, the preparations of the test item at different concentrations chosen according to the preliminary cytotoxicity assay are added. The tubes are

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closed, gently stirred using a vortex and then incubated at *ca.* 37 °C in a tilted position without shaking.

Concurrently to the different concentrations of the test item a solvent control and a positive control, mitomycin C at 0.15 µg/mL, in the absence of S9 because it does not need metabolic activation to express its clastogenic effect, will be studied under the same conditions.

After a period of 4-hour treatment, the culture medium is discarded and the cells washed twice with culture medium containing 10 % inactivated fetal calf serum. Fresh complete medium containing cytochalasin B (Sigma) at a final concentration of 6 µg/mL is then added and the lymphocytes are incubated again at *ca.* 37 °C and harvest 1.5 – 2.0 normal cell cycles after the beginning of treatment (*i.e.* around 24 hours after the end of treatment).

For each culture, the cells are collected by centrifugation for 5 min at 1000 rpm and washed twice with the culture medium containing 2% of inactivated fetal calf serum. Thereafter, the cells are subjected to hypotonic shock for 10 min using RPMI 1640 containing 2% of inactivated fetal calf serum and sterile water: RPMI 1640 medium – sterile water (1 vol / 4 vol). A step of pre-fixation is realized by adding 2 mL of a Carnoy-ethanol mix: ethanol-acetic acid (3:1). After centrifugation, as much as possible of the supernatant is eliminated and the cells are fixed for 10 min using 10 mL of a Carnoy-ethanol mix.

The cells are centrifuged, spread on slides, exposed to air for 24 hours and then stained with a dilution of Giemsa reagent in mineral water.

After coding the slides by a person not involved in the reading, the cells are examined under the immersion microscope and screened for micronuclei in binucleated cells. Two slides per culture are coded, and the incidence of micronucleated cells out of 1000 binucleated cells per culture are counted (2000 binucleated cells/concentration).

This number of 1000 binucleated cells may not be reached in case of strong toxicity.

The micronuclei among mononucleates may be observed separately, in accordance with the Sponsor, in case(s) of biologically and/or statistically significant increases in the number of micronucleated binucleates for mechanistic purpose.

3.7.2 With metabolic activation

The assay is performed as described above but S9 is added at 5 % to the culture medium during treatment, *i.e.* 2% of S9 in final concentration in culture medium.

After 44 to 48-hour induction of cell division, the preparations of the test item at different concentrations chosen according to the preliminary cytotoxicity assay and 5% S9-mix are added to the culture medium for 4-hour treatment with shaking. After that, the culture medium is discarded and the cells washed twice with the culture medium containing 10% of inactivated calf fetal serum. A volume of 5 mL of fresh complete culture medium containing cytochalasin B (Sigma) at a final concentration of 6 µg/mL is then added and the lymphocytes are incubated again at *ca.* 37 °C for 1.5 – 2.0 normal cell cycles after the beginning of treatment (*i.e.* around 24 hours after the end of treatment).

Concurrently to the different concentrations of the test item, a solvent control and a positive control, cyclophosphamide at 10 µg/mL, in the presence of S9 because it needs metabolic activation to express its clastogenic effect, will be studied under the same conditions.

For each culture, the cells are collected and slides are prepared as mentioned above.

3.7.3 Without metabolic activation: extended exposure

For this assay, the same protocol above mentioned without metabolic activation is used, but a continuous treatment is performed for 1.5 – 2.0 normal cell cycles in the presence of cytochalasin B *(i.e.* around 24 hours).

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Concurrently to the different concentrations of the test item, a solvent control and 2 positive controls (both a clastogenic test compound, mitomycin C at 0.075 µg/mL and an aneugenic test compound, griseofulvin at 10 µg/mL), which do not need metabolic activation, will be studied under the same conditions.

At the end of the treatment period, the cells are harvested and slides are prepared.

3.8 Duplicate cultures

Duplicate cultures will be conducted since the Guidelines of OECD (2016) have strongly recommended that for each treatment, treated and control cultures should be duplicated throughout the entire experiment.

4. EXPRESSION OF THE RESULTS AND STATISTICAL ANALYSIS

4.1 Identification of micronuclei

The frequency of the number of incidence of micronucleated cells is assessed in binucleated cells. Micronuclei are identified according to the criteria of Fenech *et al.* (2000, 2003).

Micronuclei are morphologically identical, but smaller, than nuclei. They also have the following characteristics:

- The diameter of micronuclei in human lymphocytes usually varies between $1/16^{th}$ and $1/3^{rd}$ of the mean diameter of the main nuclei, which corresponds to $1/256^{th}$, and $1/9^{th}$ of the area of one of the main nuclei in a binucleated cell, respectively.
- Micronuclei are non-refractile and they can therefore be readily distinguished from artefact such as staining particles;
- Micronuclei are not linked or connected to the main nuclei;
- Micronuclei may touch but not overlap the main nuclei and the micronuclear boundary should be distinguishable from the nuclear boundary;

Micronuclei usually have the same staining intensity as the main nuclei but occasionally staining may be more intense.

4.2 Expression of the results

The results obtained in the different treatment cultures are presented in tabular form giving the mean number of micronuclei for 2000 binucleated cells per duplicate cell culture, and the relative CBPI per concentration, the percentage of cytostasis and the replication index.

4.3 Statistical analysis

Statistical analysis of the results obtained in the cells treated at each concentration level is performed using the χ^2 test in comparison with those in control groups. ANOVA trend test is also applied, using the statistical software Stat view®, version 5.

5. ACCEPTANCE CRITERIA FOR THE RESULTS

A study is accepted if the following criteria are fulfilled:

- Concurrent negative controls are within the 95% control limits of the distribution of the laboratory's historical negative control database,
- In the positive control groups, the mean number of micronuclei must be statistically different from negative controls reference substances. The values observed must be close to those of historical data of reference substances,
- The response of the positive control should not be compromised by cytotoxicity exceeding the limits specified ,
- An adequate number of cells (ideally 2000 cells) must be analysed for at least 3 concentrations of the test item for the assessment of genotoxicity,

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- For toxic test items, the highest concentration of the test item presents a replication index of 45+/-5% of solvent controls, *i.e.* 55 +/-5% of relative cytotoxicity,
- The top concentration of a poorly soluble test item is precipitating, without interfering with the test system.

6. INTERPRETATION OF THE RESULTS

Providing that all acceptability criteria are fulfilled, a test item is considered to be clearly positive if, in any of the experimental conditions examined

- at least one of the test concentrations exhibits a statistically significant increase (χ^2) compared with the concurrent negative control and,
- the increase is dose-related in at least one experimental condition when evaluated with an appropriate trend test and,
- any of the results are outside the distribution of the historical negative control data.

When all of these criteria are met, the test item is then considered able to induce chromosome breaks and/or gain or loss in this test system.

Providing that all acceptability criteria are fulfilled, a test item is considered clearly negative if, in all experimental conditions examined:

- none of the test concentrations exhibits a statistically significant increase compared with the concurrent negative control and,
- there is no concentration-related increase when evaluated with an appropriate trend test and
- all results are inside the distribution of the historical negative control data (95% control limits).

The test item is then considered unable to induce chromosome breaks and/or gain or loss in this test system.

In case the response is neither clearly negative or clearly positive as described above and/or in order to assist in establishing the biological relevance of a result, scoring additional cells (where appropriate) or performing a repeat experiment possibly using modified experimental conditions (*e.g.* concentration spacing, other metabolic activation conditions (*i.e.* S9 concentration or S9 origin) could be useful.

Any complementary assay will be the subject of an amendment or a new study plan.

7. CONTROL OF CONCENTRATIONS IN TREATMENT PREPARATIONS

No assessment of concentration in treatment preparations will be performed (treatment preparations will be eliminated after treatment). This will be justified in the report or will be indicated as a deviation in the final report GLP statement.

8. MODIFICATIONS TO THE STUDY PLAN

Any intended change to the study plan should be notified in the form of an amendment to the study plan and signed by the Study Director, the Test Facility Management, the Quality Assurance, the Sponsor Representative.

9. TREATMENT OF DEVIATIONS

No deviations from the Study Plan are anticipated.

Deviations from the Study Plan or Standard Operating Procedures (SOPs) related to the study should be documented and acknowledged by the Study Director who will assess the impact of each event on the quality and/or the integrity of the study.

The deviation, its possible reason(s) and the corrective actions should be fully described in the study. The deviation should be included in the report.

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10. DATA RECORDING

The computer applications programs used to acquire and derive data for this study included:

- Microsoft® Word,
- Microsoft® Excel.

These applications have been validated in the laboratory. The conformity certificate number will be added in the Study Report.

11. REPORT

11.1 Draft report

A complete draft report will be issued for comments. The report will be prepared, in accordance with the Good Laboratory Practice regulations, to contain the following information:

- The name and address of the sponsor and name of the sponsor representative.
- The name and address of the test facility.
- The name of the Study Director.
- The names of all scientists involved in the study.
- The name of the Quality Assurance Unit responsible and the dates of inspections and reports inspections.
- The location and conditions of archives of raw data, documentation, study plan and final report.
- A summary of results and conclusions of the study.
- The test system used and the procedure for identification of the test system.
- The objectives and procedures stated in the approved study plan including any changes made to the original study plan, any unforeseen circumstances which may have affected the quality or integrity of the study.
- The name of the test item (or its code number) and its identification sheet (if furnished by the sponsor).
- The concentration levels used.
- The experimental starting and completion dates.
- The detailed results of the study and statistical analysis of the data (if possible).
- The discussion of results and conclusion of the study.

This draft report will be sent by email to the Sponsor Representative in word format. It will be accepted without correction, or after introducing corrections or remarks of the sponsor in the final report or a new draft report will be requested.

In absence of ongoing communication 4 months after the sending of the Draft Study Report, and after writing notification(s) to the Sponsor, all materials and data will be archived as described in the relative paragraph.

11.2 Final report

The final report will be issued following Q.A.U. evaluation of the complete draft report. This report will include all the details described above, the remarks or corrections to be made by the sponsor with the following additions:

- The Study Director Statement of compliance as authentication of the report.
- The name of others scientists involved in the study.
- A Quality Assurance Unit Statement dated and signed by the Q.A.U. responsible. This Statement list the type of audits made and their dates, and the dates any audit results were reported to management and to the Study Director.

The final report will be sent in English in PDF format. After definitively sending final report, any correction will form the subject of an amendment to the final report.

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12. ARCHIVES

The following documents will be retained in the archives of the Toxicology Laboratory of the Institut Pasteur de Lille (1, rue du Professeur Calmette, BP 245, 59019 Lille Cedex - FRANCE):

- The study plan and any amendments,
- All raw data and slides,
- The final report and any amendments,
- Correspondence and documentation,
- The Quality Assurance Unit inspection report.

Archives are conserved at the Institut Pasteur de Lille during at least 10 years after the end of the study. After the end of this period, they will be returned to the sponsor or destroyed at sponsor's written request.

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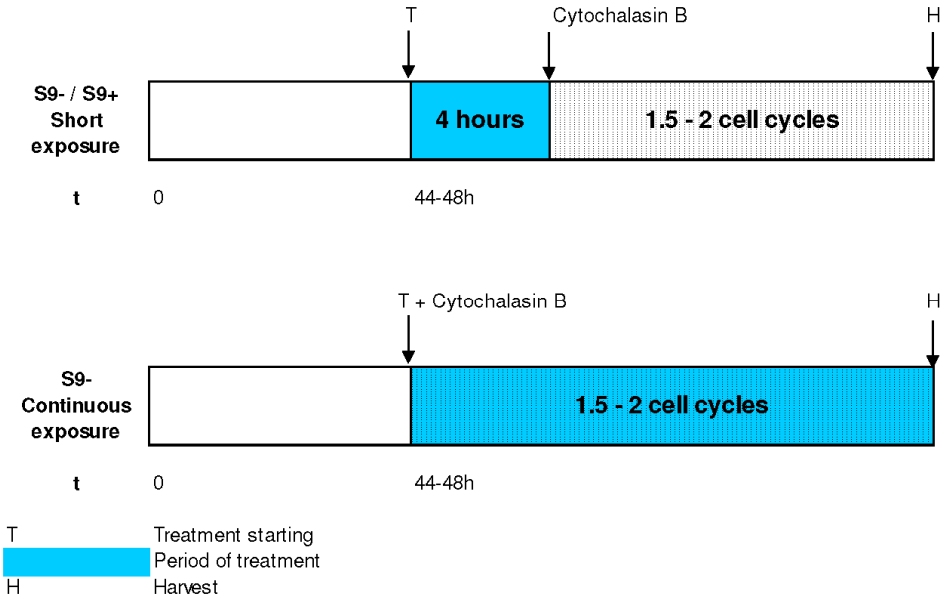
FIGURE 1

***In Vitro* Mammalian Cell Micronucleus Test**

On cultured human lymphocytes

STUDY DESIGN (following OECD 487, July, 29th 2016)



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B. SPECIFIC PROTOCOL

SPONSOR	**Sederma**	
TEST ITEM	**PENTA 18 479 – TX 19006**	
OTHER NAME / CODE	/	
BATCH NUMBER	**0001684789**	
APPEARANCE	☒ Solid	Color: white
	☐ Liquid	Homogeneity:-
	☐ Other, please specify:-	Volatility:-
WATER CONTENT	**2.5%**	
PURITY / COMPOSITION	**>96%**	
SALT / BASE RATIO	unknown	
CORRECTION FACTOR	None (at the Sponsor's request)	
MOLECULAR WEIGHT	base form: 788.04 g/mol	
DENSITY	unknown	
STORAGE CONDITIONS	☒ Room temperature (+15 to +28°C)*	☐ Protected from light
		☐ Protected from humidity
	☐ Refrigerator (+5±3°C)	☐ Protected from air
	☐ Deep freeze (-14° to -28°C)	*If yes, under inert gas* ☐ *Yes* ☐ *No*
		☐ Other, please specify:

**The Sponsor confirms that the conditions of conservation at IPL are compatible with the ones required for the test item regarding the storage temperature* ☒ Yes ☐ No

MANUFACTURING DATE	30/03/2020
☐EXPIRY DATE	
☒RETEST DATE	30/03/2022
ANALYSIS DATE	will be indicated in the Final Study Report, if available
STABILITY UNDER STORAGE CONDITIONS	at least 2 years, up to 30/03/2022 for the batch **0001684789**
SOLVENT TO BE USED	will be indicated in the Final Study Report
STABILITY IN SOLVENT	unknown

Solvent	Solubility g/L	Stability		
		Yes/No/Unknown	Temperature	Duration
Water	-	-	-	-
Ethanol	-	-	-	-
DMSO	-	-	-	-
Acetone	-	-	-	-
Others	-	-	-	-

INCOMPATIBILITY WITH	Glass	unknown
	Plastics	unknown
	Other	unknown

The sponsor certifies that the test item provided for testing will be identical to the test item described in the Study Plan and in the Analytical certificate
This test item will be investigated in accordance with the general study plan with the special features, which result from its solubility and toxicity.

FSP-IPL 210103 / PENTA 18 479 – TX 19006 / Sederma

SPECIFIC REQUIREMENTS

Formulation of the test item

The test item will be dissolved in the appropriate solvent compatible with the test system: in water (the final concentration in the culture medium must not be higher than 10%) or if the test item is not soluble in water, it will be dissolved in dimethylsulfoxide, ethanol or other (in that case, the final concentration of the selected vehicle in the culture medium must not be higher than 1%). The initial concentration may thus reach 20 mg/mL or 10 mM if soluble in water or 200 mg/mL or 1000 mM if soluble in organic solvent.

Preparations for treatments will be performed just before use unless stability data demonstrate the acceptability of storage.

Determination of the concentrations in treatment preparations

The determination will not be performed

Is the Certificate of analysis (CoA) available?

☒ **Yes** ☐ **No**

The laboratory in charge of analysis of the test item is in compliance with?

☐ **GLP**
☐ **GMP**
☒ **ISO, please specify standard: 9001**
☐ **Other. Please specify:**

Study plan information must match exactly with the CoA and with the labelling of the test item.

Is the MSDS available?

☐ **Yes** ☒ **No**

Quantity of test item to provide:

- **1.5 g**

Category of the test item:

*NB: This part **must be** fulfilled in order to adequately complete the Study Plan regarding regulations (GLP, ICH…)*

☐ Drug
☐ Veterinary drug
☐ Chemical product
☒ Cosmetic product
☐ Other, please specify:

IMPORTANT

- **The following information must be supplied before the beginning of the study preferably in the form of a dated and signed certificate of analysis:**
  - Test item identity,
  - Batch number,
  - Appearance,
  - Composition (or concentration),
  - Purity,
  - Expiry date
  - Storage conditions
  - Stability in storage conditions (or retest date)
  - Stability in the solvent.

if one (or several) of this information is lacking, it will be notified in the Study Director statement as a deviation.

- **The study report will be edited in English.**

- **This study is a short-term assay, and according to OECD's GLP Guidelines, the test item will not be preserved.**

After the archiving of the study:

☒ the test item will be destroyed by IPL
☐ the test item will be sent back. The name and the address for shipment will be asked to the Sponsor in timely manner.

In case of other studies on the same test item, the test item will be destroyed by IPL after the archiving of the whole package of studies.

FSP-IPL 210103 / PENTA 18 479 – TX 19006 / Sederma

GOOD LABORATORY PRACTICE

STUDY	:	***In Vitro* MAMMALIAN CELL MICRONUCLEUS TEST On Cultured Human Lymphocytes**
TEST ITEM	:	**PENTA 18 479 – TX 19006**
SPONSOR	:	**Sederma**
STUDY CARRIED OUT AT	:	**INSTITUT PASTEUR DE LILLE Genetic Toxicology Laboratory B.P. 245 59019 LILLE CEDEX FRANCE**

This study will be carried out in accordance with Good Laboratory Practice:

- OECD Principles of Good Laboratory Practice (as revised in 1997), ENV/MC/CHEM(98)17;
- GLP departmental order 10/8/2004 (Official Journal of 18th September 2004);
- EC Commission Directive 2004/10/EC of 11th February 2004 (Official Journal No. L050);
- The standard operating procedures of the INSTITUT PASTEUR DE LILLE (SOP).

FSP-IPL 210103 / PENTA 18 479 – TX 19006 / Sederma

CONFIDENTIALITY AGREEMENT

CONCERNING ***In Vitro* MAMMALIAN CELL MICRONUCLEUS TEST On Cultured Human Lymphocytes**

TEST ITEM **PENTA 18 479 – TX 19006**

Since the Genetic Toxicology Laboratory of the Institut Pasteur de Lille will, in the context of the work entrusted to it, receive confidential information concerning the nature and properties of the test item prior to the studies, which are to be performed, it is agreed as follows:

The Genetic Toxicology Laboratory of the Institut Pasteur de Lille undertakes:

1. To use the test item only for the studies listed above and after approval eg. by email by the Sponsor,
2. To communicate all results of the experiments carried out using this test item to the Sponsor immediately as soon as they become available,
3. To keep secret and not to divulge to any third party all specific data concerning the test item. This includes all technical data (chemical, pharmacological, economic) which have been or will be revealed to the laboratory by the sponsor, whether in writing or orally for an unlimited period,
4. To keep secret, and not to divulge to any third party, all specific data of a physico-chemical, pharmacologic, toxicologic or other nature which may be discovered during the scheduled studies or other observations revealing them only to the sponsor who alone assesses the opportuneness of divulging such information, in particular no communication to the scientific community (whether writer or oral) may be made without the formal, written consent of the sponsor,
5. Not to have any claim from any member of the laboratory personnel to any industrial, intellectual, literary or commercial copyright based on any of the information received from the divulging party.

Confidential information does not include information that would, at the time of disclosure or later, became publicly known under circumstances involving no breach of this agreement.

Since the Sponsor will, in the context of the work entrusted to it, receive confidential information concerning, without this limitation, technical, organizational, methods, processes, plans, tools… to perform the study at the Genetic Toxicology Laboratory of the Institut Pasteur de Lille, the Sponsor undertakes not to use them oral or written, as well as the name of the Genetic Toxicology Laboratory of the Institut Pasteur de Lille, for promotional purposes or for corporate communications, regardless of the medium used (advertising brochure, posters, video, external activity report, publication press release, internet ...) without the prior written consent of the Institut Pasteur de Lille.

The Genetic Toxicology Laboratory of the Institut Pasteur de Lille, the Sponsor shall use its best efforts to ensure that all of its employees, agents, subcontractors and affiliates to whom information is disclosed take all necessary precautions to safeguard and preserve the confidential status of the information.

XENOMETRIX
Contract Research Laboratory Report

XenoScreen YES/YAS Endocrine Disruptor Testing

Date of test: 08–10.12.2020

Test samples:

- TX 19005 – GREY 13386
- TX 19011 – PENTA 18479
- TX 20020 – EYEZ 18483

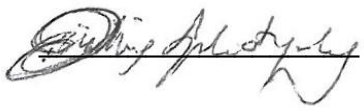
Study Number: SEDE2010

Sponsor:	Testing facility:
Vincente VICEDO Sederma 29 rue de Chemin Vert – BP 33 78612 Le Perray en Yvelines Cedex France	Client Research Laboratory Services Xenometrix AG Gewerbestrasse 25 CH-4123 Allschwil Switzerland and GenEvolutioN ZI de Limay-Porcheville 2-8 rue de Rouen 78440 Porcheville France

XENOMETRIX CLIENT RESEARCH LABORATORY SERVICES
LABORATORY PRACTICES

The assays reported in this document were performed according to Xenometrix' Quality Assurance SOP's.

Study Director: Dimitrios Spiliotopoulos, Ph.D.
Product Specialist

Signed: 

Date: December 14th, 2020

Final Distribution List:
Copy 1 - Sponsor (pdf file)
Copy 2 - Xenometrix AG (on file)

Xenometrix AG, Gewerbestrasse 25, CH-4123 Allschwil, Switzerland
Phone 0041-61-482 14 34, Fax 0041-61-482 20 72, Email info@xenometrix.ch; www.xenometrix.ch

Table of Contents

1. Summary

Three samples were tested for estrogenic and androgenic activities using the XenoScreen Yeast Estrogen Screen (YES) and Yeast Androgen Screen (YAS). The assays are able to identify both activating (agonist) and inhibiting (antagonist) activities of test compounds.

The assays were performed according to the Xenometrix SOP's.

TX 19011 was toxic (growth inhibition ≥50% of yeast cells) in the tests with the YAS strain at the two top concentrations tested.
The samples **TX 19005** and **TX 19011** showed estrogenic agonist activity in the YES strain in the XenoScreen assay presented here. No decisive indication of androgenic agonist, estrogenic antagonist, or androgenic antagonist activity was observed.

2. Introduction

The purpose of this study was to assess the estrogenic and androgenic activity of three test samples. The protocol used allow to identify both activating (agonistic) and inhibiting (antagonistic) activities.

3. Principle of the YES/YAS Assay

The common Baker's or Brewer's yeast (*Saccharomyces cerevisiae*) was genetically modified to identify compounds that can interact with the human estrogen and androgen receptors hERα and hAR. For this purpose the DNA sequences of hERα or hAR were stably integrated into the main chromosome of yeast cells. Additionally, the cells also contain an expression plasmid carrying the reporter gene lacZ encoding the enzyme β-galactosidase and estrogen (YES) or androgen (YAS) responsive elements (Routledge, E.J. and Sumpter, J.P. 1996. *Environ Toxicol Chem*. **13**:241–8; Sohoni, P. and Sumpter, J.P. 1998. *J. Endocrinol*. **158**:327-39)

Upon binding of a ligand, the hERα and hAR interact with the corresponding response elements on the expression plasmid and modulate the transcription of the lacZ reporter gene. The β-galactosidase is secreted into the medium and converts the yellow substrate chlorophenol red-β-D-galactopyranoside (CPRG) into red product which can be quantified colorimetrically at 570 nm. The measured OD_{570}, corrected for unspecific absorption and light scattering at OD_{690}, correlates directly with the amount of secreted β-galactosidase and thus with the activity of the test substance which binds to the corresponding receptor.

In order to detect antagonistic activities sample dilutions are tested in the presence of a fixed amount of agonist reference substance which allows to detect inhibitory activities of test compounds.

4. Materials and Methods

4.1 Test Sample

The test items were delivered to Xenometrix AG in Allschwil on September 18th, 2020. They were stored at room temperature in the dark and shipped to the testing facility on September 28th, 2020. Tests were run starting December 7th, 2020.

Sample		Appearance
TX 19005	GREY 13386	clear liquid
TX 19011	PENTA 18479	viscous clear liquid
TX 20020	EYEZ 18483	viscous clear liquid

Their initial concentration was set to 1.

4.2 Yeast Cells

The tests were performed with exponentially growing cultures of yeast cells *(Saccharomyces cerevisiae)* transformed with the human receptors for estrogen (hERα, YES) and androgen (hAR, YAS), respectively. Additionally, the cells also contain an expression plasmid carrying the reporter gene lacZ encoding the enzyme β-galactosidase and estrogen (YES) or androgen (YAS) responsive elements (Routledge, E.J. and Sumpter, J.P. 1996. *Environ Toxicol Chem*. **13**:241–8; Sohoni, P. and Sumpter, J.P. 1998. *J Endocrinol*. **158**:327–39).

4.3 Procedures

The assay was run according to Xenometrix SOP's, corresponding to the XenoScreen YES/YAS Instructions for Use, Version 3.11.

4.3.1 Test Sample Dilutions

Test samples were serially diluted in 8 steps (half-log steps, 1:3.16) in DMSO. This results in a final test dilution range in the assay of 1×10^{-2} – 3.16×10^{-6}.

All sample dilutions were done immediately before use. All manipulations were done under sterile conditions.

4.3.2 Control chemicals

Positive controls:

For the agonist assays 17-β estradiol (E2) for YES and 5α-dihydrotestosterone (DHT) for YAS were used as positive controls in 7 final concentrations between $1x10^{-8}$ and $1x10^{-11}$ M (YES), and $1x10^{-6}$ and $1x10^{-9}$ M (YAS), using half-log dilution steps.

The antagonist assays were done in the presence of $1x10^{-9}$ M E2 and $3x10^{-8}$ M DHT, respectively. Serial dilutions of 4-hydroxytamoxifen (4-HT) starting at $1x10^{-5}$ M and flutamide (FL) starting at $1x10^{-4}$ M were used as antagonist positive controls.

DMSO at 1% served as solvent control.

4.3.3 Plate Layout

Schematic representation of the YES agonist assay plate

	1	2	3	4	5	6	7	8	9	10	11	12
A	SC	SC	SP1 Dil 8	SP1 Dil 8	SP2 Dil 8	SP2 Dil 8	SP3 Dil 8	SP3 Dil 8				
B	E2 $1x10^{-11}$	E2 $1x10^{-11}$	SP1 Dil 7	SP1 Dil 7	SP2 Dil 7	SP2 Dil 7	SP3 Dil 7	SP3 Dil 7				
C	E2 $3x10^{-11}$	E2 $3x10^{-11}$	SP1 Dil 6	SP1 Dil 6	SP2 Dil 6	SP2 Dil 6	SP3 Dil 6	SP3 Dil 6				
D	E2 $1x10^{-10}$	E2 $1x10^{-10}$	SP1 Dil 5	SP1 Dil 5	SP2 Dil 5	SP2 Dil 5	SP3 Dil 5	SP3 Dil 5				
E	E2 $3x10^{-10}$	E2 $3x10^{-10}$	SP1 Dil 4	SP1 Dil 4	SP2 Dil 4	SP2 Dil 4	SP3 Dil 4	SP3 Dil 4				
F	E2 $1x10^{-9}$	E2 $1x10^{-9}$	SP1 Dil 3	SP1 Dil 3	SP2 Dil 3	SP2 Dil 3	SP3 Dil 3	SP3 Dil 3				
G	E2 $3x10^{-9}$	E2 $3x10^{-9}$	SP1 Dil 2	SP1 Dil 2	SP2 Dil 2	SP2 Dil 2	SP3 Dil 2	SP3 Dil 2				
H	E2 $1x10^{-8}$	E2 $1x10^{-8}$	SP1 Dil 1	SP1 Dil 1	SP2 Dil 1	SP2 Dil 1	SP3 Dil 1	SP3 Dil 1				
	E2	E2	SP1	SP1	SP2	SP2	SP3	SP3	SP4	SP4	SP5	SP5

E2 = 17β-estradiol SP1…3 = Samples1–3 SC = Solvent control

Schematic representation of the YES antagonist assay plate

	1	2	3	4	5	6	7	8	9	10	11	12
A	E2-9	E2-9	E2-9+ SP1 Dil 8	E2-9+ SP1 Dil 8	E2-9+ SP2 Dil 8	E2-9+ SP2 Dil 8	E2-9+ SP3 Dil 8	E2-9+ SP3 Dil 8				
B	E2-9+ HT $1x10^{-8}$	E2-9+ HT $1x10^{-8}$	E2-9+ SP1 Dil 7	E2-9+ SP1 Dil 7	E2-9+ SP2 Dil 7	E2-9+ SP2 Dil 7	E2-9+ SP3 Dil 7	E2-9+ SP3 Dil 7				
C	E2-9+ HT $3x10^{-8}$	E2-9+ HT $3x10^{-8}$	E2-9+ SP1 Dil 6	E2-9+ SP1 Dil 6	E2-9+ SP2 Dil 6	E2-9+ SP2 Dil 6	E2-9+ SP3 Dil 6	E2-9+ SP3 Dil 6				
D	E2-9+ HT $1x10^{-7}$	E2-9+ HT $1x10^{-7}$	E2-9+ SP1 Dil 5	E2-9+ SP1 Dil 5	E2-9+ SP2 Dil 5	E2-9+ SP2 Dil 5	E2-9+ SP3 Dil 5	E2-9+ SP3 Dil 5				
E	E2-9+ HT $3x10^{-7}$	E2-9+ HT $3x10^{-7}$	E2-9+ SP1 Dil 4	E2-9+ SP1 Dil 4	E2-9+ SP2 Dil 4	E2-9+ SP2 Dil 4	E2-9+ SP3 Dil 4	E2-9+ SP3 Dil 4				
F	E2-9+ HT $1x10^{-6}$	E2-9+ HT $1x10^{-6}$	E2-9+ SP1 Dil 3	E2-9+ SP1 Dil 3	E2-9+ SP2 Dil 3	E2-9+ SP2 Dil 3	E2-9+ SP3 Dil 3	E2-9+ SP3 Dil 3				
G	E2-9+ HT $3x10^{-6}$	E2-9+ HT $3x10^{-6}$	E2-9+ SP1 Dil 2	E2-9+ SP1 Dil 2	E2-9+ SP2 Dil 2	E2-9+ SP2 Dil 2	E2-9+ SP3 Dil 2	E2-9+ SP3 Dil 2				
H	E2-9+ HT $1x10^{-5}$	E2-9+ HT $1x10^{-5}$	E2-9+ SP1 Dil 1	E2-9+ SP1 Dil 1	E2-9+ SP2 Dil 1	E2-9+ SP2 Dil 1	E2-9+ SP3 Dil 1	E2-9+ SP3 Dil 1				
	HT	HT	SP1	SP1	SP2	SP2	SP3	SP3	SP4	SP4	SP5	SP5

E2-9 at $1.3x10^{-9}$ M 17β-estradiol HT = 4-hydroxytamoxifen SP1…3 = Samples1–3

Schematic representation of the YAS agonist assay plate

	1	2	3	4	5	6	7	8	9	10	11	12
A	DHT $2.1x10^{-11}$	DHT $2.1x10^{-11}$	SP1 Dil 8	SP1 Dil 8	SP2 Dil 8	SP2 Dil 8	SP3 Dil 8	SP3 Dil 8				
B	DHT $6.7x10^{-11}$	DHT $6.7x10^{-11}$	SP1 Dil 7	SP1 Dil 7	SP2 Dil 7	SP2 Dil 7	SP3 Dil 7	SP3 Dil 7				
C	DHT $2.1x10^{-10}$	DHT $2.1x10^{-10}$	SP1 Dil 6	SP1 Dil 6	SP2 Dil 6	SP2 Dil 6	SP3 Dil 6	SP3 Dil 6				
D	DHT $6.7x10^{-10}$	DHT $6.7x10^{-10}$	SP1 Dil 5	SP1 Dil 5	SP2 Dil 5	SP2 Dil 5	SP3 Dil 5	SP3 Dil 5				
E	DHT $2.1x10^{-9}$	DHT $2.1x10^{-9}$	SP1 Dil 4	SP1 Dil 4	SP2 Dil 4	SP2 Dil 4	SP3 Dil 4	SP3 Dil 4				
F	DHT $6.7x10^{-9}$	DHT $6.7x10^{-9}$	SP1 Dil 3	SP1 Dil 3	SP2 Dil 3	SP2 Dil 3	SP3 Dil 3	SP3 Dil 3				
G	DHT $2.1x10^{-8}$	DHT $2.1x10^{-8}$	SP1 Dil 2	SP1 Dil 2	SP2 Dil 2	SP2 Dil 2	SP3 Dil 2	SP3 Dil 2				
H	DHT $6.7x10^{-8}$	DHT $6.7x10^{-8}$	SP1 Dil 1	SP1 Dil 1	SP2 Dil 1	SP2 Dil 1	SP3 Dil 1	SP3 Dil 1				
	DHT	DHT	SP1	SP1	SP2	SP2	SP3	SP3	SP4	SP4	SP5	SP5

DHT = 5α-dihydrotestosterone SP1…3 = Samples1–3 SC = Solvent Control

Schematic representation of the YAS antagonist assay plate

	1	2	3	4	5	6	7	8	9	10	11	12
A	DHT-8	DHT-8	DHT-8+ SP1 Dil 8	DHT-8+ SP1 Dil 8	DHT-8+ SP2 Dil 8	DHT-8+ SP2 Dil 8	DHT-8+ SP3 Dil 8	DHT-8+ SP3 Dil 8				
B	DHT-8+FL $1x10^{-7}$	DHT-8+FL $1x10^{-7}$	DHT-8+ SP1 Dil 7	DHT-8+ SP1 Dil 7	DHT-8+ SP2 Dil 7	DHT-8+ SP2 Dil 7	DHT-8+ SP3 Dil 7	DHT-8+ SP3 Dil 7				
C	DHT-8+FL $3x10^{-7}$	DHT-8+FL $3x10^{-7}$	DHT-8+ SP1 Dil 6	DHT-8+ SP1 Dil 6	DHT-8+ SP2 Dil 6	DHT-8+ SP2 Dil 6	DHT-8+ SP3 Dil 6	DHT-8+ SP3 Dil 6				
D	DHT-8+FL $1x10^{-6}$	DHT-8+FL $1x10^{-6}$	DHT-8+ SP1 Dil 5	DHT-8+ SP1 Dil 5	DHT-8+ SP2 Dil 5	DHT-8+ SP2 Dil 5	DHT-8+ SP3 Dil 5	DHT-8+ SP3 Dil 5				
E	DHT-8+FL $3x10^{-6}$	DHT-8+FL $3x10^{-6}$	DHT-8+ SP1 Dil 4	DHT-8+ SP1 Dil 4	DHT-8+ SP2 Dil 4	DHT-8+ SP2 Dil 4	DHT-8+ SP3 Dil 4	DHT-8+ SP3 Dil 4				
F	DHT-8+FL $1x10^{-5}$	DHT-8+FL $1x10^{-5}$	DHT-8+ SP1 Dil 3	DHT-8+ SP1 Dil 3	DHT-8+ SP2 Dil 3	DHT-8+ SP2 Dil 3	DHT-8+ SP3 Dil 3	DHT-8+ SP3 Dil 3				
G	DHT-8+FL $1x10^{-5}$	DHT-8+FL $1x10^{-5}$	DHT-8+ SP1 Dil 2	DHT-8+ SP1 Dil 2	DHT-8+ SP2 Dil 2	DHT-8+ SP2 Dil 2	DHT-8+ SP3 Dil 2	DHT-8+ SP3 Dil 2				
H	DHT-8+FL $1x10^{-4}$	DHT-8+FL $1x10^{-4}$	DHT-8+ SP1 Dil 1	DHT-8+ SP1 Dil 1	DHT-8+ SP2 Dil 1	DHT-8+ SP2 Dil 1	DHT-8+ SP3 Dil 1	DHT-8+ SP3 Dil 1				
	FL	FL	SP1	SP1	SP2	SP2	SP3	SP3	SP4	SP4	SP5	SP5

DHT-8 = $3x10^{-8}$ M FL = flutamide SP1…3 = Samples1–3

4.3.4 Assay Procedure

The procedure followed the XenoScreen YES/YAS Instructions for Use, Version 3.11, which is analogous to Xenometrix SOP's. Briefly:

- The dilutions were prepared separately on a 96-well plate
- 100 µl of assay medium was pipetted into 2 96 well plates
- 100 µl of assay medium with fixed concentrations of E2 or DHT was pipetted
- into 2 96 well plates for the antagonist assays
- 2 µl of sample and control dilutions were added to the 4 assay plates
- YES and YAS yeast cells were added to the assay plates to give a final
- volume of 200 µl
- The plates were sealed with gas-permeable plate sealers and incubated for 48 hrs at 32°C in a humidified chamber with agitation at 100 rpm
- After 48 hrs the wells were mixed and the optical densities were measured at 570 and 690 nm.

4.4 Calculations and Data Analysis

- For each well the OD_{690} values were subtracted from the OD_{570} values.
- The mean OD values for every test sample concentration and for the controls were calculated.
- Growth Factor G and Induction Ratio I_R were calculated based on the definitions given below
- Dose-response curves were drawn which served as the basis for conclusions on the samples' activities

Growth factor G: $G = \frac{A_{690,S}}{A_{690,N}}$

where $A_{690,S}$ is the absorbance of the sample S at 690 nm
$A_{690,N}$ is the absorbance of the solvent control at 690 nm

Induction Ratio I_R: $I_R = \frac{1}{G} \times \frac{A_{570,S}}{A_{570,N}}$

where $A_{570,S}$ is the corrected absorbance of the sample S at 570 nm - 690 nm
$A_{570,N}$ is the corrected absorbance of the solvent control at 570 nm - 690 nm

Limit of Detection LoD = Mean of solvent control + 3 Standard Deviations
Limit of Quantification LoQ = Mean of solvent control + 9 Standard Deviations

All calculations and graphs were made using the XenoScreen Excel calculation Sheet, version 3.42.

Data interpretation

Growth factors G ≤ 0.5 are indicative of a toxic effect. Results obtained at sample concentrations where G is ≤ 0.5 are ignored for the interpretation of hormonal activity.

For agonist activities, Induction Ratios ≥ IR10 are considered positive. IR10 is defined as the IR which is 10% of (IR max – IR solvent) above the IR solvent.

For antagonistic effects Induction Ratios ≤ IR50 are considered positive. IR50 is defined as the IR which is at 50% of the IR of the solvent control with a fixed amount of agonist E2 or DHT. Induction ratios at toxic concentrations (G ≤ 0.5) are ignored.

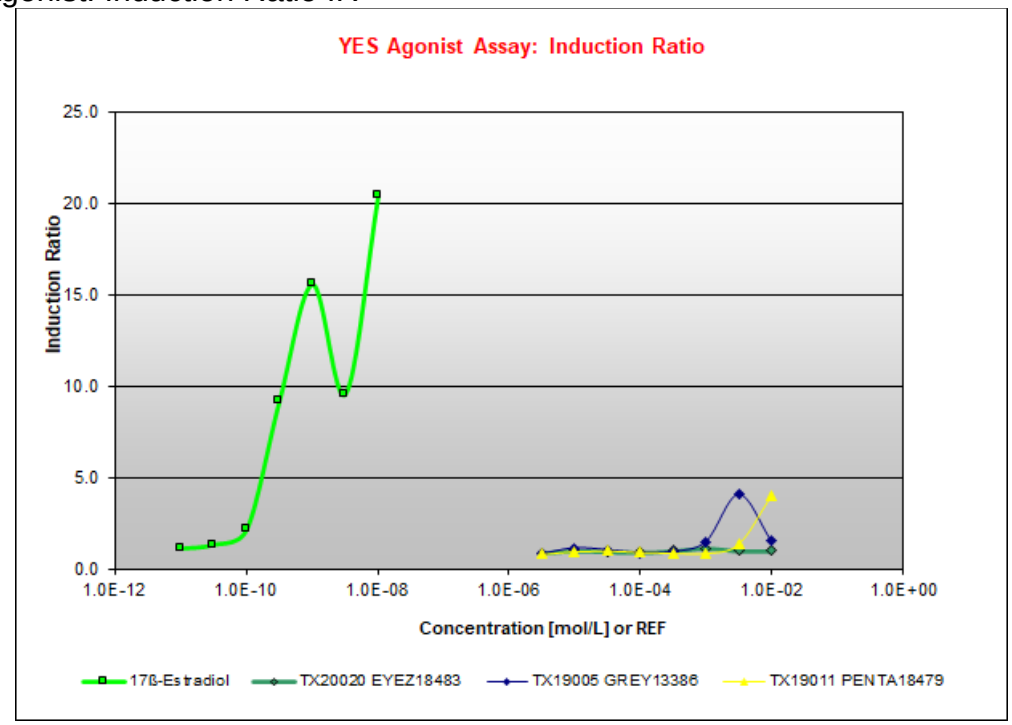
Inhibition may also be caused by non-specific effects which lead to a reduction of the response induced by the fixed concentrations of E2 and DHT. Parallel inhibition in the YES and YAS assay may be indicative of such non-specific inhibition which is not a true estrogen or androgen antagonistic effect.

5. Results

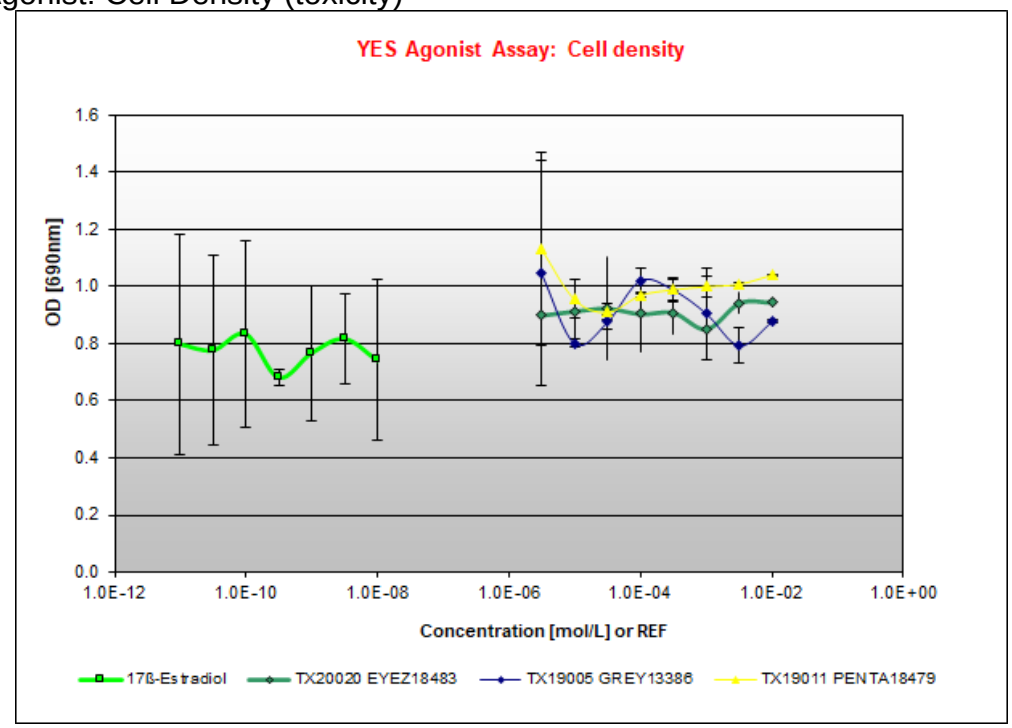
5.1 Estrogenic Agonist and Antagonist Assay (YES)

(For detailed data please see Appendix I + II)

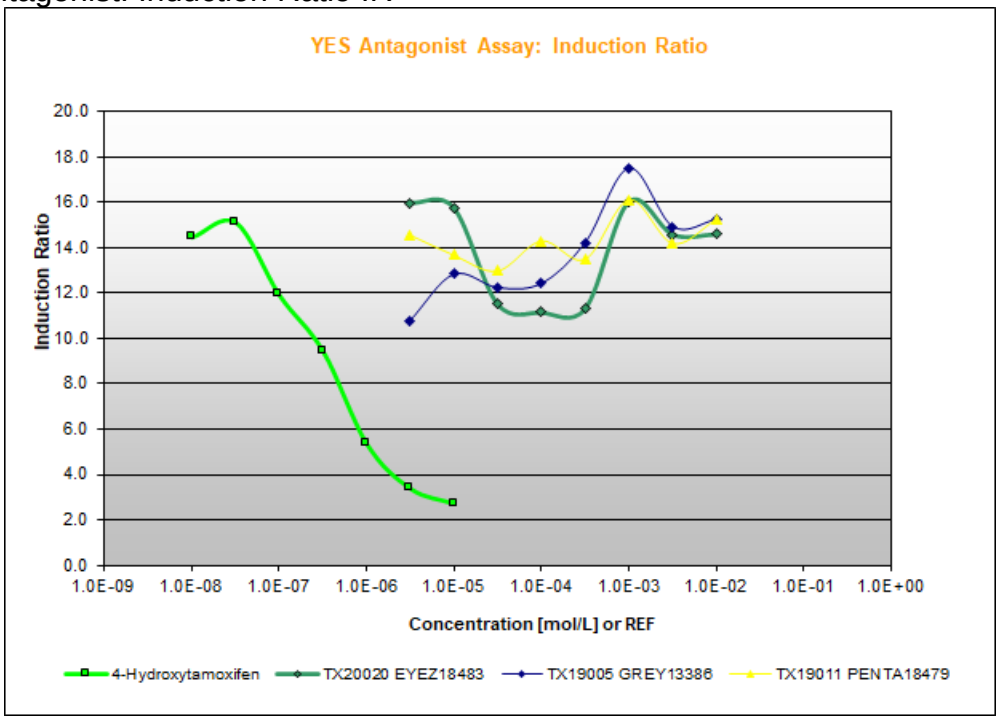
YES Agonist: Induction Ratio IR



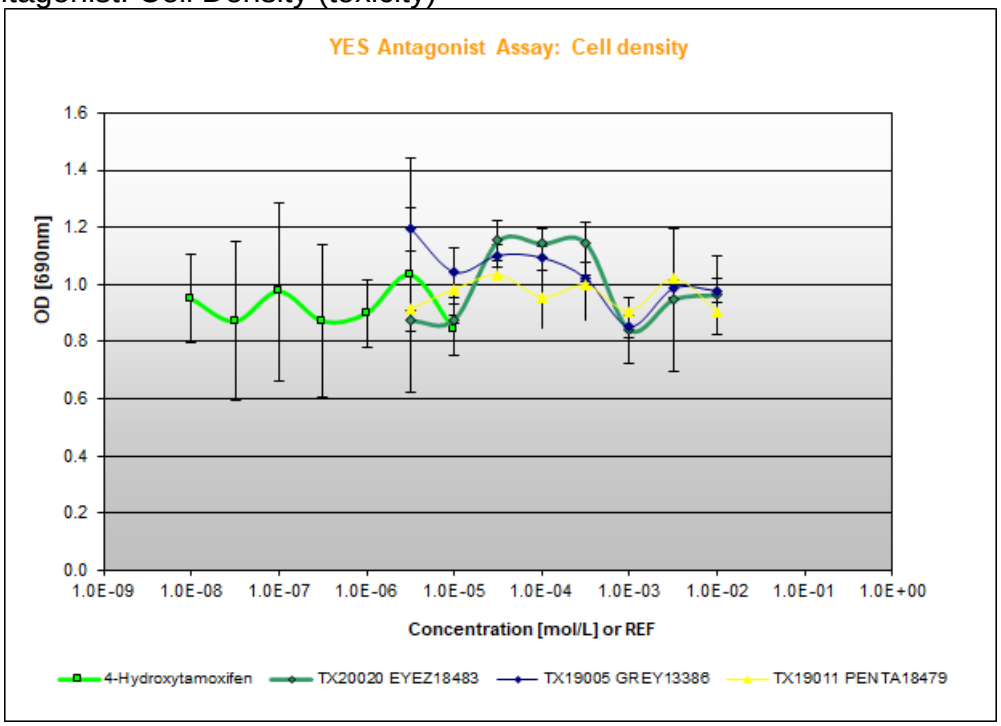
YES Agonist: Cell Density (toxicity)



YES Antagonist: Induction Ratio IR



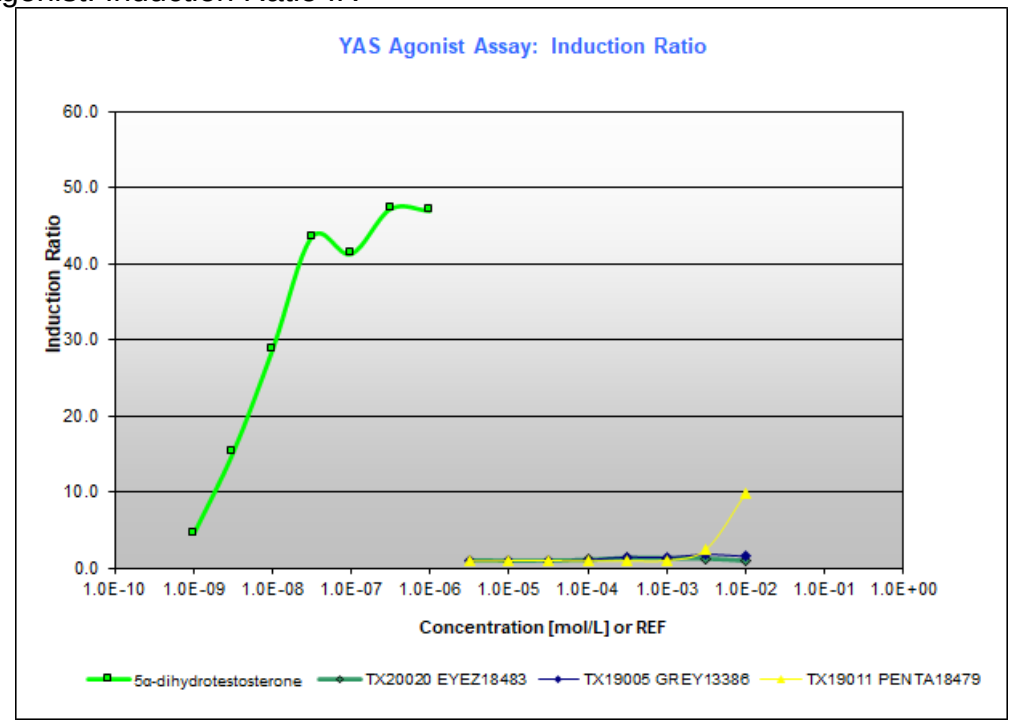
YES Antagonist: Cell Density (toxicity)



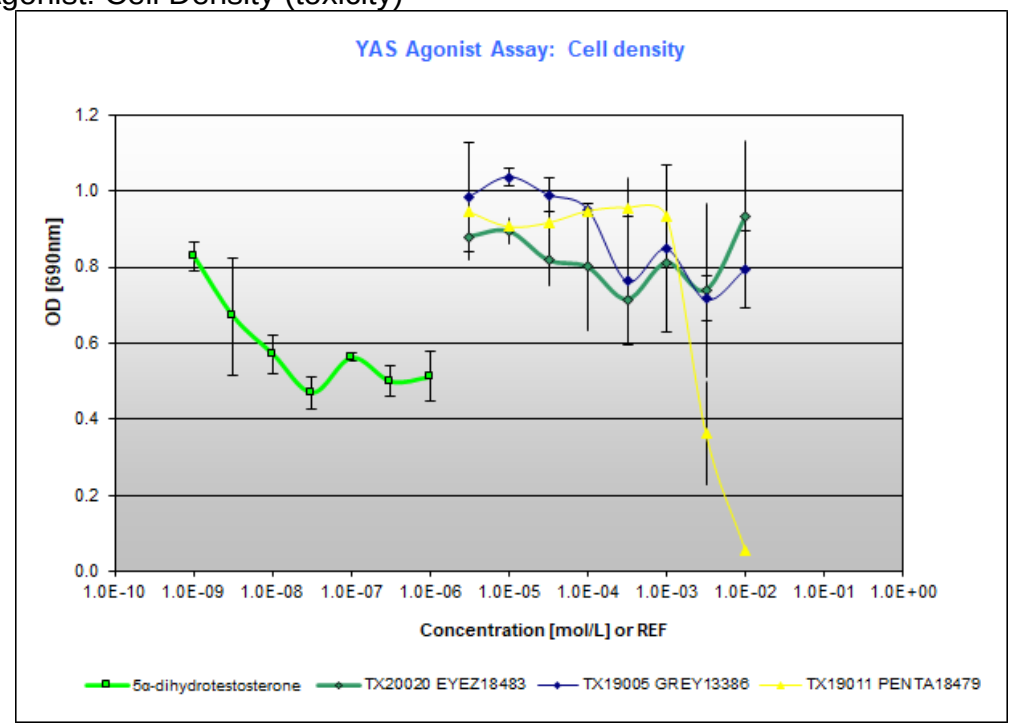
5.2 Androgenic Agonist and Antagonist Assay (YAS)

(For detailed data please see Appendix III + IV)

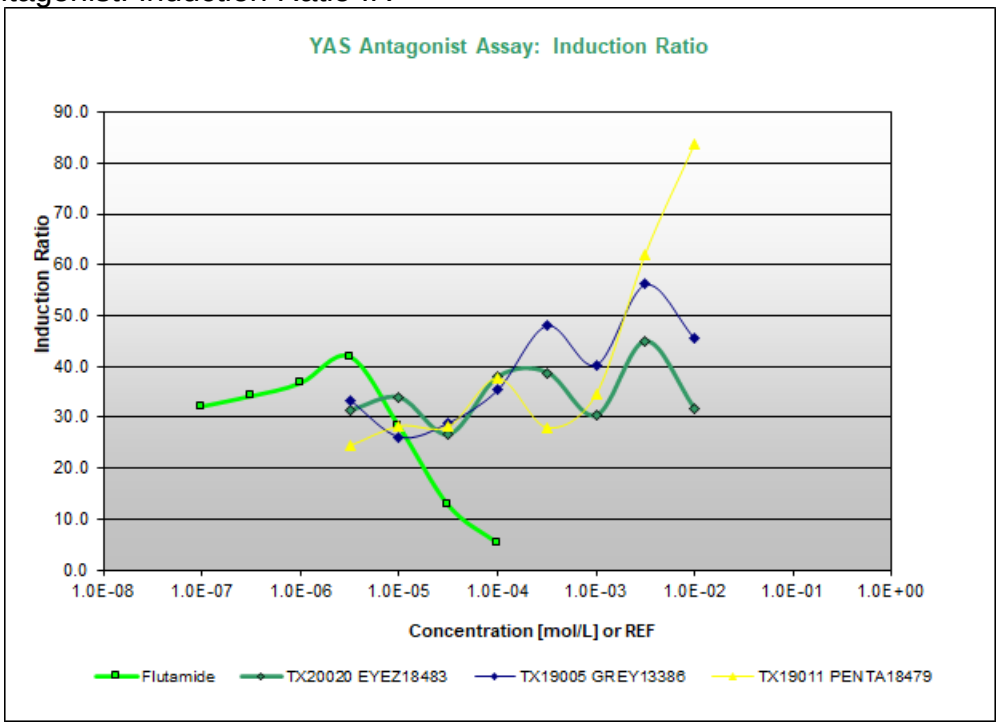
YAS Agonist: Induction Ratio IR



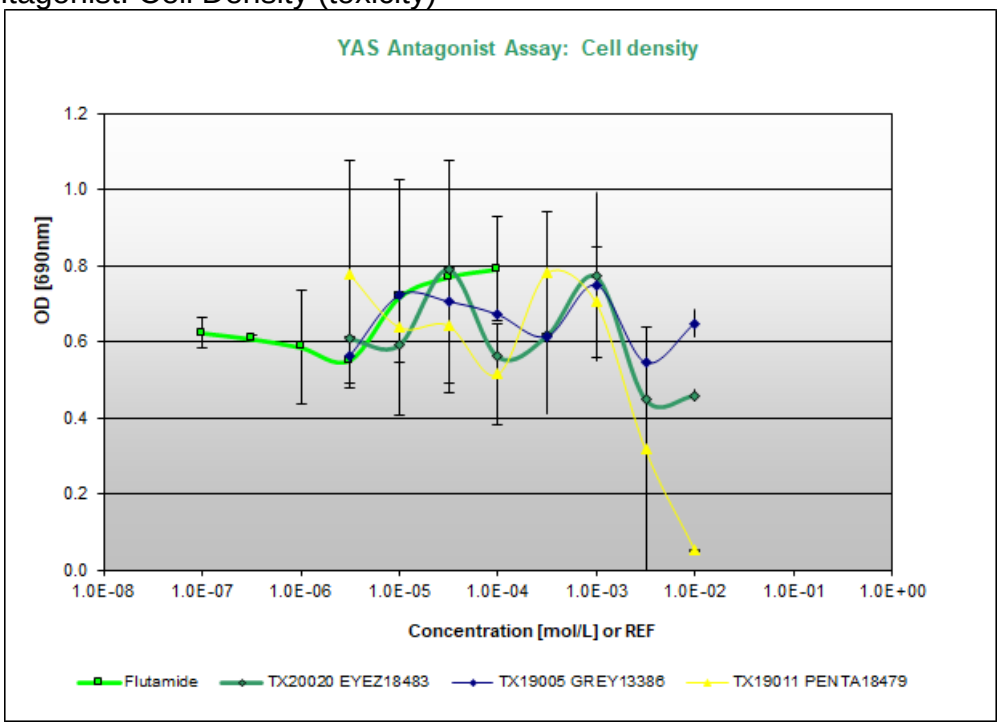
YAS Agonist: Cell Density (toxicity)



YAS Antagonist: Induction Ratio IR



YAS Antagonist: Cell Density (toxicity)



5.3 Summary Table

	YES (Estrogenic activity)			YAS (Androgenic activity)		
	Toxicity[1)]	Agonist potential activity[2)]	Antagonist activity EC50[3)]	Toxicity[1)]	Agonist potential activity[2)]	Antagonist activity EC50[3)]
TX 19005	nt	agonist	inactive	nt	–	inactive
TX 19011	nt	agonist	inactive	1.0E–3	–	inactive
TX 20020	nt	inactive	inactive	nt	–	inactive

nt = not toxic at the concentrations tested; inactive = no agonist or antagonist activity at any tested non-toxic concentration.

