SUPPLEMENT

Brown Algae

Parabens

Polyol Phosphates

Titanium

Vinylpyrrolidone Polymers

Xanthine Alkaloids

CIR EXPERT PANEL MEETING
SEPTEMBER 24-25, 2018

Cosmetic Ingredient Review

Commitment & Credibility since 1976

Memorandum

To: CIR Expert Panel Members and Liaisons
From: Priya Cherian, Scientific Writer/Analyst
Date: September 14, 2018
Subject: Wave 2 – Brown Algae

Since the draft report was issued at mail date, the Council has provided an ample amount of additional information regarding ingredients in the Brown Algae report. The following table provides an overview of the data received. An updated data profile has also been included (*broalg092018prof_wave2*).

Data Point	Data	Data Source
Alaria Esculenta Extract		
Method of Manufacture	trade name mixture consisting of Alaria Esculenta Extract in butylene glycol and water: harvesting/identification → washing → grinding → extraction with the solvents and butylene glycol and water → filtration → quality control → packaging → quality control	*broalg092018data7_wave2*
	trade name mixture consisting of Alaria Esculenta Extract in butylene glycol and water – dried before extraction: harvesting/identification → washing → drying → grinding → extraction with the solvents butylene glycol and water → filtration → quality control → packaging → quality control	*broalg092018data7_wave2*
	trade name mixture containing Alaria Esculenta Extract in Caprylic/Capric Triglycerides: harvesting/identification → drying → grinding → extraction with solvent caprylic/capric triglyceride → filtration → quality control → packaging → quality control	*broalg092018data7_wave2*
Heavy Metal Impurities	trade name mixture consisting of Alaria Esculenta Extract in butylene glycol and water: < 5 ppm arsenic, < 3 ppm cadmium, < 5 ppm lead, < 2 ppm nickel, < 5 silver, < 10 ppm iodine	*broalg092018data7_wave2*
	trade name mixture consisting of Alaria Esculenta Extract in butylene glycol and water – dried before extraction: < 5 ppm arsenic, < 3 ppm cadmium, < 5 ppm lead, < 2 ppm nickel, < 5 silver, < 10 ppm iodine	*broalg092018data7_wave2*

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	trade name mixture containing Alaria Esculenta Extract in Caprylic/Capric Triglycerides: < 2 ppm arsenic, < 3 ppm cadmium, < 5 ppm lead, < 2 ppm nickel, < 5 ppm silver, < 1 ppm mercury, < 1 ppm iodine	*broalg092018data7_wave2*
Concentration of Use	The maximum leave-on/dermal contact concentration decreased from 1% to 0.05%	*broalg092018data3_wave2*
Dermal Irritation – In Vivo – Human	test substance: trade name mixture consisting of Alaria Esculenta Extract in butylene glycol and water: 50 volunteers; 25 µL pure test substance applied 3 times a week; non-irritating	*broalg092018data7_wave2*
	test substance: trade name mixture containing Alaria Esculenta Extract in Caprylic/Capric Triglycerides: 50 volunteers; 25 µL pure test substance applied 3 times a week; non-irritating	*broalg092018data8_wave2*
	test substance: trade name mixture containing Alaria Esculenta Extract in Caprylic Capric Triglycerides: 10 volunteers; pure product applied to occlusive patch (20 µL) and then applied to skin for 24 hours; non-irritating	*broalg092018data8_wave2*
	test substance: trade name mixture containing Alaria Esculenta Extract in Caprylic Capric Triglycerides – dried before extraction: 50 volunteers; 25 µL pure test substance applied 3 times a week; non-irritating	*broalg092018data9_wave2*
	test substance: trade name mixture containing < 5% Alaria Esculenta Extract and > 95% Caprylic/Capric Triglycerides 10 volunteers; pure product (20 µL) applied for 24 hours using an occlusive patch ; non-irritating	*broalg092018data9_wave2*
Sensitization – In Vivo – Human	test substance: trade name mixture consisting of Alaria Esculenta Extract in butylene glycol and water: 50 volunteers; pure 25 µL test substance applied 3 times a week during the induction phase and once a week during challenge phase; non-sensitizing	*broalg092018data7_wave2*
	test substance: trade name mixture containing Alaria Esculenta Extract in Caprylic Capric Triglycerides: 50 volunteers; 25 µL pure test substance applied 3 times a week during induction phase and once during challenge phase; non-sensitizing	*broalg092018data8_wave2*
	test substance: trade name mixture containing Alaria Esculenta Extract in Caprylic Capric Triglycerides – dried before extraction: 50 volunteers; 25 µL pure test substance applied 3 times a week during the induction phase and once during challenge phase; non-sensitizing	*broalg092018data9_wave2*
Ascophyllum Nodosum Extract		
Method of Manufacture	polyphenol trapping, aqueous extract, solid/liquid separation, filtration to remove microparticles, alginate precipitation and removal, filtration (including demineralization), depolymerization, and atomization	*broalg092018data1_wave2*

Heavy Metal Specifications	Heavy metal limits were specified: cadmium (≤ 0.5); lead (≤ 5 mg/kg), mercury (≤ 0.1 mg/kg), mineral arsenic (≤ 3 mg/kg); and total heavy metals (≤ 20 mg/kg)	*broalg092018data1_wave2*
Acute Oral Toxicity	Sprague-Dawley rat; OECD 401; LD_{50} >2000 mg/kg	*broalg092018data1_wave2*
Genotoxicity	Ames; OECD 471; Non-mutagenic	*broalg092018data1_wave2*
Dermal Irritation – In Vivo – Animal	OECD 404; non-irritating; test organism experimental details not provided	*broalg092018data1_wave2*
Sensitization – In Vivo – Animal	Magnusson and Kligman (guinea pig maximization test); OECD 406; doses: 0.1 to 0.4 mL of 25% to 75% water solutions; 20 test and 10 control animals; non-sensitizing	*broalg092018data1_wave2*
Ocular Irritation – In Vivo – Animal	OECD 405; slightly irritating; test organism experimental details not provided	*broalg092018data1_wave2*
Cystoseira Baccata Extract		
Impurities	arsenic: 8.8 mg/kg (far casting cross-validation (FCCV) method); 20 ppm by inductively couples plasma-optical emission spectrometry (ICP-OES method)	*broalg092018data17_wave2*
Dermal Irritation – In Vivo – Human	test substance: water and Cystoseira Baccata Extract; 24 hour patch test; occlusive dressing; 10 subjects; 100% concentration, non-irritating	*broalg092018data17_wave2*
	test substance: water and Cystoseira Baccata Extract; 50 volunteers; repeated cutaneous applications; 100% concentration; hypoallergenic	*broalg092018data17_wave2*
Cystoseira Tamaricifolia Extract		
Method of Manufacture	Cystoseira Tamaricifolia Extract and Caprylic/Capric Triglycerides: extraction with supercritical carbon dioxide	*broalg092018data17_wave2*
Impurities	Cystoseira Tamaricifolia Extract and Caprylic/Capric Triglycerides: 1 mg/kg iodine	*broalg092018data17_wave2*
Dermal Irritation – In Vivo – Human	test substance: Cystoseira Tamaricifolia Extract and Caprylic/Capric Triglycerides; 24 hour patch test; 10 volunteers; occlusive dressing; pure test substance; non-irritating	*broalg092018data17_wave2*
Hydrolyzed Fucus Vesiculosus Protein		
Heavy Metal Specifications	trade name substance consisting of Hydrolyzed Fucus Vesiculosus Protein composition: < 20 ppm heavy metals; < 2 ppm arsenic	*broalg092018data6_wave2*
Composition	trade name substance consisting of Hydrolyzed Fucus Vesiculosus Protein composition: 98.9% Hydrolyzed Fucus Vesiculosus Protein, 1% of a trade name mixture consisting of phenoxyethanol, methylparaben, ethylparaben, butylparaben, propylparaben, and isobutylparaben, 0.10% tetrasodium EDTA	*broalg092018data6_wave2*
Fucus Vesiculosus Extract		
Method of Manufacture	trade name mixture containing water, alcohol and Fucus Vesiculosus Extract: dried raw material → extract with 30% ethanolic solution → filtrate → concentration → filtrate → packaging	*broalg092018data2_wave2*

	trade name mixture containing sodium sulfate and Fucus Vesiculosus Extract: dried raw material → extract with 30% ethanolic solution → filtrate → concentration → add anhydrous sodium sulfate → packaging	*broalg092018data2_wave2*
Composition	trade name mixture containing water, alcohol and Fucus Vesiculosus Extract: polyphenol and amino acid	*broalg092018data2_wave2*
	trade name mixture containing sodium sulfate and Fucus Vesiculosus Extract: saccharide and amino acid	*broalg092018data2_wave2*
Heavy Metal Impurities	trade name mixture containing water, alcohol and Fucus Vesiculosus Extract: not to exceed more than 30 ppm heavy metals or 10 ppm arsenic	*broalg092018data2_wave2*
	trade name mixture containing sodium sulfate and Fucus Vesiculosus Extract: not to exceed 20 ppm heavy metals or 10 ppm arsenic	*broalg092018data2_wave2*
Concentration of Use	Maximum leave-on/dermal contact concentration decreased from 6% to 5%.	*broalg092018data3_wave2*
Himanthalia Elongata Extract		
Impurities	Himanthalia Elongata Extract, water, and dipropylene glycol: < 9 mg/kg iodine	*broalg092018data17_wave2*
Concentration of Use	Dermal Contact/Leave-On: 0.2%	*broalg092018data3_wave2*
Dermal Irritation – In Vivo-Human	Himanthalia Elongata Extract, water, and dipropylene glycol: 24 hour patch test; 10 volunteers, pure test substance, occlusive dressing; non-irritating	*broalg092018data17_wave2*
Hizikia Fusiforme Extract		
Method of Manufacture	trade name mixture containing water butylene glycol and Hizikia Fusiforme Extract: dried raw material → extract with 80% ethanolic solution → filtrate → concentration → add 50% 1,3-butylene glycolic solution → filtrate → packaging	*broalg092018data2_wave2*
Composition	trade name mixture containing water butylene glycol and Hizikia Fusiforme Extract: polyphenol and amino acid	*broalg092018data2_wave2*
Heavy Metal Specifications	trade name mixture containing water butylene glycol and Hizikia Fusiforme Extract: not to exceed more than 30 ppm heavy metals or 10 ppm arsenic	*broalg092018data2_wave2*
Laminaria Digitata Extract		
Composition	Laminaria Digitata Extract, water and sea salt: 1.5 mg/kg arsenic, 62 mg/kg iodine	*broalg092018data17_wave2*
	Laminaria Digitata Extract, water, dipropylene glycol: 2.37 mg/kg arsenic, 87 mg/kg iodine (alkaline mineralization and potentiometric method), 110 ppm average	*broalg092018data17_wave2*
	Laminaria Digitata Extract and water: >10 ppm arsenic, 550 ± 150 ppm iodine	*broalg092018data17_wave2*
	Laminaria Digitata Extract and water: 19.06 mg/kg arsenic, 192 mg/kg iodine (alkaline mineralization and potentiometric method), 300 ppm average	*broalg092018data17_wave2*

Dermal Irritation – In Vivo – Animal	test substance: pure Laminaria Digitata Extract, water and sea salt: non-irritating	*broalg092018data17_wave2*
	test substance: pure Laminaria Digitata Extract, water, dipropylene glycol: non-irritating	*broalg092018data17_wave2*
	test substance: pure Laminaria Digitata Extract and water: slightly-irritating	*broalg092018data17_wave2*
Dermal Irritation – In Vivo – Human	test substance: pure Laminaria Digitata Extract and water: 24 hour patch test; occlusive dressing; 10 volunteers with sensitive skin; non-irritating	*broalg092018data17_wave2*
Laminaria Ochroleuca		
Method of Manufacture	trade name mixture consisting on Laminaria Ochroleuca extract in Caprylic/Capric Triglyceride: harvesting/identification → washing → grinding → extraction with the solvent caprylic/capric triglyceride → filtration → quality control → packaging → quality control	*broalg092018data12_wave2*
Heavy Metal Impurities	trade name mixture consisting on Laminaria Ochroleuca extract in Caprylic/Capric Triglyceride: < 2 ppm arsenic, < 3 ppm cadmium, < 5 ppm lead, < 2 ppm nickel, < 5 ppm silver, < 1ppm iodine	*broalg092018data12_wave2*
Dermal Irritation – In Vivo – Human	test substance: trade name mixture consisting on Laminaria Ochroleuca extract in Caprylic/Capric Triglyceride: 11 subjects; single 24 hour application: test substance diluted to 2% in water under occlusive dressing; dose: 0.02 mL over 50 mm^2; non-irritating	*broalg092018data12_wave2*
Laminaria Saccharina Extract		
Method of Manufacture	trade name mixture containing Laminaria Saccharina Extract in water and propylene glycol: harvesting/identification → washing → grinding → extraction with solvents: water + propylene glycol → mixture (addition of preservatives) → filtration → quality control	*broalg092018data10_wave2*
Heavy Metal Impurities	trade name mixture containing Laminaria Saccharina Extract in water and propylene glycol: < 2 ppm arsenic, < 3 ppm cadmium, < 5 ppm lead, < 2 ppm nickel, < 5 ppm silver, < 1 ppm mercury, < 200 ppm iodine	*broalg092018data10_wave2*
Genotoxicity	test substance: trade name mixture containing Laminaria Saccharina Extract in sea water and methylpropandiol; Ames test; Salmonella typhimurium strains TA1535, TA1537, TA102, TA98 and TA100; with and without metabolic activation; dose: 50 to 5000 µg/plate; non-mutagenic	*broalg092018data11_wave2*
Dermal Irritation – In Vivo – Human	test substance: trade name mixture containing Laminaria Saccharina Extract in water and propylene glycol: 50 subjects; 25 µL pure test substance applied 3 times a week under semi-occlusive patch; non-irritating	*broalg092018data10_wave2*
	test substance: 8%, 16% or 100% of a trade name mixture containing Laminaria Saccharina Extract in water and propylene glycol; 10 subjects; 6 occlusive patches (drenched with 0.02 mL test substance) per concentration were applied to the arms over a 50 mm^2 surface for 24 and 48 hours Results: 100% dose was slightly irritating; minimal	*broalg092018data10_wave2*

	erythema in 5/10 subjects; 16% dose was non-irritating; 8% dose was non-irritating	
Sensitization – In Vivo – Human	trade name mixture containing Laminaria Saccharina Extract in water and propylene glycol: 50 subjects; 25 µL pure test substance applied 3 times a week under semi-occlusive patch during the induction phase and once a week during challenge phase; non-sensitizing	*broalg092018data10_wave2*
Phyllacantha Fibrosa Extract		
Impurities	Phyllacantha Fibrosa Extract and water: 11.35 ppm arsenic, 97 mg/L iodine (method ionic chromatography), 140 ppm average	*broalg092018data17_wave2*
Sargassum Filipedula		
Sensitization – In Vivo – Human	test substance: face cream containing 1.2% Sargassum Filipedula Extract; HRIPT; 206 subjects; non-sensitizing	*broalg092018data5_wave2*
Sargassum Glaucescens Extract		
Method of Manufacture	trade name mixture containing 20% Sargassum Glaucescens Extract, 79% water and 1% phenoxyethanol: grinding → extraction → preservative addition → sterilization → filtration → packaging → storage	*broalg092018data4_wave2*
Heavy Metal Specifications	trade name mixture containing 20% Sargassum Glaucescens Extract, 79% water and 1% phenoxyethanol: < 230 ppm stannum, < 2.5 ppm arsenic, < 10 ppm copper, < 1 ppm lead and < 1 ppm chromium	*broalg092018data4_wave2*
Dermal Irritation – In Vivo – Human	test substance: trade name mixture containing 20% Sargassum Glaucescens Extract, 79% water and 1% phenoxyethanol: 10% of the test substance applied under occlusive patch for 48 hours; 10 subjects; non-irritating	*broalg092018data4_wave2*
Sargassum Muticum Extract		
Concentration of Use	Maximum concentration of use decreased from 4% to 0.076% in leave-on products	*broalg092018data3_wave2*
Sphacelaria Scoparia Extract		
Impurities	Sphacelaria Scoparia Extract, water, and dipropylene glycol: 0.73 mg/kg arsenic, 15 mg/kg iodine	*broalg092018data17_wave2*
Dermal Irritation – In Vivo-Human	test substance: Sphacelaria Scoparia Extract, water, and dipropylene glycol: 24 hour patch test, pure test substance, occlusive dressing, 11 volunteers, non-irritating	*broalg092018data17_wave2*
	test substance: Sphacelaria Scoparia Extract, water, and dipropylene glycol: repeated epicutaneous applications on 50 subjects; 100% test substance; hypoallergenic	*broalg092018data17_wave2*
Undaria Pinnatifida Extract		
Method of Manufacture	trade name mixture containing Undaria Pinnatifida Extract in water and propylene glycol: harvesting/identification → drying → grinding → extraction with solvents water and propylene glycol, and addition of preservatives (methylparaben and propylparaben) → filtration → quality control → packaging → quality control	*broalg092018data14_wave2*

	trade name mixture containing Undaria Pinnatifida Extract in Caprylic/Capric Triglyceride: harvesting of fertile sporophytes → fragment isolation of gametophyte → culture in liquid medium → gametophyte separation → freeze-dried gametophyte - → quality control → extraction with the solvent caprylic/capric triglyceride → filtration → quality control → packaging → quality control	*broalg092018data14_wave2*
Heavy Metal Impurities	trade name mixture containing Undaria Pinnatifida Extract in water and propylene glycol: < 5 ppm arsenic, < 10 ppm cadmium, < 5 ppm lead, < 2 ppm nickel, < 5 ppm silver, < 1 ppm iodine	*broalg092018data14_wave2*
	trade name mixture containing Undaria Pinnatifida Extract in caprylic/capric triglyceride: < 2 ppm arsenic, < 3 ppm cadmium, < 5 ppm lead, < 2 ppm nickel, < 5 ppm silver, < 1 ppm mercury, < 1 ppm iodine	*broalg092018data14_wave2*
Dermal Irritation – In Vitro – Human	test substance: trade name mixture containing Undaria Pinnatifida Extract in Caprylic/Capric triglyceride; reconstructed human epidermis test method; 3 samples; 10 µL dose of pure test substance for 15 minutes; non-irritating	*broalg092018data16_wave2*
Dermal Irritation – In Vivo – Human	test substance: trade name mixture containing Undaria Pinnatifida Extract in water and propylene glycol; 12 subjects; occlusive patches (0.02 mL test substance) were applied to the skin over a 50 mm^2 surface for 30 minutes and 24 hours; 100% concentration Results: moderately irritating after 30 minutes, and mildly irritating after 24 hours	*broalg092018data14_wave2*
	test substance: Undaria Pinnatifida Extract, water, and dipropylene glycol; 24 hour patch test; 10 volunteers; occlusive dressing; non-irritating	*broalg092018data17_wave2*
Sensitization – In Vivo – Human	test substance: trade name mixture containing Undaria Pinnatifida Extract in Caprylic/Capric Triglyceride; 100 subjects; Marzulli-Maibach method; 50 µL pure test substance applied 3 times a week during induction face and once during challenge phase; non-irritating and non-sensitizing	*broalg092018data15_wave2*
Pelvetia Canaliculata Extract and Laminaria Digitata Extract		
Method of Manufacture	trade name mixture containing Pelvetia Canaliculata and Laminaria Digitata extracted in propylene glycol with panthenol: harvesting/identification → washing → grinding → extraction with the solvent propylene glycol → filtration → quality control → mixture → filtration → quality control → packaging → quality control	*broalg092018data13_wave2*
	trade name mixture containing Pelvetia Canaliculata and Laminaria Digitata extracted in butylene glycol with preservatives: harvesting/identification → washing → grinding → extraction with butylene glycol → filtration → quality control → mixture → filtration → quality control → packaging → quality control	*broalg092018data13_wave2*

	trade name mixture containing Pelvetia Canaliculata and Laminaria Digitata extracted in butylene glycol without preservatives: harvesting/identification → washing → grinding → extraction with butylene glycol → filtration → quality control → mixture → filtration → quality control → packaging → quality control	*broalg092018data13_wave2*
Heavy Metals	trade name mixture containing Pelvetia Canaliculata and Laminaria Digitata extracted in propylene glycol with panthenol: < 5 ppm arsenic, < 3 ppm cadmium, < 5 ppm lead, < 2 ppm nickel, < 5 ppm silver, < 100 ppm iodine	*broalg092018data13_wave2*
	trade name mixture containing Pelvetia Canaliculata and Laminaria Digitata extracted in butylene glycol with preservatives: < 5 ppm arsenic, < 10 ppm cadmium, < 5 ppm lead, < 2 ppm nickel, < 5 ppm silver, < 100 ppm iodine	*broalg092018data13_wave2*
	trade name mixture containing Pelvetia Canaliculata and Laminaria Digitata extracted in butylene glycol without preservatives: < 5 ppm arsenic, < 10 ppm cadmium, < 5 ppm lead, < 2 ppm nickel, < 5 ppm silver, < 100 ppm iodine	*broalg092018data13_wave2*
Dermal Irritation – In Vivo – Human	test substance: trade name mixture containing Pelvetia Canaliculata Extract and Laminaria Digitata Extract extracted in propylene glycol with panthenol: 10 subjects, 5, 10, or 100% of the test substance, patches (0.02 mL test substance) were applied to skin over a 50 mm^2 surface for 24 and 48 hours; Results: mild irritation at the 100% dose level, minimal irritation at 10% dose level, no irritation at 5% dose level	*broalg092018data13_wave2*
Laminaria Japonica, Nereocystis Leutkeana, and Macrocystis Pyrifera Extract		
Method of Manufacture	trade name mixture containing Laminaria Japonica, Nereocystis Leutkeana, and Macrocystis Pyrifera Extract: test of acceptance → processing (mechanical grinding/milling) → extraction with pentaerythrityl tetraethylhexanoate at specific pH and temperature for specific duration → filtration → batch adjustments (refiltration) → sample for QC → pack → sample for Micro → shipping	*broalg092018data6_wave2*
Composition	trade name mixture containing Laminaria Japonica, Nereocystis Leutkeana, and Macrocystis Pyrifera Extract: 97% pentaerythrityl tetraethylhexanoate, 7% Laminaria Japonica Extract, 7% Nereocystis Leutkeana Extract, 7% Macrocystis Pyrifera Extract	*broalg092018data6_wave2*
Heavy Metal Impurities	trade name mixture containing Laminaria Japonica, Nereocystis Leutkeana, and Macrocystis Pyrifera Extract: < 20 ppm heavy metals; < 10 ppm lead; < 2 ppm arsenic; < 1 ppm cadmium	*broalg092018data6_wave2*
Skin Irritation – In Vitro – Animal	test substance: trade name mixture containing Laminaria Japonica, Nereocystis Leutkeana, and Macrocystis Pyrifera Extract reconstructed human epidermal model (EpiDerm), dosed with 30 µL (liquid) or 25 mg (solid) of undiluted test substance applied to 3 tissue inserts and	*broalg092018data6_wave2*

	incubated; non-irritating	
Ocular Irritation – In Vitro - Animal	test substance: trade name mixture containing Laminaria Japonica, Nereocystis Leutkeana, and Macrocystis Pyrifera Extract cornea epithelial model (EpiOcular), dosed with 50 µL (liquid) or 50 mg (solid) of undiluted test substance applied to 2 tissue inserts and incubated; non-irritating	*broalg092018data6_wave2*

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Memorandum

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

FROM: Carol Eisenmann, Ph.D.
Personal Care Products Council

DATE: August 28, 2018

SUBJECT: Ascophyllum Nodosum Extract

Solabia Group. 2018. Ascophyllum Nodosum Extract - Technical Information.

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August 2018

Ascophyllum Nodosum Extract – Technical Information Provided by SOLABIA Group

Ascophyllum Nodosum Extract is a water extract of the thallus of Ascophyllum nodosum (common names Norweian kelp, knotted kelp, knotted wrack, egg wrack). The algae is collected from "very good ecological status" zone, defined in the Water Framework Directive (CE) n° 2000/60/EC.

The following steps are used in the manufacturing process:

Polyphenol trapping
Aqueous extract
Solid/liquid separation
Filtration to remove microparticles
Alginates, precipitation and removal
Filtration, including demineralization
Depolymerization
Atomization

This Ascopyllum Nodosum Extract is a fine powder that is white to pale yellow with a light and characteristic taste and smell. It consists primarily of fucoidans (total sugars + sulfate level >30%) with an average molecular weight of 10 kDa. It contains no additives, including preservatives.

Physical/Chemical Characteristics

	Limit	Method
Apparent density (at 20°C)	0.2 to 0.6	Internal method
Loss on drying	<10%	Eur. Ph.
Solubility in water	Soluble	Eur. Ph.
Particle size <300 µm	≥90%	Internal method

Heavy Metals

	Limit	Method
Cadmium	≤0.5 mg/kg	NF EN ISO 11885
Lead	≤5 mg/kg	NF EN ISO 11885
Mercury	≤0.1 mg/kg	Atomic fluorescence (IDHESA)
Mineral Arsenic	≤3 mg/kg	NF EN 15517 modified
Total heavy metals	≤20 mg/kg	

Iodine ≤2,000 mg/kg using method NF EN 15111

Microbiology*

	Limit	Method
Total aerobic microbial count	≤100 CFU/g	Eur. Ph 9th ed §2.6.12-2.6.13
Total combine yeasts-molds count	≥100 CFU/g	Eur. Ph 9th ed §2.6.12-2.6.13

**Candida albicans*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Escherichia coli* absent using method: Eur. Ph 9th ed §2.6.12-2.6.13

Pesticide residues (carbamates, organochlorides, organophosphates) below limits of detection (<100 µg/kg/entity for carbamates and <50 µg/kg entity for organochlorides and organophosphates)

Safety Studies

Acute oral toxicity:	LD50>2000 mg/kg (OECD No. 401) (Sprague-Dawley rats)
Eye irritation:	Slightly irritating (OECD No. 405)
Skin irritation:	Not irritating (OECD No. 404)
Skin sensitization:	Non sensitizing (Magnusson and Kligman (guinea pig maximization test) OECD 406 (doses tested: 0.1 ml to 0.4 ml of 25% to 75% water solutions; 20 test and 10 control guinea pigs)
Mutagenicity:	Not mutagenic (Ames, OECD No. 471)

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Memorandum

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

FROM: Carol Eisenmann, Ph.D.
Personal Care Products Council

DATE: September 4, 2018

SUBJECT: Fucus Vesiculosis Extract and Hizikia Fusiforme Extract

Anonymous. 2018. Method of manufacture and composition brown-algae-derived ingredients (Fucus Vesiculosis Extract and Hizikia Fusiforme Extract).

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September 2018

Method of Manufacture and Composition
Brown algae-derived ingredients

The method of manufacture (including the solvent used to extract the algae)

Trade name mixture	Method of manufacture
Water, Alcohol and Fucus Vesiculosis Extract	Dried raw material⇒extract with 30vol% ethanolic solution⇒filtrate⇒concentration⇒filtrate⇒packaging
Sodium Sulfate and Fucus Vesciulosis Extract	Dried raw material⇒extract with 30vol% ethanolic solution⇒filtrate ⇒ concentration ⇒ add anhydrous sodium sulfate ⇒ packaging
Water Butylene Glycol and Hizikia Fusiforme Extract	Dried raw material⇒extract with 80vol% ethanolic solution⇒filtrate⇒concentration⇒add 50vol% 1,3-butylen glycolic solution⇒filtrate⇒packaging

Composition (components in the algae itself and impurities)

Trade name mixture	Composition (identification)
Water, Alcohol and Fucus Vesiculosis Extract	<Components> Polyphenol and amino acid <Impurities> Heavy metals: not more than 30ppm Arsenic: not more than 10ppm
Sodium Sulfate and Fucus Vesciulosis Extract	<Components> Saccharide and amino acid <Impurities> Heavy metals: not more than 20ppm Arsenic: not more than 10ppm
Water Butylene Glycol and Hizikia Fusiforme Extract	<Components> Polyphenol and amino acid <Impurities> Heavy metals: not more than 30ppm Arsenic: not more than 10ppm

Concentration of Use by FDA Product Category – Brown Algae-Derived Ingredients*

Agarum Cribrosum Extract
Alaria Esculenta Extract
Ascophyllum Nodosum Extract
Ascophyllum Nodosum Powder
Cladosiphon Novae-Caledoniae Extract
Cladosiphon Okamuranus Extract
Cystoseira Amentacea/Caespitosa/Branchycarpa Extract
Cystoseira Baccata Extract
Cystoseira Balearica Extract
Cystoseira Caespitosa Extract
Cystoseira Compressa Extract
Cystoseira Compressa Powder
Cystoseira Tamariscifolia Extract
Dictyopteris Membranacea Extract (Retired)
Dictyopteris Polypodioides Extract
Dictyota Coriacea Extract
Durvillea Antarctica Extract
Ecklonia Cava Extract
Ecklonia Cava Water
Ecklonia Kurome Extract
Ecklonia Kurome Powder
Ecklonia/Laminaria Extract
Ecklonia Maxima Extract
Ecklonia Maxima Powder
Ecklonia Radiata Extract
Eisenia Arborea Extract
Fucus Serratus Extract
Fucus Spiralis Extract
Fucus Vesiculosus Extract
Fucus Vesiculosus Powder
Halidrys Siliquosa Extract
Halopteris Scoparia Extract
Himanthalia Elongata Extract
Himanthalia Elongata Powder
Hizikia Fusiforme Extract
Hizikia Fusiformis Water
Hizikia Fusiformis Callus Culture Extract
Hydrolyzed Ecklonia Cava Extract
Hydrolyzed Fucus Vesiculosus Extract
Hydrolyzed Fucus Vesiculosus Protein
Laminaria Angustata Extract (Retired)
Laminaria Cloustoni Extract
Laminaria Diabolica Extract
Laminaria Digitata Extract
Laminaria Digitata Powder
Laminaria Hyperborea Extract
Laminaria Japonica Extract
Laminaria Japonica Powder
Laminaria Longissima Extract
Laminaria Ochotensis Extract (Retired)
Laminaria Ochroleuca Extract
Laminaria Saccharina Extract
Lessonia Nigrescens Extract
Lessonia Nigrescens Powder
Macrocystis Pyrifera (Kelp)
Macrocystis Pyrifera (Kelp) Blade/Pneumatocyst/Stipe Juice Extract
Macrocystis Pyrifera (Kelp) Extract
Macrocystis Pyrifera (Kelp) Juice
Macrocystis Pyrifera (Kelp) Protein
Nereocystis Luetkeana Extract
Pelvetia Canaliculata Extract
Pelvetia Siliquosa Extract
Phyllacantha Fibrosa Extract
Saccharina Angustata Extract
Saccharina Japonica Extract
Saccharina Longicruris Extract
Sargassum Filipendula Extract
Sargassum Fulvellum Extract
Sargassum Fusiforme Extract
Sargassum Horneri Extract
Sargassum Muticum Extract
Sargassum Pallidum Extract
Sargassum Siliquastrum Extract
Sargassum Vulgare Extract
Sphacelaria Scoparia Extract
Undaria Peterseniana Extract
Undaria Pinnatifida Extract
Undaria Pinnatifida Cell Culture Extract

Undaria Pinnatifida Leaf/Stem Extract Undaria Pinnatifida Root Powder
Undaria Pinnatifida Powder

Ingredient	Product Category	Maximum Concentration of Use
Agarum Cribrosum Extract	Other skin care preparations	0.012%
Alaria Esculenta Extract	Foundations	0.03%
Alaria Esculenta Extract	Skin cleansing (cold creams, cleansing lotions, liquids and pads)	0.0015%
Alaria Esculenta Extract	Face and neck products Not spray	 0.0015-0.05%
Alaria Esculenta Extract	Night products Not spray	 0.05%
Alaria Esculenta Extract	Indoor tanning preparations	0.0005%
Ascophyllum Nodosum Extract	Eye lotion	0.025-0.2%
Ascophyllum Nodosum Extract	Hair conditioners	0.00005-0.0002%
Ascophyllum Nodosum Extract	Shampoos (noncoloring)	0.00005-0.002%
Ascophyllum Nodosum Extract	Tonics, dressings and other hair grooming aids	0.002%
Ascophyllum Nodosum Extract	Nail polish and enamel	0.000065%
Ascophyllum Nodosum Extract	Other manicuring preparations	0.02%
Ascophyllum Nodosum Extract	Other personal cleanliness products	0.00004%
Ascophyllum Nodosum Extract	Face and neck products Not spray	 0.0032-0.03%
Ascophyllum Nodosum Extract	Body and hand products Not spray	 0.0000004-0.02%
Ascophyllum Nodosum Extract	Moisturizing products Not spray	 0.03%
Ascophyllum Nodosum Extract	Paste masks and mud packs	0.0032%
Ascophyllum Nodosum Extract	Other skin care preparations	0.045%
Cladosiphon Okamuranus Extract	Eye lotion	0.025%
Cladosiphon Okamuranus Extract	Foundation	0.05%
Cladosiphon Okamuranus Extract	Skin cleansing (cold creams, cleansing lotions, liquids and pads)	0.005%
Cladosiphon Okamuranus Extract	Face and neck products Not spray	 0.025%
Dictyopteris Membranacea Extract	Lipstick	0.01%
Durvillea Antartica Extract	Basecoats and undercoats (manicuring preparations)	0.0001%
Ecklonia Radiata Extract	Hair conditioners	0.005%
Ecklonia Radiata Extract	Hair sprays Aerosol Pump spray	 0.0051% 0.0051%
Ecklonia Radiata Extract	Shampoos (noncoloring)	0.0051%
Ecklonia Radiata Extract	Other hair preparations (noncoloring)	

	Spray	0.0051%
Fucus Serratus Extract	Eye lotions	0.05%
Fucus Serratus Extract	Hair conditioners	0.00001%
Fucus Serratus Extract	Shampoos (noncoloring)	0.00001%
Fucus Serratus Extract	Skin cleansing (cold creams, cleansing lotions, liquids and pads)	0.05%
Fucus Serratus Extract	Face and neck products Not spray	 0.05%
Fucus Vesiculosis Extract	Bath oils, tablets and salts	0.0001-0.01%
Fucus Vesiculosis Extract	Bubble baths	5%
Fucus Vesiculosis Extract	Other bath preparations	0.0051%
Fucus Vesiculosis Extract	Eyebrow pencil	0.02%
Fucus Vesiculosis Extract	Eye shadow	0.01%
Fucus Vesiculosis Extract	Eye lotion	0.017%
Fucus Vesiculosis Extract	Mascara	0.02-5%
Fucus Vesiculosis Extract	Perfume	0.01%
Fucus Vesiculosis Extract	Hair conditioners	0.0006-0.17%
Fucus Vesiculosis Extract	Hair sprays Aerosol Pump spray	 0.001% 0.00018-0.0006%
Fucus Vesiculosis Extract	Rinses (noncoloring)	0.00012-0.01%
Fucus Vesiculosis Extract	Shampoos (noncoloring)	0.0001-5%
Fucus Vesiculosis Extract	Tonics, dressings and other hair grooming aids	0.0001-0.01%
Fucus Vesiculosis Extract	Other hair preparations (noncoloring)	0.0015%
Fucus Vesiculosis Extract	Hair lighteners with color	0.0001%
Fucus Vesiculosis Extract	Lipstick	0.0005%
Fucus Vesiculosis Extract	Other makeup preparations	0.005%
Fucus Vesiculosis Extract	Other manicuring preparations	0.02%
Fucus Vesiculosis Extract	Bath soaps and detergents	0.00076-3.1%
Fucus Vesiculosis Extract	Other personal cleanliness products	0.00002%
Fucus Vesiculosis Extract	Skin cleansing (cold creams, cleansing lotions, liquids and pads)	0.00011-0.17%
Fucus Vesiculosis Extract	Face and neck products Not spray	 0.005-0.05%
Fucus Vesiculosis Extract	Body and hand products Not spray	 0.000032-0.03%
Fucus Vesiculosis Extract	Foot products Not spray or powder Spray	 0.08% 0.12%
Fucus Vesiculosis Extract	Moisturizing products Not spray	 0.0051-5%
Fucus Vesiculosis Extract	Night products Not spray	 0.016-0.05%
Fucus Vesiculosis Extract	Paste masks and mud packs	0.0025-0.05%
Fucus Vesiculosis Extract	Other skin care preparations	0.03%

Fucus Vesiculosis Extract	Suntan products Not spray	0.00098%
Himanthalia Elongata Extract	Face and neck products Not spray	0.2%
Laminaria Digitata Extract	Bath oils, tablets and salts	0.1%
Laminaria Digitata Extract	Bubble baths	5%
Laminaria Digitata Extract	Eye lotion	0.095-0.5%
Laminaria Digitata Extract	Mascara	0.0035%
Laminaria Digitata Extract	Hair conditioners	0.0007-5%
Laminaria Digitata Extract	Hair sprays Aerosol	0.0007%
Laminaria Digitata Extract	Shampoos (noncoloring)	0.0007-0.0039%
Laminaria Digitata Extract	Tonics, dressings and other hair grooming aids	0.0035%
Laminaria Digitata Extract	Other hair preparations (noncoloring)	0.0007%
Laminaria Digitata Extract	Hair rinses (coloring)	0.0007%
Laminaria Digitata Extract	Hair shampoos (coloring)	0.0007%
Laminaria Digitata Extract	Hair bleaches	0.00004%
Laminaria Digitata Extract	Foundations	0.013%
Laminaria Digitata Extract	Bath soaps and detergents	0.06%
Laminaria Digitata Extract	Skin cleansing products (cold creams, cleansing lotions, liquids and pads)	0.6%
Laminaria Digitata Extract	Face and neck products Not spray	0.0001-0.1%
Laminaria Digitata Extract	Body and hand products Not spray	0.005-0.08%
Laminaria Digitata Extract	Moisturizing products Not spray	0.01%
Laminaria Digitata Extract	Skin fresheners	5%
Laminaria Digitata Extract	Other skin care preparations	0.1-0.5%
Laminaria Digitata Powder	Face and neck products Not spray	40%
Laminaria Hyperborea Extract	Body and hand products Not spray	0.03%
Laminaria Japonica Extract	Bath oils, tablets and salts	0.011-5%
Laminaria Japonica Extract	Other eye makeup preparations	0.0005-0.007%
Laminaria Japonica Extract	Hair conditioners	0.0005-0.0006%
Laminaria Japonica Extract	Rinses (noncoloring)	0.0012%
Laminaria Japonica Extract	Shampoos (noncoloring)	0.0006%
Laminaria Japonica Extract	Tonics, dressings and other hair grooming aids	0.3%
Laminaria Japonica Extract	Blushers	0.0035%
Laminaria Japonica Extract	Face powders	0.0035%
Laminaria Japonica Extract	Foundations	0.018%
Laminaria Japonica Extract	Rouges	0.019%
Laminaria Japonica Extract	Other makeup preparations	0.018%

Laminaria Japonica Extract	Skin cleansing (cold creams, cleansing lotions, liquids and pads)	0.0055%
Laminaria Japonica Extract	Face and neck products Not spray	 0.011-5%
Laminaria Japonica Extract	Body and hand products Not spray	 0.0055%
Laminaria Japonica Extract	Paste masks and mud packs	0.0005-5%
Laminaria Japonica Extract	Skin fresheners	5%
Laminaria Japonica Extract	Other skin care preparations	0.0005%
Laminaria Ochroleuca Extract	Eyeliner	0.63%
Laminaria Ochroleuca Extract	Eye shadow	0.017%
Laminaria Ochroleuca Extract	Eye lotion	0.0034-0.02%
Laminaria Ochroleuca Extract	Hair conditioners	0.017%
Laminaria Ochroleuca Extract	Shampoos (noncoloring)	0.017%
Laminaria Ochroleuca Extract	Tonics, dressings and other hair grooming aids	0.017%
Laminaria Ochroleuca Extract	Hair dyes and colors	0.017%
Laminaria Ochroleuca Extract	Foundations	0.00017-0.02%
Laminaria Ochroleuca Extract	Rouges	0.017%
Laminaria Ochroleuca Extract	Aftershave lotions	0.00024%
Laminaria Ochroleuca Extract	Skin cleansing (cold creams, cleansing lotions, liquids and pads)	0.000024-0.017%
Laminaria Ochroleuca Extract	Face and neck products Not spray Spray	 0.0026-0.17% 0.017%
Laminaria Ochroleuca Extract	Body and hand products Not spray	 0.0005-0.017%
Laminaria Ochroleuca Extract	Moisturizing products Not spray	 0.0034-0.017%
Laminaria Ochroleuca Extract	Night products Not spray	 0.0034-0.017%
Laminaria Ochroleuca Extract	Suntan products Not spray	 0.0034-0.05%
Laminaria Saccharina Extract	Eye lotion	0.002-0.019%
Laminaria Saccharina Extract	Eye makeup remover	0.000092%
Laminaria Saccharina Extract	Shampoos (noncoloring)	0.00001-0.045%
Laminaria Saccharina Extract	Tonics, dressings and other hair grooming aids	0.001-0.002%
Laminaria Saccharina Extract	Face powders	0.0008%
Laminaria Saccharina Extract	Foundations	0.01%
Laminaria Saccharina Extract	Nail polish and enamel	0.001%
Laminaria Saccharina Extract	Bath soaps and detergents	0.51%
Laminaria Saccharina Extract	Deodorants Not spray	 0.015%
Laminaria Saccharina Extract	Aftershave lotions	0.005-0.023%
Laminaria Saccharina Extract	Preshave lotions	0.23%

Laminaria Saccharina Extract	Shaving cream	0.023%
Laminaria Saccharina Extract	Skin cleansing (cold creams, cleansing lotions, liquids and pads)	0.000092-0.01%
Laminaria Saccharina Extract	Face and neck products Not spray	 0.0031-0.1%
Laminaria Saccharina Extract	Body and hand products Not spray Not spray, not powder	 0.000092-0.0031% 0.54%
Laminaria Saccharina Extract	Moisturizing products Not spray	 0.023%
Laminaria Saccharina Extract	Night products Not spray	 0.019%
Laminaria Saccharina Extract	Paste masks and mud packs	0.0078%
Laminaria Saccharina Extract	Other skin care preparations	0.0008-0.005%
Laminaria Saccharina Extract	Indoor tanning preparations	0.005%
Lessonia Nigrescens Extract	Paste masks and mud packs	0.032%
Macrocystis Pyrifera (Kelp) Extract	Bath oils, tablets and salts	0.028-1%
Macrocystis Pyrifera (Kelp) Extract	Bubble baths	0.21%
Macrocystis Pyrifera (Kelp) Extract	Other bath preparations	0.0051-0.41%
Macrocystis Pyrifera (Kelp) Extract	Eye lotion	0.2-36.4%
Macrocystis Pyrifera (Kelp) Extract	Eye makeup remover	0.0098%
Macrocystis Pyrifera (Kelp) Extract	Other eye makeup preparations	0.007%
Macrocystis Pyrifera (Kelp) Extract	Colognes and toilet waters	0.084%
Macrocystis Pyrifera (Kelp) Extract	Other fragrance preparations	0.042%
Macrocystis Pyrifera (Kelp) Extract	Hair conditioners	0.001-0.17%
Macrocystis Pyrifera (Kelp) Extract	Shampoos (noncoloring)	0.001-5%
Macrocystis Pyrifera (Kelp) Extract	Tonics, dressings and other hair grooming aids	0.0036-5%
Macrocystis Pyrifera (Kelp) Extract	Blushers	0.0035%
Macrocystis Pyrifera (Kelp) Extract	Face powders	0.0035%
Macrocystis Pyrifera (Kelp) Extract	Foundations	0.018-0.98%
Macrocystis Pyrifera (Kelp) Extract	Lipstick	0.079%
Macrocystis Pyrifera (Kelp) Extract	Rouges	0.019%
Macrocystis Pyrifera (Kelp) Extract	Other makeup preparations	0.018%
Macrocystis Pyrifera (Kelp) Extract	Nail creams and lotions	0.0002%
Macrocystis Pyrifera (Kelp) Extract	Nail extenders	0.00044%
Macrocystis Pyrifera (Kelp) Extract	Nail polish and enamel	0.0011%
Macrocystis Pyrifera (Kelp) Extract	Bath soaps and detergents	0.01-5%
Macrocystis Pyrifera (Kelp) Extract	Aftershave lotions	0.007-0.042%
Macrocystis Pyrifera (Kelp) Extract	Shaving cream (aerosol, brushless and lather)	0.05%
Macrocystis Pyrifera (Kelp) Extract	Skin cleansing (cold creams, cleansing lotions, liquids and pads)	0.00005-1.5%
Macrocystis Pyrifera (Kelp) Extract	Depilatories	0.0002%
Macrocystis Pyrifera (Kelp) Extract	Face and neck products Not spray	 0.001-33.3%

	Spray	0.79%
Macrocystis Pyrifera (Kelp) Extract	Body and hand products Not spray	 0.42-0.98%
Macrocystis Pyrifera (Kelp) Extract	Foot products Not spray, not powder	0.17% 0.2%
Macrocystis Pyrifera (Kelp) Extract	Moisturizing products Not spray	 0.42%
Macrocystis Pyrifera (Kelp) Extract	Paste masks and mud packs	0.5-0.6%
Macrocystis Pyrifera (Kelp) Extract	Skin fresheners	5%
Macrocystis Pyrifera (Kelp) Extract	Other skin care preparations	0.41-0.98%
Macrocystis Pyrifera (Kelp) Extract	Suntan products Not spray	 0.0098%
Macrocystis Pyrifera (Kelp) Extract	Indoor tanning preparations	0.0098%
Pelvetia Canaliculata Extract	Eye lotion	0.00002%
Pelvetia Canaliculata Extract	Mascara	0.0007%
Pelvetia Canaliculata Extract	Hair conditioners	0.0007%
Pelvetia Canaliculata Extract	Hair sprays Aerosol Pump spray	 0.0007% 0.00004%
Pelvetia Canaliculata Extract	Shampoos (noncoloring)	0.0007%
Pelvetia Canaliculata Extract	Tonics, dressings and other hair grooming aids	0.0035%
Pelvetia Canaliculata Extract	Other hair preparations (noncoloring)	0.0007%
Pelvetia Canaliculata Extract	Hair rinses (coloring)	0.0007%
Pelvetia Canaliculata Extract	Hair shampoos (coloring)	0.0007%
Pelvetia Canaliculata Extract	Hair bleaches	0.00004%
Pelvetia Canaliculata Extract	Skin cleansing (cold creams, cleansing lotions, liquids and pads)	0.0004-0.0018%
Pelvetia Canaliculata Extract	Face and neck products Not spray	 0.002-0.018%
Pelvetia Canaliculata Extract	Skin fresheners	0.002%
Pelvetia Canaliculata Extract	Suntan products Not spray	 0.0044%
Sargassum Filipendula Extract	Hair conditioners	0.0048-0.29%
Sargassum Filipendula Extract	Shampoos (noncoloring)	0.15%
Sargassum Filipendula Extract	Tonics, dressings and other hair grooming aids	0.0001%
Sargassum Filipendula Extract	Hair dyes and colors	0.011-0.29%
Sargassum Filipendula Extract	Skin cleansing (cold creams, cleansing lotion, liquids and pads)	0.002%
Sargassum Filipendula Extract	Face and neck products Not spray	 0.8%
Sargassum Filipendula Extract	Moisturizing products Not spray	 1.2%
Sargassum Filipendula Extract	Paste masks and mud packs	0.15%
Sargassum Muticum Extract	Eye lotion	0.076%

Sargassum Muticum Extract	Other eye makeup preparations	2.5%
Sargassum Muticum Extract	Skin cleansing (cold creams, cleansing lotions, liquids and pads)	0.01%
Sargassum Muticum Extract	Other skin care preparations	0.076%
Sargassum Vulgare Extract	Eye lotion	0.011%
Sargassum Vulgare Extract	Shampoos (noncoloring)	0.0075%
Sargassum Vulgare Extract	Tonics, dressings and other hair grooming aids	0.009%
Sargassum Vulgare Extract	Face and neck products Not spray	0.011%
Sargassum Vulgare Extract	Other skin care preparations	0.016%
Sphacelaria Scoparia Extract	Body and hand products Not spray, not powder	0.016%
Undaria Pinnatifida Extract	Other bath preparations	0.0001%
Undaria Pinnatifida Extract	Shampoos (noncoloring)	5%
Undaria Pinnatifida Extract	Tonics, dressings and other hair grooming aids	0.002%
Undaria Pinnatifida Extract	Other hair preparations (noncoloring)	0.0031%
Undaria Pinnatifida Extract	Face powders	0.00001%
Undaria Pinnatifida Extract	Foundations	0.00001%
Undaria Pinnatifida Extract	Makeup bases	0.00001%
Undaria Pinnatifida Extract	Bath soaps and detergents	0.0001%
Undaria Pinnatifida Extract	Face and neck products Not spray	0.00001-0.001%
Undaria Pinnatifida Extract	Body and hand products Not spray	5%
Undaria Pinnatifida Powder	Shaving soap	0.1%

*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 2015

Table prepared: July 3, 2015

August 17, 2018: Alaria Esculenta Extract: night products changed from 1% to 0.05%; Fucus Vesiculosis Extract: body and hand products maximum concentration changed from 6% to 0.03%; Added Himanthalia Elongata Extract; Sargassum Muticum Extract: eye lotions changed from 4% to 0.076%; other eye makeup preparations changed from 2.5% to 0.076%; other skin care products changed from 2-4% to 0.076%

Personal Care Products Council
Committed to Safety, Quality & Innovation

Memorandum

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

FROM: Carol Eisenmann, Ph.D.
Personal Care Products Council

DATE: September 7, 2018

SUBJECT: Sargassum Glaucescens Extract

TCI Co., Ltd. 2018. Material specification Sargassum Glaucescens Extract.

Chia Nan University of Pharmacy and Science. 2015. Patch testing of Ocean White® (20% Sargassum Glaucescens Extract, 79% water, 1% phenoxyethanol).

1620 L Street, N.W., Suite 1200 | Washington, D.C. 20036 | 202.331.1770 | 202.331.1969 (fax) | www.personalcarecouncil.org

MATERIAL SPECIFICATION

Sargassum glaucescens Extract — Item Code: 30000116

General Information	
Product Name	*Sargassum glaucescens* Extract (Skincare)
INCI name	*Sargassum glaucescens* Extract
Storage Condition	Store at 0-7 ℃ for 2 year, and avoid from direct sunlight
Shelf Life	2 years
Origin	Taiwan

Analytical Details		
Characteristic	***Standard Value***	***Analytical Method***
Total Polysaccharide	≧0.01%	W-QL-024
Appearance	Brown, liquid	Visual
Brix	1.6±1.0	W-QA-040
pH	7.0±1.0	W-QL-004
Total Plate Count	< 100 CFU/mL	W-QL-003*
E. coli	Negative	W-QL-003*
Coliform	< 10 CFU/mL	W-QL-003**
Mold / Yeast	< 10 CFU/mL	W-QL-003*
Staphylococcus aureus	Negative	W-QL-003*
Pseudomonas aeruginosa	Negative	W-QL-003*
Salmonella	Negative	W-QL-003**
Stannum	< 230ppm	W-QL-005(ICP/OES)***
Arsenic	< 2.5ppm	
Copper	< 10ppm	
Antimony	< 1.0ppm	
Cadmium	< 0.1ppm	
Germanium	< 0.1ppm	
Mercury	< 0.5ppm	
Lead	< 1.0ppm	
Chromium	< 1.0ppm	

References:

***International Organization for Standardization**

****Taiwan Food and Drug Administration. Microbiological tests.**

*****MOHW Food No1021950329 Announced.**

TCI Co., LTD.
TEL: 886-2-8797-7811 FAX: 886-2-8797-3577
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Http://www.tci-bio.com/

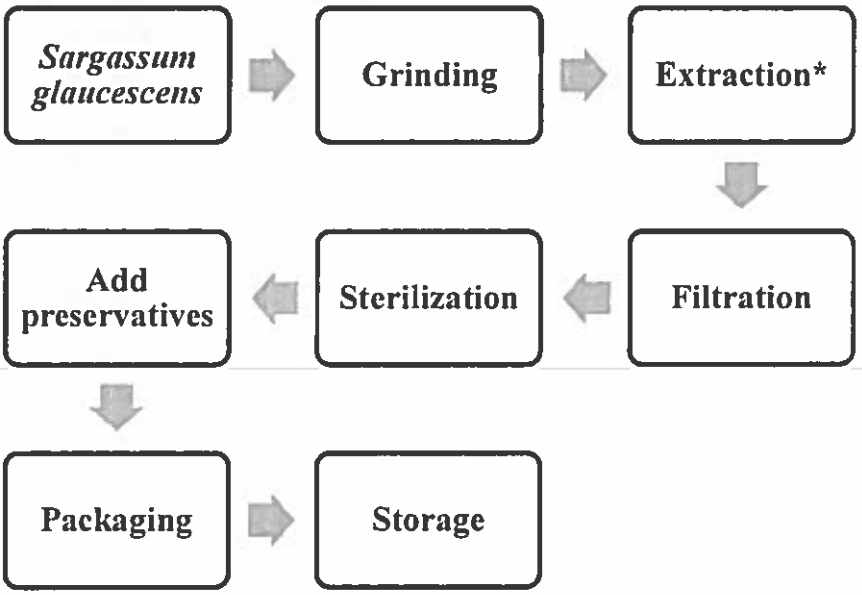
MATERIAL SPECIFICATION

Sargassum glaucescens Extract — Item Code: 30000116

Composition Break Down

Ingredient	Country of Origin	Material Source	Part Used	%
Sargassum glaucescens Extract	Taiwan	*Sargassum glaucescens*	Whole	20%
Water	Taiwan	-	-	79%
Phenoxyethanol	Germany	Chemical synthesis	-	1%

Manufacturing Process Flow



* *Sargassum glaucescens* is extracted with RO water (1 part of *Sargassum glaucescens* to 5 parts of RO water)

Date of Signature: 2018/09/06

NEXT LAB DIRECTOR

QA *DIRECTOR*

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Patch Testing of Ocean White®

Sargassum Glaucescens Extract (as described in the Material Specification)

120 % Sargassum Glaucescens Extract

79 % Water

1 % Phenoxyethanol)

Sponsor：TCI Co., Ltd.

Testing Institute：Center for Cosmetology Research and Testing ,
Chia Nan University of Pharmacy and Science

Study Director: Wei Jing Hung, PhD

Study Dates: November 01, 2015 ~ November 18, 2015

Chia Nan University of Pharmacy and Science
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1. Abstract

The study has completed the patch testing for the Ocean White®(10% Ocean White® solution), developed by TCI Co., Ltd. During the patch testing, there was no evidence of any irritant effect for the Ocean White® as shown in Table 1.

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2. Preface

This research aims at the safety assessment of the Ocean White® provided by TCI Co., Ltd.

The safety assessment included a patch testing of 48 hours to determine the irritation potential of the Ocean White® after application under occlusive patch condition to the skin of 10 subjects.

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3. Materials and Methods

A. The safety assessment of the product is shown below:

1. 10% Ocean White® solution (INCI: Sargassum glaucescens extract; diluted with water)

B. Equipment and methods are as follows:

1. Instrument:

Patch test (IQ Chamber, Chemotechnique Diagnostics, Sweden)

2.Test Area: :

Arm region

3.Test Time:

1) Before applying the sample (Baseline)

2) Applied the sample for 48 hours

4. Subjects:

10 Taiwanese Females（Age from 20 to 30 years）

5.Produces:

Volunteers applied the product for 48 hours patch testing under occlusion. We made a clinical observation of skin irritation evaluation 3 hours after removing the occlusive patches.

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4. Results

10 subjects applied the Ocean White®(10% Ocean White® solution) for 48 hours patch testing under occlusion. A clinical observation of skin irritation evaluation was conducted 3 hours after removing the occlusive patches. There was no evidence of any irritant effect of the Ocean White® evaluating by the patch testing. The results were shown in Table 1.

Table 1. Patch testing for 10% Ocean White® solution

Safety Evaluation / Skin irritation	Patch test (X/Y)*
Itch	0/10
Sting	0/10
Erythema	0/10
Eczema	0/10
Scaliness	0/10
Discontinued	0/10

*: (X/Y), X , Number of irritant effect subjects; Y, Number of total testing subjects.

Chia Nan University of Pharmacy and Science
嘉南藥理大學
Center for Cosmetology Research and Testing,
No.60, Sec. 1, Erren Rd., Rende Dist., Tainan City , R.O.C.
機能性化粧品開發與評估研究中心，台南市仁德區二仁路一段 60 號
TEL:(886-6)2664911 ext:2405, Fax:(886-6) 2667324

5. References

1. Brunner, M. J., and Smiljanic, A. Procedure for the evaluation of skin sensitizing power of new materials, Arch. Dermatol., 66:703, 1952.
2. Shelanski, M. V., The patch test. 1. The history and review of methods, capabilities and inherent limitations, J. Toxicol.Cut. Ocular Toxical., 1(2):91, 1982.
3. Schwartz, L., and Peck, S. M.,The patch test and contact dermatitis , Public Heath Rep., 59:2,1994.

Personal Care Products Council
Committed to Safety, Quality & Innovation

Memorandum

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

FROM: Carol Eisenmann, Ph.D.
Personal Care Products Council

DATE: September 7, 2018

SUBJECT: Sargassum Filipendula Extract

Anonymous. 2007. Repeated insult patch test of a face cream containing 1.2% Sargassum Filipedula Extract.

1620 L Street, N.W., Suite 1200 | Washington, D.C. 20036 | 202.331.1770 | 202.331.1969 (fax) | www.personalcarecouncil.org

[redacted]

REPEATED INSULT PATCH STUDY

[redacted]

[redacted]

Face cream containing
1.2% Sargassum Fil. pendula
Extract

CONDUCTED FOR:

[redacted]

DATE OF ISSUE:

January 18, 2007

[redacted]

[redacted] [redacted]

TABLE OF CONTENTS

APPENDICES

SIGNATURES

[redacted]

[redacted]

STATEMENT OF QUALITY ASSURANCE

This report has been reviewed by the [redacted] Corporate Quality Assurance Department and the report accurately reflects the raw data for this study.

Clinical research studies are performed by [redacted] accordance with all applicable federal regulations and proposed guidelines for Good Clinical Practices, which include:

21 CFR Part 312,	Investigational New Drug Application
21 CFR Part 50,	Protection of Human Subjects
21 CFR Part 56,	Institutional Review Boards

[redacted]

TITLE OF STUDY

Repeated Insult Patch Study

SPONSOR

[redacted]

STUDY MATERIAL

740018 11 Face Cream

DATE STUDY INITIATED

November 6, 2006

DATE STUDY COMPLETED

December 22, 2006

DATE OF ISSUE

January 18, 2007

INVESTIGATIVE PERSONNEL

[redacted]

CLINICAL SITES

[redacted]

[redacted]

SUMMARY

One study material, Formula No. [redacted] was evaluated neat to determine its ability to sensitize the skin of normal volunteer subjects using an occlusive repeated insult patch study. Two hundred six subjects completed the study.

Under the conditions employed in this study, there was no evidence of sensitization to Formula No. [redacted]

[redacted]

1.0 OBJECTIVE

The objective of this study was to determine the ability of the study material to cause sensitization by repeated applications to the skin of humans under controlled patch study conditions.

2.0 RATIONALE

Substances that come into contact with human skin need to be evaluated for their propensity to irritate and/or sensitize. Once an appropriate pre-clinical safety evaluation has been performed, a reproducible, standardized, quantitative patch evaluation procedure must be used to demonstrate that a particular material can be applied safely to human skin without significant risk of adverse reactions. The method herein employed is generally accepted for such a purpose.

Repeated insult patch evaluation is a modified predictive patch study that can detect weak sensitizers that require multiple applications to induce a cell-mediated (Type IV) immune response sufficient to cause an allergic reaction. Irritant reactions may also be detected using this evaluation method, although this is not the primary purpose of this procedure. Results are interpreted according to interpretive criteria based upon published works, as well as the clinical experience of [redacted] Inc. These interpretive criteria are periodically reviewed and amended as new information becomes available.

3.0 STUDY DESIGN

3.1 STUDY POPULATION

A sufficient number of subjects were enrolled to provide 200 completed subjects.

3.1.1 Inclusion Criteria

Individuals eligible for inclusion in the study were those who:

1. were males or females, 18 to 70 years of age, in general good health;
2. were free of any systemic or dermatologic disorder which, in the opinion of the investigative personnel, would have interfered with the study results or increased the risk of adverse events;
3. were of any skin type or race, providing the skin pigmentation would allow discernment of erythema;
4. had completed a medical screening procedure; and
5. had read, understood, and signed an informed consent agreement.

[redacted]

3.1.2 Exclusion Criteria

Individuals excluded from participation in the study were those who:

1. had any visible skin disease at the study site which, in the opinion of the investigative personnel, would have interfered with the evaluation;

2. were receiving systemic or topical drugs or medication which, in the opinion of the investigative personnel, would have interfered with the study results;

3. had psoriasis and/or active atopic dermatitis/eczema;

4. were females who were pregnant, planning to become pregnant during the study, or breast-feeding;

5. had a known sensitivity to cosmetics, skin care products, or topical drugs as related to the material being evaluated; and/or

6. were participating in another study or had been recruited to participate in another study concurrently.

3.1.3 Informed Consent

A properly executed informed consent document in compliance with FDA regulations (21 CFR Part 50) was obtained from each subject prior to entering the study. The signed informed consent document is maintained in the study file. In addition, the subject was provided with a copy of the informed consent document (see Appendix III).

3.2 Description of Study

3.2.1 Outline of Study Procedures

Subjects participated in the study over a 6-week period involving 3 phases: (1) Induction, (2) Rest, and (3) Challenge. Prior to study entry, the subjects were screened to assure that they met the inclusion/exclusion criteria. Informed consent was obtained. Each subject was provided with a schedule of the study activities. All subjects were told to avoid wetting the patches and were asked not to engage in activities that caused excessive perspiration. They were instructed to notify the staff if they experienced any discomfort beyond mild itching or observed any adverse changes at the patch sites, while on the study or within 2 weeks of completing the study.

The Induction Phase consisted of 9 consecutive applications of the study material and subsequent evaluations of the patch sites. Prior to application of the patches, the sites were outlined with a skin marker, eg, gentian violet. The subjects were required to remove the patches approximately 24 hours after application. They returned to the facility at 48-hour intervals to have the sites evaluated and identical patches applied to the same sites. Patches applied on Friday were removed by subjects

after 24 hours. The sites were evaluated on the following Monday, ie, 72 hours after patch application.*

Following the ninth evaluation, the subjects were dismissed for a rest period of approximately 10-15 days.

Subjects who were absent once during the induction phase received a make-up (MU) patch at the last induction visit. The MU applications were graded 48 hours later at the MU visit, or were recorded as N9G (no ninth grading).

The Challenge Phase was initiated during the sixth week of the study. Identical patches were applied to sites previously unexposed to the study material. The patches were removed by subjects after 24 hours and the sites graded after additional 24-hour and 48-hour periods (ie, 48 and 72 hours after application). Rechallenge was performed whenever there was evidence of possible sensitization.

To be considered a completed case, a subject must have had 9 applications and no fewer than 8 subsequent readings during induction, and a single application and 2 readings during challenge. Only completed cases were used to assess sensitization.

3.2.2 Definitions Used for Grading Responses

The symbols found in the scoring scales below were used to express the response observed at the time of examination:

SYMBOL		REACTION
-	=	No reaction
?	=	Minimal or doubtful response, slightly different from surrounding normal skin
+	=	Definite erythema, no edema
++	=	Definite erythema, definite edema
+++	=	Definite erythema, definite edema and vesiculation

SPECIAL NOTATIONS

E	=	Marked/severe erythema
S	=	Spreading of reaction beyond patch site (ie, reaction where material did not contact skin)
p	=	Papular response > 50%
pv	=	Papulovesicular response > 50%
D	=	Damage to epidermis: oozing, crusting and/or superficial erosions
I	=	Itching

* A Monday or Friday holiday could result in evaluation at 96 hours after patch application.

X = Subject absent

PD = Patch dislodged

NA = Not applied

NP = Not patched (due to reaction achieved)

N9G = No ninth grading

3.2.3 Evaluation of Responses

All responses were graded by a trained dermatologic evaluator meeting TKL's strict certification requirements to standardize the assignment of response grades.

4.0 NATURE OF STUDY MATERIAL

4.1 Study Material Specifications

Identification : [redacted] Face Cream
Amount Applied : 0.2 g

4.2 Storage, Handling, and Documentation of Study Material

Receipt of the material used in this study was documented in a general logbook, which serves as a permanent record of the receipt, storage, and disposition of all study material received by [redacted] On the basis of information provided by the sponsor, the study material was considered reasonably safe for evaluation on human subjects. A sample of the study material was reserved and will be stored for a period of 6 months. All study material is kept in a locked product storage room accessible to clinical staff members only. At the conclusion of the clinical study, the remaining study material was discarded or returned to the sponsor and the disposition documented in the logbook.

4.3 Application of Study Material

Study material was applied to the patch as instructed. The patch was applied to the infrascapular area of the back, either to the right or left of the midline, or to the upper arm.

4.4 Description of Patch Conditions

Materials evaluated under occlusive patch conditions are applied to a 2 cm x 2 cm Webril pad attached to a non-porous, plastic film adhesive bandage (3M medical tape). The patches are secured with hypoallergenic tape (Micropore), as needed.

Materials evaluated under semi-occlusive patch conditions are applied to a 2 cm x 2 cm Webril pad. The pads are affixed to the skin with hypoallergenic tape (Micropore).

5.0 INTERPRETATION

Sensitization is characterized by an acute allergic contact dermatitis. Typical sensitization reactions begin with an immunologic response in the dermis resulting in erythema, edema formation, and secondary epidermal damage (vesiculation), sometimes extending beyond the patch site and often accompanied by itching. Sensitization reactions tend to be delayed. The reaction typically becomes evident between 24 and 48 hours, peaks at 48-72 hours and subsequently subsides. The reaction is often greater at 72 hours than at 48 hours. The severity of the reaction is generally greater during the challenge phase of a Repeated Insult Patch Test (RIPT) than that seen during induction.

Irritant reactions are characterized as a non-immunologic, localized, superficial, exudative, inflammatory response of the skin due to an externally applied material. The typical initial reaction does not develop much edema or vesiculation but results in scaling, drying, cracking, oozing, crusting, and erosions. The reaction is usually sharply delineated, not spreading beyond the patch site. Irritant reactions are typically evident by 24 hours and diminish over the next 48-72 hours. Removal of the offending agent results in gradual improvement of the epidermal damage. The reaction seen at 72 hours is, therefore, less severe than that seen at 48 hours. Finally, the severity of the reaction experienced in the challenge phase is generally similar to that seen during induction.

If the results of the study indicate the likelihood of sensitization, the recommended practice is to rechallenge the subjects who have demonstrated sensitization-like reactions to confirm that these reactions are, indeed, associated with the product. Our preferred rechallenge procedure involves the application of the product to naïve sites, under both occlusive and semi-occlusive patch conditions. Use of the semi-occlusive patch condition helps to differentiate irritant and sensitization reactions. Generally speaking, if a product is a sensitizer it will produce a similar reaction under both occlusion and semi-occlusion. Whereas, if the product has caused an irritant reaction, the reactions will be less pronounced under the semi-occlusive condition.

6.0 DOCUMENTATION AND RETENTION OF DATA

The case report forms (CRFs) are designed to identify each subject by subject number and initials, and to record demographics, examination results, adverse events, and end of study status. Originals or copies of all CRFs, correspondence, study reports, and all source data will be kept on hard-copy file for a minimum of 5 years from completion of the study. Storage is maintained either at a [redacted] facility in a secured room accessible only to [redacted] or at an offsite location which provides a secure environment with burglar/fire alarm systems, camera detection and controlled temperature and humidity. Documentation will be available for the sponsor's review on the premises of [redacted]

7.0 RESULTS AND DISCUSSION

Two hundred twenty-three subjects between the ages of 18 and 70 were enrolled and 206 subjects completed the study (see Tables 1 and 2 in Appendix I and Data Listings 1 and 2 in Appendix II).

The following table summarizes subject enrollment and disposition.

Number enrolled:		223
Number discontinued:		17
Lost to follow-up:	16	
Voluntary withdrawal:	1	
Number completed:		206

Source: Table 1, Appendix I

There were no protocol deviations.

There were no adverse events reported.

A summary of response data is provided in Table 3, Appendix I. Individual dermatological response grades are provided in Data Listing 3, Appendix II.

8.0 CONCLUSION

Under the conditions employed in this study, there was no evidence of sensitization to Formula No. 740018 11.

9.0 REFERENCES

Kligman AM. The identification of contact allergens by human assay II. A critique of standard methods. J Invest Dermatol 1966; 47:369.

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Marzulli FN, Maibach HI. Contact allergy: predictive testing in man. Contact Dermatitis 1976; 2:1.

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Fisher AA. 3rd ed. Contact Dermatitis. Philadelphia:Lea & Feiberger, 1986.

Shelanski HA, Shelanski MV. A new technique of human patch tests. Proc Sci Sect Toilet Goods Assoc 1953; 204:107-110.

Jordan WP, King SF. Related hypersensitivity in families. Contact Dermatitis 1977; 3:19-26.

Kligman AM, Epstein W. Updating the maximization test for identifying contact allergens. Contact Dermatitis 1975; 1:231-239.

Stotts, J. Planning, conduct and interpretation of human predictive sensitization patch tests. In: Drill VA, Lazar P, eds. Current Concepts In Cutaneous Toxicity. New York:Academic Press, 1980:41-53.

Personal Care Products Council
Committed to Safety, Quality & Innovation

Memorandum

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

FROM: Carol Eisenmann, Ph.D.
Personal Care Products Council

DATE: September 10, 2018

SUBJECT: Hydrolyzed Fucus Vesiculosis Protein and a trade name mixture containing Laminaria Japonica Extract, Nereocystis Leutkeana Extract and Macrocystis Pyrifera Extract

Active Concepts. 2015. Compositional breakdown AC Phytogel (Hydrolyzed Fucus Vesiculosis Protein).

Active Concepts. 2014. Product specification AC Phytogel (Hydrolyzed Fucus Vesiculosis Protein).

Active Concepts. 2012. Technical data sheet AC Phytogel (Hydrolyzed Fucus Vesiculosis Protein).

Active Concepts. 2018. Compositional breakdown AC Hydrating Seaweed Complex (mixture of Laminaria Japonica, Nereocystis Leutkeana and Macrocystis Pyrifera Extracts).

Active Concepts. 2013. AC Hydrating Seaweed Complex (mixture of Laminaria Japonica, Nereocystis Leutkeana and Macrocystis Pyrifera Extracts) manufacturing flow chart.

Active Concepts. 2017. Product specification AC Hydrating Complex (mixture of Laminaria Japonica, Nereocystis Leutkeana and Macrocystis Pyrifera Extracts).

Active Concepts. 2016. Dermal and ocular irritation tests AC Hydrating Seaweed Complex (mixture of Laminaria Japonica, Nereocystis Leutkeana and Macrocystis Pyrifera Extracts).

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Compositional Breakdown

AC Phytogel
Code: 20606

Compositional Breakdown:

ingredient	%
Hydrolyzed Fucus Vesiculosus Protein	98.90
Phenonip	1.00
Tetrasodium EDTA	0.10

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Compositional Breakdown

This is to certify that the following allergens were not detected in AC Phytogel:

ALLERGENS Dir 2003 15 CEE	
INCI NAME	CAS NUMBER
Alpha-IsoMethyl Ionone	127-51-5
Amyl Cinnamal	122-40-7
Anise Alcohol	105-13-5
Benzyl Alcohol	100-51-69
Benzyl Benzoate	120-51-4
Benzyl Cinnamate	103-41-3
Benzyl Salicylate	118-58-1
Butylphenyl Methylpropional	80-54-6
Cinnamal	104-55-2
Cinnamyl Alcohol	104-54-1
Citral	5392-40-5
Citronellol	106-22-9
Coumarin	91-64-5
Eugenol	97-53-0
Farnesol	4602-84-0
Geraniol	106-24-1
Hexyl Cinnamal	101-86-0
Hydroxycitronellal	107-75-5
Hydroxymethylpentyl 3-Cyclohexene carboxaldehyde	31906-04-4
Isoeugenol	97-54-1
Limonene	5989-27-5
Linalool	78-70-6
Methyl 2 Octynoate	111-12-6
Evernia prunastri	90028-68-5
Evernia furfuracea	90028-67-4
Amylcinnamyl Alcohol	101-85-9

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Product Specification

Product Name: AC Phytogel
Code Number: 20606
CAS #'s: 84696-13-9
EINECS #'s: 283-633-7
INCI Name: Hydrolyzed Fucus Vesiculosus Protein
Status: Approved

Specification	Parameter
Appearance	Viscous Gel
Odor	Characteristic
pH (direct)	5.5 – 7.5
NVM (1g-1hr-105°C)	3.0% Minimum
Ash	1.0% Maximum
Nitrogen (Kjeldahl)	0.3% Minimum
Heavy Metals	< 20 ppm
Arsenic	< 2 ppm
Microbial Content	< 100 opg No pathogens

May Sediment upon Standing; Mix Well Prior to Use

Technical Data Sheet

Tomorrow's Vision... *Today!*®

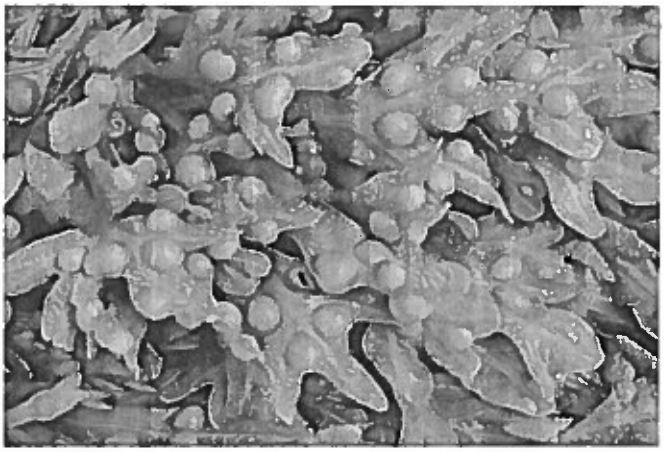
AC Phytogel

Code Number:	20606
INCI Nomenclature:	Hydrolyzed Fucus Vesiculosus Protein
INCI Status:	Approved
Suggested Use Levels:	1.0 - 5.0%
Suggested Applications:	Moisturization, Thickening, Film-Forming

Animal gelatin has long been utilized in the cosmetic industry. It is a tremendous moisturizing and film-forming agent that also helps to increase the viscosity of a formulation. In recent years, however, the use of animal derived products has dramatically decreased. Manufacturers and formulators alike are faced with the dilemma of replacing those animal derived products with alternate products derived from plant materials, products derived through biotechnology, or purely synthetic materials.

Animal gelatin is a water-soluble product that arises from the dissolution, degradation, and disorganization of the highly organized water insoluble collagen fibers. It is this disorganization of the network of linked tropocollagen units to form with a much-decreased degree of internal order that gives rise to gelatin. This disorganized state, is what gives gelatin its thick, rigid structure which provides for its much-desired cosmetic characteristics.

Since collagen is the major protein constituent found in gelatin, it is necessary to more closely examine the composition of collagen itself. Collagens, no matter the source from which they are procured, have a highly characteristic amino acid distribution. One of the most notable features of collagen is the amino acid content. Collagen consists of the imino acids proline and hydroxyproline which comprise approximately 15-30% of the overall residue content of collagen. Hydroxyproline is the key-identifying agent in this particular class of proteins, since it is found almost exclusively in collagens. Collagens found in lower organisms typically contain less praline and hydroxyproline than do the higher organisms, such as animals. It is important to note that hydroxyproline is present in significant amounts in major glycoprotein of primary plant cell walls. This unusual amino acid is produced in plant cell wall in response to injury.



The exact structure of gelatin is difficult to mimic without employing an animal derived material. However, achieving the functionality of an animal gelatin by applying another source as well as novel chemistry, is attainable.

AC Phytogel has been developed to meet the need of today's formulator. By utilizing fractions of the cell walls of *Fucus vesiculosus*, we are able to closely mimic the functionality of animal gelatin. *Fucus vesiculosus* cell walls are rich in polyguluronate subunits. It is the arrangement of these carbohydrate units that allow for the gelatin-like properties of **AC Phytogel**.

Active Concepts
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AC Phytogel

AC Phytogel may be used in any formulation where animal gelatin needs to be replaced. Typical use levels for **AC Phytogel** are 1-5% and can be applied in formulations as a thickening and moisturizing agent, a film former, viscosity builder or suspending agent. **AC Phytogel** will provide a formulation with the same aesthetic properties as that of animal gelatin, but in a "greener" more environmentally conscious fashion.

Moisturization Comparison

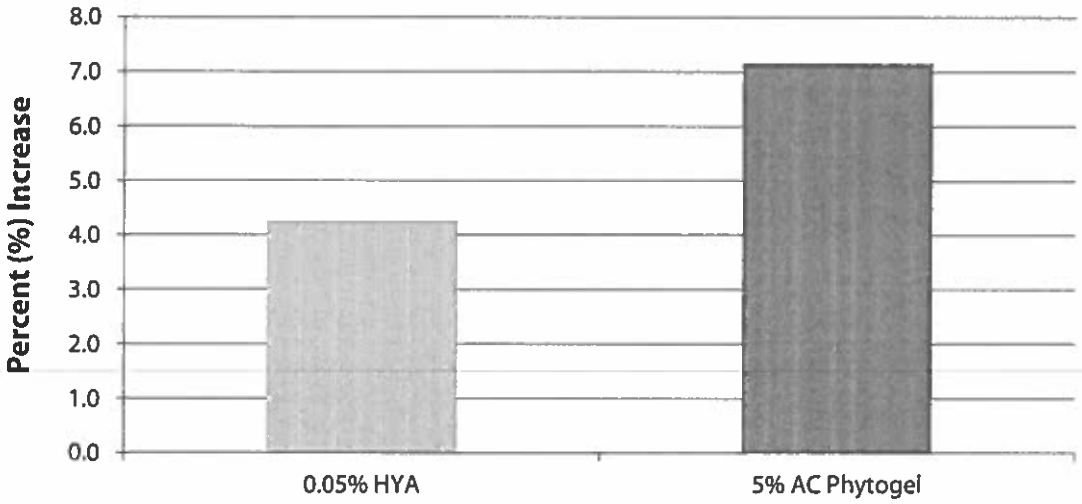

Figure 1. Results of the increase in moisturization based on cost contribution of the test materials.

Protocol for Moisturization Test:

Skin Moisturization:	10 m/f subjects (between the ages of 23 and 42)
Duration of Study:	6 weeks
Equipment:	9003 DPM Nova Impendance Meter
Site:	3 sites tested - Sodium Hyaluronate, Base Lotion and Test Material (AC Phytogel) on Volar Forearm
Conditions:	Subjects applied twice daily

References:

1) Cole, CGB (2000), "Gelatin", In Francis, FJ, Encyclopedia of Food Science and Technology, 2nd edition, John Wiley & Sons, pp. 1183–1188
2) U.S. Food and Drug Administration. "The Sourcing and Processing of Gelatin to Reduce the Potential Risk Posed by Bovine Spongiform Encephalopathy (BSE) in FDA-Regulated Products for Human Use
3) The Scientific Steering Committee (6–7 March 2003). "Updated Opinion On The Safety With Regard To TSE Risks Of Gelatine Derived From Ruminant Bones or Hides".
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6) R.H.A. Plimmer (1912) [1908]. R.H.A. Plimmer & F.G. Hopkins. ed. The chemical composition of the proteins. Monographs on biochemistry. Part I. Analysis (2nd ed.). London: Longmans, Green and Co.. p. 132. Retrieved January 18, 2010.

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Compositional Breakdown

AC Hydrating Seaweed Complex
Code: 16504

Compositional Breakdown:

Ingredient	%
Pentaerythrityl Tetraethylhexanoate	79.00
Laminaria Japonica Extract	7.00
Nereocystis Leutkeana Extract	7.00
Macrocystis Pyrifera Extract	7.00

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Compositional Breakdown

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This is to certify that AC Hydrating Seaweed Complex does not contain, neither directly nor through cross contamination, any of the 26 allergenic flavors or fragrances (Gas Chromatography-Mass Spectrometer Coupled):

ALLERGENS listed in Annex III of EU Cosmetic Regulation(EC) No. 1223/2009 amending EU Directive 2003/15/EC		
INCI NAME	CAS NUMBER	Limit (ppm)
Alpha-IsoMethyl Ionone	127-51-5	< 0.02
Amyl Cinnamal	122-40-7	< 0.10
Anise Alcohol	105-13-5	< 0.00
Benzyl Alcohol	100-51-6	< 0.01
Benzyl Benzoate	120-51-4	< 0.09
Benzyl Cinnamate	103-41-3	< 0.30
Benzyl Salicylate	118-58-1	< 0.06
Butylphenyl Methylpropional	80-54-6	< 0.50
Cinnamal	104-55-2	< 0.01
Cinnamyl Alcohol	104-54-1	< 0.30
Citral	5392-40-5	< 1.00
Citronellol	106-22-9	< 1.00
Coumarin	91-64-5	< 0.00
Eugenol	97-53-0	< 0.70
Farnesol	4602-84-0	< 0.04
Geraniol	106-24-1	< 0.08
Hexyl Cinnamal	101-86-0	< 0.40
Hydroxycitronellal	107-75-5	< 1.00
Hydroxymethylpentyl 3-Cyclohexene carboxaldehyde	31906-04-4	< 0.00
Isoeugenol	97-54-1	< 0.06
Limonene	5989-27-5	< 0.05
Linalool	78-70-6	< 0.00
Methyl 2 Octynoate	111-12-6	< 0.20
Evernia prunastri	90028-68-5	< 0.02
Evernia furfuracea	90028-67-4	< 0.00
Amylcinnamyl Alcohol	101-85-9	< 1.00

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Compositional Breakdown

This is to certify that AC Hydrating Seaweed Complex does not contain pesticide levels exceeding the following (Reverse Phase High Performance Liquid Chromatography-Mass Spectrometer Coupled):

EPA Pesticide Levels	
INCI NAME	LIMIT (mg/kg)
Alachlor	< 0.02
Aldrin and Dieldrin	< 0 .05
Azinphos-methyl	< 1. 00
Bromopropylate	< 3.0 0
Chlordane(cis and trans)	< 0.05
Chlorfenvinphos	< 0.50
Chlorpyrifos	< 0.20
Chlorpyrifos-methyl	< 0.10
Cypermethrin	< 1.00
DDT	< 1.00
Deltamethrin	< 0.50
Diazinon	< 0.50
Dichlorvos	< 1.00
Dithiocarbamates	< 2.00
Endosulfan	< 3.00
Endrin	< 0.05
Ethion	< 2.00
Fenitrothion	< 0.50
Fenvalerate	< 1.50
Fonofos	< 0.05
Heptachlor	< 0.05
Hexachlorobenzene	< 0.10
Hexachlorocyclohexane	< 0.30
Lindane	< 0.60
Malathion	< 1.00
Methidathion	< 0.20
Parathion	< 0.50
Parathion-methyl	< 0.20

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Compositional Breakdown

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Permethrin	< 1.00
Phosalone	< 0.10
Piperonyl butoxide	< 3.00
Pirimiphos-methyl	< 4.00
Pyrethrins	< 3.00
Quintozene(sum of 3 items)	< 1.00

16504-AC Hydrating Seaweed Complex- Manufacturing Flow Chart

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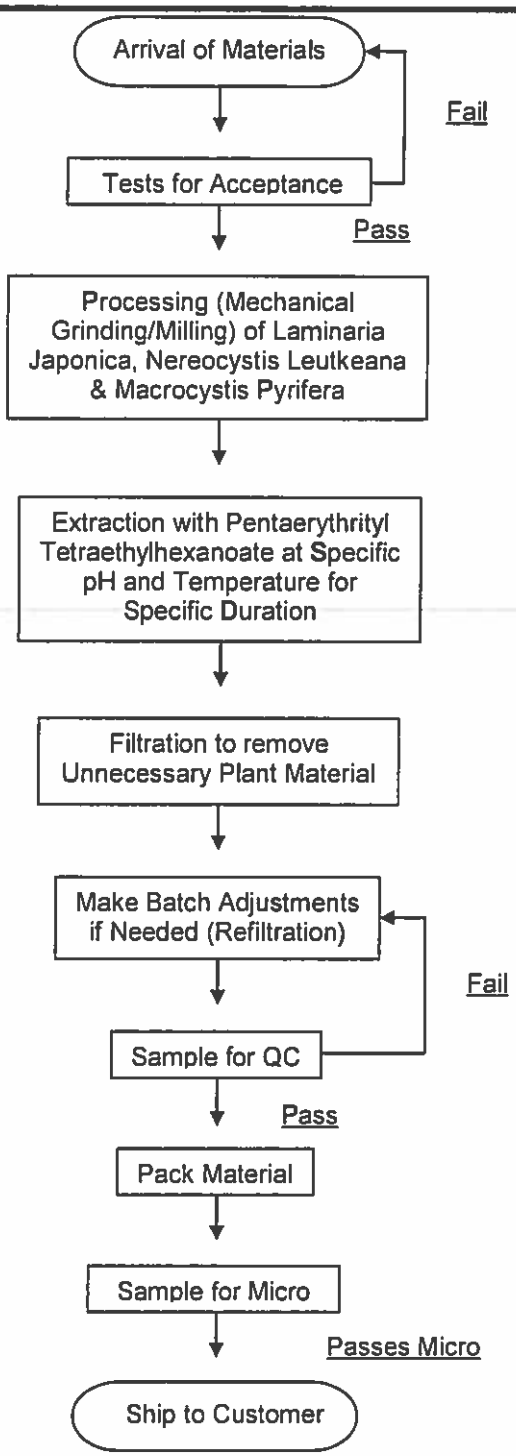

Active Concepts

Product Specification

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Product Name: AC Hydrating Seaweed Complex
Code Number: 16504
CAS #'s: 7299-99-2 & 92128-82-0 & 84696-13-9 & 84696-13-9
EINECS #'s: 230-743-8 & 295-780-4 & 283-633-7 & 283-633-7
INCI Name: Pentaerythrityl Tetraethylhexanoate & Laminaria Japonica Extract & Nereocystis Leutkeana Extract & Macrocystis Pyrifera Extract
Status: Approved

Specification	Parameter
Appearance	Clear Liquid
Color	Colorless to Light Yellow
Odor	Characteristic
Refractive Index (25°C)	1.4470 – 1.4570
Specific Gravity (25°C)	0.920 – 1.020
Iodine	< 50 ppm
Infrared Spectrum	To Match Standard
Heavy Metals	< 20 ppm
Lead	< 10 ppm
Arsenic	< 2 ppm
Cadmium	< 1 ppm
Microbial Content Yeast & Mold Gram Negative Bacteria	< 100 CFU/g; No pathogens < 100 CFU/g 0 CFU/g

May Sediment upon Standing; Mix Well Prior to Use

**Note: Product may change appearance if exposed to cold temperatures during shipment or storage. If this happens, please gently warm to 45-50°C and mix until normal appearance is restored.

Active Concepts

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Dermal and Ocular Irritation Tests

Sample: AC Hydrating Seaweed Complex

Code: 16504

CAS #: 7299-99-2 & 92128-82-0 & 84696-13-9 & 84696-13-9

Test Request Form/Submission #: 2664

Lot #: NC161005-C

Sponsor: *Active Concepts, LLC; 107 Technology Drive Lincolnton, NC 28092*
Study Director: *Maureen Danaher*
Principle Investigator: *Jennifer Goodman*

Test Performed:
In Vitro EpiDerm™ Dermal Irritation Test (EPI-200-SIT)
EpiOcular™ Eye Irritation Test (OCL-200-EIT)

SUMMARY

In vitro dermal and ocular irritation studies were conducted to evaluate whether **AC Hydrating Seaweed Complex** would induce dermal or ocular irritation in the EpiDerm™ and EpiOcular™ model assays.

The product was tested according to the manufacture's protocol. The test article solution was found to be **non-irritating**. Reconstructed human epidermis and cornea epithelial model were incubated in growth media overnight to allow for tissue equilibration after shipping from MatTek Corporation, Ashland, MA. Test substances were applied to the tissue inserts and incubated for 60 minutes for liquid and solid substances in the EpiDerm™ assay and 30 minutes for liquid substances and 90 minutes for solid substances in the EpiOcular™ assay at 37°C, 5% CO_2, and 95% relative humidity (RH). Tissue inserts were thoroughly washed and transferred to fresh plates with growth media. After post substance dosing incubation is complete, the cell viability test begins. Cell viability is measured by dehydrogenase conversion of MTT [(*3-4,5-dimethyl thiazole 2-yl*)], present in the cell mitochondria, into blue formazan salt that is measured after extraction from the tissue. The irritation potential of the test chemical is dictated by the reduction in tissue viability of exposed tissues compared to the negative control.

Under the conditions of this assay, the test article was considered to be **non-irritant**. The negative and positive controls performed as anticipated.

Active Concepts

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Dermal and Ocular Irritation Tests

I. Introduction

A. Purpose

In vitro dermal and ocular irritation studies were conducted to evaluate whether a test article would induce dermal or ocular irritation in the EpiDerm™ and EpiOcular™ model assays. MatTek Corporation's reconstructed human epidermal and human ocular models are becoming a standard in determining the irritancy potential of test substances. They are able to discriminate between irritants and non-irritants. The EpiDerm™ assay has accuracy for the prediction of UN GHS R38 skin irritating and no-label (non-skin irritating) test substances. The EpiOcular™ assay can differentiate chemicals that have been classified as R36 or R41 from the EU classifications based on Dangerous Substances Directive (DSD) or between the UN GHS Cat 1 and Cat 2 classifications.

II. Materials

A. Incubation Conditions:	37°C at 5% CO_2 and 95% relative humidity
B. Equipment:	Forma humidified incubator, ESCO biosafety laminar flow hood, Synergy HT Microplate reader; Pipettes
C. Media/Buffers:	DMEM based medium; DPBS; sterile deionized H_2O
D. Preparation:	Pre-incubate (37°C) tissue inserts in assay medium; Place assay medium and MTT diluent at 4°C, MTT concentrate at -20°C, and record lot numbers of kit components
E. Tissue Culture Plates:	Falcon flat bottom 96-well, 24-well, 12-well, and 6-well tissue culture plates
F. Reagents:	MTT (1.0mg/mL); Extraction Solution (Isopropanol); SDS (5%); Methyl Acetate
G. Other:	Nylon Mesh Circles (EPI-MESH); Cotton tip swabs; 1mL tuberculin syringes; Ted Pella micro-spatula; 220mL specimen containers; sterile disposable pipette tips; Parafilm

III. Test Assay

A. Test System

The reconstructed human epidermal model, EpiDerm™, and cornea epithelial model, EpiOcular™, consist of normal human-derived epidermal keratinocytes which have been cultured to form a multilayer, highly differentiated model of the human epidermis and cornea epithelium. These models consist of organized basal, spinous, and granular layers, and the EpiDerm™ systems also contains a multilayer stratum corneum containing intercellular lamellar lipid layers that the EpiOcular™ system is lacking. Both the EpiDerm™ and EpiOcular™ tissues are cultured on specially prepared cell culture inserts.

B. Negative Control

Sterile DPBS and sterile deionized water are used as negative controls for the EpiDerm™ and EpiOcular™ assays, respectfully.

C. Positive Control

Known dermal and eye irritants, 5% SDS solution and Methyl Acetate, were used as positive controls for the EpiDerm™ and EpiOcular™ assays, respectfully.

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D. Data Interpretation Procedure

a. EpiDerm™

An irritant is predicted if the mean relative tissue viability of the 3 tissues exposed to the test substance is reduced by 50% of the mean viability of the negative controls and a non-irritant's viability is > 50%.

b. EpiOcular™

An irritant is predicted if the mean relative tissue viability of the 2 tissues exposed to the test substance is reduced by 60% of the mean viability of the negative controls and a non-irritant's viability is > 40%.

IV. Method

A. Tissue Conditioning

Upon MatTek kit arrival at Active Concepts, LLC the tissue inserts are removed from their shipping medium and transferred into fresh media and tissue culture plates and incubated at 37°C at 5% CO_2 and 95% relative humidity for 60 minutes. After those 60 minutes the inserts are transferred into fresh media and tissue culture plates and incubated at 37°C at 5% CO_2 and 95% relative humidity for an additional 18 to 21 hours.

B. Test Substance Exposure

a. EpiDerm™

30µL (liquid) or 25mg (solid) of the undiluted test substance is applied to 3 tissue inserts and allowed to incubate for 60 minutes in a humidified incubator (37°C, 5% CO_2, 95% RH).

b. EpiOcular™

Each tissue is dosed with 20µL DPBS prior to test substance dosing. 50µL (liquid) or 50mg (solid) of the undiluted test substance is applied to 2 tissue inserts and allowed to incubate for 90 minutes in a humidified incubator (37°C, 5% CO_2, 95% RH).

C. Tissue Washing and Post Incubation

a. EpiDerm™

All tissue inserts are washed with DPBS, dried with cotton tipped swab, and transferred to fresh media and culture plates. After 24 hours the inserts are again transferred into fresh media and culture plates for an additional 18 to 20 hours.

b. EpiOcular™

Tissue inserts are washed with DPBS and immediately transferred into 5mL of assay medium for 12 to 14 minutes. After this soak the inserts are transferred into fresh media and tissue culture plates for 120 minutes for liquid substances and 18 hours for solid substances.

D. MTT Assay

Tissue inserts are transferred into 300µL MTT media in pre-filled plates and incubated for 3 hours at 37°C, 5% CO_2, and 95% RH. Inserts are then removed from the MTT medium and placed in 2mL of the extraction solution. The plate is sealed and incubated at room temperature in the dark for 24 hours. After extraction is complete the tissue inserts are pierced with forceps and 2 x 200µL aliquots of the blue formazan solution is transferred into a 96 well plate for Optical Density reading. The spectrophotometer reads the 96-well plate using a wavelength of 570 nm.

V. Acceptance Criterion

A. Negative Control

The results of this assay are acceptable if the mean negative control Optical Density (OD_{570}) is ≥ 1.0 and ≤ 2.5 (EpiDerm™) or ≥ 1.0 and ≤ 2.3 (EpiOcular™).

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B. Positive Control

a. EpiDerm™

The assay meets the acceptance criterion if the mean viability of positive control tissues expressed as a % of the negative control is ≤ 20%.

b. EpiOcular™

The assay meets the acceptance criterion if the mean viability of positive control tissues is < 60% of control viability.

C. Standard Deviation

Since each irritancy potential is predicted from the mean viability of 3 tissues for EpiDerm™ and 2 tissues for EpiOcular™, the variability of the replicates should be < 18% for EpiDerm™ and < 20% EpiOcular™.

VI. Results

A. Tissue Characteristics

The tissue inserts included in the MatTek EpiDerm™ and EpiOcular™ assay kits were in good condition, intact, and viable.

B. Tissue Viability Assay

The results are summarized in Figure 1. In no case was the tissue viability ≤ 50% for EpiDerm™ or ≤ 60% for EpiOcular™ in the presence of the test substance. The negative control mean exhibited acceptable relative tissue viability while the positive control exhibited substantial loss of tissue viability and cell death.

C. Test Validity

The data obtained from this study met criteria for a valid assay.

VII. Conclusion

Under the conditions of this assay, the test article substance was considered to be **non-irritating**. The negative and positive controls performed as anticipated.

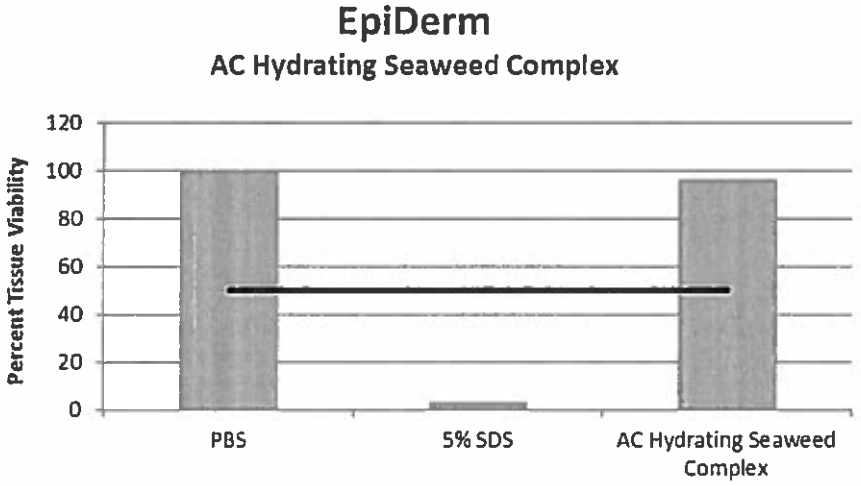

Figure 1: EpiDerm tissue viability

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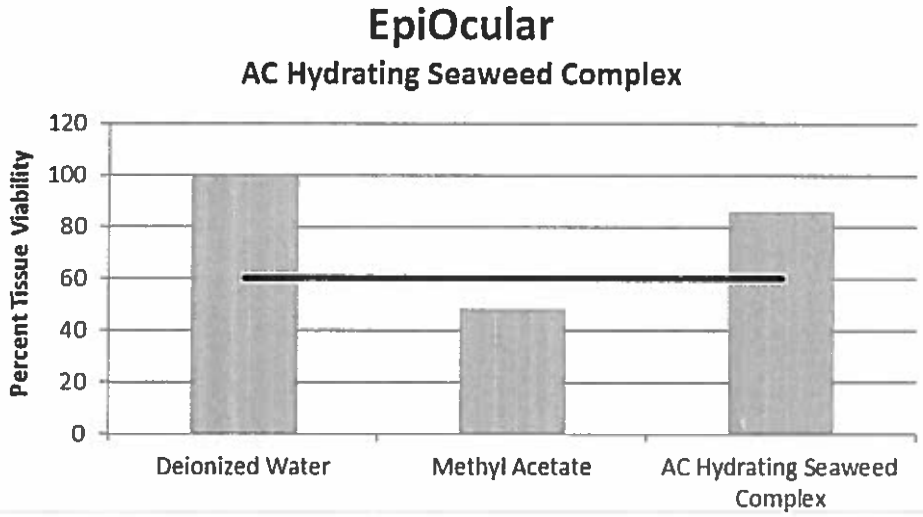


Figure 2: EpiOcular tissue viability

Personal Care Products Council
Committed to Safety, Quality & Innovation

Memorandum

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

FROM: Carol Eisenmann, Ph.D.
Personal Care Products Council

DATE: September 10, 2018

SUBJECT: Alaria Esculenta Extract

Biotech Marine. 2015. Manufacturing process Alariane™ (Alaria Esculenta Extract in butylene glycol and water).

Biotech Marine. 2015. Alariane™ Physico-chemical data (Alaria Esculenta Extract in butylene glycol and water).

Liskin. 2008. Study of the sensitizing capacity of a product according to the Marzulli-Maibach Method (Alariane - Alaria Esculenta Extract in butylene glycol and water).

Laboratoire Cosderma. 2007. Verification chez l'homme de la compatibilite cutanee d'un produit cosmetique apres application unique sous pansement. Patch test 24 h (Alariane - Alaria Esculenta Extract in butylene glycol and water).

Biotech Marine. 2015. Manufacturing process Alariane™ AD (Alaria Esculenta Extract in butylene glycol and water - dried before extraction).

Biotech Marine. 2015. Alariane™ AD Physico-chemical data (Alaria Esculenta Extract in butylene glycol and water - dried before extraction).

Biotech Marine. 2015. Manufacturing process Juvenessence™ (Alaria Esculenta Extract in Caprylic Capric Triglycerides).

Biotech Marine. 2017. Juvenessence™ Physicochemical data (Alaria Esculenta Extract in Caprylic Capric Triglycerides).

1620 L Street, N.W., Suite 1200 | Washington, D.C. 20036 | 202.331.1770 | 202.331.1969 (fax) | www.personalcarecouncil.org

Liskin. 2012. Sensitizing potential study of a product according to Marsulli-Maibach method (Juvenessence™ - Alaria Esculenta Extract in Caprylic Capric Triglycerides).

Laboratoire Cosderma. 2012. Checking in human of the skin compatibility of a cosmetic product after single application under patch (Patchtest 24 h) (Juvenessence™ - Alaria Esculenta Extract in Caprylic Capric Triglycerides).

Biotech Marine. 2015. Manufacturing process Kalpariane™ (Alaria Esculenta Extract in Caprylic Capric Triglycerides).

Biotech Marine. 2016. Kalpariane™ Physicochemical data (Alaria Esculenta Extract in Caprylic Capric Triglycerides).

Liskin. 2008. Study of the sensitizing capacity of a product according to the Marzulli-Maibach method (Kalpariane™ - Alaria Esculenta Extract in Caprylic Capric Triglycerides).

Biotech Marine. 2015. Manufacturing process Kalpariane™ AD (Alaria Esculenta Extract in Caprylic Capric Triglycerides - dried before extraction).

Biotech Marine. 2016. Kalpariane™ AD Physicochemical data (Alaria Esculenta Extract in Caprylic Capric Triglycerides - dried before extraction).

'Laboratoire Cosderma. 2007. Verification in humans of the skin compatibility of a cosmetic product after single application under dressing 24 hour patch test Olea Alaria (<5% Alaria Esculenta Extract; >95% Caprylic/Capric Triglycerides).

MANUFACTURING PROCESS
ALARIANE™

HARVESTING / IDENTIFICATION(*Alaria Esculenta*)

↓

WASHING

↓

GRINDING

↓

EXTRACTION WITH THE SOLVENTS
BUTYLENE GLYCOL AND WATER

↓

FILTRATION

↓

QUALITY CONTROL

↓

PACKAGING

↓

QUALITY CONTROL

Production Manager
Jean-Marc CATROUX

ALARIANE™

INCI NAME : Aqua/water – Butylene glycol – Alaria Esculenta extract
CAS N°: 7732-18-5 – 107-88-0 -
EINECS N°: 231-791-2 – 203-529-7 -

DONNEES PHYSICOCHIMIQUES
PHYSICO-CHEMICAL DATA
Numéro de référence / *Reference number* : STANDARD

CARACTERISTIQUES *CHARACTERISTICS*	**METHODES** *METHODS*	**STANDARD** *STANDARD*
Aspect *Aspect*	MO PHY 002	**Liquide limpide à légèrement opalescent** *Limpid to slightly opalescent liquid*
Couleur *Colour*	MO PHY 002	**Jaune à brun** *Yellow to brown*
Odeur *Odour*	MO PHY 002	**Caractéristique** *Characteristic*
pH	MO PHY 009	5,5 – 7,5
Densité (20°C) *Density*	MO PHY 024	1,010 - 1,040
Indice de réfraction(20°C) *Refractive index*	MO PHY 008	$1{,}385_0$ - $1{,}405_0$
Extrait sec (1g – 4 heures – 105°C) *Dry extract*	MO PHY 033	0,5 - 2,5 %
Teneur en eau *Water content*	MO PHY 018	48,0 – 52,0 %
Butylene glycol *Butylene glycol*	MO PHY 001	48,0 – 52,0 %
Conservateur* *Preservative**		**Absence** *None*
Spectre UV *UV spectrum*	MO PHY 013	**Conforme au témoin** *Similar to the standard*
Spectre IR *IR spectrum*	MO PHY 011	**Conforme au témoin** *Similar to the standard*

* *Sous contrôle statistique / Under statistical control*

ALARIANE™

INCI NAME : Aqua/water – Butylene glycol – Alaria Esculenta extract
CAS N°: 7732-18-5 – 107-88-0 -
EINECS N°: 231-791-2 – 203-529-7 -

DONNEES PHYSICOCHIMIQUES
PHYSICO-CHEMICAL DATA
Numéro de référence / *Reference number* : STANDARD

CARACTERISTIQUES *CHARACTERISTICS*	STANDARD *STANDARD*
Métaux lourds** *Heavy metals** (ppm)*	
• **Arsenic mineral** *Mineral Arsenic*	< 5
• **Cadmium** *Cadmium*	< 3
• **Plomb** *Lead*	< 5
• **Nickel** *Nickel*	< 2
• **Argent** *Silver*	< 5

** Teneurs garanties sous contrôle statistique / *Contents guaranteed under statistical control*

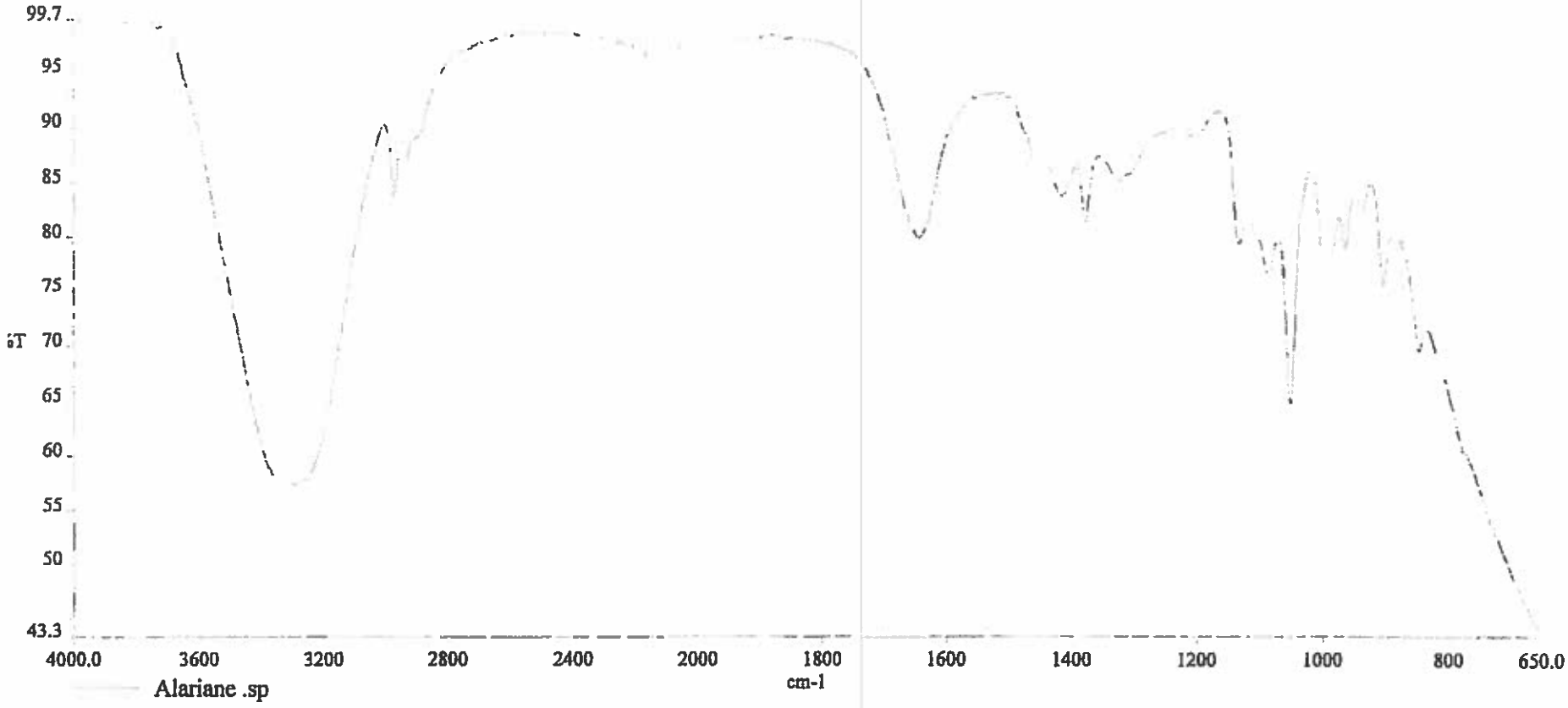
Iodine content < 10 ppm

Date: vendredi 3 juillet 2015

SPECTRE IRFT

SPECTROPHOTOMETRE SPECTRUM 100 N° de série 77774 - PERKIN ELMER

Accessoire ATR Universel N° 7031330



ALARIANE ...

INCI NAME : Aqua/water – Butylene glycol – Alaria Esculenta extract
CAS N°: 7732-18-5 – 107-88-0 -
EINECS N°: 231-791-2 – 203-529-7 -

DONNEES MICROBIOLOGIQUES
MICROBIOLOGICAL DATA
Numéro de référence / *Reference number* : STANDARD

CARACTERISTIQUES *CHARACTERISTICS*	**METHODES** *METHODS*	**STANDARD** *STANDARD*
Germes totaux* ** *Total germs****	MO MIC 002	< 100
Germes Pathogènes *Pathogens*		
- *Staphylococcus aureus*	MO MIC 012	**Absence** *None*
- *Candida albicans*	MO MIC 010	**Absence** *None*
- *Pseudomonas aeruginosa*	MO MIC 011	**Absence** *None*
- *Enterobacteriaceae*	MO MIC 020	**Absence** *None*
Levures / Moisissures* ** *Yeasts / Moulds****	MO MIC 021	< 100

*** Les résultats sont indiqués en UFC/mL pour les liquides et en UFC/g pour les solides
*** *Results are indicated in CFU/mL for the liquids and in CFU/g for the solids*

CONFORME
CERTIFIED TRUE AND CORRECT
RESPONSABLE CONTROLE QUALITE: **P. SOUBIES**
QUALITY CONTROL MANAGER
26 OCT. 2015

CONFORME
CERTIFIED TRUE AND CORRECT
COORDINATRICE ASSURANCE QUALITE : **M. TANNIOU**
QUALITY ASSURANCE COORDINATOR
26 OCT. 2015

LISKIN

STUDY OF THE SENSITIZING CAPACITY OF A PRODUCT ACCORDING TO THE MARZULLI-MAIBACH METHOD

REPORT

STUDY REF.	**ET-319**
PRODUCT	**"ALARIANE BATCH 6.04.134"**
NUMBER OF SUBJECTS	**50**
INSTIGATOR	**EUROTEST**
MONITOR	**M. Bogdan WICHROWSKI** **LISKIN** **Immeuble Fontenay Affaires** **91 Rue Boucicaut** **92260 FONTENAY-AUX-ROSES** **Tel. 33 (0)9 50 27 08 28** **Fax. 33 (0)1 49 73 66 80**
INVESTIGATOR	**Dr Marlena Nowakowska, Dermatologist**

Document containing 23 pages (21 pages in English version)

TABLE OF CONTENTS

SUMMARY OF STUDY

TITLE: STUDY OF THE SENSITIZING CAPACITY OF A PRODUCT « ALARIANE BATCH 6.04.134», ACCORDING TO THE MARZULLI-MAIBACH METHOD ON 50 SUBJECTS DURING 6 WEEKS.

STUDY REFERENCE: ET-319

PRODUCT: ALARIANE BATCH 6.04.134

STUDY LOCATION: The study was carried out and the numerical values recorded by the clinical unit 'PROCOS' in Poland: ul. Stowackiego 27/33 llok. 33/34, 01-592 Varsovie.

INVESTIGATOR: Dr Marlena NOWAKOWSKA

STUDY MONITOR: Dr Ing. Bogdan WICHROWSKI

PROTOCOL: MAXIMALISATION TEST ACCORDING TO MARZULLI-MAIBACH FOR A PRODUCT

PURPOSE: To evaluate the irritation and sensitization potential of a given product under dermatological control in the conditions established by the sponsor of the study.

SUBJECTS: 50 volunteers with normal skin corresponding to the inclusion and non-inclusion criteria established by LISKIN.

TEST DATES: 11/08/08 – 19/09/08

EXPERIMENTAL PLAN: Monocentric study in simple blind trial.

MAIN TOLERANCE PARAMETERS:

- Irritation potential (induction phase)
  - Erythema, oedema, dryness, blisters, evaluated by a dermatologist according to a scale of 0 to 3
- Sensitizing potential (revelation phase)
  - Reaction evaluated by the dermatologist according to a scale of 0 to 3 established by the ICDRG (International Contact Dermatitis Research Group)

RESULTS

Name of Product	Irritation Potential	Sensitizing Potential
ALARIANE BATCH 6.04.134	Average Score 0.000 = non irritant	Total absence of allergic reaction

CONCLUSION:

Under the conditions of this study, the product « ALARIANE BATCH 6.04.134 » proved to be non irritant and non sensitizing.

1. QUALITY ASSURANCE

The study was carried out according the 'Bonnes Pratiques Cliniques' (Good Clinical Practices) rules defined by the FDA (FR of 08/08/1978. Part V, Decree number 77N-0278), by the EEC (Directives number 91/507 and III 3976/88 EN of 11/07/1990) and by the Minstry of Health of the French Republic.

The study carried about according to the standard operational procedures and according to the protocol of the study as defined by the instigator of the study. The observation books and daily notes were checked as were the exactitude of the facts.

The authenticity and truth of the experimental facts collected were confirmed by the people having taken part in the study. See APPENDIX I.

2. CERTIFICATE OF CONFORMITY

To my knowledge, study ET-319 was carried out in accordance with the **"Quality Assurance"** quoted above.

No event liable to affect the quality or integrity of the facts occurred.

Dr Eng. B WICHROWSKI

Monitor

1 October 2008

3. METHODOLOGY

3.1 DESCRIPTION OF THE STUDY

3.1.1 Product studied

The product supplied by EUROTEST, has the following characteristics:

Name Of Product DH	Nature Of Product	Code Of Product Studied
ALARIANE BATCH 6.04.134	Transparent, brown oil	DB

The product was received 05/08/2008.

3.2 CLINICAL METHODS

3.2.1 Study Objectives

To appreciate the irritation and sensitization capacity of the product using the sensitization method Marzulli-Maibach.

3.2.2 Experimental Plan

This was an open study.

3.2.3 Subjects studied

Inclusion Criteria

- Healthy volunteer of Caucasian origin
- Age between 18 and 65
- Phototype II, III and IV
- Normal skin
- Subject having given a willing, informed consent in writing
- Co-operative subject advised about the necessity and duration of the controls enabling the expectation of a perfect adhesion to the protocol put in place by LISKIN.

Exclusion Criteria

- Pregnant or breast-feeding woman
- Person having been exposed to the sun or UV since less than a month and/or having received photo patch tests since less than two months
- Subject presenting a hyper irritable skin
- Subject allergic to adhesive bandages and/or cosmetic products
- Subject have a cutaneous pathology in the experimental zone
- Subject having a serious or evolutive illness
- Subject receiving a topical or systematic medical treatment: - anti-inflammatory, anti-histaminic, immune-suppressors, cortisone and retinoidal

Inclusion

Fifty volunteer subjects were chosen according to the inclusion and exclusion criteria, and 50 subjects completed the whole study. The following table summarizes the information concerning the participation in the study of all the selected subjects.

	Not included	Included	Stopped during study	Loss of contact
Number of subjects		50		
Reason				
Day of event				

Characteristics of the subjects

The table below gives a summary of the observations concerning only those volunteers included in the analysis of data.

Number of volunteers	Sex	Age (Av ±MES)	Phototype	Medical or surgical intervention and medical treatment	
				Before study	During study
50	37F 13M	43 ± 2	I : 0 II : 50 III : 0	Cf Table in Appendix II	

3.3 MATERIAL

The patch tests (or epidermotests) used are the FINN CHAMBERS ON SCANPOR®. The FINN CHAMBER is made up of an isolation cup that ensures good occlusion.

4. APPLICATION OF PRODUCTS

The product is applied to the back.

Application Zone	Scapular zones: homolateral (induction zone) and heterolateral (revelation zone)
Quantity applied	25µl pure
Frequency	Induction phase: 3 times a week during 48 hours Revelation phase: once a week during 48 hours
Duration	Induction phase: 3 weeks Latent phase: 2 weeks Revelation phase: 1 week
Conditions of application	The product "ALARIANE BATCH 6.04.134" was placed in a cup and applied to the back of the volunteer. A patch containing no product at all was applied in the same conditions and served as a non-treated control. Throughout the induction phase, the homolateral zone was kept dry. The volunteers had a shower the Sunday following withdrawl of the patches, being careful not to put any detergent on the sites. At the revelation phase, no washing or application of any product was carried out on the heterolateral zone.

5. STUDY PROCESS

The study was carried out according to the following schema:

Induction phase – three weeks (W1, W2, W3)

W1:

Day of the Week	Mon	Tues	Wed	Thurs	Fri	Sat	Sun
Day of the study	D1	D2	D3	D4	D5	D6	D7
Application of Product	↓		↓		↓		

W2:

Day of the Week	Mon	Tues	Wed	Thurs	Fri	Sat	Sun
Day of the study	D8	D9	D10	D11	D12	D13	D14
Application of Product	↓		↓		↓		

W3:

Day of the Week	Mon	Tues	Wed	Thurs	Fri	Sat	Sun
Day of the study	D15	D16	D17	D18	D19	D20	D21
Application of Product	↓		↓		↓		

Latent phase – two weeks (W4, W5)

W4:

Day of the Week	Mon	Tues	Wed	Thurs	Fri	Sat	Sun
Day of the study	D22	D23	D24	D25	D26	D27	D28

W5:

Day of the Week	Mon	Tues	Wed	Thurs	Fri	Sat	Sun
Day of the study	D29	D30	D31	D32	D33	D34	D35

Revelation phase (double challenge test) – one week (W6)

W6:

Day of the week	Mon	Tues	Wed	Thurs	Fri
Day of the study	D36	D37	D38	D39	D40
Application of Product	↓				
Readings			**L**		**L**

6. EVALUATION CRITERIA

6.1 Clinical criteria concerning the irritant potential (Induction phase)

After each application, the patch is removed and the reading taken 30 minutes later to eliminate the effect of pressure, occlusion and removal due to the material.
The test is negative if the skin maintains a normal aspect.