[1)]: Highest non-toxic concentration, G > 0.5 in agonist assay
[2)]: IR ≥ IR10. IR10 is defined as the IR which is 10% of (IR max - IR solvent) above the Induction Ratio IR of the solvent control.
[3)]: EC50 values are only calculated when RPCMin is ≤50%, with RPCMin defined as the percentage of IRMin relative to fitted IR(SCcalc) (IRMin is defined as the lowest Induction Ratio IR at non-toxic concentration for the compound under investigation in the antagonist assay).

5.4 Interpretation

Appearance:
All samples were clear liquids with sample **TX 19011** having a pronounced viscosity.

Toxicity:
Sample **TX 19011** showed toxicity (growth inhibition, defined as G ≤ 0.5) in the tests at the two top doses in the YAS strain.

YES/YAS Activity:
Samples **TX 19005** and **TX 19011** showed estrogenic agonist activity with EC10 values of 2.2E–3 and 6.9E–3, respectively. No estrogenic antagonist, androgenic agonist, or androgenic antagonist activities were observed in the tests.

6. Conclusions

In these XenoScreen assays one of the tested samples (**TX 19001**) was toxic in the YAS strain and two of the tested samples (**TX 19005** and **TX 19001**) showed estrogenic agonist activity.

Appendix I: Raw and Calculated Data - YES Agonist Assay

ß-Gal expression

REF or Conc.	17ß-Estradiol	SD	TX20020 EYEZ18483	SD	TX19005 GREY133	SD	TX19011 PENTA18479	SD
Solv./Contr.	0.140	0.026	0.162	0.021	0.176	0.003	0.187	0.006
1.00E-11	0.175	0.009	0.187	0.003	0.186	0.003	0.188	0.003
3.16E-11	0.204	0.008	0.179	0.008	0.185	0.005	0.186	0.002
1.00E-10	0.360	0.019	0.169	0.000	0.185	0.008	0.182	0.000
3.16E-10	1.266	0.157	0.184	0.001	0.189	0.002	0.178	0.002
1.00E-09	2.399	0.234	0.198	0.018	0.273	0.128	0.177	0.004
3.16E-09	1.567	1.972	0.192	0.023	0.657	0.002	0.281	0.011
1.00E-08	3.045	0.202	0.192	0.030	0.269	0.016	0.846	0.099

Cell density

REF or Conc.	17ß-Estradiol	SD	TX20020 EYEZ18483	SD	TX19005 GREY133	SD	TX19011 PENTA18479	SD
Solv./Contr.	0.699	0.206	0.899	0.012	1.046	0.393	1.131	0.337
1.00E-11	0.798	0.385	0.913	0.097	0.802	0.015	0.957	0.067
3.16E-11	0.777	0.331	0.923	0.179	0.876	0.028	0.911	0.029
1.00E-10	0.833	0.327	0.904	0.134	1.020	0.041	0.970	0.005
3.16E-10	0.684	0.028	0.908	0.077	0.987	0.041	0.990	0.038
1.00E-09	0.767	0.236	0.852	0.090	0.905	0.159	1.001	0.036
3.16E-09	0.816	0.160	0.942	0.037	0.794	0.063	1.009	0.006
1.00E-08	0.742	0.282	0.944	0.010	0.879	0.006	1.042	0.000

Growth factor G (for the definition see Instructions for Use)

REF or Conc.	17ß-Estradiol	TX20020 EYEZ18483	TX19005 GREY13386	TX19011 PENTA18479
Solv./Contr.	1.000	1.287	1.497	1.618
1.00E-11	1.142	1.307	1.148	1.369
3.16E-11	1.112	1.322	1.253	1.305
1.00E-10	1.192	1.294	1.460	1.389
3.16E-10	0.978	1.300	1.412	1.417
1.00E-09	1.098	1.220	1.295	1.432
3.16E-09	1.168	1.348	1.137	1.444
1.00E-08	1.062	1.351	1.258	1.491

G ≤ 0.5

β-galactosidase activity U_S (for the definition see Instructions for Use)

REF or Conc.	17ß-Estradiol	TX20020 EYEZ18483	TX19005 GREY13386	TX19011 PENTA18479
Solv./Contr.	0.201	0.180	0.168	0.165
1.00E-11	0.219	0.204	0.232	0.196
3.16E-11	0.262	0.194	0.211	0.204
1.00E-10	0.432	0.187	0.182	0.187
3.16E-10	1.853	0.203	0.191	0.180
1.00E-09	3.126	0.233	0.302	0.177
3.16E-09	1.920	0.203	0.827	0.279
1.00E-08	4.104	0.203	0.306	0.812

Induction Ratio I_R (for the definition see Instructions for Use)

REF or Conc.	17ß-Estradiol	TX20020 EYEZ18483	TX19005 GREY13386	TX19011 PENTA18479
Solv./Contr.	1.000	0.896	0.837	0.823
1.00E-11	1.092	1.017	1.155	0.977
3.16E-11	1.305	0.967	1.051	1.014
1.00E-10	2.150	0.932	0.905	0.932
3.16E-10	9.225	1.009	0.954	0.894
1.00E-09	15.566	1.159	1.504	0.879
3.16E-09	9.560	1.013	4.120	1.389
1.00E-08	20.439	1.013	1.524	4.042

IR ≥ IR10 (≥ 2.439)

IR10 = 10 percent increase

Appendix II: Raw and Calculated Data - YES Antagonist Assay

ß-Gal expression

REF or Conc.	4-Hydroxytamoxifen	SD	TX20020 EYEZ1848:	SD	:19005 GREY133	SD	TX19011 PENTA18479	SD
E2 contr.	2.742	0.018	2.798	0.109	2.583	0.095	2.671	0.002
1.00E-08	2.761	0.025	2.773	0.102	2.688	0.082	2.699	0.063
3.16E-08	2.642	0.011	2.672	0.131	2.709	0.130	2.692	0.042
1.00E-07	2.334	0.078	2.566	0.068	2.729	0.038	2.742	0.009
3.16E-07	1.653	0.162	2.609	0.014	2.917	0.100	2.702	0.028
1.00E-06	0.967	0.063	2.701	0.004	3.001	0.130	2.926	0.091
3.16E-06	0.706	0.064	2.769	0.086	2.953	0.048	2.910	0.098
1.00E-05	0.463	0.005	2.823	0.016	2.997	0.077	2.769	0.046

Cell density

REF or Conc.	4-Hydroxytamoxifen	SD	TX20020 EYEZ1848:	SD	:19005 GREY133	SD	TX19011 PENTA18479	SD
E2 contr.	0.818	0.203	0.873	0.035	1.194	0.076	0.914	0.190
1.00E-08	0.950	0.155	0.877	0.012	1.043	0.087	0.983	0.148
3.16E-08	0.870	0.277	1.153	0.068	1.101	0.038	1.033	0.021
1.00E-07	0.975	0.311	1.144	0.053	1.093	0.043	0.955	0.111
3.16E-07	0.871	0.267	1.146	0.071	1.021	0.011	0.997	0.122
1.00E-06	0.899	0.117	0.840	0.115	0.855	0.040	0.905	0.016
3.16E-06	1.033	0.410	0.947	0.251	0.986	0.031	1.022	0.029
1.00E-05	0.841	0.091	0.964	0.138	0.979	0.043	0.904	0.022

Growth factor G (for the definition see Instructions for Use)

REF or Conc.	4-Hydroxytamoxifen	TX20020 EYEZ18483	TX19005 GREY13386	TX19011 PENTA18479
E2 contr.	1.170	1.249	1.708	1.308
1.00E-08	1.360	1.255	1.493	1.407
3.16E-08	1.246	1.651	1.576	1.479
1.00E-07	1.396	1.638	1.564	1.367
3.16E-07	1.247	1.640	1.461	1.427
1.00E-06	1.286	1.202	1.223	1.295
3.16E-06	1.479	1.355	1.412	1.463
1.00E-05	1.203	1.379	1.401	1.293

<50% of E2 control

β-galactosidase activity U_S (for the definition see Instructions for Use)

REF or Conc.	4-Hydroxytamoxifen	TX20020 EYEZ18483	TX19005 GREY13386	TX19011 PENTA18479
E2 contr.	3.354	3.206	2.165	2.924
1.00E-08	2.905	3.162	2.577	2.746
3.16E-08	3.036	2.317	2.460	2.605
1.00E-07	2.393	2.242	2.497	2.871
3.16E-07	1.897	2.277	2.857	2.710
1.00E-06	1.077	3.217	3.512	3.234
3.16E-06	0.683	2.926	2.994	2.847
1.00E-05	0.551	2.930	3.061	3.064

Induction Ratio I_R (for the definition see Instructions for Use)

REF or Conc.	4-Hydroxytamoxifen	TX20020 EYEZ18483	TX19005 GREY13386	TX19011 PENTA18479
E2 contr.	16.703	15.967	10.779	14.559
1.00E-08	14.468	15.747	12.833	13.674
3.16E-08	15.118	11.537	12.251	12.972
1.00E-07	11.918	11.163	12.433	14.296
3.16E-07	9.446	11.338	14.225	13.493
1.00E-06	5.361	16.020	17.490	16.102
3.16E-06	3.403	14.569	14.910	14.178
1.00E-05	2.742	14.590	15.245	15.259

IR<=IR50 (≤ 8.76)

IR50 = 50 percent decrease from fitted maximum IR

Appendix III: Raw and Calculated Data - YAS Agonist Assay

ß-Gal expression

REF or Conc.	5α-dihydrotestosterc	SD	TX20020 EYEZ18483	SD	:19005 GREY133	SD	TX19011 PENTA18479	SD
Solv./Contr.	0.098	0.002	0.105	0.008	0.101	0.007	0.103	0.001
1.00E-09	0.401	0.043	0.099	0.004	0.102	0.001	0.101	0.002
3.16E-09	1.082	0.024	0.093	0.002	0.099	0.002	0.104	0.000
1.00E-08	1.741	0.050	0.097	0.002	0.107	0.005	0.103	0.002
3.16E-08	2.175	0.031	0.104	0.009	0.120	0.000	0.107	0.008
1.00E-07	2.466	0.043	0.115	0.008	0.129	0.013	0.107	0.005
3.16E-07	2.503	0.021	0.100	0.002	0.141	0.002	0.098	0.014
1.00E-06	2.550	0.117	0.102	0.008	0.132	0.010	0.057	0.003

Cell density

REF or Conc.	5α-dihydrotestosterc	SD	TX20020 EYEZ18483	SD	:19005 GREY133	SD	TX19011 PENTA18479	SD
Solv./Contr.	0.924	0.066	0.880	0.035	0.984	0.143	0.945	0.124
1.00E-09	0.827	0.037	0.894	0.034	1.036	0.022	0.906	0.016
3.16E-09	0.670	0.154	0.819	0.069	0.990	0.044	0.916	0.029
1.00E-08	0.572	0.051	0.800	0.169	0.952	0.016	0.947	0.009
3.16E-08	0.471	0.042	0.716	0.086	0.764	0.167	0.956	0.077
1.00E-07	0.563	0.010	0.812	0.168	0.848	0.220	0.934	0.070
3.16E-07	0.500	0.039	0.740	0.228	0.716	0.059	0.363	0.135
1.00E-06	0.512	0.065	0.932	0.200	0.795	0.100	0.055	0.005

Growth factor G (for the definition see Instructions for Use)

REF or Conc.	5α-dihydrotestosterone	TX20020 EYEZ18483	TX19005 GREY13386	TX19011 PENTA18479
Solv./Contr.	1.000	0.952	1.065	1.022
1.00E-09	0.895	0.968	1.121	0.980
3.16E-09	0.724	0.885	1.071	0.991
1.00E-08	0.618	0.866	1.030	1.025
3.16E-08	0.509	0.774	0.827	1.034
1.00E-07	0.609	0.878	0.917	1.010
3.16E-07	0.540	0.801	0.775	0.393
1.00E-06	0.554	1.009	0.860	0.059

G <= 0.5

β-galactosidase activity U_S (for the definition see Instructions for Use)

REF or Conc.	5α-dihydrotestosterone	TX20020 EYEZ18483	TX19005 GREY13386	TX19011 PENTA18479
Solv./Contr.	0.106	0.119	0.102	0.109
1.00E-09	0.484	0.111	0.099	0.112
3.16E-09	1.616	0.113	0.100	0.113
1.00E-08	3.046	0.121	0.112	0.109
3.16E-08	4.619	0.145	0.157	0.112
1.00E-07	4.383	0.142	0.152	0.115
3.16E-07	5.010	0.136	0.197	0.269
1.00E-06	4.982	0.109	0.166	1.034

Induction Ratio I_R (for the definition see Instructions for Use)

REF or Conc.	5α-dihydrotestosterone	TX20020 EYEZ18483	TX19005 GREY13386	TX19011 PENTA18479
Solv./Contr.	1.000	1.122	0.966	1.032
1.00E-09	4.576	1.044	0.932	1.054
3.16E-09	15.264	1.069	0.945	1.071
1.00E-08	28.778	1.147	1.062	1.027
3.16E-08	43.638	1.368	1.487	1.058
1.00E-07	41.404	1.337	1.432	1.087
3.16E-07	47.333	1.282	1.857	2.543
1.00E-06	47.070	1.034	1.573	9.766

IR>=IR10 (= 5.546)

IR10 = 10 percent increase

Appendix IV: Raw and Calculated Data - YAS Antagonist Assay

ß-Gal expression

REF or Conc.	Flutamide	SD	TX20020 EYEZ1848:	SD	:19005 GREY133	SD	TX19011 PENTA18479	SD
DHT control	2.060	0.146	2.020	0.044	1.988	0.055	2.022	0.031
1.00E-07	2.122	0.049	2.122	0.024	1.995	0.027	1.922	0.077
3.16E-07	2.200	0.014	2.233	0.101	2.161	0.113	1.932	0.111
1.00E-06	2.278	0.026	2.256	0.064	2.521	0.180	2.061	0.103
3.16E-06	2.450	0.124	2.531	0.044	3.129	0.061	2.324	0.053
1.00E-05	2.142	0.016	2.482	0.076	3.196	0.004	2.582	0.186
3.16E-05	1.041	0.005	2.134	0.039	3.251	0.055	2.097	0.544
1.00E-04	0.444	0.033	1.541	0.121	3.130	0.035	0.487	0.144

Cell density

REF or Conc.	Flutamide	SD	TX20020 EYEZ1848:	SD	:19005 GREY133	SD	TX19011 PENTA18479	SD
DHT control	0.650	0.070	0.609	0.070	0.564	0.052	0.779	0.298
1.00E-07	0.625	0.041	0.592	0.053	0.722	0.067	0.641	0.093
3.16E-07	0.609	0.007	0.792	0.052	0.706	0.044	0.645	0.152
1.00E-06	0.587	0.148	0.562	0.062	0.672	0.009	0.516	0.133
3.16E-06	0.553	0.060	0.616	0.202	0.615	0.129	0.783	0.159
1.00E-05	0.718	0.309	0.773	0.220	0.749	0.075	0.705	0.146
3.16E-05	0.772	0.306	0.449	0.034	0.547	0.012	0.319	0.319
1.00E-04	0.793	0.136	0.459	0.015	0.649	0.036	0.055	0.001

Growth factor G (for the definition see Instructions for Use)

REF or Conc.	Flutamide	TX20020 EYEZ18483	TX19005 GREY13386	TX19011 PENTA18479
DHT control	0.703	0.659	0.610	0.843
1.00E-07	0.676	0.641	0.781	0.693
3.16E-07	0.659	0.857	0.764	0.698
1.00E-06	0.635	0.608	0.727	0.558
3.16E-06	0.598	0.667	0.665	0.847
1.00E-05	0.776	0.836	0.810	0.763
3.16E-05	0.835	0.486	0.591	0.345
1.00E-04	0.858	0.497	0.702	0.060

<50% of DHT control

β-galactosidase activity U_S (for the definition see Instructions for Use)

REF or Conc.	Flutamide	TX20020 EYEZ18483	TX19005 GREY13386	TX19011 PENTA18479
DHT control	3.171	3.317	3.527	2.594
1.00E-07	3.397	3.582	2.764	3.000
3.16E-07	3.610	2.818	3.060	2.995
1.00E-06	3.881	4.015	3.752	3.994
3.16E-06	4.430	4.106	5.090	2.968
1.00E-05	2.984	3.211	4.268	3.663
3.16E-05	1.349	4.754	5.946	6.572
1.00E-04	0.559	3.356	4.824	8.853

Induction Ratio I_R (for the definition see Instructions for Use)

REF or Conc.	Flutamide	TX20020 EYEZ18483	TX19005 GREY13386	TX19011 PENTA18479
DHT control	29.957	31.342	33.324	24.507
1.00E-07	32.090	33.839	26.114	28.343
3.16E-07	34.102	26.624	28.914	28.292
1.00E-06	36.663	37.932	35.447	37.735
3.16E-06	41.849	38.791	48.092	28.038
1.00E-05	28.190	30.332	40.323	34.604
3.16E-05	12.742	44.916	56.175	62.094
1.00E-04	5.285	31.705	45.575	83.639

IR<=IR50 (≤ 20.562)

IR50 = 50 percent decrease from fitted maximum IR

XENOMETRIX
Contract Research Laboratory Report

XenoScreen XL YES Endocrine Disruptor Testing

Date of test: 01.06.2021 – 02.06.2021

Test samples:

- TX 19005 – GREY 13386
- TX 19011 – PENTA 18479

Study Number: SEDE2105

Sponsor:

Vincente VICEDO
Sederma
29 rue de Chemin Vert – BP 33
78612 Le Perray en Yvelines Cedex
France

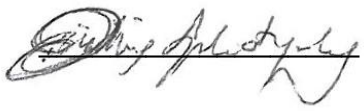
Testing facility:

Client Research Laboratory Services
Xenometrix AG
Gewerbestrasse 25
CH-4123 Allschwil
Switzerland

XENOMETRIX CLIENT RESEARCH LABORATORY SERVICES
LABORATORY PRACTICES

The assays reported in this document were performed according to Xenometrix' Quality Assurance SOP's.

Study Director: Dimitrios Spiliotopoulos, Ph.D.
Product Specialist

Signed: 

Date: June 3rd, 2021

Final Distribution List:
Copy 1 - Sponsor (pdf file)
Copy 2 - Xenometrix AG (on file)

Xenometrix AG, Gewerbestrasse 25, CH-4123 Allschwil, Switzerland
Phone 0041-61-482 14 34, Fax 0041-61-482 20 72, Email info@xenometrix.ch; www.xenometrix.ch

Table of Contents

1. Summary

Two samples were tested for estrogenic activities using the XenoScreen XL Yeast Estrogen Screen (YES). The assay is able to identify activating (agonistic) activities of test items.

The assays were performed according to the Xenometrix SOP's.

Toxic effects (growth inhibition ≥50% of yeast cells) were only observed for the sample **GREY – TX 19005** at the highest concentration tested.
None of the samples showed estrogenic agonist activity in the XenoScreen XL YES assay.

2. Introduction

The purpose of this study was to assess the estrogenic activity of two test samples. The protocol used allow to identify activating (agonistic) activities.

3. Principle of the YES Assay

The common Baker's or Brewer's yeast (*Saccharomyces cerevisiae*) was genetically modified to identify compounds that can interact with the human estrogen receptor hERα. For this purpose the DNA sequence of hERα were stably integrated into the main chromosome of yeast cells. Additionally, the cells also contain an expression plasmid carrying the reporter gene lacZ encoding the enzyme β-galactosidase and estrogen (YES) responsive elements (Routledge, E.J. and Sumpter, J.P. 1996. *Environ Toxicol Chem*. **13**:241–8).

Upon binding of a ligand, the hERα interacts with the corresponding response elements on the expression plasmid and modulate the transcription of the lacZ reporter gene. The β-galactosidase is secreted into the medium and converts the yellow substrate chlorophenol red-β-D-galactopyranoside (CPRG) into red product which can be quantified colorimetrically at 570 nm. The measured OD_{570}, corrected for unspecific absorption and light scattering at OD_{690}, correlates directly with the amount of secreted β-galactosidase and thus with the activity of the test substance which binds to the corresponding receptor.

The XenoScreen XL YES assay system can identify both activating (agonistic) and inhibitory (antagonistic) activities of test compounds. Only agonistic activities were assessed in these tests.

The XenoScreen XL YES uses lyticase and a detergent (=LYES) to facilitate the secretion of the intracellularly synthesized β-galactosidase (Schultis T. and Metzger J.W., 2004. *Chemosphere* **57**:1649–55). This allows to reduce the incubation time from 48 hrs in the standard YES assay to 18 hours. In addition, the accelerated protocol leads also to enhanced sensitivities for estrogenic compounds.

4. Materials and Methods

4.1 Test Sample

The test items were delivered to Xenometrix AG in Allschwil on April 29th, 2021.They were stored at room temperature in the dark. Tests were run starting June 1st, 2021.

Sample		Appearance
TX 19005	GREY 13386	clear liquid
TX 19011	PENTA 18479	clear liquid

Their initial concentration was set to 1.

4.2 Yeast Cells

The tests were performed with exponentially growing cultures of yeast cells *(Saccharomyces cerevisiae)* transformed with the human receptor for estrogen (hERα, YES). Additionally, the cells also contain an expression plasmid carrying the reporter gene lacZ encoding the enzyme β-galactosidase and estrogen (YES) responsive element (Routledge, E.J. and Sumpter, J.P. 1996. *Environ Toxicol Chem*. **13**:241–8; Sohoni, P. and Sumpter, J.P. 1998. *J Endocrinol*. **158**:327–39).

4.3 Procedures

The assay was run according to Xenometrix SOP's, corresponding to the XenoScreen XL YES/YAS Instructions for Use, Version 3.11.

4.3.1 Test Sample Dilutions

Test samples were serially diluted in 8 steps (half-log steps, 1:2) in water with 1% DMSO. All control dilutions contained 1% DMSO as well. The highest final concentration in the assay was 6.7E–3 due to the mixing of 80 µl sample with 40 µl cells. The other final concentrations thus were 6.7E–3, 3.33E–3, 1.67E–3, 8.33E–4, 4.17E–4, 2.08E–4, 1.04E–4, and 5.21E–5.

All sample dilutions were done immediately before use. All manipulations were done under sterile conditions.

4.3.2 Control chemicals

Positive controls:

For the YES agonist assay 17-β estradiol (E2) was used as positive controls in 8 final concentrations between 6.7 x 10^{-9} and 2.1 x 10^{-12} M using half-log dilution steps.

DMSO at 1% served as solvent control.

4.3.3 Plate Layout

Schematic representation of the <u>YES agonist</u> assay plate

	1	2	3	4	5	6	7	8	9	10	11	12
A	E2 $2.1x10^{-12}$	E2 $2.1x10^{-12}$	SP1 Dil 8	SP1 Dil 8	SP2 Dil 8	SP2 Dil 8					Solv. Control	Solv. Control
B	E2 $6.7x10^{-12}$	E2 $6.7x10^{-12}$	SP1 Dil 7	SP1 Dil 7	SP2 Dil 7	SP2 Dil 7					Solv. Control	Solv. Control
C	E2 $2.1x10^{-11}$	E2 $2.1x10^{-11}$	SP1 Dil 6	SP1 Dil 6	SP2 Dil 6	SP2 Dil 6					Solv. Control	Solv. Control
D	E2 $6.7x10^{-11}$	E2 $6.7x10^{-11}$	SP1 Dil 5	SP1 Dil 5	SP2 Dil 5	SP2 Dil 5					Solv. Control	Solv. Control
E	E2 $2.1x10^{-10}$	E2 $2.1x10^{-10}$	SP1 Dil 4	SP1 Dil 4	SP2 Dil 4	SP2 Dil 4					Solv. Control	Solv. Control
F	E2 $6.7x10^{-10}$	E2 $6.7x10^{-10}$	SP1 Dil 3	SP1 Dil 3	SP2 Dil 3	SP2 Dil 3					Solv. Control	Solv. Control
G	E2 $2.1x10^{-9}$	E2 $2.1x10^{-9}$	SP1 Dil 2	SP1 Dil 2	SP2 Dil 2	SP2 Dil 2					Solv. Control	Solv. Control
H	E2 $6.7x10^{-9}$	E2 $6.7x10^{-9}$	SP1 Dil 1	SP1 Dil 1	SP2 Dil 1	SP2 Dil 1					Solv. Control	Solv. Control
	E2	E2	SP1	SP1	SP2	SP2	SP3	SP3	SP4	SP4	SC	SC

E2 = 17β-estradiol SP1…4 = Samples1-4 SC = Solvent control

4.3.4 Assay Procedure

The procedure followed the XenoScreen XL YES/YAS Instructions for Use, Version 3.11, which is analogous to Xenometrix SOP's. Briefly:

- The samples and controls dilutions (80 µl each) were prepared in 1% DMSO in water directly in the assay plates
- Yeast cells in concentrated medium were added to give a final volume of 120 µl
- The plates were sealed with gas-permeable plate sealers and incubated for 18 hrs at 31°C in a humidified chamber with agitation at 100 rpm
- After 18 hrs the wells were shaken to homogenize their content and the optical density was measured at 690 nm. These data served to determine Growth Factors which were used to determine toxicity.
- 30 µl of the cell suspensions were added to 50 µl of a lysis buffer containing lyticase to facilitate the release of β-galactosidase.
- After incubation at 31°C for 30 – 60 minutes OD_{570} and OD_{690} were measured.

4.4 Calculations and Data Analysis

- For each well the OD_{690} values were subtracted from the OD_{570} values.
- The mean OD values for every test sample concentration and for the controls were calculated.
- Growth Factor G and Induction Ratio I_R were calculated based on the definitions given below
- Dose-response curves were drawn which served as the basis for conclusions on the samples' activities

Growth factor G: $G = \frac{A_{690,S}}{A_{690,N}}$

where $A_{690,S}$ is the absorbance of the sample S at 690 nm
$A_{690,N}$ is the absorbance of the solvent control at 690 nm

Induction Ratio I_R: $I_R = \frac{1}{G} \times \frac{A_{570,S}}{A_{570,N}}$

where $A_{570,S}$ is the corrected absorbance of the sample S at 570 nm - 690 nm
$A_{570,N}$ is the corrected absorbance of the solvent control at 570 nm - 690 nm

Limit of Detection LoD = Mean of solvent control + 3 Standard Deviations
Limit of Quantification LoQ = Mean of solvent control + 9 Standard Deviations

All calculations and graphs were made using the XenoScreen XL Excel calculation Sheet, version 3.41.

<u>Data interpretation</u>

Growth factors G ≤ 0.5 are indicative of a toxic effect. Results obtained at sample concentrations where G is ≤ 0.5 are ignored for the interpretation of hormonal activity.

For agonist activities, Induction Ratios ≥ IR10 are considered positive. IR10 is defined as the IR which is 10% of (IR max - IR solvent) above the IR solvent.

For antagonistic effects Induction Ratios ≤ IR50 are considered positive. IR50 is defined as the IR which is at 50% of the IR of the solvent control with a fixed amount of agonist E2 or DHT. Induction ratios at toxic concentrations (G ≤ 0.5) are ignored.

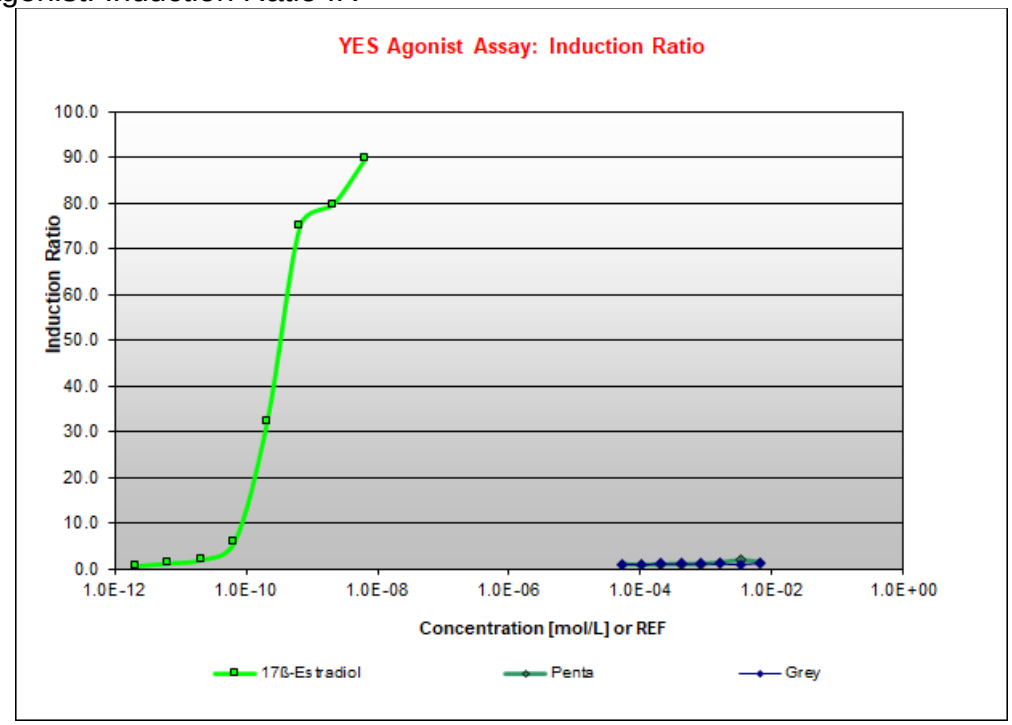
Inhibition may also be caused by non-specific effects which lead to a reduction of the response induced by the fixed concentrations of E2 and DHT. Parallel inhibition in the YES assay may be indicative of such non-specific inhibition which is not a true estrogen or androgen antagonistic effect.

5. Results

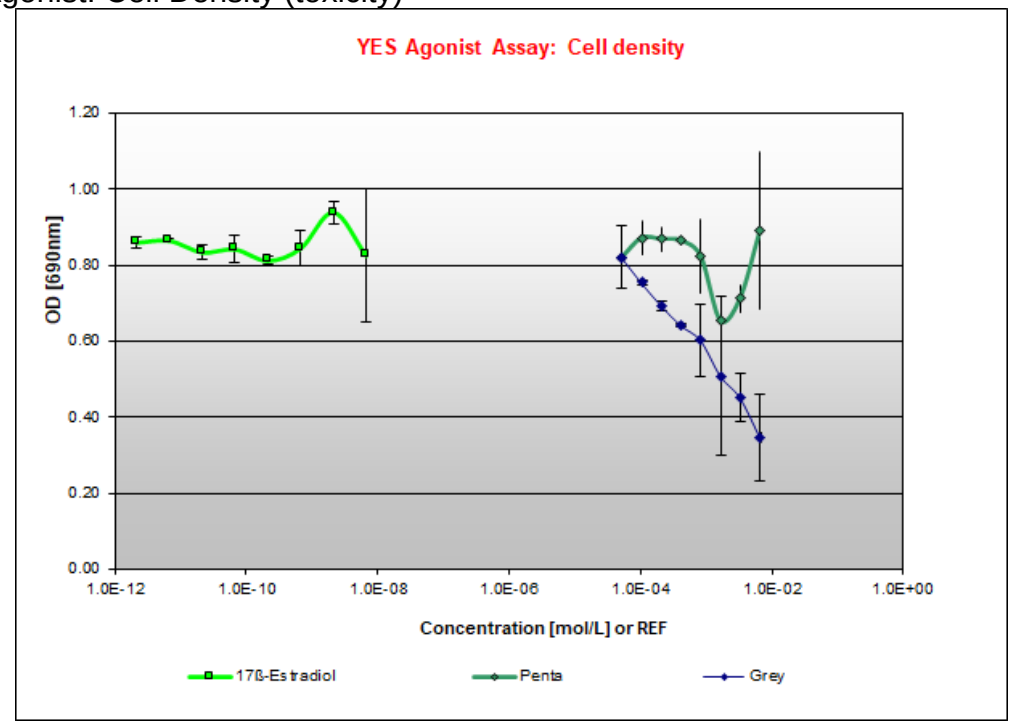
5.1 Estrogenic Agonist and Antagonist Assay (YES)

(For detailed data please see Appendix I)

YES Agonist: Induction Ratio IR



YES Agonist: Cell Density (toxicity)



5.3 Summary Table

	Toxicity[1)]	Estrogenic agonist activity?	Agonist activity EC50[2)]	average EEQ (ng/L)
TX 19005	nt	inactive	–	–
TX 19011	nt	inactive	–	–

nt = not toxic at the concentrations tested. inactive = no agonist or antagonist activity at any tested non-toxic concentration; n.m.: not measured.

[1)]: Highest non-toxic concentration, G > 0.5 in agonist assay
[2)]: IR ≥ IR10. IR10 is defined as the IR which is 10% of (IR max - IR solvent) above the Induction Ratio IR of the solvent control.

5.4 Interpretation

Toxicity:
No sample showed toxicity (growth inhibition, defined as G ≤ 0.5) at any tested concentrations.

YES Activity:
No sample showed estrogenic agonist activity on the YES strain in our experiments. The limit of detection (LoD) for estrogenic activity was 1.49×10^{-11} M 17β-estradiol.

6. Conclusions

In this XenoScreen XL YES assay (i) the tested samples were not toxic and (ii) the tested samples showed no estrogenic agonist activity at the tested concentrations.

Appendix I: Raw and Calculated Data - YES Agonist Assay

ß-Gal expression

REF or Conc.	17ß-Estradiol	SD	Penta	SD	Grey	SD	Solvent Control	SD
2.11E-12	0.019	0.013	0.031	0.004	0.030	0.001	0.030	0.002
6.67E-12	0.037	0.005	0.026	0.002	0.029	0.001	0.029	0.002
2.11E-11	0.056	0.008	0.030	0.002	0.030	0.004	0.031	0.001
6.67E-11	0.164	0.056	0.031	0.004	0.027	0.001	0.027	0.001
2.11E-10	0.868	0.221	0.030	0.001	0.027	0.001	0.026	0.003
6.67E-10	2.095	0.218	0.031	0.001	0.022	0.000	0.027	0.001
2.11E-09	2.470	0.151	0.046	0.001	0.017	0.001	0.025	0.001
6.67E-09	2.450	0.107	0.044	0.010	0.018	0.001	0.026	0.000

Cell density

REF or Conc.	17ß-Estradiol	SD	Penta	SD	Grey	SD	Solvent Control	SD
2.11E-12	0.860	0.016	0.820	0.018	0.821	0.082	0.829	0.001
6.67E-12	0.865	0.006	0.871	0.044	0.754	0.007	0.830	0.045
2.11E-11	0.835	0.018	0.869	0.032	0.694	0.013	0.815	0.054
6.67E-11	0.843	0.035	0.865	0.008	0.642	0.005	0.861	0.023
2.11E-10	0.813	0.010	0.824	0.097	0.602	0.095	0.809	0.028
6.67E-10	0.845	0.045	0.654	0.046	0.509	0.210	0.842	0.006
2.11E-09	0.939	0.029	0.713	0.035	0.451	0.063	0.798	0.011
6.67E-09	0.827	0.175	0.890	0.206	0.347	0.112	0.849	0.023

Growth factor G (for the definition see Instructions for Use)

REF or Conc.	17ß-Estradiol	Penta	Grey	Solvent Control
2.11E-12	1.038	0.989	0.990	1.000
6.67E-12	1.044	1.051	0.910	1.001
2.11E-11	1.007	1.048	0.837	0.983
6.67E-11	1.016	1.043	0.774	1.038
2.11E-10	0.981	0.994	0.726	0.976
6.67E-10	1.019	0.788	0.613	1.015
2.11E-09	1.132	0.860	0.544	0.963
6.67E-09	0.997	1.074	0.418	1.024

G ≤ 0.5

β-galactosidase activity U_S (for the definition see Instructions for Use)

REF or Conc.	17ß-Estradiol	Penta	Grey	Solvent Control
2.11E-12	0.022	0.037	0.037	0.036
6.67E-12	0.042	0.029	0.038	0.034
2.11E-11	0.066	0.034	0.043	0.037
6.67E-11	0.194	0.036	0.042	0.031
2.11E-10	1.067	0.036	0.045	0.032
6.67E-10	2.479	0.047	0.043	0.032
2.11E-09	2.631	0.065	0.038	0.031
6.67E-09	2.964	0.049	0.051	0.031

Induction Ratio I_R (for the definition see Instructions for Use)

REF or Conc.	17ß-Estradiol	Penta	Grey	Solvent Control
2.11E-12	0.651	1.126	1.106	1.078
6.67E-12	1.278	0.886	1.165	1.040
2.11E-11	2.013	1.028	1.288	1.133
6.67E-11	5.876	1.086	1.274	0.950
2.11E-10	32.308	1.085	1.358	0.973
6.67E-10	75.051	1.413	1.310	0.972
2.11E-09	79.673	1.953	1.143	0.930
6.67E-09	89.755	1.497	1.529 Toxicity!	0.928

IR ≥ IR10 (≥ 9.498)

IR10 = 10 percent increase

IEC FRANCE

REPORT: IN VITRO SAFETY STUDY

CUTANEOUS PRIMARY IRRITATION

STUDY PERFORMED ON AN EPISKIN® RECONSTRUCTED HUMAN EPIDERMIS MODEL
MTT conversion assay
Evaluation of the interaction of the test item with MTT and staining power
(According to the OECD guideline n° 439 of 28 July 2015)

TEST ITEM	:	**PENTA 18 479** (ref.: TX 19011 – batch n° B1 of 06/01/2020)
I.E.C. CODE NUMBER	:	200840 00025001
GENERAL STUDY PLAN	:	N° 20200038
SPECIFIC STUDY PLAN (GLP STUDY NUMBER)	:	N° 200840SSP
SCHEDULE	:	Experiment starting date: 07/07/2020 Experiment completion date: 10/07/2020
REPORT (STUDY COMPLETION)	:	**N° 200840RD**
SPONSOR	:	**SEDERMA** 29 rue du Chemin Vert 78612 Le Perrey-en-Yveline - France
SPONSOR'S REPRESENTATIVE	:	Vincent VICEDO
TEST FACILITY	:	**I.E.C. France** 88 Bd des Belges - 69006 Lyon - France
STUDY DIRECTOR	:	Nathalie CANNAMELA Graduate in Applied Biology and Biotechnology

Template: RD-cutaneous irritation-OCDE-03-13-2019

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AUTHENTICATION

The study described in the present report was conducted under my responsibility, in compliance with the General and Specific Study Plans, and in accordance with I.E.C. Standard Operating Procedures and Good Laboratory Practices (GLP) for cosmetic products (decree of 10 august 2004 published in the Journal Officiel de la République Française of 18 September 2004).

All observations and data obtained during this study are reported in the present document.
I have read this report, I certify that these data are an accurate reflection of the results obtained and I agree with its content.

JUDGMENT
(degree of compliance of the study with the Good Laboratory Practices)

The study was conducted in compliance with the principles of the Good Laboratory Practices and **is declared in compliance with the G.L.P.**

Lyon,

23/07/2020

Nathalie CANNAMELA
Study Director

QUALITY ASSURANCE

A Facility audit is conducted every two years by an external consultant and **audits of in vitro quality system** are regularly carried out by the Quality Assurance.

The audit of the General Study Plan was performed when the Quality Assurance signed it.

The process audit of this type of study is carried out at least once a semester.

The Specific Study plan, the raw data and the study report were audited by the Quality Assurance.
This report is an accurate account of the procedures followed, and accurately records the original raw laboratory data generated in this study.

The date of each audit and the transmission's dates of each audit report to the Study Director and to the Test Facility Management are given below:

	Audit	Study Director	Test Facility Management
Facility	13 & 14/02/2020	09/06/2020	09/06/2020
In vitro quality system	21/05/2019	28/05/2019	28/05/2019
General Study Plan	23/06/2020	23/06/2020	23/06/2020
Process	14/01/2020	16/01/2020	16/01/2020
Specific Study plan, raw data and study report	17/07/2020	17/07/2020	17/07/2020

23/07/2020

Lyon,

Etienne DALACHE
Quality Director

1. PROTOCOL

1.1. Study objective

To predict and classify the skin irritation potential of a test item, by assessment of its effect on an EPISKIN® reconstructed human epidermis (RhE) model (EPISKIN/S/13) according to the UN GHS classification.

1.2. Type of study

In vitro study performed on an EPISKIN® reconstructed human epidermis model (EPISKIN/S/13), according to the OECD Guideline n° 439 of 28 July 2015.
Given our historical data and the geographical proximity of the supplier, the chosen model is the one proposed by EPISKIN Laboratories.

1.3. Study relevance

Current guidelines include OECD guideline n° 404 for acute dermal irritation and corrosion of chemicals (Anon., 1992). This guideline is based on the method described by Draize (Draize and al., 1994), and generally involves the rabbit as the experimental animal. An ECVAM workshop allowed to take stock on the various methods for in vitro assessment of the cutaneous irritant potential of chemicals (J. Van de Sandt and al., 1999) and an ECVAM validation of related methods was undertaken (P.A. Botham and al., 1998). During the prevalidation step, methods and subsequent refinements were proposed (V. Zuang and al., 2002; P. Portes and al., 2002; J. Cotovio and al., 2005).

Pre-validation, optimisation and validation studies have been completed for three commercially available *in vitro* test methods, including the one using the model Episkin (H. Spielmann and al., 2007; J. Cotovio and al., 2007), which has been used to develop the present guideline and is referred as Validated Reference Methods (VRM).
In April 2007, the ECVAM Scientific Advisory Committee endorsed the scientific validity of the EPISKIN test as a replacement for the rabbit skin irritation method (H. Spielmann et al., 2007)

This test (Episkin™ SOP, version 1.8, February 2009) was adopted by OECD (July 2010), to identify labelled substances, as defined by the United Nations Globally Harmonized System of Classification and Labelling of Chemicals (UN GHS) (Category 2).
This OECD guideline was then updated in July 2013, to add a fourth method on a new reconstructed human epidermis model and updated in July 2015, to refer to the IATA guidance document and to introduce the use of an alternative procedure to measure viability.

The Guideline is based on the in vitro test system of reconstructed human epidermis, which closely mimics the biochemical and physiological properties of the upper parts of the human skin, i.e. the epidermis. This test system uses human derived non-transformed keratinocytes as cell source to reconstruct an epidermal model with representative histology and cytoarchitecture.

The test chemical is applied topically to a three-dimensional RhE model.
Cell viability in RhE models is measured by enzymatic conversion of the vital dye MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] (T. Mosmann, 1983), into a blue formazan salt that is quantitatively measured after extraction from tissues. The MTT measures the mitochondrial succinate dehydrogenase activity by the conversion of a dye, MTT, into formazan. Relative viability will be evaluated by comparison to a negative control and expressed as a percentage.

This MTT conversion method has been used to evaluate irritancy potential of several products on monolayer cultures and three-dimensional cultures models (Gay et al., 1992; Roguet et al., 1992; Decker et al., 1994).

Irritant test items are identified by their ability to decrease cell viability below defined threshold levels (≤ 50%). The viability measurement is not performed immediately after exposure to the test item, but after a sufficiently long post-treatment incubation period (42 hours) of the rinsed tissue in fresh medium. This period allows both for recovery from weak cytotoxic effects and for appearance of clear cytotoxic effects.

1.4. Test system

EPISKIN® kit: three-dimensional reconstructed human epidermis SMALL model (EPISKIN/S/13 - size of 0.38 cm^2), supplied by EPISKIN Laboratories (69007 Lyon - France) constituted by:
- a collagen type I matrix, coated with type IV collagen
- a differentiated and stratified epidermis model from human keratinocytes (adult donors), obtained after 13-day culture period.

The maintenance and assay medium were provided by EPISKIN Laboratories.
All biological components of the epidermis and of the medium were tested by EPISKIN Laboratories (absence of viruses, bacteria and mycoplasma).
The quality was assessed by EPISKIN Laboratories by a MTT cytotoxicity test (to evaluate barrier function with SDS) and by histological examination. A certificate of compliance was provided for each batch of test system and is annexed to the report. A technical data sheet was provided for each batch of maintenance and assay medium and is annexed to the report.
Epidermises were treated at D15.
This test system is adapted to evaluate the cutaneous irritancy potential and is recommended by the method published in the OECD Guideline n° 439 of 28 July 2015.

1.5. Methodology

CHECKING THE NON-SPECIFIC REDUCTION OF MTT

Since the MTT conversion is the parameter studied in this trial, the possible interaction between the test item and the MTT was studied. If the test item has interacted with the MTT, an additional test on dead epidermises was performed, in order to deduce the non-specific MTT conversion by the test item.

Preparation of the staining solution

♦ MTT main solution (CAS N° 298-93-1):
- prepare extemporaneously a 3 mg/mL solution (W/V) in PBS^+ (Phosphate Buffered Saline with Ca^{2+} and Mg^{2+}) preheated to 37 ± 2 °C during at least 30 minutes
- stir, away from light, for 15 ± 2 minutes using a stirring rod

♦ ready to use solution:
- main solution to be diluted to 1/10th (V/V) in PBS^+ preheated to 37 ± 2 °C during at least 30 minutes (final concentration of 0.3 mg/mL)
- protect from light, to use within one hour.

Checking the non-reduction of the MTT by the test item

- using a micropipette, deposit, in a 12-well plate, 2 mL per well of the MTT ready to use solution at 0.3 mg/mL
- add 10 µL of the negative control (PBS^+) and of the test item with a micropipette and stir for about one minute
- incubation 3 hours ± 5 minutes in the CO_2 incubator (37 ± 1 °C, 5 ± 1% of CO_2, 95 ± 5% of humidity)
- the solution didn't turn into blue or violet (naked eye), the test item has not interacted with the MTT. The non-specific MTT reduction using dead epidermis was not necessary.

CHECKING THE STAINING POWER OF THE TEST ITEM

The intrinsic staining power of the test item was studied. If the test item has had a staining power in water and/or in Dimethlysulfoxide DMSO (extraction solvent), an additional trial (without MTT) was performed in order to determine the ability of the test item to stain the epidermises in a non-specific way.

- deposit, into 2 microtubes, 10 µL of the test item using a micropipette
- using a micropipette, add 90 µL of sterile water (CAS N° 7732-18-5) in on tube and 90 µL of DMSO (CAS N° 67-68-5) in the other and stir during 15 ± 0.5 minutes at room temperature
- the solutions were not colored (to the naked eye): the test item has no staining power in the vehicle: the trial without MTT colouring was not necessary.

MTT CONVERSION ASSAY

After having assessed the possible staining power and the possible interaction of the test item with the MTT, the irritancy potential of the test item was studied.

Receipt and preparation of the epidermises

- upon receipt (14th day of culture): check the shipping date, the temperature indicator and the gel medium colour then check the compliance certificate upon receipt
- keep the assay medium at 5 ± 3 °C (refrigerator)
- using a multistep pipette, deposit 2 mL of maintenance medium (balanced at room temperature) per well in a 12-well plate
- transfer the epidermises into the maintenance medium using sterile pliers
- incubation until the following day (CO_2 incubator).

Preparation of controls and of the test item

- negative control: PBS$^+$

- positive control: Sodium Dodecyl Sulfate (SDS – CAS N° 151-21-3) to 5% (W/V) in sterile water

- test item:
. information to characterize the test item provided by the Sponsor and verified by I.E.C.
. tested as supplied

- verification of the homogeneity and of the stability of controls and of the test item under trial conditions (observation and measurement of the pH before application and after rinsing on a sample placed in the same trial conditions).

Application of controls and of the test item

- using a multistep pipette, deposit 2 mL per well of maintenance medium (room temperature) in a new 12-well plate
- each control and test item were deposited on 3 epidermises
- deposit of 10 µL of the negative control using a positive displacement micropipette and gently spread
- deposit 10 µL of the positive control using a positive displacement micropipette and gently spread. Re-spreading with a curved spatula after a 7 minutes contact timepoint
- deposit 10 µL of the test item using a micropipette and gently spread
- contact timepoint: 15 ± 0.5 minutes at room temperature

Rinsing and post incubation

- remove the units using sterile pliers
- rinse thoroughly with about 25 mL of preheated PBS$^+$ with a multistep pipette
- remove the remaining PBS$^+$ by gently taping on absorbent paper
- transfer the epidermis gradually in well containing 2 mL of maintenance medium using sterile pliers
- incubation of the plates during 42 ± 1 hours (CO_2 incubator).

MTT colouring

♦ MTT main solution (CAS N° 298-93-1):
- prepare extemporaneously a 3 mg/mL solution (W/V) in preheated PBS*
- stir, away from light, for 15 ± 2 minutes using a stirring rod

♦ ready to use solution:
- main solution to be diluted to 1/10th (V/V) in assay medium, balanced at room temperature during at least 30 minutes (final concentration of 0.3 mg/mL)
- protect from light, to use within one hour.

- using a multistep pipette, deposit 2 mL of ready to use MTT solution per well in a 12-well plate
- remove the excess of maintenance medium from the units with absorbent paper
- deposit the epidermises in the wells containing the MTT ready to use solution using pliers
- incubate for 3 hours ± 5 minutes (CO_2 incubator).

Extraction of formazan crystals

- transfer each epidermis, using pliers, in an empty new 12-well plate, to stop MTT staining
- place each epidermis on absorbent paper using pliers
- place each epidermis units on the plate lid and make a total biopsy of the epidermis by using a special biopsy punch (0.7 cm diameter)
- gently separate the epidermis from the collagen matrix using pliers, and place both parts into microtubes
- add 500 µL of DMSO per microtube using a multistep pipette
- close each microtube and shake until all the test system is immerged
- verification that all the biological material is in the solvent
- extract for 1 hour ± 10 minutes at room temperature and away from light.

Reading of the optical density (O.D.)

- shake each microtube until obtaining a homogeneous colour
- using a micropipette, transfer 2 x 200 µL of each sample (2 wells per epidermis) into a 96-well microplate
- measure the O.D. at 570 nm on a plates reader (blank = DMSO)

Determination of the linearity range of plate reader (with MTT formazan solution):
Every year, plotting a standard curve connecting the O.D. to the formazan concentration (12.5-25-40-60-80-100-120-140-150-155-160-180 and 200 µg/mL).
It also permitted to determine a value of optical density (3.000) beyond which samples have to be diluted (beginning of the plate stage). Each sample of which the O.D. exceeded this value were diluted to 1/4 in DMSO. The O.D. value obtained were then multiplied by 4, during the final calculations.

1.6. Data analysis

Abbreviations:
$O.D._{NC}$ = mean O.D. of the 3 negative control living epidermises
$O.D._{TV}$ = mean O.D. of each treated living epidermis

Calculation of viability (normal calculation method)

- data acquisition (subtracted blanks) under a Microsoft Excel form (current version)
- calculation of the mean, standard deviation and Coefficient of variation (C.V.) of the O.D. per epidermis for each control and test item
- calculation of the mean, standard deviation and C.V. of the O.D. for 3 epidermises for each control and test item

- calculation of the viability % per epidermis for positive control and test item ("normal calculation method")

viability % = [O.D. treated / Mean O.D. negative control] x 100

- calculation of the mean, standard deviation and C.V. of viability % for positive control and test item

Smoothing of the viability percentage

The viability percentages, which were superior to 100%, were smoothed to 100%.