The four following criteria are evaluated by the dermatologist according to a scale of 0 to 3.

Score	Grade	**CRITERIA** : description			
		ERYTHEMA	OEDEMA	DRYNESS	VESICLES
0	Absent	Normal appearance	Normal appearance	Normal appearance	Normal appearance
1	Slight	Discreet pinkish coloration over all the tested surface or noticeable on part of the surface	More palpable than visible	Fine, discreet desquamation, rough appearance	Palpable rather than visible vesicles
2	Definite	Definite rash covering the whole of tested surface	Visible oedema	Visible desquamation, scaly appearance	Visible vesicules
3	Significant	Intense rash covering the whole of the tested surface or spreading beyond the surface	Possibly spreading beyond the tested surface	Significant desquamation, cracks in the skin	Vesicles going beyond the tested zone, or blisters

6.2 CLINICAL CRITERIA CONCERNING THE SENSITIZING POTENTIONAL (REVELATION PHASE)

In the event of an allergic reaction during the induction or revelation phases, this is scored according to the criteria of the ICDRG (International Contact Dermatitis Research Group)/

Criteria	ICDRG Score	Numerical Score
No reaction	0	0
Suspected reaction	?	?
Erythma and Oedema	+	1
Erythma, Oedema and vesicles	++	2
Strong reaction with presence of blisters or post bulbous ulcers	+++	3

6.3 EVALUATION METHOD

6.3.1 Irritation Capacity – Induction phase

After the 8 readings taken in the induction phase, the average score of each volunteer is calculated by adding the scores obtained to each of the readings and dividing this sum by the effective number of readings (a reading will not be taken into account if there is a reaction to the control or general irritation).

The irritation capacity of the product will be evaluated during the induction phase, by taking the average of the reactions.

The irritation capacity of the product is determined according to the following formula:

$$\text{Average score} = \frac{\left[\left(\Sigma \text{scores D1}\ldots\frac{\text{D19}}{\text{n°}}\text{readings}\right)\text{vol1}+\cdots+\left(\Sigma \text{scores D1}\ldots\frac{\text{D19}}{\text{n°}}\text{readings}\right)\text{volN}\right]}{\text{number of volunteers (N)}}$$

Average Score	Irritation Capacity
0 – 0.08	Non irritant
0.081 – 0.16	Very slightly irritant
0.161 – 0.56	Slightly irritant
0.561 - 1	Moderately irritant
1.001 – 1.6	Definitely irritant
>1.6	Very definitely irritant

6.3.2 Sensitizing Capacity – Revelation Phase

An eventual allergic reaction during the induction or revelation phases will be noted from 0 to 3 according to the ICDRG (International Contact Dermatitis Research Group) criteria – see table in paragraph 6.2.

During revelation, one reading will be taken 30 minutes after removal of the patch tests, then 48 hours later.

The sensitizing capacity of the product will be evaluated during readings on D38 and D40 (revelation phase) according to the following criteria : reaction ++ (2) or +++ (3).

The appearance of only one case of active sensitization on the heterolateral side will lead to the conclusion: "Product potentially sensitizing".

6.4 PREMATURE STOPPAGE

The subjects have the right to withdraw from the trial at any moment for whatever reason.

Premature stoppage may be due to multiple reasons:

- Non respect of the calendar of visits by the subject
- Undesirable events (including intercurrent diseases)
- Violations and deviations from the protocol
- Exits after withdrawal of the subject's consent

6.5 PROTOCOL AMMENDMENTS

None.

7 RESULTS

7.1 IRRITATION CAPACITY: INDUCTION PHASE

The TABLE OF READINGS during the induction phase is presented in APPENDIX III.

These readings, carried out 30 minutes after withdrawal of the patch-tests showed the following results:

Product DH	D3	D5	D8	D10	D12	D15	D17	D19	Conclusion
ALARIANE BATCH 6.04.134	T+:0 0:50	T+:0 0:50	T+:0 0:50	T+:0 0:50	T+:0 0:50	T+:0 0:50	T+:0 0:50	T+:0 0:50	Non irritant (IRR=0.000)

T+ = Positive Control IRR = Global irritation MV = Missing Value

Given the results, the product "ALARIANE BATCH 6.04.134" may therefore be considered as <u>non irritant</u> under the conditions of this study (score below 0.080).

7.2 SENSITIZING POTENTIAL: REVELATION PHASE

The TABLE OF READINGS during the revelation phase is presented in APPENDIX IV.

The readings carried out 30 minutes and 48hours after withdrawal of the patch tests of revelation gave the following results:

Product DH	Zone	Day of Reading		Global Result
		D38	D40	
ALARIANE BATCH 6.04.134	Reading Homolateral zone	T+:0 **DB** : 0:50 ?:0 1:0 2:0 3:0	T+:0 **DB** : 0:50 ?:0 1:0 2:0 3:0	Non sensitizing
	Readings heterolateral zone	T+:0 **DB** : 0:50 ?:0 1:0 2:0 3:0	T+:0 **DB** : 0:50 ?:0 1:0 2:0 3:0	

T+ = Positive control
DB = ALARIANE BATCH 6.04.134
IRR = global irritation
MV = Missing value

The product "ALARIANE BATCH 6.04.134" can therefore be considered as <u>non sensitizing</u> under the conditions of this study.

8 CONCLUSION

Under the conditions of this study, the product "ALARIANE BATCH 6.04.134" proved to be <u>non irritant</u> and <u>non sensitizing</u>.

APPENDICES

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APPENDIX I

AUTHENTIFICATION PAGE

IROCOS

KARTA AUTENTYCZNOŚCI REZULTATÓW
FICHE D'AUTHENTIFICATION DES RESULTATS
AUTHENTIFICATION PAGE

Według posiadanych przeze mnie informacji badanie Nr. :
A ma connaissance l'étude N :
I am aware that the study N° :

ET – 319

było przeprowadzone zgodnie PROTOKOŁEM oraz KARTĄ PARAMETRÓW TESTU.
a été conduite en accord avec le PROTOCOLE et la FICHE DES PARAMETRES D'ETUDE
has been conducted according to the PROTOCOL and to the STUDY PARAMETERS PAGE.

Mgr inż. Barbara WAŁEJKO
Odpowiedzialna za badania i jakość
Responsable d'unité Responsible qualité
Unit head, Responsible for quality control
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APPENDIX II

CHARACTERISTICS OF VOLUNTEERS

CHARACTERISTICS OF VOLUNTEERS

Subject N°	Subject Code	Age	Sex: M/F	Phototype	Skin type (Normal or Sensitive	Surgical or medical events and treatments	
						Before study	During study
1	LENMA	40	F	II	N	-	-
2	OLEST	57	M	II	N	-	-
3	ZAJAR	20	M	II	N	-	-
4	MARMA	44	M	II	N	-	-
5	SWIAD	28	M	II	N	-	-
6	LUKRE	20	M	II	N	-	-
7	WASBO	25	M	II	N	-	-
8	KOSZO	31	F	II	N	-	-
9	BRYAN	58	F	II	N	-	-
10	CIBMA	61	F	II	N	-	-
11	DABPA	23	F	II	N	-	-
12	FROLE	51	F	II	N	-	-
13	SZCJA	50	M	II	N	-	-
14	KORWA	58	F	II	N	-	-
15	RUSPI	28	M	II	N	-	-
16	RUDMI	61	F	II	N	-	-
17	SKABR	65	F	II	N	-	-
18	SADHA	65	F	II	N	-	-
19	GALFE	58	F	II	N	-	-
20	ROMWI	62	F	II	N	-	-
21	KOZEW	20	F	II	N	-	-
22	SWIAN	56	F	II	N	-	-
23	OTWIW	49	F	II	N	-	-
24	STAEL	61	F	II	N	-	-
25	STRWA	62	F	II	N	-	-
26	HOPWI	31	F	II	N	-	-
27	KOWHU	27	M	II	N	-	-
28	MUSWL	63	F	II	N	-	-
29	SOKKR	21	M	II	N	-	-
30	SZEHE	65	F	II	N	-	-

Subject N°	Subject Code	Age	Sex: M/F	Phototype	Skin type (Normal or Sensitive	Surgical or medical events and treatments	
						Before study	During study
31	WYSAN	57	F	II	N	-	-
32	RUSBA	37	F	II	N	-	-
33	BIABA	51	F	II	N	-	-
34	WISLI	49	F	II	N	-	-
35	IWAMA	44	F	II	N	-	-
36	GLUJU	19	F	II	N	-	-
37	NIEAN	53	F	II	N	-	-
38	BANBO	35	F	II	N	-	-
39	LUBZO	45	F	II	N	-	-
40	WODIR	42	F	II	N	-	-
41	KOSWE	24	F	II	N	-	-
42	OLSMA	33	F	II	N	-	-
43	ZIOZO	45	F	II	N	-	-
44	STYJA	57	F	II	N	-	-
45	STAAL	20	F	II	N	-	-
46	KOSJA	30	M	II	N	-	-
47	SZPAL	26	F	II	N	-	-
48	PACJA	35	M	II	N	-	-
49	ARERO	19	M	II	N	-	-
50	KEPJO	58	F	II	N	-	-

APPENDIX III

TABLE OF READINGS

INDUCTION PHASE

TABLE OF READINGS – Induction Phase

Volunteer N°	D3		D5		D8		D10		D12		D15		D17		D19	
	C	DB	C	DB	C	DB	C	DB	C	DB	C	DB	C	DB	C	DB
1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
8	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
9	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
10	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
11	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
12	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
13	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
14	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
15	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
16	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
17	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
18	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
19	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
20	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
21	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
22	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
23	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
24	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
25	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
26	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
27	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
28	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
29	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
30	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
31	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
32	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
33	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
34	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
35	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
36	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
37	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
38	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
39	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
40	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
41	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
42	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
43	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
44	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
45	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
46	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
47	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
48	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
49	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
50	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

L.S. = Lost Sight C = control DB = ALARIANE BATCH 6.04.134

APPENDIX IV

TABLE OF READINGS

REVELATION PHASE

TABLE OF READINGS – Revelation phase

Volunteer N°	D38 Homolateral zone		D38 controlateral zone		D40 homolateral zone		D40 controlateral zone	
	C	DB	C	DB	C	DB	C	DB
1	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0
4	0	0	0	0	0	0	0	0
5	0	0	0	0	0	0	0	0
6	0	0	0	0	0	0	0	0
7	0	0	0	0	0	0	0	0
8	0	0	0	0	0	0	0	0
9	0	0	0	0	0	0	0	0
10	0	0	0	0	0	0	0	0
11	0	0	0	0	0	0	0	0
12	0	0	0	0	0	0	0	0
13	0	0	0	0	0	0	0	0
14	0	0	0	0	0	0	0	0
15	0	0	0	0	0	0	0	0
16	0	0	0	0	0	0	0	0
17	0	0	0	0	0	0	0	0
18	0	0	0	0	0	0	0	0
19	0	0	0	0	0	0	0	0
20	0	0	0	0	0	0	0	0
21	0	0	0	0	0	0	0	0
22	0	0	0	0	0	0	0	0
23	0	0	0	0	0	0	0	0
24	0	0	0	0	0	0	0	0
25	0	0	0	0	0	0	0	0
26	0	0	0	0	0	0	0	0
27	0	0	0	0	0	0	0	0
28	0	0	0	0	0	0	0	0
29	0	0	0	0	0	0	0	0
30	0	0	0	0	0	0	0	0
31	0	0	0	0	0	0	0	0
32	0	0	0	0	0	0	0	0
33	0	0	0	0	0	0	0	0
34	0	0	0	0	0	0	0	0
35	0	0	0	0	0	0	0	0
36	0	0	0	0	0	0	0	0
37	0	0	0	0	0	0	0	0
38	0	0	0	0	0	0	0	0
39	0	0	0	0	0	0	0	0
40	0	0	0	0	0	0	0	0
41	0	0	0	0	0	0	0	0
42	0	0	0	0	0	0	0	0
43	0	0	0	0	0	0	0	0
44	0	0	0	0	0	0	0	0
45	0	0	0	0	0	0	0	0
46	0	0	0	0	0	0	0	0
47	0	0	0	0	0	0	0	0
48	0	0	0	0	0	0	0	0
49	0	0	0	0	0	0	0	0
50	0	0	0	0	0	0	0	0

LS = Lost sight C = Control DB = ALARIANE BATCH 6.04.134

RAPPORT

VERIFICATION CHEZ L'HOMME DE LA COMPATIBILITE CUTANEE D'UN PRODUIT COSMETIQUE APRES APPLICATION UNIQUE SOUS PANSEMENT. Patch test 24h

Produit testé :

ALARIA BG/PF LOT 604134

ALARIANE BG PF

Promoteur

BIOTECHMARINE
ZI – BP 65
2260 PONTRIEUX

Bordeaux, le 5 janvier 2007

JA/CL

SOMMAIRE

Laboratoire COSDERMA - Service de dermatologie Pr. Taïeb - Groupe Hospitalier Saint André - 1 rue Jean Burguet - BP 50057 - 33023 Bordeaux Cedex
Tel : 05 56 94 75 40 - Fax : 05 56 79 49 75 - email : laboratoire@cosderma.com

4- Produit étudié

4.1 Informations

L'information transmise par le promoteur accompagnant l'échantillon a été la lettre d'engagement concernant en particulier la conformité de la formule aux règlementations en vigueur et sa sécurité.

4.2 Identification

Nom	Réf.	Quantité pour l'étude	Nb conditionnement
ALARIA BG/PF	**LOT 604134**	5 ml	1 x 5 ml

4.3 Conditions normales d'emploi

Nom	Mode d'emploi	
ALARIA BG/PF LOT 604134	site	/
	Matière première cosmétique	

4.4 Conditions d'utilisation pendant l'étude

Pour l'étude, la zone d'application choisie est le dos.

Produit	Type de pansement	Conditions d'application	Temps de contact	Quantité appliquée	Temps de contrôle à J1
ALARIA BG/PF LOT 604134	Occlusif (Finn chambers®)	Pur	24h	20 µl	15 minutes après dépatchage

Finn Chambers® : Pansement occlusif composé d'une cupule d'aluminium de 8 mm de diamètre (surface 50 mm²) sur laquelle 20 µl (20 mg) de produit est déposé.

Un patch témoin, correspondant au type de pansements utilisés, contenant une quantité *ad hoc* d'eau pour préparation injectable, a été appliqué parallèlement.
Le dépatchage a été effectué par l'investigateur ou la technicienne sous sa responsabilité.
Les quantités de produit ont été mesurées à l'aide d'une seringue à usage unique.

5- Volontaires

5-1 Panel

Le panel de volontaires participant à l'étude est représentatif de la population susceptible d'utiliser le produit.
Tous les volontaires sélectionnés ont répondu aux critères d'inclusion et de non inclusion.

5-2 Effectif

Le nombre de volontaires participant à l'étude a été de **10**.
Le nombre de volontaires dont les données sont présentées est de **10**.

5-3 Critères d'inclusion

Les critères d'inclusion étaient les suivants :

- âge : de 18 à 65 ans,
- sexe : féminin,
- phototype (Fitzpatrick) : I à III,
- tous types de peau,
- apte à donner son consentement écrit lu et signé,
- affiliée à la sécurité sociale

Tous les volontaires ont correspondu à ces critères d'inclusion. Les caractéristiques typologiques des volontaires sont présentées en **Annexe 1.**

5-4 Critères de non inclusion

Les critères de non inclusion étaient les suivants :

- Marques cutanées au niveau de la zone expérimentale pouvant interférer avec l'évaluation des réactions de la peau (troubles de la pigmentation, éléments cicatriciels, pilosité trop développée, éphélides et nævi en trop grande quantité, coup de soleil....)
- Réaction eczématiforme non encore complètement disparue, séquelles cicatricielles ou pigmentaires de tests antérieurs au niveau de la zone expérimentale
- Allergie à la colophane ou au nickel
- Allergie ou réactivité à la même catégorie de produits
- Hyper-réactivité cutanée
- Réactivité à l'alcool éthylique, au sparadrap
- Participation dans les 12 mois qui précèdent l'étude, à plus de 5 tests utilisant la maximalisation dont 3 au plus à visée de recherche d'hypoallergénicité
- Exposition intensive au soleil dans le mois qui précède l'étude
- Prévision d'une exposition solaire intensive (au soleil naturel ou en cabine UVA) pendant la durée de l'étude
- Intention de se baigner en baignoire, en mer ou en piscine, de faire du sauna ou du hammam pendant l'étude
- Pratique d'un ou plusieurs sports de façon intensive ou régulière dont l'interruption momentanée pose problème
- Arrêt de traitement à base de vitamine A acide ou de ses dérivés depuis moins de 3 mois avant le début de l'étude
- Arrêt de traitement par corticoïde topique sur la zone expérimentale de moins de 8 jours avant l'étude
- Arrêt de traitement par PUVA ou UVB depuis moins d'un mois avant l'étude
- Prévision de vaccination pendant la durée du test, dernière vaccination dans les 3 semaines précédant l'étude

Aucun volontaire correspondant à ces critères n'a été inclus.

5-5 Contraintes de l'étude

Les contraintes de l'étude étaient les suivantes :

- Pas d'application de produits autres que ceux testés sur la zone expérimentale
- Pas de port de vêtements trop serrés ou responsables d'une contention au niveau de la zone expérimentale, susceptibles d'occasionner des frottements et le décollement du pansement
- Pas de bain en baignoire, en mer ou en piscine et pas de sauna ou de hammam durant l'étude
- Protection de la zone expérimentale lors de la prise de douche, pas de projection violente d'eau et pas de savonnage sur cette zone pour éviter le décollement du pansement ou l'apparition de phénomènes intercurrents, et essuyage très délicat si nécessaire
- Pas de sudation excessive et pas d'activité physique intensive susceptibles d'entraîner le décollement du pansement
- Pas d'exposition au solaire intensive, (au soleil naturel ou en cabine UVA) pendant la durée de l'étude, surtout lorsque le pansement a été enlevé
- Conservation des habitudes d'hygiène sur le visage et le corps,
- Pas de traitement anti-allergique, anti-inflammatoire (corticoïde systémique ou topique) ou par des spécialités à base de vitamine A acide ou de ses dérivés le jour de l'étude (si nécessité thérapeutique : sortie d'étude envisagée)

Toutes les contraintes de l'étude ont été respectées par les volontaires.

5-6 Contrôle de l'observance des modalités du protocole

L'investigateur a vérifié si les **contraintes** avaient été respectées.

La synthèse des réponses aux différentes questions posées est jointe en **Annexe 2.**

En cas de déviations au protocole, celles-ci ont été analysées et l'investigateur a apprécié leur incidence sur la validité des résultats.

Toutes les contraintes de l'étude, définies au protocole, ont été respectées par les volontaires.

6- Evaluation

6-1 Calendrier

Déroulement de l'étude	début	Temps de contact
	T0	T24h ± 2h
Sélection des volontaires	X	
Attribution n° des volontaires	X	
Information volontaire	X	
Consentement éclairé signé *	X	
Application des pansements par la technicienne	X	
Dépatchage par la technicienne		X
Critères évaluation (15 mn après dépatchage)		X

Le fait que l'application du produit ainsi que les examens cliniques aient été parfaitement contrôlés, l'effectif de volontaires et la durée de l'étude ont permis de vérifier la compatibilité cutanée du produit étudié et d'apprécier les éventuels phénomènes irritatifs.

* Un double du consentement de participation sera remis aux volontaires le jour de la visite d'inclusion pour l'étude. L'original sera conservé par l'investigateur.

6-2 Evaluation de la compatibilité cutanée

• *Principe et bibliographies*

La compatibilité cutanée est vérifiée par l'intermédiaire de l'application de pansements sur la peau qui créent une certaine occlusion des produits et favorisent leur pénétration. Dans ces conditions expérimentales maximalisantes, le potentiel irritant des produits peut se révéler plus facilement.

La méthodologie a fait l'objet de nombreuses publications, dont :

Comment tester les produits cosmétiques ?, Dermatologie Pratique, 2003, n° 273, 1-4

Reactive changes in human epidermis following simple occlusion with water, Contact Dermatitis, Mikulowska A, 1992, 26, 224-227

Test strategies for development of cosmetic products using dermatological test models, Seifen-öle-fette-wachse, Matthies W, 1991, 117, 42-43

The Duhring Chamber: an inproved technique for epicutaneous testing of irritant and allergic rections, Contact Dermatitis,Frosch PJ & Klingmann AM, 1979, 5, 73-81

Appraisal of the safety of chemicals in Food, Drugs and Cosmetics, FDA (ed), Draize JH, 1959, 46-48

• *Méthodologie, matériel de patchage*

Les produits sont déposés sur les pansements, extemporanément, à l'aide d'une seringue de 1 ml. Les pansements sont appliqués par la suite sur la peau le plus rapidement possible en évitant soigneusement les zones exposées au frottement ou compressions diverses. L'investigateur ou la technicienne sous son autorité vérifiera que la zone de peau concernée est vierge de toute présence de grains de beauté, cicatrices et accidents cutanés. Le type de pansement, le nombre maximum de produits possibles à tester, la quantité de produit à appliquer, la méthodologie d'application et de retrait des pansements et l'examen clinique visuel sont conformes aux procédures du laboratoire référencées pour ce type d'étude.
Le site d'application des produits choisi est le dos.

• *Conditions environnementales*

Les conditions environnementales imposées aux volontaires sont les suivantes :

- *température contrôlée : t ° = 20°C ± 2°C*
- *humidité relative : HR = 45 % ± 15 %*

• *Examen clinique*

- Sites

L'investigateur ou la technicienne sous son autorité effectue un contrôle visuel de chaque zone expérimentale sous un éclairage standardisé type « lumière du jour ».

- Fréquences

L'examen visuel est réalisé à T24h ± 2h, 15 minutes après dépatchage (ou plus si des rougeurs sont apparues à l'enlèvement du patch).

• *Critères d'évaluation*

- *signes cliniques*

Description	Code laboratoire	intensité	aspect	note
Erythème	E	- échelle ordinale en 3 points : • légère • modérée • sévère	- érythème : • diffus • ponctué • périphérique	• légère = 1 • modérée = 2 • sévère = 3 • diffus = d • ponctué = p • périphérique = peri
Oedème	Oe			
Dessèchement	D			
Coloration	C			
Comédon, microkyste	Co, Mi	- dénombrés		
Vésicule, papule	V, Pa	- échelle ordinale en 2 points : • 1 à 2 vésicules • vésicules en nombre >2		• 1 à 2 = 1 • nb >2 = 2
Bulle, croutelle	Bu, Cr	- décrits		• si décrits = 2

L'investigateur, ou la technicienne sous son autorité, ont noté tout signe clinique, sa localisation, son intensité, son évolution, le traitement médicamenteux éventuellement entrepris. Il a établi le caractère habituel ou inhabituel du signe clinique, en questionnant le volontaire sur ce qu'il observe dans la vie courante, lors de l'utilisation de produits similaires.

– *Sensations d'inconfort*

Description	Code laboratoire	intensité	note
Echauffement	Ech	- échelle ordinale en 3 points : • légère • modérée • sévère	• légère = 1 • modérée = 2 • sévère = 3
Picotement	Pi		
Prurit (démangeaison)	Pr		
Tiraillement	Ti		
Brûlure	Br		

- *Expression des résultats*

Tous les volontaires ayant fait l'objet de la visite T0 ont été pris en compte pour l'évaluation de la compatibilité cutanée. L'expression des résultats de l'examen cutané et de l'interrogatoire a été conforme à la procédure du laboratoire référencée pour ce type d'étude.

Les résultats individuels sont exprimés:

- **en pourcentage de volontaires réactifs** en tenant compte pour ce calcul uniquement des signes cliniques décelables visuellement à type d'érythème, œdème, vésicule, bulle, papule, croutelle.
- **de façon descriptive** pour les autres signes décelables visuellement ou les sensations d'inconfort, le pourcentage de volontaires chez qui ils ont été observés, pouvant éventuellement être calculé si la fréquence d'apparition de ces signes le justifiait.
- **en score d'irritation cutanée** calculé à partir des « notes » attribuées aux signes cliniques décelables visuellement.

Pour chaque volontaire et à chaque temps d'observation, a été calculé un score d'irritation journalier individuel (SijI) qui est la somme des notes obtenues pour les signes observés.

Pour le panel et à chaque temps d'observation, a été calculé un score d'irritation journalier moyen (SijM) qui correspond à la formule :

$$SijM = \Sigma (SijI) / \text{Nombre de volontaires pris en compte}$$

- *Interprétation des résultats*

L'Investigateur a conclu en terme de **très bonne, bonne, moyenne ou mauvaise** compatibilité cutanée de façon absolue. L'interprétation des résultats de l'examen cutané et de l'interrogatoire a été conforme à la procédure du laboratoire référencée pour ce type d'étude.

7- Résultats et discussions

Les données individuelles de l'examen cutané et de l'interrogatoire des volontaires sont jointes en **Annexe 3.**

En résumé :

Produit	Temps de contrôle à J1	Nombre de volontaires réactifs	Types de réaction	Score d'irritation journalier moyen SIjM	% de volontaires réactifs
ALARIA BG/PF LOT 604134	15 minutes après dépatchage	2	E0,5 (2)	0,1	20%
	24h après dépatchage	0	/	0	0%

8- Conclusion

Dans les conditions expérimentales adoptées, le produit **« ALARIA BG/PF LOT 604134» a une bonne compatibilité cutanée.**

Signatures et dates :

Pr Alain Taïeb (Dermatologue)
Investigateur
16-1-2007

Chrystelle Latxague
Assistante Clinique
08/01/07

Jérôme Asserin
Directeur d'étude
9/01/07

Amour ADJADOHOUN
Responsable qualité
09/01/07

Laboratoire COSDERMA - Service de dermatologie Pr. Taïeb - Groupe Hospitalier Saint André - 1 rue Jean Burguet - BP 50057 - 33023 Bordeaux Cedex
Tel : 05 56 94 75 40 - Fax : 05 56 79 49 75 - email : laboratoire@cosderma.com

ANNEXES

Laboratoire COSDERMA - Service de dermatologie Pr. Taïeb - Groupe Hospitalier Saint André - 1 rue Jean Burguet - BP 50057 - 33023 Bordeaux Cedex
Tel : 05 56 94 75 40 - Fax : 05 56 79 49 75 - email : laboratoire@cosderma.com

Annexe 1

CARACTERISTIQUES TYPOLOGIQUES DES VOLONTAIRES

Volontaires		Age (ans)	Sexe F=féminin	Phototype *	Peau saine au niveau du dos
Réf.	Nom prénom				
1	PANT/E	22	F	II	x
2	CARR/E	54	F	III	x
3	PLAZ/I	49	F	III	x
4	MEZA/A	39	F	III	x
5	SAIN/R	26	F	I	x
6	DRUI/S	37	F	II	x
7	BOUS/C	42	F	II	x
8	NAKA/G	32	F	II	x
9	MORS/G	18	F	I	x
10	LARR/D	46	F	II	x

Légendes : / = non x = oui

***phototype selon Fitzpatrick,** établi sur le principe d'une première exposition de 30 à 40 minutes au soleil après l'hiver ou une période sans exposition d'une durée équivalente :

TYPE	CHEVEUX	PEAU	EPHELIDES	COUPS DE SOLEIL
I	roux	laiteuse	+++	constant bronzage nul
II	blonds	claire	++	fréquent bronzage léger
III	blonds châtains	claire	+	inconstant bronzage léger à mat
IV	bruns	mate	o	nul bronzage mat foncé
V	noirs et crépus	noire	o	o

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Annexe 2

CONTROLE DE L'OBSERVANCE
Contraintes

Contraintes (10 résultats exploitables)	Nombre de volontaires ayant respecté les contraintes	Pourcentage de volontaires ayant respecté les contraintes
Pas d'application de produits (autres que celui testé) sur la zone expérimentale Déviation : aucune	10	100 %
Pas de port de vêtements trop serrés ou responsables d'une contention au niveau de la zone expérimentale, susceptibles d'occasionner des frottements et le décollement du pansement Déviation : aucune	10	100 %
Pas de bain (en baignoire ou en piscine ou en mer) et pas de hammam ou de sauna pendant l'étude Déviation : aucune	10	100 %
En cas de douche, protection de la zone expérimentale ou pas de projection violente d'eau et pas de savonnage sur cette zone pour éviter le décollement du pansement ou l'apparition de phénomènes intercurrents et essuyage très délicat si nécessaire Déviation : aucune	10	100 %
Pas de sudation excessive et de sport intensif, susceptibles d'entrainer le décollement du pansement Déviation : aucune	10	100 %
Pas d'exposition solaire intensive (au soleil naturel ou en cabine UVA) pendant la durée de l'étude, surtout lorsque le pansement était enlevé Déviation : aucune	10	100 %
Pas de traitement anti-allergique, anti-inflammatoire (corticothéraple systémique ou topique...) ou par des spécialités à base de vitamine A acide ou de ses dérivés pendant l'étude – pas de médication pouvant interférer avec l'étude Déviation : aucune	10	100 %
Pas de vaccination pendant l'étude Déviation : aucune	10	100 %

Annexe 3-1

VERIFICATION DE LA COMPATIBILITE CUTANEE

Produit N°1: **« ALARIA BG/PF LOT 604134 »** (Finn chambers)

Volontaires		Examen cutané (15 min après dépatchage)	SijI
Réf.	**Nom / prénom**		
1	PANT/E	/	/
2	CARR/E	/	/
3	PLAZ/I	/	/
4	MEZA/A	/	/
5	SAIN/R	/	/
6	DRUI/S	E0,5	0,5
7	BOUS/C	/	/
8	NAKA/G	E0,5	0,5
9	MORS/G	/	/
10	LARR/D	/	/
SijM		0,1	

Légendes : / = aucun signe clinique

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Tel : 05 56 94 75 40 - Fax : 05 56 79 49 75 - email : laboratoire@cosderma.com

Annexe 3-2

VERIFICATION DE LA COMPATIBILITE CUTANEE

Produit N°2: Témoin (Finn chambers)

Volontaires		Examen cutané (15 min après dépatchage)	SijI
Réf.	Nom / prénom		
1	PANT/E	/	/
2	CARR/E	/	/
3	PLAZ/I	/	/
4	MEZA/A	/	/
5	SAIN/R	/	/
6	DRUI/S	/	/
7	BOUS/C	/	/
8	NAKA/G	/	/
9	MORS/G	/	/
10	LARR/D	/	/
SijM		0	

Légendes : / = aucun signe clinique

Laboratoire COSDERMA - Service de dermatologie Pr. Taïeb - Groupe Hospitalier Saint André - 1 rue Jean Burguet - BP 50057 - 33023 Bordeaux Cedex
Tel : 05 56 94 75 40 - Fax : 05 56 79 49 75 - email : laboratoire@cosderma.com

MANUFACTURING PROCESS
ALARIANE™ AD

HARVESTING / IDENTIFICATION(*Alaria Esculenta*)

↓

WASHING

↓

DRYING

↓

GRINDING

↓

EXTRACTION WITH THE SOLVENTS
BUTYLENE GLYCOL AND WATER

↓

FILTRATION

↓

QUALITY CONTROL

↓

PACKAGING

↓

QUALITY CONTROL

Production Manager
Jean-Marc CATROUX

ALARIANE™ AD

INCI name : Aqua/Water – Butylene glycol – Algae extract*

**Retired INCI names will be retained for publication for an interim period of time. During this transition period, trade names maintain their assignment to retired names, but are also designated by the new nomenclature so that users may update product labels, documentation, technical literature, etc. when economically feasible.*

CAS N°: 7732-18-5 – 107-88-0 - 92128-82-0

EINECS N°: 231-791-2 – 203-529-7 - 295-780-4

DONNEES PHYSICOCHIMIQUES
PHYSICO-CHEMICAL DATA
Numéro de référence / *Reference number* : STANDARD

CARACTERISTIQUES *CHARACTERISTICS*	METHODES *METHODS*	STANDARD *STANDARD*
Aspect *Aspect*	MO PHY 002	**Liquide limpide à légèrement opalescent** *Limpid to slightly opalescent liquid*
Couleur *Colour*	MO PHY 002	**Jaune à brun** *Yellow to brown*
Odeur *Odour*	MO PHY 002	**Caractéristique** *Characteristic*
pH	MO PHY 009	5,5 – 7,5
Densité (20°C) *Density*	MO PHY 024	1,010 - 1,040
Indice de réfraction(20°C) *Refractive index*	MO PHY 008	$1,385_0$ - $1,405_0$
Extrait sec (1g – 4 heures – 105°C) *Dry extract*	MO PHY 033	0,5 - 2,5 %
Teneur en eau *Water content*	MO PHY 018	48,0 – 52,0 %
Butylene glycol *Butylene glycol*	MO PHY 001	48,0 – 52,0 %
Conservateur* *Preservative**		**Absence** *None*
Spectre UV *UV spectrum*	MO PHY 013	**Conforme au témoin** *Similar to the standard*
Spectre IR *IR spectrum*	MO PHY 011	**Conforme au témoin** *Similar to the standard*

** Sous contrôle statistique / Under statistical control*

ALARIANE™ AD

INCI name : Aqua/Water – Butylene glycol – Algae extract*

**Retired INCI names will be retained for publication for an interim period of time. During this transition period, trade names maintain their assignment to retired names, but are also designated by the new nomenclature so that users may update product labels, documentation, technical literature, etc. when economically feasible.*

CAS N°: 7732-18-5 – 107-88-0 - 92128-82-0

EINECS N°: 231-791-2 – 203-529-7 - 295-780-4

DONNEES PHYSICOCHIMIQUES
PHYSICO-CHEMICAL DATA

Numéro de référence / *Reference number* : STANDARD

CARACTERISTIQUES *CHARACTERISTICS*	**STANDARD** *STANDARD*
Métaux lourds** *Heavy metals** (ppm)*	
• **Arsenic mineral** *Mineral Arsenic*	< 5
• **Cadmium** *Cadmium*	< 3
• **Plomb** *Lead*	< 5
• **Nickel** *Nickel*	< 2
• **Argent** *Silver*	< 5

** Teneurs garanties sous contrôle statistique / *Contents guaranteed under statistical control*

Iodine content < 10 ppm

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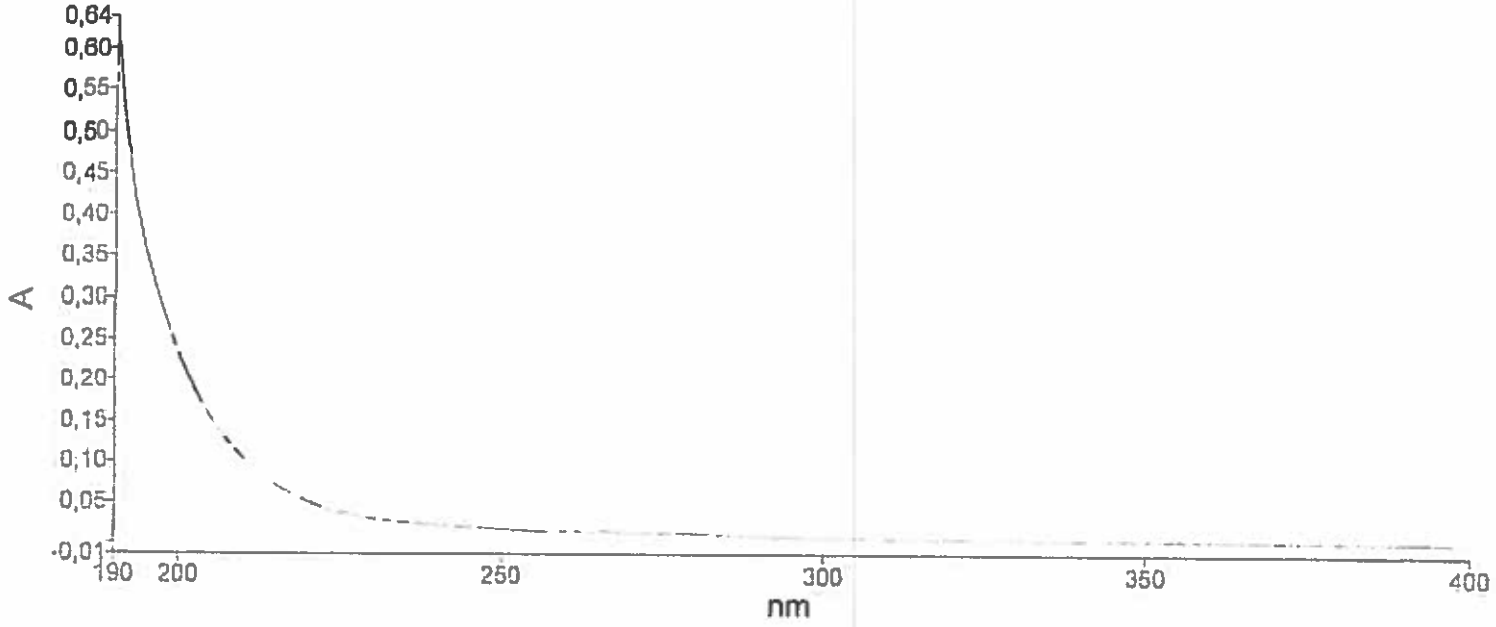
03/07/2015 09:16

Analyst
Date

contrôle qualité
vendredi 3 juillet 2015 09:16



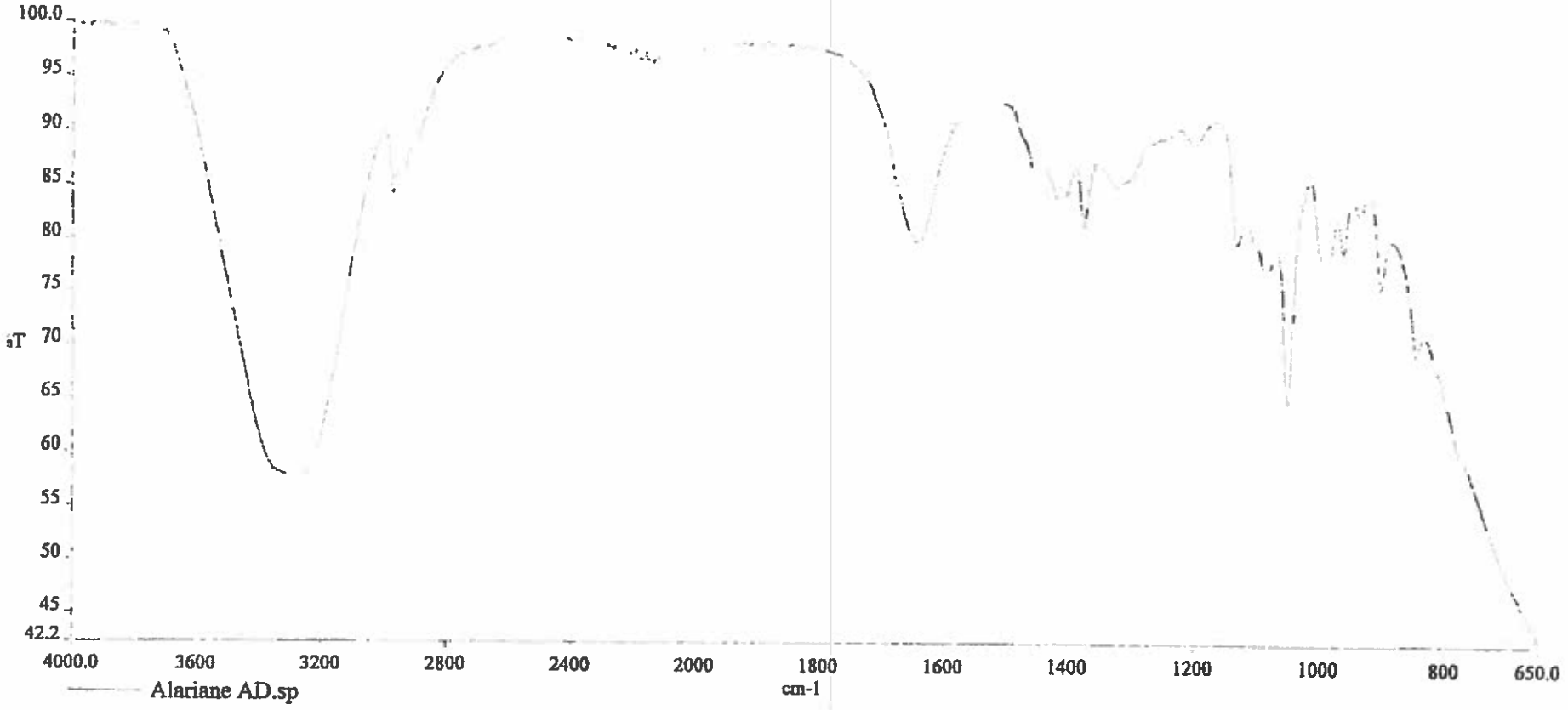
Sample Name	Description
Atarlane AD .Sample	

Date: vendredi 3 juillet 2015

SPECTRE IRFT

SPECTROPHOTOMETRE SPECTRUM 100 N° de série 77774 - PERKIN ELMER

Accessoire ATR Universel N° 7031330



INCI name : Aqua/Water – Butylene glycol – Algae extract*
**Retired INCI names will be retained for publication for an interim period of time. During this transition period, trade names maintain their assignment to retired names, but are also designated by the new nomenclature so that users may update product labels, documentation, technical literature, etc. when economically feasible.*
CAS N°: 7732-18-5 – 107-88-0 - 92128-82-0
EINECS N°: 231-791-2 – 203-529-7 - 295-780-4

DONNEES MICROBIOLOGIQUES
MICROBIOLOGICAL DATA
Numéro de référence / *Reference number* : STANDARD

CARACTERISTIQUES *CHARACTERISTICS*	**METHODES** *METHODS*	**STANDARD** *STANDARD*
Germes totaux*** *Total germs****	MO MIC 002	**< 100**
Germes Pathogènes *Pathogens*		
- *Staphylococcus aureus*	MO MIC 012	**Absence** *None*
- *Candida albicans*	MO MIC 010	**Absence** *None*
- *Pseudomonas aeruginosa*	MO MIC 011	**Absence** *None*
- *Enterobacteriaceae*	MO MIC 020	**Absence** *None*
Levures / Moisissures*** *Yeasts / Moulds****	MO MIC 021	**< 100**

*** Les résultats sont indiqués en UFC/mL pour les liquides et en UFC/g pour les solides
*** *Results are indicated in CFU/mL for the liquids and in CFU/g for the solids*

CONFORME
CERTIFIED TRUE AND CORRECT
RESPONSABLE CONTROLE QUALITE: **P. SOUBIES**
QUALITY CONTROL MANAGER
26 OCT. 2015

CONFORME
CERTIFIED TRUE AND CORRECT
COORDINATRICE ASSURANCE QUALITE : **M. TANNIOU**
QUALITY ASSURANCE COORDINATOR
26 OCT. 2015

MANUFACTURING PROCESS
JUVENESSENCE™

HARVESTING / IDENTIFICATION (Alaria Esculenta)

↓

DRYING

↓

GRINDING

↓

EXTRACTION WITH THE SOLVENT
CAPRYLIC/CAPRIC TRIGLYCERIDE

↓

FILTRATION

↓

QUALITY CONTROL

↓

PACKAGING

↓

QUALITY CONTROL

Production Manager
Jean-Marc CATROUX

Date de mise à jour / *Updated date : 01/01/2017*

JUVENESSENCE™

INCI NAME : Caprylic/Capric Triglyceride – Alaria Esculenta extract
CAS : 73398-61-5
EINECS : 277-452-2
Produit conforme Mass Balance (RSPO) BVC-RSPO-1-1972708497 / *Product Mass Balance compliant(Rountable for Sustainable Palm Oil (RSPO)) BVC-RSPO-1-1972708497*

4.5% Alaria Esculenta Extract 79.5% caprylic/capric Triglycerides

DONNEES PHYSICO-CHIMIQUES
PHYSICOCHEMICAL DATA
Numéro de référence / *Reference number* : STANDARD

CARACTERISTIQUES *CHARACTERISTICS*	METHODES *METHODS*	STANDARD *STANDARD*
Conformité Mass Balance *Mass Balance conformity*	PO-HSE-004	**Conforme** *Conform*
Aspect *Aspect*	MO PHY 002	**Liquide limpide huileux** *Oily limpid liquid*
Couleur *Colour*	MO PHY 002	**Jaune-vert pâle à vert foncé (1 à 7UG)** *Pale yellow green to dark green (1 to 7UG)*
Odeur *Odour*	MO PHY 002	**Faible** *Slight*
Densité (20°C) *Density*	MO PHY 024	0,920 - 0,950
Indice de réfraction(20°C) *Refractive index*	MO PHY 008	$1,440_0$ - $1,460_0$
Indice de péroxyde* *Peroxyd index**	MO PHY 034	<3 meq d'oxygène actif/kg <3 *meq of active oxygen/kg*
Spectre UV visible *Visible UV spectrum*	MO PHY 013	**Conforme au témoin** *Similar to the standard*
Spectre IR *IR spectrum*	MO PHY 011	**Conforme au témoin** *Similar to the standard*

Solvant / *Solvent* : **Mélange de triglycérides d'acides gras saturés (C8C10) d'origine végétale** / *Mixture of triglycerides of saturated fatty acids (C8C10) of vegetal origin.*

Métaux lourds*(ppm) / *Heavy metals* (ppm)*

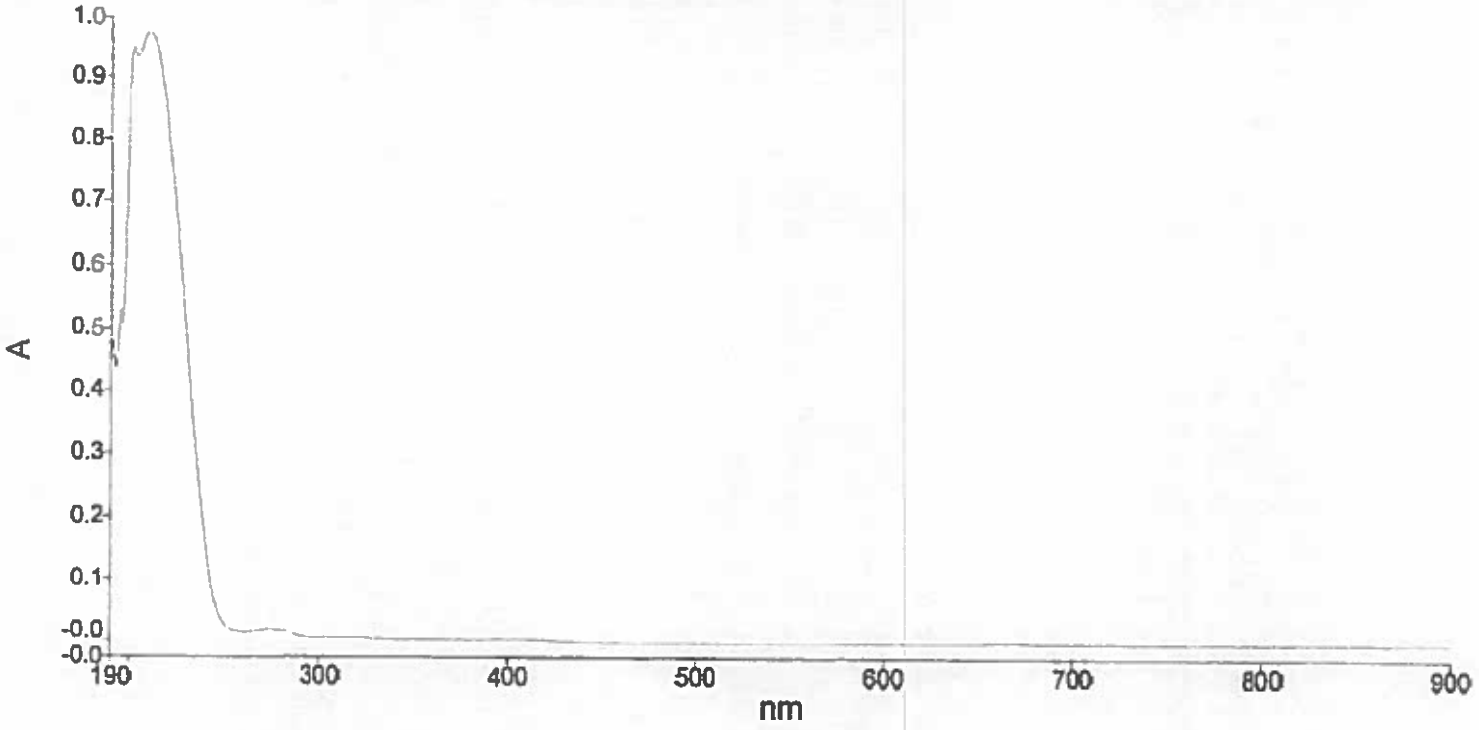
• **Arsenic** / *Arsenic*	< 2
• **Cadmium** / *Cadmium*	< 3
• **Plomb** / *Lead*	< 5
• **Nickel** / *Nickel*	< 2
• **Argent** / *Silver*	< 5
• **Mercure** / *Mercury*	< 1

Iodine < 1 ppm

* *Teneurs garanties sous contrôle statistique* / Contents guaranteed under statistical control

Analyst
Date

contrôle qualité
jeudi 24 novembre 2016 11:37



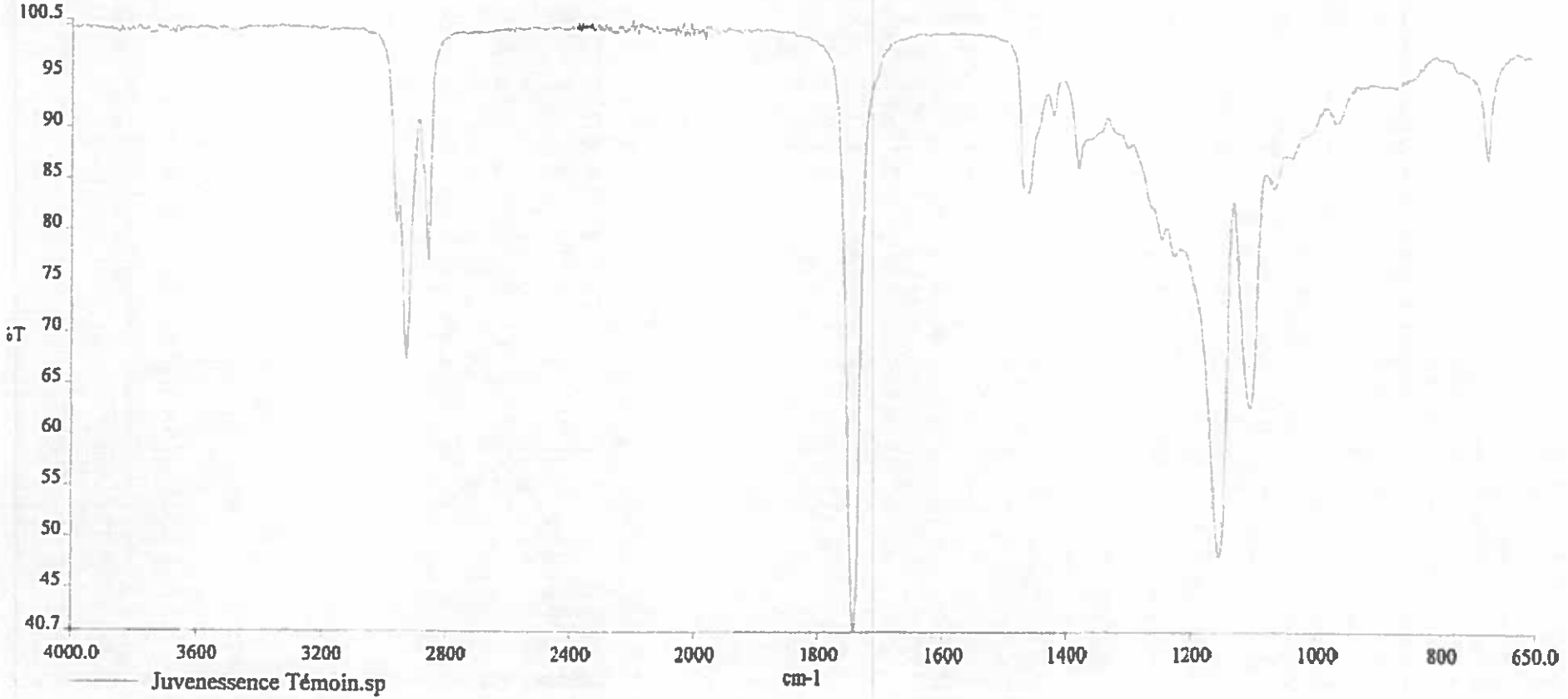
- BiotechMarine Z.I – B.P.72 – 22360 Pontrieux - FRANCE Tél : +33 (0) 2 96 95 31 32

Date: jeudi 24 novembre 2016

SPECTRE IRFT

SPECTROPHOTOMETRE SPECTRUM 100 N° de série 77774 - PERKIN ELMER

Accessoire ATR Universel N° 7031330



- BiotechMarine Z.I. – B.P.72 – 22360 Pontrieux - FRANCE Tél : +33 (0) 2 96 95 31 32

JUVENESSENCE™

INCI NAME : Caprylic/Capric Triglyceride – Alaria Esculenta extract
CAS : 73398-61-5
EINECS : 277-452-2
Produit conforme Mass Balance (RSPO) BVC-RSPO-1-1972708497 / *Product Mass Balance compliant(Roundtable for Sustainable Palm Oil (RSPO)) BVC-RSPO-1-1972708497*

DONNEES MICROBIOLOGIQUES
MICROBIOLOGICAL DATA
Numéro de référence / *Reference number* : STANDARD

CARACTERISTIQUES *CHARACTERISTICS*	METHODES *METHODS*	STANDARD *STANDARD*
Germes totaux** *Total germs***	MO MIC 002 / NF EN ISO 21149	**< 100**
Germes Pathogènes *Pathogens*		
- *Staphylococcus aureus*	MO MIC 012 / NF EN ISO 22718	**Absence** *None*
- *Candida albicans*	MO MIC 010 / NF EN ISO 18416	**Absence** *None*
- *Pseudomonas aeruginosa*	MO MIC 011 / NF EN ISO 22717	**Absence** *None*
- *Escherichia coli*	MO MIC 025 / NF EN ISO 21150	**Absence** *None*
Levures / Moisissures** *Yeasts / Moulds***	MO MIC 021 / NF EN ISO 16212	**< 100**

** Les résultats sont indiqués en UFC/mL pour les liquides et en UFC/g pour les solides
** *Results are indicated in CFU/mL for the liquids and in CFU/g for the solids*

CONFORME
CERTIFIED TRUE AND CORRECT
RESPONSABLE CONTROLE QUALITE: **P. SOUBIES**
QUALITY CONTROL MANAGER
0 1 JAN. 2017

CONFORME
CERTIFIED TRUE AND CORRECT
COORDINATRICE ASSURANCE QUALITE : **M. TANNIOU**
QUALITY ASSURANCE COORDINATOR
0 1 JAN. 2017

LISKIN

SENSITIZING POTENTIAL STUDY OF A PRODUCT ACCORDING TO MARZULLI-MAIBACH METHOD

REPORT

STUDY REF.	ET-359
PRODUCT	«JUVENESSENCE LOT 8.05.149»
NUMBER OF SUBJECTS	50
SPONSOR	EUROTEST
MONITOR	Mr Bogdan WICHROWSKI LISKIN IMMEUBLE FONTENAY AFFAIRES 91, rue Boucicaut 92260 FONTENAY-AUX-ROSES ☎ : 33 (0)9 50 27 08 28 🖷 : 33 (0)1 49 73 66 80
INVESTIGATOR	Doctor Marlena Nowakowska, Dermatologist

Y

Document including 22 pages

TABLE OF CONTENT

STUDY SUMMARY

TITLE : SENSITIZING POTENTIAL STUDY OF A PRODUCT «JUVENESSENCE LOT 8.05.149» ACCORDING TO MARZULLI-MAIBACH METHOD, ON 50 SUBJECTS DURING 6 WEEKS.

STUDY REFERENCE : ET-359

PRODUCT : JUVENESSENCE LOT 8.05.149

STUDY IMPLEMENTATION: The study was carried out and all test values recorded by the Clinical Unit PROCOS, localized in Poland; ul. Słowackiego 27/33 lok. 33/34; 01-592 Warsaw.

INVESTIGATOR : Dr Marlena NOWAKOWSKA

MONITOR : Dr ing. Bogdan WICHROWSKI

PROTOCOL : CLINICAL EVALUATION OF THE SENSITIZING POTENTIAL OF A PRODUCT ACCORDING TO MARZULLI-MAIBACH METHOD.

AIM OF THE STUDY : To evaluate the sensitizing potential of a product under dermatological control and under the conditions defined by study's sponsor.

SUBJECTS : 50 healthy volunteers with normal skin corresponding to the inclusion and non-inclusion criteria defined by LISKIN.

STUDY SCHEDULE : August 11th to September 19th, 2008

EXPERIMENTAL DESIGN : simple blind and monocentric study.

MAIN TOLERANCE PARAMETERS :

- Irritation potential (Induction Phase)
  Erythema, edema, desquamation, vesicles rated from 0 to 3 by the dermatologist
- Sensitizing potential (Challenge Phase)
  Reaction rated from 0 to 3 by the dermatologist according to ICDRG (International Contact Dermatitis Research Group)

RESULTS :

PRODUCT DA	**Irritation potential**	**Sensitizing potential**
JUVENESSENCE LOT 8.05.149	Mean rate of 0,000 = non-irritating	No allergic reaction

CONCLUSION :

Under these study conditions, the product «JUVENESSENCE LOT 8.05.149» can be considered non-irritating and non-sensitizing.

1. QUALITY POLICY

The study described has been conducted according to the Good Clinical Practice Guidelines from FDA (FR of 8/08/1978 Part V - Decree n° 77N-0278), EEC (Directives n° 91/507 and III 3976/88 of 11/07/1990) and to the Ministry of Health of the French Republic.
The study has been conducted according to Standard Operating Procedures and to the study protocol defined by the sponsor. Every study events recorded during the study is reported.
Controls on data veracity and conformity with the protocol, have been performed and confirmed by persons participating to the study (APPENDIX I).

This report is a translation of an original report written in French.

2. CONFORMITY CERTIFICATE

I am aware that the study ET-359 has been conducted according to the **«Quality Assurance»** described before.
There was no event which may have affected the quality or integrity of the data.

August 7 2012

Dr ing. B WICHROWSKI
Monitor

date

3. METHOD

3.1. STUDY PRODUCT

The product supplied by EUROTEST, has the following characteristics :

Product name	Product presentation	Product code in the study
JUVENESSENCE LOT 8.05.149	transparent greenish oil	DA

The product was delivered on August 5th, 2008.

3.2. CLINICAL METHODS

3.2.1. Aim of the study

To assess irritation potential and the sensitizing potential of a product under dermatological control and according to Marzulli-Maibach method.

3.2.2. Experimental design

This was an open study.

3.2.3. Study subjects

Inclusion criteria

- Healthy volunteer of Caucasian origin, male or female,
- Age between 18 and 65,
- Phototype II, III or IV,
- Normal skin,
- Subjects having given their informed, written consent,
- Cooperative subjects, aware of the necessity and duration of controls so that perfect adhesion to the protocol established by LISKIN could have been expected.

Non-inclusion criteria

- Pregnancy or nursing women,
- Sun exposure or UV exposure 15 days before study and/or photopatchtests from less than 2 months,
- Hyper irritable skin,
- Known allergies or sensitivities to cosmetics product and Elastoplast,
- skin pathology on the test zones,
- Subjects afflicted with serious or progressive diseases,
- Volunteers undergoing a topical or systemic treatment: anti-inflammatories, antihistamines, immuno-suppressors, corticoids and retinoids.

Inclusion

50 healthy volunteers have been selected according to the inclusion and the non-inclusion criteria, and 50 subjects completed the study. The table below presents the informations concerning all the volunteers included.

	Non included	Included	Drop out	Untraceable
Number of subjects	0	50	0	0
Reason				
Day occurence				

Subjects characteristics

The summary table below presents a synthesis of the observations concerning exclusively the volunteers taken into account for data analysis.

Number of subjects	Sex	Age (mean±SEM)	Phototype (subject number)	Medical or surgical events and medical treatments	
				Before study	During the study
50	37 F 13 M	43 ± 2	II : 50 III : 0 IV : 0	cf. Table in APPENDIX II	

3.3. MATERIAL

The patchs used are "FINN CHAMBERS ON SCANPOR®" which ensures a good occlusion.

4. PRODUCT APPLICATION

Application area	Scapular zones: homolateral (induction zone) and controlateral (challenge zone)
Quantity and Concentration applied	25 µl pure
Frequency	Induction Phase: 3 times a week during 48 hours Challenge Phase: once during 48 hours
Contact time	Induction Phase: 3 weeks Rest Phase: 2 weeks Challenge Phase: 1 week
Application conditions	The product «JUVENESSENCE LOT 8.05.149» was applied like an occlusive patch to the volunteer's back. The patch containing no product was applied under the same conditions to serve as a non-treated control. During all Induction Phase, the homolateral zone was not wet. The volunteers take a shower on Sunday, after patches removing, and pay attention not to put a detergent product on all tested zones. During all Challenge Phase, no washing and no any product application take place on controlateral zone.

5. STUDY SCHEDULE

The study was carried out according to the following diagram:

Induction Phase - 3 weeks (W1, W2, W3)

W1:

Day of the week	Mo	Tu	We	Th	Fr	Sa	Su
Study day	D1	D2	D3	D4	D5	D6	D7
Product application	↓		↓		↓		

W2:

Day of the week	Mo	Tu	We	Th	Fr	Sa	Su
Study day	D8	D9	D10	D11	D12	D13	D14
Product application	↓		↓		↓		

W3:

Day of the week	Mo	Tu	We	Th	Fr	Sa	Su
Study day	D15	D16	D17	D18	D19	D20	D21
Product application	↓		↓		↓		

Rest Phase - 2 weeks (W4, W5)
W4:

Day of the week	Mo	Tu	We	Th	Fr	Sa	Su
Study day	D22	D23	D24	D25	D26	D27	D28

W5:

Day of the week	Mo	Tu	We	Th	Fr	Sa	Su
Study day	D29	D30	D31	D32	D33	D34	D35

Challenge Phase - 1 week (W6)
W6:

Day of the week	Mo	Tu	We	Th	Fr
Study day	D36	D37	D38	D39	D40
Product application	↓				
Reading			L		L

6. ASSESSMENT CRITERIA

6.1. CLINICAL CRITERIA REGARDING THE IRRITATING POTENTIAL (INDUCTION PHASE)

After each application, the patch is removed and the clinical examination is performed by the investigator 30 minutes later in order to eliminate the pressure and the occlusion effects.
The result of examination is negative if the skin looks normal.

The clinical examination is made on the back using the following criteria and scale:

Score	Cotation	CRITERIA : description			
		ERYTHEMA	EDEMA	DRYNESS	VESICLES
0	Absent	Normal aspect	Normal aspect	Normal aspect	Normal aspect
1	Slight	Discreet pink coloration of the whole tested area or rather visible on part of the tested area	More palpable than visible oedema	Discreet thin desquamation, tarnished aspect	More palpable than visible vesicles
2	Marked	Marked erythema covering the whole tested area	Visible oedema	Visible desquamation, flaky aspect.	Visible vesicles
3	Important	Severe erythema covering the whole tested area or erythema diffusing beyond the tested area	Oedema diffusing beyond the tested area	Important desquamation, cracking	Vesicles diffusing beyond the tested area or blisters.

6.2. CLINICAL CRITERIA REGARDING THE SENSITIZING POTENTIAL (CHALLENGE PHASE)

The allergic reactions are evaluated according to the following scale:

Criterion	Quotation ICDRG (*)	Score noted in all tables
No reaction	0	0
Doubtful reaction	?	?
Erythema and edema	+	1
Erythema, edema and vesicles	++	2
Severe reaction with blisters	+++	3

(*) - International Contact Dermatitis Research Group

6.3. ASSESSMENT METHOD

6.3.1. Irritating potential - Induction Phase

At the conclusion of 8 reading of the Induction Phase, the average score of every volunteer is calculated by adding the scores obtained for each reading and by dividing this sum by the actual number of readings (a reading will not be taken into account if there is reaction of the control or global irritation).
The irritating potential of the product will be estimated during the Induction Phase, by calculating the mean of the reactions observed.

The irritating potential of the product is determined according to the following formula:

$$\text{Average score} = \frac{[(\sum \text{scores D1...D19/ nb of readings) vol1} + ... + (\sum \text{scores D1...D19/ nb of readings) volN}]}{\text{nb of volunteers (N)}}$$

Average score	Irritating Potential
score < 0,080	Non-irritating
0,080 ≤ score < 0,160	Very slightly irritating
0,160 ≤ score < 0,560	Slightly irritating
0,560 ≤ score < 1,000	Moderately irritating
1,000 ≤ score < 1,600	Strongly irritating
1,600 ≤ score	Very strongly irritating

6.3.2. Sensitizing potential - Challenge Phase

The possible allergic reaction, during the Induction or Challenge Phase, will be rated from 0 to 3 according to ICDRG (International Contact Dermatitis Research Group).
During the Challenge Phase, the reading will take place 30 minutes after patch-tests removal and 48 hours later.
The sensitizing potential of the product will be assessed by the reading D38 and D40 (Challenge Phase) as a function of the following criteria: reaction ++ (2) or +++ (3) in the absence of added irritation phenomenon.

The presence of only one case of active sensitizing on controlateral side leads to conclusion "Potentially sensitive product".

6.4. PREMATURE STUDY TERMINATION

The subjects have the right to leave the study at any time whatever the reason.
The premature study termination can be for multiple reasons:

- non-compliance with the visits schedule by the subject,
- adverse events (including intercurrent diseases),
- protocol non-adherence/departures from protocol,
- Withdrawal of subject's consent.

6.5. PROTOCOL AMENDMENT

None

7. RESULTS

7.1. IRRITATING POTENTIAL: INDUCTION PHASE

The TABLE OF READINGS regarding the Induction Phase is presented in APPENDIX III.

These reading made 30 min. after having removed the patch-tests showed the following results:

Product DA	D3	D5	D8	D10	D12	D15	D17	D19	Conclusion
JUVENESSENCE LOT 8.05.149	C+ : 0 0 : 50	C+ : 0 0 : 50	C+ : 0 0 : 50	C+ : 0 0 : 50	C+ : 0 0 : 50	C+ : 0 0 : 50	C+ : 0 0 : 50	C+ : 0 0 : 50	**non-irritating** (IRR = 0,000)

C+ = Positive control IRR = global irritation MV = missing value

Under these study conditions, the product «JUVENESSENCE LOT 8.05.149» showed a score lower than 0.080, so it can be considered <u>non-irritating</u>.

7.2. SENSITIZING POTENTIAL: CHALLENGE PHASE

The TABLE OF READING regarding the Challenge Phase is presented in APPENDIX IV.

These reading made 30 min. and 48h after having removed the patch-tests showed the following results:

Product DA	Zone	Day of the reading		Global result
		D38	D40	
JUVENESSENCE LOT 8.05.149	Reading homolateral zone	C+: 0 DA: 0: 50 ?: 0 1: 0 2: 0 3: 0	C+: 0 DA: 0: 50 ?: 0 1: 0 2: 0 3: 0	**non-sensitizing**
	Reading controlateral zone	C+: 0 DA: 0: 50 ?: 0 1: 0 2: 0 3: 0	C+: 0 DA: 0: 50 ?: 0 1: 0 2: 0 3: 0	

C+= Positive control
DA = JUVENESSENCE LOT 8.05.149
MV= missing value

The product «JUVENESSENCE LOT 8.05.149» can be considered <u>non-sensitizing</u> under these study conditions.

8. CONCLUSION

Under these study conditions, the product «JUVENESSENCE LOT 8.05.149» can be considered <u>non-irritating</u> and <u>non-sensitizing</u>.

APPENDICES

APPENDIX I

RESULTS AUTHENTIFICATION SHEET

APPENDIX II

VOLUNTEERS CHARACTERISTICS

VOLUNTEERS CHARACTERISTICS

Subject number	Subject code	Age	Sex	Photo-type	Skin type (Normal or Sensitive)	Medical or surgical events and medical treatments	
			F or M			before the study	during the study
1	LENMA	40	F	II	N	-	-
2	OLEST	57	M	II	N	-	-
3	ZAJAR	20	M	II	N	-	-
4	MARMA	44	M	II	N	-	-
5	SWIAD	28	M	II	N	-	-
6	LUKRE	20	M	II	N	-	-
7	WASBO	25	M	II	N	-	-
8	KOSZO	31	F	II	N	-	-
9	BRYAN	58	F	II	N	-	-
10	CIBMA	61	F	II	N	-	-
11	DABPA	23	F	II	N	-	-
12	FROLE	51	F	II	N	-	-
13	SZCJA	50	M	II	N	-	-
14	KORWA	58	F	II	N	-	-
15	RUSPI	28	M	II	N	-	-
16	RUDMI	61	F	II	N	-	-
17	SKABR	65	F	II	N	-	-
18	SADHA	65	F	II	N	-	-
19	GALFE	58	F	II	N	-	-
20	ROMWI	62	F	II	N	-	-
21	KOZEW	20	F	II	N	-	-
22	SWIAN	56	F	II	N	-	-
23	OTWIW	49	F	II	N	-	-
24	STAEL	61	F	II	N	-	-
25	STRWA	62	F	II	N	-	-
26	HOPWI	31	F	II	N	-	-
27	KOWHU	27	M	II	N	-	-
28	MUSWL	63	F	II	N	-	-
29	SOKKR	21	M	II	N	-	-
30	SZEHE	65	F	II	N	-	-

UN = untraceable

VOLUNTEERS' CHARACTERISTICS - (continuation)

Subject number	Subject code	Age	Sex	Photo-type	Skin type (Normal or Sensitive)	Medical or surgical events and medical treatments	
			F or M			before the study	during the study
31	WYSAN	57	F	II	N	-	-
32	RUSBA	37	F	II	N	-	-
33	BIABA	51	F	II	N	-	-
34	WISLI	49	F	II	N	-	-
35	IWAMA	44	F	II	N	-	-
36	GLUJU	19	F	II	N	-	-
37	NIEAN	53	F	II	N	-	-
38	BANBO	35	F	II	N	-	-
39	LUBZO	45	F	II	N	-	-
40	WODIR	42	F	II	N	-	-
41	KOSWE	24	F	II	N	-	-
42	OLSMA	33	F	II	N	-	-
43	ZIOZO	45	F	II	N	-	-
44	STYJA	57	F	II	N	-	-
45	STAAL	20	F	II	N	-	-
46	KOSJA	30	M	II	N	-	-
47	SZPAL	26	F	II	N	-	-
48	PACJA	35	M	II	N	-	-
49	ARERO	19	M	II	N	-	-
50	KEPJO	58	F	II	N	-	-

UN = untraceable

APPENDIX III

TABLE OF READING

INDUCTION PHASE

TABLE OF READING - Induction Phase

Subject number	D3		D5		D8		D10		D12		D15		D17		D19	
	C	DA	C	DA	C	DA	C	DA	C	DA	C	DA	C	DA	C	DA
1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
8	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
9	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
10	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
11	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
12	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
13	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
14	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
15	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
16	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
17	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
18	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
19	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
20	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
21	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
22	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
23	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
24	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
25	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
26	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
27	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
28	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
29	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
30	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

UN = untraceable
C = control
DA = JUVENESSENCE LOT 8.05.149

TABLE OF READING - Induction Phase (continuation)

Subject number	D3		D5		D8		D10		D12		D15		D17		D19	
	C	DA	C	DA	C	DA	C	DA	C	DA	C	DA	C	DA	C	DA
31	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
32	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
33	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
34	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
35	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
36	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
37	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
38	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
39	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
40	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
41	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
42	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
43	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
44	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
45	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
46	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
47	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
48	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
49	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
50	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

UN = untraceable
C = control
DA = JUVENESSENCE LOT 8.05.149

APPENDIX IV

TABLE OF READING
CHALLENGE PHASE

TABLE OF READING - Challenge Phase

Subject number	D38 Homolateral zone		D38 Controlateral zone		D40 Homolateral zone		D40 Controlateral zone	
	C	DA	C	DA	C	DA	C	DA
1	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0
4	0	0	0	0	0	0	0	0
5	0	0	0	0	0	0	0	0
6	0	0	0	0	0	0	0	0
7	0	0	0	0	0	0	0	0
8	0	0	0	0	0	0	0	0
9	0	0	0	0	0	0	0	0
10	0	0	0	0	0	0	0	0
11	0	0	0	0	0	0	0	0
12	0	0	0	0	0	0	0	0
13	0	0	0	0	0	0	0	0
14	0	0	0	0	0	0	0	0
15	0	0	0	0	0	0	0	0
16	0	0	0	0	0	0	0	0
17	0	0	0	0	0	0	0	0
18	0	0	0	0	0	0	0	0
19	0	0	0	0	0	0	0	0
20	0	0	0	0	0	0	0	0
21	0	0	0	0	0	0	0	0
22	0	0	0	0	0	0	0	0
23	0	0	0	0	0	0	0	0
24	0	0	0	0	0	0	0	0
25	0	0	0	0	0	0	0	0
26	0	0	0	0	0	0	0	0
27	0	0	0	0	0	0	0	0
28	0	0	0	0	0	0	0	0
29	0	0	0	0	0	0	0	0
30	0	0	0	0	0	0	0	0

UN = untraceable
C = control
DA = JUVENESSENCE LOT 8.05.149

TABLE OF READING - Challenge Phase (continuation)

Subject number	D38 Homolateral zone		D38 Controlateral zone		D40 Homolateral zone		D40 Controlateral zone	
	C	DA	C	DA	C	DA	C	DA
31	0	0	0	0	0	0	0	0
32	0	0	0	0	0	0	0	0
33	0	0	0	0	0	0	0	0
34	0	0	0	0	0	0	0	0
35	0	0	0	0	0	0	0	0
36	0	0	0	0	0	0	0	0
37	0	0	0	0	0	0	0	0
38	0	0	0	0	0	0	0	0
39	0	0	0	0	0	0	0	0
40	0	0	0	0	0	0	0	0
41	0	0	0	0	0	0	0	0
42	0	0	0	0	0	0	0	0
43	0	0	0	0	0	0	0	0
44	0	0	0	0	0	0	0	0
45	0	0	0	0	0	0	0	0
46	0	0	0	0	0	0	0	0
47	0	0	0	0	0	0	0	0
48	0	0	0	0	0	0	0	0
49	0	0	0	0	0	0	0	0
50	0	0	0	0	0	0	0	0

UN = untraceable
C = control
DA = JUVENESSENCE LOT 8.05.149

REPORT

CHECKING IN HUMAN OF THE SKIN COMPATIBILITY OF A COSMETIC PRODUCT AFTER SINGLE APPLICATION UNDER PATCH
Patch test 24 h

Test Product :

JUVENESSENCE LOT 604136

Promotor

BIOTECHMARINE
ZI – BP 65
2260 PONTRIEUX

JA/CL

Bordeaux, 9 October 2012

TABLE OF CONTENTS

Laboratoire COSDERMA - Service de dermatologie Pr. Taïeb - Groupe Hospitalier Saint André - 1 rue Jean Burguet - BP 50057 - 33023 Bordeaux Cedex
Tel : 05 56 94 75 40 - Fax : 05 56 79 49 75 - email : laboratoire@cosderma.com

1- Aim of the study

This study intends to check the skin compatibility of the cosmetic product " **JUVENESSENCE LOT 604136** ", after single application to the skin, during 24h, under exaggerated experimental conditions for **10** volunteers.

The skin compatibility of the test product is checked, after patch removal and visual examination of the experimental area by the investigator or the responsible technician.

The study aiming at a better knowledge of the skin compatibility of the test product and the foreseeable risk incurred by the volunteers who take part in the study being minor, there is a suitability between the aim of the study, its possible risks and the possible troubles related to the modalities planned in the protocol.

2- Methodology

2.1 Expérimental plan

This study was without direct individual benefits, each subject was used as own control.
This monocentric study was performed in open.

The delay of the study was 24h and consisted in 2 visits at the Institute (T0, T24h).

2.2 Investigateur Centre

Laboratoire COSDERMA
Service de dermatologie du Pr Taïeb - Groupe Hospitalier Saint André
1 rue Jean Burguet - BP 50057
33023 Bordeaux Cedex
tel : 05 56 94 75 40
email : laboratoire@cosderma.com

2.3 Investigation place

Service de dermatologie du Pr Taïeb
Groupe Hospitalier Saint André
1 rue Jean Burguet
33000 Bordeaux

2.4 Technical staff

Investigator : Pr Alain Taïeb

Technician : Chrystelle Latxague

3- Dates of performance

Beginning on : 3 January 2007
Ending on : 4 January 2007

4- Test product

4.1 Information

The document relating to the test product supplied with the samples was the Sponsor's letter of agreement particularly concerning the conformity of each formula the regulations in force.

4.2 Identification

Product	Ref.	Quantity for the study	Nb samples
JUVENESSENCE	**LOT 604136**	5 ml	1 x 5 ml

4.3 Normal conditions of use

Product		Conditions of Use
JUVENESSENCE LOT 604136	site	/
		Cosmetic raw material

4.4 Experimental conditions of use of the test product

For the study, the selected area of application is the back.

Product	Patch material	Experimental conditions of use	Quantity applied	Contact time	Control time on D1
JUVENESSENCE LOT 604136	Occlusive (Finn chambers®)	Pure	20 µl	24h	15 minutes after patch removal

- *Finn Chamber standard® : aluminium cupula in which the product is put down (20 µl or approximately 20 mg), kept in position by an hypoallergenic adhesive : Scanpor® (inner diameter : 8 mm, surface : 50 mm²)*

One control patch, corresponding to the type of patch material used, containing an ad hoc quantity of water for injectable preparation, is applied at the same time.

The patch removal is performed by the investigator or the responsible technician.

The quantities of product are measured with a micropipette with single use tips or with a single use syringe.

5- Volunteers

5-1 Panel

The panel is representative of a large population.
All the volunteers corresponded to these specific inclusion and non inclusion criteria.

5-2 Number

The number of volunteers participating to the study was **10**.
The number of volunteers whose data are exploitable at the end of the study was **10**.

5-3 Inclusion criteria

The specific inclusion criteria were the following ones :

- age : 18 to 65,
- sex : female,
- phototype (Fitzpatrick) : I to III.
- all types of skin
- capable of looking her assent writes read and signed,
- affiliated to the Social Security.

All the volunteers corresponded to these specific inclusion criteria. Their typological characteristics are defined in **Appendix 1.**

5-4 Non inclusion criteria

The specific non inclusion criteria are those defined for this kind of methodology in accordance with the corresponding procedure and the following ones for this study particularly :

- cutaneous marks on the experimental area which could interfere with the assessment of skin reactions (pigmentation troubles, scar elements, over-developed pilosity, ephelides and naevi in too great quantity, sunburn.....),
- eczematoid reaction still visible, scar or pigmentary sequelae of previous tests on the experimental area,
- allergy to colophony, to nickel,
- allergy or reactivity to sun care products,
- skin hyper-reactivity,
- reactivity to ethanol,
- reactivity to adhesive plaster,
- participation in more than 5 tests under exaggerated use conditions (under patch) within 12 months before the study, including 3 hypoallergenicity tests at the most,
- intensive sun exposure within the month before the study,
- forecast of intensive sun or UVA exposure (UV lamps) during the test period,
- forecast of bath (bathtub, sea or swimming-pool), sauna or hammam sessions during the test period,
- intensive or regular practice of one or several sports whose temporary interruption creates difficulties,
- treatment with Vitamin A acid or its derivatives within 3 months before the beginning of the study,
- treatment with topical corticoids on the experimental area within 8 days before the study,
- treatment with PUVA or UVB within 1 month before the study,
- forecast of vaccination during the test period or last vaccination within 3 weeks before the study.

All the volunteers corresponded to these specific non inclusion criteria

5-5 Constraints of the study

The constraints imposed on the volunteers are those defined for this kind of methodology in accordance with the corresponding procedure :

- no application of other products (than the tested ones) to the experimental area,
- no wearing of too thigh or restraining clothes on the experimental area, liable to produce frictions and to cause unsticking of the patches,
- no bath (bathtub or swimming-pool or sea), no hammam or sauna sessions during the study,
- if shower, protection of the experimental area or no violent projection of water and no application of soap to the experimental area to avoid patch removal or appearance of intercurrent phenomena and very gentle wiping if necessary,
- no excessive sweating and no intensive sport liable to cause unsticking of the patches,
- no intensive sun or UVA exposure (UV lamps) during the study, especially after patch removal,
- neither anti-allergic, anti-inflammatory (systemic or topical corticotherapy...) treatment nor treatment with patent medicines containing Vitamin A acid or its derivatives during the study (if therapeutic requirement : exclusion foreseen),
- no vaccination during the study.

5-6 Control of the observance

The Investigator checked the respect of the **constraints**.

The synthesis of the answers obtained was enclosed in **Appendix 2.**

In case of deviations from the protocol, they were analysed and the investigator assessed their effect on the validity of the results.

All the constraints of the study, defined in the protocol, were respected by the volunteers.

6- Assessment

6-1 Time table

Study process	Beginning T0	Contact time T24h ± 2h
Selection of the volunteers	X	
Assignment of the volunteer number	X	
Volunteer information	X	
IVCP *	X	
Application of the patch by the technician	X	
Removal of the patch by the technician		X
Assessment criteria (15 mn after removal patch)		X

The fact that the the application of the product and also that examinations were perfectly controlled, the number of volunteers and the delay of the study have allowed to check the cutaneous tolerance of the product and appreciate the possible Irritation.

* A duplicate of the IVCP will be given to the volunteer the day of the inclusion visit. The original has been kept by the investigator.

6-2 Checking of the skin compatibility

- *Principle and bibliography*

The skin compatibility of the test products is checked using patch application which create a certain occlusion and favour the penetration of the ingredients through the skin. In these experimental conditions, an Irritative potential is more easily proved.

Numerous publications support this methodology, notably :

Comment tester les produits cosmétiques ?, Dermatologie Pratique, 2003, n° 273, 1-4

Reactive changes in human epidermis following simple occlusion with water, Contact Dermatitis, Mikulowska A, 1992, 26, 224-227

Test strategies for development of cosmetic products using dermatological test models, Seifen-öle-fette-wachse, Matthies W, 1991, 117, 42-43

The Duhring Chamber: an improved technique for epicutaneous testing of irritant and allergic reactions, Contact Dermatitis,Frosch PJ & Klingmann AM, 1979, 5, 73-81

Appraisal of the safety of chemicals in Food, Drugs and Cosmetics, FDA (ed), Draize JH, 1959, 46-48

• *Methodology, patch material*

The products are applied on the patch using a single use syringe. The patch are applied afterward rapidly on the skin in avoiding the areas of friction or compression with clothes. The skin examination is performed by the investigator or the responsible technician who will check that the skin area chosen not contained cutaneous marks which could interfere with the assessment of skin reactions (pigmentation troubles, scar elements, over-developed pilosity, ephelides and naevi in too great quantity, sunburn.....). The maximal number of test products, the patch material, the quantity of product to apply, the methodology, the removal of the patch and the skin examination complies with the corresponding procedure of the laboratory.
The experimental area is the back.

• *Environmental conditions*

The environmental conditions imposed on the volunteers, were the following ones :

- *controlled temperature : t ° = 20°C ± 2°C*
- *controlled relative humidity : HR = 45 % ± 15 %*

• *Clinical control*

- Sites

The skin examination is visually performed by the investigator or the responsible technician under standard "daylight" source.

- Frequency

The skin examination is realised at T24h ± 2h, 15 minutes (or more if some redness appeared after patch removal) after patch removal.

• *Assessment criteria*

- *Clinical signs*

Denominations	Laboratory code	intensity	form	note
Erythema	E	- ordinal scale in 3 points : • slight • moderate • severe	- erythema : • diffuse • ponctuated • peripheral	• slight = 1 • moderate = 2 • severe = 3 • diffuse = d • ponctuated = p • peripheral = peri
Oedema	Oe			
Dryness	D			
Colouration	C			
Comedone, microcyst	Co, Mi	- cnumbered		
Vesicle, papule	V, Pa	- ordinal scale in 2 points : • 1 to 2 vesicles • number of vesicles >2		• 1 to 2 = 1 • nb >2 = 2
Bleb, croutelle	Bu, Cr	- described		• If described = 2

The investigator or responsible technician noted for any clinical sign described its location, frequency, intensity, evolution, medical treatment possibly undertaken. He has established the the usual or unusual characteristic of the clinical sign, by questioning the volunteers about the effects observed when applying similar products.

– *Discomfort sensations*

Denomination	Laboratory code	intensity	note
Heating	Ech	- ordinal scale in 3 points : • slight • moderate • severe	• slight = 1 • moderate = 2 • severe = 3
Stinging	Pi		
Pruritus (itching)	Pr		
pulling	Ti		
burning	Br		

- *Expression of the results*

All the volunteers included in the study are taken into account to check the skin compatibility of the test products as long as they are submitted at least to one post application examination at the defined time or else.
The expression of the results of the skin examination and questioning is that defined for this type of study in accordance with the corresponding procedure.

The results are expressed :

- **in percentage of reactive volunteers** : for this calculation only the visible signs of reactivity : erythema, oedema, vesicle, bulla, papule are taken into account.
- **in a descriptive manner** for the other visible signs or for the sensations of discomfort : if the frequency of appearance of these signs justifies it, the percentage of reactive volunteers is calculated.
- **in score of skin irritation**, calculated from the "marks" allocated to the visible signs : erythema, oedema, vesicle, papule (from 1 to 2 or 3) which takes into account the intensity of skin reactions.

For each volunteer, an **individual daily irritation score (Idis)** is calculated : sum of all the marks obtained for the observed signs.

For the panel, a **mean daily irritation score (Mdis)** is calculated according to the formula :

$$\textbf{Mdis} = \Sigma \textbf{ (Idis) / number of volunteers (exploitable data)}$$

- *Interpretation of the results*

The investigator has concluded in terms of **very good, good, quite good or bad skin compatibility.** The interpretation of the results of the skin examination is that defined for this type of study in accordance with the corresponding procedure.

7- Results and discussions

The individual values for the skin examination and the questionning of the volunteers are reported in **Appendix 3.**

In summary :

Product	Control time on D1	Number of reactive volunteers	Types of reaction	Mean daily irritation score Mdis	% of reactive volunteers
JUVENESSENCE LOT 604136	**15 minutes after patch removal**	**0**	**/**	**0**	**0%**

8- Conclusion

Under the experimental conditions adopted, the product " **JUVENESSENCE LOT 604136** " **has a very good skin compatibility.**

Dates and signatures :

Investigator
Pr Alain Taïeb (dermatologist)

17/10/2012

Technician
Chrystelle Latxague

P/o N. DURAND
17/10/12

Study director
Jérôme Asserin

10/19/12

Person in charge of quality
P/o DUBATON
10/19/2012

APPENDICES

Appendix 1

TYPOLOGICAL CHARACTERISTICS OF THE VOLUNTEERS

Volunteers		Age (years)	Sex F=female	Phototype *	Skin in good health in the back
Ref.	Name surname				
1	PANT/E	22	F	II	x
2	CARR/E	54	F	III	x
3	PLAZ/I	49	F	III	x
4	MEZA/A	39	F	III	x
5	SAIN/R	26	F	I	x
6	DRUI/S	37	F	II	x
7	BOUS/C	42	F	II	x
8	NAKA/G	32	F	II	x
9	MORS/G	18	F	I	x
10	LARR/D	46	F	II	x

Legends : / = no x = yes

***phototype according to Fitzpatrick,** established on the principle of a first 30 to 40-minute sun exposure after the winter or a period without exposure of an equivalent duration :*

TYPE	HAIRS	SKIN	LENTIGOS	SUN BURNING
I	redhead	milky	+++	Always burns easily, never tans
II	Fair-haired	Light coloured	++	Burns moderately, tans gradually
III	Fair-haired brown	Light coloured	+	Burns slightly, always tans easily
IV	brown	Coloured skin	o	Burns rarely, tans intensely
V	Black hair and frizzy	black	o	Never burns, strongly pigmented

Laboratoire COSDERMA - Service de dermatologie Pr. Taïeb - Groupe Hospitalier Saint André - 1 rue Jean Burguet - BP 50057 - 33023 Bordeaux Cedex
Tel : 05 56 84 25 40 - Fax : 05 56 79 49 75 - email : laboratoire@cosderma.com

Appendix 2

CONTROL OF THE OBSERVANCE
Constraints

Constraints (10 exploitable results)	Number of volunteers who respected the constraints	Percentage of volunteers who respected the constraints
No application of products similar to the tested ones to the experimental areas Deviation : none	10	100 %
no wearing of too thigh or restraining clothes on the experimental area, liable to produce frictions and to cause unsticking of the patches, Deviation : none	10	100 %
no bath (bathtub or swimming-pool or sea), no hammam or sauna sessions during the study Deviation : none	10	100 %
if shower, protection of the experimental area or no violent projection of water and no application of soap to the experimental area to avoid patch removal or appearance of intercurrent phenomena and very gentle wiping if necessary Deviation : none	10	100 %
no excessive sweating and no intensive sport liable to cause unsticking of the patches Deviation : none	10	100 %
no intensive sun or UVA exposure (UV lamps) during the study, especially after patch removal Deviation : none	10	100 %
neither anti-allergic, anti-inflammatory (systemic or topical corticotherapy...) treatment nor treatment with patent medicines containing Vitamin A acid or its derivatives during the study (if therapeutic requirement : exclusion foreseen), Deviation : none	10	100 %
no vaccination during the study Deviation : none	10	100 %

Appendix 3-1

CHECKING OF THE CUTANEOUS COMPATIBILITY

Product N°1: **"JUVENESSENCE LOT 604136"** (Finn chambers)

Volunteers		Skin examination 15 min after patch removal	Idis
Ref.	Name / surname		
1	PANT/E	/	/
2	CARR/E	/	/
3	PLAZ/I	/	/
4	MEZA/A	/	/
5	SAIN/R	/	/
6	DRUI/S	/	/
7	BOUS/C	/	/
8	NAKA/G	/	/
9	MORS/G	/	/
10	LARR/D	/	/
	Mdis	0	

Legends : / = no clinical sign

Laboratoire COSDERMA - Service de dermatologie Pr. Taïeb - Groupe Hospitalier Saint André - 1 rue Jean Burguet - BP 50057 - 33023 Bordeaux Cedex
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Appendix 3-2

CHECKING OF THE CUTANEOUS COMPATIBILITY

Product N°2: control patch (Finn chambers)

Volunteers		Skin examination 15 min after patch removal	Idis
Ref.	Name / surname		
1	PANT/E	/	/
2	CARR/E	/	/
3	PLAZ/I	/	/
4	MEZA/A	/	/
5	SAIN/R	/	/
6	DRUI/S	/	/
7	BOUS/C	/	/
8	NAKA/G	/	/
9	MORS/G	/	/
10	LARR/D	/	/
Mdis		0	

Legends : / = no clinical sign

MANUFACTURING PROCESS
KALPARIANE™

HARVESTING / IDENTIFICATION*(Alaria Esculenta)*

↓

DRYING

↓

GRINDING

↓

EXTRACTION WITH THE SOLVENT
CAPRYLIC/CAPRIC TRIGLYCERIDE

↓

FILTRATION

↓

QUALITY CONTROL

↓

PACKAGING

↓

QUALITY CONTROL

Production manager
Jean-Marc CATROUX

Date de mise à jour / *Updated date : 01/12/2016*

KALPARIANE™

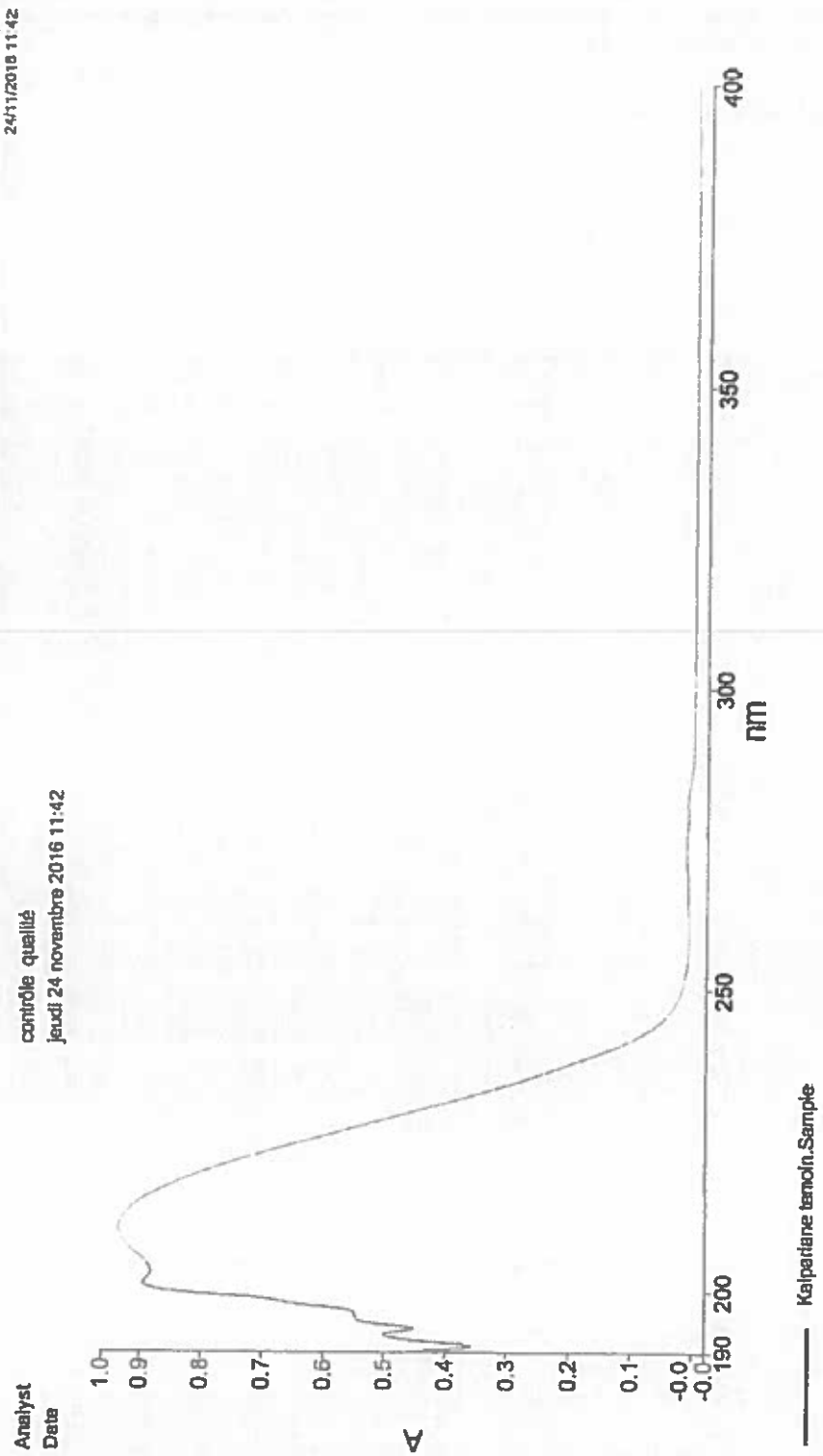
INCI NAME : Caprylic/Capric Triglyceride – Alaria Esculenta Extract
CAS : 73398-61-5
EINECS : 277-452-2
25% Alaria Esculenta Extract 75% Caprylic / Capric Triglyceride

Produit conforme Mass Balance (RSPO) BVC-RSPO-1-1972708497 / *Product Mass Balance compliant(Rountable for Sustainable Palm Oil (RSPO)) BVC-RSPO-1-1972708497*

DONNEES PHYSICOCHIMIQUES
PHYSICO-CHEMICAL DATA
Numéro de référence / *Reference number* : STANDARD

CARACTERISTIQUES *CHARACTERISTICS*	METHODES *METHODS*	STANDARD *STANDARD*
Conformité Mass Balance *Mass Balance conformity*	PO-HSE-004	**Conforme** *Conform*
Aspect *Aspect*	MO PHY 002	**Liquide limpide huileux** *Oily limpid liquid*
Couleur *Colour*	MO PHY 002	**Jaune-vert pâle à vert foncé** (1 à 7UG) *Pale yellow green to dark green (1 to 7UG)*
Odeur *Odour*	MO PHY 002	**Faible** *Slight*
Densité (20°C) *Density*	MO PHY 024	0,920 - 0,950
Indice de réfraction(20°C) *Refractive index*	MO PHY 008	$1,440_0$ - $1,460_0$
Indice de péroxyde* *Peroxyd index**	MO PHY 034	<3 meq d'oxygène actif/kg <3 *meq of active oxygen/kg*
Spectre UV *UV spectrum*	MO PHY 013	**Conforme au témoin** *Similar to the standard*
Spectre IR *IR spectrum*	MO PHY 011	**Conforme au témoin** *Similar to the standard*
Solvant *Solvent*	**Mélange de triglycérides d'acides gras saturés (C8C10) d'origine végétale** *Mixture of triglycerides of saturated fatty acids (C8C10) of vegetal origin.*	
Métaux lourds*(mg/kg) *Heavy metals* (mg/kg)*		
• **Arsenic** *Arsenic*		< 2
• **Cadmium** *Cadmium*		< 3
• **Plomb** *Lead*		< 5
• **Nickel** *Nickel*		< 2
• **Argent** *Silver*		< 5 Iodine 21 ppm
• **Mercure** *Mercury*		< 1

* Teneurs garanties sous contrôle statistique / *Contents guaranteed under statistical control*

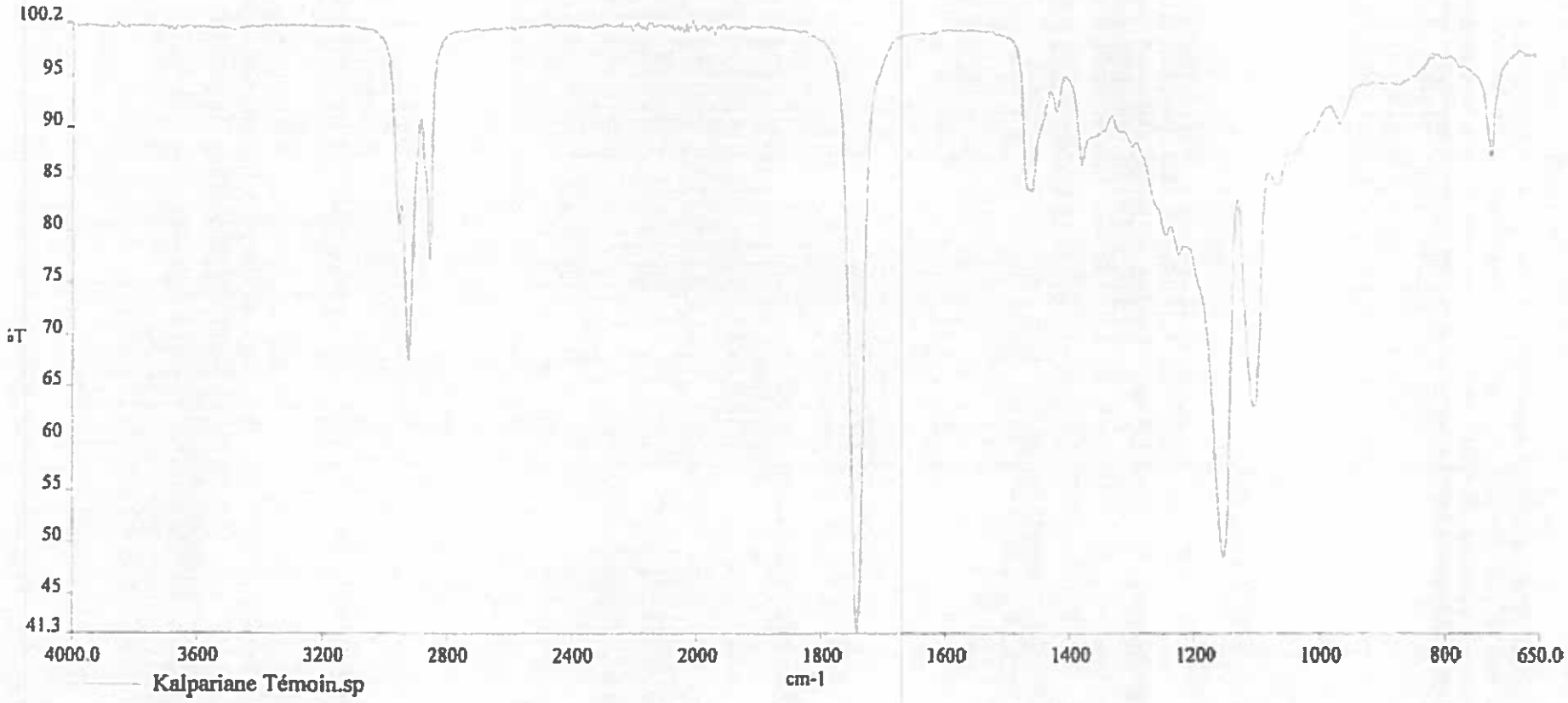


Date: jeudi 24 novembre 2016

SPECTRE IRFT

SPECTROPHOTOMETRE SPECTRUM 100 N° de série 77774 - PERKIN ELMER

Accessoire ATR Universel N° 7031330



KALPARIANE™

INCI NAME : Caprylic/Capric Triglyceride – Alaria Esculenta Extract
CAS : 73398-61-5
EINECS : 277-452-2
Produit conforme Mass Balance (RSPO) BVC-RSPO-1-1972708497 / *Product Mass Balance compliant(Rountable for Sustainable Palm Oil (RSPO)) BVC-RSPO-1-1972708497*

DONNEES MICROBIOLOGIQUES
MICROBIOLOGICAL DATA
Numéro de référence / *Reference number* : STANDARD

CARACTERISTIQUES *CHARACTERISTICS*	**METHODES** *METHODS*	**STANDARD** *STANDARD*
Germes totaux** *Total germs***	MO MIC 002 / NF EN ISO 21149	**< 100**
Germes Pathogènes *Pathogens*		
- *Staphylococcus aureus*	MO MIC 012 / NF EN ISO 22718	**Absence** *None*
- *Candida albicans*	MO MIC 010 / NF EN ISO 18416	**Absence** *None*
- *Pseudomonas aeruginosa*	MO MIC 011 / NF EN ISO 22717	**Absence** *None*
- *Escherichia coli*	MO MIC 025 / NF EN ISO 21150	**Absence** *None*
Levures / Moisissures** *Yeasts / Moulds***	MO MIC 021 / NF EN ISO 16212	**< 100**

** Les résultats sont indiqués en UFC/mL pour les liquides et en UFC/g pour les solides
** *Results are indicated in CFU/mL for the liquids and in CFU/g for the solids*

CONFORME
CERTIFIED TRUE AND CORRECT
RESPONSABLE CONTROLE QUALITE: **P. SOUBIES**
QUALITY CONTROL MANAGER
01 DEC. 2016

CONFORME
CERTIFIED TRUE AND CORRECT
ASSURANCE QUALITE : **A. HAMON**
QUALITY ASSURANCE
01 DEC. 2016

LISKIN

STUDY OF THE SENSITIZING CAPACITY OF A PRODUCT ACCORDING TO THE MARZULLI-MAIBACH METHOD

REPORT

STUDY REF.	ET-319
PRODUCT	"KALPARIANE BATCH 8.05.149"
NUMBER OF SUBJECTS	50
INSTIGATOR	EUROTEST
MONITOR	M. Bogdan WICHROWSKI LISKIN Immeuble Fontenay Affaires 91 Rue Boucicaut 92260 FONTENAY-AUX-ROSES Tel. 33 (0)9 50 27 08 28 Fax. 33 (0)1 49 73 66 80
INVESTIGATOR	Dr Marlena Nowakowska, Dermatologist

Document containing 23 pages

TABLE OF CONTENTS

SUMMARY OF STUDY

TITLE: STUDY OF THE SENSITIZING CAPACITY OF A PRODUCT « KALPARIANE BATCH 8.05.149», ACCORDING TO THE MARZULLI-MAIBACH METHOD ON 50 SUBJECTS DURING 6 WEEKS.

STUDY REFERENCE: ET-319

PRODUCT: KALPARIANE BATCH 8.05.149

STUDY LOCATION: The study was carried out and the numerical values recorded by the clinical unit 'PROCOS' in Poland: ul. Stowackiego 27/33 llok. 33/34, 01-592 Varsovie.

INVESTIGATOR: Dr Marlena NOWAKOWSKA

STUDY MONITOR: Dr Eng. Bogdan WICHROWSKI

PROTOCOL: MAXIMALISATION TEST ACCORDING TO MARZULLI-MAIBACH FOR A PRODUCT

PURPOSE: To evaluate the irritation and sensitization potential of a given product under dermatological control in the conditions established by the sponsor of the study.

SUBJECTS: 50 volunteers with normal skin corresponding to the inclusion and non-inclusion criteria established by LISKIN.

TEST DATES: 11/08/08 – 19/09/08

EXPERIMENTAL PLAN: Monocentric study in simple blind trial.

MAIN TOLERANCE PARAMETERS:

- Irritation potential (induction phase)
  - Erythema, oedema, dryness, blisters, evaluated by a dermatologist according to a scale of 0 to 3
- Sensitizing potential (revelation phase)
  - Reaction evaluated by the dermatologist according to a scale of 0 to 3 established by the ICDRG (International Contact Dermatitis Research Group)

Name of Product	Irritation Potential	Sensitizing Potential
KALPARIANE BATCH 8.05.149	Average Score 0.000 = non irritant	Total absence of allergic reaction

CONCLUSION:

Under the conditions of this study, the product « KALPARIANE BATCH 8.05.149 » proved to be non irritant and non sensitizing.

1. QUALITY ASSURANCE

The study was carried out according the 'Bonnes Pratiques Cliniques' (Good Clinical Practices) rules defined by the FDA (FR of 08/08/1978. Part V, Decree number 77N-0278), by the EEC (Directives number 91/507 and III 3976/88 EN of 11/07/1990) and by the Minstry of Health of the French Republic.

The study carried about according the the standard operational procedures and according to the protocol of the study as defined by the instigator of the study. The observation books and daily notes were checked as were the exactitude of the facts.

The authenticity and truth of the experimental facts collected were confirmed by the people having taken parT in the study. See APPENDIX I.

2. CERTIFICATE OF CONFORMITY

To my knowledge, study ET-319 was carried out in accordance with the "Quality Assurance" quoted above.

No event liable to affect the quality or integrity of the facts occurred.

Dr Eng. B WICHROWSKI

Monitor

1 October 2008

3. METHODOLOGY

3.1 DESCRIPTION OF THE STUDY

3.1.1 Product studied

The product supplied by EUROTEST, has the following characteristics:

Name Of Product DA	Nature Of Product	Code Of Product Studied
KALPARIANE BATCH 8.05.149	Transparent, greenish oil	DA

The product was received 05/08/2008.

3.2 CLINICAL METHODS

3.2.1 Study Objectives

To appreciate the irritation and sensitization capacity of the product using the sensitization method Marzulli-Maibach.

3.2.2 Experimental Plan

This was an open study.

3.2.3 Subjects studied

Inclusion Criteria

- Healthy volunteer of Caucasian origin
- Age between 18 and 65
- Phototype II, III and IV
- Normal skin
- Subject having given a willing, informed consent in writing
- Co-operative subject advised about the necessity and duration of the controls enabling the expectation of a perfect adhesion to the protocol put in place by LISKIN.

Exclusion Criteria

- Pregnant or breast-feeding woman
- Person having been exposed to the sun or UV since less than a month and/or having received photo patch tests since less than two months
- Subject presenting a hyper irritable skin
- Subject allergic to adhesive bandages and/or cosmetic products
- Subject have a cutaneous pathology in the experimental zone
- Subject having a serious or evolutive illness
- Subject receiving a topical or systematic medical treatment: - anti-inflammatory, anti-histaminic, immune-suppressors, cortisone and retinoidal

Inclusion

Fifty volunteer subjects were chosen according to the inclusion and exclusion criteria, and 50 subjects completed the whole study. The following table summarises the information concerning the participation in the study of all the selected subjects.

	Not included	Included	Stopped during study	Loss of contact
Number of subjects		50		
Reason				
Day of event				

Characteristics of the subjects

The table below gives a summary of the observations concerning only those volunteers included in the analysis of data.

Number of volunteer	Sex	Age (Av ±MES)	Phototype	Medical or surgical intervention and medical treatment	
				Before study	During study
50	37F 13M	43 ± 2	I : 0 II : 50 III : 0	Cf Table in Appendix II	

3.3 MATERIAL

The patch tests (or epidermotests) used are the FINN CHAMBERS ON SCANPOF®. The FIN CHAMBER is made up of an isolation cup that ensures good occlusion.

4. APPLICATION OF PRODUCTS

The product is applied to the back.

Application Zone	Scapular zones: homolateral (induction zone) and heterolateral (revelation zone)
Quantity applied	25µl pure
Frequency	Induction phase: 3 times a week during 48 hours Revelation phase: once a week during 48 hours
Duration	Induction phase: 3 weeks Latent phase: 2 weeks Revelation phase: 1 week
Conditions of application	The product "KALPARIANE BATCH 8.05.149" was placed in a cup and applied to the back of the volunteer. A patch containing no product at all was applied in the same conditions and served as a non-treated control. Throughout the induction phase, the homolateral zone was kept dry. The volunteers had a shower the Sunday following withdrawl of the patches, being careful not to put any detergent on the sites. At the revelation phase, no washing or application of any product was carried out on the heterolateral zone.

5. STUDY PROCESS

The study was carried out according to the following schema:

Induction phase – three weeks (W1, W2, W3)

W1:

Day of the Week	Mon	Tues	Wed	Thurs	Fri	Sat	Sun
Day of the study	D1	D2	D3	D4	D5	D6	D7
Application of Product	↓		↓		↓		

W2:

Day of the Week	Mon	Tues	Wed	Thurs	Fri	Sat	Sun
Day of the study	D8	D9	D10	D11	D12	D13	D14
Application of Product	↓		↓		↓		

W3:

Day of the Week	Mon	Tues	Wed	Thurs	Fri	Sat	Sun
Day of the study	D15	D16	D17	D18	D19	D20	D21
Application of Product	↓		↓		↓		

Latent phase – two weeks (W4, W5)

W4:

Day of the Week	Mon	Tues	Wed	Thurs	Fri	Sat	Sun
Day of the study	D22	D23	D24	D25	D26	D27	D28

W5:

Day of the Week	Mon	Tues	Wed	Thurs	Fri	Sat	Sun
Day of the study	D29	D30	D31	D32	D33	D34	D35

Revelation phase (double challenge test) – one week (W6)

W6:

Day of the week	Mon	Tues	Wed	Thurs	Fri
Day of the study	D36	D37	D38	D39	D40
Application of Product	↓				
Readings			L		L

6. EVALUATION CRITERIA

6.1 Clinical criteria concerning the irritant potential (Induction phase)

After each application, the patch is removed and the reading taken 30 minutes later to eliminate the effect of pressure, occlusion and removal due to the material.
The test is negative if the skin maintains a normal aspect.

The four following criteria are evaluated by the dermatologist according to a scale of 0 to 3.

Score	Grade	CRITERIA : description			
		ERYTHEMA	OEDEMA	DRYNESS	VESICLES
0	Absent	Normal appearance	Normal appearance	Normal appearance	Normal appearance
1	Slight	Discreet pinkish coloration over all the tested surface or noticeable on part of the surface	More palpable than visible	Fine, discreet desquamation, rough appearance	Palpable rather than visible vesicles
2	Definite	Definite rash covering the whole of tested surface	Visible oedema	Visible desquamation, scaly appearance	Visible vesicules
3	Significant	Intense rash covering the whole of the tested surface or spreading beyond the surface	Possibly spreading beyond the tested surface	Significant desquamation, cracks in the skin	Vesicles going beyond the tested zone, or blisters

6.2 CLINICAL CRITERIA CONCERNING THE SENSITIZING POTENTIONAL (REVELATION PHASE)

In the event of an allergic reaction during the induction or revelation phases, this is scored according to the criteria of the ICDRG (International Contact Dematitis Research Group)/

Criteria	ICDRG Score	Numerical Score
No reaction	0	0
Suspected reaction	?	?
Erythma and Oedema	+	1
Erythma, Oedema and vesicles	++	2
Strong reaction with presence of blisters or post bulbous ulcers	+++	3

6.3 EVALUATION METHOD

6.3.1 Irritation Capacity – Induction phase

After the 8 readings taken in the induction phase, the average score of each volunteer is calculated by adding the scores obtained to each of the readings and divising this sum by the effective number of readings (a reading will not be taken into account if there is a reaction to the control or general irritation).

The irritation capacity of the product will be evaluated during the induction phase, by taking the average of the reactions.

The irritation capacity of the product is determined according to the following formula:

$$\text{Average score} = \frac{\left[\left(\sum \text{scores D1...}\frac{D19}{n^{\circ}}\text{readings}\right)\text{vol1}+\cdots+\left(\sum \text{scores D1...}\frac{D19}{n^{\circ}}\text{readings}\right)\text{volN}\right]}{\text{number of volunteers (N)}}$$

Average Score	Irritation Capacity
0 – 0.08	Non irritant
0.081 – 0.16	Very slightly irritant
0.161 – 0.56	Slightly irritant
0.561 - 1	Moderately irritant
1.001 – 1.6	Definitely irritant
>1.6	Very definitely irritant

6.3.2 Sensitizing Capacity – Revelation Phase

An eventual allergic reaction during the induction or revelation phases will be noted from 0 to 3 according to the ICDRG (International Contact Dermatitits Reasearch Group) criteria – see table in paragraph 6.2.

During revelation, one reading will be taken 30 minutes after removal of the patch tests, then 48 hours later.

The sensitizing capacity of the product will be evaluated during readings on D38 and D40 (revelation phase) according to the following criteria : reaction ++ (2) or +++ (3).

The appearance of only one case of active sensitization on the heterolateral side will lead to the conclusion: "Product potentially sensitizing".

6.4 PREMATURE STOPPAGE

The subjects have the right to withdraw from the trial at any moment for whatever reason.

Premature stoppage may be due to multiple reasons:

- Non respect of the calendar of visits by the subject
- Undesirable events (including intercurrent diseases)
- Violations and deviations from the protocol
- Exits after withdrawal of the subject's consent

6.5 PROTOCOL AMMENDMENTS

None.

7 RESULTS

7.1 IRRITATION CAPACITY: INDUCTION PHASE

The TABLE OF READINGS during the induction phase is presented in APPENDIX III.

These readings carried out 30 minutes after withdrawal of the patch-tests showed the following results:

Produit DA	D3	D5	D8	D10	D12	D15	D17	D19	Conclusion
KALPARIANE Batch 8.05.149	T+:0 0:50	T+:0 0:50	T+:0 0:50	T+:0 0:50	T+:0 0:50	T+:0 0:50	T+:0 0:50	T+:0 0.50	Non irritant (IRR=0.000)

Given the results, the product "KALPARIANE BATCH 8.05.149" may therefore be considered as <u>non irritant</u> under the conditions of this study (score below 0.080).

7.2 SENSITIZING POTENTIAL: REVELATION PHASE

The TABLE OF READINGS during the revelation phase is presented in APPENDIX IV.

The readings carried out 30 minutes and 48hours after withdrawal of the patch tests of revelation gave the following results:

Product DA	Zone	Day of Reading		Global Result
		D38	D40	
KALPARIANE BATCH 8.05.149	Reading Homolateral zone	T+:0 **DA** : 0:50 ?:0 1:0 2:0 3:0	T+:0 **DA** : 0:50 ?:0 1:0 2:0 3:0	Non sensitizing
	Readings heterolateral zone	T+:0 **DA** : 0:50 ?:0 1:0 2:0 3:0	T+:0 **DA** : 0:50 ?:0 1:0 2:0 3:0	

T+ = Positive control
DA = KALPARIANE BATCH 8.05.149
IRR = global irritation
MV = Missing value

The product "KALPARIANE BATCH 8.05.149" can therefore be considered as non sensitizing under the conditions of this study.

8 CONCLUSION

Under the conditions of this study, the product "KALPARIANE BATCH 8.05.149" proved to be non irritant and non sensitizing.