Classification

Irritancy potential of the test item, according to the UN GHS and EU CLP, was predicted by the mean viability percentage:

VIABILITY	CLASSIFICATION
Mean viability ≤ 50%	Irritant (Category 2)
Mean viability > 50%	Non-Irritant (No category)

1.7. Validation of the trial

- O.D. of blank (DMSO) was < 0.100
- for living epidermises: mean O.D. of the negative control: was ≥ 0.600 and ≤ 1.500
- mean viability percentage of the positive control ≤ 40%
- for a same epidermis, C.V. of the 2 O.D. was < 20%
- for 3 epidermises treated on the same way, the C.V of the O.D. was ≤ 20%
- for 3 epidermises treated on the same way, the C.V of the viability percentage was ≤ 18%.

1.8. Interpretation of the results

Under the experimental conditions adopted, basing on the obtained results.

2. CHARACTERISATION OF THE TEST ITEM

By the Sponsor

Designation	**PENTA 18 479** (ref.: TX 19011 – batch n° B1 of 06/01/2020)
Type of product	Ingredients combination
Storage conditions	Room temperature
Stability under the storage conditions	confirmed by the Sponsor
Manufacturing date	06/01/2020
Shelf life	06/01/2022
Aspect of the provided product	Colorless liquid
pH	4
Qualitative composition	Glycerin, aqua, Pal-KTSKS
Other characterization data	Pal KTSKS-OH content (HPLC): 1040 ppm (see appendix)
Conformity of the manufacturing with the composition	certified by the Sponsor

By I.E.C.

Homogeneity under storage conditions	yes (checked by I.E.C.)
pH of the test item	3.92
Aspect of the test item	Colorless liquid
Homogeneity and stability under the trial conditions	yes

3. RESULTS AND CONCLUSION

(see appendix for compiled data)

3.1. Results

CHECKING THE NON-SPECIFIC REDUCTION OF MTT BY THE TEST ITEM

The test item, as supplied, didn't interact with MTT

CHECKING THE STAINING POWER OF THE TEST ITEM

The test item, as supplied has not a staining power.

IRRITANT POTENTIAL

	O.D. (570 nm)	Viability (%)
Negative control: PBS*	0.895 ± 0.041	100%
Positive control: SDS to 5% (W/V)	0.310 ± 0.029	33.3 ± 3.4%

Test item	Viability (%)	Classification
As supplied	100.0 ± 4.4%	Non-Irritant (No category)

3.2. Conclusion

From the results obtained under the experimental conditions adopted, the test item designated as "PENTA 18 479 (ref.: TX 19011 – batch n° B1 of 06/01/2020)", applied as supplied on a reconstructed human epidermis, must be LABELLED and can be considered as NON-IRRITANT (No Category).

Lyon,

23/07/2020

Nathalie CANNAMELA
Study Director

4. AMENDMENTS AND COMPLIANCE TO THE STUDY PLAN

No amendment to the study plan was issued during the course of this study.

During the experiment, no incident that could have affected the quality or the interpretation of the results obtained was observed.

It should be noted that archiving outsourced to the company EVERIAL is not performed under GLP conditions. However, I.E.C. regularly audits this company according to ISO 9001 requirements and OECD monograph No. 15 and ensures the good traceability, security and durability of these archives. This deviation has no impact on the study.

5. STORAGE AND ELIMINATION OF THE TEST ITEM, OF THE CONTROL PRODUCTS AND OF THE TEST SYSTEM

The test item is kept according to the Sponsor's recommendations, under lock and key at I.E.C France facilities for approximately 4 months from the date of the final report.
From this date onward, and barring contrary advice from the Sponsor, the test item will be destroyed by a company approved for this service.
The reagents used for the controls will be stored under appropriate conditions and will be destroyed by a company approved for this service.
All the test systems will be destroyed by a company approved for this service.

6. DATA RECORDING AND ARCHIVING

All hand-written data were immediately transcribed in Case Report Form (CRF), paginated and stapled before the beginning of the experiment.

Once the final report is signed and sent, the Study Director will transfer the original documents for archiving, including the General and Specific Study Plans, the final report, the possible amendments and all raw data, which will be kept for 5 years by:
. for a maximum of 6 months following dispatch of report: in the archives of I.E.C. France (88 boulevard des Belges - 69006 Lyon - France)
. for the following years: in the premises of the EVERIAL company – site of Rillieux-la-Pape (69140) – France.

Once this period is over, the Sponsor will be contacted regarding its archives. No archive document will be destroyed without written and signed agreement from the Sponsor.

7. GUIDELINES

Application of the GLP Principles to short term studies, ENV/JM/MONO(99)23.

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Arrêté du 10 août 2004 pris pour l'application de l'article L.5131-5 du code de la santé publique relatif aux Bonnes Pratiques de Laboratoire pour les produits cosmétiques (Journal Officiel n° 218 du 18 septembre 2004), Ministère de la Santé et de la Protection sociale.

Avis aux fabricants ou aux responsables de la mise sur le marché d'un produit cosmétique relatif à l'information concernant l'évaluation de la sécurité pour la santé humaine prévue à l'article R.5131-2 (4) du code de la santé publique au regard notamment des essais de sécurité devant être réalisés selon les principes de bonnes pratiques de laboratoire (BPL) figurant en annexe de l'arrêté du 10 août 2004.
Bulletin officiel Santé – Protection Sociale – Solidarité (N° 2010/5 - 15 juin 2010).

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Spielmann, H., Hoffmann, S., Liebsch, M., Botham, P., Fentem, J., Eskes, C., Roguet, R., Cotovio, J., Cole, T., Worth, A., Heylings, J., Jones, P., Robles, C., Kandárová, H., Gamer, A., Remmele, M., Curren, R., Raabe, H., Cockshott, A., Gerner, I. and Zuang, V. (2007), The ECVAM international validation study on in vitro tests for acute skin irritation: Report on the validity of the EPISKIN and EpiDerm assays and on the skin integrity function test, ATLA 35, 559-601.

Validation of the Episkin® skin irritation test$^{42\ hours}$ assay for the prediction of acute skin irritation of chemicals – Determination of IL-1α concentration in the culture medium; version 1.2 of September 2005.

Validation of the Episkin™ test method$^{15min\text{-}42\ hours}$ for the prediction of acute skin irritation of chemicals; version 1.8 of February 2009.

Van de Sandt J., Roguet R., Cohen C., Esdaille D., Ponec M., Corsini E., Barker C., Fusenig N., Liebsch M., Benford D., de Brugerolle de Fraissinette A., Fartash M. (1999) The use of human keratinocytes and human skin models for predicting skin irritation. The report and recommendations of ECVAM Workshop. *ATLA* **27**, 723-743.

Zuang V., Balls M., Botham P.A., Coquettte A., Corsini E., Curren R.D., Elliot G.R., Fentem J.H., Heylings J.R., Liebsch M., Medina J., Roguet R., Van de Sandt J.J.M., Wiemann C., Worth A.P. (2002) Follow-up to the ECVAM prevalidation study on in vitro tests for acute skin irritation. ECVAM Skin irritation Task Force Report 2. *ATLA* **30**, 109-1029.

APPENDIX

O.D. MEASUREMENT AND CALCULATION

EXPERIMENTAL TECHNICIAN: Caroline SING NGUYEN VAN BACH

Starting date: 07/07/2020

End date: 10/07/2020

	Epidermis 1					Epidermis 2					Epidermis 3				
	OD 1	OD 2	Mean	Standard deviation	CV	OD 1	OD 2	Mean	Standard deviation	CV	OD 1	OD 2	Mean	Standard deviation	CV
TN living (DO_{TN})	0.826	0.870	0.848	0.031	3.7	0.906	0.904	0.905	0.001	0.2	0.928	0.938	0.905	0.001	0.2
TP	0.348	0.319	0.334	0.021	6.1	0.261	0.300	0.281	0.028	9.8	0.307	0.327	0.281	0.028	9.8
viability % per epidermis	37.2					31.3					31.3				
P1 living (DO_{P1})	0.921	0.978	0.950	0.040	4.2	0.901	0.882	0.892	0.013	1.5	0.869	0.881	0.875	0.008	1.5
viability % per epidermis	106.0					99.6					97.7				

	Mean	Standard deviation	CV	Mean viability %	Mean smooth viability %	Standard deviation	CV
TN living (DO_{TN})	0.895	0.041	4.6	100.0			
TP	0.310	0.029	9.5				
viability % per epidermis				33.3	33.3	3.4	10.3
P1 living (DO_{P1})	0.905	0.040	4.4				
viability % per epidermis				101.1	100.0	4.4	4.3

OD = Optical Density
PC = Positive Control
CV = Coefficient of Variation

NC = Negative Control
P1 = Test item

DATA ABOUT TEST ITEM CHARACTERISATION

sederma	ENREGISTREMENT QSHE	Codification EE1/33 Date : 02/04/15 Révision : c
	BULLETIN D'ANALYSES *CERTIFICATE OF ANALYSIS*	Page 1 sur 1

PRODUIT / *PRODUCT* : TX 19011

N° de LOT / *BATCH Nr* : B1

Stockage / *Storage* : **Stockage recommandé longue durée entre +15°C et +25°C.**
Long storage recommended between +15°C and +25°C

Date de fabrication / *Date of manufacturing* : 06 janvier 2020 **Date de retest** / *Retest date* : 06 janvier 2022

Les analyses effectuées sont garanties quand le produit est stocké dans son emballage d'origine et à la température recommandée
The performed analysis are guaranteed when product is stored in original packaging and at recommended temperature

	Résultats *Results*
Aspect *Appearance*	Liquide limpide incolore *Clear liquid colourless*
Teneur en Pal KTSKS-OH (HPLC) *Pal KTSKS-OH content (HPLC)*	1040ppm

Liliane IACUZZI
Responsable Assurance Qualité Produit / Product Quality Assurance Manager

Ce document est une copie informatique et de ce fait ne porte pas de signature / This certificate is a computer printout and therefore has no signature

SEDERMA 29 rue du Chemin Vert – 78610 LE PERRAY EN YVELINES – France
Tél. : 01.34.84.10.10 – Fax : 01.34.84.11.30

CERTIFICATE OF ANALYSIS OF THE TEST SYSTEM AND TECHNICAL DATA SHEET OF THE MAINTENANCE AND ASSAY MEDIUM

NAME

EpiSkin™ Small / Human Epidermis (SM/13)

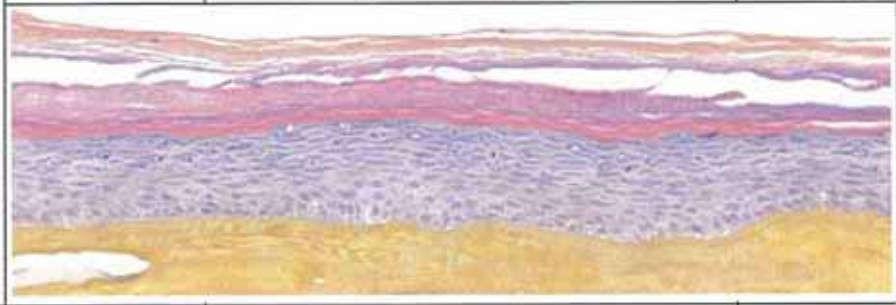
DESCRIPTION

0.38 cm² reconstructed epidermis of normal human keratinocytes. Cells are grown on a collagen matrix, for 13 days

BATCH : **20-EKIN-028**
ORIGIN : Adult donors
USAGE : FOR SCIENTIFIC USE ONLY - PRODUCT OF HUMAN ORIGIN
STORAGE : This product was prepared and packaged using aseptic techniques. Store in an incubator at 37°C, 5% CO2 with saturated humidity

QUALITY CONTROLS

Control # E200548

	Process	Specification	Result
HISTOLOGY	HES stained paraffin section	Multi-layered, highly differentiated epidermis consisting of organized basal, spinous and granular layers, and a multilayered *stratum corneum*	Satisfactory
		Number of cell layers ≥ 4	8 cell layers
			
IC50 DETERMINATION	SDS concentration, MTT test.	1.5 mg/mL ≤ IC50 ≤ 3.0 mg/mL	2.2 mg/mL

BIOLOGICAL SAFETY:

On blood of the donors, we have verified the absence of HIV1 and 2 antibodies, hepatitis C antibodies and hepatitis B antigen HBs.
On cells from the donors, we have verified the absence of bacteria, fungus and mycoplasma.

SUGGESTED EXPIRATION DATE:

July 13, 2020

Lyon, July 7, 2020
Certified and released by **Anaïs JENSEN**, Quality Control Manager

Manufactured in accordance to the ISO9001 quality system of Episkin.

The use of this human tissue is strictly limited to *in vitro* testing. All other manipulations of this tissue such as: extraction and maintenance of single cells in culture, use of the tissue for diagnostic or therapeutic purposes and in human subjects, are strictly prohibited.

ISO 9001 Certified

4, rue Alexander Fleming - 69366 Lyon Cedex 07 - France - Tél : +33 (0)4 37 28 72 00 - Fax : +33 (0)4 37 28 72 28
S.A. au capital de 13 608 807 € - 412 127 565 R.C.S. Lyon - NAF : 7211 Z - N° TVA Intracommunautaire FR 46 412 127 565
www.episkin.com

EPISKIN

TECHNICAL DATA SHEET

MAINTENANCE MEDIUM FOR

RECONSTRUCTED HUMAN TISSUE:

EPISKIN Large EPISKIN Small

Batch N° :	**20-MAIN3-018**
Expiration date :	**15 July 2020**
Storage :	**Store at 2-8 °C, protected from light.**
Usage:	**FOR SCIENTIFIC USE ONLY, use the medium at room temperature**
Handling :	**See Directions for use**

This medium has been prepared under aseptic conditions

ISO 9001 Certified
4, rue Alexander Fleming - 69366 Lyon Cedex 07 - France - Tél : +33 (0)4 37 28 72 00 - Fax : +33 (0)4 37 28 72 28
S.A. au capital de 11 608 807 € - 412 127 565 R.C.S. Lyon - NAF : 7211 Z - N° TVA Intracommunautaire FR 46 412 127 565
www.episkin.com

EPISKIN

TECHNICAL DATA SHEET

ASSAY MEDIUM FOR

RECONSTRUCTED HUMAN TISSUE:

EPISKIN Large | EPISKIN Small

Batch N° :	20-ESSC-018
Expiration date :	15 July 2020
Storage :	Store at 2-8 °C, protected from light.
Usage:	FOR SCIENTIFIC USE ONLY, use the medium at room temperature
Handling :	See Directions for use

This medium has been prepared under aseptic conditions

STATEMENT OF COMPLIANCE WITH GLP (SAFETY TEST)

ansm
Agence nationale de sécurité du médicament et des produits de santé

RÉPUBLIQUE FRANÇAISE

Direction de l'inspection

EVALUATION DE LA CONFORMITE AUX BONNES PRATIQUES DE LABORATOIRE

selon la directive 2004/9/CE (ESSAIS DE SECURITE)

STATEMENT OF COMPLIANCE WITH GOOD LABORATORY PRACTICES

according to Directive 2004/9/CE (SAFETY TESTS)

Nom et adresse de l'installation d'essai : *Name and location of the test facility*
IEC
88, Boulevard des Belges
69006 LYON

Objet de l'inspection : *Purpose of the inspection*
état des lieux : ☒ *Test facility inspection*
vérification d'étude(s) : ☒ *Study audit*

Date(s) d'inspection : *Date(s) of the inspection* 15 au 16 octobre 2018

Degré de conformité aux B.P.L. * *Status* A

Degré de conformité aux BPL valant pour les études achevées entre le 24 juin 2016 et le 16 octobre 2018
Endorsement of the claim of compliance with GLP for studies performed between

Catégorie(s) d'éléments d'essai : *Types of Test items* Produits cosmétiques

Domaine d'activité : *Areas of expertise* Tests de cytotoxicité, d'irritation et de corrosion cutanées, oculaires et sur muqueuses, phototoxicité.

Catégorie OCDE (appendice à l'annexe III de C(89)87(Final)/révisée dans C(95)8(Final))
OECD category

1	2	3	4	5	6	7	8	9

Commentaires éventuels : *Observations (if applicable)* Néant

Fait à Saint-Denis (France), le 18 DEC. 2018
Date of the statement

Le chef de pôle inspection des essais et des vigilances
Direction de l'inspection

Régis ANDRÉ

* A : conformité aux B.P.L. (*in conformity with GLP*) ;
B : conformité partielle aux B.P.L. avec déviations mineures ne remettant pas en cause la fiabilité des études réalisées (*deviations which do not affect the validity of studies conducted in the laboratory*) ;
C : absence de conformité aux B.P.L. (*not in conformity with GLP*)

Code : DOC_027_v07

SPECIFIC STUDY PLAN AND AMENDMENTS IF ANY

I E C
FRANCE

IN VITRO SPECIFIC STUDY PLAN

SPECIFIC STUDY PLAN	:	**N° 200840SSP**
SPONSOR	:	**SEDERMA**
SPONSOR'S REPRESENTATIVE	:	VINCENT VICEDO
IN VITRO STUDY	:	**CUTANEOUS PRIMARY IRRITATION ON EPISKIN® MODEL**
GENERAL STUDY PLAN	:	20200038
TEST ITEM	:	**PENTA 18 479** (ref. : TX 19011 – batch n° B1 of 06/01/2020)
I.E.C. CODE NUMBER	:	200840 00025001
CONCENTRATION	:	As supplied

ESTIMATED STUDY SCHEDULE

- Experiment starting and completion date: week of 06 July 2020
- Sending of the preliminary results by e-mail: week of 13 July 2020
- Sending of the final report: week of 27 July 2020

Lyon,
02/07/2020

NATHALIE CANNAMELA
Study Director

C.C. : Test Facility Management, Quality Assurance, Experimentators

This Specific Study Plan must be approved by the Sponsor's Representative by e-mail.
Agreement e-mail is kept with the present document.

1 page document

HUMAN PATCH TEST UNDER DERMATOLOGICAL CONTROL

Study report – version n°1 of 07/04/2021

STUDY REFERENCES — **EUROFINS EVIC france – P21 0044**
EUROFINS EVIC romania – ER 21/049

INVESTIGATIONAL PRODUCT	
Denomination	**PENTA 18 479**
Reference	**TX 19011**
Batch number	**B3**

SPONSOR	**SEDERMA** 29, rue du Chemin Vert BP 33 78612 LE PERRAY EN YVELINES
STUDY MONITOR	**Vincent VICEDO**
COORDINATING CENTRE	**EUROFINS EVIC product testing france SAS** 122, rue Croix de Seguey 33000 BORDEAUX – France Tel: +33 5 56 95 59 95 Fax: +33 5 56 95 05 22 e-mail: evic-blanquefort@evic.fr
INVESTIGATING CENTRE	**EUROFINS EVIC PRODUCT TESTING ROMANIA S.R.L** 64-66, Marasesti Boulevard, S4 040256 - Bucharest - Romania Tel.: +40 21 335 70 90 Fax: +40 21 335 70 91 e-mail: evicromania@evic.ro
SCIENTIFIC MANAGER OF THE INVESTIGATING CENTRE	**Dr Chem Eng Elena Alina Nanu** 64-66, Marasesti Avenue 040256 - Bucharest - Romania Tel: +40 21 335 70 90 Fax: +40 21 335 70 91 e-mail: ananu@evic.ro
MAIN INVESTIGATOR	**Dr Rozalia Olsavszky** (dermatologist) Registered N° (Romanian ministry of health): 461524 (specialist in dermato-venerology doctor, doctor in medical science) Tel: +40 21 335 70 90;Fax: +40 21 335 70 91 e-mail: research@evic.ro
CO-INVESTIGATORS	**Dr Monica Grigore** (resident dermatologist) and **Dr Irina Blanariu** (physician) Tel: +4 021 335 70 90; Fax: +4 021 335 70 91 e-mail: research@evic.ro

Initiation date of study performance	**22/03/2021**
Completion date of study performance	**24/03/2021**

Date of the study report: 07/04/2021

HUMAN PATCH TEST UNDER DERMATOLOGICAL CONTROL

Table of contents

HUMAN PATCH TEST UNDER DERMATOLOGICAL CONTROL

English synopsis

STUDY OBJECTIVE	To confirm the skin compatibility of the investigational product in a panel of healthy human subjects after a single application under maximising and controlled experimental conditions
SPONSOR	**SEDERMA** 29, rue du Chemin Vert BP 33 78612 LE PERRAY EN YVELINES
STUDY MONITOR	**Vincent VICEDO**
COORDINATING CENTRE	**EUROFINS EVIC product testing france SAS** 122, rue Croix de Seguey 33000 BORDEAUX – France Tel: +33 5 56 95 59 95 Fax: +33 5 56 95 05 22 *e-mail: evic-blanquefort@evic.fr*
INVESTIGATING CENTRE	**EUROFINS EVIC PRODUCT TESTING ROMANIA S.R.L** 64-66, Marasesti Boulevard, S4 040256 - Bucharest - Romania Tel.: +40 21 335 70 90 Fax: +40 21 335 70 91 *e-mail: evicromania@evic.ro*
SCIENTIFIC MANAGER OF THE INVESTIGATING CENTRE	**Dr Chem Eng Elena Alina Nanu** 64-66, Marasesti Avenue 040256 - Bucharest - Romania Tel: +40 21 335 70 90 Fax: +40 21 335 70 91 *e-mail: ananu@evic.ro*
MAIN INVESTIGATOR	**Dr Rozalia Olsavszky** (dermatologist) Registered N° (Romanian ministry of health): 461524 (specialist in dermato-venerology doctor, doctor in medical science) Tel: +40 21 335 70 90;Fax: +40 21 335 70 91 *e-mail: research@evic.ro*
CO-INVESTIGATORS	**Dr Monica Grigore** (resident dermatologist) and **Dr Irina Blanariu** (physician) Tel: +4 021 335 70 90; Fax: +4 021 335 70 91 *e-mail: research@evic.ro*
TYPE OF THE STUDY	Monocentric study performed in simple blind Study project previously approved by a survey committee
DATES OF STUDY PERFORMANCE	From March 22nd to March 24th 2021
INVESTIGATIONAL PRODUCT	**PENTA 18 479 – Ref. TX 19011 - Lot: B3**
	Modalities of use in the study: Diluted at 15% with distilled water, under semi-occlusive patch - 160 µl

English synopsis (continuation)

STUDY POPULATION	**Number of test subjects: 10** valid cases **Specific inclusion criteria: test subjects** • aged from 18 to 70 • female and/or male • with a phototype (Fitzpatrick): II to IV • with all types of skin on body • agree to wear the equipment provided by the investigating center and follow the instructions of the staff during the study **Specific non-inclusion criteria: test subjects** • with personal history of adverse reaction to: ethanol, colophony, rubber, nickel, aluminium, patch materials, adhesive plaster, • with family or personal history of atopy

METHODOLOGY	**Application of the investigational product**, by the technician in charge of the study at the investigating center to the upper back of the test subject:
	
	- diluted at 15% with distilled water,
	
	- under semi-occlusive patch: absorbent support in Webril® kept in position by a non woven medical adhesive (surface: 400 mm²) - quantity applied=160µl
	
	- during a defined time (48 +/- 4 hours)
	
	Application of distilled water to a skin site on the upper back under semi-occlusive patch and during a defined time (48 +/- 4 hours) = control site.
	
	Checking of the skin compatibility based on:
	
	• a skin examination of the treated and control areas by the same investigator or technician, supervised by the investigator, at the investigating center:
	  on D1, before patching
	  on D3, 15 to 30 minutes after patch removal
	  the analysis of the sensations of discomfort reported directly by the test subjects to the investigator or technician during the study
	
	Descriptive analysis – Percentage of reactive test subjects (erythema and other visible signs of reactivity)
	
	Expression of the results:
	
	For each test subject and each observation time, calculation of an individual daily irritation score (IDIS) = sum of the marks obtained for the visible clinical signs observed on the application area of the investigational product
	
	For the panel and each observation time, calculation of a mean daily irritation score (MDIS) according to the formula: **MDIS = Σ (IDIS) / No. of valid cases**

HUMAN PATCH TEST UNDER DERMATOLOGICAL CONTROL

English synopsis (continuation)

RESULTS

Characteristics of the included panel

Number of included subjects: 11
Number of exclusions: None
Number of withdrawals: None
Number of valid cases: 11

- Age: 27 to 69 (Mean 49)
- Sex: F/M
- Phototype: II to IV
- Skin type on the application site: with all types of skin on body

Checking of the skin compatibility

No reaction was noted on the control site.

Control time after patch removal	Types of reaction	Number of reactive subjects	% of reactive test subjects	Mean daily irritation score MDIS	Skin compatibility of the product
T 15 minutes (D3)	None	0	0%	0	Very good skin compatibility

OVERALL CONCLUSION

Under the experimental conditions adopted:

- single application of the investigational product, **PENTA 18 479 – Ref. TX 19011 - Lot: B3, diluted to 15% with distilled water,** under semi-occlusive patch on a panel of 11 female and male test subjects aged between 27 and 69 years old, with phototype II to IV and with all types of skin on body,

- the product induced no reaction of irritation and has a very good skin compatibility.

HUMAN PATCH TEST UNDER DERMATOLOGICAL CONTROL

Signatures and dates

Investigator: Dr Rozalia OLSAVSZKY (dermatologist)

I the undersigned, Dr Rozalia Olsavszky, declare that the overall conduct of the study was carried out under my responsibility in accordance with the protocol, the internal procedures and in the spirit of the principles of Good Clinical Practices (International recommendations ICH E6(R2) of 09/11/2016, Directive of the European Parliament and Council 2001/20/EC – OJ/EC of 01/05/2001), Romanian Order No. 904/25.07.2006
I assume the responsibility of the validity of all the raw data obtained during the study which are reported in the present study report.

Date: 08/04/2021

Signature:

General Manager of the investigating centre: Dr Chem Eng Elena Alina Nanu

I the undersigned, Dr Chem Eng Elena Alina Nanu, declare that the overall conduct of the study was carried out under my responsibility in accordance with the protocol and the internal procedures and in the spirit of the principles of Good Clinical Practices (International recommendations ICH E6(R2) of 09/11/2016, Directive of the European Parliament and Council 2001/20/EC.
I assume the responsibility of the validity of all the raw data obtained during the study which are reported in the present study report.

Date: 08/04/2021

Signature:

Person in charge of the quality control: Cristina Borlescu

I the undersigned, Cristina Borlescu, declare that:

- the draft of the report was audited, on 07/04/2021
- the final report was audited, on 07/04/2021
- the reported results accurately and completely reflected the raw data of the study.

Date: 08/04/2021

Signature:

HUMAN PATCH TEST UNDER DERMATOLOGICAL CONTROL

I – INITIAL PROTOCOL DESIGN

I.1. STUDY OBJECTIVE

This study intended to confirm, in a panel of healthy human subjects, the skin compatibility of the investigational product, after a single skin application under maximising and controlled experimental conditions.

I.2. ETHICS

I.2.1. Ethical conduct of the study

The study was performed in the spirit of:

- the general principles of medical ethics in clinical research coming from the Declaration of Helsinki (June 1964) and its successive amendments,
- the international recommendations relating to Good Clinical Practices for conducting clinical trials for drugs ICH E6(R2) of 09/11/2016
- the Directive of the European Parliament and Council 2001/20/EC concerning the harmonization of legislative, statutory and administrative provisions of the member States relating to the application of good clinical practices when conducting clinical trials for drugs for human use – OJ/EC of 01/05/2001,
- the recommendations of Colipa - August 1997: "guidelines for the assessment of human skin compatibility",
- the Romanian Order No. 904/25.07.2006 on approval of rules relating to the implementation of good clinical practice in the conduct of clinical trials on medicinal products for human use.

and was in accordance with the REGULATION (EU) 2016/679 OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 27 April 2016 on the protection of natural persons with regard to the processing of personal data and on the free movement of such data.

1.2.2. Relevance of the study

Based on the existing data, the aim of the study being a better knowledge of the skin safety of the investigational product and this product being applied under perfectly controlled conditions, the foreseeable risk incurred by the test subjects was minor.

The single application was performed at the investigating centre by the technician and supervised by the investigator.

Generally, in this type of study, the possible adverse effects (as erythema, vesicles…) are limited on the application area and decrease in some days.

The investigator had to ensure the clinical follow-up of the test subject(s) concerned, as long as it was necessary.

So, there was suitability between the aim of the study and its eventual risks and the foreseeable troubles related to the experimental conditions of the protocol.

The experimental conditions of product application created a certain occlusion and favoured the penetration of the product through the skin. The potential of irritation was more easily proved by this kind of approach.

A control area (without investigational product) served as control to take into account the possible effects not directly related to the investigational product but due to the patch material.

The product was applied in fixed position.

The observance of the experimental conditions by the test subjects was assessed by a questionnaire at the end of the study.

The skin examination was performed by the same investigator having the appropriate experience.

I.2.3. Survey committee

The study had to be devoid of any foreseeable serious risk for the safety of the test subjects.

According to the procedure of the investigating centre, the protocol and the informed consent form were submitted to the opinion of an Institutional Ethics Committee, formed with members living in the country where the study has been carried out.

The committee got sure that the project meets the conditions of optimal scientific rigour, assessed its general relevance, the suitability between the aim followed and the means implemented and gave an opinion on the protection of the test subjects.

The Institutional Ethics Committee gave the approval on March 19th 2021.

The study began after the approval of the Institutional Ethics Committee.

I.2.4. Information of the test subject and informed consent form

The information about the study was given to each test subject before the start of the study.

This information was accessible, understandable and suitable for each test subject. It was orally given and then in a written specific document.

This information was completed, if necessary, by the investigator (and the competent person designated) who answered all the questions asked by the test subject.

The informed consent form was personal and previous to the start of the study.

It was clear, informed and explicit. It was written and given on the same support as the information on the study, in order to avoid any risk of dispute about its content.

The content of this document particularly specified:

- that the test subject declares to have a health coverage,
- the aim of the study,
- the study design and the experimental conditions of the study,
- the investigational product conditions of use,
- the approximate number of test subjects involved in the study,
- the expected duration of the study (for the test subject),

- the number of visits to the investigating centre, their dates and their duration,
- the agreement to wear the equipment provided by the investigating center and follow the instructions of the staff during the study,
- the study constraints (obligations, restrictions and troubles),
- the reasonable foreseeable risks,
- that skin site photographs can be taken and in this case, that the test subject will not be recognizable,
- that the test subject will be requested, if necessary, to take part in a complementary test to complete the study,
- the opinion of the Institutional Ethics Committee,
- the person to contact and the contact telephone number,
- that the personal data of the test subject will be confidentially treated by the study staff, available for the study monitor and possibly consulted (with the authorization of the test subject) by the auditors, the members of the ethics committee and the Health Authorities (subject to non-divulgation),
- the ban on taking part simultaneously in other clinical studies, that could interfere with the current study
- the amount of the compensation for the constraints to be undergone,
- the form of compensation in case of possible harm caused by the study (all the costs of health care assumed through the investigating centre),
- the period of exclusion at the end of the study during which the test subject will not be allowed to take part in another clinical study,
- the confidential treatment of the study data,
- that the anonymity of the test subject will be preserved,
- the freedom for the test subject to refuse to participate or to stop his participation at any time without any justification and any legal consequences.

This document was previously approved by the Institutional Ethics Committee.

At the beginning of the study, the inform consent form will be dated and signed by the test subject and by the investigator or the competent person designated. The subject will receive a copy of informed consent form. The signed informed consent form will be kept at the investigating centre.

I.2.5. Confidentiality and identification of the test subject

The information concerning the test subject, required for his recruitment, his inclusion and particularly that related to his health, obtained during the medical examination prior to his admission in the general panel of the investigating centre, formed part of medical secret and was confidentially treated.

The test subject was coded when included in the general panel of the investigating centre (according to the corresponding procedure of the investigating centre) in order to preserve his anonymity.

If photographs of the skin had to be taken, the test subject had to be non-recognizable.

I.2.6. Insurances

<u>Insurance of the investigating centre and of the coordinating centre</u>

The investigating centre and coordinating centre are covered by an insurance guaranteeing its civil responsibility towards the test subjects: HDI-Global SE, Policy no.: 110-01325685-14023 as lead insurer and Axa Corporate Solutions as co-insurer: XFR0074974LI.

I.3. INVESTIGATING CENTRE AND STAFF

I.3.1. Investigating center

The study was performed at EUROFINS EVIC Romania, certified ISO 9001, ISO 14001 and OHSAS 18001, equipped with material and technical means suitable for clinical researches on cosmetic products and compatible with the safety requirements for human subjects.

I.3.2. Technical staff

The test was performed by a competent investigator and a trained and qualified technical staff.

Main Investigator: Dr Rozalia Olsavszky (dermatologist)

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I.3.3. Scientific management

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I.3.4. Quality assurance staff

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I.4. COORDINATING CENTRE AND STAFF

The coordinating centre ensured the liaison between the sponsor and the investigating centre.

I.5. DATES OF STUDY PERFORMANCE

Initiation date of study performance: 22/03/2021

Completion date of study performance: 24/03/2021

I.6. OVERALL STUDY DESIGN

I.6.1. Type of the study

This monocentric clinical study was performed in simple blind, in a panel of healthy human subjects.

The test subject was used as own control.

I.6.2. General principle of the study

The study was performed on the basis of the quotation 21-0219/0.

The investigational product had to be applied, in a panel of test subjects, once (on D1), to a skin area, by the technician in charge of the study at the investigating centre, under maximising conditions of exposure (under patch) and during a defined time = treated area.

A patch without investigational product but with distilled water had to be applied in parallel to a skin area during a defined time = control area.

The checking of the skin compatibility was based on:

- a skin examination of the treated and control areas, before application and after removal by the same investigator
- the analysis of the sensations of discomfort reported directly by the test subjects.

The results of the skin compatibility were mainly descriptively expressed.

I.6.3. Chronology of the study

Experimental times	Operations
D1	At the investigating centre: Delivery of the inform consent form Signature of the informed consent form Checking of the inclusion and non-inclusion criteria Inclusion of the subject in the study Clinical examination of the experimental area by the same investigator Application of the investigational product and the control patch
D3	At the investigating centre: Control of the observance Removal of the investigational product and the control patch Clinical examination of the experimental areas and questioning of the test subject by the same investigator

I.7. STUDY POPULATION

I.7.1. Constitution of the panel of test subjects and mode of recruitment

The investigating centre has at its disposal a general panel of subjects constantly renewed. These subjects come from all social categories. They either volunteer spontaneously to the investigating centre or reply to a direct call from the latter. Prior to their admission in this general panel, they are subjected to a clinical examination and a detailed cosmetological questionnaire, according to the corresponding procedure of the investigating centre.

All the data concerning the panel are computerized and on paper.
For the study, the test subjects were selected from this general panel on the basis of inclusion criteria and non-inclusion criteria specific to the study and on their ability to respect the constraints required by the protocol. They were definitely included in the study after a specific questioning and a clinical examination.

I.7.2. Number of test subjects

The number of test subjects with exploitable data (valid cases[(1)]) at the end of the study had to be at least **10**.
[(1)]valid case = test subject that respected the protocol with no significant deviation which could have some influence on the study results.

This empirically defined number was sufficient to achieve the study objectives.

At the beginning of the study, one complementary test subject (+1) was included to compensate the possible withdrawals or exclusions from the study independent of the investigational product.

The test subjects excluded from the study for reasons dependent of the investigational product had to be taken into account in the study results and did not have to be replaced.

If during the study, there was a risk not to have the required number of valid cases (great number of withdrawals…), the study monitor had to be informed and an additional quota of subjects had to be possibly included to reach the target.

At the end of the study, in spite of the precautions taken by the investigating centre, if the number of valid cases was less than the number of test subjects requested by the sponsor, the study monitor had to be informed.

I.7.3. Inclusion criteria

I.7.3.1. General inclusion criteria

According to the protocol, had to be included in the study, the subjects:

- suitable to participate in the study (after the clinical examination and questioning) and corresponding to the quality of "healthy subject" as defined in the corresponding procedure of the investigating center,
- declaring to have a health coverage,
- signing an "informed consent form" for this study,
- certifying not to take part in another clinical study,
- certifying the truth of the personal information declared to the investigator,
- capable of following directions and reliable to respect the constraints of the protocol (living not too far from the investigating centre, no linguistic and intellectual barrier),
- free to ensure the visits to the investigating centre,
- declaring not to have exposed themselves to a risk of pregnancy for at least 3 months before the beginning of the study and committing themselves to use effective contraceptive method throughout the study (for the women of childbearing potential).

I.7.3.2. Specific inclusion criteria

Subjects:

- aged from 18 to 70,
- female / male,
- with all types of skin on body,
- with a phototype (Fitzpatrick): II to IV
- agree to wear the equipment provided by the investigating center and follow the instructions of the staff during the study.

I.7.4. Non inclusion criteria

I.7.4.1. General non- inclusion criteria

According to the protocol, did not have to be included in the study, the subjects:

- being in exclusion period,
- deprived of freedom by administrative or legal decision or under guardianship,
- who cannot be contacted in case of emergency,
- admitted in a residential care,
- planning an hospitalisation during the study,
- belonging to the staff of the investigating centre,
- being of age but protected by law,
- having received vaccination or last rappel of vaccination within the 4 weeks prior to the study or intending to be vaccinated during the course of the study,
- with personal history of adverse reactions to the same type of product as the investigational products,
- with documented history of contact allergy,
- exhibiting skin marks and/or moles and/or freckles in too great quantity and/or hyperpilosity on the experimental area able to interfere with the assessment of the possible skin reactions,
- under treatment, prior to the study, able to interfere with the interpretation of the study results, particularly:
  - systemic retinoids (isotretinoin per os …) within the 6 months,
  - topical retinoids within the 2 months,
  - other topical anti-acne medication within the month,
  - topical or systemic medication with anti-inflammatory or antihistamine products within the 2 weeks,
  - medication for malignancy (of any kind) within the 5 years,
  - antibiotics within 2 weeks
  - desensitisation treatment within the 6 months,

- foreseeing, during the study, a treatment able to interfere with the interpretation of the study results (systemic or topical anti-acne medication, anti-acne cosmetic products, topical or systemic medication with anti-inflammatory or antihistamine, antibiotics, desensitisation treatment, …),
- having had a fever lasting more than 24 hours, within the 8 days prior to the study,
- having had any invasive aesthetic cares on chest and back (peeling, laser…) by a dermatologist within the 2 months prior to the study or foreseeing it for the duration of the study,
- having had any non-invasive aesthetic cares on chest and back (scrub, skin cleansing…) by an aesthetician within the month prior to the study or foreseeing it for the duration of the study,
- having received excessive or intensive exposure to sunlight (natural or artificial) within the month prior to the study or foreseeing UV exposures for the duration of the study,
- under treatment with PUVA or UVB within the month prior to the study,
- having participated in a human repeated insult patch test with challenge with or without sun exposure within the 3 months prior to the study,
- having participated in a cumulative irritability test within the 2 months prior to the study or in a single patch test within the month prior to the study,
- having already participated in 5 clinical studies involving patch test, including 3 human repeated patch tests maximum with or without challenge within the year prior to the study,
- foreseeing bath (in bathtub, sea or swimming-pool), sauna or Turkish bath during the study period,
- regularly practicing intensive sport causing sweating and requiring frequent showers.

I.7.4.2. Specific non-inclusion criteria

Subjects:

- with personal history of adverse reaction to colophony, rubber, nickel, aluminium, patch materials, adhesive plaster,
- with family or personal history of atopy.

I.7.5. Specific information concerning the test subjects and medication

Skin reactivity, history of atopy, contraception (type) and possible current medication were documented by the technician, supervised by the investigator, in the case report form.

No medication likely to interfere with the study was allowed during the study; however, if the health state of the subjects justified some medication (particularly anti-inflammatory drugs), any information relating to this concomitant medication had to be carefully documented in the case report form.

The investigator had to exclude the test subjects taking concomitant medication likely to interfere with the study and the interpretation of the results.

I.7.6. Exclusion criteria

According to the study protocol and to the procedures of the investigating centre, had to be excluded from the study, the test subjects:

- who did not comply with the protocol and created deviation resulting in un-exploitable results,
- who took part in another clinical study that could interfere with the current study,
- who had adverse event (for example: inter-current disease requiring a concomitant medication interfering with the study and the interpretation of the results or severe skin intolerance to the investigational product), incompatible with a good protocol observance.

The temporary or definitive discontinuations decided by the investigator and their dates and reasons had to be carefully documented in the case report form.

I.7.7. Withdrawal criteria

According to the study protocol and to the procedures of the investigating centre, had to be considered as withdrawals, the test subjects:

- who discontinued the study for personal reasons independent of the study (for example: moving house, new job),
- who did not come to the investigating centre for the checking in spite of phone calling.

The withdrawals and their dates and reasons had to be carefully documented by the investigator in the collective case report form (CRF).

I.7.8. Study constraints imposed on the test subjects

The constraints defined by the procedures of the investigating centre and in the study protocol, imposed on the test subjects, related the study, were the following ones:

- to wear the equipment provided by the investigating center and follow the instructions of the staff during the study,
- if justified and asked by the investigator, participation in a complementary test (additional visits to the investigating centre),
- exclusion period at the end of the study (according to the corresponding procedure of the investigating centre and 3 months minimum before starting a human repeated insult patch test with challenge, 1 month minimum before starting another type of study),
- no participation simultaneously in another clinical study which could interfere with the study,
- if justified, description of any concomitant medical treatment not excluded by the inclusion and non-inclusion criteria,
- no drug able to interfere with the study and the interpretation of the results, *e.g.* aspirin (except low dose maintenance therapy), products containing aspirin, antihistamine drugs, anti-inflammatory drugs, antibiotics… (however, if therapeutic requirement: possible exclusion from the study),
- neither anti-acne nor anti seborrheic local treatment,
- neither invasive body aesthetic cares (peeling, laser…) nor non-invasive body aesthetic cares (scrub, skin cleansing…) on chest and back, by a dermatologist or an aesthetician in Beauty Salon,

- neither initiation of a hormonal treatment nor changes of the usual hormonal treatment,
- respect of the dates of visit to the investigating centre and possibly the defined hours,
- no change of the mode of contraception,
- no application of cosmetic care products to the back, other than the investigational ones
- no change in usual body hygiene products,
- no introduction of new cosmetic products,
- no significant change in lifestyle: diet, smoking, sport,
- no intensive sun or UVA exposure (U.V. lamps) during the study and 2 weeks after the end of the study,
- no wearing of too tight or restraining clothes liable to produce frictions on the experimental area and to cause the un-sticking of the patch(es),
- neither Turkish bath nor sauna nor bath (in bathtub or swimming-pool or sea), liable to cause excessive sweating and/or the un-sticking of the patch(es),
- during shower, protection of the experimental area (no violent projection of water, no application of soap, very gentle wiping if necessary) to avoid the un-sticking of the patch(es) or the appearance of inter-current skin irritation,
- no intensive sport liable to cause excessive sweating and the un-sticking of the patch(es),
- in case of detachment or moving of the patch(es), contact with the investigating center,
- no vaccination.

The test subjects were questioned at the end of the study about the respect of the study constraints. The investigator had to assess the importance of the possible deviations in comparison with the experimental conditions required at the beginning of the study and their incidence on the validity of the results.

I.8. INVESTIGATIONAL PRODUCT

I.8.1. Identification of the investigational product

Product Denomination	PENTA 18 479
Category	Face care
Reference	TX 19011
Batch number	B3
Galenic form and organoleptic characteristics	Colourless gel
Normal foreseeable conditions of use	Diluted to 1%

The product units were sent to the investigating centre. Upon receipt, the investigating centre noted the date of product receipt and checked the supplied quantities.

The product units were coded and labelled in Romanian, according to the corresponding procedure of the investigating centre.

Number and type of product units	2 plastic phials
Content of product unit	250 ml
Eurofins EVIC romania code	21-0184

Before starting the study, the storage of the investigational product units was carried out according to the conditions defined by the sponsor, in the product storage area and a product sample was taken and kept in the sample storage area of the investigating centre for at least 3 years after the end of the study then destroyed, according to the corresponding procedure of the investigating centre.

Apart from the specific demand of the sponsor, the used product unit will be kept at least 4 weeks after the end of the study then destroyed, according to the corresponding procedure of the investigating centre.

I.8.3. Information concerning the investigational product

The investigational product unit had to be supplied with a certificate that particularly referred to:

- the compliance of the ingredients of the investigational product formula with the European Regulation N° 1223/2009 of the European Parliament,
- the safety of the finished investigational product and the absence of foreseeable serious risk for the health of the test subjects.

The qualitative formula of the product had to be supplied to the coordinating centre and the investigating centre by the study monitor.

I.8.4. Experimental conditions of use of the investigational product

The skin areas had to be defined by the technician in charge of the study, on the upper back of the test subject, taking into account the skin appearance and avoiding the areas of friction with clothes.

The quantity of investigational product had to be applied, by the technician in charge of the study, with a micropipette (with a single use tip) and put into the patch.

Before application, the skin had to be wiped with a cotton pad.

The patch containing the investigational product had to be applied, by the technician in charge of the study, to the defined skin area.

The experimental conditions of application at the investigating centre had to be the following ones:

Patch material	Experimental conditions of use of the investigational product	Quantity to be applied
Semi-occlusive patch: absorbent support in Webril® kept in position by a non woven medical adhesive (surface: 400 mm²)	Diluted at 15% with distilled water	160µl

A semi- occlusive patch, containing 160 µl of distilled water had to be applied in parallel as control to eliminate, when the results would be interpreted, the possible inter-current effects due to the patch material.

The investigational product and the control patch were applied on D1 and had to be worn for 48 hours ± 4 hours then removed at the investigating centre.

I.9. CHECKING OF THE SKIN COMPATIBILITY

I.9.1. Recording of the skin reactions

A skin examination of the treated and control areas was performed at the investigating centre:

- visually, by the same investigator, under standard "daylight" source,
- on D1, before patching,
- on D3, 15 to 30 minutes (or more, if redness appeared after the removal of the adhesive) after patch removal

Operations at the investigating centre	**Experimental times**	
	D1	**D3**
Application of the investigational product to the defined area (upper back)	●	/
Removal of the investigational product and control patch	/	● after 48h of contact
Skin examination Questioning of the test subjects	● Before patching	● 15-30 minutes after patch removal

Concurrently with the clinical examinations performed, the test subjects were questioned about the possible sensations of discomfort they felt.

In case of strong sensations of discomfort felt during wearing at home, the test subjects had to inform by phone the investigator. If necessary, the investigational product had to be removed and a skin examination had to be quickly performed by the investigator (before the next planned visit to the investigating centre).

An area where erythema was graded 2 or more (with or without infiltration) had to be evaluated on subsequent days to note whether the reaction diminishes or increases, in order to differentiate between an allergic reaction and an irritation.

Digital photographs of the skin had to be systematically taken when justified (adverse effects).

All the data were recorded in the case report form.

I.9.2. Expression of the results

All the reactions had to be accurately described at each experimental time using the criteria and the scale hereafter.

E = Erythema d=diffuse p=punctuated peri=peripheral	**0** – no visible erythema **0.5** – very slight erythema – barely perceptible **1** – mild erythema – faint pink **2** – moderate erythema – well defined **3** – severe erythema **4** – caustic effect – erosive aspect and/or necrotic aspect

In case of erythema, the investigator had to proceed to palpation to assess infiltration / oedema.

The other clinical visible signs, if present, were assigned a mark for the calculation of the individual daily irritation score (IDIS).

M = Complementary mention: Other reactions		
Abbreviation	**Reactions**	**Score**
Sv	Soap effect (shiny skin with possibly wrinkles)	**1**
D	Desquamation	**1**
Dr	Dryness	**1**
Hy	Hypopigmentation	**1**
C	Skin coloration – hyperpigmentation	**1**
Oe	Homogeneous infiltration / oedema	**2**
P	Papules	**2**
V	Vesicles	**2**
Pe	Petechiae	**2**
Fr	Follicular reaction	**2**
I	Itching at the test site	**2**
F	Fissuring	**3**
Cr	Exudation and/or Surface encrustation	**3**
B	Bullae	**3**
Sc	Scab	**1**
He	Heating	**1**
Br	Burning	**1**
/	No reaction	**0**

Note: the other visible clinical signs had to be described.

M = Complementary mention: additional comments	
NA	Product not applied
T	Tape reaction
L	Loss of patch during the first 12 hours
Abs	Test subject absent

The presence of a reaction on the control area leads the investigator to temper the skin compatibility conclusion of the investigational product.

The results were expressed in percentage of reactive test subjects: for this calculation only the erythema and other visible signs of reactivity were taken into account.

For each test subject and each observation time, an individual daily irritation score (IDIS) was calculated (sum of the marks obtained for the visible clinical signs observed on the application area of each investigational product).

For the panel and each observation time, a mean daily irritation score (MDIS) was calculated according to the formula:

MDIS = Σ (IDIS) / No of valid cases

I.9.3. Interpretation of the results

All the test subjects included in the study were taken into account to appreciate the skin compatibility of the investigational product as long as they were submitted at least to one post application examination at the defined time or any other time.

The nature, intensity, appearance period from the application, disappearance period from the application, location (just on the application area or spreading beyond the application area) of the skin reaction was taken into account for the interpretation of the results.

In case of skin reactivity, the reaction observed could be an irritation reaction or an allergic reaction (if the test subject has been previously in contact with the allergen).

A quick decreasing reaction can be indicative of irritation. A reaction with infiltration / oedema and possibly spreading, itching, that persists or increases over time usually can indicate an allergic reaction.

If a possible allergic reaction is observed, it can be the revelation of an allergy previously contracted.

So, the investigator had to classify the reaction according to the following scale:

A = ICDRG scale	
IR	Irritation reaction
-	No allergic reaction
?+	Doubtful reaction (only slight erythema)
(+)	Weak positive reaction (without vesicle): slight erythema and infiltration with presence of small papular elevations, possibly papules
(++)	Strong positive reaction: erythema, papules, vesicles, infiltration
(+++)	Extreme positive reaction: intense erythema, oedema, coalescent vesicles (bullae)

To appreciate the skin compatibility of the investigational product, the interpretation of the results was based on the experience of the investigator in this field.

The investigator concluded according to the scale: very good, good, moderate or bad skin compatibility.

In case of suspected allergic reaction or strong irritation, the study monitor had to be quickly informed.

If justified in case of reactivity in some test subjects, a complementary study was possibly carried out in these test subjects, after agreement of the sponsor. The experimental conditions of this study had to be defined by the investigator, case by case.

I.10. SUSPENSION OF THE STUDY

The investigator had to stop the study if it showed a risk for the health or the integrity of the test subjects.

The date of the suspension and the reasons had to be carefully documented by the investigator in the case report form.

The investigator centre had to inform promptly the study monitor, by phone, fax or e-mail.

The sponsor was able to stop the study at any time for administrative reasons or other ones.

I.11. ADVERSE EVENTS

I.11.1. Definitions

Any topical product can induce, when used in Human, according to individual sensitivities, a local and minor reactivity, defined as follows: any slight local reaction of intolerance or sensation of discomfort, occurring in a test subject during a clinical study, completely reversible, expected, due to the investigational product and which does not question the observance of the study protocol or the good implementation of the study.

- **adverse event**: any harmful event with or without relationship with the investigational product, occurring in a test subject during a clinical study.
- **suspicion of adverse effect**: any adverse event with a quite possible relationship with the investigational product.
- **adverse effect**: any harmful and unwanted reaction, due to the investigational product, occurring in a test subject during a clinical study.
- **unexpected adverse effect**: any adverse effect due to the investigational product, the nature, the intensity and/or the evolution of which do not agree with the product information.
- **serious adverse event / effect**: any adverse event or adverse effect that causes death, endangers test subject's life, induces an hospitalisation or the prolongation of the hospitalisation, causes severe and lasting incapacity or handicap or induces congenital anomaly or malformation.

I.11.2. Data collection

The investigator had to accurately describe the adverse event and had to appreciate its seriousness.

According to the corresponding procedure of the investigating centre, he had to define the link of causality between this event and the investigational product, on the basis of the symptoms, the chronology, the results of the possible specific complementary tests undertaken and any available information.

The imputability of the product had to be assessed according to the scale: very likely, likely, possible, questionable, excluded (in accordance with the recommendations of the European Council in its resolution ResAP (2006)1 of 08/11/2006 and the method of imputability of the adverse effects linked to the cosmetic products published by AFSSAPS in December 2009).

I.11.3. Conduct to be adopted in case of adverse event

Faced with an adverse event, the investigator had to freely define, case by case, the conduct to be adopted and the suitable steps to ensure the safety of the test subject concerned and of the other test subjects included in the study.

In case of suspicion of adverse effect (with a quite possible relationship with the investigational product), the investigator had to ensure the clinical follow-up of the test subject concerned, as long as necessary.

I.11.4. Communication with the study monitor

According to the corresponding procedure, the serious adverse events and the adverse effects had to be notified as soon as possible and within 24 hours at the latest, by the investigator centre to the study monitor, by phone, fax or e-mail.

The investigator had to send an adverse event form to the study monitor and to the coordinating centre.

If justified, the investigator had to give to the study monitor and to the coordinating centre complementary information when available.

I.12. RAW DATA RECORDING AND STUDY REPORT FILING

All the data gathered during the study were recorded accurately, legibly and indelibly by the investigator and the technician in charge of the study, under his control, in the case report form.

Each page of this document was initialled and dated by the technician; the whole was verified and validated by the investigator.

The content of this study report took into account the recommendations of the Colipa related to the assessment of the efficacy of cosmetic products (May 2008) and the explanatory note related to the structure and the content of the reports of clinical studies – ICH E3, of 28/11/1995.

At the end of the study, the information concerning the investigational product, the information concerning the test subjects (CRF(s), daily logs, informed consent forms) and the information related to the conduct of the study (protocol signed by the sponsor, copy of this study report….) were filed and will be kept for 10 years, in the filing area of the investigating centre and/or coordinating centre.

At the end of this period, the sponsor will choose among the 3 options:

- return of the study documentation to the sponsor,
- filing of the study documentation in the filing area of the investigating centre and/or coordinating centre, based on a specific contract,
- destruction of the study documentation (after sponsor's written and signed authorization).

I.13. REFERENCES

Numerous publications supported this methodology, notably:

- Cosmetic product test / Guidelines for the assessment of human skin compatibility – COLIPA – August 1997
- Mikulowska A., Reactive changes in human epidermis following simple occlusion with water, Contact Dermatitis, 1992, 26, pp. 224-227
- Matthies W., Test strategies for development of cosmetic products using dermatological test models, Seifen-Öle-Fette-Wachse, 1991, 117, pp. 42-43
- Frosch P.J. & Kligmann A.M., The Duhring Chamber: an improved technique for epicutaneous testing of irritant and allergic reactions, Contact Dermatitis, 1979, 5, pp. 73-81
- Draize J.H., Appraisal of the safety of chemicals in Food, Drugs and Cosmetics, edited by the FDA, USA, 1959, pp. 46-48

II – PRACTICAL CONDITIONS OF STUDY PERFORMANCE

II.1. PROTOCOL ADHERENCE

II.1.1 . Study population

II.1.1.1. Number of test subjects

Number of test subjects included in the study	**11**	
Withdrawals	**Test subjects concerned**	**Date and reasons**
	None	Non- applicable
Exclusion	**Test subjects concerned**	**Date and reasons**
	None	Non- applicable
Valid cases	**Skin compatibility**	
	11	

The number of recruited test subjects took into account the inclusion criteria, the constraints of the study and the period of the study performance.

At the beginning of the study, one complementary test subject (+1) was included to compensate the possible withdrawals or exclusions from the study independent of the investigational product.

II.1.1.2. Inclusion and non inclusion criteria

All the test subjects corresponded to the inclusion and non-inclusion criteria.

The individual typological characteristics of the test subjects are reported in **Appendix 1**, and recapitulated below for the whole panel:

Age (years old)	Included test subjects	Valid cases
Minimum	27	27
Maximum	69	69
Mean	49	49
Median	52	52

Criteria	Included test subjects		Valid cases	
	Nb	%	Nb	%
Phototype				
II	2	18%	2	18%
III	7	64%	7	64%
IV	2	18%	2	18%
Sex				
Male	5	45%	5	45%
Famale	6	55%	6	55%

II.1.1.3. Specific information concerning the test subjects and medication

The answers of the test subjects concerning the skin reactivity, the history of atopy, the type of contraception (for the women) and the current medication are reported in **Appendix 2**.

II.1.1.4. Study constraints imposed on the test subjects

All the constraints of the study, defined in the protocol, were respected by the test subjects.

The answers of the test subjects concerning the respect of the constraints defined in the protocol are reported in the CRF.

II.1.2. Experimental conditions of use of the investigational product

All the experimental conditions of application at the investigating centre on D1 defined in the protocol were respected.

II.1.3. Checking of the skin compatibility: recording of the skin reactions

All the skin examinations and questioning of the test subjects were performed in accordance with the conditions defined in the protocol.

III – RESULTS

III.1. RESULTS / DISCUSSION

III.1.1. Checking of the skin compatibility

The individual data of the skin examination and questioning of the test subjects are reported in **Appendix 3.**

For the control area:

Control time after patch removal	Types of reaction	Number of reactive subjects	% of reactive test subjects	Mean daily irritation score MDIS
T 15 minutes (D3)	None	0	0%	0

For the investigational product:

Control time after removal	Types of reaction	Number of reactive subjects	% of reactive test subjects	Mean daily irritation score MDIS	Skin compatibility of the product
T 15 minutes (D3)	None	0	0%	0	**Very good skin compatibility**

III.2. OVERALL CONCLUSION

Under the experimental conditions adopted:

- single application of the investigational product, **diluted at 15% with distilled water**, under semi-occlusive patch on a panel of 11 subjects aged between 27 and 69 years old, with phototype II to IV and with all types of skin on body,

- the product **PENTA 18 479 – Ref. TX 19011 - Lot: B3**, induced no reaction of irritation and has a very good skin compatibility.

III.3. QUALITY CONTROL AND QUALITY ASSURANCE

The study was performed in compliance with the procedures of the investigating centre, established according to the regulations in force.

The investigator, in charge of the performance of the study, made sure of the quality of the work of the technical staff, particularly concerning the respect of the protocol and its appendices, the collection of raw data, the management of the investigational product.

The personnel of the Quality Assurance department controlled that the study documentation was present, dated and signed.
The personnel of the Quality Assurance department regularly control that the protocol and working procedures relevant to this type of study are duly applied.

Any significant change brought to the protocol was the subject of a protocol amendment, signed by the study monitor and the investigator.

Any significant anomaly, occurring during the study, was documented in the case report form and reported to the study monitor.

Any deviation from the protocol was reported in the study report.

APPENDICES

Appendix 1

TYPOLOGICAL CHARACTERISTICS OF THE TEST SUBJECTS

Test subjects		**age** *(years)*	**sex** *F=female M=male*	**phototype**(1)
Ref.	**Code** *initials of the surname and of the first name*			
1	IF	50	F	III
2	LC	54	M	III
3	PD	36	M	III
4	PI	27	M	III
5	CF	69	F	III
6	HM	52	F	II
7	PA	33	F	III
8	CP	68	M	III
9	BA	55	F	II
10	AE	61	F	IV
11	IN	36	M	IV

Legends:
(1) ***phototype: Type I****: Always burns easily, never tans,* ***Type II****: Always burns easily, tans minimally,* ***Type III****: Burns moderately, tans gradually,* ***Type IV****: Burns slightly, always tans easily,* ***Type V****: Burns rarely, tans intensely,* ***Type VI****: Never burns, strongly pigmented*

Appendix 2/1

SPECIFIC INFORMATION CONCERNING THE TEST SUBJECTS

Test subjects		Sensitive (declarative) / reactive skin on body	Atopy	Current medication, except for contraceptive pills		Contraception
				If yes *(commercial denomination, active substance and dosage)*		If yes *(to be specified)*
Ref.	Code *initials of the surname and of the first name*			At the inclusion	During the study	
1	IF	/	/	None	None	CONDOM
2	LC	/	/	None	None	NC (MALE)
3	PD	/	/	None	None	NC (MALE)
4	PI	/	/	None	None	NC (MALE)
5	CF	/	/	None	None	NC (MENOPAUSE)
6	HM	/	/	None	None	CONDOM
7	PA	/	/	None	None	PILL
8	CP	/	/	None	None	NC (MALE)
9	BA	/	/	None	None	NC (MENOPAUSE)
10	AE	/	/	None	None	NC (MENOPAUSE)
11	IN	/	/	None	None	NC (MALE)

Legends: / = no *NC: Not Concerned*

Appendix 3/1

SKIN COMPATIBILITY - SKIN EXAMINATION AND QUESTIONING
INVESTIGATIONAL PRODUCT PENTA 18 479 – Ref. TX 19011 - Lot: B3

REACTIONS: ***Clinical signs*** ***Dr =*** *Dryness* ***D*** *= Desquamation* ***C*** *= Coloration – hyperpigmentation* ***Hy*** *= Hypopigmentation* ***Oe*** *= Homogeneous infiltration/Oedema* ***P*** *= Papules* ***V*** *= Vésicules* ***Pe*** *= Petechiae* ***Fr*** *= Follicular reaction* ***I*** *= Itching at the test site* ***F*** *= Fissuring* ***B*** *= Bullae* ***Cr*** *= Exudation and/or surface encrustation* ***Sc*** *= Scab* ***Sv*** *= Soap effect (shiny skin with possibly wrinkles)* ***He*** *= Heating* ***B*** *= Burning*	***E =*** *Erythema:* ***E0.5*** *= very slight,* ***E1*** *= slight,* ***E2*** *= moderate,* ***E3*** *= severe* ***E4*** *= caustic* ***d****: diffuse* ***p****: punctuated* ***peri****: peripheral* ***/: no reaction***

Test subjects		**Experimental times**			
		D1/T0		**D3**	
Ref.	**Code** *initials of the surname and of the first name*	Reactions + intensity	IDIS	Reactions + intensity	IDIS
1	IF	None	0	None	0
2	LC	None	0	None	0
3	PD	None	0	None	0
4	PI	None	0	None	0
5	CF	None	0	None	0
6	HM	None	0	None	0
7	PA	None	0	None	0
8	CP	None	0	None	0
9	BA	None	0	None	0
10	AE	None	0	None	0
11	IN	None	0	None	0
MDIS		SC		0	

SKIN COMPATIBILITY – SKIN EXAMINATION AND QUESTIONING CONTROL AREA

REACTIONS: ***Clinical signs*** ***Dr =*** *Dryness* ***D*** *= Desquamation* ***C*** *= Coloration – hyperpigmentation* ***Hy*** *= Hypopigmentation* ***Oe*** *= Homogeneous infiltration/Oedema* ***P*** *= Papules* ***V*** *= Vésicules* ***Pe*** *= Petechiae* ***Fr*** *= Follicular reaction* ***I*** *= Itching at the test site* ***F*** *= Fissuring* ***B*** *= Bullae* ***Cr*** *= Exudation and/or surface encrustation* ***Sc*** *= Scab* ***Sv*** *= Soap effect (shiny skin with possibly wrinkles)* ***He*** *= Heating* ***B*** *= Burning*	***E =*** *Erythema:* ***E0.5*** *= very slight,* ***E1*** *= slight,* ***E2*** *= moderate,* ***E3*** *= severe* ***E4*** *= caustic* ***d****: diffuse* ***p****: punctuated* ***peri****: peripheral* ***/: no reaction***

Test subjects		**Experimental times**			
		D1/T0		**D3**	
Ref.	**Code** *initials of the surname and of the first name*	Reactions + intensity	IDIS	Reactions + intensity	IDIS
1	IF	None	0	None	0
2	LC	None	0	None	0
3	PD	None	0	None	0
4	PI	None	0	None	0
5	CF	None	0	None	0
6	HM	None	0	None	0
7	PA	None	0	None	0
8	CP	None	0	None	0
9	BA	None	0	None	0
10	AE	None	0	None	0
11	IN	None	0	None	0
MDIS		0		0	

Test item
PENTA 18 479 - REF : TX 19006

In chemico skin sensitization: Direct Peptide Reactivity Assay (DPRA) according to the OECD 442C guideline

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FINAL REPORT

Study number: 6.53-52287-ID-19/09966

Sponsor

SEDERMA
29, rue du Chemin-Vert
BP 33
78612 LE PERRAY EN YVELINES CEDEX
FRANCE

Test Facility

IDEA Lab
Technopôle Montesquieu
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33652 MARTILLAC Cedex
FRANCE

CONTENTS

Abbreviations/ SOP

OECD	Organisation for Economic Co-operation and Development
GLP	Good Laboratory Practice
HPLC	High Pressure Liquid Chromatography
ECVAM	European Center for Validation of Alternative Method
DB-ALM	DataBase Alternative Method
ρ	Density
Fv	Final Volume
MW	Molecular Weight

FL REAC 01	Record of purchased reagents and consumables
FL REAC 05	Expiration of products.
FL REAC 06	Reagents and consumables tracking book
IL MAT 24	HPLC
IL MAT 25	Use of Empower software

Note: Internal standard operating procedures are audited by ANSM according to the Good Laboratory Practice (GLP) principles described in the decree dated August 10th, 2004 from the JOFR and inspected by GIPC according to the GLP principles described in the article annex II to article D523-8 of the French Environment Code.

GLP conformity statement

The study 6.53-52287-ID-19/09966 was performed in the IDEA Lab company laboratory, in agreement with the French Good Laboratory Practice (GLP) principles, the European Directive 2004/10/CE and the decree dated August 10th, 2004 from the JOFR.

All relevant Standard Operating Procedures have been followed and raw data have been recorded accurately.

For confidentiality concerns some characterisation data related to the test item composition are not shown in this report. This is a deviation to GLP. However, this characterisation had been provided by the Sponsor, brought to my attention, then stored in a secure environment in accordance with the company procedures,

The lack of verification of the concentration of the test item in various dilutions has no impact on the reliability of the results generated for the following reasons:

- the test item preparation in its vehicle is controlled, particularly with micropipette and precision scales regularly controlled, calibrated and traceable with national or international standards of measurement,
- the control of the homogeneity of the test item dilutions in the vehicle is performed using organoleptic criteria and is documented in the study log book,
- the test item dilutions are prepared extemporaneously.

This report accurately reflects the study carried out and the results obtained.
I declare this study compliant with the good laboratory practice and assume responsibility for the data validity of the study.

Date: 26 NOV 2019

Study Director
BSc Biotechnology
Fabien NECA

Quality Assurance statement

According to the Good Laboratory Practices, I state that:

- The General Study Plan was audited by the Quality Assurance and that the Specific Study Plan was verified before the beginning of the study

- The different technical phases of the study 6.53 are regularly audited by the Quality Assurance. Facility audits are also carried out. The audit frequency is defined in the corresponding procedure

  At the last technical audit (A-17/19), the following activities have been inspected:
  - Preparation of the test item and positive control
  - Preparation of the peptide solutions
  - Preparation of the samples for the test
  - Preparation of the calibration curve
  - Preparation and manipulation of the HPLC system
  - HPLC data analysis

- The final report was audited by the Quality Assurance of IDEA Lab. It accurately reflects the raw data from the study and the application of the Standard Operating Procedures and the Study Plan

Audit nature	Audits dates	Transmission dates of the audit report to the Study Director and the General Management
Technical phases of the study	From 21/10/2019 to 24/10/2019	22/11/2019
General Study Plan	19/09/2019	19/09/2019
Draft report	22/11/2019	22/11/2019
Final Report	25 NOV. 2019	25 NOV. 2019

Date: 25 NOV. 2019

Quality Assurance

Nelly TEYSSANDIER

Study presentation

1. Study principle

The aim of the study is the skin sensitization potential evaluation, by an *in chemico* test, of a test item according to the current General Study Plan 6.53. This study is performed according to the OECD Guideline 442C and the Protocol N°154 from ECVAM DB ALM.

The principle is based on the chemical reactivity of the test item with proteins. The interaction between the test item and lysine or cysteine-rich peptides is detected with a high performance liquid chromatography (HPLC). The remaining concentration of peptides is measured after 24 hours of incubation with the test item at room temperature. It is measured with the UV detector of the HPLC system, after gradient elution, at 220 nm. The depletion rates of lysine and cysteine peptides are then used to distinguish the skin sensitizer and non-sensitizer so as to sort and label chemicals according to SGH.

2. Test item

PENTA 18 479 - REF : TX 19006

Internal code	: ID-19/09966
Batch number	: B1
Aspect	: Solid
Colour	: White
Storage conditions	: Room temperature (20°C ± 5°C)
Test item nature	: Cosmetic ingredient
Retest date	: 27/02/2020

Physico-chemical properties

Purity	: 81.6%
Physical state at 20°C	: Solid
Homogeneity	: Yes
Molecular Weight	: 859.50 g/mol

Solubility and stability

According to the Study Plan, the solubility of the test item in an appropriate solvent was assessed before performing the assay. The chosen solvent, shown in the Specific Study Plan, was water.

Information linked to the identification, purity and stability of the test item is under the responsibility of the Sponsor of the study. The characterisation of the test item was provided by the Sponsor of the study.

The test item will be stored at least 2 months after the end of the study in the product room of the Martillac location before to be destroyed or sent back to the Sponsor according to his choice.

The analysis certificate is shown at the end of this report.

3. Reference items

- Positive control: 100 mM cinnamaldehyde (CAS N°: 104-55-2), purity ≥ 95% (i.e. 5 or 25 mM as final concentration in the reaction mixtures, respectively with cysteine and lysine).

Internal code	: ID2696
CAS number	: 104-55-2
Batch number	: MKBT8955V
Aspect	: Liquid
Color	: Colorless to yellow
Storage conditions	: Room temperature
Expiry date	: 28/02/2020
Purity	: 99.1%
Molecular weight	: 859.50 g/mol
Density	: 1.048
Solubility	: Acetonitrile

- Co-elution control: 100 mM test item in the appropriate buffer (cf § 7.5) (i.e. 5 or 25 mM as final concentration in the reaction mixtures, respectively with cysteine and lysine).

- 4 references, made with 0.667 mM peptides solutions are included in each set of analysis: (i.e. 0.500 mM as final concentration in the reaction mixtures):
  - **Reference control A:** prepared with acetonitrile in order to verify the suitability of HPLC system,
  - **Reference control B:** prepared with acetonitrile and included at the beginning and at the end of the sequence in order to check the stability of peptide over time,
  - **Reference control C:** prepared with acetonitrile, the positive control solvent to check its influence on the peptide stability.
  - **Reference control C':** prepared with water, the test item solvent, in order to check its influence on the peptide stability

4. Test system

	Cysteine peptide	Lysine peptide
Supplier	RS Synthesis, LLC	
Reference	Ac RFAACAA-COOH	Ac-RFAAKAA-COOH
Batch number	180622	180622
Purity	94.35%	93.68%

5. Reagents

Stored at 5°C ± 3°C
• Ammonium hydroxide – CAS number: 1336-21-6

Stored at room temperature 20°C ± 5°C
• HPLC grade solvents:
  - Acetonitrile - CAS number 75-05-8- purity > 99.9%
  - Purified water - Ultrapure type I
• Ammonium acetate – CAS number: 631-61-8
• Sodium phosphate monobasic 0.1 M (13.8 g/l NaH_2PO_4 H_2O) - CAS number : 10049-21-5
• Sodium phosphate dibasic 0.1 M (26.8 g/l Na_2HPO_4 $7H_2O$) – CAS number: 7782-85-6
• Trifluoroacetic acid - CAS number: 76-05-1
• HPLC mobile phase A: trifluoroacetic acid at 0.1% (v/v) in water
• HPLC mobile phase B: trifluoroacetic acid at 0.085 % (v/v) in acetonitrile

FL REAC 01 and FL REAC 06 forms ensure the traceability of media and reagents used in the study.

The expiry after opening of the media and reagents used in the study is defined in the form FL REAC 05. Reagent are stored at room temperature 20°C ± 5°C.

6. Material and consumables

- Waters e2695 HPLC; Waters 2489 UV detector
- Xbridge Peptide BEH C18 3.5 µm ; dimensions 2.1 x 100 mm
- pH meter; 0.01 pH unit accuracy, Mettler Toledo Seven Multi
- Analytical weight; 0.1 mg accuracy, Denver Instrument APX-60
- Micropipettes; 3 to 1000 µl
- 2 ml glass vials with septum for HPLC
- 5 ml and 3 ml glass vials (for test item or reference preparation)

FL REAC 01 and FL REAC 06 forms ensure the consumables traceability used in the study.

The material used was recorded in the study notebook.

7. Study course

7.1. Experimentation dates

The experimentation was carried out from 21/10/2019 to 24/10/2019.

7.2. Series definition

The test item was prepared at 100 mM in water and the positive control was prepared at 100 mM in acetonitrile.

They were incubated in excess with the peptides at 1:10 and 1:50 ratio for cysteine and lysine peptides respectively.

Each sample was tested 3 times from 3 independent solutions.

7.3. Preparation of the test item and positive control

Test item
The test item was diluted at 100mM in water.

The weight of the test item is calculated according to the following formula:

$$\text{Weight (g)} = \frac{0.01 \times Fv \times MW}{\text{Purity}}$$

where MW = 859.50 g/mol and purity = 81.6%.

316 mg was pre-weighed in a glass vial in order to prepare, right for use, 3 ml of a limpid 100 mM solution.

Positive control
The volume of the positive control was calculated according to the following formula:

$$\text{Volume (ml)} = \frac{0.01 \times fV \times MW}{\text{Purity} \times \rho}$$

MW= 132.16 g/mol
Purity = 99.1%
Density ρ = 1.048
fV (final volume) = 3 ml

38.2 µl of the positive control were distributed in a 5 ml glass vial in order to prepare, right before use, 3 ml of a limpid 100 mM solution.

7.4. Preparation of the peptide solution

Peptide solutions were prepared at 0.667 mM:

- 13.9 mg of cysteine peptide were pre-weighed then dissolved, right before the incubation, in 27.8 ml of phosphate buffer (100 mM; pH 7.5 ± 0.05).
- 13.5 mg of lysine peptide were pre-weighed then dissolved, right before the incubation, in 26.1 ml of ammonium acetate buffer (100 mM; pH 10.2 ± 0.05).

7.5. Preparation of the sample for the test

Samples were dissolved immediately before use (100 mM positive control solution was kept for the 2 runs). Peptides were incubated with each sample (test item and positive control) at 1:10 and 1:50 ratio for cysteine and lysine respectively. All the replicates were prepared with the same peptide stock solutions.

1 ml of solution was prepared according to the following table:

Ratio cysteine:test item 1:10 0.5 mM Peptide, 5 mM Sample	**Ratio lysine:test item 1:50** 0.5 mM Peptide, 25 mM Sample
- 750 µl of cysteine peptide solution (buffer only to check co-elution)	- 750 µl of lysine peptide solution (buffer only to check co-elution)
- 50 µl of sample or solvent for Reference controls	- 250 µl of sample or solvent for Reference controls
- 200 µl of acetonitrile	

The vials were capped and mixed carefully avoiding bubbling, then placed in the HPLC system sampler at room temperature (≥ 19°C to ≤ 27.5°C). HPLC analysis started 24 hours ± 2 hours after addition of peptides.

Immediately upon addition of the test item solution to the peptide solution, just prior the beginning of the HPLC analysis and at the end of the analysis, homogeneity of the samples vials were checked.

7.6. Preparation of calibration curve

A calibration curve was generated for cysteine and lysine peptides. Peptides standards were prepared in 20% acetonitrile in buffer solution (phosphate buffer for cysteine peptide and ammonium acetate for lysine peptide).
Six standard solutions between 0.534 mM and 0.0167 mM (dilution factor 2) were prepared from the stock solution (0.667 mM) by serial dilution. The dilution buffer was also included as blank in the standard calibration curve.

7.7. Preparation and manipulation of the HPLC system

The HPLC system was used according to the current working instruction IL MAT 24.

According to the guideline, the set-up parameters were adjusted to guarantee an appropriate elution and integration of the cysteine and lysine peptides.
Proficiency substances recommended in the OECD guideline were performed in these conditions. Results are shown at the end of the report.

The HPLC column was installed and equilibrated at 30°C with 50% of phase A and 50% of phase B, for at least 20 minutes before use. The gradient (cf. § 7.8) was performed at least one time before to use the column.

7.8. HPLC analysis

After the equilibration phase, 7 µl of each sample are automatically injected and the HPLC analysis was performed with a flow of 0.40 ml per minute according to the sequence described in the table below.

Time	Flow	Phase A (%)	Phase B (%)
0 minute	0.40 ml/minute	90	10
13.5 minutes	0.40 ml/minute	10	90
14 minutes	0.40 ml/minute	90	10
19 minutes	End of analyse	90	10

A complete sequence was performed for each sample.
The column was re-equilibrated to initial conditions (90% Phase A and 10% of phase B) at least 4 minutes between each injection.

7.9. Sequence of the analysis

The analysis was programmed according to the following principles:

- The standards of the calibration curve and the reference controls A are placed at the beginning of the sequence.
- The reference controls B are placed at the beginning and at the end of the analysis (3 repetitions).
- The reference controls C are placed at the beginning of each repetition.

Lysine and cysteine analyses were conducted on separate days and the test item was freshly prepared for both assays on each day. The analysis was timed to assure that the injection of the first sample starts 22 to 26 hours after the test item was mixed with the peptide solution. The HPLC analysis time was less than 30 hours.

8. Results calculation and interpretation

8.1. Results calculation

Results were processed by the Empower 3 software according to the current working instruction IL MAT 25.

The depletion rate was calculated for each type of peptide according to the following formula:

$$\text{Depletion \%} = \left(1 - \frac{\text{Peptide peak area with the replicate injection}}{\text{Mean peptide peak area with the C base control}}\right) \times 100$$

Then, the mean depletion percentage for cysteine and lysine peptides was calculated for the test item and the positive control.

The following table allows a determination of reactivity: the 6.38% threshold is used in order to differentiate between non-sensitizers and sensitizers.

Mean of cysteine and lysine% depletion	Reactivity Class	DPRA Prediction
0.00 % ≤ mean% depletion ≤ 6.38 %	No or minimal reactivity	Negative
6.38 % < mean% depletion ≤ 22.62 %	Low reactivity	Positive
22.62 % < mean% depletion ≤ 42.47 %	Moderate reactivity	
42.47 % < mean% depletion ≤ 100 %	High reactivity	

8.2. Test validation

The following criteria must be checked in order to validate the test.

Run validation criteria

Calibration curve:
Standard curve must have a coefficient r^2 higher than 0.99.

Reference control A:
The mean concentration of the peptide must be equal to 0.50 ± 0.05 mM.

Reference controls B and C:
The CV of the 9 controls B and C must be less than 15%.

Positive Control (cinnamaldehyde):
The standard deviation of measurements must be lower than 14.9% and 11.6% for cysteine and lysine peptides respectively. The mean depletion rate must be between 60.8% and 100% for the cysteine peptide and between 40.2% and 69.4% for the lysine peptide.

Test item validation criteria

Reference control C:
The mean concentration of the peptide must be equal to 0.50 ± 0.05 mM.

Test item:
The standard deviation of measurements must be lower than 14.9% and 11.6% for cysteine and lysine peptides respectively.

9. Observations, deviations and Study Plan amendments

No observation, deviation or amendment to the Study Plan has been observed during this study.

10. Results

Raw data from Empower software analysis are shown at the end of the report.

10.1. Reference Control

Positive Control

Cinnamaldehyde	Depletion in Lysine Peptide %	Depletion in Cysteine Peptide %
Repetition 1	58.76	72.62
Repetition 2	59.62	75.55
Repetition 3	57.55	73.13
Mean	**58.64**	**73.77**
Depletion Validity criteria	*40.2 < Depletion < 69.4*	*60.8 < Depletion < 100*

Reference Controls

	Lysine Peptide	Cysteine Peptide	
	Concentration (mM)	**Concentration (mM)**	*Concentration validity criteria (mM)*
Reference A	0.477	0.503	*0.500 ± 0.050*
*** Reference C**	0.474	0.508	
**** Reference C'**	0.460	0.493	

*Reference C: Positive control diluant, i.e. acetonitrile
**Reference C': Test item diluant, i.e. = water

	CV %	CV %	*CI' validity criteria*
Reference B/C	0.48	0.47	*< 15 %*

10.2. Test Item

	Depletion in Lysine Peptide %	Depletion in Cysteine Peptide %
Repetition 1	0.76	5.30
Repetition 2	0	6.73
Repetition 3	9.67	5.03
Mean	**3.48**	**5.69**
SD (Standard Deviation)	5.38	0.92
SD Validity criteria	*< 11.6%*	*< 14.9%*

Mean Depletion %
4.58

No coelution of the test item with the peptides has been highlighted.

10.3. Validity criteria

All validity criteria are fulfilled which allow us to validate the study.

11. Conclusion

The sensitivity of the test item is determined by calculating the mean percentage of depletion of Lysine and Cysteine.
The test item, **PENTA 18 479 - REF : TX 19006** code **ID-19/09966**, shows mean depletion of 3.48% for Lysine and 5.69% for Cysteine, i.e. an overall average of 4.58% reflecting no or minimal reactivity and therefore a negative prediction of DPRA.

However, the mean percent depletion falling in the range of 3% to 10% for the cysteine 1:10/lysine 1:50 prediction model, a second run was considered.

The test method DPRA is considered scientifically valid to be used **as part of an integrated approach** to testing and assessment, to support the identification of the sensitization potential of test item for hazard classification and labeling purposes.

12. Archive

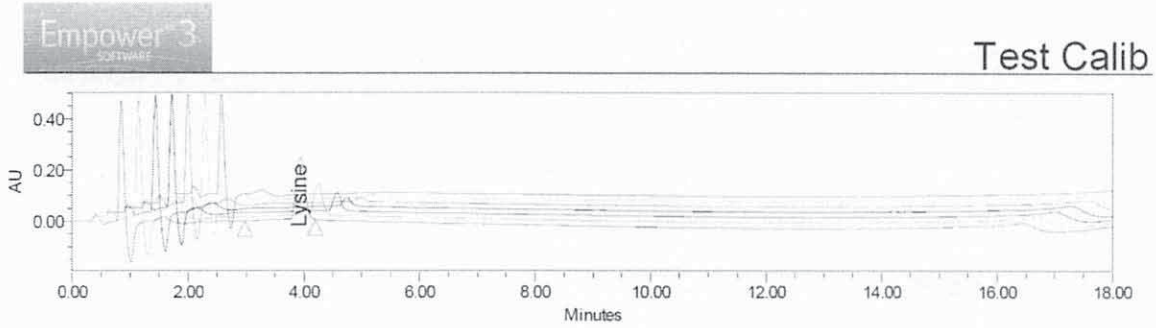
The total storage of the study folder is 10 years (Study Plan and amendment, report, raw data). The folder will be stored at least 6 months in the IDEA Lab archive room, on the Martillac location, and would be susceptible to be transferred to the non GLP premises of EVERIAL CHARTRES MEGASTORE Avenue Gustave EIFFEL 28000 CHARTRES, archiving specialist.

The reference item samples will be stored 10 years, or until their expiry date on the Martillac location, in the storage condition described in the quality form FL REAC 07.

Empower 3 analysis raw data

LYSINE

Calibration curve



SampleName: STD 1; Injection: 1; Channel: W2489 ChA; Date Acquired: 10/22/2019 11:50:54 AM CEST

SampleName: STD 2; Injection: 1; Channel: W2489 ChA; Date Acquired: 10/22/2019 12:13:47 PM CEST

SampleName: STD 3; Injection: 1; Channel: W2489 ChA; Date Acquired: 10/22/2019 12:36:38 PM CEST

SampleName: STD 4; Injection: 1; Channel: W2489 ChA; Date Acquired: 10/22/2019 12:59:28 PM CEST

SampleName: STD 5; Injection: 1; Channel: W2489 ChA; Date Acquired: 10/22/2019 1:22:19 PM CEST

SampleName: STD 6; Injection: 1; Channel: W2489 ChA; Date Acquired: 10/22/2019 1:45:12 PM CEST

SampleName: Tampon Dilution; Injection: 1; Channel: W2489 ChA; Date Acquired: 10/22/2019 2:08:07 PM CEST

Component Summary Table
Name: Lysine

	SampleName	Inj	Channel	Name	RT	Area	Height	Units
1	STD 1	1	W2489 ChA	Lysine	3.938	2677374	242447	
2	STD 2	1	W2489 ChA	Lysine	3.952	1393535	121298	
3	STD 3	1	W2489 ChA	Lysine	3.985	769297	70131	
4	STD 4	1	W2489 ChA	Lysine	3.860	467742	33950	
5	STD 5	1	W2489 ChA	Lysine	3.793	286233	17725	
6	STD 6	1	W2489 ChA	Lysine	3.719	208809	9894	
7	Tampon Dilution	1	W2489 ChA	Lysine	3.513	123395	2822	
Mean						846626	71181	
Std. Dev.						916897	86137	
% RSD						108.3	121.0	

Reported by User: Technicien Fabien NECA (TechFNE)
Report Method: Test Calib
Report Method ID: 1045
Page: 1 of 2

Project Name: DPRA 20191
Date Printed: 10/24/2019
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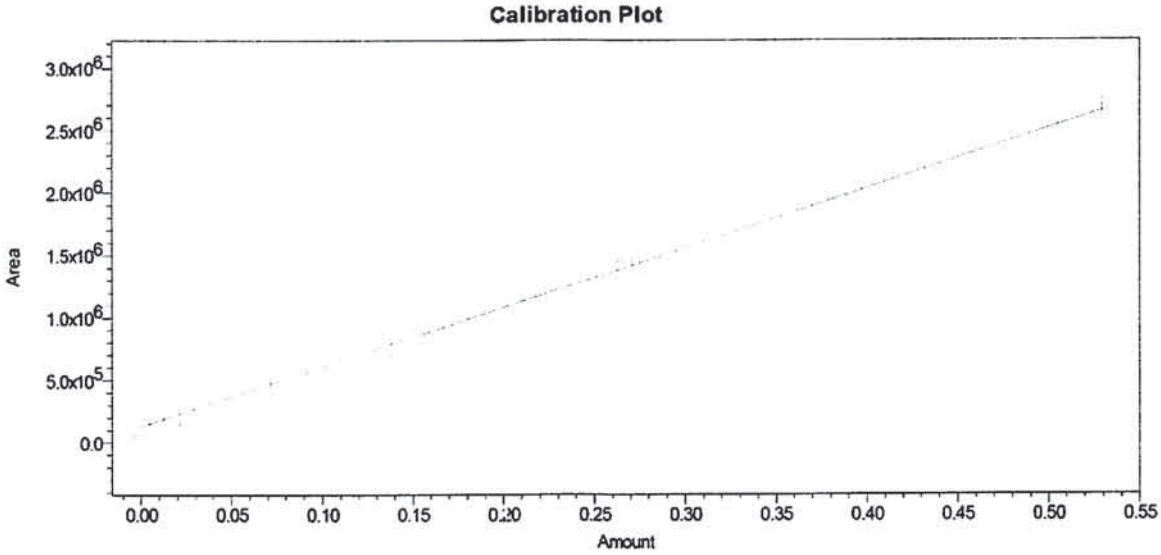

Name: Lysine; Processing Method: Lysine Nv Gdt; Fit Type: Linear (1st Order); R^2: 0.999894;
Equation Y = 4.76e+006 X + 1.31e+005

Reported by User: Technicien Fabien NECA (TechFNE)
Report Method: Test Calib
Report Method ID: 1045
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Date Printed:
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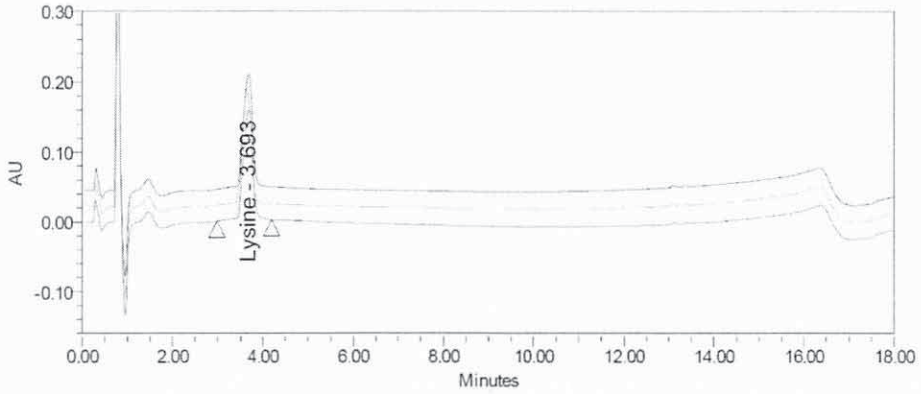
Reference control A

Empower 3 SOFTWARE

Ref A et C



Sample Name: Ref A I; Date Acquired: 10/22/2019 2:31:03 PM CEST
Sample Name: Ref A II; Date Acquired: 10/22/2019 2:53:58 PM CEST
Sample Name: Ref A III; Date Acquired: 10/22/2019 3:16:57 PM CEST

Component Summary Table
Name: Lysine

	SampleName	Inj	Channel	Name	RT	Area	Height	Amount	Units	Vial
1	Ref A I	1	W2489 ChA	Lysine	3.693	2404869	158010	0.477		8
2	Ref A II	1	W2489 ChA	Lysine	3.648	2412686	158997	0.479		9
3	Ref A III	1	W2489 ChA	Lysine	3.683	2396430	162662	0.475		10
Mean						2404662	159890	0.477		
Std. Dev.						8130	2451	0.002		
% RSD						0.3	1.5	0.4		

Reported by User: Technicien Fabien NECA (TechFNE)
Report Method: Ref A et C
Report Method ID: 1159
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Project Name: DPRA 20191
Date Printed:
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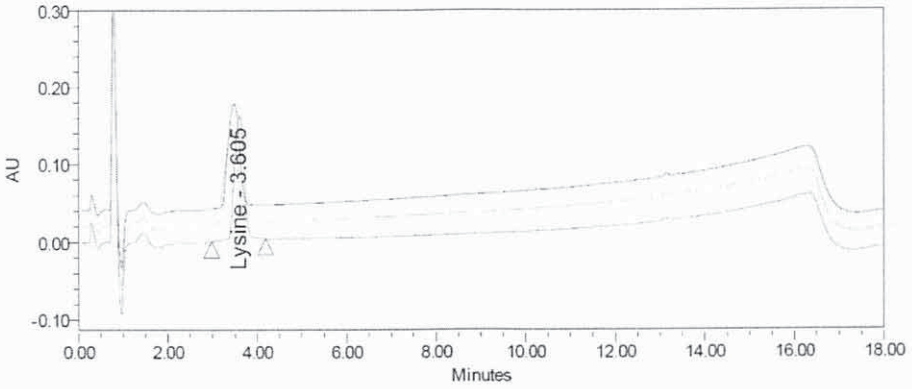
Reference control C

Empower 3 SOFTWARE

Ref A et C



Sample Name: Ref C I; Date Acquired: 10/22/2019 5:34:08 PM CEST
Sample Name: Ref C II; Date Acquired: 10/22/2019 7:28:46 PM CEST
Sample Name: Ref C III; Date Acquired: 10/22/2019 9:23:22 PM CEST

Component Summary Table
Name: Lysine

	SampleName	Inj	Channel	Name	RT	Area	Height	Amount	Units	Vial
1	Ref C I	1	W2489 ChA	Lysine	3.605	2388422	159129	0.474		17
2	Ref C II	1	W2489 ChA	Lysine	3.431	2390628	117336	0.474		22
3	Ref C III	1	W2489 ChA	Lysine	3.480	2386994	133780	0.474		27
Mean						2388681	136748	0.474		
Std. Dev.						1831	21054	0.000		
% RSD						0.1	15.4	0.1		

Reported by User: Technicien Fabien NECA (TechFNE)
Report Method: Ref A et C
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Project Name: DPRA 20191
Date Printed:
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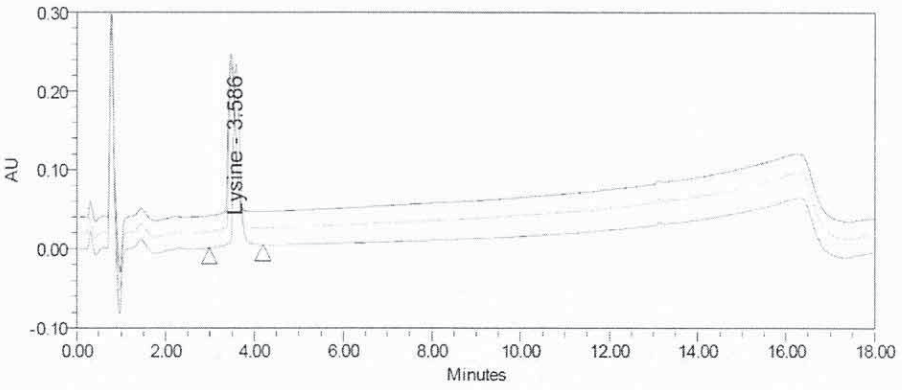
Reference control C'

Empower 3 SOFTWARE

Ref A et C



Sample Name: Ref C' I; Date Acquired: 10/22/2019 5:57:02 PM CEST
Sample Name: Ref C' II; Date Acquired: 10/22/2019 7:51:38 PM CEST
Sample Name: Ref C' III; Date Acquired: 10/22/2019 9:46:19 PM CEST

Component Summary Table
Name: Lysine

	SampleName	Inj	Channel	Name	RT	Area	Height	Amount	Units	Vial
1	Ref C' I	1	W2489 ChA	Lysine	3.586	2317672	230470	0.459		18
2	Ref C' II	1	W2489 ChA	Lysine	3.493	2317016	216749	0.459		23
3	Ref C' III	1	W2489 ChA	Lysine	3.468	2329286	204247	0.461		28
Mean						2321324	217155	0.460		
Std. Dev.						6903	13116	0.001		
% RSD						0.3	6.0	0.3		

Reported by User: Technicien Fabien NECA (TechFNE)
Report Method: Ref A et C
Report Method ID: 1159
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Project Name: DPRA 20191
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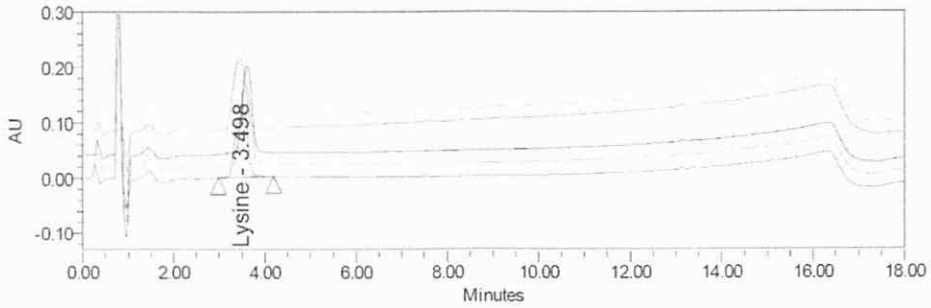
Reference control B

Empower 3 SOFTWARE

Ref B



Sample Name: Ref B I; Date Acquired: 10/22/2019 4:25:36 PM CEST
Sample Name: Ref B II; Date Acquired: 10/22/2019 4:48:25 PM CEST
Sample Name: Ref B III; Date Acquired: 10/22/2019 5:11:19 PM CEST
Sample Name: Ref B IV; Date Acquired: 10/22/2019 11:17:54 PM CEST
Sample Name: Ref B V; Date Acquired: 10/22/2019 11:40:45 PM CEST
Sample Name: Ref B VI; Date Acquired: 10/23/2019 12:03:37 AM CEST

Component Summary Table
Name: Lysine

	SampleName	Inj	Channel	Name	RT	Area	Height	Amount	Units	Vial
1	Ref B I	1	W2489 ChA	Lysine	3.498	2354367	131022	0.467		14
2	Ref B II	1	W2489 ChA	Lysine	3.583	2382681	144838	0.473		15
3	Ref B VI	1	W2489 ChA	Lysine	3.454	2384673	132031	0.473		34
4	Ref B IV	1	W2489 ChA	Lysine	3.423	2391513	128227	0.474		32
5	Ref B V	1	W2489 ChA	Lysine	3.443	2383218	126122	0.473		33
6	Ref B III	1	W2489 ChA	Lysine	3.608	2389322	158164	0.474		16
Mean						2380962	136734	0.472		
Std. Dev.						13493	12367	0.003		
% RSD						0.6	9.0	0.6		

Reported by User: Technicien Fabien NECA (TechFNE)
Report Method: Ref B
Report Method ID: 1151
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Project Name: DPRA 20191
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Positive control

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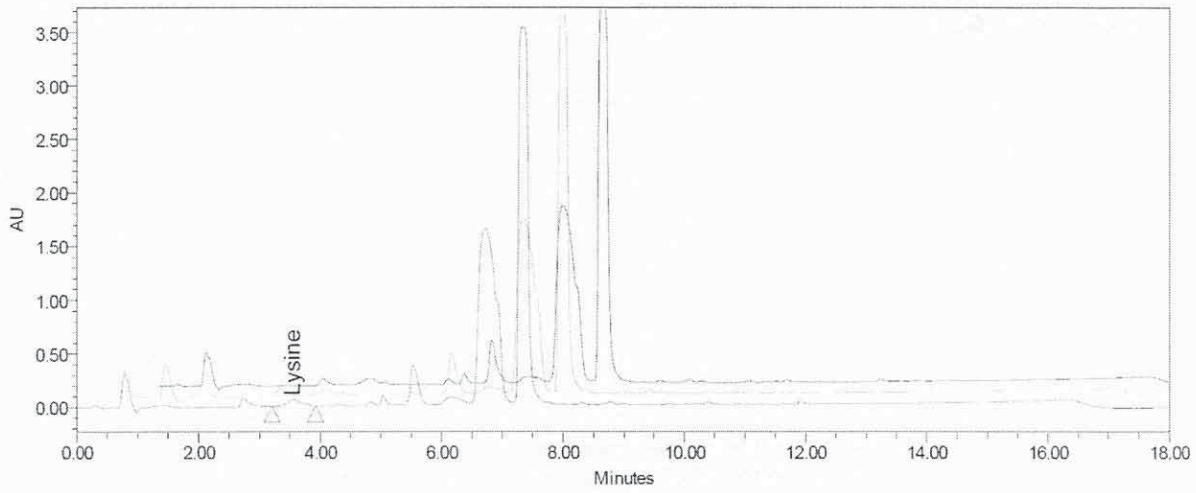
Témoin Positif



SampleName: Témoin Pos I; Vial: 19; Injection: 1; Channel: W2489 ChA; Date Acquired: 10/22/2019 6:19:56 PM CEST

SampleName: Témoin Pos II; Vial: 24; Injection: 1; Channel: W2489 ChA; Date Acquired: 10/22/2019 8:14:26 PM CEST

SampleName: Témoin Pos III; Vial: 29; Injection: 1; Channel: W2489 ChA; Date Acquired: 10/22/2019 10:09:09 PM CEST

Component Summary Table
Name: Lysine

	SampleName	Inj	Channel	Name	RT	Area	Height	Amount	Units	Vial
1	Témoin Pos I	1	W2489 ChA	Lysine	3.566	985186	58593	0.179		19
2	Témoin Pos II	1	W2489 ChA	Lysine	3.428	964515	49628	0.175		24
3	Témoin Pos III	1	W2489 ChA	Lysine	3.482	1014096	55586	0.185		29
Mean						987932	54602	0.180		
Std. Dev.						24904	4562	0.005		
% RSD						2.5	8.4	2.9		

Reported by User: Technicien Fabien NECA (TechFNE)
Report Method: Témoin Positif
Report Method ID 1148
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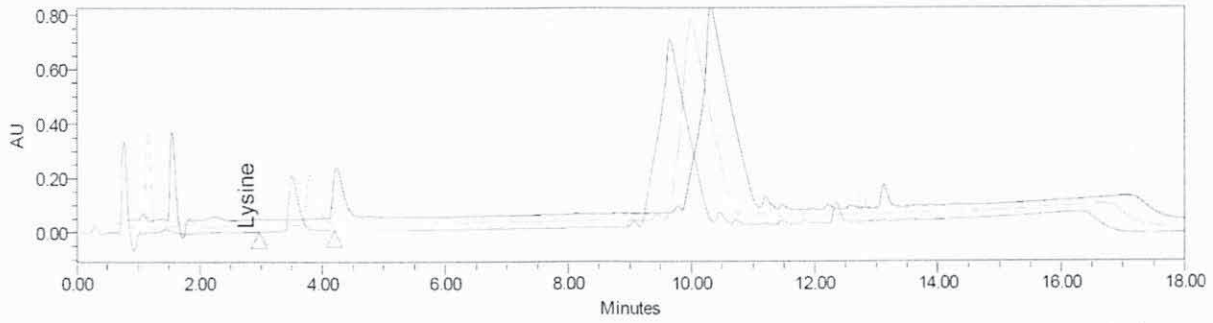
10:33:59 AM Europe/Paris

Test item

Empower 3 SOFTWARE

Elément dessai



SampleName: ID-19/09966 I; Vial: 21; Injection: 1; Channel: W2489 ChA; Date Acquired: 10/22/2019 7:05:51 PM CEST
SampleName: ID-19/09966 II; Vial: 26; Injection: 1; Channel: W2489 ChA; Date Acquired: 10/22/2019 9:00:29 PM CEST
SampleName: ID-19/09966 III; Vial: 31; Injection: 1; Channel: W2489 ChA; Date Acquired: 10/22/2019 10:55:00 PM CEST

Component Summary Table
Name: Lysine

	SampleName	Inj	Channel	Name	RT	Area	Height	Amount	Units	Vial
1	ID-19/09966 I	1	W2489 ChA	Lysine	3.512	2303746	208028	0.456		21
2	ID-19/09966 II	1	W2489 ChA	Lysine	3.413	2341126	186664	0.464		26
3	ID-19/09966 III	1	W2489 ChA	Lysine	3.449	2096753	184450	0.413		31
Mean						2247208	193047	0.444		
Std. Dev.						131632	13021	0.028		
% RSD						5.9	6.7	6.2		

Reported by User: Technicien Fabien NECA (TechFNE)
Report Method: Elément dessai
Report Method ID: 2021
Page: 1 of 1

Project Name: DPRA 20191
Date Printed: 10/24/2019
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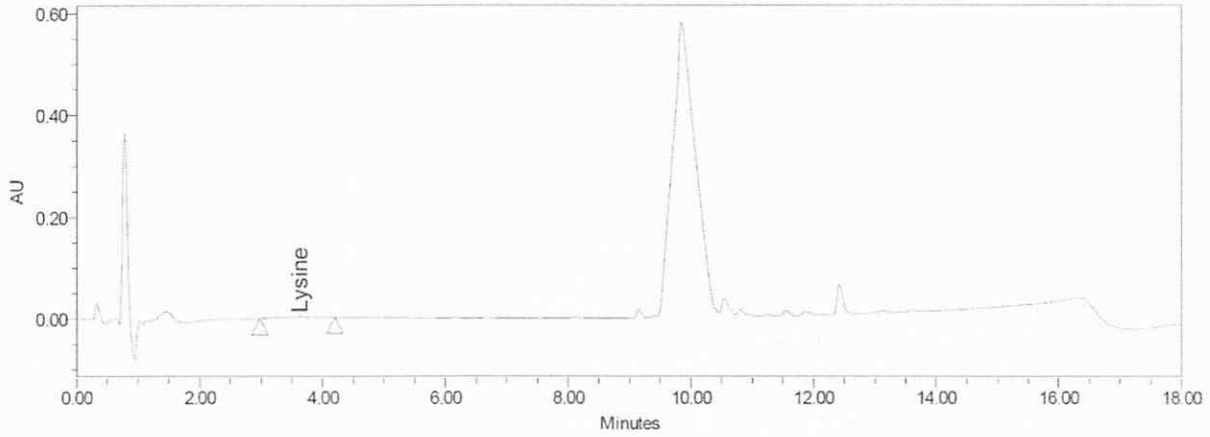
Coelution control

Empower 3 SOFTWARE

Coelution



SampleName: Coel ID-19/09966; Vial: 12; Injection: 1; Channel: W2489 ChA; Date Acquired: 10/22/2019 4:02:39 PM CEST

Component Summary Table
Name: Lysine

	SampleName	Inj	Channel	Name	RT	Area	Height	Amount	Units	Vial
1	Coel ID-19/09966	1	W2489 ChA	Lysine	3.643	83638	1992			12
Mean						83638	1992			
Std. Dev.										
% RSD										

Reported by User: Technicien Fabien NECA (TechFNE)
Report Method: Coelution
Report Method ID: 1173
Page: 1 of 1

Project Name: DPRA 20191
Date Printed:
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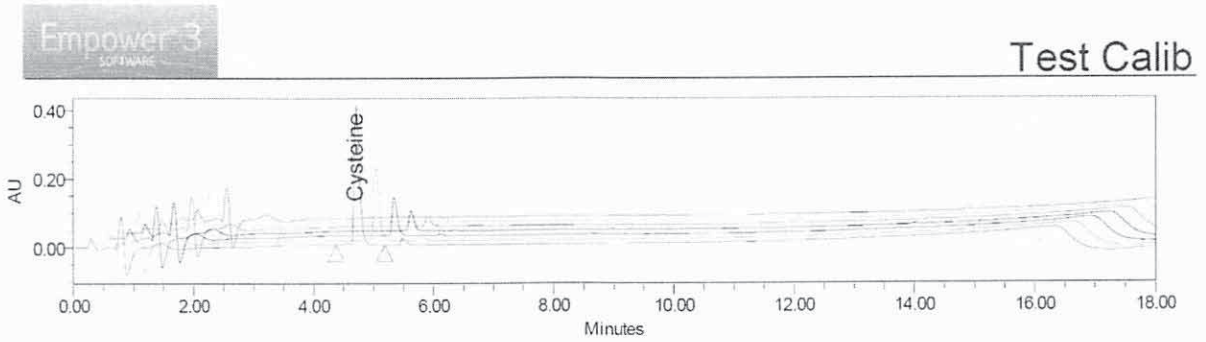
10:32:34 AM Europe/Paris

CYSTEINE

Calibration curve



SampleName: STD 1; Injection: 1; Channel: W2489 ChA; Date Acquired: 10/23/2019 11:36:15 AM CEST

SampleName: STD 2; Injection: 1; Channel: W2489 ChA; Date Acquired: 10/23/2019 11:59:09 AM CEST

SampleName: STD 3; Injection: 1; Channel: W2489 ChA; Date Acquired: 10/23/2019 12:22:01 PM CEST

SampleName: STD 4; Injection: 1; Channel: W2489 ChA; Date Acquired: 10/23/2019 12:44:56 PM CEST

SampleName: STD 5; Injection: 1; Channel: W2489 ChA; Date Acquired: 10/23/2019 1:07:46 PM CEST

SampleName: STD 6; Injection: 1; Channel: W2489 ChA; Date Acquired: 10/23/2019 1:30:38 PM CEST

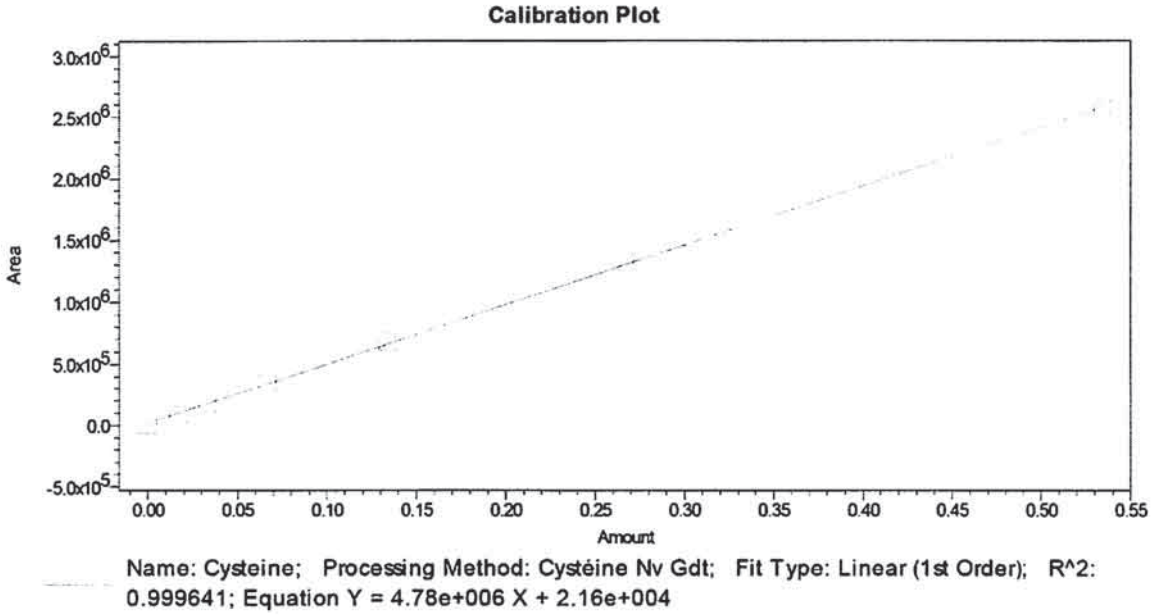
SampleName: Tampon Dilution; Injection: 1; Channel: W2489 ChA; Date Acquired: 10/23/2019 1:53:34 PM CEST