APPENDICES

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CHARACTERISTICS OF VOLUNTEERS

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TABLE OF READINGS – INDUCTION PHASE

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TABLE OF READINGS - REVELATION PHASE

APPENDIX I

AUTHENTIFICATION PAGE

PROCOS

KARTA AUTENTYCZNOŚCI REZULTATÓW
FICHE D'AUTHENTIFICATION DES RESULTATS
AUTHENTIFICATION PAGE

Według posiadanych przeze mnie informacji, badanie Nr. :
A ma connaissance l'étude N° :
I am aware that the study N° :

ET – 319

było przeprowadzone zgodnie PROTOKOŁEM oraz KARTĄ PARAMETRÓW TESTU.
a été conduite en accord avec le PROTOCOLE et la FICHE DES PARAMETRES D'ETUDE
has been conducted according to the PROTOCOL and to the STUDY PARAMETERS PAGE.

Mgr inż. Barbara WAŁEJKO Odpowiedzialna za badania i jakość *Responsable d'unité, Responsable qualité* Unit head, Responsible for quality control	B. Wałejko podpis / signature	19/09/2008 data / date
Dr Marlena NOWAKOWSKA Lekarz dermatolog *Médecin dermatologue* Dermatologist	M. Nowakowska podpis / signature	19/09/2008 data / date
Anna MAREK Technik *Technicienne* Technician	A. Marek podpis / signature	19/09/2008 data / date
Anna MIŚKIEWICZ Asystentka medyczna *Assistante médicale* Medical assistant	A. Miśkiewicz podpis / signature	19/09/2008 data / date

APPENDIX II

CHARACTERISTICS OF VOLUNTEERS

CHARACTERISTICS OF VOLUNTEERS

Subject N°	Subject Code	Age	Sex: M/F	Phototype	Skin type (Normal or Sensitive	Surgical or medical events and treatments	
						Before study	During study
1	LENMA	40	F	II	N	-	-
2	OLEST	57	M	II	N	-	-
3	ZAJAR	20	M	II	N	-	-
4	MARMA	44	M	II	N	-	-
5	SWIAD	28	M	II	N	-	-
6	LUKRE	20	M	II	N	-	-
7	WASBO	25	M	II	N	-	-
8	KOSZO	31	F	II	N	-	-
9	BRYAN	58	F	II	N	-	-
10	CIBMA	61	F	II	N	-	-
11	DABPA	23	F	II	N	-	-
12	FROLE	51	F	II	N	-	-
13	SZCJA	50	M	II	N	-	-
14	KORWA	58	F	II	N	-	-
15	RUSPI	28	M	II	N	-	-
16	RUDMI	61	F	II	N	-	-
17	SKABR	65	F	II	N	-	-
18	SADHA	65	F	II	N	-	-
19	GALFE	58	F	II	N	-	-
20	ROMWI	62	F	II	N	-	-
21	KOZEW	20	F	II	N	-	-
22	SWIAN	56	F	II	N	-	-
23	OTWIW	49	F	II	N	-	-
24	STAEL	61	F	II	N	-	-
25	STRWA	62	F	II	N	-	-
26	HOPWI	31	F	II	N	-	-
27	KOWHU	27	M	II	N	-	-
28	MUSWL	63	F	II	N	-	-
29	SOKKR	21	M	II	N	-	-
30	SZEHE	65	F	II	N	-	-

Subject N°	Subject Code	Age	Sex: M/F	Phototype	Skin type (Normal or Sensitive	Surgical or medical events and treatments	
						Before study	During study
31	WYSAN	57	F	II	N	-	-
32	RUSBA	37	F	II	N	-	-
33	BIABA	51	F	II	N	-	-
34	WISLI	49	F	II	N	-	-
35	IWAMA	44	F	II	N	-	-
36	GLUJU	19	F	II	N	-	-
37	NIEAN	53	F	II	N	-	-
38	BANBO	35	F	II	N	-	-
39	LUBZO	45	F	II	N	-	-
40	WODIR	42	F	II	N	-	-
41	KOSWE	24	F	II	N	-	-
42	OLSMA	33	F	II	N	-	-
43	ZIOZO	45	F	II	N	-	-
44	STYJA	57	F	II	N	-	-
45	STAAL	20	F	II	N	-	-
46	KOSJA	30	M	II	N	-	-
47	SZPAL	26	F	II	N	-	-
48	PACJA	35	M	II	N	-	-
49	ARERO	19	M	II	N	-	-
50	KEPJO	58	F	II	N	-	-

APPENDIX III

TABLE OF READINGS

INDUCTION PHASE

TABLE OF READINGS -- Induction Phase

Volunteer N°	D3		D5		D8		D10		D12		D15		D17		D19	
	C	DA	C	DA	C	DA	C	DA	C	DA	C	DA	C	DA	C	DA
1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
8	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
9	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
10	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
11	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
12	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
13	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
14	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
15	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
16	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
17	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
18	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
19	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
20	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
21	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
22	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
23	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
24	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
25	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
26	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
27	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
28	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
29	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
30	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
31	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
32	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
33	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
34	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
35	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
36	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
37	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
38	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
39	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
40	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
41	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
42	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
43	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
44	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
45	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
46	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
47	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
48	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
49	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
50	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

L.S. = Lost Sight C = control DA = KALPARIANE BATCH 8.05.149

APPENDIX IV

TABLE OF READINGS

REVELATION PHASE

TABLE OF READINGS – Revelation phase

Volunteer N°	D38 Homolateral zone		D38 heterolateral zone		D40 homolateral zone		D40 heterolaeral zone	
	C	DA	C	DA	C	DA	C	DA
1	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0
4	0	0	0	0	0	0	0	0
5	0	0	0	0	0	0	0	0
6	0	0	0	0	0	0	0	0
7	0	0	0	0	0	0	0	0
8	0	0	0	0	0	0	0	0
9	0	0	0	0	0	0	0	0
10	0	0	0	0	0	0	0	0
11	0	0	0	0	0	0	0	0
12	0	0	0	0	0	0	0	0
13	0	0	0	0	0	0	0	0
14	0	0	0	0	0	0	0	0
15	0	0	0	0	0	0	0	0
16	0	0	0	0	0	0	0	0
17	0	0	0	0	0	0	0	0
18	0	0	0	0	0	0	0	0
19	0	0	0	0	0	0	0	0
20	0	0	0	0	0	0	0	0
21	0	0	0	0	0	0	0	0
22	0	0	0	0	0	0	0	0
23	0	0	0	0	0	0	0	0
24	0	0	0	0	0	0	0	0
25	0	0	0	0	0	0	0	0
26	0	0	0	0	0	0	0	0
27	0	0	0	0	0	0	0	0
28	0	0	0	0	0	0	0	0
29	0	0	0	0	0	0	0	0
30	0	0	0	0	0	0	0	0
31	0	0	0	0	0	0	0	0
32	0	0	0	0	0	0	0	0
33	0	0	0	0	0	0	0	0
34	0	0	0	0	0	0	0	0
35	0	0	0	0	0	0	0	0
36	0	0	0	0	0	0	0	0
37	0	0	0	0	0	0	0	0
38	0	0	0	0	0	0	0	0
39	0	0	0	0	0	0	0	0
40	0	0	0	0	0	0	0	0
41	0	0	0	0	0	0	0	0
42	0	0	0	0	0	0	0	0
43	0	0	0	0	0	0	0	0
44	0	0	0	0	0	0	0	0
45	0	0	0	0	0	0	0	0
46	0	0	0	0	0	0	0	0
47	0	0	0	0	0	0	0	0
48	0	0	0	0	0	0	0	0
49	0	0	0	0	0	0	0	0
50	0	0	0	0	0	0	0	0

I.S = Lost sight C = Control DA = KALPARIANE BATCH 8.05.149

MANUFACTURING PROCESS

KALPARIANE™ AD

HARVESTING / IDENTIFICATION *(Alaria Esculenta)*

↓

DRYING

↓

GRINDING

↓

EXTRACTION WITH THE SOLVENT
CAPRYLIC/CAPRIC TRIGLYCERIDE

↓

FILTRATION

↓

QUALITY CONTROL

↓

PACKAGING

↓

QUALITY CONTROL

Production Manager
Jean-Marc CATROUX

Date de mise à jour / *Updated date* : 01/12/2016

KALPARIANE™ AD

INCI NAME : Caprylic/Capric Triglyceride – Algae extract*

**Retired INCI names will be retained for publication for an interim period of time. During this transition period, trade names maintain their assignment to retired names, but are also designated by the new nomenclature so that users may update product labels, documentation, technical literature, etc. when economically feasible.*

CAS : 73398-61-5 – 92128-82-0

EINECS : 277-452-2 – 295-780-4

5% Alaria Esculenta Extract 95%

Produit conforme Mass Balance (RSPO) BVC-RSPO-1-1972708497 / *Product Mass Balance compliant(Roundtable for Sustainable Palm Oil (RSPO)) BVC-RSPO-1-1972708497*

Caprylic/Capric Triglycerides

DONNEES PHYSICOCHIMIQUES
PHYSICO-CHEMICAL DATA

Numéro de référence / *Reference number* : STANDARD

CARACTERISTIQUES *CHARACTERISTICS*	METHODES *METHODS*	STANDARD *STANDARD*
Conformité Mass Balance *Mass Balance conformity*	PO-HSE-004	**Conforme** *Conform*
Aspect *Aspect*	MO PHY 002	**Liquide limpide huileux** *Oily limpid liquid*
Couleur *Colour*	MO PHY 002	**Jaune-vert pâle à vert foncé (1 à 7 UG)** *Pale yellow green to dark green (1 to 7 UG)*
Odeur *Odour*	MO PHY 002	**Faible** *Slight*
Densité (20°C) *Density*	MO PHY 024	0,920 - 0,950
Indice de réfraction (20°C) *Refractive index*	MO PHY 008	$1,440_0$ - $1,460_0$
Indice de péroxyde* *Peroxyd index**	MO PHY 034	<3 meq d'oxygène actif/kg <*3 meq of active oxygen/kg*
Spectre UV *UV spectrum*	MO PHY 013	**Conforme au témoin** *Similar to the standard*
Spectre IR *IR spectrum*	MO PHY 011	**Conforme au témoin** *Similar to the standard*
Solvant *Solvent*	**Mélange de triglycérides d'acides gras saturés (C8C10) d'origine végétale** *Mixture of triglycerides of saturated fatty acids (C8C10) of vegetal origin.*	
Métaux lourds* *Heavy metals* (ppm)*		
• **Arsenic** *Arsenic*		< 2
• **Cadmium** *Cadmium*		< 3
• **Plomb** *Lead*		< 5
• **Nickel** *Nickel*		< 2
• **Argent** *Silver*		< 5
• **Mercure** *Mercury*		< 1

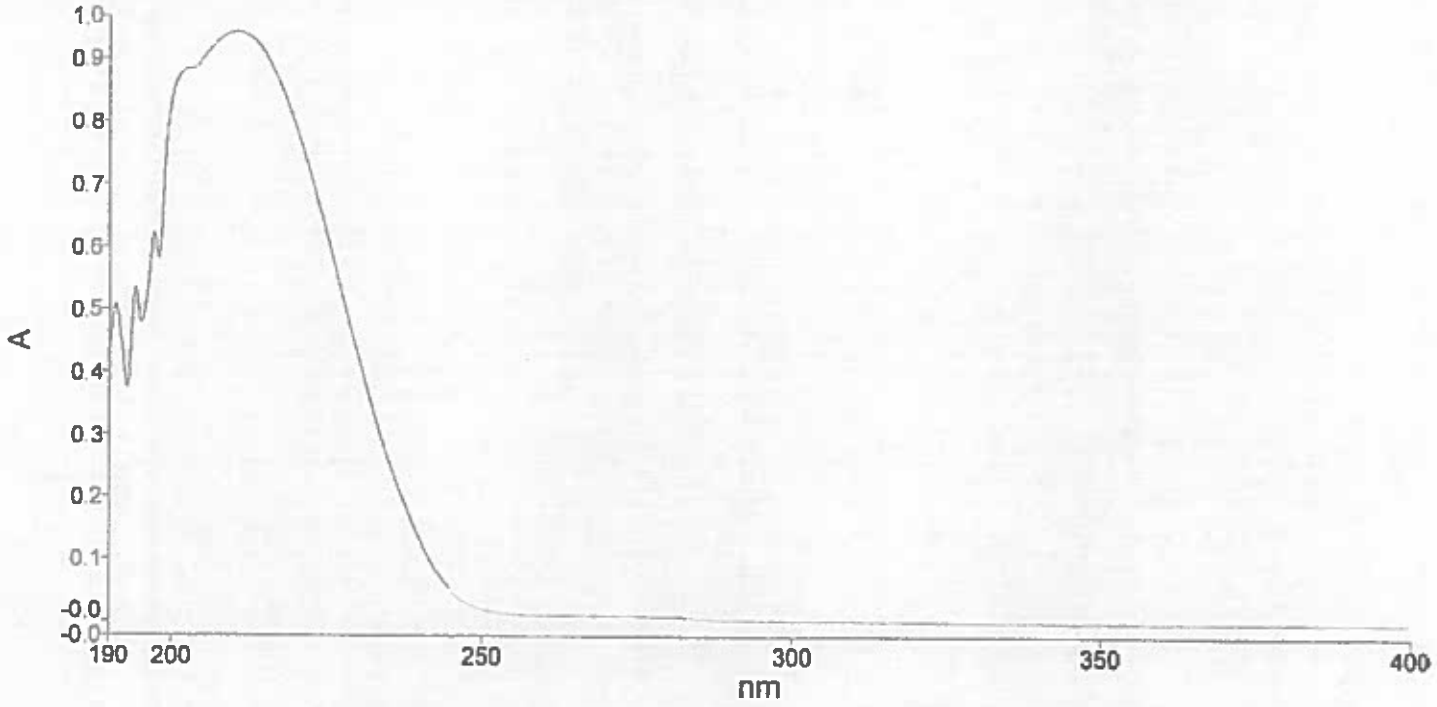
Iodine < 1 ppm

* Teneurs garanties sous contrôle statistique / *Contents guaranteed under statistical control*

PerkinElmer UV WinLab Data Processor and Viewer Version 1.00.03
24/11/2016 15:53

Analyst
Date

contrôle qualité
jeudi 24 novembre 2016 15:53



Date: jeudi 24 novembre 201

SPECTRE IRFT

SPECTROPHOTOMETRE SPECTRUM 100 N° de série 77774 - PERKIN ELMER

Accessoire ATR Universel N° 7031330

KALPARIANE™ AD

INCI NAME : Caprylic/Capric Triglyceride – Algae extract*
**Retired INCI names will be retained for publication for an interim period of time. During this transition period, trade names maintain their assignment to retired names, but are also designated by the new nomenclature so that users may update product labels, documentation, technical literature, etc. when economically feasible.*
CAS : 73398-61-5 – 92128-82-0
EINECS : 277-452-2 – 295-780-4
Produit conforme Mass Balance (RSPO) BVC-RSPO-1-1972708497 / *Product Mass Balance compliant(Rountable for Sustainable Palm Oil (RSPO)) BVC-RSPO-1-1972708497*

DONNEES MICROBIOLOGIQUES
MICROBIOLOGICAL DATA
Numéro de référence / *Reference number* : STANDARD

CARACTERISTIQUES *CHARACTERISTICS*	METHODES *METHODS*	STANDARD *STANDARD*
Germes totaux** *Total germs***	MO MIC 002 / NF EN ISO 21149	**< 100**
Germes Pathogènes *Pathogens*		
- *Staphylococcus aureus*	MO MIC 012 / NF EN ISO 22718	**Absence** *None*
- *Candida albicans*	MO MIC 010 / NF EN ISO 18416	**Absence** *None*
- *Pseudomonas aeruginosa*	MO MIC 011 / NF EN ISO 22717	**Absence** *None*
- *Escherichia coli*	MO MIC 025 / NF EN ISO 21150	**Absence** *None*
Levures / Moisissures** *Yeasts / Moulds***	MO MIC 021 / NF EN ISO 16212	**< 100**

**Les résultats sont indiqués en UFC/mL pour les liquides et en UFC/g pour les solides
*** Results are indicated in CFU/mL for the liquids and in CFU/g for the solids*

CONFORME
CERTIFIED TRUE AND CORRECT
RESPONSABLE CONTROLE QUALITE: **P. SOUBIES**
QUALITY CONTROL MANAGER
01 DEC. 2016

CONFORME
CERTIFIED TRUE AND CORRECT
ASSURANCE QUALITE : **A. HAMON**
QUALITY ASSURANCE
01 DEC. 2016

BiotechMarine Z.I. - B.P.72 - 22260 Pontrieux - FRANCE Tél. +33 (0) 2 96 95 31 32

COSderma
LABORATOIRE

REPORT

VERIFICATION IN HUMANS OF THE SKIN COMPATIBILITY OF A COSMETIC PRODUCT AFTER SINGLE APPLICATION UNDER DRESSING
24 hour Patch Test

Product tested:

OLEA ALARIA LOT 604136

5% Alaria Esculenta Extract

95% Caprylic / Capric Triglycerides

Sponsor

BIOTECHMARINE
ZI – BP 65
2260 PONTRIEUX

JA/CL

Bordeaux, 5 January 2007

LABORATOIRE COSDERMA
SERVICE DERMATOLOGIE DU PR ALAIN TAIEB
GROUPE HOSPITALIER SAINT ANDRE
1 RUE JEAN BURGUET BP 50057 33023 BORDEAUX CEDEX

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TEL: 05 56 94 75 40
FAX: 05 56 79 49 75

CONTENTS

1- Aim of the study

The aim of this study was to evaluate the skin compatibility of the product "**OLEA ALARIA LOT 604136**" after single application on the skin for 24 hours in 10 volunteers.

The skin compatibility of the product was verified by a visual examination of the experimental area carried out by the investigator, or by the technician under his authority.

Since this study aimed at a better knowledge of the skin compatibility of the product studied and the foreseeable risk for the participating volunteers was tiny, there was a good accord between the aim of the study and the potential risks.

2- Methodology

2.1 Experimental Plan

This study had no direct individual benefit; each participating volunteer was his own control.
This study was single centre, conducted openly.
The duration of the study was 24 hours and comprised 2 visits to the Institute (T0, T24 hours).

- inclusion
- fitting of patch
- removal of patch
- visual examination, 15 minutes after removal of patch

2.2 Investigating Centre

Laboratoire COSDERMA
Service de dermatologie du Pr Taïeb – Groupe Hospitalier Saint André
1 rue Jean Burguet – BP 50057
33023 Bordeaux Cedex
Tel: 05 56 94 75 40
email: laboratoire@cosderma.com

2.3 Place of the Investigation

Service de dermatologie du Pr Taïeb
Groupe Hospitalier Saint André
1 rue Jean Burguet
33000 Bordeaux

2.4 Technical Team

Investigator: Prof Alain Taïeb

Technician: Chrystelle Latxague

3- Date the study was carried out

Start: 3 January 2007

End: 4 January 2007

4- Product studied

4.1 Information

The information passed on by the sponsor accompanying the sample was the letter of undertaking concerning in particular the conformity of the formula with the regulations in force and its safety.

4.2 Identification

Name	Ref	Quantity for the study	Packaging quantity
OLEA ALARIA	LOT 604136	5 ml	1 x 5 ml

4.3 Normal conditions of use

Name	Method of use	
OLEA ALARIA LOT 604136	site	/
	Cosmetic raw material	

4.4 Conditions of use during the study

For the study, the area of application chosen was the back.

Product	Type of dressing	Conditions of application	Contact time	Quantity applied	Control time on D1
OLEA ALARIA LOT 604136	Occlusive (Finn chambers®)	Pure	24 hours	20 µl	15 minutes after removal of patch

Finn Chambers®: Occlusive dressing composed of an aluminium cup of 8mm diameter (surface 50 mm²) on which 20 µl (20 mg) of product is deposited.

A control patch, corresponding to the type of dressings used, containing an ad hoc quantity of water for injectable preparation, was applied at the same time.
The patch removal was carried out by the investigator, or the technician under his responsibility.
The quantities of product were measured using a single-use syringe.

5 – Volunteers

5-1 Panel

The panel of volunteers participating in the study was representative of the population that may use the product. All volunteers selected met the inclusion and non-inclusion criteria.

5-2 Population

The number of volunteers participating in the study was **10**.
The number of volunteers whose data is presented is **10**.

5-3 Inclusion criteria

The inclusion criteria were as follows:

- age: 18 to 65
- sex: female
- phototype (Fitzpatrick): I to III
- all skin types
- able to give written informed consent
- affiliated to Social Security

All volunteers met these inclusion criteria. The typological characteristics of the volunteers are presented in **Annex 1**.

5-4 Non-inclusion criteria

The non-inclusion criteria were as follows:

- Skin marks in the experimental area that may interfere with the evaluation of skin reactions (pigmentation problems, elements of scars, too much hair, too many freckles and moles, sunburn, etc.)
- Eczema-type reaction not yet completely disappeared, pigment or healing sequelae of previous tests in the experimental area
- Allergy to gum rosin or nickel
- Allergy or reactivity to the same category of products
- Skin hyper-reactivity
- Reactivity to ethyl alcohol or adhesive plaster
- Participation in the 12 months preceding the study in over 5 tests using maximisation, of which no more than 3 for hypoallergenicity research
- Intensive sun exposure within the month preceding the study
- Planned intensive sun exposure (natural sun or UVA sunbed) during the period of the study
- Intention to bathe in a bathtub, sea or swimming pool or use a sauna or Turkish bath during the study
- Intensive or regular practice of one or more sports whereby temporary interruption would cause a problem
- Withdrawal of treatment based on Vitamin A acid or its derivatives less than 3 months before the start of the study
- Withdrawal of topical corticosteroid treatment on the experimental area less than 8 days before the study
- Withdrawal of PUVA or UVB treatment less than one month before the study
- Planned vaccination during the period of the test, last vaccination within the 3 weeks preceding the study

No volunteer corresponding to these criteria was included.

5-5 Constraints of the study

The constraints of the study were as follows:

- No application of products other than those tested on the experimental area
- No wearing of clothing that is too tight or responsible for compression in the experimental area, or that may cause rubbing and loosening of the dressing
- No bathing in bathtub, sea or swimming pool and no sauna or Turkish bath during the study
- Protection of the experimental area when taking a shower, no violent water projection and no soaping this area to avoid loosening the dressing or the appearance of intercurrent phenomena, and very careful wiping if necessary
- No excessive sweating and no intensive physical activity that may entail loosening of the dressing
- No exposure to intensive sun (natural sun or UVA sunbed) throughout the duration of the study, particularly when the dressing has been removed
- Keep to usual hygiene habits on the face and body

- No anti-allergy or anti-inflammatory treatment (systemic or topical corticosteroid) or those based on Vitamin A acid or its derivatives on the day of the study (if therapeutic need: withdrawal from the trial envisaged)

All constraints of the study were respected by the volunteers.

5.6 Control of observance of the terms of the protocol

The investigator verified whether the **constraints** had been respected.

The summary of the responses to the various questions asked is attached in Annex 2.

In the event of deviations to the protocol, they were analysed and the investigator assessed their impact on the validity of the results.

All constraints of the study defined in the protocol were respected by the volunteers.

6- Evaluation

6-1 Schedule

Sequencing of the study	Start	Contact time
	T0	T24 hours ± 2 hours
Selection of volunteers	X	
Allocation of volunteer n°	X	
Volunteer information	X	
Informed consent signed*	X	
Application of dressings by the technician	X	
Removal of patch by the technician		X
Evaluation criterion (15 mins after removal of patch)		X

The fact that the application of the product and the clinical examinations had been perfectly controlled, the population of volunteers and the duration of the study made it possible to verify the skin compatibility of the product studied and to assess any irritation.

*A duplicate of the participation consent was given to the volunteers on the day of the inclusion visit for the study. The original will be kept by the investigator.

6-2 Evaluation of skin compatibility

- *Principle and bibliographies*

The skin compatibility was verified via the application of dressings on the skin which create a certain occlusion of the products and encourage their penetration. Under these maximising experimental conditions, the irritant potential of the products can be revealed more easily.

The methodology has been the subject of many publications, including:

Comment tester les produits cosmétiques?, Dermatologie Pratique, 2003, n° 273, 1-4

Reactive changes in human epidermis following simple occlusion with water, Contact Dermatitis, Mikulowska A, 1992, 26, 224-227

Test strategies for development of cosmetic products using dermatological test models, Seifen-öle-fette-wachse, Matthies W, 1991, 117, 42-43

The Duhring Chamber: an improved technique for epicutaneous testing of irritant and allergic reactions, Contact Dermatitis, Frosch PJ & Klingmann AM, 1979, 5, 73-81

Appraisal of the safety of chemicals in Food, Drugs and Cosmetics, FDA (ed), Draize JH, 1959, 46-48

- *Methodology, patching material*

The products were deposited on the dressings extemporaneously using a 1ml syringe. The dressings were applied subsequently on the skin as quickly as possible, carefully avoiding the areas exposed to rubbing or various compressions. The investigator, or the technician under his authority, verified that the skin area concerned was clear of the presence of any beauty spots, scars and skin trauma. The type of dressing, the maximum number of products possible to be tested, the quantity of the product to be applied, the methodology of application and removal of the dressings and the visual clinical examination complied with the benchmark laboratory procedures for this type of study. The site of application of the products chosen was the back.

- *Environmental conditions*

The environmental conditions imposed on the volunteers were as follows:

- *controlled temperature: t° = 20°C ± 2°C*
- *relative humidity: RH = 45% ± 15%*

- *Clinical examination*

- Sites

The investigator, or the technician under his authority, carried out a visual check of each experimental area under standardised "daylight" lighting.

- Frequencies

The visual check was carried out at T24 hours ± 2 hours, 15 minutes after removal of the patch (or more if redness appeared upon removing the patch).

- *Evaluation criteria*

- *clinical signs*

description	laboratory code	intensity	appearance	score
Erythema	E	-ordinal 3-point scale * mild * moderate * severe	-erythema: * diffuse * isolated * peripheral	* mild = 1 * moderate = 2 * severe = 3 * diffuse = d * isolated = p * peripheral = peri
Oedema	Oe			
Drying	D			
Colouring	C			
Blackhead, microcyst	Co, MI	-counted		
Vesicle, weal	V, Pa	-ordinal 2-point scale: * 1 to 2 vesicles * vesicles > 2		* 1 to 2 = 1 * > 2 = 2
Blister, crust	Bu, Cr	-described		* if described = 2

The investigator, or the technician under his authority, noted every clinical sign, its location, its intensity, its evolution, and any drug treatments taken. He established the usual or unusual nature of the clinical sign, questioning the volunteer on what he observes in everyday life upon using similar products.

- *Feelings of discomfort*

description	laboratory code	intensity	score
Warmth	Ech	-ordinal 3-point scale: * mild * moderate * severe	* mild = 1 * moderate = 2 * severe = 3
Tingling	Pi		
Pruritus (itching)	Pr		
Pulling	Ti		
Burning	Br		

- *Expression of the results*

All volunteers having been the subject of visit T0 was taken into account for the evaluation of the skin compatibility. The expression of the results of the skin examination and the questioning complied with the benchmark laboratory procedure for this type of study.

The individual results were expressed:

- **as a percentage of reactive volunteers**, taking account for this calculation only of the clinically detectable clinical signs such as erythema, oedema, ulcer, blister, papule, crust;

- **descriptively** for the other signs detectable visually or the feelings of discomfort; the percentage of volunteers in whom they were observed able to be calculated if the frequency of onset of these signs so justified;

- **as a skin irritation score**, calculated from "scores" attributed to the visually detectable clinical signs.

For each volunteer and at each observation time, a daily individual irritation score (Sijl) was calculated, being the sum of the scores obtained for the signs observed.

For the panel and at each observation time, an average daily irritation score (SijM) was calculated, which corresponds to the formula:

SijM = □ (Sijl) / Number of volunteers included

- *Interpretation of the results*

The investigator concluded absolutely in terms of **very good, good, medium or poor** skin compatibility. The interpretation of the results of the skin examination and the questionnaire complied with the benchmark laboratory procedure for this type of study.

7- Results and discussions

The individual data of the skin examination and the volunteer questionnaire are annexed in **Annex 3.**

To sum up:

Product	Control time on D1	Number of reactive volunteers	Types of reaction	Average daily irritation score SijM	% of reactive volunteers
OLEA ALARIA LOT 604136	**15 minutes after removal of patch**	**0**	**/**	**0**	**0%**

8- Conclusion

Under the experimental conditions adopted, the product **"OLEA ALARIA LOT 604136" has very good skin compatibility**.

Signatures and dates:

Prof Alain Taïeb (Dermatologist)

Investigator

16/1/2007

[*signature*]

Chrystelle Latxague

Clinical Assistant

08/01/07

[*signature*]

Jérôme Asserin
Study Director

9/01/07

[*signature*]

Amour ADJADOHUN
Quality Manager

09/01/07

[*signature*]

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contact@biotechmarine.com

guenole LE CALVEZ
General Manager
Biotechmarine.

ANNEXES

TYPOLOGICAL CHARACTERISTICS OF THE VOLUNTEERS

Volunteers		Age (years)	Sex F=female	Phototype*	Healthy skin on back
Ref	Surname forename				
1	PANT/E	22	F	II	X
2	CARR/E	54	F	III	X
3	PLAZ/I	49	F	III	X
4	MEZA/A	39	F	III	X
5	SAIN/R	26	F	I	X
6	DRUI/S	37	F	II	X
7	BOUS/C	42	F	II	X
8	NAKA/G	32	F	II	X
9	MORS/G	18	F	I	X
10	LARR/D	46	F	II	X

Keys: / = no X = yes

***phototype according to Fitzpatrick**, established on the principle of first exposure of 30 to 40 minutes to the sun after winter or a period without exposure of an equivalent period:

TYPE	HAIR	SKIN	FRECKLES	SUNBURN
I	red	pale	+++	constant never tans
II	blond	fair	++	frequent slight tan
III	blond fair	fair	+	sometimes light to olive tan
IV	brown	olive	o	none dark olive tan
V	black and frizzy	black	o	o

CONTROL OF OBSERVANCE
Constraints

Constraints (10 exploitable results)	Number of volunteers having respected the constraints	Percentage of volunteers having respected the constraints
No application of products (other than that tested) on the experimental area Deviation: none	10	100%
No wearing of clothing that is too tight or responsible for compression in the experimental area, or that may cause rubbing and loosening of the dressing Deviation: none	10	100%
No bathing (in bathtub, sea or swimming pool) and no sauna or hammam during the study Deviation: none	10	100%
Protection of the experimental area when taking a shower, no violent water projection and no soaping this area to avoid loosening the dressing or the appearance of intercurrent phenomena, and very careful wiping if necessary Deviation: none	10	100%
No excessive sweating or intensive sport that may entail loosening of the dressing Deviation: none	10	100%
No exposure to intensive sun (natural sun or UVA sunbed) throughout the duration of the study, particularly when the dressing has been removed Deviation: none	10	100%
No anti-allergy or anti-inflammatory treatment (systemic or topical corticosteroid) or those based on Vitamin A acid or its derivatives throughout the study – no medication that may interfere with the study Deviation: none	10	100%
No vaccination during the study Deviation: none	10	100%

VERIFICATION OF SKIN COMPATIBILITY

Product Nº 1: **"OLEA ALARIA LOT 604136"** (Finn chambers)

Volunteers		Skin examination (15 minutes after removal of patch)	Sijl
Ref	Surname forename		
1	PANT/E	/	/
2	CARR/E	/	/
3	PLAZ/I	/	/
4	MEZA/A	/	/
5	SAIN/R	/	/
6	DRUI/S	/	/
7	BOUS/C	/	/
8	NAKA/G	/	/
9	MORS/G	/	/
10	LARR/D	/	/
SijM		0	

Keys: / = no clinical sign

VERIFICATION OF SKIN COMPATIBILITY

Product Nº 2: Control (Finn chambers)

Volunteers		Skin examination (15 minutes after removal of patch)	Sijl
Ref	Surname forename		
1	PANT/E	/	/
2	CARR/E	/	/
3	PLAZ/I	/	/
4	MEZA/A	/	/
5	SAIN/R	/	/
6	DRUI/S	/	/
7	BOUS/C	/	/
8	NAKA/G	/	/
9	MORS/G	/	/
10	LARR/D	/	/
SijM		0	

Keys: / = no clinical sign

Personal Care Products Council
Committed to Safety, Quality & Innovation

Memorandum

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

FROM: Carol Eisenmann, Ph.D.
Personal Care Products Council

DATE: September 11, 2018

SUBJECT: Laminaria Saccharina Extract

Biotech Marine. 2014. Manufacturing process Phlorogine (Laminaria Saccharina in Water and Propylene Glycol).

Biotech Marine. 2016. Phlorogine™ (Laminaria Saccharina Extract in Water and Propylene Glycol) Physico-chemical data.

Liskin. 2004. Evaluation of the irritating and sensitizing potential by 48-hours repeated application (Marzulli-Maibach method) Phlorgine (Laminaria Saccharina Extract in Water and Propylene Glycol).

Palmer Research. 1996. Etude de la tolérance cutanée aiguë chez 10 volontaires adultes: Patch tests 24 et 48 heures, uniques: Phlorogine (Laminaria Saccharina Extract in Water and Propylene Glycol).

Biotech Marine. 2014. Manufacturing process Phlorogine BG (Laminaria Saccharina in Water and Butylene Glycol).

Biotech Marine. 2016. Phlorogine™ BG (Laminaria Saccharina Extract in Water and Butylene Glycol) Physico-chemical data.

Biotech Marine. 2018. Synopsis de Fabrication - Laminaria Saccharina DJ (Laminaria Saccharina Extract in Sea Water and Methylpropandiol).

Biotech Marine. 2013. Laminaria Saccharina DJ (Laminaria Saccharina Extract in Sea Water and Methylpropanediol) Physico-chemical data.

1620 L Street, N.W., Suite 1200 | Washington, D.C. 20036 | 202.331.1770 | 202.331.1969 (fax) | www.personalcarecouncil.org

EuroTest. 2005. Evaluation de la tolérance oculaire par étude de la cytotoxicité après diffusion en ged'agarose sur lignée (Laminaria Saccharina DJ - Laminaria Saccharina Extract in Sea Water and Methylpropanediol).

Laboratoire Cosderma. 2005. Verification chez l'homme de la compatibilite cutanee d'un produit cosmetique apres application unique sous pansement: Patch test (Laminaria Saccharina DJ - Laminaria Saccharina Extract in Sea Water and Methylpropanediol).

SafePharm Laboratories. 2005. Reverse mutation assay "Ames test" using *Salmonella typhimurium* (Laminaria Saccharina DJ - Laminaria Saccharina Extract in Sea Water and Methylpropanediol).

MANUFACTURING PROCESS

PHLOROGINE

HARVESTING/IDENTIFICATION (Laminaria Saccharina)

↓

WASHING

↓

GRINDING

↓

EXTRACTION WITH
THE SOLVENTS: WATER + PROPYLENE GLYCOL

↓

MIXTURE ← ADDITION OF PRESERVATIVES

↓

FILTRATION

↓

QUALITY CONTROL

CARRY OVER :

- VITAMINE B6 CHLORYDRATE PYRIDOXINE
- ZINC SULFATE

Production Manager
Jean Marc CATROUX

BIOTECHMARINE (5/21/2014)

Date de mise à jour / *Updated date : 01/12/2016*

PHLOROGINE™

INCI NAME : Water / Aqua – Propylene Glycol – Laminaria Saccharina Extract
CAS : 7732-18-5 – 57-55-6 –90046-14-3
EINECS : 231-791-2 – 200-338-0 – 289-982-1

DONNEES PHYSICOCHIMIQUES
PHYSICO-CHEMICAL DATA
Numéro de référence/ *Reference number* **: STANDARD**

CARACTERISTIQUES *CHARACTERISTICS*	METHODES *METHODS*	STANDARD *STANDARD*
Aspect Aspect	MO PHY 002	**Liquide limpide à légèrement opalescent*** *Limpid to slightly opalescent liquid**
Couleur *Colour*	MO PHY 002	**Jaune à brun orangé** *Yellow to brown orange*
Odeur *Odour*	MO PHY 002	**Caractéristique** *Characteristic*
pH	MO PHY 009	4,5 – 6,5
Densité (20°C) *Density*	MO PHY 024	1,030 – 1,050
Indice de réfraction (20°C) *Refractive index*	MO PHY 008	$1,370_0 – 1,390_0$
Extrait sec (1g – 4 heures à 105°C) *Dry extract*	MO PHY 033	1,0 – 3,0 %
Teneur en eau *Water content*	MO PHY 018	58,0 – 62,0 %
Propylène glycol *Propylene glycol*	MO PHY 001	38,0 – 42,0 %
Spectre UV *UV spectrum*	MO PHY 013	**Conforme au témoin** *Similar to the standard*
Spectre IR *IR spectrum*	MO PHY 011	**Conforme au témoin** *Similar to the standard*
Conservateurs *Preservatives*		
- POB Methyle *Methyl Paraben*	MO PHY 020	0,16 – 0,20 %
- POB Propyle *Propyl Paraben*	MO PHY 020	0,03 – 0,06 %
Vitamine B6 *Vitamin B6*	MO PHY 055	**Présence** *Presence*
Teneur en Zn *Zn content*	MO PHY 037	800 – 1 200 ppm
Polyphénols totaux *Total Polyphenols*	MO PHY 038	**> 500 mg d'équivalent d'acide Gallique / litre** *> 500 mg of equivalent of gallic acid / litre*

* un léger dépôt peut apparaître. Il s'agit d'un phénomène naturel qui n'altère pas la qualité du produit / *a light deposit may appear. This is a natural phenomenon which does not affect product quality.*

PHLOROGINE™

INCI NAME : Water / Aqua – Propylene Glycol – Laminaria Saccharina Extract
CAS : 7732-18-5 – 57-55-6 –90046-14-3
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DONNEES PHYSICOCHIMIQUES
PHYSICO-CHEMICAL DATA
Numéro de référence/ *Reference number* **: STANDARD**

CARACTERISTIQUES *CHARACTERISTICS*	STANDARD *STANDARD*
Métaux lourds** (ppm) *Heavy metals***	
• **Arsenic mineral** *Mineral Arsenic*	< 2
• **Cadmium** *Cadmium*	< 3
• **Plomb** *Lead*	< 5
• **Nickel** *Nickel*	< 2
• **Argent** *Silver*	< 5
• **Mercure** *Mercury*	< 1

Iodine < 200 ppm

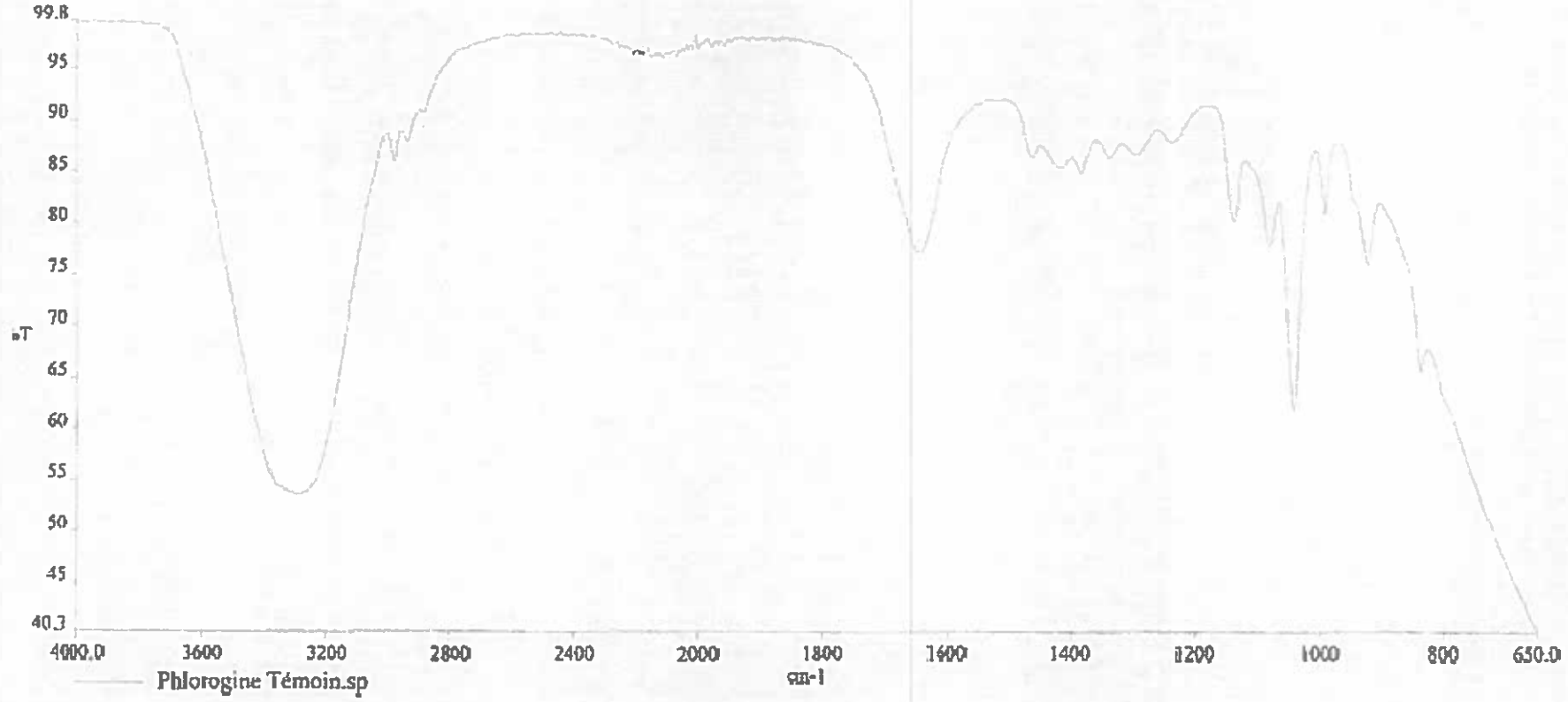
** Teneurs garanties sous contrôle statistique / *Contents guaranteed under statistical control*

Date: jeudi 24 novembre 2016

SPECTRE IRFT

SPECTROPHOTOMETRE SPECTRUM 100 N° de série 77774 - PERKIN ELMER

Accessoire ATR Universel N° 7031330



PHLOROGINE™

INCI NAME : Water / Aqua – Propylene Glycol – Laminaria Saccharina Extract
CAS : 7732-18-5 – 57-55-6 –90046-14-3
EINECS : 231-791-2 – 200-338-0 – 289-982-1

DONNEES MICROBIOLOGIQUES
MICROBIOLOGICAL DATA
Numéro de référence / *Reference number*: STANDARD

CARACTERISTIQUES *CHARACTERISTICS*	METHODES *METHODS*	STANDARD *STANDARD*
Germes totaux*** *Total germs****	MO MIC 002 / NF EN ISO 21149	< 100
Germes Pathogènes *Pathogens*		
- *Staphylococcus aureus*	MO MIC 012 / NF EN ISO 22718	**Absence** *None*
- *Candida albicans*	MO MIC 010 / NF EN ISO 18416	**Absence** *None*
- *Pseudomonas aeruginosa*	MO MIC 011 / NF EN ISO 22717	**Absence** *None*
- *Escherichia coli*	MO MIC 025 / NF EN ISO 21150	**Absence** *None*
Levures / Moisissures*** *Yeasts / Moulds****	MO MIC 021 / NF EN ISO 16212	< 100

*** Les résultats sont indiqués en UFC/mL pour les liquides et en UFC/g pour les solides
*** *Results are indicated in CFU/mL for the liquids and in CFU/g for the solids*

CONFORME
CERTIFIED TRUE AND CORRECT
RESPONSABLE CONTROLE QUALITE: **P. SOUBIES**
QUALITY CONTROL MANAGER
01 DEC. 2016

CONFORME
CERTIFIED TRUE AND CORRECT
COORDINATRICE ASSURANCE QUALITE : **M. TANNIOU**
QUALITY ASSURANCE COORDINATOR
01 DEC. 2016

PALMER RESEARCH

Evaluation of the irritating and sensitizing potential by 48-hours repeated applications (Marzulli – Maibach method)

GROUPE DERMSCAN

SIEGE SOCIAL - LYON
27, bd du 11 Novembre 1918
B.P. 2132
69603 VILLEURBANNE Cedex
FRANCE
Tél. : 33 (0)4 72 82 60 88
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BORDEAUX
Parc Innolin - 3, rue du Golf
33700 MERIGNAC
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Tél. : 33 (0)5 56 34 75 56
Fax : 33 (0)5 56 34 75 54

e-mail : palmer@dermscan.com
internet : www.palmer-research.com

ρ Promotor : SECMA BIOTECHNOLOGIE MARINE
ZI
BP 65
22260 PONTRIEUX

ρ Product : PHLOROGINE R20% H2O (P/P)
LOT 3.10.262

ρ Reference : LISKIN-DN-091PALMER 1040062PA

Lyon, May 25th 2004

AUTHENTICATION OF THE RESULTS

I, the undersigned, Dominique SABOUREAU, Doctor of Pharmacy, certify that :

- The study referenced **LISKIN-DN-091PALMER 1040062** which is the object of this report, was carried out, in accordance with the Good Practice of Laboratory (G.P.L.), by Laboratory LISKIN (VARSOVIE 01-601 UL. Krasinskiego 49 POLOGNE) partner of DERMSCAN Group;

- In the scope of this partnership, our quality service audits the laboratory LISKIN according to our internal procedures.

- All of the observations and numerical data collected during this trial are included in this document made by LISKIN laboratory with 23 pages and including a summary page 2 and 3/23.

Date : May 24, 2004

Signature :

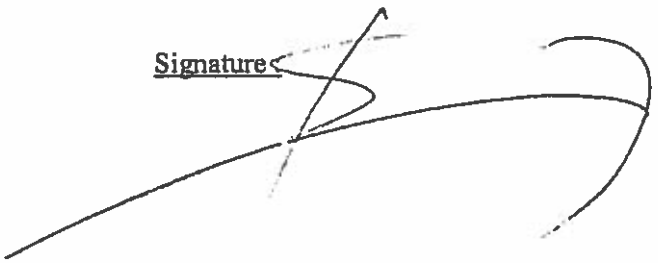

TABLE OF CONTENT

LISKIN

SENSITIZING POTENTIAL STUDY OF A PRODUCT ACCORDING TO MARZULLI-MAIBACH METHOD

REPORT

STUDY REF.	**DN 091**
PRODUCT	**PRODUCT:** **«PHLOROGINE R 20% H2O (P/P) LOT 3.10.262 - 59051»**
NUMBER OF SUBJECTS	**50**
SPONSOR	**DERMSCAN Group**
MONITOR	**Mr. Bogdan WICHROWSKI** **LISKIN** **38 bis, Av. Gabriel Péri** **92260 FONTENAY-AUX-ROSES** **☎/🖷 : 33 (0)1 46 83 15 83**
INVESTIGATOR	**Doctor Marlena Nowakowska, Dermatologist**

Document including 23 pages

LISKIN Eurl au capital de 8 000 €
38 bis, av. Gabriel Péri
92260 Fontenay-aux-Roses
Tél. / fax : (33) 01 46 83 15 83

RCS NANTERRE B 429 820 365
SIREN : 429 820 365
SIRET : 429 820 365 00014
N° TVA FR 91 429 820 365

STUDY SUMMARY

TITLE : SENSITIZING POTENTIAL STUDY OF A PRODUCT «PHLOROGINE R 20% H2O (P/P) LOT 3.10.262 - 59051» ACCORDING TO MARZULLI-MAIBACH METHOD, ON 50 SUBJECTS DURING 6 WEEKS.

STUDY REFERENCE: DN 091

PRODUCT: PHLOROGINE R 20% H2O (P/P) LOT 3.10.262 - 59051

STUDY IMPLEMENTATION: The study was carried out and all test values recorded by the Clinical Unit PROCOS, localized in Poland; ul. Słowackiego 27/33 lok. 33/34; 01-572 Warsaw.

INVESTIGATOR: Dr Marlena NOWAKOWSKA

MONITOR: Dr ing. Bogdan WICHROWSKI

PROTOCOL: CLINICAL EVALUATION OF THE SENSITIZING POTENTIAL OF A PRODUCT ACCORDING TO MARZULLI-MAIBACH METHOD.

AIM OF THE STUDY: To evaluate the sensitizing potential of a product under dermatological control and under the conditions defined by study's sponsor.

SUBJECTS: 50 healthy volunteers corresponding to the inclusion and non-inclusion criteria defined by the DERMSCAN Group.

STUDY SCHEDULE: March 01 to April 09, 2004

EXPERIMENTAL DESIGN: simple blind and monocentric study.

MAIN TOLERANCE PARAMETERS:

- Irritation potential (Induction Phase)
  Erythema, edema, desquamation, vesicles rated from 0 to 3 by the dermatologist
- Sensitizing potential (Challenge Phase)
  Reaction rated from 0 to 3 by the dermatologist according to ICDRG (International Contact Dermatitis Research Group)

RESULTS :

PRODUCT	Irritation potential	Sensitizing potential
PHLOROGINE R 20% H2O (P/P) LOT 3.10.262 - 59051	Mean rate of 0.000 = non-irritating	No allergic reaction

CONCLUSION :

Under these study conditions, the product «PHLOROGINE R 20% H2O (P/P) LOT 3.10.262 - 59051» can be considered non-irritating and non-sensitizing.

1. QUALITY ASSURANCE

The study described has been conducted according to the Good Clinical Practice Guidelines from FDA (FR of 8/08/1978 Part V - Decree n° 77N-0278), EEC (Directives n° 91/507 and III 3976/88 of 11/07/1990) and to the Ministry of Health of the French Republic.
The study has been conducted according to Standard Operating Procedures and to the study protocol defined by the sponsor. Every study events recorded during the study are reported.
Controls on data veracity and conformity with the protocol have been performed and confirmed by persons participating to the study (APPENDIX I).

2. CONFORMITY CERTIFICATE

I am aware that the study DN-091 has been conducted according to the «**Quality Assurance**» described before.
There was no event which may have affected the quality or integrity of the data.

Dr ing. B WICHROWSKI
Monitor

10/05/2004
date

3. METHOD

3.1. STUDY DESCRIPTION

3.1.1. Study product

The product supplied by Group DERMSCAN, has the following characteristics :

Product name	Product presentation	Product code in the study
PHLOROGINE R 20% H2O (P/P) LOT 3.10.262 - 59051	Orange transparent liquid	MF

The product was delivered on February 24, 2004.

3.2. CLINICAL METHODS

3.2.2. Aim of the study

To assess irritation potential and the sensitizing potential of a product under dermatological control and according to Marzulli-Maibach method.

3.2.3. Experimental design

This was an open study.

3.2.4. Study subjects

Inclusion criteria

- Healthy volunteer of Caucasian origin, male or female
- Age between 18 and 65
- Phototype II, III or IV
- Normal skin
- Subjects having given their informed, written consent
- Cooperative subjects, aware of the necessity and duration of controls so that perfect adhesion to the protocol established by the DERMSCAN Group could have been expected.

Non-inclusion criteria

- Pregnancy or nursing women
- Sun exposure or UV exposure 15 days before study and/or photopatchtests from less than 2 months
- Hyper Irritable skin
- Known allergies or sensitivities to s and Elastoplast
- Cutaneous pathology on the test zones
- Subjects afflicted with serious or progressive diseases
- Volunteers undergoing a topical or systemic treatment:
  - anti-inflammatories, anti-histamines, immunosuppressors, corticoids and retinoids

Inclusion

50 healthy volunteers have been selected according to the inclusion and the non-inclusion criteria, and 50 subjects completed the study. The table below presents the informations concerning all the volunteers included.

	Non included	Included	Drop out	Untraceable
Number of subjects		50		
Reason				
Day occurence				

Subjects characteristics

The summary table below presents a synthesis of the observations concerning exclusively the volunteers taken into account for data analysis.

Number of subjects	Sex	Age (mean±SEM)	Phototype	Medical or surgical events and medical treatments	
				Before study	During the study
50	41 F 9 M	33 ± 2	II: 49 III: 1	cf. **Table** In APPENDIX II	

3.3. MATERIAL

The patch-tests used are FINN CHAMBERS ON SCANPOR®.
The FINN CHAMBER constitutes a cupule to ensure a good occlusion.
In this study, the cupule was replaced by the disk of filter paper (same diameter) in order to create a semi-occlusion.

4. PRODUCT APPLICATION

Application area	Scapular zones: homolateral (induction zone) and controlateral (challenge zone)
Quantity and Concentration applied	25 µl pure
Frequency	Induction Phase: 3 times a week during 48 hours Challenge Phase: once during 48 hours
Contact time	Induction Phase: 3 weeks Rest Phase: 2 weeks Challenge Phase: 1 week
Application conditions	The product «PHLOROGINE R 20% H2O (P/P) LOT 3.10.262 - 59051» was applied like a semi-occlusive patch (filter paper) and applied to the volunteer's back. The patch containing no product was applied under the same conditions to serve as a non-treated control. During all Induction Phase, the homolateral zone was not wet. The volunteers take a shower on Sunday, after patches removing, and pay attention not to put a detergent product on all tested zones. During all Challenge Phase, no washing and no any product application take place on controlateral zone.

5. STUDY SCHEDULE

The study was carried out according to the following diagram :

Induction Phase - 3 weeks (W1, W2, W3)

W1 :

Day of the week	Mo	Tu	We	Th	Fr	Sa	Su
Study day	D1	D2	D3	D4	D5	D6	D7
Product application	↓		↓		↓		

W2 :

Day of the week	Mo	Tu	We	Th	Fr	Sa	Su
Study day	D8	D9	D10	D11	D12	D13	D14
Product application	↓		↓		↓		

W3 :

Day of the week	Mo	Tu	We	Th	Fr	Sa	Su
Study day	D15	D16	D17	D18	D19	D20	D21
Product application	↓		↓		↓		

Rest Phase - 2 weeks (W4, W5)

W4 :

Day of the week	Mo	Tu	We	Th	Fr	Sa	Su
Study day	D22	D23	D24	D25	D26	D27	D28

W5 :

Day of the week	Mo	Tu	We	Th	Fr	Sa	Su
Study day	D29	D30	D31	D32	D33	D34	D35

Challenge Phase - 1 week (W6)

W6 :

Day of the week	Mo	Tu	We	Th	Fr
Study day	D36	D37	D38	D39	D40
Product application	↓				
Reading			L		L

6. ASSESSMENT CRITERIA

6.1. CLINICAL CRITERIA REGARDING THE IRRITATING POTENTIAL (INDUCTION PHASE)

After each application, the patch is removed and the clinical examination is performed by the investigator 30 minutes later in order to eliminate the pressure and the occlusion effects.
The result of examination is negative if the skin looks normal.

The clinical examination is made on the back using the following criteria and scale:

Score	Cotation	CRITERIA : description			
		ERYTHEMA	EDEMA	DRYNESS	VESICLES
0	absent	Normal aspect	Normal aspect	Normal aspect	Normal aspect
1	slight	Discreet pink coloration of the whole tested area or rather visible on part of the tested area	More palpable than visible edema	Discreet thin desquamation, tarnished aspect	More palpables than visible vesicles
2	marked	Marked erythema covering the whole tested area	Visible edema	Visible desquamation, flaky aspect.	Visible vesicles
3	important	Severe erythema covering the whole tested area or erythema diffusing beyond the tested area	Edema diffusing beyond the tested area	Important desquamation, cracking	Vesicles diffusing beyond the tested area or blisters.

6.2. CLINICAL CRITERIA REGARDING THE SENSITIZING POTENTIAL (CHALLENGE PHASE)

The allergic reactions are evaluated according to the following scale:

Criterion	Quotation ICDRG (*)	Score noted in all tables
No reaction	0	0
Doubtful reaction	?	?
Erythema and edema	+	1
Erythema, edema and vesicles	++	2
Severe reaction with blisters	+++	3

(*) - International Contact Dermatitis Research Group

6.3. ASSESSMENT METHOD

6.3.1. Irritating potential - Induction Phase

At the conclusion of 8 reading of the Induction Phase, the average score of every volunteer is calculated by adding the scores obtained for each reading and by dividing this sum by the actual number of readings (a reading will not be taken into account if there is reaction of the control or global irritation).
The irritating potential of the product will be estimated during the Induction Phase, by calculating the mean of the reactions observed.

The irritating potential of the product is determined according to the following formula:

$$\text{Average score} = \frac{[(\sum \text{scores D1...D19/ nb of readings) vol1} + ... + (\sum \text{scores D1...D19/ nb of readings) volN}]}{\text{nb of volunteers (N)}}$$

Average score	Irritating Potential
0.000 - 0.080	Non-Irritating
0.081 - 0.160	Very slightly irritating
0.161 - 0.560	Slightly irritating
0.561 - 1.000	Moderately irritating
1.001 - 1.600	Strongly irritating
> 1.600	Very strongly irritating

6.3.2. Sensitizing potential - Challenge Phase

The possible allergic reaction, during the Induction or Challenge Phase, will be rated from 0 to 3 according to ICDRG (International Contact Dermatitis Research Group).
During the Challenge Phase, the reading will take place 30 minutes after patch-tests removal and 48 hours later.
The sensitizing potential of the product will be assessed by the reading D38 and D40 (Challenge Phase) as a function of the following criteria: reaction ++ (2) or +++ (3) in the absence of added irritation phenomenon.

The presence of only one case of active sensitizing leads to conclusion "Potentially sensitive product".

6.4. PREMATURE STUDY TERMINATION

The subjects have the right to leave the study at any time whatever the reason.
The premature study termination can be for multiple reasons :

- non-compliance with the visits schedule by the subject,
- adverse events (including intercurrent diseases),
- protocol non-adherence/departures from protocol,
- withdrawal of subject's consent.

6.5. AMENDMENT TO PROTOCOL

None

7. RESULTS

7.1. IRRITATING POTENTIAL : INDUCTION PHASE

The TABLE OF READINGS regarding the Induction Phase is presented in APPENDIX III.

These reading made 30 min. after having removed the patch-tests showed the following results:

Product	D3	D5	D8	D10	D12	D15	D17	D19	Conclusion
PHLOROGINE R 20% H2O (P/P) LOT 3.10.262 - 59051	C+ : 0 0 : 50	C+ : 0 0 : 50	C+ : 0 0 : 50	C+ : 0 0 : 50	C+ : 0 0 : 50	C+ : 0 0 : 50	C+ : 0 0 : 50	C+ : 0 0 : 50	Non-irritating (IRR = 0.000)

C+ = Positive control IRR = global irritation MV = missing value

Under these study conditions, the product «PHLOROGINE R 20% H2O (P/P) LOT 3.10.262 - 59051» showed a score below 0.08, so it can be considered to be non-irritating.

7.2. SENSITIZING POTENTIAL : CHALLENGE PHASE

The TABLE OF READING regarding the Challenge Phase is presented in APPENDIX IV.