Component Summary Table
Name: Cysteine

	SampleName	Inj	Channel	Name	RT	Area	Height	Units
1	STD 1	1	W2489 ChA	Cysteine	4.720	2560024	404408	
2	STD 2	1	W2489 ChA	Cysteine	4.743	1319985	216919	
3	STD 3	1	W2489 ChA	Cysteine	4.754	681352	112269	
4	STD 4	1	W2489 ChA	Cysteine	4.737	350705	57858	
5	STD 5	1	W2489 ChA	Cysteine	4.719	178398	28412	
6	STD 6	1	W2489 ChA	Cysteine	4.707	89444	14304	
7	Tampon Dilution	1	W2489 ChA	Cysteine	4.698	1114	193	
Mean						740146	119195	
Std. Dev.						920908	146123	
% RSD						124.4	122.6	

Reported by User: Technicien Fabien NECA (TechFNE)
Report Method: Test Calib
Report Method ID: 1045
Page: 1 of 2

Project Name: DPRA 20191
Date Printed: 10/24/2019
10:53:03 AM Europe/Paris



Name: Cysteine; Processing Method: Cystéine Nv Gdt; Fit Type: Linear (1st Order); R^2: 0.999641; Equation Y = 4.78e+006 X + 2.16e+004

Reported by User: Technicien Fabien NECA (TechFNE)
Report Method: Test Calib
Report Method ID: 1045
Page: 2 of 2

Project Name: DPRA 20191
Date Printed:
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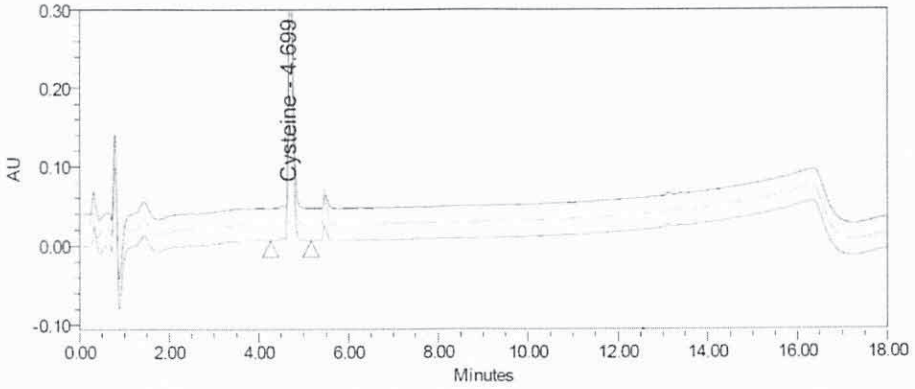
10:53:03 AM Europe/Paris

Reference control A

Empower 3 SOFTWARE

Ref A et C



Sample Name: Ref A I; Date Acquired: 10/23/2019 2:16:22 PM CEST
Sample Name: Ref A II; Date Acquired: 10/23/2019 2:39:19 PM CEST
Sample Name: Ref A III; Date Acquired: 10/23/2019 3:02:14 PM CEST

Component Summary Table
Name: Cysteine

	SampleName	Inj	Channel	Name	RT	Area	Height	Amount	Units	Vial
1	Ref A I	1	W2489 ChA	Cysteine	4.699	2412928	360831	0.500		56
2	Ref A II	1	W2489 ChA	Cysteine	4.696	2436861	376536	0.505		57
3	Ref A III	1	W2489 ChA	Cysteine	4.717	2430944	373655	0.504		58
Mean						2426911	370341	0.503		
Std. Dev.						12466	8361	0.003		
% RSD						0.5	2.3	0.5		

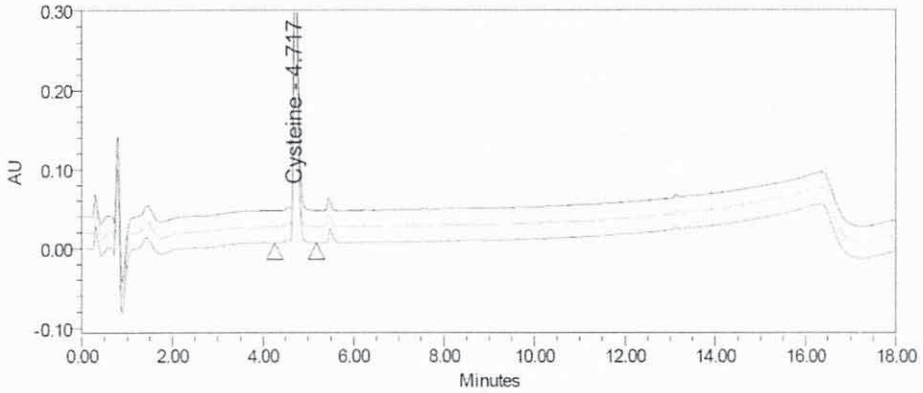
Reported by User: Technicien Fabien NECA (TechFNE)
Report Method: Ref A et C
Report Method ID: 1159
Page: 1 of 1

Project Name: DPRA 20191
Date Printed: 10/24/2019
10:53:44 AM Europe/Paris

Reference control C

Empower 3 SOFTWARE

Ref A et C



Sample Name: Ref C I; Date Acquired: 10/23/2019 5:19:24 PM CEST
Sample Name: Ref C II; Date Acquired: 10/23/2019 7:13:39 PM CEST
Sample Name: Ref C III; Date Acquired: 10/23/2019 9:08:25 PM CEST

Component Summary Table
Name: Cysteine

	SampleName	Inj	Channel	Name	RT	Area	Height	Amount	Units	Vial
1	Ref C I	1	W2489 ChA	Cysteine	4.717	2432203	368600	0.504		65
2	Ref C II	1	W2489 ChA	Cysteine	4.712	2458916	380784	0.509		70
3	Ref C III	1	W2489 ChA	Cysteine	4.730	2461088	381430	0.510		75
Mean						2450736	376938	0.508		
Std. Dev.						16087	7228	0.003		
% RSD						0.7	1.9	0.7		

Reported by User: Technicien Fabien NECA (TechFNE)
Report Method: Ref A et C
Report Method ID: 1159
Page: 1 of 1

Project Name: DPRA 20191
Date Printed:
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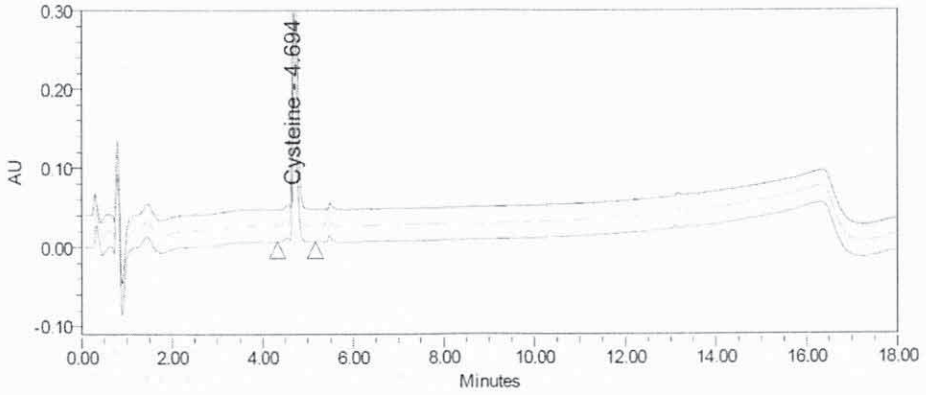
Reference control C'

Empower 3 SOFTWARE

Ref A et C



Sample Name: Ref C' I; Date Acquired: 10/23/2019 5:42:15 PM CEST
Sample Name: Ref C' II; Date Acquired: 10/23/2019 7:36:28 PM CEST
Sample Name: Ref C' III; Date Acquired: 10/23/2019 9:31:17 PM CEST

Component Summary Table
Name: Cysteine

	SampleName	Inj	Channel	Name	RT	Area	Height	Amount	Units	Vial
1	Ref C' I	1	W2489 ChA	Cysteine	4.694	2383878	379943	0.494		66
2	Ref C' II	1	W2489 ChA	Cysteine	4.662	2334843	390404	0.484		71
3	Ref C' III	1	W2489 ChA	Cysteine	4.722	2424124	384262	0.502		76
Mean						2380948	384870	0.493		
Std. Dev.						44713	5257	0.009		
% RSD						1.9	1.4	1.9		

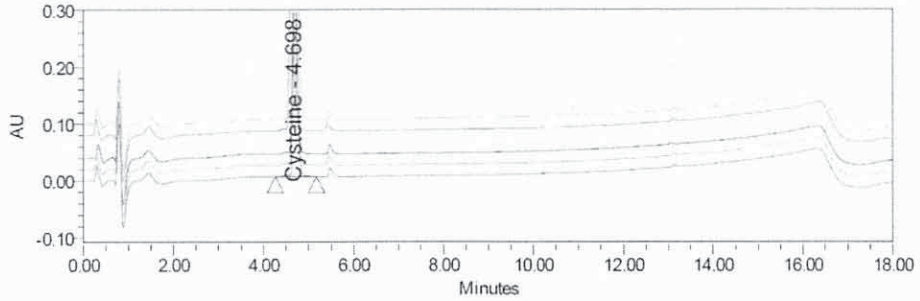
Reported by User: Technicien Fabien NECA (TechFNE)
Report Method: Ref A et C
Report Method ID: 1159
Page: 1 of 1

Project Name: DPRA 20191
Date Printed: 10/24/2019
10:55:38 AM Europe/Paris

Reference control B

Empower 3 SOFTWARE

Ref B



Sample Name: Ref B I; Date Acquired: 10/23/2019 4:10:51 PM CEST
Sample Name: Ref B II; Date Acquired: 10/23/2019 4:33:41 PM CEST
Sample Name: Ref B III; Date Acquired: 10/23/2019 4:56:33 PM CEST
Sample Name: Ref B IV; Date Acquired: 10/23/2019 11:02:45 PM CEST
Sample Name: Ref B V; Date Acquired: 10/23/2019 11:25:44 PM CEST
Sample Name: Ref B VI; Date Acquired: 10/23/2019 11:48:35 PM CEST

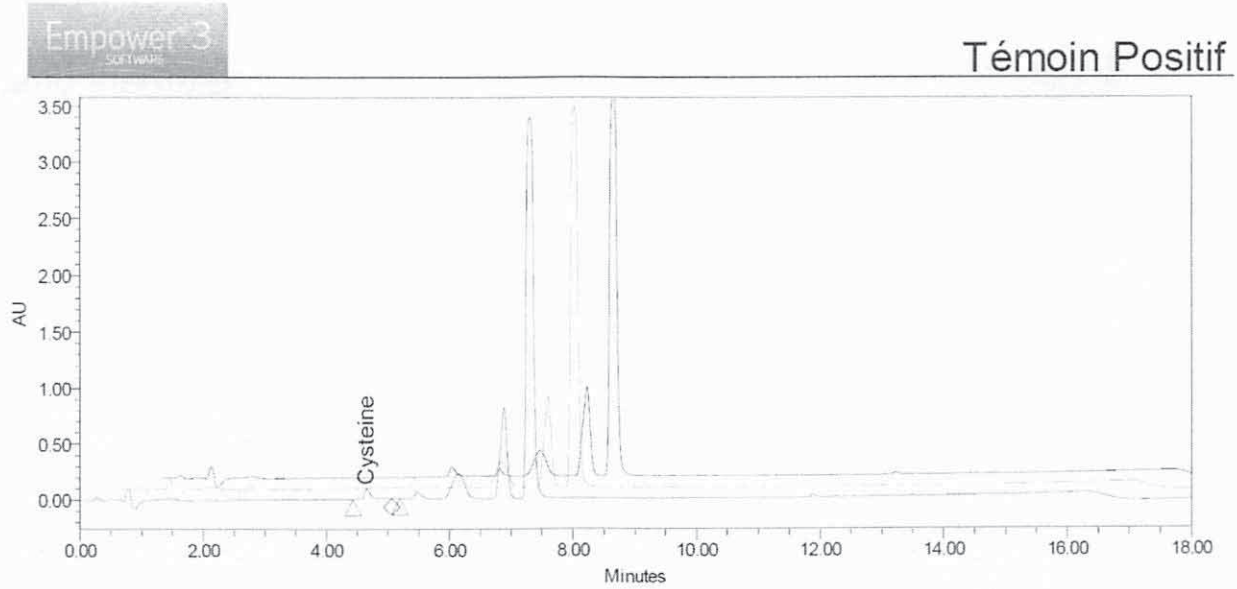
Component Summary Table
Name: Cysteine

	SampleName	Inj	Channel	Name	RT	Area	Height	Amount	Units	Vial
1	Ref B I	1	W2489 ChA	Cysteine	4.698	2435035	371636	0.504		62
2	Ref B II	1	W2489 ChA	Cysteine	4.680	2437632	369234	0.505		63
3	Ref B VI	1	W2489 ChA	Cysteine	4.729	2429819	373127	0.503		82
4	Ref B IV	1	W2489 ChA	Cysteine	4.721	2442606	368722	0.506		80
5	Ref B V	1	W2489 ChA	Cysteine	4.621	2453636	365069	0.508		81
6	Ref B III	1	W2489 ChA	Cysteine	4.684	2442113	363591	0.506		64
Mean						2440140	368563	0.506		
Std. Dev.						8136	3679	0.002		
% RSD						0.3	1.0	0.3		

Reported by User: Technicien Fabien NECA (TechFNE)
Report Method: Ref B
Report Method ID: 1151
Page: 1 of 1

Project Name: DPRA 20191
Date Printed:
10/24/2019
10:54:50 AM Europe/Paris

Positive control



SampleName: Témoin Pos I; Vial: 67; Injection: 1; Channel: W2489 ChA; Date Acquired: 10/23/2019 6:05:11 PM CEST
SampleName: Témoin Pos II; Vial: 72; Injection: 1; Channel: W2489 ChA; Date Acquired: 10/23/2019 7:59:20 PM CEST
SampleName: Témoin Pos III; Vial: 77; Injection: 1; Channel: W2489 ChA; Date Acquired: 10/23/2019 9:54:07 PM CEST

Component Summary Table
Name: Cysteine

	SampleName	Inj	Channel	Name	RT	Area	Height	Amount	Units	Vial
1	Témoin Pos I	1	W2489 ChA	Cysteine	4.661	671024	90747	0.136		67
2	Témoin Pos II	1	W2489 ChA	Cysteine	4.741	599266	90464	0.121		72
3	Témoin Pos III	1	W2489 ChA	Cysteine	4.701	658445	90374	0.133		77
Mean						642912	90528	0.130		
Std. Dev.						38318	194	0.008		
% RSD						6.0	0.2	6.2		

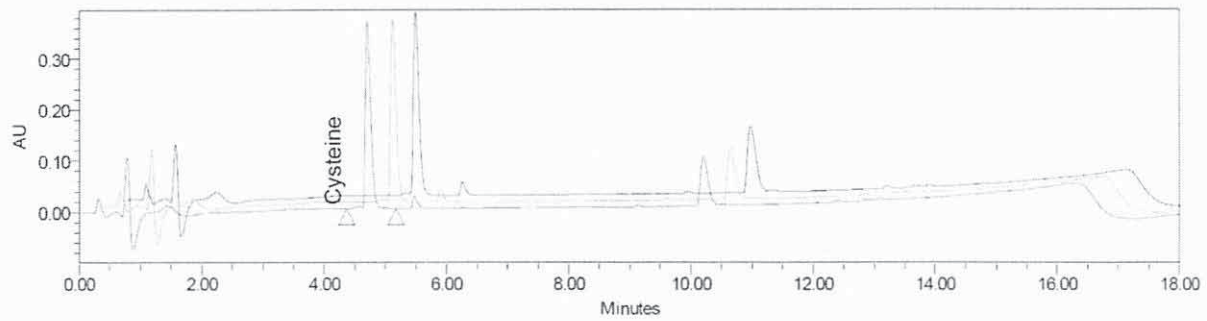
Reported by User: Technicien Fabien NECA (TechFNE)
Report Method: Témoin Positif
Report Method ID 1148
Page: 1 of 1

Project Name: DPRA 20191
Date Printed: 10/24/2019
10:56:00 AM Europe/Paris

Test item

Empower 3 SOFTWARE

Elément dessai



SampleName: ID-19/09966 I; Vial: 69; Injection: 1; Channel: W2489 ChA; Date Acquired: 10/23/2019 6:50:52 PM CEST

SampleName: ID-19/09966 II; Vial: 74; Injection: 1; Channel: W2489 ChA; Date Acquired: 10/23/2019 8:45:29 PM CEST

SampleName: ID-19/09966 III; Vial: 79; Injection: 1; Channel: W2489 ChA; Date Acquired: 10/23/2019 10:39:54 PM CEST

Component Summary Table
Name: Cysteine

	SampleName	Inj	Channel	Name	RT	Area	Height	Amount	Units	Vial
1	ID-19/09966 I	1	W2489 ChA	Cysteine	4.711	2254843	365543	0.467		69
2	ID-19/09966 II	1	W2489 ChA	Cysteine	4.731	2220613	357928	0.460		74
3	ID-19/09966 III	1	W2489 ChA	Cysteine	4.709	2261247	358720	0.468		79
Mean						2245568	360731	0.465		
Std. Dev.						21848	4187	0.005		
% RSD						1.0	1.2	1.0		

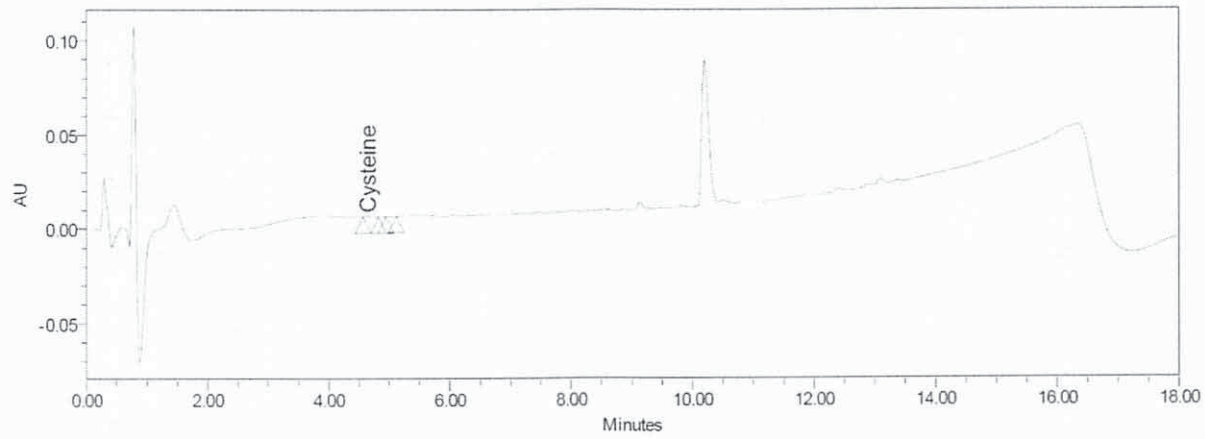
Reported by User: Technicien Fabien NECA (TechFNE)
Report Method: Elément dessai
Report Method ID: 2021
Page: 1 of 1

Project Name: DPRA 20191
Date Printed: 10/24/2019
10:56:44 AM Europe/Paris

Coelution control

Empower 3 SOFTWARE

Coelution



SampleName: Coel ID-19/09966; Vial: 60; Injection: 1; Channel: W2489 ChA; Date Acquired: 10/23/2019 3:48:01 PM CEST

Component Summary Table
Name: Cysteine

	SampleName	Inj	Channel	Name	RT	Area	Height	Amount	Units	Vial
1	Coel ID-19/09966	1	W2489 ChA	Cysteine	4.669	2335	373			60
Mean						2335	373			
Std. Dev.										
% RSD										

Reported by User: Technicien Fabien NECA (TechFNE)
Report Method: Coelution
Report Method ID: 1173
Page: 1 of 1

Project Name: DPRA 20191
Date Printed: 10/24/2019
10:54:25 AM Europe/Paris

Proficiency Substances

Recommended substances for demonstrating technical proficiency with the DPRA test method were tested in order to validate the method in our laboratory. Results are shown in the table below:

Proficiency substances	IDEA Internal code	OECD Specifications					IDEA LAb performances			
		N°CAS	OCDE In vivo prediction	DPRA prediction	Range of % cysteine peptide	Range of % lysine peptide	% cysteine peptide depletion	% lysine peptide depletion	% mean depletion	DPRA Prediction
2,4 – Dinitrochlorobenzene	ID3097	97-00-7	Sensitiser (extreme)	POS	90-100	15-45	99.35	21.16	**60.26**	POS
Oxazolone	ID2397	15646-46-5	Sensitiser (extreme)	POS	60-80	10-55	30.56	51.63	**41.10**	POS
Formaldehyde	ID3098	50-00-0	Sensitiser (strong)	POS	30-60	0-24	49.82	0.46	**25.14**	POS
Benzylidene-acetone	ID2394	122-57-6	Sensitiser (moderate)	POS	80-100	0-7	89.85	0	**44.93**	POS
Farmesal	ID2393	19317-11-4	Sensitiser (weak)	POS	15-55	0-25	14.07	4.78	**9.42**	POS
2,3 – Butanedione	ID2398	431-03-8	Sensitiser (weak)	POS	60-100	10-45	66.49	25.98	**46.24**	POS
1-Butanol	ID2392	71-36-3	Non-sensitiser	NEG	0-7	0-5.5	0.52	0	**0.26**	NEG
6-Methylcoumarine	ID2395	92-48-8	Non-sensitiser	NEG	0-7	0-5.5	0.22	0	**0.11**	NEG
Lactic Acid	ID3099	50-21-5	Non-sensitiser	NEG	0-7	0-5.5	1.68	0	**0.84**	NEG
4-Methixyacetophenone	ID2396	100-06-1	Non-sensitiser	NEG	0-7	0-5.5	0	0.15	**0.08**	NEG

Laboratories should demonstrate technical proficiency by correctly obtaining the expected DPRA prediction for the 10 proficiency substances recommended in Table 1 and by obtaining cysteine and lysine depletion values that fall within the respective reference range for 8 out of the 10 proficiency substances for each peptide.

Certificate of analysis

sederma	ENREGISTREMENT QSHE	Codification **EE1/33** Date : 02/04/15 Révision : c
	BULLETIN D'ANALYSES *CERTIFICATE OF ANALYSIS*	Page 1 sur 1

PRODUIT / *PRODUCT* : TX 19006

N° de LOT / *BATCH Nr* : B1

Stockage / *Storage* : **Stockage entre +15°C et +25°C**
Storage between +15°C and +25°C

Date de fabrication : 27 mai 2019
Date of manufacturing

Date de retest : 27 février 2020
Retest date

Les analyses effectuées sont garanties quand le produit est stocké dans son emballage d'origine et à la température recommandée
The performed analysis are guaranteed when product is stored in original packaging and at recommended temperature

	Résultats *Results*
Aspect *Appearance*	Poudre blanche *White powder*
Teneur en Pal KTSKS (HPLC) *Pal KTSKS content*	81.6%

Liliane IACUZZI
Responsable Assurance Qualité Produit / Product Quality Assurance Manager

Ce document est une copie informatique et de ce fait ne porte pas de signature / This certificate is a computer printout and therefore has no signature

SEDERMA 29 rue du Chemin Vert – 78610 LE PERRAY EN YVELINES – France
Tél. : 01.34.84.10.10 – Fax : 01.34.84.11.30

IDEA TESTS GROUP

IDEA Lab

Test item
PENTA 18 479 - REF : TX 19006

In vitro sensitization test: KeratinoSens™ Keratinocytes test based on the signaling pathway Keap1-Nrf2-ARE coupled to the luciferase reporter gene

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FINAL REPORT

Study number: 6.52-52291-ID-19/09966

Sponsor

SEDERMA
29, rue du Chemin-Vert
BP 33
78612 LE PERRAY EN YVELINES CEDEX
FRANCE

Test Facility

IDEA Lab
Technopôle Montesquieu
5, rue Jacques Monod
CS 60077
33652 MARTILLAC Cedex
FRANCE

CONTENTS

GLP conformity statement

The study 6.52-52291-ID-19/09966 was performed in the IDEA Lab company laboratory, in agreement with the French Good Laboratory Practice (GLP) principles, the European Directive 2004/10/CE and the decree dated August 10th, 2004 from the JOFR.

All relevant Standard Operating Procedures have been followed and raw data have been registered accurately.

For confidentiality concerns, some characterisation data related to the test item composition are not shown in this report. This is a deviation to GLP. However, this characterization had been provided by the Sponsor, brought to my attention, then stored in a secure environment in accordance with the company procedures.

The lack of verification of the concentration of the test item in various dilutions has no impact on the reliability of the results generated for the following reasons:

- the test item preparation in its vehicle is controlled, particularly with micropipette and precision scales regularly controlled, calibrated and traceable with national or international standards of measurement,
- the control of the homogeneity of the test item dilutions in the vehicle is performed using organoleptic criteria and is documented in the study log book,
- test item dilutions are prepared extemporaneously.

This report accurately reflects the study carried out and the results obtained.

I declare this study compliant with the Good Laboratory Practice and assume responsibility for the data validity of the study.

Date: 27 NOV. 2019

Study Director
Michèle PARMANTIER
High level technician in biochemistry

Quality Assurance statement

According to the Good Laboratory Practices, I state that:

The General Study Plan was audited by the Quality Assurance and that the Specific Study Plan was verified before the beginning of the study.

The different technical phases of the study 6.52 are regularly audited by the Quality Assurance. Facility audits are also carried out. The audit frequency is defined in the corresponding procedure.

At the last technical audit A-18/03, the following activities have been inspected:

- Cells seeding
- Preparation of test and reference items dilutions
- Contact of cells with test and reference items
- Luciferase activity reading
- Cytotoxicity reading

The final report was audited by the Quality Assurance of IDEA Lab. It accurately reflects the raw data from the study and the application of the Standard Operating Procedures and the Study Plan.

Audit nature	**Audits dates**	**Transmission dates of the audit report to the Study Director and the General Management**
Technical phases of the study	From 26/03/2018 to 30/04/2018	30/04/2018
General Study Plan	29/09/2016	29/09/2016
Draft report	21/11/2019	21/11/2019
Final Report	25 NOV. 2019	25 NOV. 2019

Date: 25 NOV. 2019

Quality Assurance

Nelly TEYSSANDIER

Study presentation

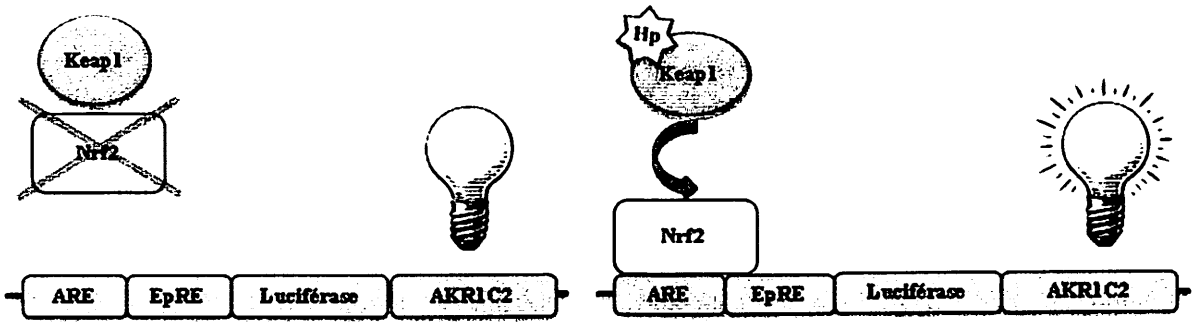
1. Study objective

At the Sponsor's request, we have evaluated, by an *in vitro* test, the sensitizing potential of a test item according to the General Study Plan 6.52 version 03A.

A skin sensitizer refers to a substance that will lead to an allergic response following skin contact.
One of the biological key events takes place in the keratinocytes and includes inflammatory responses as well as gene expression associated with specific cell signaling pathways such as the antioxidant/electrophile response element (ARE) dependent pathways.
The genes under the ARE control, including AKR1C2 gene identified as a target gene for detecting skin sensitizers in keratinocytes, are induced by the protein Nrf2 via Keap1.
The test consists in evaluating the activation of AKR1C2 in transformed keratinocytes (KeratinoSens™), by monitoring the induction of the luciferase gene fused to AKR1C2. The luciferase produced by the cells complexes with luciferin which, in the presence of ATP, produces light measured in relative light units (RLU).

After contact between a potentially sensitizing element with a KeratinoSens™ monolayer, the induction of the luciferase is quantified. In parallel, the cytotoxicity is measured, in order to exclude a false positive generated by skin irritation.

The principle of the test is shown in the schema below.



Since activation of the Keap1-Nrf2-ARE pathway addresses only the second key event of the skin sensitization AOP, information from test methods based on the activation of this pathway is unlikely to be sufficient when used on its own to conclude on the skin sensitization potential of chemicals.
Therefore data generated should be considered in the context of integrated approaches.

This study is carried out according to the OECD Guideline 442D dated February, 04th, 2015 and the ECVAM DB-ALM protocol 155: KeratinoSens™.

2. Experimentation dates

Repetition 1 was carried out from 30/09/2019 to 04/10/2019.
Repetition 2 was carried out from 07/10/2019 to 11/10/2019.

3. Series definition

Test item
The test item was tested at 12 concentrations according to a geometric progression of ratio 2 from 0.98 µM to 2000 µM.

Reference item
Negative control: 6 wells of solvent control (1% DMSO in treatment medium) with cells and 1 well of solvent control without cell by culture plate.
Positive control: 5 concentrations of cinnamaldehyde on each culture plate. The concentration varies from 4 to 64 µM according to a geometric progression of ratio 2.

The study was composed of two independent repetitions. For each repetition the test item and the reference items were replicated on three independent plates for the measurement of induction and two plates for the measurement of cytotoxicity. Each repetition was performed on a different day with fresh stock solution.

4. Test item

PENTA 18 479 - REF : TX 19006

Internal code	: ID-19/09966
Batch number	: B1
Storage conditions	: Room temperature (20°C ± 5°C)
Test item nature	: Cosmetic ingredient
Retest date	: 27/02/2020

Physico-chemical properties

Color	: White
Physical state at 20°C	: Solid
Homogeneity	: Yes
Purity	: 81.6%
Molecular weight	: 859.50 g/mol
pH	: NA

Solubility and stability

Solvent	: Ethanol
Stability in the solvent	: Unspecified*
Maximal concentration in solvent	: 2.5 g/l

* The technical data sheet provided by the Sponsor did not indicate information about the stability of the test item in its solvent. The homogeneity and stability of the dilutions have been checked during the study and recorded in the study file.

Information linked to the identification, purity and stability of the test item are under the responsibility of the Sponsor of the study. The technical data sheet of the test item was provided by the Sponsor of the study.

The test item will be stored at least 2 months in the product room of the Martillac location before to be destroyed or sent back to the Sponsor according to his choice.

The certificate of analysis is shown at the end of the report.

5. Reference items

5.1. Positive control

Cinnamaldehyde (SIGMA ALDRICH Ref W228613):

Internal code : ID 2696
CAS number : 104-55-2
Batch number : MKBT8955V
Aspect : Liquid
Color : Colorless to yellow
Storage conditions : Room temperature
Expiry date : 28/02/2020
Purity : 99.1%
Molecular weight : 132.16 g/mol
Density : 1.049
Solubility : DMSO

5.2. Negative control

Treatment culture medium, 1% DMSO, 1% Non-heat inactivated foetal calf serum:

Repetition 1 and 2

	DMEM 1 g/l glucose	DMSO	Non-heat inactivated foetal calf serum
Internal code	ID 3691	ID 3590	ID 3398
Supplier reference	Ref 21885025 (Fisher Bioblock)	41640 (Sigma Aldrich)	Ref 11563397 (Fisher Bioblock)
Batch number	2095710	STBH8906	42F6480K
Expiry date	31/03/2020	30/10/2021	30/04/2023
CAS number	-	67-68-5	-
Purity	-	99.9%	-

The negative control is prepared just prior the test and used within the day.

6. Test system

Cells: KeratinoSens™ (Givaudan) maintained according to the current working instruction IL 09. Cells are cultured in maintenance medium (cf. § Media and reagents) at 37°C, 5% CO_2.

Cells are exempt of mycoplasma. Assessment of mycoplasma was performed according to the current working instruction IL 07.

Cells were used at passage 18 in repetition 1 and passage 20 in repetition 2.

7. Media and reagents

- Maintenance medium: DMEM 1 g/l glucose, 9.1% non-heat inactivated foetal calf serum, 0.05% geneticin - stored at 5°C ± 3°C
- Seeding medium: DMEM 1 g/l glucose, 9.1% non-heat inactivated foetal calf serum - stored at 5°C ± 3°C
- Treatment medium: DMEM 1 g/l glucose, 1% non-heat inactivated foetal calf serum - stored at 5°C ± 3°C
- Trypsin (0.5 g/l) - EDTA (0.2 g/l) - stored at -20°C ± 5°C
- Diluent for the test item: treatment culture medium 4% DMSO - prepared extemporaneously and used within the day
- Diluent for the positive control: DMSO - stored at room temperature 20°C ± 5°C
- Luciferase substrate: Bright Glo™ Luciferase Assay System (Promega) - stored at -80°C after reconstitution
- Cell washing solution: Dulbecco's PBS Ca^{2+} and Mg^{2+} free with 0.05% EDTA - stored at 5°C ± 3°C
- Dulbecco's PBS Ca^{2+} and Mg^{2+} free - stored at room temperature 20°C ± 5°C
- Staining solution: 5 mg/ml MTT* (3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide) solution in PBS - prepared extemporaneously and used within the day
- Desorption solution: 10% SDS in water - stored at room temperature 20°C ± 5°C

FL REAC 01 and FL REAC 06 forms ensure the traceability of media and reagents used in the study.

** Note: MTT powder is stored at 5°C ± 3°C.*

8. Equipment and consumables

- Luminometer: GloMax™ (Promega)
- MULTISKAN EX plate reader (Thermo life sciences) - reading range 0 - 3.5 units of Absorbance - linearity range 0 - 2.200 units of Absorbance
- White cell culture 96-well plates for luminescence reading
- Transparent cell culture 96-well plates for absorbance reading
- Plastic adhesive foils
- 0.2 µm filter
- Conventional cell culture laboratory equipment.

FL REAC 01 and FL REAC 06 forms ensure the consumables traceability used in the study.

The equipment used is recorded in the study notebook.

9. Test protocol

9.1. Cells seeding (first day)

The cells were trypsinized according to the current working instruction IL 09. Cells suspension were adjusted to a density of 8.10^4 cells/ml in seeding medium.
125 µl of the cell suspension at 8.10^4 cells/ml (i.e. 10^4 cells per well) were distributed in three white plates for the induction measurement and two transparent plates to assess the cytotoxicity. The seeded plates were incubated 24 hours ± 1 hour at 37°C, 5% CO_2.
Note: the H12 wells were left without cells and allowed the measurement of blanks.

9.2. Preparation of test item and positive control dilutions (second day)

Preparation of the test item stock solution

Given the slight solubility of the test item stock solution it was prepared at 8 mM (4X) in treatment medium, 4% DMSO instead of 200 mM (100X).

A volume V of treatment medium, 4% DMSO was calculated according to the following formula:

$$V = 125 \times \frac{(p \div 100) \times w}{MW} - \frac{w}{1000}$$

p is the purity of the test item in %
MW is the molecular weight of the test item in g/mol
w is the exact weight of the test item in mg.

Preparation of the positive control stock solution
The positive control stock solution was prepared at 200 mM in DMSO according to the following formula, then diluted to the concentration of 6.4 mM.

$$V = 5 \times \frac{(p \div 100) \times w}{MW} - \frac{w}{1000}$$

V is the volume of DMSO in ml to be added
p is the purity of the positive control in %
MW is the molecular weight of the positive control in g/mol
w is the exact weight of the positive control in mg.

Preparation of the 100 X plate *(positive and negative control)*
A 100-fold concentrated dilutions series was prepared in 96-well plate.

Positive control
100 µl of DMSO were distributed in row G from columns 7 to 10. 200 µl of the 6.4 mM stock solution were placed in column 11 then the series dilutions were prepared by transferring 100 µl of the column 11 in the column 10 and so on until the column 7. Dilutions were mixed by repeated pipetting, at least 3 times, between each concentration.

Negative control
100 µl of DMSO were distributed in row G columns 1 to 6 and 12 and in the well H12.

Preparation of the 4 X dilution plate

Test item
The test item was placed in one of the rows B to F.
100 µl of treatment culture medium 4% DMSO were distributed from columns 1 to 11. 200 µl of the 8 mM stock solution were placed in column 12 then the series dilutions were prepared by transferring 100 µl of the column 12 in the column 11 and so on until the column 1. Dilutions were mixed by repeated pipetting, at least 3 times, between each concentration.

Positive and negative control
The 100 X DMSO plate was diluted 25 fold in a 4X plate in treatment medium.

9.3. Contact between the cells and the test and reference items (second day)

In the 5 seeded plates, the medium was aspirated and replaced with 150 µl of treatment medium. Then the 4 X plate was replicated 5 times: 50 µl from the 4 X plate were placed in each of the three white plates and in the two transparent plates. The plates (1 X) were covered with an adhesive plastic foil to prevent evaporation and incubated for 48 hours ± 1 hour (37°C, 5% CO_2).

9.4. Luciferase activity (day 4)

After 48 hours, the medium was aspirated and each well was gently washed once with 200 µl of PBS. Then 100 µl of luciferase substrate (luciferin + ATP + lysing agent) were then added in each well. The plates were incubated at least 15 minutes at room temperature to ensure cells lysis.
The plates were placed in the luminometer then the luciferase activity was measured with an integration time of 2 seconds.

9.5. Cell viability assessment with MTT method (day 4)

After 48 hours, the medium was aspirated and each well was gently washed once with 200 µl of PBS. Then, 225 µl of staining solution diluted at 0.6 mg/ml in treatment medium (from the 5 mg/ml stock solution) were distributed in each well. The plates were covered with an adhesive plastic foil and incubated for 4 hours ± 30 minutes (37°C, 5% CO_2).
After this contact time, the staining solution was eliminated and the cells were treated with 200 µl of 10% SDS one night in the dark (37°C, 5% CO_2). After a 10 minutes homogenization, the absorbances were measured at 540 nm.

10. Results calculation and interpretation

10.1. Expression of results

Two parameters are measured, the luciferase induction and the cytotoxicity.

10.1.1. Luciferase induction

I_{max}, maximal fold induction of luciferase activity value observed at any concentration of the test item and positive control. The induction value I is calculated according to the following formula:

$$I = \frac{\text{Luminescence}_{\text{Test item}} - \text{Luminescence}_{\text{Blank}}}{\text{Luminescence}_{\text{Negative control}} - \text{Luminescence}_{\text{Blank}}}$$

The I_{max} of an item is the average of the I_{max} calculated for each of the repetitions.

EC1.5, value representing the concentration for which induction of luciferase activity is above 1.5 threshold, is obtained according to the following equation:

$$EC1.5 = (C_b - C_a) \times \left[\frac{(1.5 - I_a)}{(I_b - I_a)}\right] + C_a$$

Where:
C_a = the lowest concentration with more than 1.5 fold the induction
C_b = the highest concentration with less than 1.5 fold the induction
I_a = induction factor for the lowest concentration with more than 1.5 fold the induction.
I_b = induction factor for the highest concentration with less than 1.5 fold the induction

The EC1.5 of a test item is the geometric average of the EC1.5 calculated for each of the repetitions

10.1.2. Cytotoxicity

The viability V is calculated according to the following formula:

$$V = \frac{\text{Absorbance}_{\text{Test item}} - \text{Absorbance}_{\text{Blank}}}{\text{Absorbance}_{\text{Negative control}} - \text{Absorbance}_{\text{Blank}}} \times 100$$

IC_{70}, concentration for which we obtained 70% cell viability:

$$ICx = (C_b - C_a) \times \left[\frac{(x) - V_a)}{(V_b - V_a)}\right] + C_a$$

where
x is the % viability at the concentration to be calculated (70)
C_a is the lowest concentration for which the % viability is lower than X%
C_b is the highest concentration for which the % viability is higher than X%
V_a is the % viability at the lowest concentration for which the % viability is lower than X%
V_b is the % viability at the highest concentration for which the % viability is higher than X%

The data processing is carried out by a locked Excel sheet provided by Givaudan. The raw data generated from the reading of the plates are directly introduced into the dedicated fields, and a data processing is performed automatically.

A graph showing the gene induction and the cytotoxicity of each element, the I_{max} and EC1.5 value are automatically generated.
The treatment of the data generated by the matrix Excel is carried out according to the current working instruction IL 04.

10.2. Test validation / historical data

To validate the test, it is essential to check the validity criteria for the test:

Positive Control:
• the gene induction must be statistically significant above the threshold of 1.5 in at least one dose,
• the EC1.5 value should be between IDEA Lab historical data: mean EC1.5 value ± 2 SD and the average induction, in each repetition, for cinnamaldehyde at 64 µM should be between 2 and 8. If the latter criterion is not fulfilled, the dose-response of cinnamaldehyde should be carefully checked, and tests may be accepted only if there is a clear dose-response with increasing luciferase activity induction at increasing concentrations for the positive control.

Follow-up of positive control: Cinnamaldehyde (CAS N°104-55-2):
Updated on 19/04/2019 - N = 344

	Imax	EC1.5	IC_{70}
Mean value	5.12	11.01µM	> 64 µM
Standard deviation	2.57	4.51	-

Mean EC1.5 value ± 2 SD	-	2.0 µM ≤ EC1.5 ≤ 20 µM	-

OECD validation dataset: between 7 µM and 30 µM.

Negative Control
For each repetition, the coefficient of variation of the solvent controls (3 x 6 wells) must be less than 20%.

If for one repetition the validity criteria are not met, a third repetition should be considered.
The validation of the results is carried out by the Study Director in accordance with the current working instruction IL 04.

10.3. Interpretation

The test item is identified as potential skin sensitizer if the 4 following conditions are met in 2 of 2 or in 2 of 3 repetitions. Otherwise the Keratinosens™ prediction is considered as negative:

• the I_{max} is strictly 1.5 fold higher of the basal luciferase activity* statistically significantly to the value obtained for the negative control (as determined by a two-tailed, unpaired Student's t-test on the raw RLU values),
** If the I_{max} is exactly equal to 1.5, the test item is rated as negative and no EC1.5 value is calculated*

• the EC1.5 value is strictly below 1000 µM,

• at the lowest concentration with a gene induction above 1.5, the cell viability must be strictly above 70% (i.e. EC1.5 < IC70),

• there is an apparent overall dose-response for luciferase induction, which is similar between the repetitions.

In rare cases, the test items which induce the gene activity at a concentration very close to the cytotoxic levels, are positive in some repetitions at non-cytotoxic levels, and in other repetitions only at cytotoxic levels. In this case, the test item must be tested again with a narrower range using a dilution factor of 4/3 instead of 2.

Test items that only induce the gene activity at cytotoxic levels are not rated as positive, as it is the case for some non-sensitizing skin irritants.

If, in a given repetition, all of the three first conditions are met but a clear dose-response for the luciferase induction cannot be observed, then the result of that repetition should be considered inconclusive and further testing may be required. In addition, in case of a test item with poor solubility tested at a concentration lower than 1000 µM, a negative result obtained should also be considered as inconclusive.

Negative results should be interpreted with caution as substances with an exclusive reactivity towards lysine-residues can be detected as negative by the test method. Furthermore, because of the limited metabolic capability of the cell line used and because of the experimental conditions, pro-haptens (i.e.chemicals requiring enzymatic activation for example via P450 enzymes) and pre-haptens (i.e. chemicals activated by auto-oxidation) in particular with a slow oxidation rate may also provide negative results.
Test chemicals that do not act as a sensitizer but are nevertheless chemical stressors may lead on the other hand to false positive results. Furthermore, highly cytotoxic test chemicals cannot always be reliably assessed.
Finally, test chemicals that interfere with the luciferase enzyme can confound the activity of luciferase in cell-based assays causing either apparent inhibition or increased luminescence.

11. Study plan deviations and amendments

No deviation or amendment to the Study Plan has been observed during this study.

12. Results

12.1. Reference item

Cinnamaldehyde	**4 µM**	**8 µM**	**16 µM**	**32 µM**	**64 µM**	**EC1.5**	**Imax**
Rep 1	*1.18*	*1.43*	*1.76*	*2.91*	*4.26*	**9.76**	**4.26**
Rep 2	*1.12*	*1.32*	*1.44*	*1.75*	*3.45*	**18.89**	**3.45**
Mean	**1.15**	**1.37**	**1.60**	**2.33**	**3.85**	**13.58***	**3.85**

*geometric mean

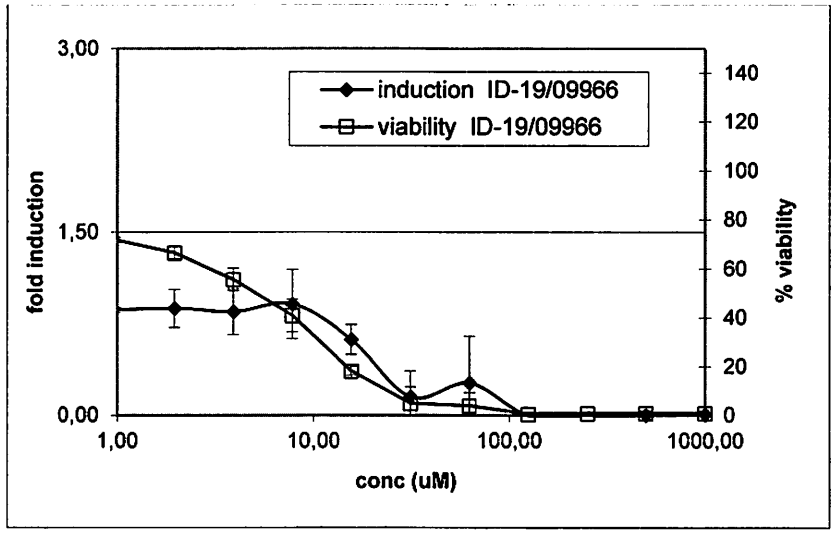
Control solvent	**CV % control solvent**
Rep 1	11.0
Rep 2	16.7

Since all validity criteria are met, study is considered as valid.

12.2. Test item

ID-19/09966	VIABILITY	INDUCTION		
	IC_{70} µM	Imax	Linear EC1.5 µM	EC1.5 Lin/Log µM
Rep 1	*1.14*	0.88	-	-
Rep 2	*1.59*	1.11	-	-
Mean	-	**1.00**	-	-
Geometric mean	**1.35**	-	-	-

Imax is less than 1.5, the EC1.5 is not determined.



13. Conclusion

Under the retained experimental conditions **PENTA 18 479 - REF : TX 19006** code **ID-19/09966** may be classified as **not skin sensitizer.**

The test method KeratinoSens™ is considered scientifically valid to be used **as part of an integrated approaches** to testing and assessment, to support the identification of the sensitization potential of test item for hazard classification and labeling purposes.

14. Archive

The total storage of the study folder is 10 years (Study Plan and amendment, report, raw data). The folder will be stored at least 6 months in the IDEA Lab archive room, on the Martillac location, and would be susceptible to be transferred to the non GLP premises of EVERIAL CHARTRES MEGASTORE Avenue Gustave EIFFEL 28000 CHARTRES, archiving specialist.

The reference item samples will be stored 10 years, or until their expiry date on the Martillac location, in the storage condition described in the quality form FL REAC 07.

Results table

ID-19/09966

MEAN VIABILITY PERCENTAGE

Concentration µM	0.98	1.95	3.91	7.81	15.63	31.25	62.5	125	250	500	1000	2000
Rep 1	*71.07*	*64.83*	*58.99*	*36.24*	*19.53*	*9.66*	*7.65*	*1.01*	*1.21*	*1.61*	*1.61*	*2.42*
Rep 2	*72.60*	*68.45*	*52.41*	*45.77*	*16.87*	*0.00*	*0.00*	*0.00*	*0.28*	*0.00*	*0.00*	*0.00*
Viability	**71.8**	**66.6**	**55.7**	**41.0**	**18.2**	**4.8**	**3.8**	**0.5**	**0.7**	**0.8**	**0.8**	**1.2**

MEAN INDUCTION

Concentration µM	0.98	1.95	3.91	7.81	15.63	31.25	62.5	125	250	500	1000	2000
Rep 1	*0.88*	*0.77*	*0.72*	*0.71*	*0.54*	*0.30*	*0.54*	*0.00*	*0.00*	*0.00*	*0.01*	*-0.01*
Rep 2	*0.85*	*0.99*	*0.98*	*1.11*	*0.71*	*0.00*	*-0.01*	*-0.01*	*0.00*	*0.00*	*0.01*	*-0.01*
Induction	**0.87**	**0.88**	**0.85**	**0.91**	**0.62**	**0.15**	**0.27**	**0.00**	**0.00**	**0.00**	**0.01**	**-0.01**
SD	*0.02*	*0.15*	*0.19*	*0.28*	*0.12*	*0.21*	*0.38*	*0.00*	*0.00*	*0.00*	*0.00*	*0.00*

Student t-test

Rep 1	0.174	0.001	0.001	0.006	0.000	0.000	0.003	0.000	0.000	0.000	0.000	0.000
Rep 2	0.163	0.955	0.856	0.414	0.016	0.000	0.000	0.000	0.000	0.000	0.000	0.000

Proficiency Substances

Recommended substances for demonstrating technical proficiency with the KeratinoSensTM test method were tested in order to validate the method in our laboratory. Results are shown in the table below:

Chemical substances	IDEA Internal code	CAS	In Vivo Prediction	Expected $EC_{1.5}$ (µM)	Expected IC_{70} (µM)	KeratinoSens™ Prediction	IDEA LAb performances		
							$EC_{1.5}$ (µM)	IC_{70} (µM)	Result
Isopropanol	ID1646	67-63-0	Non-sensitizer	> 1000	> 1000	Negative	> 1000	> 1000 > 1000	Negative
Salicylic acid	ID1980	69-72-7	Non-sensitizer	> 1000	> 1000	Negative	> 1000	> 1000	Negative
Lactic acid	ID1981	50-21-5	Non-sensitizer	> 1000	> 1000	Negative	> 1000	> 1000	Negative
Glycerol	ID1951	56-81-5	Non-sensitizer	> 1000	> 1000	Negative	> 1000	> 1000 > 1000	Negative
Cinnamyl alcohol	ID1983	104-54-1	Sensitizer (weak)	25 - 175	> 1000	Positive	25.78	694.69	Positive
Ethylene glycol dimethacrylate	ID1888	97-90-5	Sensitizer (weak)	5 - 125	> 500	Positive	14.58 11.53	617 641	Positive
2-Mercaptobenzothiazole	ID1974	149-30-4	Sensitizer (moderate)	25 - 250	> 500	Positive	21.84 44.02	457 570	Positive
Methyldibromo glutaronitrile	ID1984	35691-65-7	Sensitizer (strong)	< 20	20 - 100	Positive	4.70	35.82	Positive
4-Methylaminophenol sulfate	ID1985	55-55-0	Sensitizer (strong)	< 12.5	20 - 200	Positive	5.06	22.99	Positive
2.4-Dinitro-chlorobenzene	ID1982	97-00-7	Sensitizer (extreme)	< 12.5	5 - 20	Positive	1.70	6	Positive

Analysis certificate

sederma	ENREGISTREMENT QSHE	Codification EE1/33 Date : 02/04/15 Révision : c
	BULLETIN D'ANALYSES *CERTIFICATE OF ANALYSIS*	Page 1 sur 1

PRODUIT / *PRODUCT* : TX 19006

N° de LOT / *BATCH Nr* : B1

Stockage / *Storage* : **Stockage entre +15°C et +25°C**
Storage between +15°C and +25°C

Date de fabrication / *Date of manufacturing* : 27 mai 2019

Date de retest : 27 février 2020 / *Retest date*

Les analyses effectuées sont garanties quand le produit est stocké dans son emballage d'origine et à la température recommandée
The performed analysis are guaranteed when product is stored in original packaging and at recommended temperature

	Résultats *Results*
Aspect / *Appearance*	Poudre blanche / *White powder*
Teneur en Pal KTSKS (HPLC) / *Pal KTSKS content*	81.6%

Liliane IACUZZI
Responsable Assurance Qualité Produit / Product Quality Assurance Manager

Ce document est une copie informatique et de ce fait ne porte pas de signature / This certificate is a computer printout and therefore has no signature.

SEDERMA 29 rue du Chemin Vert – 78610 LE PERRAY EN YVELINES – France
Tél. : 01.34.84.10.10 – Fax : 01.34.84.11.30

HUMAN REPEATED INSULT PATCH TEST WITH CHALLENGE

Study report – final version nº 1.0 of 27/05/2021

STUDY REFERENCES **EUROFINS EVIC france – P21 0045**
EUROFINS EVIC romania – ER 21/048-14

INVESTIGATIONAL PRODUCT	
Denomination	**PENTA 18 479**
Formula number	**TX 19011**
Batch number	**B3**

SPONSOR	**SEDERMA** 29, rue du Chemin Vert BP 33 78612 LE PERRAY EN YVELINES
STUDY MONITOR	**Vincent VICEDO**
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CO-INVESTIGATORS	**Dr. Monica Grigore** (resident dermatologist) & **Dr. Irina Blanariu** (physician) Tel: +40 21 335 70 90 Fax: +40 21 335 70 91 *e-mail: research@evic.ro*

Initiation date of study performance	**22/03/2021**
Completion date of study performance	**30/04/2021**

Date of the study report 27/05/2021

HUMAN REPEATED INSULT PATCH TEST WITH CHALLENGE

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HUMAN REPEATED INSULT PATCH TEST WITH CHALLENGE

English synopsis

STUDY OBJECTIVES	Mainly, to confirm that the application of the investigational product under maximising conditions of exposure in a panel of healthy human adult subjects does not induce delayed contact sensitisation Secondarily, to assess the skin compatibility of the investigational product during the study
SPONSOR	**SEDERMA** 29, rue du Chemin Vert BP 33 78612 LE PERRAY EN YVELINES
STUDY MONITOR	**Vincent VICEDO**
COORDINATING CENTRE	**EUROFINS EVIC product testing france SAS** 122, rue Croix de Seguey 33000 BORDEAUX – France Tel: +33 5 56 95 59 95 Fax: +33 5 56 95 05 22 *e-mail: evic-blanquefort@evic.fr* **Contact: Dr Clotilde TRARIEUX-FOURAULT** General practitioner
INVESTIGATING CENTRE	**EUROFINS EVIC PRODUCT TESTING ROMANIA S.R.L** 64-66, Marasesti Avenue 040256 - Bucharest - Romania Tel.: +40 21 335 70 90 Fax: +40 21 335 70 91 *e-mail: evicromania@evic.ro*
MAIN INVESTIGATOR	**Dr Rozalia Olsavszky** (dermatologist) Registered N° (Romanian ministry of health): 461524 (specialist in dermato-venerology doctor, doctor in medical science) Tel: +40 21 335 70 90 Fax: +40 21 335 70 91 *e-mail: research@evic.ro*
CO-INVESTIGATORS	**Dr. Monica Grigore** (resident dermatologist) & **Dr. Irina Blanariu** (physician) Tel: +40 21 335 70 90 Fax: +40 21 335 70 91 *e-mail: research@evic.ro*
TYPE OF THE STUDY	Monocentric randomised study performed in simple blind, corresponding to the "Test Clinique Final de Sécurité" as defined by the France's Agency for the safety of health products (ANSM) Study project previously approved by a survey committee
DATES OF STUDY PERFORMANCE	From March 22nd to April 30th 2021
INVESTIGATIONAL PRODUCT	**PENTA 18 479 – Ref. TX 19011 - Lot: B3**
	Modalities of application in the study: Diluted at 15% with distilled water, under semi-occlusive patch - 160µl

English synopsis (continuation)

STUDY POPULATION	**Number of test subjects: 100** valid cases **Specific inclusion criteria: test subjects** • aged from 18 to 70, • female and/or male, • with a phototype (Fitzpatrick): from II to IV, • with all types of skin on body, • pandemic preventive medical safety measures, when the case (agree to perform a rapid blood test to identify anti-SARS-CoV-2 IgG / IgM antibodies (COVID-19), • agree to wear the equipment provided by the investigating center and follow the instructions of the staff during the study. In case of a new state of emergency and new legal rules adopted in the context of SARS-CoV2 pandemic, agree to receive the patches with products at home, to apply them at home according to the instruction received from the center, to remove them and send the pictures with experimental area to the investigating center **Specific non-inclusion criteria: test subjects** • with personal history of adverse reaction to colophony, rubber, nickel, aluminium, patch materials, adhesive plaster, • with family or personal history of atopy
METHODOLOGY	**Application of the investigational product**, in healthy human subjects, by a technician, at the investigating centre, to a skin site on the upper back, under maximising conditions of exposure (semi-occlusive patch) for a defined time Repeated applications 9 times to the same site (induction site) over a period of 3 consecutive weeks, period necessary to induce a possible allergy (induction period) After a minimal 2-weeks rest period, with no product application, single application of the investigational product, under patch, to the induction site and to a virgin site and for a defined time, enabling to reveal a possible induced allergy (challenge) **Application in parallel of distilled water** under semi-occlusive patch at the same defined times as the investigational product = control site Skin examination of the application site, before the 1st product application of the induction period and the application of the challenge phase and after each patch removal by the same investigator or technician, supervised by the investigator Reporting of the sensations of discomfort directly by the test subjects to the investigator or technician, during the study Assessment of the allergic potential - checking of the skin compatibility: • Accurate description of the skin reactions observed • Evaluation of the allergic reaction according to the ICDRG scale: ?+, (+), (++), (+++) • Calculation of the percentage of reactive test subjects during the challenge phase and the induction period

English synopsis (continuation)

RESULTS

Characteristics of the included panel

Number of included subjects: 111
Number of exclusions: none
Number of withdrawals (reason): 4 (ref. 7a, 52a, 1b and 4b) - for personal reasons independent from the study, 1 (ref. 29b) - discontinuation from reason independent of the will of the subject (direct contact of a person infected with SARS-CoV 2)
Number of valid cases: 106

- Age: 19 to 69 (Mean: 51)
- Sex: F/M
- Phototype: II and III
- Skin types on the application site: with all types of skin on body

Checking of the skin compatibility

No reaction was noted on the control site.

For the investigational product:

Induction period			
Type of reaction	**Description of the reaction on the induction site**	**Number and percentage of reactive test subjects**	**Total number and percentage of reactive test subjects**
E: Erythema	None	0 / 0%	0 / 0%
M: Complementary mention	None	0 / 0%	

Challenge phase			
Type of reaction	**Description of the reactions on the induction site and the virgin site**	**Number and percentage of reactive test subjects**	**Total number and percentage of reactive test subjects**
E: Erythema	None	0 / 0%	0 / 0%
M: Complementary mention	None	0 / 0%	
A: ICDRG scale	None	0 / 0%	

OVERALL CONCLUSION

Under the experimental conditions adopted:

- During the induction period, the repeated applications of the product PE**NTA 18 479 – Ref. TX 19011 - Lot: B3, diluted at 15% with distilled water**, under semi-occlusive patch on a panel of 106 test subjects with all types of skin on body induced no reaction of irritation.

- During the challenge phase, the single application of the investigational product to the induction site and virgin site induced no allergic reaction.

Based on these results, the product has a very good skin compatibility and does not show a sensitizing effect.

HUMAN REPEATED INSULT PATCH TEST WITH CHALLENGE

Signatures and dates

Investigator: Dr Rozalia OLSAVSZKY (dermatologist)

I the undersigned, Dr Rozalia OLSAVSZKY, declare that the overall conduct of the study was carried out under my responsibility in accordance with the protocol, the internal procedures and in the spirit of the principles of Good Clinical Practices (International recommendations ICH E6(R2) of 09/11/2016, Directive of the European Parliament and Council 2001/20/EC – OJ/EC of 01/05/2001, Romanian Order No. 904/25.07.2006).
I assume the responsibility of the validity of all the raw data obtained during the study which are reported in the present study report.

Date: 02/06/2021

Signature:

Head Manager of the investigating center: Dr Chem Eng. Elena Alina Nanu

I the undersigned, Dr Chem Eng. Elena Alina NANU, declare that the overall conduct of the study was carried out under my responsibility in accordance with the protocol and the internal procedures and in the spirit of the principles of Good Clinical Practices (International recommendations ICH E6(R2) of 09/11/2016, Directive of the European Parliament and Council 2001/20/EC.
I assume the responsibility of the validity of all the raw data obtained during the study which are reported in the present study report.

Date: 02/06/2021

Signature:

Person in charge of the quality control: Cristina Borlescu

I the undersigned, Cristina Borlescu, declare that:

- the draft of the report was audited, on 26/05/2021; 27/05/2021
- the final report was audited, on 27/05/2021
- the reported results accurately and completely reflected the raw data of the study.

Date: 02/06/2021

Signature:

HUMAN REPEATED INSULT PATCH TEST WITH CHALLENGE

I – INITIAL PROTOCOL DESIGN

I.1. STUDY OBJECTIVES

Mainly, this study intended to confirm that the application of the investigational product under maximising conditions of exposure in a panel of healthy human adult subjects did not induce delayed contact sensitisation.

Secondarily, the skin compatibility of the investigational product was assessed during the study.

I.2. ETHICS

I.2.1. Ethical conduct of the study

The study was performed in the spirit of:

- the general principles of medical ethics in clinical research coming from the Declaration of Helsinki (June 1964) and its successive amendments,
- the international recommendations relating to Good Clinical Practices for conducting clinical trials for drugs ICH E6(R2) of 09/11/2016,
- the Directive of the European Parliament and Council 2001/20/EC concerning the harmonization of legislative, statutory and administrative provisions of the member States relating to the application of good clinical practices when conducting clinical trials for drugs for human use – OJ/EC of 01/05/2001,
- the recommendations of Colipa - August 1997: "guidelines for the assessment of human skin compatibility",
- the Romanian Order No. 904/25.07.2006 on approval of rules relating to the implementation of good clinical practice in the conduct of clinical trials on medicinal products for human use.

and was in accordance with the REGULATION (EU) 2016/679 OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 27 April 2016 on the protection of natural persons with regard to the processing of personal data and on the free movement of such data.

and according to the recommendations of the France's Agency for the safety of health products *("Agence Française de Sécurité Sanitaire des Produits de Santé – AFSSAPS")* – December 2008: "Test clinique final de sécurité d'un produit cosmétique en vue de confirmer son absence de potentiel sensibilisant cutané retardé : recommandations aux promoteurs de recherche et aux prestataires de service".

I.2.2. Relevance of the study

On the one hand, the aims of the study were a better knowledge of the skin safety of the investigational product and the confirmation of the absence of allergic potential and the investigational product was not applied under normal conditions of use. So, the test subject had no direct benefit from this study.

On the other hand, the foreseeable risk incurred by the test subjects were a possible allergic reaction to one or several ingredients of the investigational product or a skin irritation due to the finished product applied under maximising conditions (under patch).

Generally in this type of study, the possible adverse effects (as erythema, vesicles…) are limited on the application sites and decrease in some days.

The applications were performed at the investigating centre and supervised by a dermatologist, so the application had to be quickly stopped if necessary, and the clinical follow-up of the reactive test subject(s) had to be done by a competent person.

So, according to the nature and the severity of the possible reaction, the investigator had to define the conduct to be adopted and the suitable steps to ensure the safety of the test subject(s) (for example definitive or temporary exclusion of the test subject(s) concerned from the study, modification of the application conditions of the product…) and had to ensure the clinical follow-up of the test subject(s) concerned, as long as it was necessary.

All the test subjects were included in the study the same day.

So, there was suitability between the aim of the study and its eventual risks and the foreseeable troubles related to the experimental conditions of the protocol.

The skin examination was performed by the investigator or technician, supervised by the investigator, having the appropriate experience.

The experimental conditions of product application created a certain occlusion and favoured the penetration of the ingredients through the skin. If some of them had an allergenic potential, this one was more easily proved by this kind of approach.

The product dose was perfectly controlled and the patch material and the conditions of use of the product were adapted to the product category.

A control site (without investigational product) served as control to take into account the possible effects not directly related to the investigational product but due to the patch material.

The investigational product was tested with other products at the same time, the experimental area chosen (back) enabling to test easily several products (maximum 17 product sites at least 1 cm far apart). The sites of application of the different products and the control site(s) were chosen according to a clockwise distribution, altering of one rank from a test subject to another, to take into account the variability of the skin reactivity according to the site.

The observance of the experimental conditions by the subjects, who took part in the study, was assessed by a questionnaire at the end of the induction period and at the end of the challenge phase.

I.2.3. Survey committee

The study had to be devoid of any foreseeable serious risk for the safety of the test subjects.

According to the procedure of the investigating centre, the protocol, the informed consent form and the preclinical information concerning the investigational product (particularly referring to its safety) were submitted to the opinion of an Institutional Ethics Committee, formed with members belonging to the staff of the investigating centre, but not directly involved in the study.

The Institutional Ethics Committee gave the approval on March 19th 2021.

The study began after the approval of the Institutional Ethics Committee.

I.2.4. Information of the test subject and informed consent form

The information about the study was given to each test subject before the start of the study.

This information was accessible, understandable and suitable for each test subject. It was orally given and then in a written specific document (in Romanian).

This information was completed, if necessary, by the investigator (or the competent person designated by him) who answered all the questions asked by the test subject.

The informed consent form was personal and previous to the start of the study.

It was clear, informed and explicit. It was written and given on the same support as the information on the study, in order to avoid any risk of dispute about its content.

The content of this document particularly specified:

- that the test subject declares to have a health coverage,
- the aim of the study,
- the study design and the experimental conditions of the study,
- the investigational product conditions of use,
- the approximate number of test subjects involved in the study,
- the expected duration of the study (for the test subject),
- the number of visits to the investigating centre, their dates and their duration,
- the agreement to wear the equipment provided by the investigating center and follow the instructions of the staff during the study,
- the study constraints (obligations, restrictions and troubles),
- the reasonable foreseeable risks,
- that skin site photographs can be taken and in this case, that the test subject will not be recognizable,
- that the test subject will be requested, if necessary, to take part in a complementary test to complete the study,
- the opinion of the Ethics Committee,
- the person to contact and the contact telephone number,
- that the personal data of the test subject will be confidentially treated by the study staff, available for the study monitor and possibly consulted (with the authorization of the test subject) by the auditors, the members of the Ethics Committees and the Health Authorities (subject to non-divulgation),
- the ban on taking part simultaneously in other clinical studies that could interfere with the current study,
- the amount of the compensation for the constraints to be undergone,
- the form of compensation in case of possible harm caused by the study (all the costs of health care assumed through the investigating centre),
- the follow-up of all test subjects after the end of the study by delivering an oral and written information note,

- the period of exclusion at the end of the study during which the test subject will not be allowed to take part in another clinical study,
- the confidential treatment of the study data,
- that the anonymity of the test subject will be preserved,
- the freedom for the test subject to refuse to participate or to stop his participation at any time without any justification and any legal consequences.

This document was previously approved by Institutional Ethics Committee.

At the beginning of the study, the inform consent form had to be dated and signed by the test subject and by the investigator or the competent person designated. The subject received a copy of informed consent form. The signed informed consent form was kept at the investigating centre.

I.2.5. Confidentiality and identification of the test subject

The information concerning the test subject, required for his recruitment, his inclusion and particularly that related to his health, obtained during the medical examination prior to his admission in the general panel of the investigating centre, formed part of medical secret and was confidentially treated.

The test subject was coded when included in the current study (according to the corresponding procedure of the investigating centre) in order to preserve his anonymity.

If photographs of the skin had to be taken, the test subject had to be non-recognizable.

I.2.6. Insurances

Insurance of the coordinating centre

The coordinating centre is covered by an insurance: HDI Global SE, Policy no.: 01012182-14009 as lead insurer.

Insurance of the investigating centre

The investigating centre is covered by an insurance guaranteeing its civil responsibility towards the test subjects: HDI Global SE, Policy no.: 110-01325685-14023 as lead insurer and Axa Corporate Solutions as co-insurer: XFR0074974LI.

I.3. COORDINATING CENTRE AND STAFF

The coordinating centre ensured the liaison between the sponsor and the investigating centre.

I.4. INVESTIGATING CENTRE AND STAFF

I.4.1. Investigating centre

The study was performed at EUROFINS EVIC romania, certified ISO 9001, ISO 14001 and OHSAS 18001, with material and technical means suitable for clinical researches on cosmetic products and compatible with the safety requirements for human subjects.

I.4.2. Technical staff

The test was performed by a competent investigator and a trained and qualified technical staff.

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I.4.3. Scientific management

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I.4.4. Quality assurance staff

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I.5. DATES OF STUDY PERFORMANCE

Initiation date of study performance: 22/03/2021

Completion date of study performance: 30/04/2021

I.6. OVERALL STUDY DESIGN

I.6.1. Type of the study

This monocentric clinical study was randomized and performed in simple blind, in a panel of healthy human subjects.

This study corresponds to the "Test Clinique Final de Sécurité" as defined by the France's Agency for the safety of health products (ANSM).

The test subject was used as own control.

I.6.2. General principle of the study

The study was performed on the basis of the specific experimental conditions defined in the quotation reference 21-0220/0.

The investigational product had to be applied in **100** test subjects, by a technician, at the investigating centre, under maximized conditions of exposure (under patch) for a defined time. The applications had to be repeated 9 times to the same site (induction site) over a period of 3 consecutive weeks, period necessary to induce a possible allergy (induction period).

After a minimal 2-week rest period, with no product application, a single application of the investigational product, under patch, to the induction site and to a virgin site and for a defined time, enabling to reveal a possible induced allergy (challenge phase), had to be performed.

A skin examination of the application site had to be performed before the 1st product application of the induction period and the application of the challenge phase and after each patch removal, by the same investigator or technician, supervised by the investigator.

The sensations of discomfort had to be directly reported by the test subjects to the investigator or technician, during the study.

The results of the skin compatibility were descriptively expressed.

Since sensitisation is not a matter of quantification, the possible reactions had to be classified as allergic or not, according to the observation done during the challenge phase compared with the observation done during the induction period.

I.6.3. Chronology of the study

Induction period: 3 consecutive weeks			
Operations at the investigating centre	**Experimental times**		
	D1	**D3 - D5 - D8 - D10 - D12 - D15 - D17 - D19**	**D22**
Delivery of the informed consent form Signature of the informed consent form Checking of the inclusion and non-inclusion criteria	●		
Clinical examination of the application site and questioning of the test subject by the investigator or technician supervised by the investigator, before product application	●		
Final inclusion	●		
Application of the investigational product under patch to the defined induction site	●	●	
Removal of the patch at the investigating centre		●	●
Clinical examination of the application site and questioning of the test subject by the investigator or technician supervised by the investigator		●	●
Control of the observance			●

Rest period : 2 consecutive weeks at least (4 weeks at the most)	**No product application**

Challenge phase: 1 week			
Operations at the investigating centre	**Experimental times**		
	D36	**D38**	**D40**
Clinical examination of the application site and questioning of the test subject by the investigator or technician supervised by the investigator, before product application	●		
Application of the investigational product under patch to the defined induction site and to a defined virgin site	●		
Removal of the patch at the investigating centre		●	
Clinical examination of the application sites and questioning of the test subject by the investigator or technician supervised by the investigator		●	●
Control of the observance			●

I.7. STUDY POPULATION

I.7.1. Constitution of the panel of test subjects and mode of recruitment

The investigating centre has at its disposal a general panel of subjects constantly renewed. These subjects come from all social categories. They either volunteer spontaneously to the investigating centre or reply to a direct call from the latter. Prior to their admission in this general panel, they are subjected to a medical examination and a detailed medical and cosmetological questionnaire, performed by a general practitioner according to the internal procedure of the investigating centre.

All the data concerning the panel are computerized and on paper.

For the study, the test subjects were selected from this general panel on the basis of inclusion criteria and non-inclusion criteria specific to the study and on their ability to respect the constraints required by the protocol. They were definitely included in the study after a specific questioning and a clinical examination.

I.7.2. Number of test subjects

The number of test subjects with exploitable data (valid cases[(1)]) at the end of the study had to be at least **100**.

[(1)]valid case = test subject that respected the protocol with no significant deviation which could have some influence on the study results.

The number of test subjects necessary to allow a reliable prediction of the sensitising potential of an investigational product depends on the methods used. The statistical considerations involved in extrapolating from a small test population to a large number of users were discussed in the following publication:

- Henderson C.R., Riley E., Certain statistical considerations in patch testing, J. Invest. Dermatology, 1945, 6, pp. 227-232

It is obvious that studies with numbers of test subjects sufficient to obtain statistically valid data applicable to several thousand consumers are not feasible. Therefore, the value of predictive patch testing does not lie in the precision of the prediction but in screening out the rare sensitising products.

So, referring to the experience acquired in the field of contact allergy to cosmetic products, the number of test subjects, empirically defined in the protocol, was sufficient to confirm, before product launching, the absence of allergenic potential of the investigational product and to achieve the study objectives.

At the beginning of the study, complementary test subjects (+11) had to be included to answer the demand and to compensate the possible withdrawals or exclusions from the study independent of the investigational product.

The test subjects excluded from the study for reasons dependent of the investigational product had to be taken into account in the study results and did not have to be replaced.

If during the study, there was a risk not to have the required number of valid cases (great number of withdrawals…), the study monitor had to be informed and an additional quota of subjects had to be possibly included to reach the target.

At the end of the study, in spite of the precautions taken by the investigating centre, if the number of valid cases was less than the number of test subjects requested by the sponsor, the study monitor had to be informed and mentioned in the study report.

I.7.3. Inclusion criteria

I.7.3.1. General inclusion criteria

According to the protocol, had to be included in the study, the subjects:

- suitable to participate in the study (after the clinical examination and questioning) and corresponding to the quality of "healthy subject" as defined in the corresponding procedure of the investigating centre,
- declaring to have a health coverage,
- signing an "informed consent form" for this study,
- certifying not to take part in another clinical study that could interfere with the current study,
- certifying the truth of the personal information declared to the investigator,
- capable of following directions and reliable to respect the constraints of the protocol (living not too far from the investigating centre, no linguistic and intellectual barrier),
- free to ensure the visits to the investigating centre,
- declaring not to have exposed themselves to a risk of pregnancy for at least 3 months before the beginning of the study and committing themselves to use effective contraceptive method throughout the study (for the women of childbearing potential).

I.7.3.2. Specific inclusion criteria

Subjects:

- aged from 18 to 70,
- female and/or male,
- with all types of skin on body,
- with a phototype (Fitzpatrick): II-IV,
- pandemic preventive medical safety measures, when the case (agree to perform a rapid blood test to identify anti-SARS-CoV-2 IgG / IgM antibodies (COVID-19),
- agree to wear the equipment provided by the investigating center and follow the instructions of the staff during the study.

I.7.4. Non inclusion criteria

I.7.4.1 General non inclusion criteria

According to the protocol, did not have to be included in the study, the subjects:

- being in exclusion period,
- deprived of freedom by administrative or legal decision or under guardianship,
- who cannot be contacted in case of emergency,
- admitted in a residential care,
- planning an hospitalisation during the study,
- belonging to the staff of the investigating centre,
- being of age but protected by law,
- having received vaccination within the 3 weeks prior to the study or intending to be vaccinated during the course of the study,
- with personal history of adverse reactions to the same type of product as the investigational product,
- with documented history of contact allergy,
- exhibiting skin marks and/or moles and/or freckles in too great quantity, hyperpilosity on the experimental area able to interfere with the assessment of the possible skin reactions,
- with still visible eczematous reaction, scar or pigmentary after-effects of previous tests on the experimental area,
- under treatment, prior to the study, able to interfere with the interpretation of the study results, particularly:
  - systemic retinoids (isotretinoin per os …) within the 6 months,
  - other systemic anti-acne medication within the 3 months,
  - topical retinoids within the 2 months,
  - other topical anti-acne medication within the month,
  - anti-acne cosmetic products within the 2 weeks (excluding face anti acne products),
  - topical or systemic medication with anti-inflammatory or antihistamine products within the 2 weeks,
  - antibiotics within the 2 weeks,
  - medication for malignancy (of any kind) within the 5 years,
  - desensitisation treatment within the 6 months,
- foreseeing, during the study, a treatment able to interfere with the interpretation of the study results (systemic or topical anti-acne medication, anti-acne cosmetic products, topical or systemic medication with anti-inflammatory or antihistamine, antibiotics, desensitisation treatment, …),
- having had a fever lasting more than 24 hours, within the 8 days prior to the study,
- breastfeeding or pregnant or planning a pregnancy during the study (for the women of childbearing potential),
- having started or changed oestrogen-progesterone contraception or hormonal treatment, within the 3 months prior to the study or foreseeing it for the duration of the study,

- having had any invasive aesthetic cares on chest and back (peeling, laser…) by a dermatologist within the 2 months prior to the study or foreseeing it for the duration of the study,
- having had any non-invasive aesthetic cares on chest and back (scrub, skin cleansing…) by an aesthetician within the month prior to the study or foreseeing it for the duration of the study,
- having received excessive or intensive exposure to sunlight (natural or artificial) within the month prior to the study or foreseeing UV exposures for the duration of the study,
- under treatment with PUVA or UVB within the month prior to the study,
- having participated in a human repeated insult patch test with challenge with or without sun exposure 3 months minimum prior to the study,
- having participated in a cumulative irritability test within the 2 months prior to the study or in a single patch test within the month prior to the study,
- having already participated in 5 clinical studies involving patch test, including 3 human repeated patch tests with or without challenge within the year prior to the study
- foreseeing bath (in bathtub, sea or swimming-pool), sauna or Turkish bath during the study period,
- regularly practicing intensive sport causing sweating and requiring frequent showers.

I.7.4.2. Specific non-inclusion criteria

Subjects:

- with personal history of adverse reaction to: colophony, ethanol, rubber, nickel, aluminium, patch materials, adhesive plaster,
- with family or personal history of atopy.

I.7.5. Specific information concerning the test subjects and medication

Skin reactivity, history of atopy, contraception (type) and possible current medication were documented at the inclusion by the technician, supervised by the investigator or the competent person designated, in the collective case report form (CRF) and mentioned in the study report.

No medication likely to interfere with the study was allowed during the study; however, if the health state of the subjects justified some medication (particularly anti-inflammatory drugs), any information relating to this concomitant medication had to be carefully documented in the case report form and mentioned in the study report.

The investigator had to exclude the test subjects taking concomitant medication likely to interfere with the study and the interpretation of the results.

I.7.6. Exclusion criteria

According to the study protocol and to the procedures of the investigating centre, had to be excluded from the study, the test subjects:

- who did not comply with the protocol and created deviation resulting in un-exploitable results,
- who took part in another clinical study that could interfere with the current study,
- who had adverse event (for example: inter-current disease requiring a concomitant medication interfering with the study and the interpretation of the results or severe skin intolerance to the investigational product), incompatible with a good protocol observance.

The temporary or definitive discontinuations decided by the investigator and their dates and reasons had to be carefully documented in the collective case report form and mentioned in the study report.

I.7.7. Withdrawal criteria

According to the study protocol and to the procedures of the investigating centre, had to be considered as withdrawals, the test subjects:

- who discontinued the study for personal reasons independent of the study (for example: moving house, new job),
- who did not come to the investigating centre for the checking in spite of phone calling.

The withdrawals and their dates and reasons had to be carefully documented by the investigator in the collective case report form (CRF) and mentioned in the study report.

I.7.8. Study constraints imposed on the test subjects

The constraints defined by the procedures of the investigating centre and partly in the study protocol, imposed on the test subjects during the study, were the following ones:

- to wear the equipment provided by the investigating center and follow the instructions of the staff during the study,
- in case of a new state of emergency and new legal rules adopted in the context of SARS-CoV2 pandemic, agree to receive, apply and remove the patches with products at home and send the pictures with experimental area to the investigating center,
- if justified and asked by the investigator, participation in a complementary test (additional visits to the investigating centre),
- exclusion period at the end of the study (according to the corresponding procedure of the investigating centre and 3 months minimum before starting a human repeated insult patch test with challenge, 1 month minimum before starting another type of study),
- no participation in another clinical study that could interfere with the current study,
- if justified, description of any concomitant medical treatment not excluded by the inclusion and non-inclusion criteria,

- no drug liable to interfere with the study and the interpretation of the results, *e.g.* aspirin (except low dose maintenance therapy), products containing aspirin, antihistamine drugs, anti-inflammatory drugs, antibiotics… (however, if therapeutic requirement: possible exclusion from the study),
- neither initiation of an hormonal treatment nor change of the usual hormonal treatment,
- no change of the mode of contraception,
- no significant change in lifestyle: diet, smoking, sport,…,
- visit to the investigating centre (13/14 times) and respect of the dates and hours of visits,
- neither anti-acne nor anti seborrheic local treatment,
- neither invasive body aesthetic cares (peeling, laser…) nor non-invasive body aesthetic cares (scrub, skin cleansing…) on chest and back, by a dermatologist or an aesthetician in Beauty Salon,
- no application of cosmetic care products to the back,
- no change in usual body hygiene products,
- no introduction of new cosmetic products,
- no intensive sun or UVA exposure (U.V. lamps) during the study and 2 weeks after the end of the study,
- no wearing of too tight or restraining clothes liable to produce frictions on the experimental area and to cause the un-sticking of the patch(es),
- neither Turkish bath nor sauna nor bath (in bathtub or swimming-pool or sea), liable to cause excessive sweating and/or the un-sticking of the patch(es),
- during shower, protection of the experimental area (no violent projection of water, no application of soap, very gentle wiping if necessary) to avoid the un-sticking of the patch(es) or the appearance of inter-current skin irritation,
- no intensive sport liable to cause excessive sweating and the un-sticking of the patch(es),
- no vaccination.

The test subjects were questioned at the end of the induction period and at the end of the challenge phase about the respect of the study constraints. These data were documented in the case report form (CRF). The investigator had to assess the importance of the possible deviations in comparison with the experimental conditions required at the beginning of the study and their incidence on the validity of the results.

I.8. INVESTIGATIONAL PRODUCT

I.8.1. Identification of the investigational product

Denomination	PENTA 18 479
Cosmetic category	Face care
Formula number	TX 19011
Batch number	B3
Galenic form and organoleptic characteristics	Colourless liquid
Normal foreseeable conditions of use	Diluted to 1%

I.8.2. Coding and storage

The product units were sent to the investigating centre. Upon receipt, the investigating centre noted the date of product receipt and checked the supplied quantities.

The product unist were coded and labelled in Romania, according to the corresponding procedure of the investigating centre.

Number and type of product unit	2 plastic flasks
Content of product unit	250ml
EUROFINS EVIC Romania code / EUROFINS EVIC coordinating centre code	21-0184 / 21-0645

Before starting the study, the storage of the investigational product units was carried out according to the conditions defined by the sponsor, in the product storage area and a product sample was taken and kept in the sample storage area of the investigating centre for at least 3 years after the end of the study then destroyed, according to the corresponding procedure of the investigating centre.

Apart from the specific demand of the sponsor, the used product unit will be kept at least 2 weeks after the sending of the final report then destroyed, according to the corresponding procedure of the investigating centre.

I.8.3. Information concerning the investigational product

The investigational product units had to be supplied with a certificate that particularly referred to:

- the compliance of the ingredients of the investigational product formula with the European Regulation N° 1223/2009 of the European Parliament,
- the safety of the finished investigational product and the absence of foreseeable serious risk for the health of the test subjects.

The qualitative formula of the product had to be supplied to the coordinating centre and the investigating centre by the study monitor.

I.8.4. Experimental conditions of application of the investigational product

I.8.4.1. Induction period

The skin site had to be defined by the technician in charge of the study, on the upper back, on a surface free from scars, moles, freckles and any other skin anomaly, and avoiding the areas of friction with clothes.

The quantity of investigational product had to be applied, by the technician in charge of the study, with a micropipette (with a single use tip) and put into the patch.

Before patching, the skin site had to be wiped with a cotton pad and dried.

The patch containing the investigational product had to be applied, by the technician in charge of the study, on the defined skin site.

The experimental conditions of application had to be the following ones:

Patch material	Experimental conditions of use of the investigational product	Quantity to be applied
Semi-occlusive patch: absorbent support in Webril® kept in position by a non woven medical adhesive (surface: 400 mm²)	Diluted at 15% with distilled water	160µl

A semi-occlusive patch containing 160 µl of distilled water had to be applied in parallel as control to eliminate, when the results were interpreted, the possible inter-current effects due to the patch material.

The induction consisted of 9 patches applied 3 times a week (for example: Monday, Wednesday and Friday) for a 3 week period.

Patches applied on Mondays and Wednesdays had to be worn for 48 hours ± 4 hours and patches applied on Fridays had to be worn for 72 hours ± 4 hours.

The application was possibly performed on other days of the week, subject to the respect of the three 72-hour contact patches and the six 48-hour contact patches.

Induction period				
Operations	**Experimental times**			
	D1	**D3 - D5 D10 - D12 D17 - D19**	**D8 - D15**	**D22**
Application of the investigational product under patch to the defined induction site	●	●	●	/
Removal of the patch at the investigating centre	/	● After 48h of contact	● After 72h of contact	● After 72h of contact

The applications had to be repeated on the same site, except in the case of significant irritation/sensitisation reaction.

In case of moderate or severe skin erythema or mild erythema with oedema/infiltration, the study monitor had to be quickly informed and the product application had to be stopped to the induction site defined and continued to a new adjacent site (the change of site being done once only).

In case of suspected allergic reaction the product did not have to be applied again and the case had to be quickly discussed with the coordinating centre and the study monitor. Then, the decision to reapply or not the product had to be jointly taken by the investigator and the study monitor.

During the induction period, the technician had to precisely locate the test site to be able to retrieve it after the rest period, according to the procedure of the investigating centre.

I.8.4.2. Rest period

No product application had to be performed for a period of 2 weeks minimum (4 weeks maximum) following the end of the induction period.

The test subjects had to inform the investigator of any reaction occurring during this period.

I.8.4.3. Challenge phase

The challenge patches had to be applied once after the rest period. The investigational product and the control product had to be applied using the same patching conditions as those used for the induction period, on 2 sites: a virgin site and the induction site, symmetrically located, if possible. The patches had to be removed 48 hours ± 4 hours after application.

Challenge phase			
Operations	**Experimental times**		
	D36	**D38**	**D40**
Application of the investigational product under patch to the defined induction site and to a defined virgin site	●	/	/
Removal of the patch at the investigating centre	/	● After 48h of contact	/

I.9. CHECKING OF THE SKIN COMPATIBILITY

I.9.1. Recording of the skin reactions

Skin examinations of the application sites had to be performed visually, by the same investigator or technician, supervised by the investigator, under standard "daylight" source:

- during the induction period:
  - before patching on D1
  - 15 to 30 minutes after patch removal (or more, if redness appeared after the removal of the adhesive) on D3, D5, D8, D10, D12, D15, D17, D19, D22
- during the challenge phase:
  - before patching on D36
  - 15 to 30 minutes after patch removal on D38 (or more, if redness appeared after the removal of the adhesive)
  - 48h +/- 4 hours after patch removal on D40.

In case of delayed skin reaction occurring after the 96h grading, the test subject had to contact the investigating centre and the site had to be re-examined by the investigator as long as necessary until reactions disappear.

All adverse reactions had to be graded until resolution.

Concurrently with the clinical examinations performed, the test subjects had to be questioned about the possible sensations of discomfort they feel.

In case of strong sensations of discomfort felt during the patch wearing at home, the test subjects had to inform by phone the investigator. If necessary, the patch was removed and a skin examination was quickly performed by the investigator (before the next planned visit to the investigating centre).

In case of application to a new adjacent site, the original test site had to be scored in parallel with the new test site until completion of the study and the skin scores of this original test site had to be distinctly documented.

Digital photographs of the skin had to be systematically taken when justified (adverse effects).

All the data were recorded in the collective case report form (CRF).

I.9.2. Expression of the results

All the reactions had to be accurately described at each experimental time using the criteria and the scale hereafter.

E = Erythema d= diffuse p = punctuated peri = peripheral	**0** – no visible erythema **0.5** – very slight erythema – barely perceptible **1** – mild erythema – faint pink **2** – moderate erythema – well defined **3** – severe erythema **4** – caustic effect – erosive aspect and/or necrotic aspect

If any visible response, the investigator had to proceed to palpation to assess infiltration / oedema.

M = Complementary mention: Other reactions	
Sv	Soap effect (shiny skin with possibly wrinkles)
D	Desquamation
Dr	Dryness
Hy	Hypopigmentation
C	Skin coloration – hyperpigmentation
Oe	Homogeneous infiltration / oedema
P	Papules
V	Vesicles
Pe	Petechiae
Fr	Follicular reaction
I (Pr)	Itching at the test site
S	Spreading beyond the patch area (infiltration or erythema)
F	Fissuring
Cr	Exudation and/or Surface encrustation
B	Bullae
Sc	Scab
He	Heating
Pu	Pustules
/	No reaction

The other visible clinical signs had to be presented descriptively

M = Complementary mention: Additional comments	
NA	Product not applied
T	Tape reaction
L	Loss of patch during the first 12 hours
N9G	No 9th grading
X	Succeeding patch not applied and succeeding grade (in brackets) denotes a residual reaction
Abs	Test subject absent

For the induction period and the challenge phase, the results were expressed in percentage of reactive test subjects: for this calculation only the visible signs of reactivity were taken into account: erythema, oedema, vesicle, bulla, papule…

I.9.3. Interpretation of the results

I.9.3.1. Allergy

All the test subjects included in the study were taken into account to appreciate the skin allergic potential of the investigational product as long as they were submitted at least to one post application examination at the defined time or else.

The nature, intensity, appearance period from the application, disappearance period from the application, location (induction site and/or virgin site) of the skin reaction and the phase of the study were taken into account for the interpretation of the results.

A = ICDRG scale	
IR	Irritation reaction
-	No allergic reaction
?+	Doubtful reaction (only slight erythema)
(+)	Weak positive reaction (without vesicle): slight erythema and infiltration with presence of small papular elevations, possibly papules
(++)	Strong positive reaction: erythema, papules, vesicles, infiltration
(+++)	Extreme positive reaction: intense erythema, oedema, coalescent vesicles (bullae)

A site where erythema was graded 2 or more during the challenge phase (with or without infiltration) had to be evaluated on subsequent days to note whether the reaction diminished or increased, in order to differentiate between an allergic and an irritant reaction. A quick decreasing reaction could be indicative of irritation. A reaction with infiltration / oedema that persisted or increased over time usually could indicate an allergic reaction.

If the possible allergic reaction was observed during the induction period, it could be the revelation of an allergy previously contracted or the revelation of an allergy precociously induced by the investigational product.

If the possible allergic reaction was observed during the challenge phase (similar responses observed on the virgin site and on the induction site), it could be the revelation of an allergy induced during the induction period by the investigational product.

In case of suspected allergic reaction, the study monitor had to be quickly informed.

In order to confirm the possible allergic reaction, an additional application (rechallenge) had to be proposed to the test subject with the agreement of the study monitor, at least 3 weeks after complete disappearance of the reaction. The experimental conditions of this complementary test had to be jointly defined case by case by the investigator and the study monitor.

I.9.3.2. Irritation

All the test subjects included in the study were taken into account to appreciate the skin irritant potential of the investigational product as long as they were submitted at least to one post application examination at the defined time or any other time.

To appreciate the skin irritant potential, the interpretation of the results was based on the experience of the investigator in this field. The skin compatibility of the investigational product was classified as: very good, good, moderate or bad, in the study conditions.

If justified in case of reactivity in some test subjects, a complementary study will be possibly carried out in these test subjects, after agreement of the sponsor. The experimental conditions of this study will be defined by the investigator, case by case.

I.10. FOLLOW-UP OF THE TEST SUBJECTS

The test subjects having, in totality or partially, participated in this study received at the end of the study an oral and written information, asking them to contact the investigating centre in case of any abnormal skin reaction, occurring with the use of a cosmetic product after the study.

The test subjects having presented a confirmed allergic reaction during the study were definitely crossed off the general panel of the investigating centre.

An information note was given to them with the following information:

- title and objective of the study, and study reference of the investigating centre,
- dates of the study and of the complementary test(s) performed,
- description of the reaction, conclusion of the investigator, imputability of the investigational product,
- category of the investigational product,
- when identified, substance(s) responsible for the reaction or suspected substances if the link cannot be established with certainty between the reaction and the product ingredients: INCI name(s) and function(s),
- type of products containing or likely to contain the concerned substance(s),
- recommendations and cautions for the future use of certain categories of cosmetic products, advices in case of reaction (general practitioner / dermatologist / allergologist consultation),
- necessity to inform the investigating centre in case of future reaction with cosmetic products.

The investigating centre has to keep a copy of this document and an updated record of these test subjects.

I.11. SUSPENSION OF THE STUDY

The investigator had to stop the study if it showed a risk for the health or the integrity of the test subjects.

The date of the suspension and the reasons had to be carefully documented by the investigator in the case report form.

The coordinating centre had to inform promptly the study monitor, by phone, fax or e-mail.

The sponsor was able to stop the study at any time for administrative reasons or other ones.

I.12. ADVERSE EVENTS

I.12.1. Definitions

Any topical product can induce, when used in Human, according to individual sensitivities, a local and minor reactivity, defined as follows: any slight local reaction of intolerance or sensation of discomfort, occurring in a test subject during a clinical study, completely reversible, expected, due to the investigational product and which does not question the observance of the study protocol or the good implementation of the study.

- **adverse event**: any harmful event with or without relationship with the investigational product, occurring in a test subject during a clinical study.
- **suspicion of adverse effect**: any adverse event with a quite possible relationship with the investigational product.
- **adverse effect**: any harmful and unwanted reaction, due to the investigational product, occurring in a test subject during a clinical study.
- **unexpected adverse effect**: any adverse effect due to the investigational product, the nature, the intensity and/or the evolution of which do not agree with the product information.
- **serious adverse event / effect**: any adverse event or adverse effect that causes death, endangers test subject's life, induces an hospitalisation or the prolongation of the hospitalisation, causes severe and lasting incapacity or handicap or induces congenital anomaly or malformation.

I.12.2. Data collection

The investigator had to accurately describe the adverse event and had to appreciate its seriousness. According to the corresponding procedure of the investigating centre, he had to define the link of causality between this event and the investigational product, on the basis of the symptoms, the chronology, the results of the possible specific complementary tests undertaken and any available information.

The imputability of the product had to be assessed according to the scale: very likely, likely, possible, questionable, excluded (in accordance with the recommendations of the European Council in its resolution ResAP (2006)1 of 08/11/2006 and the method of imputability of the adverse effects linked to the cosmetic products published by AFSSAPS in December 2009).

I.12.3. Conduct to be adopted in case of adverse event

Faced with an adverse event, the investigator had to freely define, case by case, the conduct to be adopted and the suitable steps to ensure the safety of the test subject concerned and of the other test subjects included in the study.

In case of suspicion of adverse effect (with a quite possible relationship with the investigational product), the investigator had to ensure the clinical follow-up of the test subject concerned, as long as necessary.

I.12.4. Communication with the study monitor

According to the corresponding procedure, the serious adverse events and the adverse effects had to be notified as soon as possible and within 24 hours at the latest, by the investigating centre to the study monitor, by phone, fax or e-mail.

The investigator had to send an adverse event form to the study monitor and to the coordinating centre.

If justified, the investigator had to give to the study monitor and to the coordinating centre complementary information when available.

I.13. RAW DATA RECORDING AND STUDY REPORT FILING

All the data gathered during the study were recorded accurately, legibly and indelibly by the investigator or the technician in charge of the study, under his control, in the collective case report form.

Each page of this document was initialled by the technician; the whole was verified and validated by the investigator.

The content of this study report took into account the recommendations of the Colipa related to the assessment of the efficacy of cosmetic products (May 2008) and the explanatory note related to the structure and the content of the reports of clinical studies – ICH E3, of 28/11/1995 and is in accordance with the French decree of 25/08/2006 concerning the content of the final report and of the final report summary of a biomedical research on cosmetic product.

At the end of the study, the information concerning the investigational product, the information concerning the test subjects (collective CRF, informed consent forms) were filed and will be kept for 10 years, in the filing area of the investigating centre and the information related to the conduct of the study (protocol signed by the sponsor, copy of this study report….) were filed and will be kept for 10 years, in the filing area of the investigating centre and /or the coordinating centre.

At the end of this period, the sponsor will choose among 3 options:

- return of the study documentation to the sponsor,
- filing of the study documentation in the filing area of the investigating and/or coordinating centre, based on a specific contract,
- destruction of the study documentation (after sponsor's written and signed authorization).

I.14. REFERENCE

The methodology used was an adaptation from that described by Marzulli and Maibach (Human Repeated Insult Patch Test for delayed contact hypersensitivity: HRIPT)

- Marzulli F.N., Maibach H.I., Contact allergy: predictive testing in man, Contact Dermatitis, 1976, 2, pp. 1-17
- V.T. Politano, A.M. Api / Regulatory Toxicology and Pharmacology 52 (2008) 35–38

II – PRACTICAL CONDITIONS OF STUDY PERFORMANCE

II.1. PROTOCOL ADHERENCE

Preamble: No serious adverse effect or intercurrent event justified the suspension of the study.

II.1.1. Study population

Number of test subjects included in the study	**111**	
Withdrawals	**Test subjects concerned**	**Date and reasons**
	Ref. 7a	26/04/2021 - for personal reasons independent from the study
	Ref. 52a	29/03/2021 - for personal reasons independent from the study
	Ref. 1b	31/03/2021 - for personal reasons independent from the study
	Ref. 4b	29/03/2021 - for personal reasons independent from the study
	Ref. 29b	02/04/2021 - discontinuation from reason independent of the will of the subject
Exclusion	**Test subjects concerned**	**Date and reasons**
	None	Not applicable
Valid cases	**106**	

The number of recruited test subjects took into account the inclusion criteria, the constraints of the study and the period of the study performance.

At the beginning of the study, complementary test subjects (+11) were included to compensate the possible withdrawals or exclusions from the study independent of the investigational product.

II.1.1.2. Inclusion and non-inclusion criteria

All the test subjects corresponded to the inclusion and non-inclusion criteria.

The individual typological characteristics of the test subjects are reported in **Appendices 1**, and recapitulated below for the whole panel:

Age (years old)	**Included test subjects**	**Valid cases**
Minimum	19	19
Maximum	69	69
Mean	50	51
Median	53	53

Criteria	Included test subjects		Valid cases	
	Nb	%	Nb	%
II	24	22%	24	23%
III	87	78%	82	77%
Sex				
Male	31	28%	28	26%
Female	80	72%	78	74%

II.1.1.3. Specific information concerning the test subjects

The answers of the test subjects concerning the skin reactivity, the history of atopy, contraception (type) and the current medication are reported in **Appendices 2**.

II.1.1.4. Study constraints imposed on the test subjects

All the constraints of the study, defined in the protocol, were respected by the test subjects who completed the study.

The answers of the test subjects concerning the respect of the constraints defined in the protocol were reported in the CRF.

II.2. INVESTIGATIONAL PRODUCT

All the experimental conditions of application at the investigating centre were respected, as defined in the protocol.

II.3. CHECKING OF THE SKIN COMPATIBILITY: RECORDING OF THE SKIN REACTIONS

All the skin examinations and questioning of the test subjects were performed in accordance with the conditions defined in the protocol.

III – RESULTS

III.1. RESULTS / DISCUSSION

III.1.1. Checking of the skin compatibility

For the investigational product, the individual data of the skin examination and questioning of the test subjects are reported in **Appendices 3**.

Induction period			
Type of reaction	**Description of the reaction on the induction site**	**Number and percentage of reactive test subjects**	**Total number and percentage of reactive test subjects**
E: Erythema	None	0 / 0%	0 / 0%
M: Complementary mention	None	0 / 0%	

Challenge phase			
Type of reaction	**Description of the reactions on the induction site and the virgin site**	**Number and percentage of reactive test subjects**	**Total number and percentage of reactive test subjects**
E: Erythema	None	0 / 0%	0 / 0%
M: Complementary mention	None	0 / 0%	
A: ICDRG scale	None	0 / 0%	

For the control product, the individual data of the skin examination and questioning of the test subjects are reported in **Appendices 4**.

Induction period			
Type of reaction	**Description of the reaction on the induction site**	**Number and percentage of reactive test subjects**	**Total number and percentage of reactive test subjects**
E: Erythema	None	0 / 0%	0 / 0%
M: Complementary mention	None	0 / 0%	

Challenge phase			
Type of reaction	**Description of the reactions on the induction site and the virgin site**	**Number and percentage of reactive test subjects**	**Total number and percentage of reactive test subjects**
E: Erythema	None	0 / 0%	0 / 0%
M: Complementary mention	None	0 / 0%	
A: ICDRG scale	None	0 / 0%	

III.2. OVERALL CONCLUSION

Under the experimental conditions adopted:

- During the induction period, the repeated applications of the product **PENTA 18 479 – Ref. TX 19011 - Lot: B3, diluted at 15% with distilled water**, under semi-occlusive patch on a panel of 106 test subjects with all types of skin on body induced no reaction of irritation.

- During the challenge phase, the single application of the investigational product to the induction site and virgin site induced no allergic reaction.

Based on these results, the product has a very good skin compatibility and does not show a sensitizing effect.

III.3. QUALITY CONTROL AND QUALITY ASSURANCE

The study was performed in compliance with the procedures of the investigating centre, established according to the regulations in force.

The investigator, in charge of the performance of the study, made sure of the quality of the work of the technical staff, particularly concerning the respect of the protocol and its appendices, the collection of raw data, the management of the investigational product.

The personnel of the Quality Assurance department controlled that the study documentation was present, dated and signed.

The personnel of the Quality Assurance department regularly controls that the protocol and working procedures relevant to this type of study are duly applied.