These reading made 30 min. and 48h after having removed the patch-tests showed the following results:

Product	Zone	Day of the reading		Global result
		D38	D40	
PHLOROGINE R 20% H2O (P/P) LOT 3.10.262 - 59051	Reading homolateral zone	C+: 0 MF: 0: 50 ?: 0 1: 0 2: 0 3: 0	C+: 0 MF: 0: 50 ?: 0 1: 0 2: 0 3: 0	Non-sensitizing
	Reading controlateral zone	C+: 0 MF:0: 50 ?: 0 1: 0 2: 0 3: 0	C+: 0 MF: 0: 50 ?: 0 1: 0 2: 0 3: 0	

C+= Positive control
MF = PHLOROGINE R 20% H2O (P/P) LOT 3.10.262 - 59051
MV= missing value

The product «PHLOROGINE R 20% H2O (P/P) LOT 3.10.262 - 59051» can be considered to be <u>not-sensitizing</u> under these study conditions.

8. CONCLUSION

Under these study conditions, the product «PHLOROGINE R 20% H2O (P/P) LOT 3.10.262 - 59051» can be considered <u>not-irritating</u> and <u>not-sensitizing</u>.

APPENDICES

APPENDIX I

RESULTS AUTHENTIFICATION SHEET

PROCOS

KARTA AUTENTYCZNOŚCI REZULTATÓW
FICHE D'AUTHENTIFICATION DES RESULTATS
AUTHENTIFICATION PAGE

Według posiadanych przeze mnie informacji, badanie Nr. :
A ma connaissance l'étude N :
I am aware that the study N° :

DN- 091

było przeprowadzone zgodnie PROTOKOŁEM oraz KARTĄ PARAMETRÓW TESTU.
a été conduite en accord avec le PROTOCOLE et la FICHE DES PARAMETRES D'ETUDE.
has been conducted according to the PROTOCOL and to the STUDY PARAMETERS PAGE.

Mgr inż. Barbara WAŁEJKO Odpowiedzialna za badania i jakość *Responsable d'unité; Responsable qualité* Unit head; Responsible for quality control	B. Wałejko podpis / *signature*	09.04.2004 data /*date*
Dr Marlena NOWAKOWSKA Lekarz dermatolog *Médecin dermatologue* Dermatologist	4177024 MARLENA NOWAKOWSKA specjalista dermatolog podpis / *signature*	09.04.2004 data /*date*
Anna MAREK Technik *Technicienne* Technician	A. Marek podpis / *signature*	09.04.2004. data /*date*
Anna MIŚKIEWICZ Asystentka medyczna *Assistante médicale* Medical assistant	A. Miśkiewicz podpis / *signature*	9.04.04 data /*date*

APPENDIX II

VOLUNTEERS' CHARACTERISTICS

VOLUNTEERS' CHARACTERISTICS

Subject number	Subject code	Age	Sex	Photo-type	Medical or surgical events and medical treatments	
			F or M		before the study	during the study
1	WASRE	18	F	II		
2	BEDMA	22	M	II		
3	WYSHE	55	F	II		
4	ZABEW	45	F	II		
5	PRZEW	19	F	II		
6	KOWJO	58	F	II		
7	FOLBA	43	F	II		
8	RYNJO	52	F	II		
9	BERMO	43	F	II		
10	NATHO	25	F	II		
11	RENMA	40	F	II		
12	HAWMA	47	F	II		
13	STUEL	50	F	II		
14	SZEJU	18	F	II		
15	KICLI	33	F	II		
16	WOJDO	20	F	II		
17	PAWMO	18	F	II		
18	SZUJA	19	M	II		
19	SYPPR	19	M	II		
20	JANBA	60	F	II		
21	STEJO	32	F	III		
22	SZYMA	22	F	II		
23	SOKMO	22	F	II		
24	URBMA	37	F	II		
25	WILKL	26	F	II		
26	JEGST	23	M	II		
27	LUISL	23	F	II		
28	ZIEZU	21	F	II		
29	WILLE	21	M	II		
30	ROZRE	28	M	II		

UN = untraceable

VOLUNTEERS' CHARACTERISTICS - (continuation)

Subject number	Subject code	Age	Sex	Photo-type	Medical or surgical events and medical treatments	
			F or M		before the study	during the study
31	KEDUR	18	F	II		
32	PACEW	18	F	II		
33	BARDO	18	F	II		
34	ZANMI	20	F	II		
35	SLOBE	42	F	II		
36	ANDRA	37	M	II		
37	PRUBE	44	F	II		
38	JULZA	40	M	II		
39	MALMO	20	F	II		
40	LUKSZ	21	M	II		
41	WALAN	57	F	II		
42	KRUEL	60	F	II		
43	JOLPR	47	F	II		
44	GLAEL	28	F	II		
45	IWORY	34	F	II		
46	GRAMA	21	F	II		
47	KRZWI	35	F	II		
48	MARAN	35	F	II		
49	OBSJA	43	F	II		
50	BABAL	60	F	II		

UN = untraceable

APPENDIX III

TABLE OF READING

INDUCTION PHASE

TABLE OF READING - Induction Phase

Subject number	D3		D5		D8		D10		D12		D15		D17		D19	
	C	MF	C	MF	C	MF	C	MF	C	MF	C	MF	C	MF	C	MF
1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
8	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
9	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
10	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
11	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
12	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
13	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
14	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
15	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
16	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
17	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
18	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
19	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
20	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
21	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
22	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
23	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
24	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
25	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
26	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
27	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
28	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
29	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
30	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

UN = untraceable
C = control
MF= PHLOROGINE R 20% H2O (P/P) LOT 3.10.262 - 59051

TABLE OF READING - Induction Phase (continuation)

Subject number	D3		D5		D8		D10		D12		D15		D17		D19	
	C	MF	C	MF	C	MF	C	MF	C	MF	C	MF	C	MF	C	MF
31	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
32	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
33	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
34	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
35	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
36	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
37	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
38	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
39	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
40	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
41	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
42	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
43	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
44	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
45	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
46	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
47	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
48	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
49	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
50	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

UN = untraceable
C = control
MF = PHLOROGINE R 20% H2O (P/P) LOT 3.10.262 - 59051

APPENDIX IV

TABLE OF READING
CHALLENGE PHASE

TABLE OF READING - Challenge Phase

Subject number	D38 Homolateral zone		D38 Controlateral zone		D40 Homolateral zone		D40 Controlateral zone	
	C	MF	C	MF	C	MF	C	MF
1	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0
4	0	0	0	0	0	0	0	0
5	0	0	0	0	0	0	0	0
6	0	0	0	0	0	0	0	0
7	0	0	0	0	0	0	0	0
8	0	0	0	0	0	0	0	0
9	0	0	0	0	0	0	0	0
10	0	0	0	0	0	0	0	0
11	0	0	0	0	0	0	0	0
12	0	0	0	0	0	0	0	0
13	0	0	0	0	0	0	0	0
14	0	0	0	0	0	0	0	0
15	0	0	0	0	0	0	0	0
16	0	0	0	0	0	0	0	0
17	0	0	0	0	0	0	0	0
18	0	0	0	0	0	0	0	0
19	0	0	0	0	0	0	0	0
20	0	0	0	0	0	0	0	0
21	0	0	0	0	0	0	0	0
22	0	0	0	0	0	0	0	0
23	0	0	0	0	0	0	0	0
24	0	0	0	0	0	0	0	0
25	0	0	0	0	0	0	0	0
26	0	0	0	0	0	0	0	0
27	0	0	0	0	0	0	0	0
28	0	0	0	0	0	0	0	0
29	0	0	0	0	0	0	0	0
30	0	0	0	0	0	0	0	0

UN = untraceable
C = control
MF = PHLOROGINE R 20% H2O (P/P) LOT 3.10.262 - 59051

TABLE OF READING - Challenge Phase (continuation)

Subject number	D38 Homolateral zone		D38 Controlateral zone		D40 Homolateral zone		D40 Controlateral zone	
	C	MF	C	MF	C	MF	C	MF
31	0	0	0	0	0	0	0	0
32	0	0	0	0	0	0	0	0
33	0	0	0	0	0	0	0	0
34	0	0	0	0	0	0	0	0
35	0	0	0	0	0	0	0	0
36	0	0	0	0	0	0	0	0
37	0	0	0	0	0	0	0	0
38	0	0	0	0	0	0	0	0
39	0	0	0	0	0	0	0	0
40	0	0	0	0	0	0	0	0
41	0	0	0	0	0	0	0	0
42	0	0	0	0	0	0	0	0
43	0	0	0	0	0	0	0	0
44	0	0	0	0	0	0	0	0
45	0	0	0	0	0	0	0	0
46	0	0	0	0	0	0	0	0
47	0	0	0	0	0	0	0	0
48	0	0	0	0	0	0	0	0
49	0	0	0	0	0	0	0	0
50	0	0	0	0	0	0	0	0

UN = untraceable
C = control
MF = PHLOROGINE R 20% H2O (P/P) LOT 3.10.262 - 59051

PALMER Research

Etude de la tolérance cutanée aiguë chez 10 volontaires adultes Patch-tests 24 et 48 heures,uniques

Etude référence TC.aiguë.5-SEC/P/PALM 95
réalisée du 19/12/95 au 21/12/95

Société : **SECMA Biotechnologies Marines**
B.P. 65
22260 PONTRIEUX

Produit : **PHLOROGINE (3 concentrations)**

Arbanats, Février 1996

SOCIÉTÉ DE CONSEIL-EXPERTISE PHARMACEUTIQUE & COSMÉTOLOGIQUE

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1 - INTRODUCTION

A la demande de la société **SECMA Biotechnologies Marines - B.P. 65 ; 22260 PONTRIEUX** -, nous avons évalué sur 10 volontaires adultes la tolérance cutanée aiguë du produit **PHLOROGINE** aux 3 concentrations suivantes :

- **P : PHLOROGINE à 100 %**
- **P1 : 8 % de PHLOROGINE**
- **P2 : 16 % de PHLOROGINE**

après application unique sur la peau de la face antérieure d'un bras, sous pansements occlusifs maintenus pendant 24 et 48 heures (Patch-Tests 24 et 48 heures).

Cet essai a été réalisé "en ouvert" selon la méthodologie des tests épicutanés sous occlusion.

Pour réaliser cette étude, nous avons reçu le 18/12/95 trois échantillons de 60 ml de chaque concentration du produit PHLOROGINE.

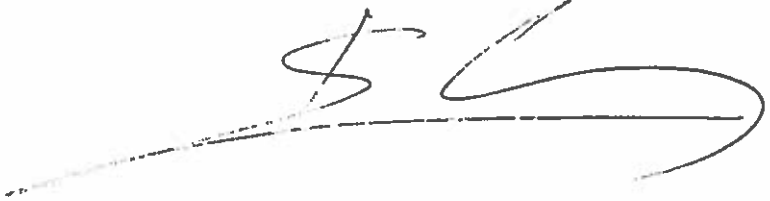
L'essai a commencé le19/12/95 pour s'achever le 21/12/95.

2 - AUTHENTIFICATION DES RESULTATS

L'étude faisant l'objet du présent rapport a été conduite sous ma responsabilité, en conformité avec le protocole expérimental et dans le respect des règles des Bonnes Pratiques Cliniques. Toutes les observations et les données numériques recueillies au cours de cet essai sont rapportées dans le présent document.

Après relecture, je certifie ces données conformes à la réalité des résultats obtenus.
Docteur Pascale DENIS, Investigateur et Directeur de l'Etude.

En tant que Moniteur de l'Etude, je certifie avoir relu ce rapport et je suis en accord avec son contenu.
Dominique SABOUREAU, Docteur en Pharmacie, Gérant de PALMER RESEARCH.



3 - PROTOCOLE EXPERIMENTAL

3.1 - Volontaires

3.1.1 - Caractéristiques des sujets inclus

- 10 sujets, dont 5 de sexe masculin et 5 de sexe féminin, ont été inclus dans l'essai,
- âgés de 21 à 46 ans.

Tous les sujets devaient répondre aux critères d'inclusion et ne présenter aucun critère de non inclusion.

3.1.2 - Critères d'inclusion

- aucun antécédent d'intolérance ou d'allergie à un produit cosmétique,
- acceptation de signature du consentement éclairé de participation.

3.1.3 - Critères de non inclusion

- pathologie cutanée, quelque soit son site,
prise d'un traitement interférant avec le métabolisme cutané, en particulier isotrétinoïne, acitrétine et étrétinate.

3.2 - Méthodologie

3.2.1 - Matériel, dose, durée

Le produit a été appliqué aux 3 concentrations suivantes :

- **P : PHLOROGINE à 100 %**
- **P1 : 8 % de PHLOROGINE**
- **P2 : 16 % de PHLOROGINE**

une seule fois, sur une surface d'environ 50mm de peau de la face antérieure d'un bras de chaque volontaire, à la dose d'environ 0,02ml imbibant la rondelle de papier filtre.

Nota : La raison du choix de la dose est conditionnée par la capacité de la cupule, indiquée par le fabricant dans "Finn Chambers".

Six pansements occlusifs, correspondant à chaque concentration et à chaque temps de contact, ont été posés et maintenus en contact avec la peau pendant respectivement 24 et 48 heures consécutives.

Ces applications sont effectuées parallèlement et dans les mêmes conditions avec un patch-test seul (sans produit) en tant que témoin négatif.

3.2.2 - Lectures

Les examens macroscopiques cutanés ont été réalisés immédiatement, 30 minutes et 24 heures après l'enlèvement des patch.

L'évaluation des réactions cutanées (érythème, oedème, ...) a été effectuée selon la nomenclature proposée par l'International Contact Dermatitis Research Group (I.C.D.R.G) :

NT	:	Non testé.
?+	:	Réaction douteuse. Léger érythème seulement.
+	:	Réaction positive faible (non vésiculeuse) : érythème, infiltration, parfois quelques papules.
++	:	Forte réaction positive : présence d'érythème, de papules, de vésicules.
+++	:	Réaction positive violente, avec présence de bulles.
-	:	Réaction négative.
IR	:	Réaction d'irritation = Erythème (E) **E = 0,5 érythème très léger** **E = 1 érythème léger** **E = 2 érythème net** **E = 3 érythème important**

Nota : En l'absence de toute réaction cutanée locale à la lecture de 24 heures, l'essai est arrêté. Dans le cas de réactions nettes ou douteuses, une lecture est effectuée 48 heures et si nécessaire 72 heures après la dépose des patchs.

3.2.3 - Interprétation des résultats

Référence bibliographique :"Les essais cliniques en dermatologie", Thérapie, 1991, Tome 46, page 183-7.

L'indice d'irritation moyen à chaque temps de lecture est calculé selon le rapport :

$$IM = \frac{\sum \text{des cotations érythémateuses}}{\text{nombre de sujets}}$$

Le barême d'interprétation de l'irritation cutanée est le suivant :

Si IM≤0,20 non irritant
Si 0,20≤IM≤0,5 légèrement irritant
Si 0,50≤IM≤1 moyennement irritant
Si IM>1 irritant

4 - RESULTATS

Les résultats individuels des lectures à chaque expérimental et à chacune des 3 concentrations du produit sont regroupés dans les tableaux ci-dessous.

***Tableau 1* : PHLOROGINE à 100 %**

SUJETS		Produit à l'essai : PHLOROGINE 100% Patch 24 heures		Produit à l'essai : PHLOROGINE 100% Patch 48 heures		Témoin Négatif
Identification	Age et Sexe	Lecture 30 min après enlèvement du patch	Lecture 24 heures après enlèvement du patch	Lecture 30 min après enlèvement du patch	Lecture 24 heures après enlèvement du patch	Lecture 30 min après enlèvement du patch
BA.FR	40 M	-	-	E1	-	-
CR.FR	28 M	E0,5	-	E1	-	-
RA.AM	30 F	-	-	-	-	-
RI.JP	46 M	E0,5	-	E1	-	-
KA.LA	21 F	E0,5	-	-	-	-
AH.RO	31 F	-	-	-	-	-
EN.RA	31 M	-	-	E0,5	-	-
KA.AL	21 F	-	-	-	-	-
MO.NA	35 F	E0,5	-	-	-	-
KA.TH	37 M	E0,5	E1	E0,5	-	-
II/I		0,25	0,10	0,30	0	0
Résultats		légèrement irritant	non irritant	légèrement irritant	non irritant	non irritant

***Tableau 2* : 16 % de PHLOROGINE**

SUJETS		Produit à l'essai : 16 % PHLOROGINE Patch 24 heures		Produit à l'essai : 16 % PHLOROGINE Patch 48 heures		Témoin Négatif
Identification	Age et Sexe	Lecture 30 min après enlèvement du patch	Lecture 24 heures après enlèvement du patch	Lecture 30 min après enlèvement du patch	Lecture 24 heures après enlèvement du patch	Lecture 30 min après enlèvement du patch
BA.FR	40 M	-	-	-	-	-
CR.FR	28 M	-	-	E0,5	-	-
RA.AM	30 F	-	-	-	-	-
RI.JP	46 M	E1	-	E1	-	-
KA.LA	21 F	-	-	E0,5	-	-
AH.RO	31 F	-	-	E0,5	-	-
EN.RA	31 M	-	-	E0,5	-	-
KA.AL	21 F	-	-	-	-	-
MO.NA	35 F	-	-	-	-	-
KA.TH	37 M	-	-	-	-	-
TSI		0,5/0	0	0,5/0	0	0
Résultats		non irritant	non irritant	légèrement irritant	non irritant	non irritant

***Tableau 3* : 8 % de PHLOROGINE**

SUJETS		Produit à l'essai : 8 % PHLOROGINE Patch 24 heures		Produit à l'essai : 8 % PHLOROGINE Patch 48 heures		Témoin Négatif
Identification	Age et Sexe	Lecture 30 min après enlèvement du patch	Lecture 24 heures après enlèvement du patch	Lecture 30 min après enlèvement du patch	Lecture 24 heures après enlèvement du patch	Lecture 30 min après enlèvement du patch
BA.FR	40 M	-	-	-	-	-
CR.FR	28 M	-	-	E0,5	-	-
RA.AM	30 F	-	-	-	-	-
RI.JP	46 M	E0,5	-	E0,5	-	-
KA.LA	21 F	-	-	-	-	-
AH.RO	31 F	-	-	-	-	-
EN.RA	31 M	-	-	-	-	-
KA.AL	21 F	-	-	-	-	-
MO.NA	35 F	-	-	E0,5	-	-
KA.TH	37 M	E1	-	-	-	-
[illegible]		0, 1.5	0	0, 1.5	0	0
[illegible]		non irritant	non irritant	non irritant	non irritant	non irritant

5 - CONCLUSION

Dans les conditions expérimentales retenues, le produit **PHLOROGINE** s'est révélé :

- appliqué pur, après 24 et 48 heures de contact avec la peau, ***Légèrement Irritant***, induisant chez 5 sujets sur 10, à chaque temps de contact, un érythème très léger à léger. La réversibilité des réactions a été globalement bonne, excepté chez un sujet chez lequel l'érythème coté 0,5, 30 minutes après l'enlèvement du pansement occlusif, avait augmenté 24 heures après (cotation 1). 96 heures après, cette réaction cutanée avait totalement régressée.

- appliqué dilué à 16 %,
  ⇒ après 24 heures de contact avec la peau, ***Non Irritant***..
  ⇒ après 48 heures de contact, ***Légèrement Irritant.***
  La réversibilité a été bonne et aucun effet secondaire n'a été observé.

- appliqué dilué à 8 %, après 24 et 48 heures de contact avec la peau, ***Non Irritant***, malgré un très léger érythème passager enregistré 30 minutes après l'enlèvement des pansements occlusifs.

Il s'avère nécessaire de vérifier, dans les mêmes conditions expérimentales, la tolérance cutanée du véhicule de dilution afin de lever toute ambiguité sur le produit.

D. SABOUREAU
Docteur en Pharmacie

P. DENIS
Docteur en Médecine

RAPPORT

VERIFICATION CHEZ L'HOMME DE LA COMPATIBILITE CUTANEE D'UN PRODUIT COSMETIQUE APRES APPLICATION UNIQUE SOUS PANSEMENT.

Patch test

Produit testé :

0 J

Laminaria saccharina dy lot 4.07.199

Promoteur

Madame Nicole Meckideche
Secma Biotechnologie Marines
ZI – BP 65
22260 PONTRIEUX

JA/ND

Bordeaux, le 29 avril 2005

SOMMAIRE

Laboratoire COSDERMA - Service de dermatologie Pr. Taïeb - Groupe Hospitalier Saint André - 1 rue Jean Burguet - BP 50057 - 33023 Bordeaux Cedex
Tel : 05 56 94 75 40 - Fax : 05 56 79 49 75 - email : laboratoire@cosderma.com

1- But de l'étude

Le but de cette étude était d'évaluer la compatibilité cutanée du produit **« Laminaria saccharina dy lot 4.07.199 »** après application unique sur la peau pendant 24h chez **10** volontaires.

La compatibilité cutanée du produit a été vérifiée par un examen visuel de la zone expérimentale réalisé par l'investigateur ou la technicienne sous son autorité.

Le fait que cette étude visait à une meilleure connaissance de la compatibilité cutanée du produit étudié et que le risque prévisible pour les volontaires qui participent à celle-ci est infime, il y a eu une bonne adéquation entre le but de l'étude et les risques potentiels.

2- Méthodologie

2.1 Plan expérimental

Cette étude a été sans bénéfice individuel direct, chaque volontaire y participant a été son propre témoin.
Cette étude était monocentrique, réalisée en ouvert.
La durée de l'étude a été de 24h et a comporté une visite à l'Institut (J0, T24h).

2.2 Centre investigateur

Laboratoire COSDERMA
Service de dermatologie du Pr Taïeb - Groupe Hospitalier Saint André
1 rue Jean Burguet - BP 50057
33023 Bordeaux Cedex
tel : 05 56 94 75 40
email : laboratoire@cosderma.com

2.3 Lieu de l'investigation

Service de dermatologie du Pr Taïeb
Groupe Hospitalier Saint André
1 rue Jean Burguet
33000 Bordeaux

2.4 Equipe technique

Investigateur : Jérôme Asserin

Technicienne : Nadège Durand

3- Date de réalisation de l'étude

Début le : 4 avril 2005

Fin le : 5 avril 2005

4- Produit étudié

4.1 Informations

L'information transmise par le promoteur accompagnant l'échantillon a été la lettre d'engagement concernant en particulier la conformité de la formule aux règlementations en vigeur et sa sécurité.

4.2 Identification

Nom	Réf.	Quantité pour l'étude	Nb conditionnement
Laminaria saccharina dy	**lot 4.07.199**	5 ml	1 x 5 ml

4.3 Conditions normales d'emploi

Nom	Mode d'emploi	
Laminaria saccharina dy lot 4.07.199	**site**	visage
	- application 2 fois par jour sur le visage	

4.4 Conditions d'utilisation pendant l'étude

Nom	Matériel de patchage	Conditions expérimentales d'utilisation	Quantité appliquée	Temps de contact	Temps de contrôle après dépatchage
Laminaria saccharina dy lot 4.07.199	Finn Chamber Standard*	Pur	20mg	24 +/- 2 heures	J1 /T15

- *Finn Chambers® : Pansement occlusif composé d'une cupule d'aluminium de 8 mm de diamètre (surface 50 mm²) sur laquelle 20 µl (20 mg) de produit est déposé.*

Un patch témoin, correspondant au type de pansements utilisés, contenant une quantité ad hoc d'eau pour préparation injectable, a été appliqué parallèlement.
Le dépatchage a été effectué par l'investigateur ou la technicienne sous sa responsabilité.
Les quantités de produit ont été mesurées à l'aide d'une seringue à usage unique.

5- Volontaires

5-1 Panel

Le panel de volontaire participant à l'étude est représentatif de la population susceptible d'utiliser le produit.
Tous les volontaires sélectionnés ont répondu aux critères d'inclusion et de non inclusion.

5-2 Effectif

Le nombre de volontaires participant à l'étude a été de **10**.
Le nombre de volontaires dont les données sont présentées est de **10**.

5-3 Critères d'inclusion

Les critères d'inclusion étaient les suivants :

- âge : 18 à 65 ans
- sexe : féminin et/ou masculin
- phototype (Fitzpatrick) : I à III
- tous types de peau

Toutes les volontaires ont correspondu à ces critères d'inclusion. Les caractéristiques typologiques des volontaires sont présentées en **Annexe 1.**

5-4 Critères de non inclusion

Les critères de non inclusion étaient les suivants :

- Pas d'application de produits autres que ceux testés sur la zone expérimentale
- Pas de port de vêtements trop serrés ou responsables d'une contention au niveau de la zone expérimentale, susceptibles d'occasionner des frottements et le décollement du pansement
- Pas de bain en baignoire, en mer ou en piscine et pas de sauna ou de hammam durant l'étude
- Protection de la zone expérimentale lors de la prise de douche, pas de projection violente d'eau et pas de savonnage sur cette zone pour éviter le décollement du pansement ou l'apparition de phénomènes intercurrents, et essuyage très délicat si nécessaire
- Pas de sudation excessive et pas d'activité physique intensive susceptibles d'entrainer le décollement du pansement
- Pas d'exposition au solaire intensive, (au soleil naturel ou en cabine UVA) pendant la durée de l'étude, surtout lorsque le pansement à été enlevé
- Conservation des habitudes d'hygiène sur le visage et le corps,
- Conservation des habitudes de maquillage,
- Pas de traitement anti-allergique, anti-inflammatoire(corticoïde systémique ou topique) ou par des spécialités à base de vitamine A acide ou de ses dérivés le jour de l'étude (si nécessité thérapeutique :sortie d'étude envisagée)

Aucun volontaire correspondant à ces critères n'a été inclu.

5-5 Contraintes de l'étude

Les contraintes de l'étude étaient les suivantes :

- Marques cutanées au niveau de la zone expérimentale pouvant interférer avec l'évaluation des réactions de la peau (troubles de la pigmentation, éléments cicatriciels, pilosité trop développée, éphélides et nævi en trop grande quantité, coup de soleil....)
- Réaction eczématiforme non encore complètement disparue, séquelles cicatricielles ou pigmentaires de tests antérieurs au niveau de la zone expérimentale
- Allergie à la colophane ou au nickel
- Allergie ou réactivité à la même catégorie de produits
- Hyper-réactivité cutanée
- Réactivité à l'alcool éthylique, au sparadrap
- Participation dans les 12 mois qui précédent l'étude, à plus de 5 tests utilisant la maximalisation dont 3 au plus à visée de recherche d'hypoallergénicité
- Exposition intensive au soleil dans le mois qui précède l'étude
- Prévision d'une exposition solaire intensive (au soleil naturel ou en cabine UVA) pendant la durée de l'étude
- Intention de se baigner en baignoire, en mer ou en piscine, de faire du sauna ou du hammam pendant l'étude
- Pratique d'un ou plusieurs sports de façon intensive ou régulière dont l'interruption momentanée pose problème
- Arrêt de traitement à base de vitamine A acide ou de ses dérivés depuis moins de 3 mois avant le début de l'étude
- Arrêt de traitement par corticoïde topique sur la zone expérimentale de moins de 8 jours avant l'étude
- Arrêt de traitement par PUVA ou UVB depuis moins d'un mois avant l'étude
- Prévision de vaccination pendant la durée du test, dernière vaccination dans les 3 semaines précédant l'étude

Toutes les contraintes de l'étude ont été respectées par les volontaires.

5-6 Contrôle de l'observance des modalités du protocole

L'investigateur a vérifié si les **contraintes** avaient été respectées.

La synthèse des réponses aux différentes questions posées est jointe en **Annexes 2-1 et 2-2.**

En cas de déviations au protocole, celles-ci ont été analysées et l'investigateur a apprécié leur incidence sur la validité des résultats.

Toutes les contraintes de l'étude, définies au protocole, ont été respectées par les volontaires.

6- Evaluation

6-1 Calendrier

	début	Temps de contact
Jour	J0	T24h ± 2h
Sélection des volontaires	X	
Attribution n° des volontaires	X	
Information volontaire	X	
Consentement éclairé signé *	X	
Application des pansements par la technicienne	X	
Dépatchage par la technicienne		X
Critères évaluation (15 mn après dépatchage)		X

Le fait que l'application du produit ainsi que les examens cliniques aient été parfaitement contrôlés, l'effectif de volontaires et la durée de l'étude ont permis de vérifier la compatibilité cutanée du produit étudié et d'apprécier les éventuels phénomènes irritatifs.

Un double du consentement de participation sera remis aux volontaires le jour de la visite d'inclusion pour l'étude. L'original sera conservé par l'investigateur.

6-2 Evaluation de la compatibilité cutanée

♦ *Principe et bibliographies*

La compatibilité cutanée est vérifiée par l'intermédiaire de l'application de pansements sur la peau qui créent une certaine occlusion des produits et favorisent leur pénétration. Dans ces conditions expérimentales maximalisantes, le potentiel irritant des produits peut se révéler plus facilement.

La méthodologie a fait l'objet de nombreuses publications, dont :

Comment tester les produits cosmétiques ?, Dermatologie Pratique, 2003, n° 273, 1-4

Reactive changes in human epidermis following simple occlusion with water, Contact Dermatitis, Mikulowska A, 1992, 26, 224-227

Test strategies for development of cosmetic products using dermatological test models, Seifen-öle-fette-wachse, Matthies W, 1991, 117, 42-43

The Duhring Chamber: an inproved technique for epicutaneous testing of irritant and allergic rections, Contact Dermatitis,Frosch PJ & Klingmann AM, 1979, 5, 73-81

Appraisal of the safety of chemicals in Food, Drugs and Cosmetics, FDA (ed), Draize JH, 1959, 46-48

♦ *Méthodologie, matériel de patchage*

Les produits sont déposés sur les pansements, extemporanément, à l'aide d'une seringue de 1ml. Les pansements sont appliqués par la suite sur la peau le plus rapidement possible en évitant soigneusement les zones exposées au frottement ou compressions diverses. L'investigateur ou la technicienne sous son autorité vérifiera que la zone de peau concernée est vierge de toutes présences de grains de beauté, cicatrices et accidents cutanés. Le type de pansement, le nombre maximum de produits possible à tester, la quantité de produit à appliquer, la méthodologie d'application et de retrait des pansements et l'examen clinique visuel sont conformes aux procédures du laboratoire référencées pour ce type d'étude.
Le site d'application des produits choisi est le dos.

♦ *Conditions environnementales*

Les conditions environnementales imposées aux volontaires sont les suivantes :

- *température contrôlée : t ° = 20°C ± 2°C*

- *humidité relative : HR = 45 % ± 15 %*

• *Examen clinique*

- Sites

L'investigateur ou la technicienne sous son autorité effectue un contrôle visuel de chaque zone expérimentale sous un éclairage standardisé type « lumière du jour ».

- Fréquences

L'examen visuel est réalisé à T24h ± 2h, 15 minutes après dépatchage (ou plus si des rougeurs sont apparues à l'enlèvement du patch).

• *Critères d'évaluation*

- *signes cliniques*

Description	Code laboratoire	Intensité	aspect	note
Erythème	E	- échelle ordinale en 3 points : • légère • modérée • sévère	- érythème : • diffus • ponctué • périphérique	• légère = 1 • modérée = 2 • sévère = 3 • diffus = d • ponctué = p • périphérique = peri
œdème	Oe			
dessèchement	D			
coloration	C			
Comédon, microkyste	Co, Mi	- dénombrés		
Vésicule, papule	V, Pa	- échelle ordinale en 2 points : • 1 à 2 vésicules • vésicules en nombre >2		• 1 à 2 = 1 • nb >2 = 2
bulle, croutelle	Bu, Cr	- décrits		• si décrits = 2

L'investigateur, ou la technicienne sous son autorité, ont noté tout signe clinique, sa localisation, son intensité, son évolution, le traitement médicamenteux éventuellement entrepris. Il a établi le caractère habituel ou inhabituel du signe clinique, en questionnant le volontaire sur ce qu'il observe dans la vie courante, lors de l'utilisation de produits similaires.

- *Sensations d'inconfort*

Description	Code laboratoire	Intensité	note
Echauffement	Ech	- échelle ordinale en 3 points : • légère • modérée • sévère	• légère = 1 • modérée = 2 • sévère = 3
Picotement	Pi		
Prurit(démangeaison)	Pr		
Tiraillement	Ti		
Brûlure	Br		

- *Expression des résultats*

Tous les volontaires ayant fait l'objet de la visite J0 ont été pris en compte pour l'évaluation de la compatibilité cutanée. L'expression des résultats de l'examen cutané et de l'interrogatoire a été conforme à la procédure du laboratoire référencée pour ce type d'étude.

Les résultats individuels sont exprimés:

- **en pourcentage de volontaires réactifs** en tenant compte pour ce calcul uniquement les signes cliniques décelables visuellement à type d'érythème, œdème, vésicule, bulle, papule, croutelle.
- **de façon descriptive** pour les autres signes décelables visuellement ou les sensations d'inconfort, le pourcentage de volontaires chez qui ils ont été observés, pouvant éventuellement être calculé si la fréquence d'apparition de ces signes le justifiait.
- **en score d'irritation cutanée** calculé à partir des « notes » attribuées aux signes cliniques décelables visuellement.

Pour chaque volontaire et à chaque temps d'observation, a été calculé un score d'irritation journalier individuel (SijI) qui est la somme des notes obtenues pour les signes observés.

Pour le panel et à chaque temps d'observation, a été calculé un score d'irritation journalier moyen (SijM) qui correspond à la formule :

$$SijM = \sum (SijI) / \text{Nombre de volontaires pris en compte}$$

Le score d'irritation moyen maximal (SimMax), défini comme étant le score d'irritation moyen le plus élevé parmi les scores moyens obtenus aux différents temps expérimentaux, a été noté.

- *Interprétation des résultats*

L'investigateur a conclu en terme de **très bonne, bonne, moyenne ou mauvaise** compatibilité cutanée de façon absolue. L'interprétation des résultats de l'examen cutané et de l'interrogatoire a été conforme à la procédure du laboratoire référencée pour ce type d'étude.

7- Résultats et discussions

Les données individuelles de l'examen cutané et de l'interrogatoire des volontaires sont jointes en **Annexes 3.**

En résumé :

Temps de contrôle après dépatchage	Nombre de volontaires réactifs	Types de réaction	Score d'irritation journalier moyen SIJM	% de volontaires réactifs
T 15	3	E0.5	0.15	30%

8- Conclusion

Dans les conditions expérimentales adoptées, le produit **« Laminaria saccharina dy lot 4.07.199 » a une compatibilité cutanée moyenne.**

Signatures et dates :

Docteur Thomas Jouary (Dermatologue)
Investigateur

Nadège Durand
Assistante Clinique

ANNEXES

Annexe 1

CARACTERISTIQUES TYPOLOGIQUES DES VOLONTAIRES

Volontaires		Age (ans)	Sexe F=féminin	Phototype *	Peau saine au niveau du dos
Réf.	Nom prénom				
1	GUED / R	24	F	II	x
2	LECOI / L	40	F	III	x
3	EMER / B	45	F	III	x
4	DA SI/ S	30	F	II	x
5	DRUI / S	35	F	II	x
6	FAVR / M	57	F	III	x
7	RATI / N	23	F	III	x
8	GIAC / C	27	F	III	x
9	RABO / S	22	F	II	x
10	COUP / J	28	F	III	x

Légendes : / = non x = oui

***phototype selon Fitzpatrick,** établi sur le principe d'une première exposition de 30 à 40 minutes au soleil après l'hiver ou une période sans exposition d'une durée équivalente :*

TYPE	CHEVEUX	PEAU	EPHELIDES	COUPS DE SOLEIL
I	roux	laiteuse	+++	constant bronzage nul
II	blonds	claire	++	fréquent bronzage léger
II	blonds châtains	claire	+	inconstant bronzage léger à mat
IV	bruns	mate	o	nul bronzage mat foncé
V	noirs et crépus	noire	o	o

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Annexe 2-1

CONTROLE DE L'OBSERVANCE
Contraintes

Contraintes (10 résultats exploitables)	Nombre de volontaires ayant respecté les contraintes	Pourcentage de volontaires ayant respecté les contraintes
Pas d'application de produits (autres que celui testé) sur la zone expérimentale Déviation : aucune	10	100 %
Pas de port de vêtements trop serrés ou responsables d'une contention au niveau de la zone expérimentale, susceptibles d'occasionner des frottements et le décollement du pansement Déviation : aucune	10	100 %
Pas de bain (en baignoire ou en piscine ou en mer) et pas de hammam ou de sauna pendant l'étude Déviation : aucune	10	100 %
En cas de douche, protection de la zone expérimentale ou pas de projection violente d'eau et pas de savonnage sur cette zone pour éviter le décollement du pansement ou l'apparition de phénomènes intercurrents et essuyage très délicat si nécessaire Déviation : aucune	10	100 %
Pas de sudation excessive et de sport intensif, susceptibles d'entraîner le décollement du pansement Déviation : aucune	10	100 %

Annexe 2-2

CONTROLE DE L'OBSERVANCE
Contraintes

Contraintes (10 résultats exploitables)	Nombre de volontaires ayant respecté les contraintes	Pourcentage de volontaires ayant respecté les contraintes
Pas d'exposition solaire intensive (au soleil naturel ou en cabine UVA) pendant la durée de l'étude, surtout lorsque le pansement était enlevé Déviation : aucune	10	100 %
Pas de traitement anti-allergique, anti-inflammatoire (corticothérapie systémique ou topique...) ou par des spécialités à base de vitamine A acide ou de ses dérivés pendant l'étude – pas de médication pouvant interférer avec l'étude Déviation : aucune	10	100 %
Pas de vaccination pendant l'étude Déviation : aucune	10	100 %

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VERIFICATION DE LA COMPATIBILITE CUTANEE

Produit N°1: **Laminaria saccharina dy lot 4.07.199** (Finn chambers)

Volontaires		T 15	
Réf.	Nom prénom	Examen cutané	SIJI
1	GUED / R	/	/
2	LECOI / L	E0.5	0.5
3	EMER / B	/	/
4	DA SI/ S	/	/
5	DRUI / S	/	/
6	FAVR / M	E0.5	0.5
7	RATI / N	/	/
8	GIAC / C	/	/
9	RABO / S	E0.5	0.5
10	COUP / J	/	/
SIJM		0.15	

Légendes : / = aucun signe clinique

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Annexe 3-2

VERIFICATION DE LA COMPATIBILITE CUTANEE

Produit N°2: Temoin (Finn chambers)

Volontaires		T 15	
Réf.	Nom prénom	Examen cutané	SIJI
1	GUED / R	/	/
2	LECOI / L	/	/
3	EMER / B	/	/
4	DA SI/ S	/	/
5	DRUI / S	/	/
6	FAVR / M	/	/
7	RATI / N	/	/
8	GIAC / C	/	/
9	RABO / S	/	/
10	COUP / J	/	/
SIJM		0	

Légendes : / = aucun signe clinique

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Dominique SABOUREAU
Docteur en Pharmacie
Expert toxicologue (Liste EUROTOX)

SECMA BIOTECHNOLOGIE MARINES
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ATTESTATION

Je soussigné, Dominique SABOUREAU, Docteur en Pharmacie, Expert Toxicologue *(Liste EUROTOX)* estime que sur la base des données du rapport d'étude *« Vérifier chez l'homme la compatibilité cutanée après application unique sous pansement – Patch test 24 heures »* référence *EC013 du 11/04/2005*, la substance **LAMINARIA SACCHARINA DJ LOT 4.07.199** appliquée sous pansement occlusif 24 heures présente une tolérance cutanée tout à fait acceptable. En effet dans les conditions expérimentales retenues à savoir maximalisées, la substance **LAMINARIA SACCHARINA DJ LOT 4.07.199** a induit un érythème léger chez 6 sujets sur 20, ce qui classe le produit comme présentant une compatibilité cutanée moyenne. Cependant, à la lecture 24 heures après enlèvement des pansements occlusifs, aucune réaction cutanée n'était notée, la réversibilité des érythèmes induits dans des conditions maximalisées étant très bonne.

De plus dans les conditions normales d'emploi préconisée par la société SECMA BIOTECHNOLOGIE, cette matière première à usage exclusif cosmétique entrant dans des préparations cosmétiques à la concentration maximale de 10% ne doit donc pas présenter de risque d'intolérance locale

Fait à Cestas le 24 juin 2005

86 route d'Arcachon - 33610 CESTAS - Tél 06.10.82.14.69 - Fax 05.56.34.75.54
Siret 397 69393800029 - APE 741G

SafePharm Laboratories

LAMINARIA SACCHARINA DJ Lot no. 407.199:

REVERSE MUTATION ASSAY "AMES TEST" USING *SALMONELLA TYPHIMURIUM*

SPL PROJECT NUMBER: 1764/008

AUTHOR: P W Thompson

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QUALITY ASSURANCE REPORT

This study type is classed as short-term. The standard test method for this study type ("General Study Plan" in OECD terminology) was reviewed for compliance once only on initial production. Inspection of the routine and repetitive procedures that constitute the study is carried out as a continuous process designed to encompass the major phases at or about the time this study was in progress.

This report has been audited by Safepharm Quality Assurance Unit, and is considered to be an accurate account of the data generated and of the procedures followed.

In each case, the outcome of QA evaluation is reported to the Study Director and Management on the day of evaluation. Audits of study documentation, and process inspections appropriate to the type and schedule of this study were as follows:

	12 July 2004	Standard Test Method Compliance Audit
	21 June 2005	Test Material Preparation
	23 June 2005	Test System Preparation
	14 June 2005	Exposure
	30 June 2005	Assessment of Response
§	08 July 2005	Draft Report Audit
§	Date of QA Signature	Final Report Audit

§ Evaluation specific to this study

..... DATE: 28 JUL 2005

For Safepharm Quality Assurance Unit*

*Authorised QA Signatures:

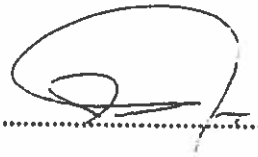
Head of Department:	JR Pateman CBiol MIBiol DipRQA AIQA FRQA
Deputy Head of Department:	JM Crowther MIScT MRQA
Senior Audit Staff:	JV Johnson BSc MRQA; G Wren ONC MRQA

GLP COMPLIANCE STATEMENT

The work described was performed in compliance with UK GLP standards (Schedule 1, Good Laboratory Practice Regulations 1999 (SI 1999/3106 as amended by SI 2004/0994)). These Regulations are in accordance with GLP standards published as OECD Principles on Good Laboratory Practice (revised 1997, ENV/MC/CHEM(98)17); and are in accordance with, and implement, the requirements of Directives 2004/9/EC and 2004/10/EC.

These international standards are acceptable to the Regulatory agencies of the following countries: Australia, Austria, Belgium, Canada, the Czech Republic, Denmark, Finland, France, Germany, Greece, Hungary, Iceland, Ireland, Israel, Italy, Japan, Republic of Korea, Luxembourg, Mexico, The Netherlands, New Zealand, Norway, Poland, Portugal, Slovenia, South Africa, Spain, Sweden, Switzerland, Turkey, the United Kingdom, and the United States of America.

This report fully and accurately reflects the procedures used and data generated.



..... DATE: 28 JUL 2005

P W Thompson HNC
Study Director

CONTENTS

LAMINARIA SACCHARINA DJ Lot no. 407.199:

REVERSE MUTATION ASSAY "AMES TEST" USING *SALMONELLA TYPHIMURIUM*

SUMMARY

Introduction. The method was designed to meet the requirements of the OECD Guidelines for Testing of Chemicals No. 471 "Bacterial Reverse Mutation Test", Method B13/14 of Commission Directive 2000/32/EC and the USA, EPA (TSCA) OPPTS harmonised guidelines.

Methods. *Salmonella typhimurium* strains TA1535, TA1537, TA102, TA98 and TA100 were treated with the test material using the Ames plate incorporation method at five dose levels, in triplicate, both with and without the addition of a rat liver homogenate metabolising system (10% liver S9 in standard co-factors). The dose range was determined in a preliminary toxicity assay and was 50 to 5000 µg/plate in the first experiment. The experiment was repeated on a separate day using the same dose range as Experiment 1, fresh cultures of the bacterial strains and fresh test material formulations.

Results. The vehicle (sterile distilled water) control plates gave counts of revertant colonies within the normal range. All of the positive control chemicals used in the test induced marked increases in the frequency of revertant colonies, both with or without metabolic activation. Thus, the sensitivity of the assay and the efficacy of the S9-mix were validated.

The test material caused no visible reduction in the growth of the bacterial background lawn at any dose level. The test material was, therefore, tested up to the maximum recommended dose level of 5000 µg/plate. No test material precipitate was observed on the plates at any of the doses tested in either the presence or absence of S9-mix.

No significant increases in the frequency of revertant colonies were recorded for any of the bacterial strains, with any dose of the test material, either with or without metabolic activation.

Conclusion. The test material was considered to be non-mutagenic under the conditions of this test.

LAMINARIA SACCHARINA DJ Lot no. 407.199:

REVERSE MUTATION ASSAY "AMES TEST" USING *SALMONELLA TYPHIMURIUM*

1. INTRODUCTION

This study was designed to assess the mutagenic potential of the test material using a bacterial/microsome test system. The study was based on the in vitro technique described by Ames and his co-workers (1, 2, 3) and Garner *et al* (4) in which mutagenic activity is assessed by exposing histidine auxotrophs of *Salmonella typhimurium* to various concentrations of the test material. The method used conforms with the OECD Guidelines for the Testing of Chemicals, No. 471, Method B13/14 in EC Commission Directive 2000/32/EC and the USA, EPA (TSCA) OPPTS harmonised guidelines. A copy of the Certificate of Compliance with GLP, issued by the UK Department of Health, is included.

The mutant strains of *Salmonella* are incapable of synthesising histidine and are, therefore, dependent on an external source of this particular amino acid for normal growth. When exposed to a mutagenic agent these bacteria may undergo a reverse mutation to histidine independent forms which are detected by their ability to grow on a histidine deficient medium. Using various strains of this organism, revertants produced after exposure to a chemical mutagen may arise as a result of base-pair substitution in the genetic material (miscoding) or frame-shift mutation in which genetic material is either added or deleted. In order to make the bacteria more sensitive to mutation by chemical and physical agents, several additional traits have been introduced. These include a deletion through the excision repair gene (uvrB: except TA102), which renders the organism incapable of DNA excision repair, and deep rough mutation (rfa), which increases the permeability of the cell wall. Since many compounds do not exert a mutagenic effect until they have been metabolised by enzyme systems not available in the bacterial cell, the test material and the bacteria are also incubated in the presence of a liver microsomal preparation (S9) prepared from rats pre-treated with a mixture known to induce an elevated level of these enzymes.

The experimental phase of this study was performed between 28 April 2005 and 16 June 2005.

2. TEST MATERIAL

Sponsor's identification	:	LAMINARIA SACCHARINA DJ Lot no. 407.199
Description	:	Pale yellow liquid
Batch number	:	4 07 199
Date received	:	06 April 2005
Storage conditions	:	Room temperature in the dark

The integrity of supplied data relating to the identity, purity and stability of the test material is the responsibility of the Sponsor.

3. METHODS

3.1 Tester Strains

The strains used in this assay were all mutants derived from *Salmonella typhimurium* LT2 and were those recommended for general screening.

TA100
TA1535 sensitive to agents inducing base-pair substitution
TA102

TA1537
TA98 sensitive to agents inducing frame-shift mutations

The strains were obtained from the University of California at Berkeley on culture discs on 4 August 1995 and were stored at -196°C in a Statebourne liquid nitrogen freezer, model SXR 34. Prior to the master strains being used, characterisation checks were carried out to determine the amino-acid requirement, presence of rfa, R factors, uvrB mutation and the spontaneous reversion rate.

In this assay, overnight sub-cultures of the appropriate coded stock cultures were prepared in nutrient broth (Oxoid Limited; lot number 350536 06/09) and incubated at 37°C for approximately 10 hours. Each culture was monitored spectrophotometrically for turbidity with titres determined by viable count analysis on nutrient agar plates.

3.2 Preparation of Test and Control Materials

The test material was fully miscible in sterile distilled water at 50 mg/ml in solubility checks performed in-house. Sterile distilled water was therefore selected as the vehicle of choice.

The test material was accurately weighed and approximate half-log dilutions prepared in sterile distilled water by mixing on a vortex mixer on the day of each experiment. Experiment 1 formulations were filter sterilised using a 0.2 µm filter. Analysis for concentration, homogeneity and stability of the test material formulations is not a requirement of the test guidelines and was, therefore, not determined.

Vehicle and positive controls were used in parallel with the test material. A solvent treatment group was used as the vehicle control and the positive control materials were as follows:

N-ethyl-N'-nitro-N-nitrosoguanidine (ENNG):	3 µg/plate for TA100 and 5 µg/plate for TA1535
9-Aminoacridine (9AA):	80 µg/plate for TA1537
Mitomycin C (MMC):	0.5 µg/plate for TA102
4-Nitroquinoline-1-oxide (4NQO):	0.2 µg/plate for TA98

In addition, 2-Aminoanthracene (2AA), Benzo(a)pyrene (BP) and 1,8-Dihydroxyanthraquinone (DAN), which are non-mutagenic in the absence of metabolising enzymes, were used in the S9 series of plates at the following concentrations:

2AA:	1 µg/plate for TA100
2AA:	2 µg/plate for TA1535 and TA1537
BP:	5 µg/plate for TA98
DAN:	10 µg/plate for TA102

3.3 Microsomal Enzyme Fraction

S9 was prepared in-house on 12 April 2002 (preliminary toxicity test only) and 13 February 2005 from the livers of male Sprague-Dawley rats weighing ~ 250g. These had each orally received three consecutive daily doses of phenobarbitone/β-naphthoflavone (80/100 mg per kg per day) prior to S9 preparation. Before use, each batch of S9 was assayed for its ability to metabolise appropriate indirect mutagens used in the Ames Test. The S9 was stored at -196°C.

3.4 S9-Mix and Agar

The S9-mix was prepared immediately before use using sterilised co-factors and maintained on ice for the duration of the test.

S9	5.0 ml
1.65 M KCl/0.4 M $MgCl_2$	1.0 ml
0.1 M Glucose-6-phosphate	2.5 ml

0.1 M NADPH	2.0 ml
0.1 M NADH	2.0 ml
0.2 M Sodium phosphate buffer (pH 7.4)	25.0 ml
Sterile distilled water	12.5 ml

A 0.5 ml aliquot of S9-mix and 2 ml of molten, trace histidine supplemented, top agar was overlaid onto a sterile Vogel-Bonner Minimal agar plate in order to assess the sterility of the S9-mix. This procedure was repeated, in triplicate, on the day of each experiment.

Top agar was prepared using 0.6% Difco Bacto agar (lot number 4348296 09/09) and 0.5% sodium chloride with 5 ml of 1.0 mM histidine and 1.0 mM biotin solution added to each 100 ml of top agar. Vogel-Bonner Minimal agar plates were purchased from ILS Limited (lot numbers 892686-02 11/09 and 899855-02 01/10).

3.5 Test Procedure

3.5.1 Preliminary Toxicity Test

In order to select appropriate dose levels for use in the main test, a preliminary test was carried out to determine the toxicity of the test material. The concentrations tested were 0, 0.15, 0.5, 1.5, 5, 15, 50, 150, 500, 1500 and 5000 µg/plate. The test was performed by mixing 0.1 ml of bacterial culture (TA100), 2 ml of molten, trace histidine supplemented, top agar, 0.1 ml of test material formulation, 0.5 ml of S9-mix or phosphate buffer and overlaying onto sterile plates of Vogel- Bonner Minimal agar (30 ml/plate). Ten doses of the test material and a vehicle control (sterile distilled water) were tested. In addition, 0.1 ml of the maximum concentration of the test material and 2 ml of molten, trace histidine supplemented, top agar was overlaid onto a sterile Nutrient agar plate in order to assess the sterility of the test material. After approximately 48 hours incubation at 37°C the plates were assessed for numbers of revertant colonies using a Domino colony counter and examined for effects on the growth of the bacterial background lawn.

3.5.2 Mutation Test - Experiment 1

Five concentrations of the test material (50, 150, 500, 1500 and 5000 µg/plate) were assayed in triplicate against each tester strain, using the direct plate incorporation method.

Measured aliquots (0.1 ml) of one of the bacterial cultures were dispensed into sets of test tubes followed by 2.0 ml of molten, trace histidine supplemented, top agar, 0.1 ml of the test material formulation, vehicle or positive control and either 0.5 ml of S9-mix or phosphate buffer. The contents of each test tube were mixed and equally distributed onto the surface of Vogel-Bonner

Minimal agar plates (one tube per plate). This procedure was repeated, in triplicate, for each bacterial strain and for each concentration of test material both with and without S9-mix.

All of the plates were incubated at 37°C for approximately 48 hours and the frequency of revertant colonies assessed using a Domino colony counter.

3.5.3 Mutation Test - Experiment 2

The second experiment was performed using methodology as described for Experiment 1, using fresh bacterial cultures, test material and control solutions. The test material dose range was the same as Experiment 1 (50 to 5000 µg/plate).

3.6 Acceptance Criteria

The reverse mutation assay may be considered valid if the following criteria are met:

All tester strain cultures exhibit a characteristic number of spontaneous revertants per plate in the vehicle and untreated controls. Acceptable ranges are presented in the standard test method section 3 with historical control ranges for 2003 and 2004 presented in Appendix 1.

The appropriate characteristics for each tester strain have been confirmed, eg rfa cell-wall mutation and pKM101 plasmid R-factor etc.

All tester strain cultures should be in the approximate range of 1 to 9.9 x 10^9 bacteria per ml.

Each mean positive control value should be at least two times the respective vehicle control value for each strain, thus demonstrating both the intrinsic sensitivity of the tester strains to mutagenic exposure and the integrity of the S9-mix. The historical control ranges for 2003 and 2004 are presented in Appendix 1.

There should be a minimum of four non-toxic test material dose levels.

There should be no evidence of excessive contamination.

3.7 Evaluation Criteria

There are several criteria for determining a positive result, such as a dose-related increase in revertant frequency over the dose range tested and/or a reproducible increase at one or more concentrations in at least one bacterial strain with or without metabolic activation. Biological relevance of the results will be considered first, statistical methods, as recommended by the

UKEMS (5) can also be used as an aid to evaluation, however, statistical significance will not be the only determining factor for a positive response.

A test material will be considered non-mutagenic (negative) in the test system if the above criteria are not met.

Although most experiments will give clear positive or negative results, in some instances the data generated will prohibit a definitive judgement about the test material activity. Results of this type will be reported as equivocal.

4. ARCHIVES

Unless instructed otherwise by the Sponsor, all original data and the final report will be retained in the Safepharm archives for five years, after which instructions will be sought as to further retention or disposal.

5. RESULTS

5.1 Preliminary Toxicity Test

The test material was non-toxic to the strain of *Salmonella* used (TA100). The test material formulation and the S9-mix used in this experiment were both shown to be sterile.

The number of revertant colonies for the toxicity assay were:

With (+) or without (-) Metabolic Activation	Strain	Dose (µg/plate)										
		0	0.15	0.5	1.5	5	15	50	150	500	1500	5000
-	TA100	121	106	128	119	117	122	121	150	150	107	118
+	TA100	163	114	122	128	112	126	117	119	110	101	100

5.2 Mutation Test

Prior to use, the master strains were checked for characteristics, viability and spontaneous reversion rate (all were found to be satisfactory). These data are not given in the report. The S9-mix used in both experiments was shown to be sterile.

Results for the negative controls (spontaneous mutation rates) are presented in Table 1 and were considered to be acceptable. These data are for concurrent untreated control plates performed on the same day as the Mutation Test.

The individual plate counts, the mean number of revertant colonies and the standard deviations, for the test material, positive and vehicle controls, both with and without metabolic activation, are presented in Table 2 to Table 5.

A history profile of vehicle and positive control values is presented in Appendix 1.

The test material caused no visible reduction in the growth of the bacterial background lawn at any dose level. The test material was, therefore, tested up to the maximum recommended dose level of 5000 µg/plate. No test material precipitate was observed on the plates at any of the doses tested in either the presence or absence of S9-mix.

No significant increases in the frequency of revertant colonies were recorded for any of the strains of *Salmonella*, at any dose level either with or without metabolic activation.

All of the positive control chemicals used in the test induced marked increases in the frequency of revertant colonies thus confirming the activity of the S9-mix and the sensitivity of the bacterial strains.

6. CONCLUSION

The test material was considered to be non-mutagenic under the conditions of this test.

7. REFERENCES

1. Ames B N, Durston W E, Yamasaki E and Lee F D (1973b) *Proc. Natl. Acad. Sci. (USA)*, **70**, 2281-2285.

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5. Kirkland D J (Ed) (1989) Statistical Evaluation of Mutagenicity Test Data. *UKEMS Sub-committee on Guidelines for Mutagenicity Testing*, **Report - Part III**, Cambridge University Press.