APPENDICES

Appendix 1/1

TYPOLOGICAL CHARACTERISTICS OF THE TEST SUBJECTS

Test subjects		age *(years)*	sex *F=female M=male*	phototype(1)
Ref.	**Code** *initials of the surname and of the first name*			
1a	IF	50	F	III
2a	LC	54	M	III
3a	PD	36	M	III
4a	PI	27	M	III
5a	CF	69	F	III
6a	HM	52	F	II
7a	PA	33	F	III
8a	CP	68	M	III
9a	BA	55	F	II
10a	AE	59	F	III
11a	IN	36	M	III
12a	AL	59	F	II
13a	IE	62	F	III
14a	VA	58	F	III
15a	MM	62	F	III
16a	MA	53	M	III
17a	TD	38	F	III
18a	IE	59	F	II
19a	PA	59	F	III
20a	NG	57	M	III

Legends: [ ] *Withdrawal*

(1) ***phototype: Type I****: Always burns easily, never tans,* ***Type II****: Always burns easily, tans minimally,* ***Type III****: Burns moderately, tans gradually,* ***Type IV****: Burns slightly, always tans easily,* ***Type V****: Burns rarely, tans intensely,* ***Type VI****: Never burns, strongly pigmented*

Appendix 1/2

TYPOLOGICAL CHARACTERISTICS OF THE TEST SUBJECTS

Test subjects		age *(years)*	sex *F=female M=male*	phototype[1]
Ref.	**Code** *initials of the surname and of the first name*			
21a	PA	59	F	III
22a	LI	66	F	III
23a	ND	19	F	II
24a	PC	47	F	III
25a	NJ	49	F	III
26a	ZA	59	F	III
27a	NE	45	F	III
28a	DM	59	M	III
29a	DA	48	M	II
30a	ND	59	F	III
31a	KV	47	F	III
32a	DA	53	F	II
33a	NG	58	F	III
34a	BE	59	F	III
35a	PM	57	M	III
36a	DM	58	F	II
37a	BM	43	M	III
38a	LZ	59	F	III
39a	AG	59	F	III
40a	SD	49	M	III

Legends:

*[1] **phototype: Type I**: Always burns easily, never tans, **Type II**: Always burns easily, tans minimally, **Type III**: Burns moderately, tans gradually, **Type IV**: Burns slightly, always tans easily, **Type V**: Burns rarely, tans intensely, **Type VI**: Never burns, strongly pigmented*

Appendix 1/3

TYPOLOGICAL CHARACTERISTICS OF THE TEST SUBJECTS

Test subjects		age *(years)*	sex *F=female M=male*	phototype[1]
Ref.	**Code** *initials of the surname and of the first name*			
41a	MG	58	F	II
42a	LC	22	M	II
43a	PF	50	F	III
44a	FM	48	F	III
45a	IN	59	F	III
46a	HA	41	F	II
47a	AI	47	F	III
48a	CM	51	M	III
49a	TA	58	F	III
50a	DM	31	F	III
51a	AA	41	M	III
52a	DI	33	M	III
53a	NA	51	F	III
54a	SM	57	F	II
55a	MA	52	F	III

Legends: *Withdrawal*

*[1] **phototype: Type I**: Always burns easily, never tans, **Type II**: Always burns easily, tans minimally, **Type III**: Burns moderately, tans gradually, **Type IV**: Burns slightly, always tans easily, **Type V**: Burns rarely, tans intensely, **Type VI**: Never burns, strongly pigmented*

Appendix 1/4

TYPOLOGICAL CHARACTERISTICS OF THE TEST SUBJECTS

Test subjects		age *(years)*	sex *F=female M=male*	phototype(1)
Ref.	**Code** *initials of the surname and of the first name*			
1b	RC	27	M	III
2b	ES	68	F	III
3b	CI	58	M	III
4b	RA	34	F	III
5b	CD	44	M	III
6b	CE	66	F	II
7b	PC	67	M	III
8b	TA	59	F	II
9b	MN	57	F	III
10b	MI	40	F	III
11b	PC	58	F	III
12b	TM	59	F	III
13b	AE	58	F	II
14b	CG	29	F	III
15b	GD	64	F	III
16b	AS	20	F	III
17b	NG	50	M	III
18b	FL	35	F	II
19b	BP	53	F	III
20b	ME	37	F	III

Legends: *Withdrawal*

(1) ***phototype: Type I****: Always burns easily, never tans,* ***Type II****: Always burns easily, tans minimally,* ***Type III****: Burns moderately, tans gradually,* ***Type IV****: Burns slightly, always tans easily,* ***Type V****: Burns rarely, tans intensely,* ***Type VI****: Never burns, strongly pigmented*

Appendix 1/5

TYPOLOGICAL CHARACTERISTICS OF THE TEST SUBJECTS

Test subjects		age *(years)*	sex *F=female M=male*	phototype[1]
Ref.	**Code** *initials of the surname and of the first name*			
21b	TM	33	F	II
22b	PM	37	F	III
23b	PD	49	M	III
24b	GG	56	F	III
25b	BM	49	F	III
26b	TS	58	M	III
27b	NC	56	F	III
28b	CL	59	F	III
29b	KV	60	M	III
30b	VA	39	M	III
31b	VS	59	F	II
32b	NA	37	F	III
33b	BR	36	M	III
34b	AL	47	F	III
35b	IL	58	F	III
36b	DM	56	M	III
37b	GC	49	F	II
38b	PM	57	F	II
39b	GC	40	M	III
40b	CV	53	F	II

Legends: *Withdrawal*

*[1] **phototype: Type I**: Always burns easily, never tans, **Type II**: Always burns easily, tans minimally, **Type III**: Burns moderately, tans gradually, **Type IV**: Burns slightly, always tans easily, **Type V**: Burns rarely, tans intensely, **Type VI**: Never burns, strongly pigmented*

Appendix 1/6

TYPOLOGICAL CHARACTERISTICS OF THE TEST SUBJECTS

Test subjects		age *(years)*	sex *F=female M=male*	phototype[1]
Ref.	**Code** *initials of the surname and of the first name*			
41b	GA	41	M	III
42b	CM	49	F	II
43b	HO	39	F	II
44b	AF	40	F	III
45b	PM	48	F	III
46b	GF	44	F	III
47b	NI	45	F	III
48b	ME	57	F	II
49b	CL	45	M	III
50b	DM	57	F	III
51b	DM	59	F	III
52b	DV	49	M	III
53b	AR	57	F	III
54b	MI	53	F	III
55b	PI	57	F	III
56b	FF	45	F	III

Legends:

*[1] **phototype: Type I**: Always burns easily, never tans, **Type II**: Always burns easily, tans minimally, **Type III**: Burns moderately, tans gradually, **Type IV**: Burns slightly, always tans easily, **Type V**: Burns rarely, tans intensely, **Type VI**: Never burns, strongly pigmented*

Appendix 2/1

SPECIFIC INFORMATION CONCERNING THE TEST SUBJECTS

Test subjects		Sensitive (declarative) / reactive skin on body	Atopy	Current medication except for contraceptive pills	Contraception
Ref.	**Code** *initials of the surname and of the first name*			**If yes** *(specify commercial denomination, active substance and dosage, pathology treated)*	**If Yes** *(Type to be specified)*
1a	IF	/	/	/	CONDOM
2a	LC	/	/	/	NC (MALE)
3a	PD	/	/	/	NC (MALE)
4a	PI	/	/	/	NC (MALE)
5a	CF	/	/	/	NC (MENOPAUSE)
6a	HM	/	/	/	CONDOM
7a	PA	/	/	/	PILL
8a	CP	/	/	/	NC (MALE)
9a	BA	/	/	/	NC (MENOPAUSE)
10a	AE	/	/	/	NC (MENOPAUSE)
11a	IN	/	/	/	NC (MALE)
12a	AL	/	/	/	NC (MENOPAUSE)
13a	IE	/	/	/	NC (MENOPAUSE)
14a	VA	/	/	/	NC (MENOPAUSE)
15a	MM	/	/	/	NC (MENOPAUSE)
16a	MA	/	/	/	NC (MALE)
17a	TD	/	/	/	TUBAL LIGATION
18a	IE	/	/	/	NC (MENOPAUSE)
19a	PA	/	/	/	NC (MENOPAUSE)
20a	NG	/	/	/	NC (MALE)

Legends: / = no *Withdrawal* *NC: Not Concerned*

Appendix 2/2

SPECIFIC INFORMATION CONCERNING THE TEST SUBJECTS

Test subjects		Sensitive (declarative) / reactive skin on body	Atopy	Current medication except for contraceptive pills	Contraception
Ref.	**Code** *initials of the surname and of the first name*			**If yes** *(specify commercial denomination, active substance and dosage, pathology treated)*	**If Yes** *(Type to be specified)*
21a	PA	/	/	/	NC (MENOPAUSE)
22a	LI	/	/	/	NC (MENOPAUSE)
23a	ND	/	/	/	CONDOM
24a	PC	/	/	/	CONDOM
25a	NJ	/	/	/	CONDOM
26a	ZA	/	/	/	NC (MENOPAUSE)
27a	NE	/	/	/	CONDOM
28a	DM	/	/	/	NC (MALE)
29a	DA	/	/	/	NC (MALE)
30a	ND	/	/	/	NC (MENOPAUSE)
31a	KV	/	/	/	CONDOM
32a	DA	/	/	/	NC (MENOPAUSE)
33a	NG	/	/	/	NC (MENOPAUSE)
34a	BE	/	/	/	NC (MENOPAUSE)
35a	PM	/	/	/	NC (MALE)
36a	DM	/	/	/	CONDOM
37a	BM	/	/	/	NC (MALE)
38a	LZ	/	/	/	NC (MENOPAUSE)
39a	AG	/	/	/	NC (MENOPAUSE)
40a	SD	/	/	/	NC (MALE)

Legends: / = no *NC: Not Concerned*

Appendix 2/3

SPECIFIC INFORMATION CONCERNING THE TEST SUBJECTS

Test subjects		Sensitive (declarative) / reactive skin on body	Atopy	Current medication except for contraceptive pills	Contraception
Ref.	**Code** *initials of the surname and of the first name*			**If yes** *(specify commercial denomination, active substance and dosage, pathology treated)*	**If Yes** *(Type to be specified)*
41a	MG	/	/	/	NC (MENOPAUSE)
42a	LC	/	/	/	NC (MALE)
43a	PF	/	/	/	CONDOM
44a	FM	/	/	/	CONDOM
45a	IN	/	/	/	NC (MENOPAUSE)
46a	HA	/	/	/	CONDOM
47a	AI	/	/	/	PILL
48a	CM	/	/	/	NC (MALE)
49a	TA	/	/	/	NC (MENOPAUSE)
50a	DM	/	/	/	CONDOM
51a	AA	/	/	/	NC (MALE)
52a	DI	/	/	/	NC (MALE)
53a	NA	/	/	/	CONDOM
54a	SM	/	/	/	NC (MENOPAUSE)
55a	MA	/	/	/	NC (MENOPAUSE)

Legends: / = no *Withdrawal* *NC: Not Concerned*

Appendix 2/4

SPECIFIC INFORMATION CONCERNING THE TEST SUBJECTS

Test subjects		Sensitive (declarative) / reactive skin on body	Atopy	Current medication except for contraceptive pills	Contraception
Ref.	**Code** *initials of the surname and of the first name*			**If yes** *(specify commercial denomination, active substance and dosage, pathology treated)*	**If Yes** *(Type to be specified)*
1b	RC	/	/	/	NC (MALE)
2b	ES	/	/	/	NC (MENOPAUSE)
3b	CI	/	/	/	NC (MALE)
4b	RA	/	/	/	CONDOM
5b	CD	/	/	/	NC (MALE)
6b	CE	/	/	/	NC (MENOPAUSE)
7b	PC	/	/	/	NC (MALE)
8b	TA	/	/	/	NC (MENOPAUSE)
9b	MN	/	/	/	NC (MENOPAUSE)
10b	MI	/	/	/	CONDOM
11b	PC	/	/	/	NC (MENOPAUSE)
12b	TM	/	/	/	NC (MENOPAUSE)
13b	AE	/	/	/	NC (MENOPAUSE)
14b	CG	/	/	/	CONDOM
15b	GD	/	/	/	NC (MENOPAUSE)
16b	AS	/	/	/	CONDOM
17b	NG	/	/	/	NC (MALE)
18b	FL	/	/	/	PILL
19b	BP	/	/	/	NC (MENOPAUSE)
20b	ME	/	/	/	CONDOM

Legends: / = no *Withdrawal* *NC: Not Concerned*

Appendix 2/5

SPECIFIC INFORMATION CONCERNING THE TEST SUBJECTS

Test subjects		Sensitive (declarative) / reactive skin on body	Atopy	Current medication except for contraceptive pills	Contraception
Ref.	**Code** *initials of the surname and of the first name*			**If yes** *(specify commercial denomination, active substance and dosage, pathology treated)*	**If Yes** *(Type to be specified)*
21b	TM	/	/	/	CONDOM
22b	PM	/	/	/	PILL
23b	PD	/	/	/	NC (MALE)
24b	GG	/	/	/	NC (MENOPAUSE)
25b	BM	/	/	/	CONDOM
26b	TS	/	/	/	NC (MALE)
27b	NC	/	/	/	NC (MENOPAUSE)
28b	CL	/	/	/	NC (MENOPAUSE)
29b	KV	/	/	/	NC (MALE)
30b	VA	/	/	/	NC (MALE)
31b	VS	/	/	/	NC (MENOPAUSE)
32b	NA	/	/	/	CONDOM
33b	BR	/	/	/	NC (MALE)
34b	AL	/	/	/	CONDOM
35b	IL	/	/	/	NC (MENOPAUSE)
36b	DM	/	/	/	NC (MALE)
37b	GC	/	/	/	CONDOM
38b	PM	/	/	/	NC (MENOPAUSE)
39b	GC	/	/	/	NC (MALE)
40b	CV	/	/	/	NC (MENOPAUSE)

Legends: / = no *Withdrawal* *NC: Not Concerned*

Appendix 2/6

SPECIFIC INFORMATION CONCERNING THE TEST SUBJECTS

Test subjects		Sensitive (declarative) / reactive skin on body	Atopy	Current medication except for contraceptive pills	Contraception
Ref.	**Code** *initials of the surname and of the first name*			**If yes** *(specify commercial denomination, active substance and dosage, pathology treated)*	**If Yes** *(Type to be specified)*
41b	GA	/	/	/	NC (MALE)
42b	CM	/	/	/	CONDOM
43b	HO	/	/	/	CONDOM
44b	AF	/	/	/	CONDOM
45b	PM	/	/	/	NC (MENOPAUSE)
46b	GF	/	/	/	NC (MENOPAUSE)
47b	NI	/	/	/	CONDOM
48b	ME	/	/	/	NC (MENOPAUSE)
49b	CL	/	/	/	NC (MALE)
50b	DM	/	/	/	NC (MENOPAUSE)
51b	DM	/	/	/	NC (MENOPAUSE)
52b	DV	/	/	/	NC (MALE)
53b	AR	/	/	/	NC (MENOPAUSE)
54b	MI	/	/	/	NC (MENOPAUSE)
55b	PI	/	/	/	NC (MENOPAUSE)
56b	FF	/	/	/	CONDOM

Legends: / = no *NC: Not Concerned*

Appendix 3-1/1

INVESTIGATIONAL PRODUCT: PENTA 18 479 – Ref. TX 19011
SKIN EXAMINATION AND QUESTIONING DURING THE INDUCTION PHASE

***E: Erythema**: **0** = no visible erythema, **0.5** = very slight erythema – barely perceptible, **1** = mild erythema – faint pink, **2** = moderate erythema – well defined, **3** = severe erythema, **4** = caustic effect – erosive aspect and/or necrotic aspect*
d= diffuse / p = punctuated / peri = peripheral

***M: Additional comments/Others reactions: H or Oe** = Homogeneous infiltration / oedema, **P** = Papules, **V** = Vesicles, **B** = Bullae, **Pe** = Petechiae, **S:** Spreading beyond the patch, **SV** = Soap effect (shiny skin with possibly wrinkles), **F** = Fissuring , **D** = Desquamation, **Dr** = Dryness, **C** = Skin coloration, hyperpigmentation, **HY** = Hypopigmentation, **Fr** = Follicular reaction, **NA** = Product not applied, **T**= Tape reaction, **I** = Itching at the test site, **Cr** = Exsudation and/or Surface encrustation, **Sc** = Scab, **Pr** = Pruritus, **He** = Heating, **Pu** = Pustules, * = Additional free comments, **N9G** = No 9th grade, **X** = Succeeding patch not applied and succeeding grade (in brackets) denotes a residual reaction, **Abs or "–"** = Subject absent, **MU** = Make-up patch*

/: no reaction

Test subjects reference	Type of reaction	Experimental times									
		D1	D3	D5	D8	D10	D12	D15	D17	D19	D22
1a	E	0	0	0	0	0	0	0	0	0	0
	M	/	/	/	/	/	/	/	/	/	/
2a	E	0	0	0	0	0	0	0	0	0	0
	M	/	/	/	/	/	/	/	/	/	/
3a	E	0	0	0	0	0	0	0	0	0	0
	M	/	/	/	/	/	/	/	/	/	/
4a	E	0	0	0	0	0	0	0	0	0	0
	M	/	/	/	/	/	/	/	/	/	/
5a	E	0	0	0	0	0	0	0	0	0	0
	M	/	/	/	/	/	/	/	/	/	/
6a	E	0	0	0	0	0	0	0	0	0	0
	M	/	/	/	/	/	/	/	/	/	/
7a	E	0	0	0	0	0	0	0	0	0	0
	M	/	/	/	/	/	/	/	/	/	/
8a	E	0	0	0	0	0	0	0	0	0	0
	M	/	/	/	/	/	/	/	/	/	/
9a	E	0	0	0	0	0	0	0	0	0	0
	M	/	/	/	/	/	/	/	/	/	/
10a	E	0	0	0	0	0	0	0	0	0	0
	M	/	/	/	/	/	/	/	/	/	/
11a	E	0	0	0	0	0	0	0	0	0	0
	M	/	/	/	/	/	/	/	/	/	/
12a	E	0	0	0	0	0	0	0	0	0	0
	M	/	/	/	/	/	/	/	/	/	/
13a	E	0	0	0	0	0	0	0	0	0	0
	M	/	/	/	/	/	/	/	/	/	/
14a	E	0	0	0	0	0	0	0	0	0	0
	M	/	/	/	/	/	/	/	/	/	/
15a	E	0	0	0	0	0	0	0	0	0	0
	M	/	/	/	/	/	/	/	/	/	/
16a	E	0	0	0	0	0	0	0	0	0	0
	M	/	/	/	/	/	/	/	/	/	/
17a	E	0	0	0	0	0	0	0	0	0	0
	M	/	/	/	/	/	/	/	/	/	/
18a	E	0	0	0	0	0	0	0	0	0	0
	M	/	/	/	/	/	/	/	/	/	/
19a	E	0	0	0	0	0	0	0	0	0	0
	M	/	/	/	/	/	/	/	/	/	/
20a	E	0	0	0	0	0	0	0	0	0	0
	M	/	/	/	/	/	/	/	/	/	/

Legends: *Withdrawal*

Appendix 3-1/2

INVESTIGATIONAL PRODUCT: PENTA 18 479 – Ref. TX 19011
SKIN EXAMINATION AND QUESTIONING DURING THE INDUCTION PHASE

Test subjects reference	Type of reaction	Experimental times									
		D1	D3	D5	D8	D10	D12	D15	D17	D19	D22
21a	E	0	0	0	0	0	0	0	0	0	0
	M	/	/	/	/	/	/	/	/	/	/
22a	E	0	0	0	0	0	0	0	0	0	0
	M	/	/	/	/	/	/	/	/	/	/
23a	E	0	0	0	0	0	0	0	0	0	0
	M	/	/	/	/	/	/	/	/	/	/
24a	E	0	0	0	0	0	0	0	0	0	0
	M	/	/	/	/	/	/	/	/	/	/
25a	E	0	0	0	0	0	0	0	0	0	0
	M	/	/	/	/	/	/	/	/	/	/
26a	E	0	0	0	0	0	0	0	0	0	0
	M	/	/	/	/	/	/	/	/	/	/
27a	E	0	0	0	0	0	0	0	0	0	0
	M	/	/	/	/	/	/	/	/	/	/
28a	E	0	0	0	0	0	0	0	0	0	0
	M	/	/	/	/	/	/	/	/	/	/
29a	E	0	0	0	0	0	0	0	0	0	0
	M	/	/	/	/	/	/	/	/	/	/
30a	E	0	0	0	0	0	0	0	0	0	0
	M	/	/	/	/	/	/	/	/	/	/
31a	E	0	0	0	0	0	0	0	0	0	0
	M	/	/	/	/	/	/	/	/	/	/
32a	E	0	0	0	0	0	0	0	0	0	0
	M	/	/	/	/	/	/	/	/	/	/
33a	E	0	0	0	0	0	0	0	0	0	0
	M	/	/	/	/	/	/	/	/	/	/
34a	E	0	0	0	0	0	0	0	0	0	0
	M	/	/	/	/	/	/	/	/	/	/
35a	E	0	0	0	0	0	0	0	0	0	0
	M	/	/	/	/	/	/	/	/	/	/
36a	E	0	0	0	0	0	0	0	0	0	0
	M	/	/	/	/	/	/	/	/	/	/
37a	E	0	0	0	0	0	0	0	0	0	0
	M	/	/	/	/	/	/	/	/	/	/
38a	E	0	0	0	0	0	0	0	0	0	0
	M	/	/	/	/	/	/	/	/	/	/
39a	E	0	0	0	0	0	0	0	0	0	0
	M	/	/	/	/	/	/	/	/	/	/
40a	E	0	0	0	0	0	0	0	0	0	0
	M	/	/	/	/	/	/	/	/	/	/
41a	E	0	0	0	0	0	0	0	0	0	0
	M	/	/	/	/	/	/	/	/	/	/
42a	E	0	0	0	0	0	0	0	0	0	0
	M	/	/	/	/	/	/	/	/	/	/
43a	E	0	0	0	0	0	0	0	0	0	0
	M	/	/	/	/	/	/	/	/	/	/
44a	E	0	0	0	0	0	0	0	0	0	0
	M	/	/	/	/	/	/	/	/	/	/
45a	E	0	0	0	0	0	0	0	0	0	0
	M	/	/	/	/	/	/	/	/	/	/

Appendix 3-1/3

INVESTIGATIONAL PRODUCT: PENTA 18 479 – Ref. TX 19011
SKIN EXAMINATION AND QUESTIONING DURING THE INDUCTION PHASE

Test subjects reference	Type of reaction	Experimental times									
		D1	D3	D5	D8	D10	D12	D15	D17	D19	D22
46a	E	0	0	0	0	0	0	0	0	0	0
	M	/	/	/	/	/	/	/	/	/	/
47a	E	0	0	0	0	0	0	0	0	0	0
	M	/	/	/	/	/	/	/	/	/	/
48a	E	0	0	0	0	0	0	0	0	0	0
	M	/	/	/	/	/	/	/	/	/	/
49a	E	0	0	0	0	0	0	0	0	0	0
	M	/	/	/	/	/	/	/	/	/	/
50a	E	0	0	0	0	0	0	0	0	0	0
	M	/	/	/	/	/	/	/	/	/	/
51a	E	0	0	0	0	0	0	0	0	0	0
	M	/	/	/	/	/	/	/	/	/	/
52a	E	0	0	0							
	M	/	/	/							
53a	E	0	0	0	0	0	0	0	0	0	0
	M	/	/	/	/	/	/	/	/	/	/
54a	E	0	0	0	0	0	0	0	0	0	0
	M	/	/	/	/	/	/	/	/	/	/
55a	E	0	0	0	0	0	0	0	0	0	0
	M	/	/	/	/	/	/	/	/	/	/
1b	E	0	0	0	0						
	M	/	/	/	/						
2b	E	0	0	0	0	0	0	0	0	0	0
	M	/	/	/	/	/	/	/	/	/	/
3b	E	0	0	0	0	0	0	0	0	0	0
	M	/	/	/	/	/	/	/	/	/	/
4b	E	0	0	0							
	M	/	/	/							
5b	E	0	0	0	0	0	0	0	0	0	0
	M	/	/	/	/	/	/	/	/	/	/
6b	E	0	0	0	0	0	0	0	0	0	0
	M	/	/	/	/	/	/	/	/	/	/
7b	E	0	0	0	0	0	0	0	0	0	0
	M	/	/	/	/	/	/	/	/	/	/
8b	E	0	0	0	0	0	0	0	0	0	0
	M	/	/	/	/	/	/	/	/	/	/
9b	E	0	0	0	0	0	0	0	0	0	0
	M	/	/	/	/	/	/	/	/	/	/
10b	E	0	0	0	0	0	0	0	0	0	0
	M	/	/	/	/	/	/	/	/	/	/
11b	E	0	0	0	0	0	0	0	0	0	0
	M	/	/	/	/	/	/	/	/	/	/
12b	E	0	0	0	0	0	0	0	0	0	0
	M	/	/	/	/	/	/	/	/	/	/
13b	E	0	0	0	0	0	0	0	0	0	0
	M	/	/	/	/	/	/	/	/	/	/
14b	E	0	0	0	0	0	0	0	0	0	0
	M	/	/	/	/	/	/	/	/	/	/
15b	E	0	0	0	0	0	0	0	0	0	0
	M	/	/	/	/	/	/	/	/	/	/
16b	E	0	0	0	0	0	0	0	0	0	0
	M	/	/	/	/	/	/	/	/	/	/

Legends: *Withdrawal*

Appendix 3-1/4

INVESTIGATIONAL PRODUCT: PENTA 18 479 – Ref. TX 19011
SKIN EXAMINATION AND QUESTIONING DURING THE INDUCTION PHASE

Test subjects reference	Type of reaction	Experimental times									
		D1	D3	D5	D8	D10	D12	D15	D17	D19	D22
17b	E	0	0	0	0	0	0	0	0	0	0
	M	/	/	/	/	/	/	/	/	/	/
18b	E	0	0	0	0	0	0	0	0	0	0
	M	/	/	/	/	/	/	/	/	/	/
19b	E	0	0	0	0	0	0	0	0	0	0
	M	/	/	/	/	/	/	/	/	/	/
20b	E	0	0	0	0	0	0	0	0	0	0
	M	/	/	/	/	/	/	/	/	/	/
21b	E	0	0	0	0	0	0	0	0	0	0
	M	/	/	/	/	/	/	/	/	/	/
22b	E	0	0	0	0	0	0	0	0	0	0
	M	/	/	/	/	/	/	/	/	/	/
23b	E	0	0	0	0	0	0	0	0	0	0
	M	/	/	/	/	/	/	/	/	/	/
24b	E	0	0	0	0	0	0	0	0	0	0
	M	/	/	/	/	/	/	/	/	/	/
25b	E	0	0	0	0	0	0	0	0	0	0
	M	/	/	/	/	/	/	/	/	/	/
26b	E	0	0	0	0	0	0	0	0	0	0
	M	/	/	/	/	/	/	/	/	/	/
27b	E	0	0	0	0	0	0	0	0	0	0
	M	/	/	/	/	/	/	/	/	/	/
28b	E	0	0	0	0	0	0	0	0	0	0
	M	/	/	/	/	/	/	/	/	/	/
29b	E	0	0	0	0	0	Direct contact of a person infected with SARS-CoV 2				
	M	/	/	/	/	/					
30b	E	0	0	0	0	0	0	0	0	0	0
	M	/	/	/	/	/	/	/	/	/	/
31b	E	0	0	0	0	0	0	0	0	0	0
	M	/	/	/	/	/	/	/	/	/	/
32b	E	0	0	0	0	0	0	0	0	0	0
	M	/	/	/	/	/	/	/	/	/	/
33b	E	0	0	0	0	0	0	0	0	0	0
	M	/	/	/	/	/	/	/	/	/	/
34b	E	0	0	0	0	0	0	0	0	0	0
	M	/	/	/	/	/	/	/	/	/	/
35b	E	0	0	0	0	0	0	0	0	0	0
	M	/	/	/	/	/	/	/	/	/	/
36b	E	0	0	0	0	0	0	0	0	0	0
	M	/	/	/	/	/	/	/	/	/	/
37b	E	0	0	0	0	0	0	0	0	0	0
	M	/	/	/	/	/	/	/	/	/	/
38b	E	0	0	0	0	0	0	0	0	0	0
	M	/	/	/	/	/	/	/	/	/	/
39b	E	0	0	0	0	0	0	0	0	0	0
	M	/	/	/	/	/	/	/	/	/	/
40b	E	0	0	0	0	0	0	0	0	0	0
	M	/	/	/	/	/	/	/	/	/	/
41b	E	0	0	0	0	0	0	0	0	0	0
	M	/	/	/	/	/	/	/	/	/	/

Legends: *Withdrawal*

Appendix 3-1/5

INVESTIGATIONAL PRODUCT: PENTA 18 479 – Ref. TX 19011
SKIN EXAMINATION AND QUESTIONING DURING THE INDUCTION PHASE

Test subjects reference	Type of reaction	Experimental times									
		D1	D3	D5	D8	D10	D12	D15	D17	D19	D22
42b	E	0	0	0	0	0	0	0	0	0	0
	M	/	/	/	/	/	/	/	/	/	/
43b	E	0	0	0	0	0	0	0	0	0	0
	M	/	/	/	/	/	/	/	/	/	/
44b	E	0	0	0	0	0	0	0	0	0	0
	M	/	/	/	/	/	/	/	/	/	/
45b	E	0	0	0	0	0	0	0	0	0	0
	M	/	/	/	/	/	/	/	/	/	/
46b	E	0	0	0	0	0	0	0	0	0	0
	M	/	/	/	/	/	/	/	/	/	/
47b	E	0	0	0	0	0	0	0	0	0	0
	M	/	/	/	/	/	/	/	/	/	/
48b	E	0	0	0	0	0	0	0	0	0	0
	M	/	/	/	/	/	/	/	/	/	/
49b	E	0	0	0	0	0	0	0	0	0	0
	M	/	/	/	/	/	/	/	/	/	/
50b	E	0	0	0	0	0	0	0	0	0	0
	M	/	/	/	/	/	/	/	/	/	/
51b	E	0	0	0	0	0	0	0	0	0	0
	M	/	/	/	/	/	/	/	/	/	/
52b	E	0	0	0	0	0	0	0	0	0	0
	M	/	/	/	/	/	/	/	/	/	/
53b	E	0	0	0	0	0	0	0	0	0	0
	M	/	/	/	/	/	/	/	/	/	/
54b	E	0	0	0	0	0	0	0	0	0	0
	M	/	/	/	/	/	/	/	/	/	/
55b	E	0	0	0	0	0	0	0	0	0	0
	M	/	/	/	/	/	/	/	/	/	/
56b	E	0	0	0	0	0	0	0	0	0	0
	M	/	/	/	/	/	/	/	/	/	/

Appendix 3-2/1

INVESTIGATIONAL PRODUCT: PENTA 18 479 – Ref. TX 19011
SKIN EXAMINATION AND QUESTIONING DURING THE CHALLENGE PHASE

E: Erythema: 0 *= no visible erythema,* ***0.5*** *= very slight erythema – barely perceptible,* ***1*** *= mild erythema – faint pink,* ***2*** *= moderate erythema – well defined,* ***3*** *= severe erythema,* ***4*** *= caustic effect – erosive aspect and/or necrotic aspect*
d= diffuse / p = punctuated / peri = peripheral

M: Additional comments/Others reactions: H or Oe *= Homogeneous infiltration / oedema,* ***P*** *= Papules,* ***V*** *= Vesicles,* ***B*** *= Bullae,* ***Pe*** *= Petechiae,* ***S:*** *Spreading beyond the patch,* ***SV*** *= Soap effect (shiny skin with possibly wrinkles),* ***F*** *= Fissuring ,* ***D*** *= Desquamation,* ***Dr*** *= Dryness,* ***C*** *= Skin coloration, hyperpigmentation,* ***HY*** *= Hypopigmentation,* ***Fr*** *= Follicular reaction,* ***NA*** *= Product not applied,* ***T***= *Tape reaction,* ***I*** *= Itching at the test site,* ***Cr*** *= Exsudation and/or Surface encrustation,* ***Sc*** *= Scab,* ***Pr*** *= Pruritus,* ***He*** *= Heating,* ***Pu*** *= Pustules,* ******* *= Additional free comments,* ***N9G*** *= No 9th grade,* ***X*** *= Succeeding patch not applied and succeeding grade (in brackets) denotes a residual reaction,* ***Abs or "–"*** *= Subject absent,* ***MU*** *= Make-up patch*

/: no reaction

A: ICDRG scale*:* ***IR*** *= Irritation reaction,* ***-*** *= No allergic reaction,* ***?+ =*** *Doubtful reaction (only slight erythema),* ***(+) =*** *Weak positive reaction (without vesicle): slight erythema and infiltration with presence of small papular elevations, possibly papules,* ***(++) =*** *Strong positive reaction: erythema, papules, vesicles, infiltration,* ***(+++) =*** *Extreme positive reaction: intense erythema, oedema, coalescent vesicles (bullae)*

Test subjects reference	Type of reaction	Experimental times					
		Induction site			Virgin site		
		D36	D38	D40	D36	D38	D40
1a	E	0	0	0	0	0	0
	M	/	/	/	/	/	/
	A	-					
2a	E	0	0	0	0	0	0
	M	/	/	/	/	/	/
	A						
3a	E	0	0	0	0	0	0
	M	/	/	/	/	/	/
	A	-					
4a	E	0	0	0	0	0	0
	M	/	/	/	/	/	/
	A	-					
5a	E	0	0	0	0	0	0
	M	/	/	/	/	/	/
	A	-					
6a	E	0	0	0	0	0	0
	M	/	/	/	/	/	/
	A	-					
7a	E						
	M						
	A						
8a	E	0	0	0	0	0	0
	M	/	/	/	/	/	/
	A	-					
9a	E	0	0	0	0	0	0
	M	/	/	/	/	/	/
	A	-					
10a	E	0	0	0	0	0	0
	M	/	/	/	/	/	/
	A	-					
11a	E	0	0	0	0	0	0
	M	/	/	/	/	/	/
	A	-					
12a	E	0	0	0	0	0	0
	M	/	/	/	/	/	/
	A	-					
13a	E	0	0	0	0	0	0
	M	/	/	/	/	/	/
	A	-					

Legends: *Withdrawal*

Appendix 3-2/2

INVESTIGATIONAL PRODUCT: PENTA 18 479 – Ref. TX 19011
SKIN EXAMINATION AND QUESTIONING DURING THE CHALLENGE PHASE

Test subjects reference	Type of reaction	Experimental times					
		Induction site			Virgin site		
		D36	D38	D40	D36	D38	D40
14a	E	0	0	0	0	0	0
	M	/	/	/	/	/	/
	A	-					
15a	E	0	0	0	0	0	0
	M	/	/	/	/	/	/
	A	-					
16a	E	0	0	0	0	0	0
	M	/	/	/	/	/	/
	A	-					
17a	E	0	0	0	0	0	0
	M	/	/	/	/	/	/
	A	-					
18a	E	0	0	0	0	0	0
	M	/	/	/	/	/	/
	A	-					
19a	E	0	0	0	0	0	0
	M	/	/	/	/	/	/
	A	-					
20a	E	0	0	0	0	0	0
	M	/	/	/	/	/	/
	A	-					
21a	E	0	0	0	0	0	0
	M	/	/	/	/	/	/
	A	-					
22a	E	0	0	0	0	0	0
	M	/	/	/	/	/	/
	A	-					
23a	E	0	0	0	0	0	0
	M	/	/	/	/	/	/
	A	-					
24a	E	0	0	0	0	0	0
	M	/	/	/	/	/	/
	A	-					
25a	E	0	0	0	0	0	0
	M	/	/	/	/	/	/
	A	-					
26a	E	0	0	0	0	0	0
	M	/	/	/	/	/	/
	A	-					
27a	E	0	0	0	0	0	0
	M	/	/	/	/	/	/
	A	-					
28a	E	0	0	0	0	0	0
	M	/	/	/	/	/	/
	A	-					
29a	E	0	0	0	0	0	0
	M	/	/	/	/	/	/
	A	-					

Appendix 3-2/3

INVESTIGATIONAL PRODUCT: PENTA 18 479 – Ref. TX 19011
SKIN EXAMINATION AND QUESTIONING DURING THE CHALLENGE PHASE

Test subjects reference	Type of reaction	Experimental times					
		Induction site			Virgin site		
		D36	D38	D40	D36	D38	D40
30a	E	0	0	0	0	0	0
	M	/	/	/	/	/	/
	A	-					
31a	E	0	0	0	0	0	0
	M	/	/	/	/	/	/
	A	-					
32a	E	0	0	0	0	0	0
	M	/	/	/	/	/	/
	A	-					
33a	E	0	0	0	0	0	0
	M	/	/	/	/	/	/
	A	-					
34a	E	0	0	0	0	0	0
	M	/	/	/	/	/	/
	A	-					
35a	E	0	0	0	0	0	0
	M	/	/	/	/	/	/
	A	-					
36a	E	0	0	0	0	0	0
	M	/	/	/	/	/	/
	A	-					
37a	E	0	0	0	0	0	0
	M	/	/	/	/	/	/
	A	-					
38a	E	0	0	0	0	0	0
	M	/	/	/	/	/	/
	A	-					
39a	E	0	0	0	0	0	0
	M	/	/	/	/	/	/
	A	-					
40a	E	0	0	0	0	0	0
	M	/	/	/	/	/	/
	A	-					
41a	E	0	0	0	0	0	0
	M	/	/	/	/	/	/
	A	-					
42a	E	0	0	0	0	0	0
	M	/	/	/	/	/	/
	A	-					
43a	E	0	0	0	0	0	0
	M	/	/	/	/	/	/
	A	-					
44a	E	0	0	0	0	0	0
	M	/	/	/	/	/	/
	A	-					
45a	E	0	0	0	0	0	0
	M	/	/	/	/	/	/
	A	-					

Appendix 3-2/4

INVESTIGATIONAL PRODUCT: PENTA 18 479 – Ref. TX 19011
SKIN EXAMINATION AND QUESTIONING DURING THE CHALLENGE PHASE

Test subjects reference	Type of reaction	Experimental times					
		Induction site			Virgin site		
		D36	D38	D40	D36	D38	D40
46a	E	0	0	0	0	0	0
	M	/	/	/	/	/	/
	A	-					
47a	E	0	0	0	0	0	0
	M	/	/	/	/	/	/
	A	-					
48a	E	0	0	0	0	0	0
	M	/	/	/	/	/	/
	A	-					
49a	E	0	0	0	0	0	0
	M	/	/	/	/	/	/
	A	-					
50a	E	0	0	0	0	0	0
	M	/	/	/	/	/	/
	A	-					
51a	E	0	0	0	0	0	0
	M	/	/	/	/	/	/
	A	-					
52a	E						
	M						
	A						
53a	E	0	0	0	0	0	0
	M	/	/	/	/	/	/
	A	-					
54a	E	0	0	0	0	0	0
	M	/	/	/	/	/	/
	A	-					
55a	E	0	0	0	0	0	0
	M	/	/	/	/	/	/
	A	-					
1b	E						
	M						
	A						
2b	E	0	0	0	0	0	0
	M	/	/	/	/	/	/
	A	-					
3b	E	0	0	0	0	0	0
	M	/	/	/	/	/	/
	A	-					
4b	E						
	M						
	A						
5b	E	0	0	0	0	0	0
	M	/	/	/	/	/	/
	A	-					
6b	E	0	0	0	0	0	0
	M	/	/	/	/	/	/
	A	-					

Legends: *Withdrawal*

Appendix 3-2/4

INVESTIGATIONAL PRODUCT: PENTA 18 479 – Ref. TX 19011
SKIN EXAMINATION AND QUESTIONING DURING THE CHALLENGE PHASE

Test subjects reference	Type of reaction	Experimental times					
		Induction site			Virgin site		
		D36	D38	D40	D36	D38	D40
7b	E	0	0	0	0	0	0
	M	/	/	/	/	/	/
	A	-					
8b	E	0	0	0	0	0	0
	M	/	/	/	/	/	/
	A	-					
9b	E	0	0	0	0	0	0
	M	/	/	/	/	/	/
	A	-					
10b	E	0	0	0	0	0	0
	M	/	/	/	/	/	/
	A	-					
11b	E	0	0	0	0	0	0
	M	/	/	/	/	/	/
	A	-					
12b	E	0	0	0	0	0	0
	M	/	/	/	/	/	/
	A	-					
13b	E	0	0	0	0	0	0
	M	/	/	/	/	/	/
	A	-					
14b	E	0	0	0	0	0	0
	M	/	/	/	/	/	/
	A	-					
15b	E	0	0	0	0	0	0
	M	/	/	/	/	/	/
	A	-					
16b	E	0	0	0	0	0	0
	M	/	/	/	/	/	/
	A	-					
17b	E	0	0	0	0	0	0
	M	/	/	/	/	/	/
	A	-					
18b	E	0	0	0	0	0	0
	M	/	/	/	/	/	/
	A	-					
19b	E	0	0	0	0	0	0
	M	/	/	/	/	/	/
	A	-					
20b	E	0	0	0	0	0	0
	M	/	/	/	/	/	/
	A	-					
21b	E	0	0	0	0	0	0
	M	/	/	/	/	/	/
	A	-					
22b	E	0	0	0	0	0	0
	M	/	/	/	/	/	/
	A	-					

Appendix 3-2/5

INVESTIGATIONAL PRODUCT: PENTA 18 479 – Ref. TX 19011
SKIN EXAMINATION AND QUESTIONING DURING THE CHALLENGE PHASE

Test subjects reference	Type of reaction	Experimental times					
		Induction site			Virgin site		
		D36	D38	D40	D36	D38	D40
23b	E	0	0	0	0	0	0
	M	/	/	/	/	/	/
	A	-					
24b	E	0	0	0	0	0	0
	M	/	/	/	/	/	/
	A	-					
25b	E	0	0	0	0	0	0
	M	/	/	/	/	/	/
	A	-					
26b	E	0	0	0	0	0	0
	M	/	/	/	/	/	/
	A	-					
27b	E	0	0	0	0	0	0
	M	/	/	/	/	/	/
	A	-					
28b	E	0	0	0	0	0	0
	M	/	/	/	/	/	/
	A	-					
29b	E	Direct contact of a person infected with SARS-CoV 2					
	M						
	A						
30b	E	0	0	0	0	0	0
	M	/	/	/	/	/	/
	A	-					
31b	E	0	0	0	0	0	0
	M	/	/	/	/	/	/
	A	-					
32b	E	0	0	0	0	0	0
	M	/	/	/	/	/	/
	A	-					
33b	E	0	0	0	0	0	0
	M	/	/	/	/	/	/
	A	-					
34b	E	0	0	0	0	0	0
	M	/	/	/	/	/	/
	A	-					
35b	E	0	0	0	0	0	0
	M	/	/	/	/	/	/
	A	-					
36b	E	0	0	0	0	0	0
	M	/	/	/	/	/	/
	A	-					
37b	E	0	0	0	0	0	0
	M	/	/	/	/	/	/
	A	-					
38b	E	0	0	0	0	0	0
	M	/	/	/	/	/	/
	A	-					

Appendix 3-2/6

INVESTIGATIONAL PRODUCT: PENTA 18 479 – Ref. TX 19011
SKIN EXAMINATION AND QUESTIONING DURING THE CHALLENGE PHASE

Test subjects reference	Type of reaction	Experimental times					
		Induction site			Virgin site		
		D36	D38	D40	D36	D38	D40
39b	E	0	0	0	0	0	0
	M	/	/	/	/	/	/
	A	-					
40b	E	0	0	0	0	0	0
	M	/	/	/	/	/	/
	A	-					
41b	E	0	0	0	0	0	0
	M	/	/	/	/	/	/
	A	-					
42b	E	0	0	0	0	0	0
	M	/	/	/	/	/	/
	A	-					
43b	E	0	0	0	0	0	0
	M	/	/	/	/	/	/
	A	-					
44b	E	0	0	0	0	0	0
	M	/	/	/	/	/	/
	A	-					
45b	E	0	0	0	0	0	0
	M	/	/	/	/	/	/
	A	-					
46b	E	0	0	0	0	0	0
	M	/	/	/	/	/	/
	A	-					
47b	E	0	0	0	0	0	0
	M	/	/	/	/	/	/
	A	-					
48b	E	0	0	0	0	0	0
	M	/	/	/	/	/	/
	A	-					
49b	E	0	0	0	0	0	0
	M	/	/	/	/	/	/
	A	-					
50b	E	0	0	0	0	0	0
	M	/	/	/	/	/	/
	A	-					
51b	E	0	0	0	0	0	0
	M	/	/	/	/	/	/
	A	-					
52b	E	0	0	0	0	0	0
	M	/	/	/	/	/	/
	A	-					
53b	E	0	0	0	0	0	0
	M	/	/	/	/	/	/
	A	-					
54b	E	0	0	0	0	0	0
	M	/	/	/	/	/	/
	A	-					
55b	E	0	0	0	0	0	0
	M	/	/	/	/	/	/
	A	-					
56b	E	0	0	0	0	0	0
	M	/	/	/	/	/	/
	A	-					

Appendix 4-1/1

CONTROL PRODUCT: DISTILLED WATER
SKIN EXAMINATION AND QUESTIONING DURING THE INDUCTION PHASE

***E: Erythema**: **0** = no visible erythema, **0.5** = very slight erythema – barely perceptible, **1** = mild erythema – faint pink, **2** = moderate erythema – well defined, **3** = severe erythema, **4** = caustic effect – erosive aspect and/or necrotic aspect*
d= diffuse / p = punctuated / peri = peripheral

***M: Additional comments/Others reactions: H or Oe** = Homogeneous infiltration / oedema, **P** = Papules, **V** = Vesicles, **B** = Bullae, **Pe** = Petechiae, **S:** Spreading beyond the patch, **SV** = Soap effect (shiny skin with possibly wrinkles), **F** = Fissuring , **D** = Desquamation, **Dr** = Dryness, **C** = Skin coloration, hyperpigmentation, **HY** = Hypopigmentation, **Fr** = Follicular reaction, **NA** = Product not applied, **T**= Tape reaction, **I** = Itching at the test site, **Cr** = Exsudation and/or Surface encrustation, **Sc** = Scab, **Pr** = Pruritus, **He** = Heating, **Pu** = Pustules, * = Additional free comments, **N9G** = No 9th grade, **X** = Succeeding patch not applied and succeeding grade (in brackets) denotes a residual reaction, **Abs or "–"** = Subject absent, **MU** = Make-up patch*

/: no reaction

Test subjects reference	Type of reaction	Experimental times									
		D1	D3	D5	D8	D10	D12	D15	D17	D19	D22
1a	E	0	0	0	0	0	0	0	0	0	0
	M	/	/	/	/	/	/	/	/	/	/
2a	E	0	0	0	0	0	0	0	0	0	0
	M	/	/	/	/	/	/	/	/	/	/
3a	E	0	0	0	0	0	0	0	0	0	0
	M	/	/	/	/	/	/	/	/	/	/
4a	E	0	0	0	0	0	0	0	0	0	0
	M	/	/	/	/	/	/	/	/	/	/
5a	E	0	0	0	0	0	0	0	0	0	0
	M	/	/	/	/	/	/	/	/	/	/
6a	E	0	0	0	0	0	0	0	0	0	0
	M	/	/	/	/	/	/	/	/	/	/
7a	E	0	0	0	0	0	0	0	0	0	0
	M	/	/	/	/	/	/	/	/	/	/
8a	E	0	0	0	0	0	0	0	0	0	0
	M	/	/	/	/	/	/	/	/	/	/
9a	E	0	0	0	0	0	0	0	0	0	0
	M	/	/	/	/	/	/	/	/	/	/
10a	E	0	0	0	0	0	0	0	0	0	0
	M	/	/	/	/	/	/	/	/	/	/
11a	E	0	0	0	0	0	0	0	0	0	0
	M	/	/	/	/	/	/	/	/	/	/
12a	E	0	0	0	0	0	0	0	0	0	0
	M	/	/	/	/	/	/	/	/	/	/
13a	E	0	0	0	0	0	0	0	0	0	0
	M	/	/	/	/	/	/	/	/	/	/
14a	E	0	0	0	0	0	0	0	0	0	0
	M	/	/	/	/	/	/	/	/	/	/
15a	E	0	0	0	0	0	0	0	0	0	0
	M	/	/	/	/	/	/	/	/	/	/
16a	E	0	0	0	0	0	0	0	0	0	0
	M	/	/	/	/	/	/	/	/	/	/
17a	E	0	0	0	0	0	0	0	0	0	0
	M	/	/	/	/	/	/	/	/	/	/
18a	E	0	0	0	0	0	0	0	0	0	0
	M	/	/	/	/	/	/	/	/	/	/
19a	E	0	0	0	0	0	0	0	0	0	0
	M	/	/	/	/	/	/	/	/	/	/
20a	E	0	0	0	0	0	0	0	0	0	0
	M	/	/	/	/	/	/	/	/	/	/

Legends: *Withdrawal*

Appendix 4-1/2

CONTROL PRODUCT: DISTILLED WATER
SKIN EXAMINATION AND QUESTIONING DURING THE INDUCTION PHASE

Test subjects reference	Type of reaction	Experimental times									
		D1	D3	D5	D8	D10	D12	D15	D17	D19	D22
21a	E	0	0	0	0	0	0	0	0	0	0
	M	/	/	/	/	/	/	/	/	/	/
22a	E	0	0	0	0	0	0	0	0	0	0
	M	/	/	/	/	/	/	/	/	/	/
23a	E	0	0	0	0	0	0	0	0	0	0
	M	/	/	/	/	/	/	/	/	/	/
24a	E	0	0	0	0	0	0	0	0	0	0
	M	/	/	/	/	/	/	/	/	/	/
25a	E	0	0	0	0	0	0	0	0	0	0
	M	/	/	/	/	/	/	/	/	/	/
26a	E	0	0	0	0	0	0	0	0	0	0
	M	/	/	/	/	/	/	/	/	/	/
27a	E	0	0	0	0	0	0	0	0	0	0
	M	/	/	/	/	/	/	/	/	/	/
28a	E	0	0	0	0	0	0	0	0	0	0
	M	/	/	/	/	/	/	/	/	/	/
29a	E	0	0	0	0	0	0	0	0	0	0
	M	/	/	/	/	/	/	/	/	/	/
30a	E	0	0	0	0	0	0	0	0	0	0
	M	/	/	/	/	/	/	/	/	/	/
31a	E	0	0	0	0	0	0	0	0	0	0
	M	/	/	/	/	/	/	/	/	/	/
32a	E	0	0	0	0	0	0	0	0	0	0
	M	/	/	/	/	/	/	/	/	/	/
33a	E	0	0	0	0	0	0	0	0	0	0
	M	/	/	/	/	/	/	/	/	/	/
34a	E	0	0	0	0	0	0	0	0	0	0
	M	/	/	/	/	/	/	/	/	/	/
35a	E	0	0	0	0	0	0	0	0	0	0
	M	/	/	/	/	/	/	/	/	/	/
36a	E	0	0	0	0	0	0	0	0	0	0
	M	/	/	/	/	/	/	/	/	/	/
37a	E	0	0	0	0	0	0	0	0	0	0
	M	/	/	/	/	/	/	/	/	/	/
38a	E	0	0	0	0	0	0	0	0	0	0
	M	/	/	/	/	/	/	/	/	/	/
39a	E	0	0	0	0	0	0	0	0	0	0
	M	/	/	/	/	/	/	/	/	/	/
40a	E	0	0	0	0	0	0	0	0	0	0
	M	/	/	/	/	/	/	/	/	/	/
41a	E	0	0	0	0	0	0	0	0	0	0
	M	/	/	/	/	/	/	/	/	/	/
42a	E	0	0	0	0	0	0	0	0	0	0
	M	/	/	/	/	/	/	/	/	/	/
43a	E	0	0	0	0	0	0	0	0	0	0
	M	/	/	/	/	/	/	/	/	/	/
44a	E	0	0	0	0	0	0	0	0	0	0
	M	/	/	/	/	/	/	/	/	/	/
45a	E	0	0	0	0	0	0	0	0	0	0
	M	/	/	/	/	/	/	/	/	/	/

Appendix 4-1/3

CONTROL PRODUCT: DISTILLED WATER
SKIN EXAMINATION AND QUESTIONING DURING THE INDUCTION PHASE

Test subjects reference	Type of reaction	Experimental times									
		D1	D3	D5	D8	D10	D12	D15	D17	D19	D22
46a	E	0	0	0	0	0	0	0	0	0	0
	M	/	/	/	/	/	/	/	/	/	/
47a	E	0	0	0	0	0	0	0	0	0	0
	M	/	/	/	/	/	/	/	/	/	/
48a	E	0	0	0	0	0	0	0	0	0	0
	M	/	/	/	/	/	/	/	/	/	/
49a	E	0	0	0	0	0	0	0	0	0	0
	M	/	/	/	/	/	/	/	/	/	/
50a	E	0	0	0	0	0	0	0	0	0	0
	M	/	/	/	/	/	/	/	/	/	/
51a	E	0	0	0	0	0	0	0	0	0	0
	M	/	/	/	/	/	/	/	/	/	/
52a	E	0	0	0							
	M	/	/	/							
53a	E	0	0	0	0	0	0	0	0	0	0
	M	/	/	/	/	/	/	/	/	/	/
54a	E	0	0	0	0	0	0	0	0	0	0
	M	/	/	/	/	/	/	/	/	/	/
55a	E	0	0	0	0	0	0	0	0	0	0
	M	/	/	/	/	/	/	/	/	/	/
1b	E	0	0	0	0						
	M	/	/	/	/						
2b	E	0	0	0	0	0	0	0	0	0	0
	M	/	/	/	/	/	/	/	/	/	/
3b	E	0	0	0	0	0	0	0	0	0	0
	M	/	/	/	/	/	/	/	/	/	/
4b	E	0	0	0							
	M	/	/	/							
5b	E	0	0	0	0	0	0	0	0	0	0
	M	/	/	/	/	/	/	/	/	/	/
6b	E	0	0	0	0	0	0	0	0	0	0
	M	/	/	/	/	/	/	/	/	/	/
7b	E	0	0	0	0	0	0	0	0	0	0
	M	/	/	/	/	/	/	/	/	/	/
8b	E	0	0	0	0	0	0	0	0	0	0
	M	/	/	/	/	/	/	/	/	/	/
9b	E	0	0	0	0	0	0	0	0	0	0
	M	/	/	/	/	/	/	/	/	/	/
10b	E	0	0	0	0	0	0	0	0	0	0
	M	/	/	/	/	/	/	/	/	/	/
11b	E	0	0	0	0	0	0	0	0	0	0
	M	/	/	/	/	/	/	/	/	/	/
12b	E	0	0	0	0	0	0	0	0	0	0
	M	/	/	/	/	/	/	/	/	/	/
13b	E	0	0	0	0	0	0	0	0	0	0
	M	/	/	/	/	/	/	/	/	/	/
14b	E	0	0	0	0	0	0	0	0	0	0
	M	/	/	/	/	/	/	/	/	/	/
15b	E	0	0	0	0	0	0	0	0	0	0
	M	/	/	/	/	/	/	/	/	/	/
16b	E	0	0	0	0	0	0	0	0	0	0
	M	/	/	/	/	/	/	/	/	/	/

Legends: *Withdrawal*

Appendix 4-1/4

CONTROL PRODUCT: DISTILLED WATER
SKIN EXAMINATION AND QUESTIONING DURING THE INDUCTION PHASE

Test subjects reference	Type of reaction	Experimental times									
		D1	D3	D5	D8	D10	D12	D15	D17	D19	D22
17b	E	0	0	0	0	0	0	0	0	0	0
	M	/	/	/	/	/	/	/	/	/	/
18b	E	0	0	0	0	0	0	0	0	0	0
	M	/	/	/	/	/	/	/	/	/	/
19b	E	0	0	0	0	0	0	0	0	0	0
	M	/	/	/	/	/	/	/	/	/	/
20b	E	0	0	0	0	0	0	0	0	0	0
	M	/	/	/	/	/	/	/	/	/	/
21b	E	0	0	0	0	0	0	0	0	0	0
	M	/	/	/	/	/	/	/	/	/	/
22b	E	0	0	0	0	0	0	0	0	0	0
	M	/	/	/	/	/	/	/	/	/	/
23b	E	0	0	0	0	0	0	0	0	0	0
	M	/	/	/	/	/	/	/	/	/	/
24b	E	0	0	0	0	0	0	0	0	0	0
	M	/	/	/	/	/	/	/	/	/	/
25b	E	0	0	0	0	0	0	0	0	0	0
	M	/	/	/	/	/	/	/	/	/	/
26b	E	0	0	0	0	0	0	0	0	0	0
	M	/	/	/	/	/	/	/	/	/	/
27b	E	0	0	0	0	0	0	0	0	0	0
	M	/	/	/	/	/	/	/	/	/	/
28b	E	0	0	0	0	0	0	0	0	0	0
	M	/	/	/	/	/	/	/	/	/	/
29b	E	0	0	0	0	0	Direct contact of a person infected with SARS-CoV 2				
	M	/	/	/	/	/					
30b	E	0	0	0	0	0	0	0	0	0	0
	M	/	/	/	/	/	/	/	/	/	/
31b	E	0	0	0	0	0	0	0	0	0	0
	M	/	/	/	/	/	/	/	/	/	/
32b	E	0	0	0	0	0	0	0	0	0	0
	M	/	/	/	/	/	/	/	/	/	/
33b	E	0	0	0	0	0	0	0	0	0	0
	M	/	/	/	/	/	/	/	/	/	/
34b	E	0	0	0	0	0	0	0	0	0	0
	M	/	/	/	/	/	/	/	/	/	/
35b	E	0	0	0	0	0	0	0	0	0	0
	M	/	/	/	/	/	/	/	/	/	/
36b	E	0	0	0	0	0	0	0	0	0	0
	M	/	/	/	/	/	/	/	/	/	/
37b	E	0	0	0	0	0	0	0	0	0	0
	M	/	/	/	/	/	/	/	/	/	/
38b	E	0	0	0	0	0	0	0	0	0	0
	M	/	/	/	/	/	/	/	/	/	/
39b	E	0	0	0	0	0	0	0	0	0	0
	M	/	/	/	/	/	/	/	/	/	/
40b	E	0	0	0	0	0	0	0	0	0	0
	M	/	/	/	/	/	/	/	/	/	/
41b	E	0	0	0	0	0	0	0	0	0	0
	M	/	/	/	/	/	/	/	/	/	/

Legends: Withdrawal

Appendix 4-1/5

CONTROL PRODUCT: DISTILLED WATER
SKIN EXAMINATION AND QUESTIONING DURING THE INDUCTION PHASE

Test subjects reference	Type of reaction	Experimental times									
		D1	D3	D5	D8	D10	D12	D15	D17	D19	D22
42b	E	0	0	0	0	0	0	0	0	0	0
	M	/	/	/	/	/	/	/	/	/	/
43b	E	0	0	0	0	0	0	0	0	0	0
	M	/	/	/	/	/	/	/	/	/	/
44b	E	0	0	0	0	0	0	0	0	0	0
	M	/	/	/	/	/	/	/	/	/	/
45b	E	0	0	0	0	0	0	0	0	0	0
	M	/	/	/	/	/	/	/	/	/	/
46b	E	0	0	0	0	0	0	0	0	0	0
	M	/	/	/	/	/	/	/	/	/	/
47b	E	0	0	0	0	0	0	0	0	0	0
	M	/	/	/	/	/	/	/	/	/	/
48b	E	0	0	0	0	0	0	0	0	0	0
	M	/	/	/	/	/	/	/	/	/	/
49b	E	0	0	0	0	0	0	0	0	0	0
	M	/	/	/	/	/	/	/	/	/	/
50b	E	0	0	0	0	0	0	0	0	0	0
	M	/	/	/	/	/	/	/	/	/	/
51b	E	0	0	0	0	0	0	0	0	0	0
	M	/	/	/	/	/	/	/	/	/	/
52b	E	0	0	0	0	0	0	0	0	0	0
	M	/	/	/	/	/	/	/	/	/	/
53b	E	0	0	0	0	0	0	0	0	0	0
	M	/	/	/	/	/	/	/	/	/	/
54b	E	0	0	0	0	0	0	0	0	0	0
	M	/	/	/	/	/	/	/	/	/	/
55b	E	0	0	0	0	0	0	0	0	0	0
	M	/	/	/	/	/	/	/	/	/	/
56b	E	0	0	0	0	0	0	0	0	0	0
	M	/	/	/	/	/	/	/	/	/	/

Appendix 4-2/1

CONTROL PRODUCT: DISTILLED WATER
SKIN EXAMINATION AND QUESTIONING DURING THE CHALLENGE PHASE

E: Erythema*: *0 *= no visible erythema,* ***0.5*** *= very slight erythema – barely perceptible,* ***1*** *= mild erythema – faint pink,* ***2*** *= moderate erythema – well defined,* ***3*** *= severe erythema,* ***4*** *= caustic effect – erosive aspect and/or necrotic aspect*
d= diffuse / p = punctuated / peri = peripheral

M: Additional comments/Others reactions: H or Oe *= Homogeneous infiltration / oedema,* ***P*** *= Papules,* ***V*** *= Vesicles,* ***B*** *= Bullae,* ***Pe*** *= Petechiae,* ***S:*** *Spreading beyond the patch,* ***SV*** *= Soap effect (shiny skin with possibly wrinkles),* ***F*** *= Fissuring ,* ***D*** *= Desquamation,* ***Dr*** *= Dryness,* ***C*** *= Skin coloration, hyperpigmentation,* ***HY*** *= Hypopigmentation,* ***Fr*** *= Follicular reaction,* ***NA*** *= Product not applied,* ***T***= *Tape reaction,* ***I*** *= Itching at the test site,* ***Cr*** *= Exsudation and/or Surface encrustation,* ***Sc*** *= Scab,* ***Pr*** *= Pruritus,* ***He*** *= Heating,* ***Pu*** *= Pustules,* ******* *= Additional free comments,* ***N9G*** *= No 9th grade,* ***X*** *= Succeeding patch not applied and succeeding grade (in brackets) denotes a residual reaction,* ***Abs or "–"*** *= Subject absent,* ***MU*** *= Make-up patch*

/: no reaction

A: ICDRG scale*: *IR *= Irritation reaction,* ***-*** *= No allergic reaction,* ***?+ =*** *Doubtful reaction (only slight erythema),* ***(+) =*** *Weak positive reaction (without vesicle): slight erythema and infiltration with presence of small papular elevations, possibly papules,* ***(++) =*** *Strong positive reaction: erythema, papules, vesicles, infiltration,* ***(+++) =*** *Extreme positive reaction: intense erythema, oedema, coalescent vesicles (bullae)*

Test subjects reference	Type of reaction	Experimental times					
		Induction site			Virgin site		
		D36	D38	D40	D36	D38	D40
1a	E	0	0	0	0	0	0
	M	/	/	/	/	/	/
	A	-					
2a	E	0	0	0	0	0	0
	M	/	/	/	/	/	/
	A						
3a	E	0	0	0	0	0	0
	M	/	/	/	/	/	/
	A	-					
4a	E	0	0	0	0	0	0
	M	/	/	/	/	/	/
	A	-					
5a	E	0	0	0	0	0	0
	M	/	/	/	/	/	/
	A	-					
6a	E	0	0	0	0	0	0
	M	/	/	/	/	/	/
	A	-					
7a	E						
	M						
	A						
8a	E	0	0	0	0	0	0
	M	/	/	/	/	/	/
	A	-					
9a	E	0	0	0	0	0	0
	M	/	/	/	/	/	/
	A	-					
10a	E	0	0	0	0	0	0
	M	/	/	/	/	/	/
	A	-					
11a	E	0	0	0	0	0	0
	M	/	/	/	/	/	/
	A	-					
12a	E	0	0	0	0	0	0
	M	/	/	/	/	/	/
	A	-					
13a	E	0	0	0	0	0	0
	M	/	/	/	/	/	/
	A	-					

Legends: *Withdrawal*

Appendix 4-2/2

CONTROL PRODUCT: DISTILLED WATER
SKIN EXAMINATION AND QUESTIONING DURING THE CHALLENGE PHASE

Test subjects reference	Type of reaction	Experimental times					
		Induction site			Virgin site		
		D36	D38	D40	D36	D38	D40
14a	E	0	0	0	0	0	0
	M	/	/	/	/	/	/
	A	-					
15a	E	0	0	0	0	0	0
	M	/	/	/	/	/	/
	A	-					
16a	E	0	0	0	0	0	0
	M	/	/	/	/	/	/
	A	-					
17a	E	0	0	0	0	0	0
	M	/	/	/	/	/	/
	A	-					
18a	E	0	0	0	0	0	0
	M	/	/	/	/	/	/
	A	-					
19a	E	0	0	0	0	0	0
	M	/	/	/	/	/	/
	A	-					
20a	E	0	0	0	0	0	0
	M	/	/	/	/	/	/
	A	-					
21a	E	0	0	0	0	0	0
	M	/	/	/	/	/	/
	A	-					
22a	E	0	0	0	0	0	0
	M	/	/	/	/	/	/
	A	-					
23a	E	0	0	0	0	0	0
	M	/	/	/	/	/	/
	A	-					
24a	E	0	0	0	0	0	0
	M	/	/	/	/	/	/
	A	-					
25a	E	0	0	0	0	0	0
	M	/	/	/	/	/	/
	A	-					
26a	E	0	0	0	0	0	0
	M	/	/	/	/	/	/
	A	-					
27a	E	0	0	0	0	0	0
	M	/	/	/	/	/	/
	A	-					
28a	E	0	0	0	0	0	0
	M	/	/	/	/	/	/
	A	-					
29a	E	0	0	0	0	0	0
	M	/	/	/	/	/	/
	A	-					

Appendix 4-2/3

CONTROL PRODUCT: DISTILLED WATER
SKIN EXAMINATION AND QUESTIONING DURING THE CHALLENGE PHASE

Test subjects reference	Type of reaction	Experimental times					
		Induction site			Virgin site		
		D36	D38	D40	D36	D38	D40
30a	E	0	0	0	0	0	0
	M	/	/	/	/	/	/
	A	-					
31a	E	0	0	0	0	0	0
	M	/	/	/	/	/	/
	A	-					
32a	E	0	0	0	0	0	0
	M	/	/	/	/	/	/
	A	-					
33a	E	0	0	0	0	0	0
	M	/	/	/	/	/	/
	A	-					
34a	E	0	0	0	0	0	0
	M	/	/	/	/	/	/
	A	-					
35a	E	0	0	0	0	0	0
	M	/	/	/	/	/	/
	A	-					
36a	E	0	0	0	0	0	0
	M	/	/	/	/	/	/
	A	-					
37a	E	0	0	0	0	0	0
	M	/	/	/	/	/	/
	A	-					
38a	E	0	0	0	0	0	0
	M	/	/	/	/	/	/
	A	-					
39a	E	0	0	0	0	0	0
	M	/	/	/	/	/	/
	A	-					
40a	E	0	0	0	0	0	0
	M	/	/	/	/	/	/
	A	-					
41a	E	0	0	0	0	0	0
	M	/	/	/	/	/	/
	A	-					
42a	E	0	0	0	0	0	0
	M	/	/	/	/	/	/
	A	-					
43a	E	0	0	0	0	0	0
	M	/	/	/	/	/	/
	A	-					
44a	E	0	0	0	0	0	0
	M	/	/	/	/	/	/
	A	-					
45a	E	0	0	0	0	0	0
	M	/	/	/	/	/	/
	A	-					

Appendix 4-2/4

CONTROL PRODUCT: DISTILLED WATER
SKIN EXAMINATION AND QUESTIONING DURING THE CHALLENGE PHASE

Test subjects reference	Type of reaction	Experimental times					
		Induction site			Virgin site		
		D36	D38	D40	D36	D38	D40
46a	E	0	0	0	0	0	0
	M	/	/	/	/	/	/
	A	-					
47a	E	0	0	0	0	0	0
	M	/	/	/	/	/	/
	A	-					
48a	E	0	0	0	0	0	0
	M	/	/	/	/	/	/
	A	-					
49a	E	0	0	0	0	0	0
	M	/	/	/	/	/	/
	A	-					
50a	E	0	0	0	0	0	0
	M	/	/	/	/	/	/
	A	-					
51a	E	0	0	0	0	0	0
	M	/	/	/	/	/	/
	A	-					
52a	E						
	M						
	A						
53a	E	0	0	0	0	0	0
	M	/	/	/	/	/	/
	A	-					
54a	E	0	0	0	0	0	0
	M	/	/	/	/	/	/
	A	-					
55a	E	0	0	0	0	0	0
	M	/	/	/	/	/	/
	A	-					
1b	E						
	M						
	A						
2b	E	0	0	0	0	0	0
	M	/	/	/	/	/	/
	A	-					
3b	E	0	0	0	0	0	0
	M	/	/	/	/	/	/
	A	-					
4b	E						
	M						
	A						
5b	E	0	0	0	0	0	0
	M	/	/	/	/	/	/
	A	-					
6b	E	0	0	0	0	0	0
	M	/	/	/	/	/	/
	A	-					

Legends: *Withdrawal*

Appendix 4-2/4

CONTROL PRODUCT: DISTILLED WATER
SKIN EXAMINATION AND QUESTIONING DURING THE CHALLENGE PHASE

Test subjects reference	Type of reaction	Experimental times					
		Induction site			Virgin site		
		D36	D38	D40	D36	D38	D40
7b	E	0	0	0	0	0	0
	M	/	/	/	/	/	/
	A	-					
8b	E	0	0	0	0	0	0
	M	/	/	/	/	/	/
	A	-					
9b	E	0	0	0	0	0	0
	M	/	/	/	/	/	/
	A	-					
10b	E	0	0	0	0	0	0
	M	/	/	/	/	/	/
	A	-					
11b	E	0	0	0	0	0	0
	M	/	/	/	/	/	/
	A	-					
12b	E	0	0	0	0	0	0
	M	/	/	/	/	/	/
	A	-					
13b	E	0	0	0	0	0	0
	M	/	/	/	/	/	/
	A	-					
14b	E	0	0	0	0	0	0
	M	/	/	/	/	/	/
	A	-					
15b	E	0	0	0	0	0	0
	M	/	/	/	/	/	/
	A	-					
16b	E	0	0	0	0	0	0
	M	/	/	/	/	/	/
	A	-					
17b	E	0	0	0	0	0	0
	M	/	/	/	/	/	/
	A	-					
18b	E	0	0	0	0	0	0
	M	/	/	/	/	/	/
	A	-					
19b	E	0	0	0	0	0	0
	M	/	/	/	/	/	/
	A	-					
20b	E	0	0	0	0	0	0
	M	/	/	/	/	/	/
	A	-					
21b	E	0	0	0	0	0	0
	M	/	/	/	/	/	/
	A	-					
22b	E	0	0	0	0	0	0
	M	/	/	/	/	/	/
	A	-					

Appendix 4-2/5

CONTROL PRODUCT: DISTILLED WATER
SKIN EXAMINATION AND QUESTIONING DURING THE CHALLENGE PHASE

Test subjects reference	Type of reaction	Experimental times					
		Induction site			Virgin site		
		D36	D38	D40	D36	D38	D40
23b	E	0	0	0	0	0	0
	M	/	/	/	/	/	/
	A	-					
24b	E	0	0	0	0	0	0
	M	/	/	/	/	/	/
	A	-					
25b	E	0	0	0	0	0	0
	M	/	/	/	/	/	/
	A	-					
26b	E	0	0	0	0	0	0
	M	/	/	/	/	/	/
	A	-					
27b	E	0	0	0	0	0	0
	M	/	/	/	/	/	/
	A	-					
28b	E	0	0	0	0	0	0
	M	/	/	/	/	/	/
	A	-					
29b	E	Direct contact of a person infected with SARS-CoV 2					
	M						
	A						
30b	E	0	0	0	0	0	0
	M	/	/	/	/	/	/
	A	-					
31b	E	0	0	0	0	0	0
	M	/	/	/	/	/	/
	A	-					
32b	E	0	0	0	0	0	0
	M	/	/	/	/	/	/
	A	-					
33b	E	0	0	0	0	0	0
	M	/	/	/	/	/	/
	A	-					
34b	E	0	0	0	0	0	0
	M	/	/	/	/	/	/
	A	-					
35b	E	0	0	0	0	0	0
	M	/	/	/	/	/	/
	A	-					
36b	E	0	0	0	0	0	0
	M	/	/	/	/	/	/
	A	-					
37b	E	0	0	0	0	0	0
	M	/	/	/	/	/	/
	A	-					
38b	E	0	0	0	0	0	0
	M	/	/	/	/	/	/
	A	-					

Appendix 4-2/6

CONTROL PRODUCT: DISTILLED WATER
SKIN EXAMINATION AND QUESTIONING DURING THE CHALLENGE PHASE

Test subjects reference	Type of reaction	Experimental times					
		Induction site			Virgin site		
		D36	D38	D40	D36	D38	D40
39b	E	0	0	0	0	0	0
	M	/	/	/	/	/	/
	A	-					
40b	E	0	0	0	0	0	0
	M	/	/	/	/	/	/
	A	-					
41b	E	0	0	0	0	0	0
	M	/	/	/	/	/	/
	A	-					
42b	E	0	0	0	0	0	0
	M	/	/	/	/	/	/
	A	-					
43b	E	0	0	0	0	0	0
	M	/	/	/	/	/	/
	A	-					
44b	E	0	0	0	0	0	0
	M	/	/	/	/	/	/
	A	-					
45b	E	0	0	0	0	0	0
	M	/	/	/	/	/	/
	A	-					
46b	E	0	0	0	0	0	0
	M	/	/	/	/	/	/
	A	-					
47b	E	0	0	0	0	0	0
	M	/	/	/	/	/	/
	A	-					
48b	E	0	0	0	0	0	0
	M	/	/	/	/	/	/
	A	-					
49b	E	0	0	0	0	0	0
	M	/	/	/	/	/	/
	A	-					
50b	E	0	0	0	0	0	0
	M	/	/	/	/	/	/
	A	-					
51b	E	0	0	0	0	0	0
	M	/	/	/	/	/	/
	A	-					
52b	E	0	0	0	0	0	0
	M	/	/	/	/	/	/
	A	-					
53b	E	0	0	0	0	0	0
	M	/	/	/	/	/	/
	A	-					
54b	E	0	0	0	0	0	0
	M	/	/	/	/	/	/
	A	-					
55b	E	0	0	0	0	0	0
	M	/	/	/	/	/	/
	A	-					
56b	E	0	0	0	0	0	0
	M	/	/	/	/	/	/
	A	-					

TEST REPORT: UVA SPECTRUM

On TX 19006

Date: Jan 2021

1. INTRODUCTION

This is a report on the test: UVA Spectrum, performed by SEDERMA. The purpose of this test is to evaluate whether an ingredient, or a formula, absorbs into ultraviolet rays type A (UVA) or ultraviolet rays type B (UVB). This allows to evaluate the phototoxicity potential of this ingredient or this formula. This evaluation method complies with the OECD guideline n°101.

2. PRODUCT DEFINITION

- Product name: TX 19006 corresponding to the peptide Pal-KTSKS present in PENTA 18 479
- Tested concentration: 15 ppm
- Request date: 01/08/2019
- Report date: 01/08/2019. Update document at 20/01/2021

3. PRINCIPLE OF THE METHOD

Light is divided into 3 main areas: infrared (>750 nm), visible light (400-750 nm) and ultraviolet (200-400 nm). UV is divided into 3 groups: UVA (320-400 nm), UVB (290-320) and UVC (200-290 nm). For a phototoxic hazard analysis, we will focus on ultraviolet light and more particularly on UVB and UVA. UVC does not pass through our atmosphere and therefore we are not exposed to these rays.

The UV rays that come into contact with us, 95% are UVA and 5% are UVB. Shorter is the wavelengths, more the energy of the wave is powerful. UV rays have shorter wavelengths than infrared rays. This energy available thanks to the UV wave can be transmitted to molecules, which become "excited" molecules.

This absorbed energy will disturb the molecule which may release one or more electrons. This molecule which becomes unstable will be able to lead the production of RoS (Reactive oxygen Species) and thus cause oxidative stress. This is phototoxicity.

Phototoxicity is a general term that includes several toxicological phenomena: photo irritation (acute reaction like sunburn), photo allergy (activation of the molecule that will become allergenic, activation of the immune system), photo genotoxicity (activation of the molecule that will become genotoxic) and photo carcinogenicity (activation of the molecule that will become carcinogenic by a genotoxic or non-genotoxic mechanism).

In order to avoid this phenomenon, the first step is to check whether a molecule is capable of absorbing UV. To do this, a UV spectrum is made with the help of a spectrophotometer. Absorption is present if these conditions are present:

- There is an absorbance peak between 290 and 400 nm
- Peak absorbance is between 0.5 and 1.5 absorbance or more
- If the Molar Extinction Coefficient (MEC) is greater than 1000 $L/mol^{-1}.cm^{-1}$

To validate the last condition, a series of calculations is necessary. You will find the calculations below:

$$Number\ of\ mole\ (Mol) = \frac{\text{Sample Mass (g)}}{\text{Molar Mass (g/mol)}}$$

$$Concentration\ (Mol/L) = \frac{Number\ of\ mole\ (Mol)}{\text{Volume (L)}}$$

TEST REPORT: UVA SPECTRUM

On TX 19006

Date: Jan 2021

$$Absorbance = MEC\ (L/mol^{-1}.cm^{-1})\ \text{x}\ Concentration\ (Mol/L)\ \text{x}\ Spectrophotometer\ cuvette\ length\ (cm)$$

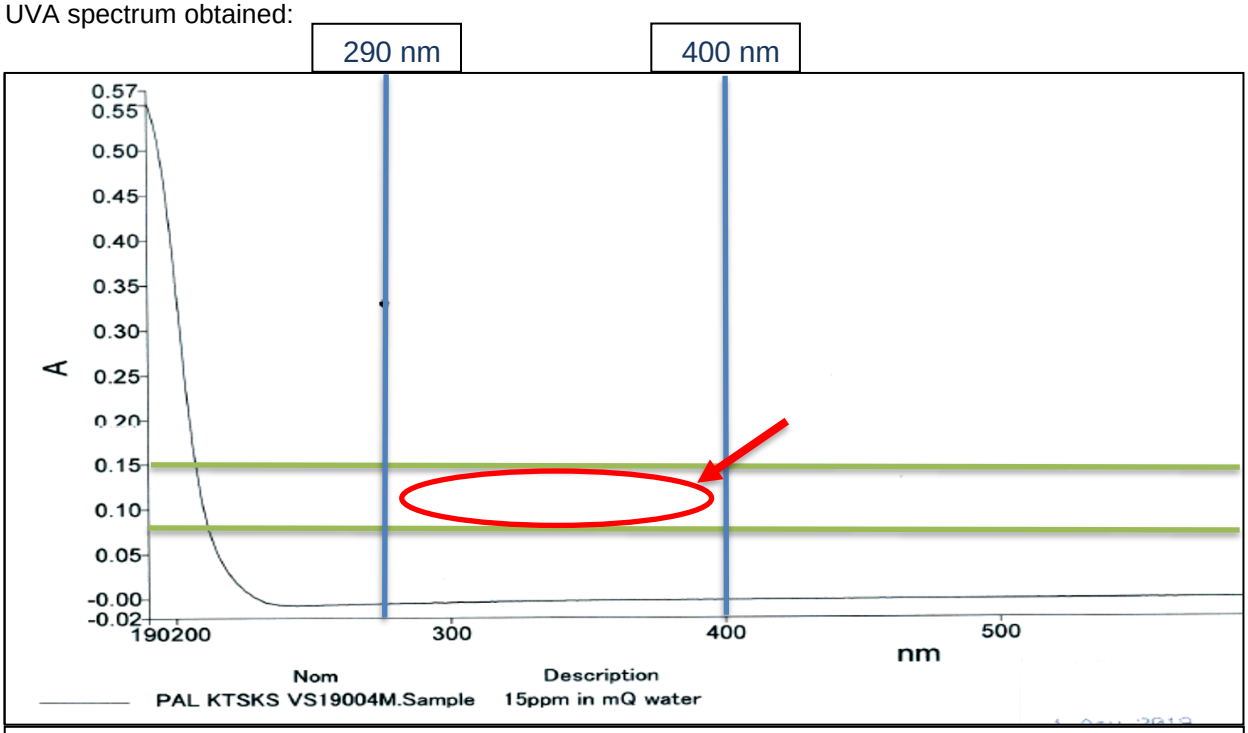
$$MEC\ (L/mol^{-1}.cm^{-1}) = \frac{Absorbance}{Concentration\ (Mol/L)\ x\ Spectrophotometer\ cuvette\ length\ (cm)}$$

To obtain the UV spectrum, the molecule is first diluted in a suitable solvent to one or more of the following concentrations. Then, an amount of ≈1 mL is placed in a spectrophotometer cuvette (length of 1 cm). The spectrophotometer is calibrated with a reference solution (solvent without molecule)

Once the spectrophotometer is calibrated, it will expose the peptide in solution at different wavelengths between 200/500 nm or 200/800 nm. The results will be retrieved in the form of graphic.

4. RESULT AND INTERPRETATION

UVA spectrum obtained:



UVA absorption spectrum is evaluated between 290 and 400 nm (see blue lines, and between absorbance values from 0.5 to 1.5 (see green lines). The peak of absorption must be located in the red circle.

"**The peptide TX 19006 (Pal KTSKS)**" is present at the final concentration of 0.0012% (12 ppm) in the PENTA 18 479 formula (present at 0.12% in PENTA 18 479 and the product is recommended at 1%). In the UVA spectrum (290-400 nm), **there is no** maximum absorbance peak in the red circle between 0.5/1.5 absorbance at the concentration of 0.1% (1000 ppm).

The absence of a maximum absorbance peak between 290-400 nm does not allow the MEC calculation. In this case, it is considered that the molecule MEC is less than 1000 L/mol-1.cm-1.

TEST REPORT: UVA SPECTRUM

On TX 19006

Date: Jan 2021

5. CONCLUSION

The results showed that there is no UVA absorption. So, the peptide has a negative prediction to be phototoxic in the PENTA 18 479 formula. Then, a 3T3 NRU test is not required.

6. REFERENCE

Brenner *et al,* (2008) The protective Role of Melanin against UV Damage in Human Skin. *Photochem. Photobiol.,* 84, 539-549.

Henry *et al,* (2009) Can light absorption and photostability data be used to assess the photosafety risks in patients for a new drug molecule. *Journal of Photochemistry and Photobiology B: Biologie,* 96, 57-62.

Kim et al, (2015) Phototoxicity: its Mechanism and Animal Alternative Test Methods. *Official Journal of Korean Society of Toxicology,* 31, 97-104.

Lee *et al,* (2017) Phototoxicity evaluation of Pharmaceutical substances with a Readctive Oxygen Species Assays Using Ultraviolet A. *Official Journal of Korean Society of Toxicology,* 33, 43-48.

OECD guideline N°101, UV-VIS absorption spectra (spectrophotometric method). *OECD guideline for testing of chemicals*, 12 May 1981.

Santé Canada (2016) LIGNE DIRECTRICE, évaluation du potentiel phototoxique des produits pharmaceutiques, ICH thème S10. *Direction générale des produits de santé et des aliments*, 1-20.

Written by:

Vicedo Vincent
Toxicologist/Risk assessor

Validated by:

Dr. Philippe MONDON, *PhD*
Scientific Director

IDEA TESTS GROUP

IDEA Lab

Test item
PENTA 18 479 - REF : TX 19011

Evaluation of a test item ocular irritant potential by application onto the hen egg chorio-allantoic membrane (HET-CAM)

FINAL REPORT

❖ Study # : 6.02-54075-ID-20/00404

Sponsor

SEDERMA
29, rue du Chemin-Vert
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FRANCE

Test Facility

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CONTENTS

Abbreviations / SOP

SOP	Standard Operating Procedures
GLP	Good Laboratory Practices
OJRF	Official Journal of the Republic of France
CAM	Chorio-Allantoic Membrane
FL REAC 01	Record of purchased reagents and consumables
FL REAC 05	Expiration of products
FL REAC 06	Reagents and consumables tracking book
FL REAC 07	Reference items tracking book
PL 04	Hygiene and security
IL 01	Management of test systems
IL 04	Determination of studies validity criteron

Note: Internal standard operating procedures are audited by ANSM according to the Good Laboratory Practice (GLP) principles described in the decree dated August 10th, 2004 from the JOFR and inspected by GIPC according to the GLP principles described in the article annex II to article D523-8 of the French Environment Code.

GLP conformity statement

The study 6.02-54075-ID-20/00404 was performed in the IDEA Lab company laboratory, in agreement with the French Good Laboratory Practice (GLP) principles, the European Directive 2004/10/CE and the decree dated August 10th, 2004 from the JOFR.

All relevant Standard Operating Procedures have been followed and raw data have been registered accurately.

The study 6.02-54075-ID-20/00404 is in conformity with the GLP principles despite of the following points which do not affect the reliability of results generated:

- the test item concentrations control in the dilution was not performed for the following reasons:
  - control of the test item preparation in its vehicle, particularly with micropipette and precision scales regularly controlled, calibrated and traceable with national or international standards of measurement
  - the control of the homogeneity of the test item dilution in the vehicle is performed using organoleptic criteria and is documented in the study log book
  - test item dilution is prepared extemporaneously

For confidentiality concerns, some characterization data related to the test item composition are not shown in this report. This is a deviation to GLP. However, this characterization had been provided by the Sponsor, brought to my attention, then stored in a secure environment in accordance with the company procedures.

This report accurately reflects the study carried out and the results obtained.

I declare this study compliant with the Good Laboratory Practice and assume responsibility for the data validity of the study.

Date: 14 FEV. 2020

Study Director

Coralie DEVIGNE

Quality Assurance statement

According to the Good Laboratory Practices, I state that:

- The General Study Plan was audited by the Quality Assurance and that the Specific Study Plan was verified before the beginning of the study,

- The different technical phases of the study 6.02 are regularly audited by the Quality Assurance. Facility audits are also carried out. The audit frequency is defined in the corresponding procedure,

  At the last technical audit (A-19/02), the following activities have been inspected:
  - Egg preparation
  - Contact with chorioallantoic membrane
  - Reading procedure

- The final report was audited by the Quality Assurance of IDEA Lab. It accurately reflects the raw data from the study and the application of the Standard Operating Procedures and the Study Plan.

Audit nature	Audits dates	Transmission dates of the audit report to the Study Director and the General Management
Technical phases of the study	From 18/03/2019 to 18/03/2019	29/03/2019
General Study Plan	07/01/2020	07/01/2020
Draft Report	13/02/2020	13/02/2020
Final Report	14 FEV. 2020	14 FEV. 2020

Date: 14 FEV. 2020

Quality Assurance

Nelly TEYSSANDIER

Study presentation

1. Study objective

At the Sponsor's request, we have evaluated the eye tolerance of a test item with the HET-CAM method according to the current General Study Plan 6.02.

2. Test item

PENTA 18 479 - REF : TX 19011

Internal code	: ID-20/00404
Batch number	: B1
Aspect	: Liquid
Color	: Colourless
Storage conditions	: Room temperature (20°C ± 5°C)
Test item nature	: Cosmetic ingredients mixture
Retest date	: 06/01/2022
CAS number	: 58-81-5 / 7731-18-5 / 521091-64-5

Physico-chemical properties

Purity	: NA (Mixture)
Physical state at 20°C	: Liquid
Homogeneity	: Yes

Solubility and stability
Recommended concentration in solvent: 10%

The technical data sheet provided by the Sponsor did not indicate information about the solubility of the test item. The point was assessed in a preliminary test and kept in the study file. The chosen diluent, water, has been shown in the Specific Study Plan.

Information linked to the identification, purity and stability of the test item are under the responsibility of the Sponsor of the study. The characterisation of the test item was provided by the Sponsor of the study.

The test item will be stored at least 2 months in the product room of the Martillac location before to be destroyed or sent back to the Sponsor according to his choice.

The analysis certificate is shown at the end of the report.

3. Study principle

The test is an *in vitro* method which evaluates the ocular irritant potential of a test item.

The principle is based on the observation of the irritant effects (hyperemia, hemorrhage, coagulation) which may occur within five minutes after placing a test item onto the chorio-allantoic membrane (CAM) of an embryonated hen egg, on the tenth day of incubation. Depending on the presence of these effects and their appearance time, a score is established. The mean scores obtained from four eggs allows to note the test item and to classify according to its eye irritant potential.

This study is carried out according to the Official Journal of the Republic of France (N° 300), December 26th, 1996.

4. Study course

Test system:

Egg hen embryonated (White Leghorn strain) with a weight of 50 to 65 g the day of reception.

At receipt, the eggs cracked or broken were removed, the other eggs were weighed. The weight of each egg was recorded on the shell and was retranscribed on the scores sheet at the time of the test. The eggs stored protected from light and at 20°C ± 5°C until placed in the incubator.

The eggs weighed were incubated with the air pocket upwards (pointed end of egg downwards).
The incubation was performed at 37.5°C ± 0.5°C with a relative humidity of 40% to 60% during 10 days in an egg incubator with automatic oscillating plates. The study started on the tenth day of incubation.

Experimentation dates:

The experimentation was carried out on 23/01/2020.

Media and reagents:

- Saline solution (NaCl 9 g/l) - stored at room temperature (20°C ± 5°C)
- Water - stored at room temperature (20°C ± 5°C)

FL REAC 01, FL REAC 06 forms and study laboratory notebook ensure the traceability of media and reagents used in the study.
The expiration after opening of media and reagents used in the study is defined in the FL REAC 05 form.

Material and consumables:

- Egg incubator
- Timer
- Forceps, scissors
- Syringes of 10 ml
- Water bath
- Precision weight
- Precision pipette
- Lamp
- Water atomizer
- Freezer -80°C
- Conventional laboratory cell culture equipment

FL REAC 01 and FL REAC 06 forms ensure the consumables traceability used in the study.

The equipment used is recorded in the notebook.

Reference items:

- **Positive control**: 3.2 and 0.4% Lauryl sulfobetaine (CAS number: 14933-08-5) in saline solution

- **Negative control**: 0.05% Lauryl sulfobetaine in saline solution

Series definition:

The test item 10% diluted in water was tested on four eggs.

The reference items were tested on two eggs.

Test protocol:

The different steps were performed rapidly under sufficient intensity lighting non heat releasing to avoid drying of the CAM (otherwise the humidity level is maintained by misting).

The egg was placed vertically on a support (air pocket upwards).
The shell was cracked at the level of the air pocket taking care not to injure the CAM. Using forceps or a pair of scissors, the shell was removed to the level of the shell membrane. The released surface was moistened with saline solution warmed to around 37°C. The saline solution was removed by tilting the egg. The shell membrane was delicately detached with forceps and then removed in order to uncover the underlying CAM. The CAM integrity was recorded for every egg on scores sheet at the time of the test. Eggs which do not have a living hen embryo were removed as well as eggs which the MCA was defective or present signs of haemorrhage.

300 µl of the warmed test item (diluted) or reference item were deposited gently on the CAM and the timer was immediately started.

After 20 seconds of contact, the membrane was rinsed with 5 ml of saline solution at around 37°C, avoiding any violent splashing. The rinse liquid was removed by tilting the egg. Any irritant effects were observed for 5 minutes (cf. § Reading procedure). The time of occurrence of each effect was recorded.

At the end of testing, the eggs are frozen at -80°C until the day of waste disposal according to the procedure PL 04.

Reading procedure:

The observations taken into account in the scoring of the test or reference items were made with naked eye. The observed effects were identified according to the law of all or nothing (the presence and not the severity of the effect is recorded). The time interval was noted for the appearance of each of the phenomena.

Hyperemia: capillaries which were invisible before adding the test or references items become visible, whereas capillaries which were already visible dilate and become redder. This phenomenon may also affect large diameter vessels.

Hemorrhage: release of blood escaping from the vessels and or capillaries, may take on different appearances, and particularly « cauliflower », patches, diffuse sheet, or punctate (the blood escapes intermittently at different places in the membrane).

It must be noted that:
- Hemorrhage may take on a transient appearance: this must nevertheless by counted,
- Masked hyperemia must be counted if massive haemorrhage occurs during the first 30 seconds.

Coagulation (Opacity and/or Thrombosis):

Opacity: apparition on a part or all of the membrane, of an opalescent sheet or direct opacification (take care that this effect is not due to the physico-chemical properties of the test item in aqueous medium, formation of a colloid, precipitate,...).

Thrombosis: discontinuation of blood flow producing a segmented appearance of the vessels (alternating areas of strangulation more or less dark turgescent areas). Changes occurring in the capillaries were not counted.

5. Results calculation and interpretation

An evaluation note was assigned to each phenomenon according to its occurrence time:

Note according to the time:

Phenomenon	Time		
	T ≤ 30 s	30 s < T ≤ 2 min	2 min < T ≤ 5 min
Hyperemia	5	3	1
Hemorrhage	7	5	3
Coagulation, opacity and/or thrombosis	9	7	5

The score for each egg is the sum of each phenomenon notes.
The test item or references items notation is the arithmetic mean, rounded to two decimal points, of the scores obtained for 4 eggs (maximum rating = 21).

The irritation potential is given by the following scale:

Note (N)	**Classification**
N < 1	Practically non irritant
1 ≤ N < 5	Slightly irritant
5 ≤ N < 9	Moderately irritant
N ≥ 9	Irritant

The result validation was performed by the Study Director in agreement with the working instruction IL 04.

In order to validate the test, it is essential that the test validity criteria are confirmed:

0.05% Lauryl Sulfobetaine: Practically non-irritant or Slightly irritant
0.4% Lauryl Sulfobetaine: Moderately irritant or Irritant
3.2% Lauryl Sulfobetaine: Irritant

6. Observations, deviations and Study Plan amendments

No observation, deviation, or amendment to the Study Plan has been observed during this study.

7. Results

The table of results is shown at the end of the report.

Test validation and results:

Results found for the reference items allow to validate the test.

The **mean score** calculated for the test item is **4.25**.

8. Conclusion

Under the retained experimental conditions, the irritant potential of the test item **PENTA 18 479 - REF : TX 19011** code **ID-20/00404** tested **10% diluted in water**, may be classified as **slightly irritant** according to the adopted scale.

9. Archive

The total storage of the study folder is 10 years (Study Plan and amendment, report, raw data). The folder will be stored at least 6 months in the IDEA Lab archive room, on the Martillac location, and would be susceptible to be transferred to the non GLP premises of EVERIAL CHARTRES MEGASTORE Avenue Gustave EIFFEL 28000 CHARTRES, archiving specialist.

The reference item samples will be stored 10 years, or until their expiry date on the Martillac location, in the storage condition described in the quality form FL REAC 07.

Results table

Experimentation from 23/01/2020

ID-20/00404

Control

Lauryl Sulfobetaine	3.20 %	score on 2 eggs	17
	0.40 %		8
	0.05 %		0

Dilution	Eggs	Observed phenomenon	Notation	Score	Score moyen Mean score	Classification
10%	1	Hyperemia	3	3	4.25	Slightly irritant
		Hemorrhage	0			
		Coagulation	0			
	2	Hyperemia	3	8		
		Hemorrhage	5			
		Coagulation	0			
	3	Hyperemia	3	3		
		Hemorrhage	0			
		Coagulation	0			
	4	Hyperemia	3	3		
		Hemorrhage	0			
		Coagulation	0			

Test item diluent: Water

Analysis certificate

ID-20/00404

sederma	**ENREGISTREMENT QSHE**	Codification **EE1/33** Date : 02/04/15 Révision : c
	BULLETIN D'ANALYSES *CERTIFICATE OF ANALYSIS*	Page 1 sur 1

PRODUIT / *PRODUCT* : TX 19011

N° de LOT / *BATCH Nr* : B1

Stockage / *Storage* : **Stockage recommandé longue durée entre +15°C et +25°C,** *Long storage recommended between +15°C and +25°C*

Date de fabrication *Date of manufacturing* : 06 janvier 2020 **Date de retest** : 06 janvier 2022 *Retest date*

Les analyses effectuées sont garanties quand le produit est stocké dans son emballage d'origine et à la température recommandée
The performed analysis are guaranteed when product is stored in original packaging and at recommended temperature

	Résultats *Results*
Aspect *Appearance*	Liquide limpide incolore *Clear liquid colourless*
Teneur en Pal KTSKS-OH (HPLC) *Pal KTSKS-OH content (HPLC)*	1040ppm

Liliane IACUZZI
Responsable Assurance Qualité Produit / Product Quality Assurance Manager

Ce document est une copie informatique et de ce fait ne porte pas de signature / This certificate is a computer printout and therefore has no signature.

SEDERMA 29 rue du Chemin Vert – 78610 LE PERRAY EN YVELINES – France
Tél. : 01.34.84.10.10 – Fax : 01.34.84.11.30

IDEA TESTS GROUP

IDEA Lab

Test item
PENTA 18 479 - REF : TX 19011

Ocular irritation evaluation on human corneal epithelial model according to the OECD guideline n°492 - SkinEthic™ model

FINAL REPORT

❖ Study # : 6.49_S-54682-ID-20/00404

Sponsor

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CONTENTS

Abbreviations and SOP

UN GHS	United Nation Globally Harmonized System of Classification and Labeling of Chemicals
GLP	Good Laboratory Practice
DPBS	Dulbecco's Phosphate Buffered Saline
MTT	3-(4.5-dimethylthiazole-2-yl)-2.5-diphenyltetrazolium bromide
Abs	Absorbance
HCE	Human corneal epithelial
IC50	Concentration giving 50% cell viability
MTT NS	Non specific reduction control on dead epithelium
KU	Negative control killed tissue
TlivingNS	Non specific coloration control on living epithelium
Tdead NS	Non specific coloration control on dead epithelium
FL REAC 01	Record of purchased reagents and consumables
FL REAC 05	Expiration of products.
FL REAC 06	Reagents and consumables tracking book
FL REAC 07	Reference elements tracking book

GLP conformity statement

The study 6.49_S-54682-ID-20/00404 was performed in the IDEA Lab company laboratory, in agreement with the French Good Laboratory Practice (GLP) principles, the European Directive 2004/10/CE and the decree dated August 10th, 2004 from the JOFR.

All relevant Standard Operating Procedures have been followed and raw data have been recorded accurately.

For confidentiality concerns, some characterisation data related to the test item composition are not shown in this report. This is a deviation from GLP. However, this characterisation had been provided by the Sponsor, brought to my attention, then stored in a secure environment in accordance with the company procedures.

The lack of verification of the concentration of the test item in various dilutions has no impact on the reliability of the results generated for the following reasons:

- the test item preparation in its vehicle is controlled, particularly with the use of micropipette and precision scales regularly controlled, calibrated and traceable with national or international standards of measurement,
- the control of the homogeneity of the test item dilution in the vehicle is performed using organoleptic criteria and is documented in the study log book,
- the test item dilution is prepared extemporaneously.

This report accurately reflects the study carried out and the results obtained.

I declare this study compliant with the Good Laboratory Practice and assumes responsibility for the data validity of the study.

Date: 13 Nov 2020

Study Director
Doctor in Bioengineering
Sophie CATOIRE

Quality Assurance statement

According to the Good Laboratory Practices, I state that:

- The General Study Plan was inspected by the Quality Assurance and that the Specific Study Plan was verified before the beginning of the study,

- The different technical phases of the study 6.49_S are regularly inspected by the Quality Assurance. Facility inspections are also carried out. The inspection frequency is defined in the corresponding procedure.

  At the last technical inspection (A-18/08), the following activities have been inspected:
  - Assessment of the potential reduction of the MTT by the test item
  - Assessment of the test item colouring potential
  - Contact between the epithelia and the test item
  - Assessment of the cell viability by the MTT method

- The final report was inspected by the Quality Assurance of IDEA Lab. It accurately reflects the raw data from the study and the application of the Standard Operating Procedures and the Study Plan.

Audit nature	Audits dates	Transmission dates of the audit report to the Study Director and the General Management
Technical phases of the study	from 03/07/2018 to 09/07/2018	27/07/2018
General Study Plan	21/07/2020	21/07/2020
Draft Report	16/10/2020	16/10/2020
Final Report	0 2 NOV. 2020	0 2 NOV. 2020

Date: 0 2 NOV. 2020

Quality Assurance

Camille CAPPE

Study presentation

1. Study objective

At the Sponsor's request, we have evaluated, by an *in vitro* test, the ocular irritation on human corneal epithelial model of a test item according to the current General Study Plan 6.49_S.

2. Test item

PENTA 18 479 - REF : TX 19011

Internal code	: ID-20/00404
Batch number	: B1
Aspect	: Liquid
Color	: Colourless
Storage conditions	: Room temperature (20°C ± 5°C)
Test item nature	: Cosmetic ingredients mixture
Retest date	: 06/01/2022
CAS number	: Glycerine (Vegetale): 58-81-5 Water: 7731-18-5 Pal-KTSKS: 521091-64-5

Physico-chemical properties

Purity	: NA (Mixture)
Physical state at 20°C	: Liquid
Homogeneity	: Yes

Solubility and stability

Recommended concentration in solvent: 30%

The technical data sheet provided by the Sponsor did not indicate information about the solubility of the test item. The point was assessed in a preliminary test and kept in the study file. The chosen diluent, water, has been shown in the Specific Study Plan.

Information linked to the identification, purity and stability of the test item is under the responsibility of the Sponsor of the study. The characterisation of the test item was provided by the Sponsor of the study.

The test item will be stored at least 2 months in the product room of the Martillac location before to be destroyed or sent back to the Sponsor according to his choice.

The analysis certificate is shown at the end of this report.

3. Study principle

The aim of the study is the evaluation of a test item capability to induce neither ocular irritation nor damage effects on in vitro reconstructed cornea epithelial model.

The SkinEthic™ HCE model is composed of human immortalized corneal epithelial cells cultivated on an inert polycarbonate filter at the air liquid interface in a chemically defined medium. The reconstructed tissue forms a stratified and well organized epithelium which is structurally, morphologically and functionally similar to the human cornea.

After the test item application, pure or in the conditions defined by the Sponsor, on epithelia for 30 minutes (liquid test item) or 4 hours followed by a post-treatment incubation (solid test item), cellular viability is evaluated by the measurement of the succinate dehydrogenase mitochondrial activity of the living cells. This enzyme is involved in the transformation of MTT (3(4.5-dimethylthiazol-2-yl)-2.5-diphenyltetrazolium bromide) into blue formazan crystal. A spectrophotometric measurement is performed after the crystal dissolution. The measured absorbances are proportional to the number of living cells (Mosmann, J. Immunol. Meth., 1983, 55-63).

This study is based on the OECD guideline N°492 dated June 25th 2018 and protocol DB ALM N°190 (liquid test item).

4. Study course

Experimentation dates:

The experimentation was carried out from 21/07/2020 to 03/09/2020.

Test system:

Human corneal epithelial model SkinEthic™ HCE 0.5 cm² provided by Episkin company.
The validity criteria of the certificate of analysis from the batch of received epithelia, with viability, barrier function, histological analysis and the determination of the absence of biological contamination are verified and a copy of the document is shown at the end of this report.
The test starts at the epithelia receipt.

Media and reagents:

Stored at 5°C ± 3°C
Culture medium: SkinEthic maintenance medium
MTT powder (CAS number: 298-93-1)

Stored at room temperature 20°C ± 5°C
Dulbecco's PBS Ca^{2+} and Mg^{2+} free (DPBS) *
Isopropanol (CAS number: 67-63-0)

Others storage conditions
MTT solution: 1 mg/ml MTT solution in culture medium - Prepared extemporaneously, protected from light and used within 3 hours

* Stored at 5°C ± 3°C after opening.

FL REAC 01 and FL REAC 06 forms ensure the traceability of media and reagents used in the study.
The expiry after opening of the media and reagents used in the study is defined in the form FL REAC 05.

Equipments and consumables:

- 6, 12 and 24 wells plate for cell culture
- 96 wells plate for absorbances reading
- Plate shaker
- Absorbent paper
- 0.5 cm^2 nylon meshes
- MULTISKAN EX plate reader (Thermo life sciences) – reading range 0 - 3.5 units of Absorbance – linearity range 0 - 2.200 units of Absorbance at 540 nm
- CO_2 incubator*
- Classical material used in cell culture laboratory

*Note: As indicated in the technical manual provided by the manufacturer, if the CO_2 incubator water pan is filled, and for a constant temperature, the relative humidity in the CO_2 incubator is 95%. The temperature and presence of water in the pan are checked daily and recorded in the environmental conditions form.

FL REAC 01 and FL REAC 06 forms ensure the consumables traceability used in the study.

The equipment used is recorded in the study notebook.

Reference items:

Positive control: Methyl acetate (CAS number: 79-20-9) pure.

Negative control: Dulbecco's PBS Ca^{2+} and Mg^{2+} free.

Series definition:

30 µl ± 2 µl of the test item diluted at 30% in water, as well as the reference items, were tested on two epithelia.

Preliminary tests to the study were conducted in order to include or not additional controls:

- Assessment of the MTT reduction potential by the test item:

30 µl ± 2 µl of the test item were added to 300 µl of MTT solution, mixed and incubated 3 hours ± 15 minutes at 37°C 5% CO_2, protected from the light. At the same time, 30 µl ± 2 µl of water are added to 300 µl of MTT solution and incubated under the same conditions.
A blue/purple coloration indicates a non-specific reduction induced by the test item. The colour was assessed visually at the end of the exposure time.

No colour was observed. No additional control for the direct MTT reduction by the test item is required.

- Assessment of the test item colouring potential:

10 µl ± 1 µl of the test item were added to 90 µl ± 2 µl of water, mixed and incubated 30 minutes ± 2 minutes at room temperature. The reading was performed visually at the end of the exposure time.

No colour was observed. No additional control for coloured and/or colouring test item is required.

Test protocol:

Inserts (filter + epithelia) were gently detached from the agar and if necessary the bottom of the insert was wiped on an absorbent paper in order to avoid leaving agar pieces onto the polycarbonate membrane.
Inserts were then placed into wells (6 wells culture plate) previously filled with 1 ml of culture medium pre-warmed at room temperature.
The absence of bubbles was verified then cultures were incubated overnight at 37°C, 5% CO_2.

The epithelia were transferred in 24 wells plate in 300 µl per well of culture medium at room temperature. The absence of bubbles was checked then the cultures were incubated at least 30 minutes at 37°C, 5% CO_2.

30 µl ± 2 µl of the negative control were deposited using a positive displacement micropipette.

Before being exposed to the test item or the positive control, the surface of the tissue was pre-treated with 10 µl ± 1 µl of DPBS then 30 µl ± 2 µl of the test item or the positive control were laid on with a positive displacement micropipette on the surface of the epithelia, ensuring that the entire surface of the epithelium is covered.

In order to overcome the problem of surface tension or to enhance the spreading of the test or references items, a nylon mesh was gently applied on the surface of the epithelia with tweezers.

The absence of bubbles was checked then the cultures were incubated for 30 minutes at 37°C, 5% CO_2.
The contact time was measured with a timer, regularly shifting the series.

The nylon meshes were removed and the epithelia were rinsed with 2 times 10 ml of PBS by epithelia using a dispenser (50 ml tip, 10 ml by push). The residual PBS was eliminated by energised reversals.

The epithelia were immediately transferred in 24 wells plate containing 750 µl per well of culture medium and then covered with 750 µl of culture medium at room temperature.
The absence of bubbles was checked then the cultures were incubated for 30 minutes ± 2 minutes at 37°C, 5% CO_2.
At the end of the incubation, the culture medium inside the insert was discarded by returning the insert. The bottom of the insert is carefully dried on absorbent paper and the surface with a coton swab.

The epithelia were transferred in 24 wells plate containing 300 µl per well of MTT solution. The absence of bubbles was checked and the epithelia are incubated 3 hours ± 15 minutes at 37°C, 5% CO_2.

At the end of incubation period, the inserts were rinsed with 300 µl of PBS and dried by a quick contact with an absorbent paper. The epithelia were transferred in a 24 wells plate containing 750 µl of isopropanol and then covered with 750 µl of isopropanol.

The plate was covered with parafilm to prevent evaporation then, incubated overnight at 5°C ± 3°C protected from light without agitation.
After a night of extraction, the inserts were homogenized at least 30 minutes (approximately 120 rpm) at room temperature before use.

The membrane of inserts was perforated with the tip of a micropipette, inserts were removed then the solution is homogenized.

The absorbances were measured at 540 nm within maximum 20 minutes in duplicate on 200 µl extract in 96 wells plates against isopropanol as blank.

5. Results calculation and interpretation

Results are expressed in viability percentage (V%) compared with the negative control:

$$V\%_{\text{Test item}} = \frac{\text{Mean Abs}_{\text{Test item}}}{\text{Mean Abs}_{\text{Negative control}}} \times 100$$

Results interpretation:

This method is recommended to identify a test item that requires no classification for eyes irritation or serious damage according to the UN GHS. However, the SkinEthic™ HCE model is not intended to differentiate between categories 1 and 2 of the UN GHS.
The prediction model classifies test item into 2 groups:
- No category
- Category 1 / category 2 without distinction

Due to the high over-prediction rate during the validation of the model, OECD 492 interprets the positive results as " No prediction can be made ".
For a full assessment of severe eye damage or irritation eyes, the test item should be submitted to further tests.

Mean viability of epithelia	**Classification**
> 60%	Non irritant / « No category »
≤ 60%	No prediction can be made

A single test is sufficient when the classification is unequivocal. However, in case of border line results such as non-concordent replicates or viability equal to 60% ± 5%, a second test must be considered, as well as a third in the case of discordance between the first two tests.
Additional tests will be proposed to the Sponsor. In the absence of confirmation the worst result will be retained.

Validity criteria:

The difference of viability between the two epithelia of the same test item or reference item must be lower than or equal to 20%.

The absorbance of the solvent for extraction (Isopropanol) must be lower than 0.100.

Negative control
The mean absorbance must be higher than 1.0 and lower than or equal to 2.5.

Positive control
The viability percentage for the positive control must be lower than or equal to 30% for a liquid test item.

The result validation is performed by the Study Director in agreement with the current working instruction IL 04.

6. Observations, deviations and Study Plan amendments

Study Plan amendment n°1
The positive control did not meet the quality criteria and the first trial was not validated.
A new trial has been planned.

MON deviation
The formalization of the overview of the General Study Plan was not carried out before the signing of the Specific Study Plan. However, the Study Director had read the old version of General Study Plan before signing the Specific Study Plan.
The new version of General Study Plan was only intended to add a Study Director.

7. Test item results

Test validation:

The viability percentage for the positive control is lower than or equal to 30% and all criteria are fulfilled, this validates the test.

Test item result:

The mean viability observed for test item is 104.3%.

8. Conclusion

Under the retained experimental conditions and according to the CLP regulation, the test item **PENTA 18 479 - REF : TX 19011** code **ID-20/00404** tested **diluted at 30% in water** must **not be classified.**
No symbol, risk phrase, signal word or hazard statement is required.

9. Archive

The total storage of the study folder is 10 years (Study Plan and amendment, report, raw data).
The folder will be stored at least 6 months in the IDEA Lab archive room, on the Martillac location, and would be susceptible to be transferred to the non GLP premises of EVERIAL CHARTRES MEGASTORE Avenue Gustave EIFFEL 28000 CHARTRES, archiving specialist.

The reference item samples will be stored 10 years, or until their expiry date on the Martillac location, in the storage condition described in the quality form FL REAC 07.

Results table

Experimentation from 21/07/2020 to 03/09/2020

ID-20/00404

		ASSESMENT OF VIABILITY						CONCLUSION
		(Abs. - blank)		Mean Abs. / Ep.	Viability / Ep. %	Difference of viability %	Viability means %	
Negative control DPBS	Ep. 1	1.351	1.430	1.390	107.4%	14.7%	**100.0%**	**Non irritant**
	Ep. 2	1.214	1.186	1.200	92.6%			
Positive control *Methyl acetate*	Ep. 1	0.387	0.391	0.389	30.0%	0.7%	**29.7%**	**Irritant**
	Ep. 2	0.377	0.383	0.380	29.3%			
ID-20/00404	Ep. 1	1.354	1.351	1.353	104.5%	0.3%	**104.3%**	**No category**
	Ep. 2	1.360	1.337	1.349	104.2%			

Mean blank : *0.035*
Mean KU : *0.001*

Certificate of analysis

Test item

sederma	ENREGISTREMENT QSHE	Codification EE1/33 Date : 02/04/15 Révision : c
	BULLETIN D'ANALYSES *CERTIFICATE OF ANALYSIS*	Page 1 sur 1

PRODUIT / *PRODUCT* : TX 19011

N° de LOT / *BATCH Nr* : B1

Stockage / *Storage* : **Stockage recommandé longue durée entre +15°C et +25°C,**
Long storage recommended between +15°C and +25°C

Date de fabrication / *Date of manufacturing* : 06 janvier 2020 **Date de retest** : 06 janvier 2022 / *Retest date*

Les analyses effectuées sont garanties quand le produit est stocké dans son emballage d'origine et à la température recommandée
The performed analysis are guaranteed when product is stored in original packaging and at recommended temperature

	Résultats *Results*
Aspect *Appearance*	Liquide limpide incolore *Clear liquid colourless*
Teneur en Pal KTSKS-OH (HPLC) *Pal KTSKS-OH content (HPLC)*	1040ppm

Liliane IACUZZI
Responsable Assurance Qualité Produit / Product Quality Assurance Manager

Ce document est une copie informatique et de ce fait ne porte pas de signature / This certificate is a computer printout and therefore has no signature.

SEDERMA 29 rue du Chemin Vert – 78610 LE PERRAY EN YVELINES – France
Tél. : 01.34.84.10.10 – Fax : 01.34.84.11.30

Reconstructed epithelia

EPISKIN

TECHNICAL DATA, SAFETY SHEET AND CERTIFICATE OF ANALYSIS

CCE-092-HCE DS-S/04

NAME

SkinEthic™ HCE / Corneal Epithelium (HCE/S/5)

DESCRIPTION

0.5 cm² reconstructed epithelium by airlifted culture of transformed human corneal keratinocytes for 5 days on inert polycarbonate filters in chemically defined medium.

BATCH : **20-HCE-037**
ORIGIN : Immortalized Human Corneal Epithelium cells (HCE).
Thickness : **55** µm (indicative value)
USAGE : FOR SCIENTIFIC USE ONLY - PRODUCT OF HUMAN ORIGIN
STORAGE : This product was prepared and packaged using aseptic techniques. Store in an incubator at 37°C, 5% CO2 with saturated humidity

QUALITY CONTROLS

Control # E200822

	Test	Specification	Result
HISTOLOGY	HES stained paraffin section	Epithelium consisting of viable layers of cells including columnar basal cells, transitional wing cells and superficial squamous cells	**Satisfactory**
		Number of cell layers ≥ 4	**5 cell layers**
			
CELL VIABILITY	570 nm optical density, MTT test.	1.0 ≤ O.D. ≤ 2.5	**O.D. = 1.8** (CV = **3.7** %)
IC 50 DETERMINATION	SDS concentration, MTT test	1.0 mg/mL ≤ IC50 ≤ 3.2 mg/mL	**2.0 mg/mL**

HANDLING:

Human Corneal Epithelium is reconstructed using transformed human cells of Class 2. Its handling requires the user to conform with local regulations applicable to transformed human cells – Class 2.

BIOLOGICAL SAFETY:

On this cell line, we have verified the absence of HIV integrated pro-viral DNA, hepatitis C viral RNA, hepatitis B viral DNA, viral contamination of the virus used for the cell line immortalization and the absence of bacteria, fungus and mycoplasma.

SUGGESTED EXPIRATION DATE:

September 7, 2020

Lyon, September 1, 2020
Certified and released by Michel BATAILLON, Quality Control Manager

Manufactured in accordance to the ISO9001 quality system of Episkin.

The use of this human tissue is strictly limited to *in vitro* testing. All other manipulations of this tissue such as: extraction and maintenance of single cells in culture, use of the tissue for diagnostic or therapeutic purposes and in human subjects, are strictly prohibited.

ISO 9001 Certified
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S.A. au capital de 13 608 807 € - 412 127 565 R.C.S. Lyon - NAF : 7211 Z - N° TVA Intracommunautaire FR 46 412 127 565
www.episkin.com

afaq ISO 9001

Personal Care Products Council
Committed to Safety, Quality & Innovation

Memorandum

TO: Bart Heldreth, Ph.D.
Executive Director - Cosmetic Ingredient Review

FROM: Carol Eisenmann, Ph.D.
Personal Care Products Council

DATE: April 28, 2023

SUBJECT: Palmitoyl Pentapeptide-4

The company completing the use study cited in the Scientific Literature Review as reference 36 has indicated that the concentration of Palmitoyl Pentapeptide-4 did not exceed the concentration of this ingredient in face and neck products reported to the PCPC concentration of use survey.