LAMINARIA SACCHARINA DJ Lot no. 407.199: REVERSE MUTATION ASSAY "AMES TEST" USING *SALMONELLA TYPHIMURIUM*

Table 1 Spontaneous Mutation Rates (Concurrent Negative Controls)

EXPERIMENT 1

Number of revertants (mean number of colonies per plate)									
Base-pair substitution type						Frameshift type			
TA100		TA1535		TA102		TA98		TA1537	
117		22		262		19		15	
111	(124)	20	(22)	242	(254)	19	(20)	8	(10)
143		25		259		22		7	

EXPERIMENT 2

Number of revertants (mean number of colonies per plate)									
Base-pair substitution type						Frameshift type			
TA100		TA1535		TA102		TA98		TA1537	
125		21		258		23		9	
91	(110)	18	(19)	262	(259)	25	(23)	13	(11)
115		18		257		20		11	

LAMINARIA SACCHARINA DJ Lot no. 407.199: REVERSE MUTATION ASSAY "AMES TEST" USING *SALMONELLA TYPHIMURIUM*

Table 2 Test Results: Experiment 1 – Without Metabolic Activation

Test Period		From: 31 May 2005						To: 03 June 2005					
With or without S9-Mix	Test substance concentration (µg/plate)	Number of revertants (mean number of colonies per plate)											
		Base-pair substitution type						Frameshift type					
		TA100		TA1535		TA102		TA98		TA1537			
-	0	121 117 119	(119) 2.0#	20 21 15	(19) 3.2	338 241 341	(307) 56.9	21 15 17	(18) 3.1	10 5 13	(9) 4.0		
-	50	114 113 105	(111) 4.9	17 18 17	(17) 0.6	293 325 373	(330) 40.3	19 23 18	(20) 2.6	13 11 14	(13) 1.5		
-	150	112 96 120	(109) 12.2	15 21 26	(21) 5.5	316 307 369	(331) 33.5	23 16 27	(22) 5.6	8 8 8	(8) 0.0		
-	500	101 99 96	(99) 2.5	21 25 27	(24) 3.1	311 310 355	(325) 25.7	32 16 20	(23) 8.3	9 9 11	(10) 1.2		
-	1500	110 97 114	(107) 8.9	21 29 21	(24) 4.6	285 326 351	(321) 33.3	20 28 23	(24) 4.0	10 8 13	(10) 2.5		
-	5000	94 115 111	(107) 11.2	27 21 21	(23) 3.5	317 294 365	(325) 36.2	20 27 26	(24) 3.8	3 11 8	(7) 4.0		
Positive controls	Name	ENNG		ENNG		MMC		4NQO		9AA			
	Concentration (µg/plate)	3		5		0.5		0.2		80			
S9-Mix -	No. colonies per plate	365 356 541	(421) 104.3	235 157 213	(202) 40.2	1299 1334 1001	(1211) 183.0	125 111 119	(118) 7.0	382 340 325	(349) 29.5		

ENNG N-ethyl-N'-nitro-N-nitrosoguanidine
4NQO 4-Nitroquinoline-1-oxide
9AA 9-Aminoacridine
MMC Mitomycin C
Standard deviation

LAMINARIA SACCHARINA DJ Lot no. 407.199: REVERSE MUTATION ASSAY "AMES TEST" USING *SALMONELLA TYPHIMURIUM*

Table 3 Test Results: Experiment 1 – With Metabolic Activation

Test Period		From: 31 May 2005						To: 03 June 2005					
With or without S9-Mix	Test substance concentration (µg/plate)	Number of revertants (mean number of colonies per plate)											
		Base-pair substitution type								Frameshift type			
		TA100		TA1535		TA102		TA98		TA1537			
+	0	112 114 118	(115) 3.1#	11 9 10	(10) 1.0	331 354 321	(335) 16.9	24 30 28	(27) 3.1	14 9 7	(10) 3.6		
+	50	78 74 104	(85) 16.3	9 10 8	(9) 1.0	323 369 371	(354) 27.2	33 16 19	(23) 9.1	6 11 10	(9) 2.6		
+	150	79 63 87	(76) 12.2	13 9 9	(10) 2.3	329 351 317	(332) 17.2	39 27 33	(33) 6.0	8 10 7	(8) 1.5		
+	500	73 110 107	(97) 20.6	9 10 18	(12) 4.9	325 376 308	(336) 35.4	32 41 23	(32) 9.0	5 5 4	(5) 0.6		
+	1500	90 88 108	(95) 11.0	13 9 9	(10) 2.3	319 368 385	(357) 34.3	29 23 22	(25) 3.8	6 8 10	(8) 2.0		
+	5000	81 88 105	(91) 12.3	11 8 9	(9) 1.5	349 329 335	(338) 10.3	37 32 24	(31) 6.6	13 10 10	(11) 1.7		
Positive controls	Name	2AA		2AA		DAN		BP		2AA			
	Concentration (µg/plate)	1		2		10		5		2			
S9-Mix +	No. colonies per plate	333 612 621	(522) 163.7	213 217 256	(229) 23.8	636 632 981	(750) 200.4	255 301 348	(301) 46.5	243 261 247	(250) 9.5		

2AA 2-Aminoanthracene
BP Benzo(a)pyrene
DAN 1,8-Dihydroxyanthraquinone
Standard deviation

LAMINARIA SACCHARINA DJ Lot no. 407.199: REVERSE MUTATION ASSAY "AMES TEST" USING *SALMONELLA TYPHIMURIUM*

Table 4 Test Results: Experiment 2 – Without Metabolic Activation

Test Period		From: 13 June 2005						To: 16 June 2005					
With or without S9-Mix	Test substance concentration (µg/plate)	Number of revertants (mean number of colonies per plate)											
		Base-pair substitution type									Frameshift type		
		TA100		TA1535		TA102				TA98		TA1537	
-	0	105 122 123	(117) 10.1#	20 22 21	(21) 1.0	322 300 344	(322) 22.0		15 31 23	(23) 8.0	11 6 11	(9) 2.9	
-	50	120 91 101	(104) 14.7	22 20 20	(21) 1.2	346 310 330	(329) 18.0		18 27 19	(21) 4.9	6 9 13	(9) 3.5	
-	150	87 104 117	(103) 15.0	19 23 18	(20) 2.6	328 319 319	(322) 5.2		19 21 26	(22) 3.6	10 9 9	(9) 0.6	
-	500	96 105 112	(104) 8.0	21 20 23	(21) 1.5	331 332 314	(326) 10.1		20 24 25	(23) 2.6	9 6 10	(8) 2.1	
-	1500	105 114 109	(109) 4.5	22 18 26	(22) 4.0	303 306 324	(311) 11.4		12 19 13	(15) 3.8	12 7 6	(8) 3.2	
-	5000	115 102 113	(110) 7.0	18 23 28	(23) 5.0	323 306 323	(317) 9.8		21 20 32	(24) 6.7	7 7 7	(7) 0.0	
Positive controls	Name	ENNG		ENNG		MMC			4NQO		9AA		
	Concentration (µg/plate)	3		5		0.5			0.2		80		
S9-Mix	No. colonies per plate	317 392 388	(366) 42.2	262 281 289	(277) 13.9	1273 1373 1295	(1314) 52.5		130 114 108	(117) 11.4	602 884 610	(699) 160.6	
-													

ENNG N-ethyl-N'-nitro-N-nitrosoguanidine
4NQO 4-Nitroquinoline-1-oxide
9AA 9-Aminoacridine
MMC Mitomycin C
Standard deviation

LAMINARIA SACCHARINA DJ Lot no. 407.199: REVERSE MUTATION ASSAY "AMES TEST" USING *SALMONELLA TYPHIMURIUM*

Table 5 Test Results: Experiment 2 – With Metabolic Activation

Test Period		From: 13 June 2005						To: 16 June 2005			
With or without S9-Mix	Test substance concentration (µg/plate)	Number of revertants (mean number of colonies per plate)									
		Base-pair substitution type						Frameshift type			
		TA100		TA1535		TA102		TA98		TA1537	
+	0	90 91 97	(93) 3.8#	11 11 11	(11) 0.0	377 347 376	(367) 17.0	31 20 34	(28) 7.4	10 17 10	(12) 4.0
+	50	88 77 77	(81) 6.4	11 11 8	(10) 1.7	364 338 377	(360) 19.9	21 20 25	(22) 2.6	13 8 10	(10) 2.5
+	150	80 78 89	(82) 5.9	12 10 10	(11) 1.2	379 346 366	(364) 16.6	31 23 23	(26) 4.6	12 13 12	(12) 0.6
+	500	95 91 92	(93) 2.1	13 15 7	(12) 4.2	374 327 369	(357) 25.8	27 32 26	(28) 3.2	12 16 10	(13) 3.1
+	1500	96 98 96	(97) 1.2	9 15 9	(11) 3.5	356 367 357	(360) 6.1	22 26 30	(26) 4.0	13 11 15	(13) 2.0
+	5000	95 90 82	(89) 6.6	11 11 12	(11) 0.6	331 338 344	(338) 6.5	32 29 24	(28) 4.0	15 9 12	(12) 3.0
Positive controls	Name	2AA		2AA		DAN		BP		2AA	
	Concentration (µg/plate)	1		2		10		5		2	
S9-Mix +	No. colonies per plate	765 1012 935	(904) 126.4	185 161 180	(175) 12.7	957 744 902	(868) 110.6	275 294 220	(263) 38.4	249 323 277	(283) 37.4

2AA 2-Aminoanthracene
BP Benzo(a)pyrene
DAN 1,8-Dihydroxyanthraquinone
Standard deviation

LAMINARIA SACCHARINA DJ Lot no. 407.199: REVERSE MUTATION ASSAY "AMES TEST" USING *SALMONELLA TYPHIMURIUM*

Appendix 1 History Profile of Vehicle and Positive Control Values

COMBINED VEHICLE AND UNTREATED CONTROL VALUES 2003

Strain	TA100		TA1535		WP2uvrA-		TA102		TA98		TA1537	
S9-Mix	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9
Mean	98	106	20	15	23	28	331	365	20	30	10	14
SD	20.7	23.4	8.5	3.9	5.6	7	38.2	29	5.1	7.1	3.9	5
Min	61	64	8	7	11	13	233	264	10	15	3	3
Max	165	191	45	39	44	57	400	422	52	53	26	32
Values	882	735	838	690	686	539	273	170	877	721	838	678

POSITIVE CONTROL VALUES 2003

Strain	TA100		TA1535		WP2uvrA-		TA102		TA98		TA1537	
S9-Mix	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9
Mean	429	1529	297	305	649	656	1068	911	174	249	1273	370
SD	108.1	493.1	156.8	82.6	175.7	264.4	230.0	183.8	64.6	72.2	623.1	109.9
Min	197	485	98	162	273	136	698	559	64	87	262	148
Max	849	3662	1099	625	1198	1373	1886	1887	410	465	3704	759
Values	160	160	156	156	150	150	102	100	162	162	155	155

COMBINED VEHICLE AND UNTREATED CONTROL VALUES 2004

Strain	TA100		TA1535		WP2uvrA-		TA102		TA98		TA1537		WP2uvrA- pKM101	
S9-Mix	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9
Mean	94	98	24	14	23	27	333	367	21	31	12	18	147	183
SD	16.5	14.8	7.0	4.8	5.3	6.7	34.9	24.5	5.1	6.5	3.4	4.0	28.5	42.9
Min	61	60	9	7	9	11	192	263	10	11	5	6	92	131
Max	163	157	40	39	43	52	397	406	49	57	26	35	190	247
Values	993	798	911	713	735	579	291	183	917	740	889	708	15	12

POSITIVE CONTROL VALUES 2004

Strain	TA100		TA1535		WP2uvrA-		TA102		TA98		TA1537		WP2uvrA- pKM101	
S9-Mix	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9
Mean	557	1124	440	265	923	482	1250	893	175	219	999	366	2386	1954
SD	163.5	417.4	275.3	80.5	240.1	202.5	239.3	124.8	52.5	68.9	511.6	130.1	867.9	188.6
Min	189	348	93	162	371	153	728	660	95	114	130	137	1120	1737
Max	1215	2942	1634	1026	1714	1087	1960	1288	435	495	2964	832	3009	2075
Values	180	178	176	175	160	159	103	103	179	178	176	175	4	3

SD = Standard deviation
Min = Minimum value
Max = Maximum value

Appendix 2 Statement of GLP Compliance in Accordance with Directive 88/320/EEC

THE DEPARTMENT OF HEALTH OF THE GOVERNMENT OF THE UNITED KINGDOM

GOOD LABORATORY PRACTICE

STATEMENT OF COMPLIANCE
IN ACCORDANCE WITH DIRECTIVE 88/320 EEC

LABORATORY
SafePharm Limited
Shardlow Business Park,
London Road,
Shardlow,
Derbyshire,
DE72 2GD

TEST TYPE
Analytical/Clinical
Chemistry
Environmental tox.
Environmental fate
Mutagenicity
Phys./Chem. tests
Toxicology

DATE OF INSPECTION

2nd December 2002

A general inspection for compliance with the Principles of Good Laboratory Practice was carried out at the above laboratory as part of UK GLP Compliance Programme.

At the time of the inspection no deviations were found of sufficient magnitude to affect the validity of non-clinical studies performed at these facilities.

13/2/03

Dr. Roger G. Alexander
Head, UK GLP Monitoring Authority

Personal Care Products Council
Committed to Safety, Quality & Innovation

Memorandum

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

FROM: Carol Eisenmann, Ph.D.
Personal Care Products Council

DATE: September 11, 2018

SUBJECT: Laminaria Ochroleuca Extract

Biotech Marine. 2014. Manufacturing process Antileukin 6 (Laminaria Ochroleuca Extract in Caprylic/Capric Triglyceride).

Biotech Marine. 2016. Antileukine6™ (Laminaria Ochroleuca Extract in Caprylic/Capric Triglyceride) Physico-chemical data.

DECS Investigation Clinique. 1999. Study of acute skin tolerance in adult volunteers: 24-hour patch test: Antileukine 6 (Laminaria Ochroleuca Extract in Caprylic/Capric Triglyceride).

Palmer Research. 1999. Evaluation du potentiel allergisant après applications épicutanées répétées sur 52 volontaires: Antileukine 6 (Laminaria Ochroleuca Extract in Caprylic/Capric Triglyceride).

1620 L Street, N.W., Suite 1200 | Washington, D.C. 20036 | 202.331.1770 | 202.331.1969 (fax) | www.personalcarecouncil.org

MANUFACTURING PROCESS

ANTILEUKINE 6

HARVESTING / IDENTIFICATION (*Laminaria Ochroleuca*)

↓

WASHING

↓

GRINDING

↓

EXTRACTION WITH THE SOLVENT
CAPRYLIC/CAPRIC TRIGLYCERIDE

↓

FILTRATION

↓

QUALITY CONTROL

↓

PACKAGING

↓

QUALITY CONTROL

Production Manager
Jean-Marc CATROUX

Date de mise à jour / *Updated date* : 01/12/2016

ANTILEUKINE 6™

INCI NAME : Caprylic / Capric Triglyceride – Laminaria Ochroleuca Extract
CAS N°: 73398-61-5 – 92128-82-0
EINECS N° : 277-452-2– 295-780-4
Produit certifié Mass Balance (RSPO) BVC-RSPO-1-1972708497 / *Product Mass Balance certified (Rountable for Sustainable Palm Oil (RSPO)) BVC-RSPO-1-1972708497*

795% Caprylic / Capric Triglyceride
25 %
Laminaria Ochroleuca Ext.

DONNEES PHYSICOCHIMIQUES
PHYSICO-CHEMICAL DATA
Numéro de référence / *Reference number* : STANDARD

CARACTERISTIQUES *CHARACTERISTICS*	METHODES *METHODS*	STANDARD *STANDARD*
Conformité Mass Balance *Mass Balance conformity*	PO-HSE-004	**Conforme** *Conform*
Aspect *Aspect*	MO PHY 002	**Liquide limpide huileux** *Oily limpid liquid*
Couleur *Colour*	MO PHY 002	**Jaune très pâle à jaune-vert (1 à 5 UG)** *Very pale yellow to green-yellow (1 to5 UG)*
Odeur *Odour*	MO PHY 002	**Faible** *Slight*
Densité relative à 20°C *Density*	MO PHY 024	0,920 – 0,980
Indice de réfraction (20°C) *Refractive index*	MO PHY 008	$1,440_0 – 1,460_0$
Spectre UV *UV spectrum*	MO PHY 013	**Conforme au témoin** *Similar to the standard*
Spectre IR *IR spectrum*	MO PHY 011	**Conforme au témoin** *Similar to the standard*
Test IL6* (inhibition)	biological test on IL6 inhibition	≥ 20 %

* Teneurs garanties sous contrôle statistique / *Contents guaranteed under statistical control*

ANTILEUKINE 6™

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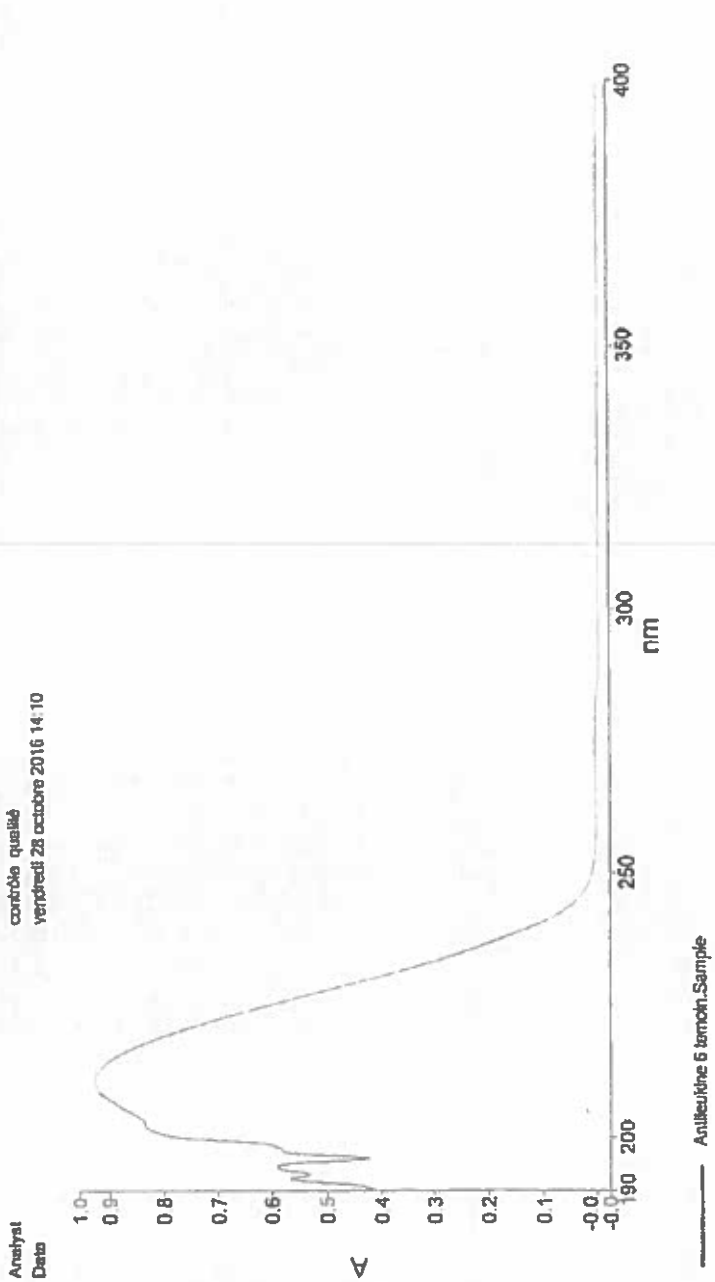
Produit certifié Mass Balance (RSPO) BVC-RSPO-1-1972708497 / *Product Mass Balance certified (Rountable for Sustainable Palm Oil (RSPO)) BVC-RSPO-1-1972708497*

DONNEES PHYSICOCHIMIQUES
PHYSICO-CHEMICAL DATA
Numéro de référence / *Reference number* : STANDARD

CARACTERISTIQUES *CHARACTERISTICS*	STANDARD *STANDARD*
Métaux lourds** *Heavy metals** (mg/kg)*	
-Arsenic *-Arsenic*	< 2
-Cadmium *-Cadmium*	< 3
-Plomb *-Lead*	< 5
-Nickel *-Nickel*	< 2
-Argent *-Silver*	< 5

Iodine < 1ppm

** Teneurs garanties sous contrôle statistique / *Contents guaranteed under statistical control*



Date: vendredi 28 octobre 2016

SPECTRE IRFT

SPECTROPHOTOMETRE SPECTRUM 100 N° de série 77774 - PERKIN ELMER

Accessoire ATR Universel N° 7031330

ANTILEUKINE 6™

INCI NAME : Caprylic / Capric Triglyceride – Laminaria Ochroleuca Extract
CAS N°: 73398-61-5 – 92128-82-0
EINECS N° : 277-452-2– 295-780-4
Produit certifié Mass Balance (RSPO) BVC-RSPO-1-1972708497 / *Product Mass Balance certified (Rountable for Sustainable Palm Oil (RSPO)) BVC-RSPO-1-1972708497*

DONNEES MICROBIOLOGIQUES
MICROBIOLOGICAL DATA
Numéro de référence / *Reference number* : STANDARD

CARACTERISTIQUES *CHARACTERISTICS*	METHODES *METHODS*	STANDARD *STANDARD*
Germes totaux*** *Total germs****	MO MIC 002 / NF EN ISO 21149	**< 100**
Germes Pathogènes *Pathogens*		
- *Staphylococcus aureus*	MO MIC 012 / NF EN ISO 22718	**Absence** *None*
- *Candida albicans*	MO MIC 010 / NF EN ISO 18416	**Absence** *None*
- *Pseudomonas aeruginosa*	MO MIC 011 / NF EN ISO 22717	**Absence** *None*
- *Escherichia coli*	MO MIC 025 / NF EN ISO 21150	**Absence** *None*
Levures / Moisissures*** *Yeasts / Moulds****	MO MIC 021 / NF EN ISO 16212	**< 100**

*** Les résultats sont indiqués en UFC/mL pour les liquides et en UFC/g pour les solides
*** *Results are indicated in CFU/mL for the liquids and in CFU/g for the solids*

CONFORME
CERTIFIED TRUE AND CORRECT
RESPONSABLE CONTROLE QUALITE: **P. SOUBIES**
QUALITY CONTROL MANAGER
01 DEC. 2016

CONFORME
CERTIFIED TRUE AND CORRECT
ASSURANCE QUALITE : **A. HAMON**
QUALITY ASSURANCE
01 DEC. 2016

Study of acute skin tolerance in adult volunteers: 24-hour patch-test

Version no. 99/001 dated 22/11/1999

Study: TCP24H/DE-99/1062

Product: ANTILEUKINE 6 REF 909 500

Sponsor: S.E.C.M.A. BIOTECHNOLOGIES MARINES
BP 65
22260 PONTRIEUX

Mérignac, November 1999

TABLE OF CONTENTS

STUDY REPORT SUMMARY

Company: S.E.C.MLA. BIOTECHNOLOGIES MARINES **Address:** BP 65 22260 PONTRIEUX		**Product:** ANTILEUKINE 6 REF 909 500 **DECS code:** DE-99/1062 **PALMER Research code:** PA.99/1244
STUDY OF ACUTE SKIN TOLERANCE IN ADULT VOLUNTEERS: 24-HOUR PATCH TEST		
Study date	The study was carried out between 16/11/99 and 17/11/99.	
Study location	DECS Investigation Clinique 1, rue du Golf Parc Innolin 33700 MERIGNAC	
Aim	To determine the primary irritation potential of a cosmetic product after a single 24-hour application in volunteers.	
Methodology	Open study.	Number of subjects: 11.
Inclusion criteria	Skin free from dermatological lesions, non-allergic subject.	• Application duration: 24 hours. • Conditions of use: 2% diluted in water.
Evaluation criteria	Calculation of primary dermal irritation index: $PDII = \frac{\text{total reaction score}}{\text{total number of volunteers}}$ Skin reactions are scored from 0 to 4.	
Analytical methods	Product classification according to PDII: If PDII < 0.20 Non Irritant If 0.20 ≤ PDII < 0.50 Slight irritant If 0.50 ≤ I.I.M < 1 Moderate irritant If PDII ≥ 1 Irritant	
Conclusion	The mean irritation index of the product DE-99/1062 is equal to 0.05. It is therefore classified as Non-irritant to human skin.	
Study Director: **Dr. Marie José Albin, Allergist**		

1 - INTRODUCTION

At the request of the company S.E.C.M.A., BP 65, 22260 PONTRIEUX-, we evaluated on 11 adult volunteers the acute skin tolerance of the product:

ANTILEUKINE 6 REF 909 500

after a single application to the skin of the front of one arm, under an occlusive dressing maintained in place for 24 hours (24-hour patch-test).

This test was conducted in an "open" manner, according to the patch-test method.

To conduct this study, we received a 60 mL sample of product that we referenced under DECS code DE-99/1062 and under PALMER Research code PA.99/1244.

The test was initiated on 16/11/1999 and ended on 17/11/1999.

2 - CERTIFICATE OF RESULT AUTHENTICITY

The study covered by this report was carried out under my responsibility, in compliance with the experimental protocol and in accordance with Good Laboratory Practice.
All the observations and numerical data collected during this trial are reported in this document.

After review, and in my position of Study Director, I hereby certify that these data accurately reflect the results obtained.
Dr. Marie José Albin, Allergist

After review, and in my position of Study Monitor, I hereby certify that these data accurately reflect the results obtained.
Dominique Saboureau, Doctor of Pharmacy.

In my position of Quality Assurer, I certify having read this report and being in agreement with its content,
Mélanie Pohe-Bollou

BiotechMarine
Z.I. BP72.
22260 Pontrieux (FR)
Tel: +33 (0) 2 96 95 31 32
Fax: +33 (0) 2 96 95 31 30
www.biotechmarine.com
contact@biotechmarine.com

04 JUIL. 2014

3 - EXPERIMENTAL PROTOCOL (ref. DECS TCP24H/DE-99/1062)

3.1 - Volunteers

3.1.1 - Included subject characteristics

- ✓ 11 subjects were included in the test,
- ✓ 7 were female and 4 were male,
- ✓ aged 19 to 48 years.

All of the subjects met the inclusion criteria and presented with none on the non-inclusion criteria.

3.1.2 - Inclusion criteria

- ✓ no history of intolerance or allergy to a cosmetic product,
- ✓ agreeing to sign the informed participation consent form.

3.1.3 - Non-inclusion criteria

- ✓ skin disease, whatever its location,
- ✓ treatment interfering with skin metabolism, in particular isotretinoin,
- ✓ acetretin and etretinate.

3.2 - Methodology

3.2.1 - Material, dose, duration

The product was applied once, diluted to 2% in water, over a skin area of approximately 50 mm^2 on the front of each volunteer's arm, at a dose of approximately 0.02 ml soaked into a filter paper disc placed in the patch chamber.

NB: The choice of dose was governed by the capacity of the chamber, specified by the "Fin Chambers" manufacturer.

The product was held in contact with the skin for 24 consecutive hours.

This application was carried out in parallel to and under the same conditions as a patch-test alone (no product) used as a negative control.

3.2.2 - Readings

Macroscopic skin examinations were performed immediately, 30 minutes after removing the patches.

Skin reaction (erythema, oedema, etc.) was evaluated according to the nomenclature proposed by the International Contact Dermatitis Research Group (ICDRG). :

NT	:	Not Tested.
?+	:	Questionable reaction Slight erythema only.
+		Weak positive reaction (non-vesicular): erythema, infiltration, occasionally some papules.
++	:	Strong positive reaction: erythema, papules, vesicles.
+++		Violent positive reaction with blistering.
-	:	Negative reaction.
IR	:	Irritation reaction: E 0.5: very mild erythema E1: mild erythema E2: distinct erythema E3: significant erythema

NB:

Failing any local skin reaction upon reading 30 minutes after removing the dressing, the test was stopped. Each volunteer was, however, asked to check for any reaction the following day. If a visible reaction was noted, the subject was to return to the centre.
In the case of distinct or questionable reactions, a reading was performed 48 hours and, if necessary 72 hours after dressing removal.

3.2.3 - Interpretation of results

Bibliographic reference: "Les essais cliniques en dermatologie", Thérapie, 1991,
Tome 46, page 183-7.

The primary dermal irritation index at each reading time was calculated by the following ratio:

$$*PDII = \frac{\Sigma \text{ of erythematous scores}}{\text{number of subjects}}$$

The skin irritation scale is as follows:

****If PDII < 0.20 Non Irritant**
If 0.20 ≤ PDII < 0.50 Slight irritant
If 0.50 ≤ I.I.M < 1 Moderate irritant
If PDII ≥ 1 Irritant

4 - RESULTS

The individual reading results at each experimental time are presented in the table below.

SUBJECTS		TEST PRODUCT: DE-99/1062		NEGATIVE CONTROL	
Identification	Age and Gender (1)	reading 30 min. after dressing removal	reading 24h after dressing removal	reading 30 min. after dressing removal	reading 24h after dressing removal
FR-RO	28 F	0	0	0	0
CH-GA	22 M	0	0	0	0
AN-AL	41 M	E <0.5	0	0	0
LA-NA	30 F	0	0	0	0
DE-CA	23 F	0	0	0	0
PO-PI	26 M	0	0	0	0
DU-MI	48 F	0	0	0	0
GI-CY	22 M	0	0	0	0
CO-NO	47 F	0	0	0	0
LU-ST	19 F	0	0	0	0
GI-VA	19 F	0	0	0	0
I.I.M*		**0.05**	**0**	**0**	**0**
Results**		**Non-irritant**	**Non-irritant**	**Non-irritant**	**Non-irritant**

(1): M = male
F = female

5 - CONCLUSION

Under the experimental conditions adopted, thirty minutes after removing the occlusive dressing, one volunteer presented with very mild erythema that had disappeared at the 24-hour reading.

No volunteers presented with irritation reactions indicative of a skin intolerance reaction.

We can therefore conclude that the product ANTILEUKINE 6 REF 909 500, code DECS DE-99/1062, diluted to 2% in water and applied locally under an occlusive dressing for 24 hours, to the skin of 11 adult volunteers, was **Non-irritant**.

Marie José Albin
Medical doctor - Allergist
Expert Toxicologist
(EUROTOX1999 Commission)

Dominique Saboureau
Doctor of Pharmacy
Expert Toxicologist
(EUROTOX List)

BiotechMarine
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04 JUIL. 2014

STUDY SUMMARY REPORT

Version no. 99/001 dated 11/22/1999

Sponsor: S.E.C.MA. BIOTECHNOLOGIES MARINES **Address:** BP 65 22260 PONTRIEUX	**Product:** ANTILEUKINE 6 REF 909 500 **DECS code:** DE-99/1062 **PALMER Research code:** PA.99/1244

DETERMINATION OF IRRITATION POTENTIAL IN 11 HUMAN SUBJECTS:
24 HOUR SINGLE PATCH-TEST

Study date:	Initiated on 11/16/99 and concluded on 11/17/99.	
Study location(s):	DECS Investigation Clinique 1, rue du Golf 33700 MERIGNAC	
Objective(s):	Determination of the acute skin tolerance of a cosmetic product by application over a 24-hour period.	
Methodology:	Open Study.	Number of subjects: 11.
Inclusion criteria:	Skin without any dermatological lesions, non-allergic volunteer.	• Duration of applications: 24 hours • Conditions of use: 2% diluted in water.
Outcome measures:	Calculation of primary dermal irritation index: $PDII = \frac{\text{total skin reaction score}}{\text{number of volunteers}}$ Skin reactions are scored from 0 to 4.	
Analysis:	Product classification according to its PDII: if PDII < 0.20 Non Irritant if 0.20 ≤ PDII < 0.50 Slight irritant if 0.50 ≤ PDII < 1 Moderate irritant if PDII ≥ 1 Irritant	
Conclusion	The irritation index of the product DE-99/1062 is equal to 0.05 and classified as Non-Irritant to human skin.	

Dominique SABOUREAU
Doctor of Pharmacy

Trial Director
Doctor Marie José Alhin, Allergist

Evaluation du potentiel allergisant après applications épicutanées répétées sur 52 volontaires

- Société : SECMA Biotechnologies Marines
  Zone Industrielle
  B.P. 65
  22260 PONTRIEUX

- Produit : ANTILEUKINE 6

- Panel : HRL #99-110 (6)

Arbanats, le 31 Mai 1999

SOCIÉTÉ DE CONSEIL-EXPERTISE PHARMACEUTIQUE & COSMETOLOGIQUE

SARL [illegible]

SIÈGE SOCIAL : 16 [illegible] 33640 ARBANATS

ETABLISSEMENT PRINCIPAL (ADRESSE DE LIVRAISON) : 13, CHEMIN DE LOU TRIBAR - Z.A. DE TOU[illegible] - B.P. 5 - 33610 CESTAS

TÉL : 05 56 67 33 02 - FAX : 05 57 97 16 81 - e-mail : phycher.palmer@wanadoo.fr

Je, soussigné, Dominique SABOUREAU, Docteur en Pharmacie, gérant de la Société **PALMER** Research (sis : 18, rue de Coulon; B.P. 15; 33640 ARBANATS), atteste que le produit :

ANTILEUKINE 6

a été confié à la Société **HARRISON Research Laboratories, INC** (sis HRL : 2497 Vauxhall Road - Union, NJ 07083), afin d'évaluer le potentiel allergisant de cette préparation, par applications épicutanées, sur 58 volontaires (52 ayant suivi le protocole jusqu'à la fin de l'essai).

Cette étude a été réalisée conformément :

- Au protocole HRL Standard, protocole # 100, Repeated Insult Patch Test (RIPT),

- Aux procédures en vigueur dans ce laboratoire,

- Dans le respect des réglementations internationales visant à la protection des personnes dans la recherche biomédicale.

- A fait l'objet d'un rapport référence : **HRL Panel #99-110 (6)**

Cette étude a été réalisée du 29 mars 1999 au 7 mai 1999 selon le schéma expérimental suivant :

METHODOLOGIE

❶ Volontaires inclus

58 volontaires de type caucasien et des deux sexes (15 hommes et 43 femmes), sans affection cutanée ni antécédent médical empêchant l'application topique de substances, et âgés de 19 à 65 ans.

❷ Traitements

Phase d'induction :

9 applications successives (lundi, mercredi et vendredi, pendant 3 semaines) du produit tel quel, à raison de 0,2 ml sous "patch" (Professional Medical Products # 4022) pendant 24 heures, au niveau du dos (côté gauche).

Nota : Si une irritation nette (cotation 2 ou plus) est observée au cours de cette phase, soit le sujet est mis au repos, soit le site d'application est changé.

Phase déclenchante :

Après une période de repos de 2 à 3 semaines, une application a été réalisée au niveau d'un site vierge (dos, côté droit). Cette application a été effectuée sous patch pendant 24 heures.

❺ Appréciation des réactions

Les lectures ont été effectuées (au niveau des deux sites induit et challenge) 24, 48 ou 72 heures après l'application du patch, selon une échelle de cotation arbitraire :

0 = pas de réaction,
+ = douteux,
1 = léger érythème,
2 = érythème net,
3 = érythème avec induration,
4 = érythème avec ulcération.

❻ Interprétation des résultats

L'importance du potentiel allergisant du produit étudié est déterminée en fonction du pourcentage de sujets ayant présenté une réponse positive lors de l'application déclenchante.

RESULTATS

Dans les conditions expérimentales retenues, après 9 applications successives, le produit **ANTILEUKINE 6** n'a induit aucune réaction significative d'intolérance cutanée locale au cours de la phase d'induction.

Au cours de la période de repos et avant la phase déclenchante, la peau des sujets au niveau des sites d'application est demeurée normale.

Après la dernière application, phase déclenchante, aucune réaction cutanée n'a été notée.

CONCLUSION

Au vu de ces résultats, on peut conclure que le produit **ANTILEUKINE 6** révélé *très bien toléré* au plan irritatif. Aucune réaction cutanée locale significative d'une réaction d'allergie de contact n'a été enregistrée.

Il peut donc être qualifié d'hypoallergénique.

Fait à Arbanats, le 31 mai 1999.

Dominique SABOUREAU,
Docteur en Pharmacie,
Expert Toxicologue
(Commission d'enregistrement EUROTOX, Avril 1997)

Personal Care Products Council
Committed to Safety, Quality & Innovation

Memorandum

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

FROM: Carol Eisenmann, Ph.D.
Personal Care Products Council

DATE: September 13, 2018

SUBJECT: Pelvetia Canaliculata Extract and Laminaria Digitata Extract

Biotech Marine. 2014. Manufacturing Process- Bioenergizer P (mixture of *Pelvetia canaliculata* and *Laminaria digitata* extracted in Propylene Glycol with Panthenol).

Biotech Marine. 2016. Bioenergizer™ P (mixture of *Pelvetia canaliculata* and *Laminaria digitata* extracted in Propylene Glycol with Panthenol) Physico-chemical data.

Palmer Research. 1996. Etude de la tolérance cutanée aiguë chez 10 volontaires adultes Patch-tests 24 et 48 heures, uniques (Bioenergizer P mixture of *Pelvetia canaliculata* and *Laminaria digitata* extracted in Propylene Glycol with Panthenol).

Biotech Marine. 2014. Manufacturing Process- Bioenergizer P BG (mixture of *Pelvetia canaliculata* and *Laminaria digitata* extracted in Butylene Glycol with Panthenol, with preservatives).

Biotech Marine. 2014. Bioenergizer P BG (mixture of *Pelvetia canaliculata* and *Laminaria digitata* extracted in Butylene Glycol with Panthenol with preservatives) Physico-chemical data.

Biotech Marine. 2014. Manufacturing Process- Bioenergizer P BG/PF (mixture of *Pelvetia canaliculata* and *Laminaria digitata* extracted in Butylene Glycol with Panthenol - no preservatives).

Biotech Marine. 2014. Bioenergizer P BG/PF (mixture of *Pelvetia canaliculata* and *Laminaria digitata* extracted in Butylene Glycol with Panthenol - no preservatives) Physico-chemical data.

1620 L Street, N.W., Suite 1200 | Washington, D.C. 20036 | 202.331.1770 | 202.331.1969 (fax) | www.personalcarecouncil.org

SYNOPSIS DE FABRICATION/ *MANUFACTURING PROCESS*

BIOENERGIZER P

RECOLTE / IDENTIFICATION
(Pelvetia Canaliculata)
HARVESTING / IDENTIFICATION
↓
LAVAGE
WASHING
↓
BROYAGE
GRINDING
↓
EXTRACTION AVEC LE SOLVANT PROPYLENE GLYCOL
EXTRACTION WITH THE SOLVENT PROPYLENE GLYCOL
↓
FILTRATION
↓
CONTRÔLE QUALITE
QUALITY CONTROL

RECOLTE / IDENTIFICATION
(Laminaria Digitata)
HARVESTING / IDENTIFICATION
↓
LAVAGE
WASHING
↓
BROYAGE
GRINDING
↓
EXTRACTION AVEC LE SOLVANT PROPYLENE GLYCOL
EXTRACTION WITH THE SOLVENT PROPYLENE GLYCOL
↓
FILTRATION
↓
CONTRÔLE QUALITE
QUALITY CONTROL

↓
MELANGE — Ajout panthenol / *Panthenol addition*
MIXTURE
↓
FILTRATION
↓
CONTRÔLE QUALITE
QUALITY CONTROL
↓
CONDITIONNEMENT
PACKAGING
↓
CONTRÔLE QUALITE
QUALITY CONTROL

Responsable production
Production Manager
Jean-Marc CAPROUX

BIOTECHMARINE (02/10/2014)

Date de mise à jour / *Updated date* : 01/12/2016

BIOENERGIZER™ P

INCI NAME : Propylene Glycol - Aqua / Water – Panthenol - Pelvetia Canaliculata Extract – Laminaria Digitata Extract
CAS N°: 57-55-6 – 7732-18-5 – 81-13-0– 223751-75-5 - 90046-12-1
EC N°: 200-338-0(EINECS) – 231-791-2(EINECS) –201-327-3 (EINECS) – 607-055-5 - 289-980-0 (EINECS)

DONNEES PHYSICOCHIMIQUES
PHYSICO-CHEMICAL DATA
Numéro de référence / *Reference number* : STANDARD

CARACTERISTIQUES *CHARACTERISTICS*	METHODES *METHODS*	STANDARD *STANDARD*
Aspect *Aspect*	MO PHY 002	**Liquide limpide à légèrement opalescent** *Limpid liquid to slightly opalescent*
Couleur *Colour*	MO PHY 002	**Brun orange à brun rouge** *Orange brown to red brown*
Odeur *Odour*	MO PHY 002	**Caractéristique** *Characteristic*
pH	MO PHY 009	5,5 – 7,0
Teneur en eau *Water content*	MO PHY 018	43,0 – 47,0 %
Densité (20°C) *Density*	MO PHY 024	1,040 – 1,060
Indice de réfraction(20°C) *Refractive index*	MO PHY 008	$1{,}395_0$ – $1{,}405_0$
Extrait sec *Dry extract*	MO PHY 017	5,5 – 9,0 %
Spectre UV *UV spectrum*	MO PHY 013	**Conforme au témoin** *Similar to the standard*
Spectre IR *IR spectrum*	MO PHY 011	**Conforme au témoin** *Similar to the standard*
Conservateurs *Preservatives*		
- **POB méthyle** *Methyl Paraben*	MO PHY 020	0,15 – 0,20 %
- **POB propyle** *Propyl Paraben*	MO PHY 020	0,03 – 0,06 %

BIOENERGIZER™ P

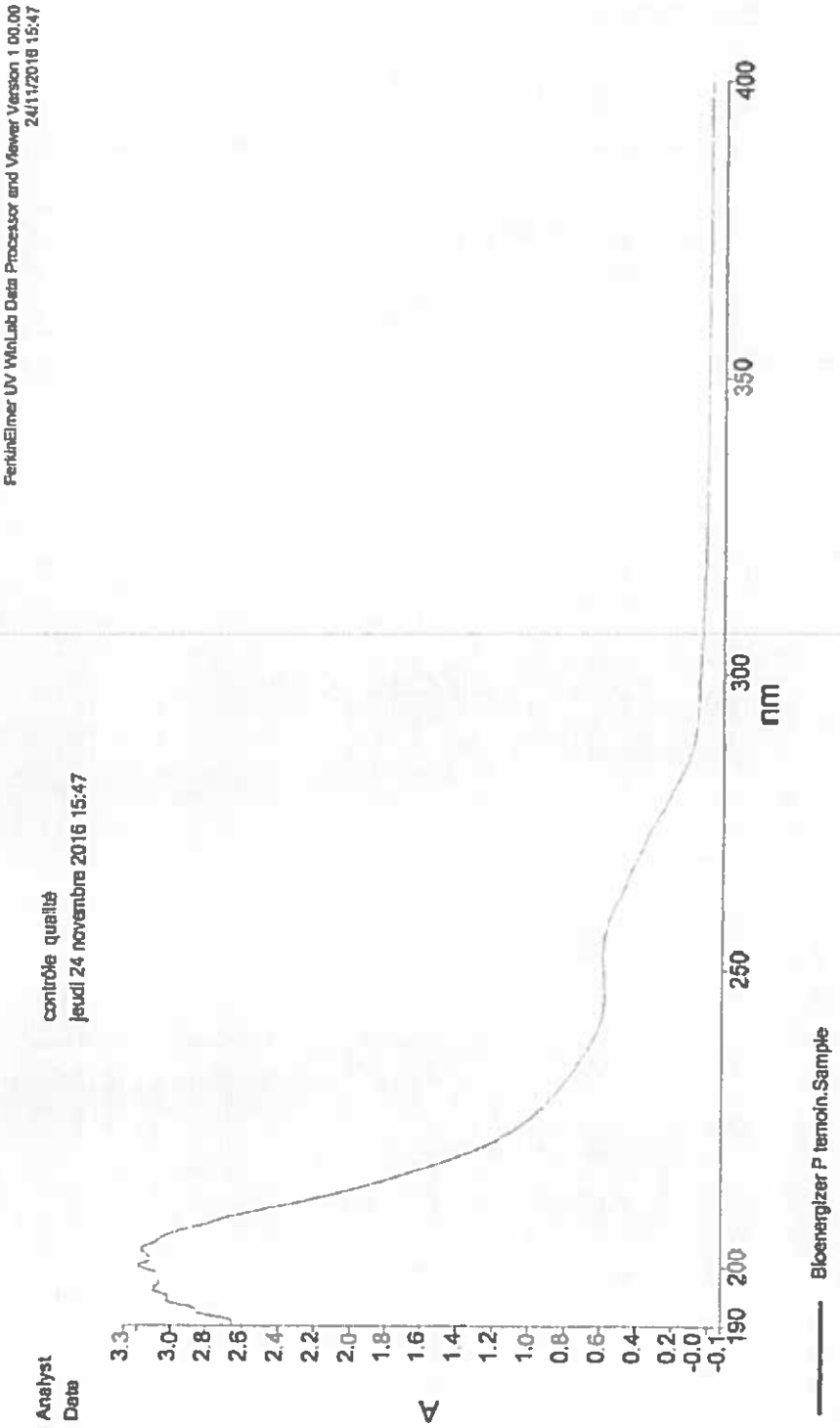
INCI NAME : Propylene Glycol - Aqua / Water – Panthenol - Pelvetia Canaliculata Extract – Laminaria Digitata Extract
CAS N°: 57-55-6 – 7732-18-5 – 81-13-0– 223751-75-5 - 90046-12-1
EC N°: 200-338-0(EINECS) – 231-791-2(EINECS) –201-327-3 (EINECS) – 607-055-5 - 289-980-0 (EINECS)

DONNEES PHYSICOCHIMIQUES
PHYSICO-CHEMICAL DATA
Numéro de référence / *Reference number* : STANDARD

CARACTERISTIQUES *CHARACTERISTICS*	STANDARD *STANDARD*
Métaux lourds* (mg/kg) *Heavy metals* (mg/kg)*	
- Arsenic *- Arsenic*	< 5
- Cadmium *- Cadmium*	< 3
- Plomb *- Lead*	< 5
- Nickel *- Nickel*	< 2
- Argent *- Silver*	< 5

Zodine L100 ppm

* Teneurs garanties sous contrôle statistique / *Contents guaranteed under statistical control*

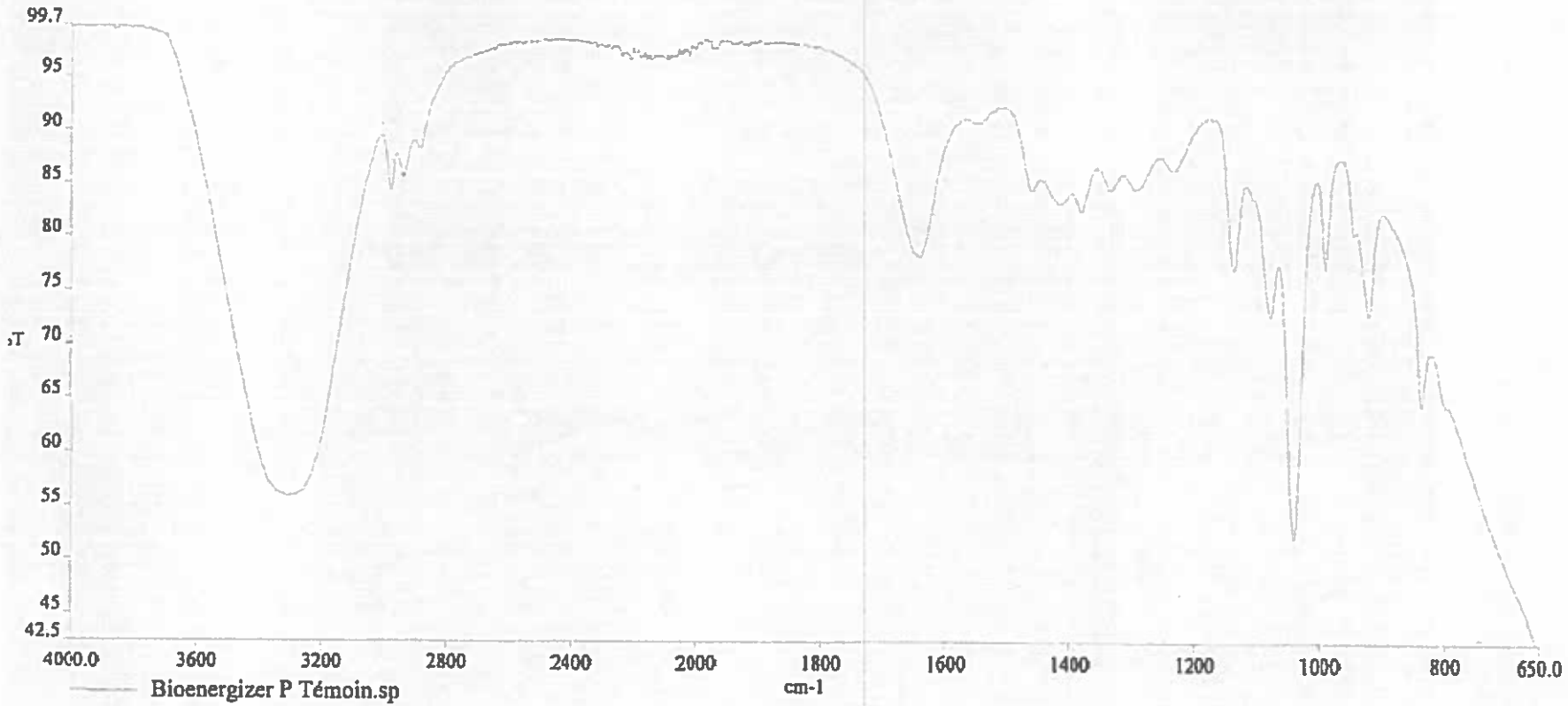


Date: jeudi 24 novembre 2016

SPECTRE IRFT

SPECTROPHOTOMETRE SPECTRUM 100 N° de série 77774 - PERKIN ELMER

Accessoire ATR Universel N° 7031330



BIOENERGIZER™ P

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CAS N°: 57-55-6 – 7732-18-5 – 81-13-0– 223751-75-5 - 90046-12-1
EC N°: 200-338-0(EINECS) – 231-791-2(EINECS) –201-327-3 (EINECS) – 607-055-5 - 289-980-0 (EINECS)

DONNEES MICROBIOLOGIQUES
MICROBIOLOGICAL DATA
Numéro de référence / *Reference number* : STANDARD

CARACTERISTIQUES *CHARACTERISTICS*	**METHODES** *METHODS*	**STANDARD** *STANDARD*
Germes totaux** *Total germs***	MO MIC 002 / NF EN ISO 21149	< 100
Germes Pathogènes *Pathogens*		
- *Staphylococcus aureus*	MO MIC 012 / NF EN ISO 22718	**Absence** *None*
- *Candida albicans*	MO MIC 010 / NF EN ISO 18416	**Absence** *None*
- *Pseudomonas aeruginosa*	MO MIC 011 / NF EN ISO 22717	**Absence** *None*
- *Escherichia coli*	MO MIC 025 / NF EN ISO 21150	**Absence** *None*
Levures / Moisissures** *Yeasts / Moulds***	MO MIC 021 / NF EN ISO 16212	< 100

** Les résultats sont indiqués en UFC/mL pour les liquides et en UFC/g pour les solides
** *Results are indicated in CFU/mL for the liquids and in CFU/g for the solids*

CONFORME
CERTIFIED TRUE AND CORRECT
RESPONSABLE CONTROLE QUALITE: **P. SOUBIES**
QUALITY CONTROL MANAGER
01 DEC. 2016

CONFORME
CERTIFIED TRUE AND CORRECT
COORDINATRICE ASSURANCE QUALITE : **M. TANNIOU**
QUALITY ASSURANCE COORDINATOR
01 DEC. 2016

PALMER Research

Etude de la tolérance cutanée aiguë chez 10 volontaires adultes Patch-tests 24 et 48 heures,uniques

Etude référence TC.aiguë.5-SEC/B/PALM 95
réalisée du 19/12/95 au 21/12/95

Société : **SECMA Biotechnologies Marines**
B.P. 65
22260 PONTRIEUX

Produit : **BIOENERGIZER P (3 concentrations)**

Propylene Glycol / water / Panteno1 /
Pelvetia Canaliculata Extrat and Laminaria
Digitata Extract

Arbanats, Février 1996

SOCIÉTÉ DE CONSEIL-EXPERTISE PHARMACEUTIQUE & COSMÉTOLOGIQUE

18 , RUE DE COULON - B.P. 15 - 33640 ARBANATS - TÉL : 56 67 33 02 – FAX : 56 67 05 60
S.A.R.L. AU CAPITAL DE 120.000 FRANCS – APE 7312 – SIRET 384 324 141 00017

1 - INTRODUCTION

A la demande de la société **SECMA Biotechnologies Marines - B.P. 65 ; 22260 PONTRIEUX** -, nous avons évalué sur 10 volontaires adultes la tolérance cutanée aiguë du produit **BIOENERGIZER** aux 3 concentrations suivantes :

- **B : BIOENERGIZER à 100 %**
- **B1 : 5 % de BIOENERGIZER**
- **B2 : 10 % de BIOENERGIZER**

après application unique sur la peau de la face antérieure d'un bras, sous pansements occlusifs maintenus pendant 24 et 48 heures (Patch-Tests 24 et 48 heures).

Cet essai a été réalisé "en ouvert" selon la méthodologie des tests épicutanés sous occlusion.

Pour réaliser cette étude, nous avons reçu le 18/12/95 trois échantillons de 60 ml de chaque concentration du produit BIOENERGIZER.

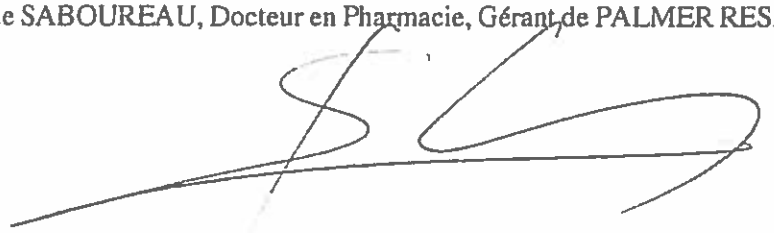
L'essai a commencé le19/12/95 pour s'achever le 21/12/95.

2 - AUTHENTIFICATION DES RESULTATS

L'étude faisant l'objet du présent rapport a été conduite sous ma responsabilité, en conformité avec le protocole expérimental et dans le respect des règles des Bonnes Pratiques Cliniques.
Toutes les observations et les données numériques recueillies au cours de cet essai sont rapportées dans le présent document.

Après relecture, je certifie ces données conformes à la réalité des résultats obtenus.
Docteur Pascale DENIS, Investigateur et Directeur de l'Etude.

En tant que Moniteur de l'Etude, je certifie avoir relu ce rapport et je suis en accord avec son contenu.
Dominique SABOUREAU, Docteur en Pharmacie, Gérant de PALMER RESEARCH.



3 - PROTOCOLE EXPERIMENTAL

3.1 - Volontaires

3.1.1 - Caractéristiques des sujets inclus

- 10 sujets, dont 5 de sexe masculin et 5 de sexe féminin, ont été inclus dans l'essai,
- âgés de21 à 46 ans.

Tous les sujets devaient répondre aux critères d'inclusion et ne présenter aucun critère de non inclusion.

3.1.2 - Critères d'inclusion

- aucun antécédent d'intolérance ou d'allergie à un produit cosmétique,
- acceptation de signature du consentement éclairé de participation.

3.1.3 - Critères de non inclusion

- pathologie cutanée, quelque soit son site,
prise d'un traitement interférant avec le métabolisme cutané, en particulier isotrétinoïne, acitrétine et étrétinate.

3.2 - Méthodologie

3.2.1 - Matériel, dose, durée

Le produit a été appliqué aux 3 concentrations suivantes :

- **B : BIOENERGIZER à 100 %**
- **B1 : 5 % de BIOENERGIZER**
- **B2 : 10 % de BIOENERGIZER**

une seule fois, sur une surface d'environ 50mm de peau de la face antérieure d'un bras de chaque volontaire, à la dose d'environ 0,02ml imbibant la rondelle de papier filtre.

Nota : La raison du choix de la dose est conditionnée par la capacité de la cupule, indiquée par le fabricant dans "Finn Chambers".

Six pansements occlusifs, correspondant à chaque concentration et à chaque temps de contact, ont été posés et maintenus en contact avec la peau pendant respectivement 24 et 48 heures consécutives.

Ces applications sont effectuées parallèlement et dans les mêmes conditions avec un patch-test seul (sans produit) en tant que témoin négatif.

3.2.2 - Lectures

Les examens macroscopiques cutanés ont été réalisés immédiatement, 30 minutes et 24 heures après l'enlèvement des patch.

L'évaluation des réactions cutanées (érythème, oedème, ...) a été effectuée selon la nomenclature proposée par l'International Contact Dermatitis Research Group (I.C.D.R.G) :

NT	:	Non testé.
?+	:	Réaction douteuse. Léger érythème seulement.
+	:	Réaction positive faible (non vésiculeuse) : érythème, infiltration, parfois quelques papules.
++	:	Forte réaction positive : présence d'érythème, de papules, de vésicules.
+++	:	Réaction positive violente, avec présence de bulles.
-	:	Réaction négative.
IR	:	Réaction d'irritation = Erythème (E) **E = 0,5 érythème très léger** **E = 1 érythème léger** **E = 2 érythème net** **E = 3 érythème important**

Nota : En l'absence de toute réaction cutanée locale à la lecture de 24 heures, l'essai est arrêté. Dans le cas de réactions nettes ou douteuses, une lecture est effectuée 48 heures et si nécessaire 72 heures après la dépose des patchs.

3.2.3 - Interprétation des résultats

Référence bibliographique :"Les essais cliniques en dermatologie", Thérapie, 1991, Tome 46, page 183-7.

L'indice d'irritation moyen à chaque temps de lecture est calculé selon le rapport :

$$IM = \frac{\sum \text{des cotations érythémateuses}}{\text{nombre de sujets}}$$

Le barême d'interprétation de l'irritation cutanée est le suivant :

Si IM≤0,20 non irritant
Si 0,20≤IM≤0,5 légèrement irritant
Si 0,50≤IM≤1 moyennement irritant
Si IM>1 irritant

4 - RESULTATS

Les résultats individuels des lectures à chaque expérimental et à chacune des 3 concentrations du produit sont regroupés dans les tableaux ci-dessous.

Tableau 1 : BIOENERGIZER à 100 %

SUJETS		Produit à l'essai : BIOENERGIZER 100% Patch 24 heures		Produit à l'essai : BIOENERGIZER 100% Patch 48 heures		Témoin Négatif
Identifica-tion	Age et Sexe	Lecture 30 min après enlèvement du patch	Lecture 24 heures après enlèvement du patch	Lecture 30 min après enlèvement du patch	Lecture 24 heures après enlèvement du patch	Lecture 30 min après enlèvement du patch
BA.FR	40 M	-	-	E1	E0,5	-
CR.FR	28 M	E1	-	-	-	-
RA.AM	30 F	E0,5	-	-	-	-
RI.JP	46 M	E1	E0,5	-	-	-
KA.LA	21 F	-	-	-	-	-
AH.RO	31 F	-	-	E0,5	-	-
EN.RA	31 M	-	-	E0,5	-	-
KA.AL	21 F	-	-	-	-	-
MO.NA	35 F	-	-	E1	-	-
KA.TH	37 M	E2	-	-	-	-
IM Résultats		0,45 légèrement irritant	0,05 non irritant	0,30 légèrement irritant	0.05 non irritant	0 non irritant

Tableau 2 : 10 % de BIOENERGIZER

SUJETS		Produit à l'essai : 10 % BIOENERGIZER Patch 24 heures		Produit à l'essai : 10 % BIOENERGIZER Patch 48 heures		Témoin Négatif
Identification	Age et Sexe	Lecture 30 min après enlèvement du patch	Lecture 24 heures après enlèvement du patch	Lecture 30 min après enlèvement du patch	Lecture 24 heures après enlèvement du patch	Lecture 30 min après enlèvement du patch
BA.FR	40 M	-	-	-	-	-
CR.FR	28 M	-	-	E0,5	-	-
RA.AM	30 F	-	-	E0,5	-	-
RI.JP	46 M	E1	-	E0,5	-	-
KA.LA	21 F	-	-	-	-	-
AH.RO	31 F	E1	E0,5	-	-	-
EN.RA	31 M	-	-	-	-	-
KA.AL	21 F	-	-	-	-	-
MO.NA	35 F	-	-	-	-	-
KA.TH	37 M	-	-	-	-	-
IM		0,20	0.05	0,15	0	0
Résultats		non irritant	non irritant	non irritant	non irritant	non irritant

***Tableau 3* : 5 % de BIOENERGIZER**

SUJETS		Produit à l'essai : 5 % BIOENERGIZER Patch 24 heures		Produit à l'essai : 5 % BIOENERGIZER Patch 48 heures		Témoin Négatif
Identification	Age et Sexe	Lecture 30 min après enlèvement du patch	Lecture 24 heures après enlèvement du patch	Lecture 30 min après enlèvement du patch	Lecture 24 heures après enlèvement du patch	Lecture 30 min après enlèvement du patch
BA.FR	40 M	-	-	-	-	-
CR.FR	28 M	-	-	-	-	-
RA.AM	30 F	-	-	-	-	-
RI.JP	46 M	E0,5	-	-	-	-
KA.LA	21 F	-	-	-	-	-
AH.RO	31 F	-	-	-	-	-
EN.RA	31 M	-	-	-	-	-
KA.AL	21 F	-	-	-	-	-
MO.NA	35 F	-	-	-	-	-
KA.TH	37 M	-	-	-	-	-
IM		**0,05**	**0**	**0**	**0**	**0**
Résultats		**non irritant**	**non irritant**	**non irritant**	**non irritant**	**non irritant**

5 - CONCLUSION

Dans les conditions expérimentales retenues, le produit **BIOENERGIZER** s'est révélé :

- appliqué pur, après 24 et 48 heures de contact avec la peau, ***Légèrement Irritant*** , induisant chez 4 sujets sur 10, à chaque temps de contact, un érythème très léger à léger. La réversibilité des réactions a été globalement bonne et aucun effet secondaire n'a été observé.

- appliqué dilué à 10 %, après 24 et 48 heures de contact avec la peau, ***Très Faiblement Irritant***, induisant à chaque temps respectivement, chez 2 sujets un érythème léger et chez 3 autres un très léger érythème. La réversibilité a été bonne et aucun effet secondaire n'a été observé.

- appliqué dilué à 5 %, après 24 et 48 heures de contact avec la peau, ***Non Irritant***, aucun des sujets n'ayant présenté de réaction d'irritation significative d'une intolérance cutanée. Aucun effet secondaire n'a été observé.

D. SABOUREAU
Docteur en Pharmacie

P. DENIS
Docteur en Médecine

SYNOPSIS DE FABRICATION/ *MANUFACTURING PROCESS*

BIOENERGIZER P BG

RECOLTE / IDENTIFICATION
(Pelvetia Canaliculata)
HARVESTING / IDENTIFICATION

↓

LAVAGE
WASHING

↓

BROYAGE
GRINDING

↓

EXTRACTION AVEC LE SOLVANT BUTYLENE GLYCOL
EXTRACTION WITH THE SOLVENT BUTYLENE GLYCOL

↓

FILTRATION

↓

CONTRÔLE QUALITE
QUALITY CONTROL

RECOLTE / IDENTIFICATION
(*Laminaria Digitata*)
HARVESTING / IDENTIFICATION

↓

LAVAGE
WASHING

↓

BROYAGE
GRINDING

↓

EXTRACTION AVEC LE SOLVANT BUTYLENE GLYCOL
EXTRACTION WITH THE SOLVENT BUTYLENE GLYCOL

↓

FILTRATION

↓

CONTRÔLE QUALITE
QUALITY CONTROL

↓

MELANGE
MIXTURE

Ajout panthenol
Panthenol addition

↓

FILTRATION

↓

CONTRÔLE QUALITE
QUALITY CONTROL

↓

CONDITIONNEMENT
PACKAGING

↓

CONTRÔLE QUALITE
QUALITY CONTROL

Responsable production
Production Manager
Jean-Marc CAZROUX

Date de mise à jour : 18/02/2014

BIOENERGIZER P BG

CTFA / INCI NAME : Aqua / Water - Butylene Glycol – Panthenol – Propylene glycol - Pelvetia Canaliculata Extract – Laminaria Digitata Extract
CAS N° : 7732-18-5 - 107.88.0 – 81-13-0 – 57-55-6 – 223751.75.5 – 90046.12.1/92128.82.0
EINECS N°: 231-791-2 – 203-529-7 – 201-327-3 – 200-338-0 – 607-055-5 – 289-980-0 – 202-785-7

DONNEES PHYSICOCHIMIQUES
PHYSICO-CHEMICAL DATA
Numéro de référence / *Reference number* : STANDARD

CARACTERISTIQUES *CHARACTERISTICS*	**METHODES** *METHODS*	**STANDARD** *STANDARD*
Aspect *Aspect*	MO PHY 002	**Liquide limpide** *Limpid liquid*
Couleur *Colour*	MO PHY 002	**Brun orange à brun rouge** *Orange brown to red brown*
Odeur *Odour*	MO PHY 002	**Caractéristique** *Characteristic*
pH	MO PHY 009	6 – 7
Teneur en eau *Water content*	MO PHY 018	43 – 49 %
Densité (20°C) *Density*	MO PHY 024	1,030 – 1,040
Indice de réfraction(20°C) *Refractive index*	MO PHY 008	$1{,}397_0$ – $1{,}405_0$
Extrait sec (MA 40 - 1g - 10 min à 160°C) *Dry extract*	MO PHY 017	5 – 9 %
Spectre UV *UV spectrum*	MO PHY 013	**Conforme au témoin** *Similar to the standard*
Spectre IR *IR spectrum*	MO PHY 011	**Conforme au témoin** *Similar to the standard*
Conservateur *Preservative* **- POB méthyle** *Methyl Paraben*	MO PHY 019	0,16 – 0,20 %

BIOENERGIZER P BG

CTFA / INCI NAME : Aqua / Water - Butylene Glycol – Panthenol – Propylene glycol - Pelvetia Canaliculata Extract – Laminaria Digitata Extract
CAS N° : 7732-18-5 - 107.88.0 – 81-13-0 – 57-55-6 – 223751.75.5 - 90046.12.1/92128.82.0
EINECS N°: 231-791-2 – 203-529-7 – 201-327-3 – 200-338-0 – 607-055-5 – 289-980-0 – 202-785-7

DONNEES PHYSICOCHIMIQUES
PHYSICO-CHEMICAL DATA
Numéro de référence / *Reference number* : STANDARD

CARACTERISTIQUES *CHARACTERISTICS*	STANDARD *STANDARD*
Métaux lourds (mg/kg) *Heavy metals (mg/kg)*	
- Arsenic mineral *- Mineral arsenic*	< 5
- Cadmium *- Cadmium*	< 10
- Plomb *- Lead*	< 5
- Nickel *- Nickel*	< 2
- Argent *- Silver*	< 5

Iodine
< 100 ppm

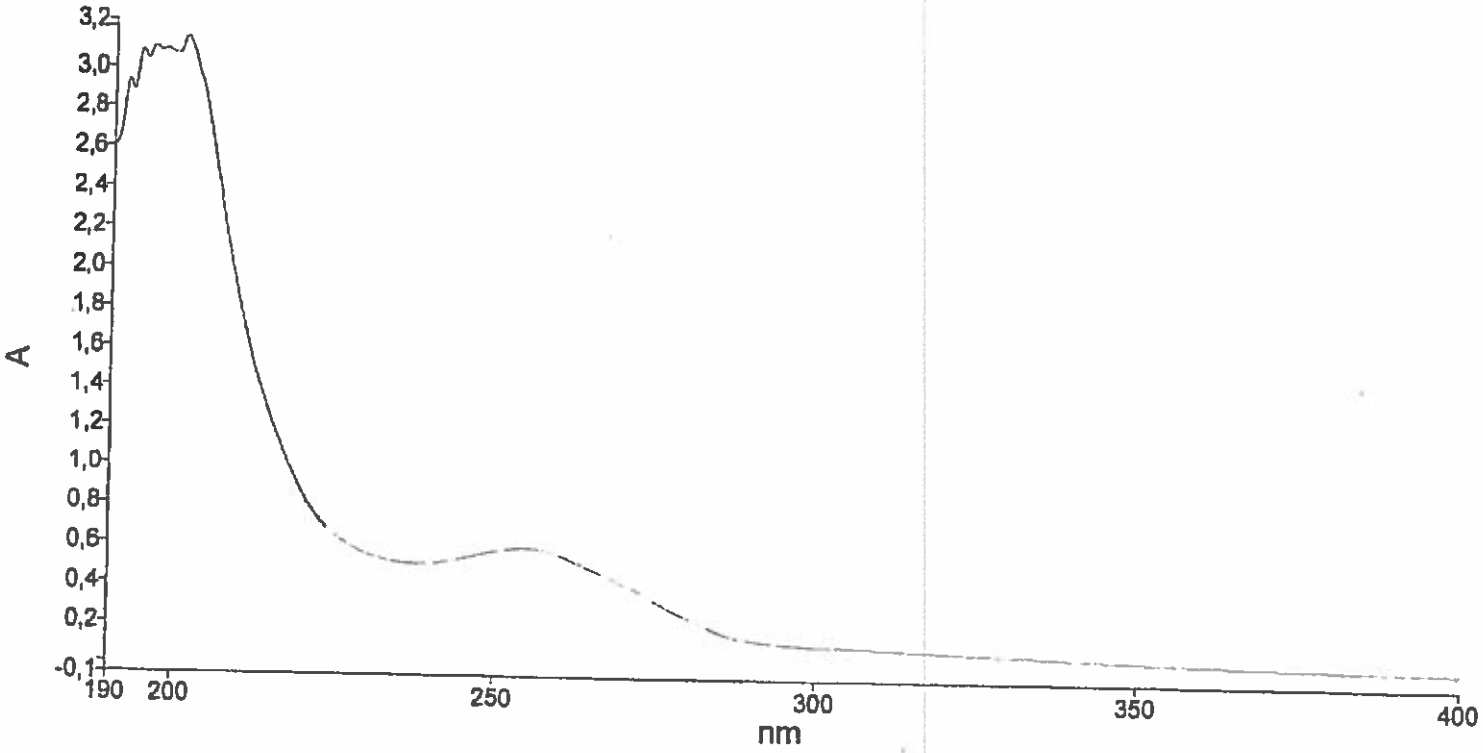
CERTIFIE CONFORME
CERTIFIED TRUE AND CORRECT
RESPONSABLE DU LABORATOIRE DE PHYSICO-CHIMIE : C. AUBRY
PHYSICOCHIMICAL LABORATORY MANAGER

- BiotechMarine Z.I. – B P 72 – 22260 Pontrieux - FRANCE Tél : +33 (0) 2 96 95 31 32

Analyst
Date

contrôle qualité
mercredi 19 février 2014 15:54

PerkinElmer UV WinLab Data Processor and Viewer Version 1.00.00
19/02/2014 15:54



Bioenergizer P BG.Sample Temoin

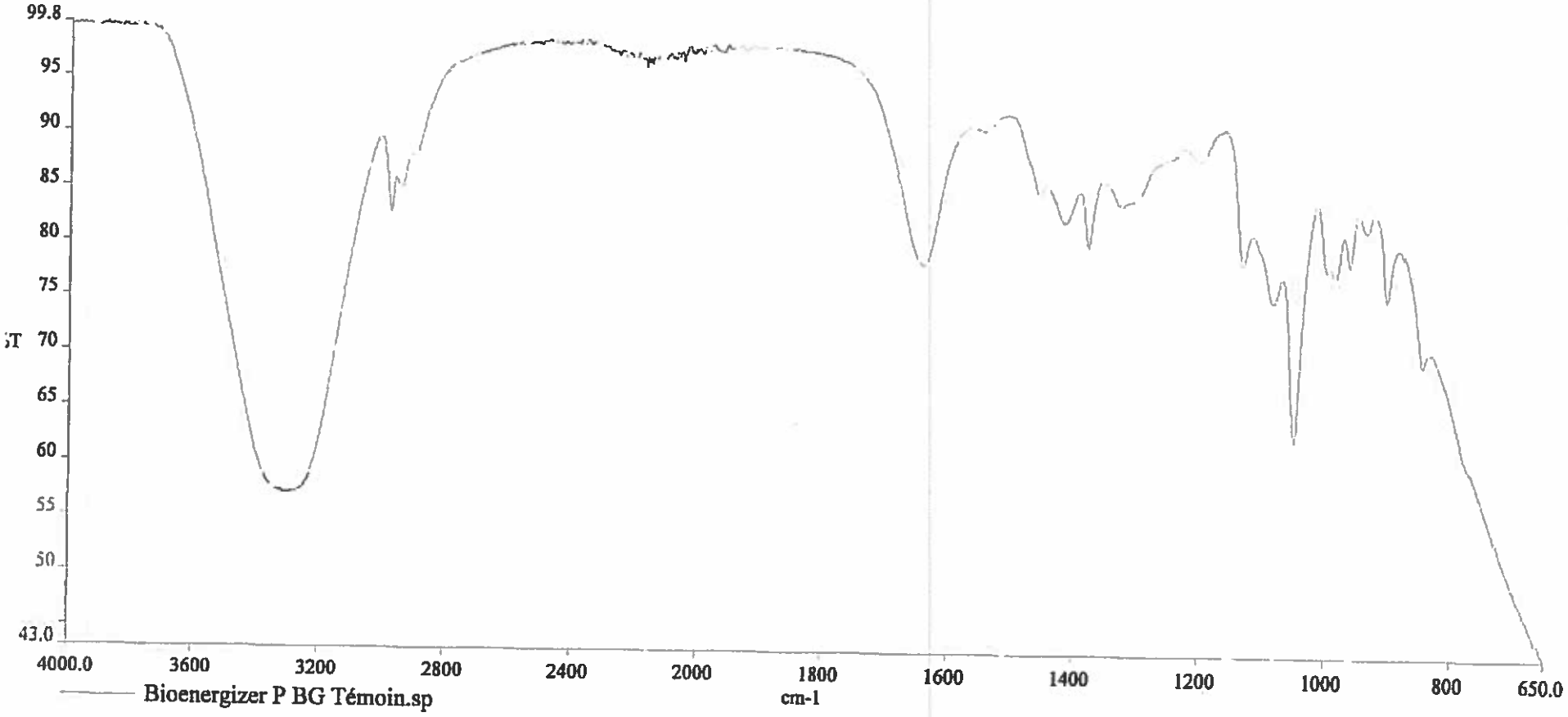
blanc = H_2O

SPECTRE IRFT

Date: mardi 18 février 2014

SPECTROPHOTOMETRE SPECTRUM 100 N° de série 77774 - PERKIN ELMER

Accessoire ATR Universel N° 7031330



BIOENERGIZER P BG

CTFA / INCI NAME : Aqua / Water - Butylene Glycol - Panthenol - Propylene glycol - Pelvetia Canaliculata Extract - Laminaria Digitata Extract
CAS N° : 7732-18-5 - 107.88.0 - 81-13-0 - 57-55-6 - 223751.75.5 - 90046.12.1/92128.82.0
EINECS N°: 231-791-2 - 203-529-7 - 201-327-3 - 200-338-0 - 607-055-5 - 289-980-0 - 202-785-7

DONNEES MICROBIOLOGIQUES
MICROBIOLOGICAL DATA
Numéro de référence / *Reference number* : STANDARD

CARACTERISTIQUES *CHARACTERISTICS*	METHODES *METHODS*	STANDARD *STANDARD*
Germes totaux * *Total germs **	MO MIC 002	**< 100**
Germes Pathogènes *Pathogens*		
- *Staphylococcus aureus*	MO MIC 012	**Absence** *None*
- *Candida albicans*	MO MIC 010	**Absence** *None*
- *Pseudomonas aeruginosa*	MO MIC 011	**Absence** *None*
- *Enterobacteriaceae*	MO MIC 020	**Absence** *None*
Levures / Moisissures* *Yeasts / Moulds**	MO MIC 021	**< 100**

*Les résultats sont indiqués en UFC/ml pour les liquides et en UFC/g pour les solides

CERTIFIE CONFORME
CERTIFIED TRUE AND CORRECT
RESPONSABLE DU LABORATOIRE DE MICROBIOLOGIE : M.TANNIOU
MICROBIOLOGICAL LABORATORY MANAGER

Le 25/02/14

- BiotechMarine Z.I. – B.P.72 – 22260 Pontrieux - FRANCE Tél : +33 (0) 2 96 95 31 32

SYNOPSIS DE FABRICATION/
MANUFACTURING PROCESS
BIOENERGIZER P BG/PF

RECOLTE / IDENTIFICATION
(Pelvetia Canaliculata)
HARVESTING / IDENTIFICATION
↓
LAVAGE
WASHING
↓
BROYAGE
GRINDING
↓
EXTRACTION AVEC LE SOLVANT
BUTYLENE GLYCOL
EXTRACTION WITH THE SOLVENT
BUTYLENE GLYCOL
↓
FILTRATION
↓
CONTRÔLE QUALITE
QUALITY CONTROL

RECOLTE / IDENTIFICATION
(*Laminaria Digitata*)
HARVESTING / IDENTIFICATION
↓
LAVAGE
WASHING
↓
BROYAGE
GRINDING
↓
EXTRACTION AVEC LE SOLVANT
BUTYLENE GLYCOL
EXTRACTION WITH THE SOLVENT
BUTYLENE GLYCOL
↓
FILTRATION
↓
CONTRÔLE QUALITE
QUALITY CONTROL

↓
MELANGE — Ajout panthenol / *Panthenol addition*
MIXTURE
↓
FILTRATION
↓
CONTRÔLE QUALITE
QUALITY CONTROL
↓
CONDITIONNEMENT
PACKAGING
↓
CONTRÔLE QUALITE
QUALITY CONTROL

Responsable production
Production Manager
Jean-Marc GATROUX

BIOTECHMARINE (02/8/2014)

Date de mise à jour : 18/02/2014

BIOENERGIZER P BG/PF

CTFA / INCI NAME : Aqua / Water - Butylene Glycol - Panthenol - Propylene glycol - Pelvetia Canaliculata Extract - Laminaria Digitata Extract
CAS N° : 7732-18-5 - 107.88.0 - 81-13-0 - 57-55-6 - 223751.75.5 - 90046.12.1/92128.82.0
EINECS N°: 231-791-2 - 203-529-7 - 201-327-3 - 200-338-0 - 607-055-5 - 289-980-0 - 202-785-7

DONNEES PHYSICOCHIMIQUES
PHYSICO-CHEMICAL DATA
Numéro de référence / *Reference number* : STANDARD PROVISOIRE

CARACTERISTIQUES *CHARACTERISTICS*	**METHODES** *METHODS*	**STANDARD** *STANDARD*
Aspect *Aspect*	MO PHY 002	**Liquide limpide** *Limpid liquid*
Couleur *Colour*	MO PHY 002	**Brun orange à brun rouge** *Orange brown to red brown*
Odeur *Odour*	MO PHY 002	**Caractéristique** *Characteristic*
pH	MO PHY 009	6 – 7
Teneur en eau *Water content*	MO PHY 018	43 – 49 %
Densité (20°C) *Density*	MO PHY 024	1,030 – 1,040
Indice de réfraction(20°C) *Refractive index*	MO PHY 008	$1,397_0$ – $1,405_0$
Extrait sec (MA 40 - 1g - 10 min à 160°C) *Dry extract*	MO PHY 017	5 – 9 %
Spectre UV *UV spectrum*	MO PHY 013	**Conforme au témoin** *Similar to the standard*
Spectre IR *IR spectrum*	MO PHY 011	**Conforme au témoin** *Similar to the standard*

BIOENERGIZER P BG/PF

CTFA / INCI NAME : Aqua / Water - Butylene Glycol - Panthenol - Propylene glycol - Pelvetia Canaliculata Extract - Laminaria Digitata Extract
CAS N° : 7732-18-5 - 107.88.0 - 81-13-0 - 57-55-6 - 223751.75.5 - 90046.12.1/92128.82.0
EINECS N°: 231-791-2 - 203-529-7 - 201-327-3 - 200-338-0 - 607-055-5 - 289-980-0 - 202-785-7

DONNEES PHYSICOCHIMIQUES
PHYSICO-CHEMICAL DATA
Numéro de référence/ *Reference number* **: STANDARD PROVISOIRE**

CARACTERISTIQUES *CHARACTERISTICS*	STANDARD *STANDARD*
Métaux lourds *Heavy metals* (ppm)	
• **Arsenic mineral** *Mineral Arsenic*	< 5
• **Cadmium** *Cadmium*	< 10
• **Plomb** *Lead*	< 5
• **Nickel** *Nickel*	< 2
• **Argent** *Silver*	< 5

Iodine < 100 ppm

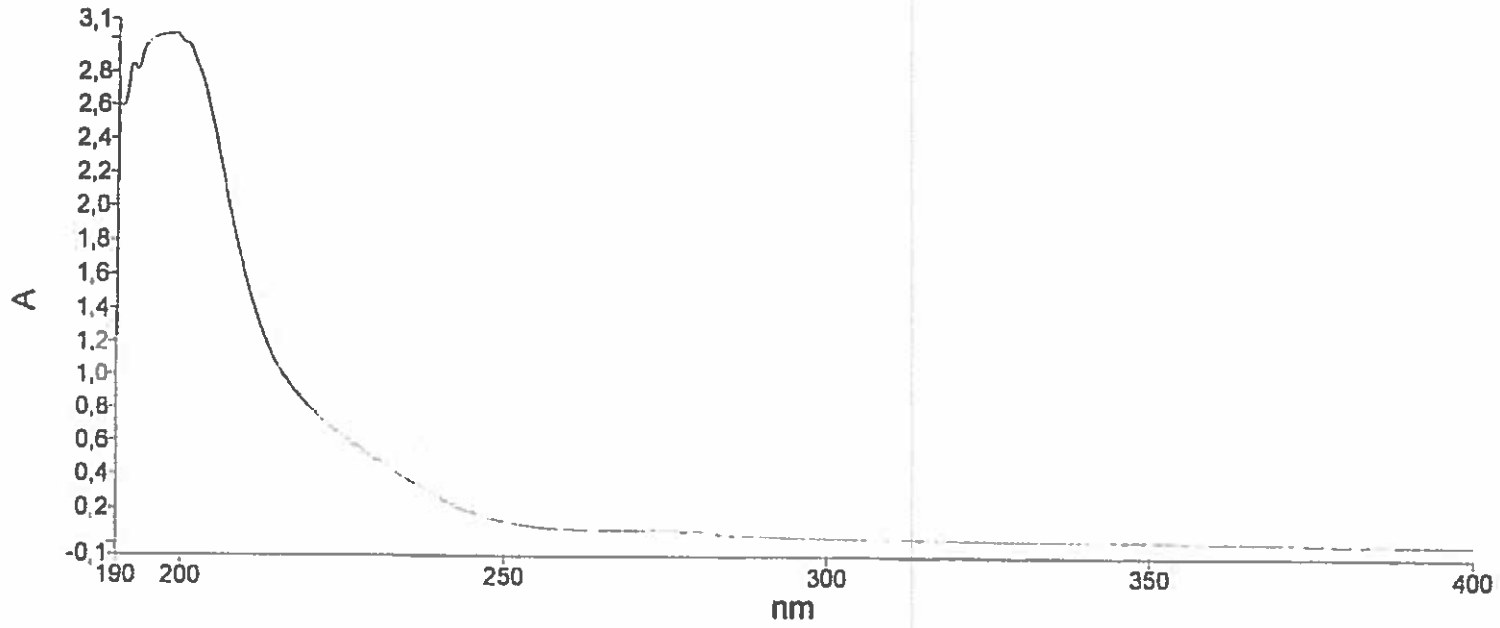
CERTIFIE CONFORME
CERTIFIED TRUE AND CORRECT
RESPONSABLE DU LABORATOIRE DE PHYSICO-CHIMIE : **C. AUBRY**
PHYSICOCHIMICAL LABORATORY MANAGER

- BiotechMarine Z.I. – B.P.72 – 22260 Pontrieux - FRANCE Tél : +33 (0) 2 96 95 31 32 -

PerkinElmer UV WinLab Data Processor and Viewer Version 1.00.00
18/02/2014 13:41

Analyst
Date

contrôle qualité
mardi 18 février 2014 13:41



Sample Name	Description
Bioenergizer P BG PF .Sample	

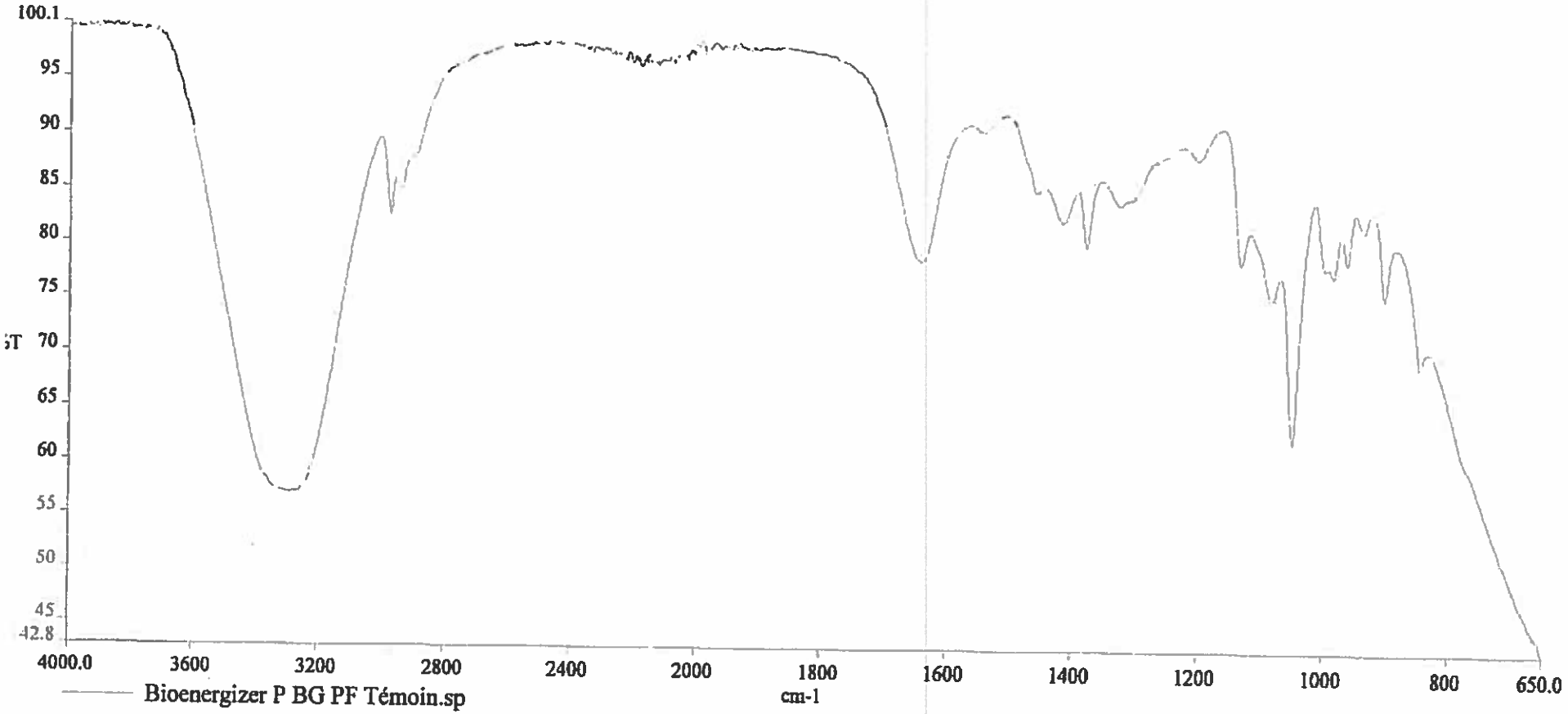
blanc = H_2O P_e = 0,2g pour 1ml H_2O

SPECTRE IRFT

Date: mardi 18 février 2014

SPECTROPHOTOMETRE SPECTRUM 100 N° de série 77774 - PERKIN ELMER

Accessoire ATR Universel N° 7031330



BIOENERGIZER P BG/PF

CTFA / INCI NAME : Aqua / Water - Butylene Glycol - Panthenol - Propylene glycol - Pelvetia Canaliculata Extract - Laminaria Digitata Extract
CAS N° : 7732-18-5 - 107.88.0 - 81-13-0 - 57-55-6 - 223751.75.5 - 90046.12.1/92128.82.0
EINECS N°: 231-791-2 - 203-529-7 - 201-327-3 - 200-338-0 - 607-055-5 - 289-980-0 - 202-785-7

DONNEES MICROBIOLOGIQUES
MICROBIOLOGICAL DATA
Numéro de référence / *Reference number* : STANDARD PROVISOIRE

CARACTERISTIQUES *CHARACTERISTICS*	METHODES *METHODS*	STANDARD *STANDARD*
Germes totaux * *Total germs **	MO MIC 002	**< 100**
Germes Pathogènes *Pathogens*		
- *Staphylococcus aureus*	MO MIC 012	**Absence** *None*
- *Candida albicans*	MO MIC 010	**Absence** *None*
- *Pseudomonas aeruginosa*	MO MIC 011	**Absence** *None*
- *Enterobacteriaceae*	MO MIC 020	**Absence** *None*
Levures / Moisissures* *Yeasts / Moulds**	MO MIC 021	**< 100**

***Les résultats sont indiqués en UFC/ml pour les liquides et en UFC/g pour les solides**

CERTIFIE CONFORME
CERTIFIED TRUE AND CORRECT
RESPONSABLE DU LABORATOIRE DE MICROBIOLOGIE : **M.TANNIOU**
MICROBIOLOGICAL LABORATORY MANAGER

Le 25/02/14

- BiotechMarine Z.I. - B.P.72 - 22260 Pontrieux - FRANCE Tél. : +33 (0) 2 96 95 31 32 -

Personal Care Products Council
Committed to Safety, Quality & Innovation

Memorandum

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

FROM: Carol Eisenmann, Ph.D.
Personal Care Products Council

DATE: September 13, 2018

SUBJECT: Undaria Pinnatifida Extract

Biotech Marine. 2016. Manufacturing Process- Phycol UP (Undaria Pinnatifida Extract in Water and Propylene Glycol).

Biotech Marine. 2008. Phycol® UP (Undaria Pinnatifida Extract in Water and Propylene Glycol) Physico-chemical data.

Palmer Research. 2004. Etude de la tolérance cutanée aiguë d'une matière première chez le volontaire adulte: Patch-test 24 heures occlusif sous contrôle dermatologique (Phycol UP - Undaria Pinnatifida Extract in Water and Propylene Glycol).

Biotech Marine. 2014. Manufacturing Process- Ephemer™ (Undaria Pinnatifida Extract in Caprylic/Capric Triglyceride).

Biotech Marine. 2016. Ephemer™ (Undaria Pinnatifida Extract in Caprylic/Capric Triglyceride) Physico-chemical data.

Groupe Dermscan. 2014. Assessment of the sensitizing potential of a cosmetic product (Ephemer Undaria Pinnatifida Extract in Caprylic/Capric Triglyceride).

Institut Pasteur de Lille. 2014. *In vitro* skin irritation: Reconstructed human epidermis test method Ephemer™ (Undaria Pinnatifida Extract in Caprylic/Capric Triglyceride).

1620 L Street, N.W., Suite 1200 | Washington, D.C. 20036 | 202.331.1770 | 202.331.1969 (fax) | www.personalcarecouncil.org

MANUFACTURING PROCESS
PHYCOL UP

HARVESTING / IDENTIFICATION (Undaria pinnatifida)

↓

DRYING

↓

GRINDING

↓

EXTRACTION WITH THE SOLVENTS WATER AND PROPYLENE GLYCOL

Addition of preservatives :
- *Methylparaben*
- *Propylparaben*

↓

FILTRATION

↓

QUALITY CONTROL

↓

PACKAGING

↓

QUALITY CONTROL

Production Manager
Jean-Marc CATROUX

24/2/16

Date de mise à jour : 19/03/2008

PHYCOL® UP

CTFA/INCI NAME : Propylene Glycol – Aqua / Water – Undaria Pinnatifida Extract
CAS : 57.55.6 – 7732.18.5 – 223751.81.3
EINECS : 200.338.0 – 231.791.2 -

DONNEES PHYSICOCHIMIQUES
PHYSICO-CHEMICAL DATA
Numéro de référence / *Reference number* : STANDARD

CARACTERISTIQUES *CHARACTERISTICS*	METHODES *METHODS*	STANDARD *STANDARD*
Aspect *Aspect*	MO PHY 002	**Liquide limpide** *Limpid liquid*
Couleur *Colour*	MO PHY 002	**Jaune (≤ 7 UG)** *Yellow*
Odeur *Odour*	MO PHY 002	**Caractéristique** *Characteristic*
pH	MO PHY 009	4,5 - 6,5
Densité (20°C) *Density*	MO PHY 024	1,030 - 1,050
Indice de réfraction (20°C) *Refractive index*	MO PHY 008	$1,382_0$ - $1,396_0$
Extrait sec (1g – 4 heures à 105°C) *Dry extract*	MO PHY 033	0,5 - 2 %
Propylène glycol *Propylene glycol*	MO PHY 001	48 - 52 %
Teneur en eau *Water content*	MO PHY 018	48 - 52 %
Acide alginique (réaction colorée) *Alginic acid (colored reaction)*	MO PHY 004	**Positive** *Positive*
Spectre UV *UV spectrum*	MO PHY 013	**Conforme au témoin** *Similar to the standard*
Spectre IR *IR spectrum*	MO PHY 011	**Conforme au témoin** *Similar to the standard*
Conservateurs *Preservatives*		
- **POB méthyle** *Methyl Paraben*	MO PHY 020	0,16 - 0,20 %
- **POB propyle** *Propyl Paraben*	MO PHY 020	0,03 - 0,06 %

PHYCOL® UP

CTFA/INCI NAME : Propylene Glycol – Aqua / Water – Undaria Pinnatifida Extract
CAS : 57 55 6 – 7732.18.5 – 223751.81.3
EINECS : 200 338 0 – 231.791.2 -

DONNEES MICROBIOLOGIQUES
MICROBIOLOGICAL DATA
Numéro de référence / *Reference number* : STANDARD

CARACTERISTIQUES *CHARACTERISTICS*	METHODES *METHODS*	STANDARD *STANDARD*
Germes totaux* *Total germs**	MO MIC 002	< 100
Germes Pathogènes *Pathogens*		
- *Staphylococcus aureus*	MO MIC 012	**Absence** *None*
- *Candida albicans*	MO MIC 010	**Absence** *None*
- *Pseudomonas aeruginosa*	MO MIC 011	**Absence** *None*
- *Enterobacteriaceae*	MO MIC 020	< 100
Levures / Moisissures* *Yeasts / Moulds**	MO MIC 021	< 100

*Les résultats sont exprimés en (UFC/ml) pour les liquides et (UFC/g) pour les solides

CERTIFIE CONFORME
CERTIFIED TRUE AND CORRECT
RESPONSABLE DU LABORATOIRE DE MICROBIOLOGIE : **M. QUINTIN**
MICROBIOLOGICAL LABORATORY MANAGER

le 19.03.08

PHYCOL® UP

CTFA/INCI NAME : Propylene Glycol – Aqua / Water – Undaria Pinnatifida Extract
CAS : 57.55.6 – 7732.18.5 – 223751.81.3
EINECS : 200.338.0 – 231.791.2 -

DONNEES PHYSICOCHIMIQUES
PHYSICO-CHEMICAL DATA
Numéro de référence / *Reference number* : STANDARD

CARACTERISTIQUES *CHARACTERISTICS*	STANDARD *STANDARD*
Métaux lourds *Heavy metals (ppm)*	
• **Arsenic mineral** *Mineral Arsenic*	< 5
• **Cadmium** *Cadmium*	< 10
• **Plomb** *Lead*	< 5
• **Nickel** *Nickel*	< 2
• **Argent** *Silver*	< 5

Iodine < 1ppm

CERTIFIE CONFORME
CERTIFIED TRUE AND CORRECT
RESPONSABLE DU LABORATOIRE DE PHYSICO-CHIMIE : **M. LE BRETON**
PHYSICOCHIMICAL LABORATORY MANAGER

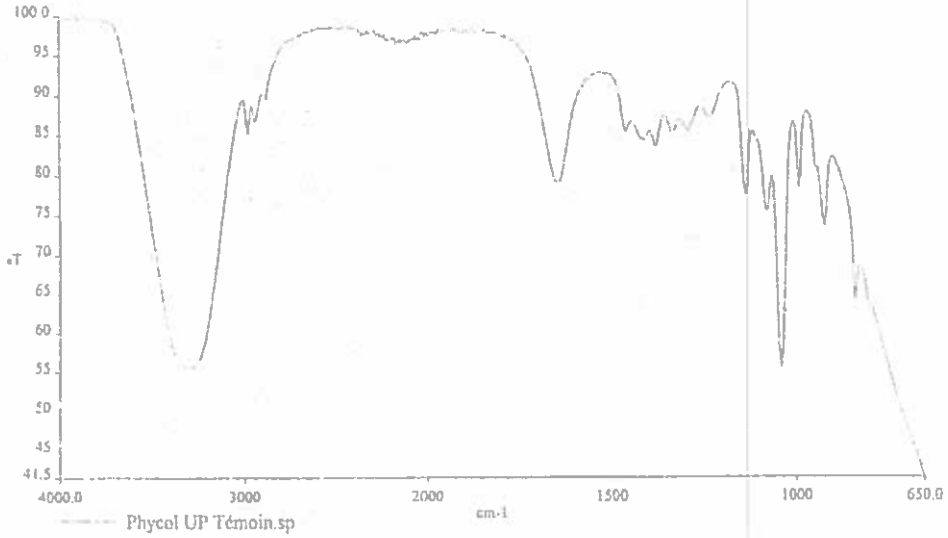
Date: lundi 28 avril 2008

SPECTRE IRFT

PERKIN ELMER

SPECTROPHOTOMETRE SPECTRUM 100 N° de série 77774

Accessoire ATR Universel N° 7031330



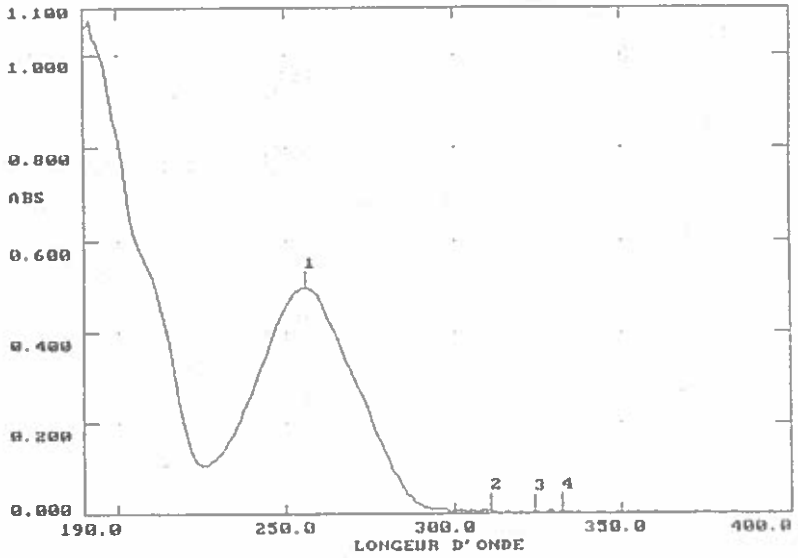
UV310 SPECTROPHOTOMÈTRE UV-VISIBLE v6.56 PAGE 1

DATE :10/06/08 SÉRIE No :104601 ID :SPECTRE UV
HEURE:13:27:04 OPÉRATEUR: N°ÉCHANTILLON:TEMOIN

TYPE SPECTRE:INTELLIGENTVITESSE:NORMAL ENTRE 2 VAL:1.0nm
LIGNE BASE:OPÉRATEUR FENTE:1.5nm CHANGER LAMPE:315nm

PICS

	1	2	3	4	5	6	7	8	9	10
λnm	256.0	311.0	324.0	332.0						
ABS	0.498	0.008	0.006	0.008						



*Blanc : H_2O

10 Jun 2008
Modèle balance
Plate-forme SAP:
Terminal SAP:
Lot Phycol UP
Signature EF

PALMER RESEARCH

Etude de la tolérance cutanée aiguë d'une matière première chez le volontaire adulte : Patch-test 24 heures occlusif sous contrôle dermatologique

Version n° 01/004 du 16 janvier 2004

GROUPE DERMSCAN

SIEGE SOCIAL - LYON
27, bd du 11 Novembre 1918
B.P. 2132
69603 VILLEURBANNE Cedex
FRANCE
Tél. : 33 (0)4 72 82 60 88
Fax : 33 (0)4 72 82 60 83

BORDEAUX
Parc Innolin - 3, rue du Golf
33700 MERIGNAC
FRANCE
Tél. : 33 (0)5 56 34 75 56
Fax : 33 (0)5 56 34 75 54

e-mail : [illegible]@dermscan.com
Internet : www.palmer-research.com

Etude : 1030478PA

Matière première : PHYCOL UP LOT 3.01.017 (58359)

Promoteur: **SECMA BIOTECHNOLOGIE MARINE**
ZI – BP 65
22260 PONTRIEUX
FRANCE

Lyon, le 16 janvier 2004

SOMMAIRE

RESUME DU RAPPORT D'ETUDE

Promoteur : **SECMA BIOTECHNOLOGIE MARINE** **Adresse : ZI – BP 65** **22260 PONTRIEUX** **FRANCE**	**Matière première:** **PHYCOL UP LOT 3.01.017** **Code PALMER Research : 58359**

ETUDE DE LA TOLERANCE CUTANEE AIGUE D'UNE MATIERE PREMIERE CHEZ LE VOLONTAIRE ADULTE : PATCH-TEST 24 HEURES OCCLUSIF SOUS CONTRÔLE DERMATOLOGIQUE

Numéro d'étude :	1030478PA	
Dates de l'étude :	du 7 au 9 janvier 2004.	
Lieu de l'étude :	PALMER RESEARCH - Groupe DERMSCAN Immeuble le CEI 2 – B.P.2132 27 Bd du 11 novembre 1918 69603 VILLEURBANNE CEDEX – FRANCE	
Objectif :	Déterminer le potentiel irritant primaire d'une matière première après application unique sous pansement occlusif pendant 24 heures chez le volontaire adulte.	
Méthodologie :	Etude en ouvert.	Nombre de sujets : 12.
Critères d'inclusion :	Peau indemne de toute lésion dermatologique, sujet non allergique.	• Durée de l'application : 24 heures. • Condition d'utilisation : pure.
Critères d'évaluation :	Détermination du score d'irritation moyen : I.I.M = score total des réactions (érythème + œdème) / nombre total de volontaires Les réactions sont cotées de 0 à 3.	
Méthodes d'analyse :	Classement de la matière première en fonction de son I.I.M : Si I.I.M < 0,20 : Non Irritante Si 0,20 ≤ I.I.M < 0,50 : Légèrement Irritante Si 0,50 ≤ I.I.M < 1 : Moyennement Irritante Si I.I.M ≥ 1 : Irritante	
Conclusion :	L'indice d'irritation moyen de la matière première **PHYCOL UP LOT 3.01.017** est égal à **0.71 (moyennement irritante)** à la lecture 30 minutes et à **0.29 (légèrement irritante)** à la lecture 24 heures.	
Investigateur : **Dr Yvette WELTERT,** ***Dermatologue***		

1 - INTRODUCTION

A la demande de la société **SECMA BIOTECHNOLOGIE MARINE - ZI - BP 65 - 22260 PONTRIEUX - FRANCE**, nous avons évalué sur 12 volontaires adultes, la tolérance cutanée aiguë ou potentiel irritant de la matière première:

PHYCOL UP LOT 3.01.017

après application unique sur la peau du dos (zone scapulaire), sous pansement occlusif maintenu pendant 24 heures (patch-test 24 heures).

Cet essai a été réalisé "en ouvert" selon la méthodologie des essais épicutanés sous occlusion.

Pour réaliser cette étude, nous avons reçu le 5 décembre 2003 un échantillon de la matière première que nous avons référencé sous le code PALMER Research 58359.

L'essai a commencé le 7 janvier pour s'achever le 9 janvier 2004.

2 - CERTIFICAT D'AUTHENTICITE DES RESULTATS

L'étude faisant l'objet du présent rapport a été conduite sous ma responsabilité, en conformité avec le protocole expérimental et dans le respect des règles des Bonnes Pratiques Cliniques.
Toutes les observations et les données numériques recueillies au cours de cet essai sont rapportées dans le présent document.

Après relecture et en tant qu'Investigateur, je certifie ces données conformes
à la réalité des résultats obtenus.
Docteur Yvette WELTERT, *Dermatologue*.

Date : 30 01 04 Signature :

Ce rapport a été audité par la personne en charge du Contrôle Qualité.
Il est considéré comme étant le reflet exact des données générées et des procédures en vigueur en rapport avec les Bonnes Pratiques Cliniques.

Date : 03.02.04 Nom : BRUNET-DUNAND Séverine

Signature :

3 - PROTOCOLE EXPERIMENTAL

L'essai a été réalisé conformément au mode opérationnel référencé « Patch test simple».

3.1 - Volontaires

3.1.1 - Caractéristiques des sujets inclus

- ✓ 12 sujets ont été inclus dans l'essai,
- ✓ dont dix de sexe féminin et deux de sexe masculin,
- ✓ âgés de 19 à 57 ans (moyenne d'âge: 36 ans).

Tous les sujets devaient répondre aux critères d'inclusion et ne présenter aucun critère de non-inclusion, dont en particulier :

3.1.2 - Critères d'inclusion

- ✓ aucun antécédent d'intolérance ou d'allergie à une matière première,
- ✓ acceptation de signature du consentement éclairé de participation,
- ✓ phototype I à III.

3.1.3 - Critères de non-inclusion

- ✓ femme enceinte ou qui allaite ou prévoyant un début de grossesse en cours d'étude,
- ✓ pathologie cutanée sur la zone d'expérience (psoriasis, eczéma, vitiligo, pytiriasis versicolor, acné, etc…),
- ✓ présence d'un traitement médicamenteux per os:
  - antihistaminiques, anti-inflammatoires et/ou antibiotiques < 1 semaine,
  - anti-tussifs et/ou corticoïdes < 4 semaines,
  - immunosuppresseur, rétinoïde et/ou anti-cancéreux < 6 mois,
- ✓ début, arrêt ou changement de traitement hormonal (y compris pilule contraceptive) < 1 mois et demi,
- ✓ exposition au soleil ou aux UV < 1 mois au niveau du dos,
- ✓ personne présentant une peau hyper irritable,
- ✓ personne présentant une pilosité importante, des taches de rousseur, des grains de beauté ou un tatouage au niveau du dos,
- ✓ sujet atteint d'une maladie grave ou évolutive,
- ✓ usage immodéré de l'alcool ou du tabac.

3.2 - Méthodologie

3.2.1 - Matériel, dose, durée

La matière première a été appliquée dans les conditions suivantes :

	PHYCOL UP LOT 3.01.017
Zone:	zone scapulaire
Type de Patch tests:	Finn Chamber® 8mm (50mm²) occlusif
Dose*:	approximativement 0.02ml
Condition de l'application:	pure, imprégnant une rondelle de papier filtre
Durée de l'application:	24 heures
Control:	patch sans produit

* Note: La raison du choix de la dose est conditionnée par la capacité de la cupule, indiquée par le fabricant des "Finn Chambers®".

3.2.2 - Lectures

Les examens macroscopiques cutanés ont été réalisés dans les mêmes conditions, en particulier au niveau de l'éclairage (lampe « lumière du jour »), 30 minutes après l'enlèvement des patchs. En l'absence de toute réaction cutanée locale à la lecture de 30 minutes après enlèvement du pansement, l'essai a été arrêté. Cependant, il a été demandé à chaque volontaire de vérifier le lendemain l'absence de réaction. Dans le cas d'une réaction visible, le sujet devait revenir au centre, des lectures pouvant être effectuées jusqu'à réversibilité des réactions cutanées.

Les cotations des éventuelles réactions d'irritation sur chaque site ayant reçu la matière première étudiée ont été réalisées comparativement au site sans produit, selon les échelles numériques suivantes :

Erythème « E » :

E = 0 : absence d'érythème.
E = 0.5: érythème très léger (à peine perceptible : coloration rosée discrète d'une partie de la surface testée).
E = 1 : érythème léger (coloration rosée discrète de toute la surface testée ou bien visible sur une partie de la surface testée).
E = 2 : érythème net (érythème net couvrant toute la surface testée).
E = 3 : érythème important (érythème intense couvrant toute la surface testée ou érythème diffusant en dehors de la surface testée

Œdème « O » :

O = 0 : absence d'œdème
O = 0.5: œdème très léger (palpable, à peine visible)
O = 1 : œdème léger (palpable et visible)
O = 2 : œdème net avec ou sans présence de papule(s) ou vésicule(s)
O = 3 : œdème important (surface débordant la zone d'application) avec ou sans présence de vésicules ou de bulle(s).

Les modifications de structure cutanée (dessèchement, rugosité, épaississement, réflectivité) pouvant être liées à la nature même de la matière première étudiée ou à l'un des ingrédients, ont fait l'objet d'une description clinique dont l'intensité de chaque modification a été appréciée selon le barème :

0,5 = douteux
1 = léger
2 = net
3 = important

3.2.3 – Interprétation des résultats

L'analyse et l'interprétation des résultats ont été réalisées en fonction des données obtenues dans les conditions expérimentales, à chaque temps de lecture.

Elles sont descriptives et complétées par le calcul d'un indice d'irritation moyen (I.I.M) à chaque temps de lecture, selon le rapport :

$$\text{I.I.M} = \frac{\sum \text{des cotations (érythème + œdème)}}{\text{Nombre de sujets}}$$

Cet indice ainsi obtenu (maximum 12), permet de classer arbitrairement la matière première étudiée selon le barème d'interprétation suivant :

I.I.M	Classe
I.I.M < 0.20	**Non irritante (NI)**
0.20 ≤ I.I.M < 0.50	**Légèrement irritante(LI)**
0.50 ≤ I.I.M < 1	**Moyennement irritante (MI)**
I.I.M ≥ 1	**Irritante (I)**

Les valeurs individuelles et la catégorie de matières premières à laquelle appartient la matière première étudiée ont également été prises en compte pour une conclusion adaptée dans les conditions de l'essai (24 heures sous pansement occlusif).

**Références bibliographiques :*

- *« Les essais cliniques en dermatologie », Thérapie, 1991, Tome 46, pages 183 à 187*
- *« Dermato-allergologie de contact », G. DUCOMBS, Editions MASSON, 1988 pages 13 à 16 ; 36-37*
- *« Dermatotoxicology Methods : The laboratory worker's VADEMECUM » ; N. MARZULLI – H. MAIBACH. Ed. Taylor & Francis, 1998.*

4 - RESULTATS

Les résultats individuels des lectures à chaque temps expérimental sont regroupés dans le tableau ci-dessous.

PHYCOL UP LOT 3.01.017
(patch test 24 heures occlusif – pure)

SUJETS					LECTURES									
N°	Identification	Age	Sexe (1)	Type de peau	Lecture 30 minutes après enlèvement du patch					Lecture 24 heures après enlèvement du patch				
					Témoin		Matière première		Modification de structure	Témoin		Matière première		Modification de structure
					E	O	E	O		E	O	E	O	
16S02	FEV Mi	55	F	Normale	0	0	1	0	-	0	0	0	0	-
18S02	LAR Cl	55	F	Normale	0	0	0.5	0	-	0	0	0	0	-
19S02	SCA La	21	F	Normale	0	0	1	0	-	0	0	0.5	0	-
20S02	SAN Va	30	F	Normale	0	0	0	0	-	0	0	0	0	-
21S02	GUI Al	19	F	Normale	0	0	0.5	0	-	0	0	0	0	-
23S02	PIC Pa	57	F	Normale	0	0	0	0	-	0	0	0	0	-
24S02	TAN So	21	F	Normale	0	0	0.5	0	-	0	0	0	0	-
25S02	SER Fr	45	F	Normale	0.5	0	0.5	0	-	0	0	0.5	0	-
26S02	BER So	21	F	Normale	0	0	0	0	-	0	0	0	0	-
27S02	FOR Gi	51	M	Normale	0	0	2	1	-	0	0	1	0	-
30S02	LOM Fr	38	F	Normale	0	0	1	0.5	-	0	0	1	0	-
31S02	KHA Gr	19	M	Normale	0	0	0.5	0	-	0	0	0.5	0	-
Age moyen		36	I.I.M		0.04		0.75		-	0		0.29		-

I.I.M	0.71	0.29
Résultats	**moyennement irritante**	**légèrement irritante**

(1) : M = masculin
F = féminin

Remarque : Le volontaire n°25S02 a présenté une très légère réaction érythémateuse au niveau de la cupule témoin 30 minutes après le retrait des patchs. Cette réaction ayant disparu à 24 heures, le sujet est inclus dans le calcul.
Le calcul de l'I.I.M est effectué par différence entre le score de la matière première et le score témoin.

5 - CONCLUSION

30 minutes après l'enlèvement du patch occlusif, huit volontaires (n°16S02, n°18S02, n°19S02, n°21S02, n°24S02, n°27S02, n°30S02 et n°31S02) ont présenté un très léger à net érythème accompagné d'un très léger à léger œdème pour les volontaires n°27S02 et n°30S02.
A la lecture 24 heures, un très léger à léger érythème était toujours observé chez les sujets n°19S02, n°27S02, n°30S02 et n°31S02. Un très léger érythème retardé était noté chez les sujets n°17S02 et n°25S02.
A la lecture 4 jours, plus aucune réaction n'était constatée.

Par ailleurs, aucun effet secondaire n'a été observé.

Dans les conditions expérimentales retenues, on peut donc conclure que la matière première **PHYCOL UP LOT 3.01.017** testée sous contrôle dermatologique, et appliquée pure et localement sous pansement occlusif pendant 24 heures, sur la peau de 12 volontaires adultes, est classée **movennement irritante** à la lecture 30 minutes et **légèrement irritante** à la lecture 24 heures selon la cotation de l'IIM.

Dr Yvette WELTERT
Dermatologue

STUDY SUMMARY REPORT

Sponsor: **SECMA BIOTECHNOLOGIE MARINE** **Address: ZI – BP 65** **22260 PONTRIEUX** **FRANCE**	**Raw material: PHYCOL UP LOT 3.01.017** **PALMER Research code: 58359**

EVALUATION OF THE ACUTE CUTANEOUS TOLERANCE OF A RAW MATERIAL ON ADULT VOLUNTEERS: 24-HOUR SINGLE PATCH TEST UNDER DERMATOLOGICAL CONTROL		
Study number:	1030478PA	
Study dates:	from January 7 to January 9, 2004.	
Study place:	PALMER RESEARCH - Groupe DERMSCAN Immeuble le CEI 2 – B.P.2132 27 Bd du 11 novembre 1918 69603 VILLEURBANNE Cedex - FRANCE	
Objective:	Determination of the acute skin tolerance of a raw material by application under occlusive patch over a 24-hour period on the adult volunteer.	
Methodology:	Open Study.	Number of subjects: 12.
Included criteria:	Skin without any dermatological lesion, non allergic volunteer.	• Application duration: 24 hours. • Condition of application: pure.
Evaluation criteria:	Calculation of the mean irritation index: $M.I.I. = \frac{\text{total cutaneous reactions score (erythema + edema)}}{\text{number of volunteers}}$ Skin responses are scored from 0 to 3.	
Analysis:	Classification of the raw material according to its M.I.I: if M.I.I. < 0.20 : Non irritating if 0.20 ≤ M.I.I. < 0.50 : Slightly irritating if 0.50 ≤ M.I.I. < 1 : Moderately irritating if M.I.I. ≥ 1 : Irritating	
Conclusion:	The irritation index of the raw material **PHYCOL UP LOT 3.01.017** is equal to **0.71 (moderately irritating)** at the 30-minute reading and to **0.29 (slightly irritating)** at the 24-hour reading.	
Dr Yvette WELTERT, ***Dermatologist***		

MANUFACTURING PROCESS

EPHEMER™

HARVESTING OF
FERTILE SPOROPHYTES OF *Undaria Pinnatifida* / IDENTIFICATION

↓

FRAGMENT ISOLATION OF GAMETOPHYTE OF *Undaria Pinnatifida*

↓

CULTURE IN LIQUID MEDIUM

↓

GAMETOPHYTE SEPARATION OF *Undaria Pinnatifida* / CULTURE MEDIUM

↓

FREEZE-DRIED GAMETOPHYTE OF *Undaria Pinnatifida*

↓

GRINDING OF GAMETOPHYTE OF *Undaria Pinnatifida* / SIEVING

↓

QUALITY CONTROL

↓

EXTRACTION WITH THE SOLVENT
CAPRYLIC/CAPRIC TRIGLYCERIDE

↓

FILTRATION

↓

QUALITY CONTROL

↓

PACKAGING

↓

QUALITY CONTROL

Production Manager
Jean-Marc CATROUX

BIOTECHMARINE (10/14/2014)

Date de mise à jour / *Updated date* : 01/12/2016

EPHEMER™

INCI NAME : Caprylic/Capric Triglyceride – Undaria Pinnatifida Extract
CAS N°: 73398-61-5 – 223751-81-3 caprylic / capric triglyceride 795%
EINECS N°: 277-452-2 –
Produit conforme Mass Balance (RSPO) BVC-RSPO-1-1972708497 / *Product Mass Balance compliant*
(Roundtable for Sustainable Palm Oil (RSPO)) BVC-RSPO-1-1972708497

Undaria Pinnatifida
<5% Extra

DONNEES PHYSICOCHIMIQUES
PHYSICO-CHEMICAL DATA

Numéro de référence / *Reference number* : Standard provisoire / *Temporary standard*

CARACTERISTIQUES *CHARACTERISTICS*	METHODES *METHODS*	STANDARD PROVISOIRE *TEMPORARY STANDARD*
Conformité Mass Balance *Mass Balance conformity*	PO-HSE-004	**Conforme** *Conform*
Aspect *Aspect*	MO PHY 002	**Liquide limpide huileux** *Oily limpid liquid*
Couleur *Colour*	MO PHY 002	**Jaune-vert pâle à vert foncé** *Pale yellow green to dark green*
Odeur *Odour*	MO PHY 002	**Faible** *Slight*
Densité (20°C) *Density*	MO PHY 024	0,920 - 0,950
Indice de réfraction (20°C) *Refractive index*	MO PHY 008	$1{,}440_0$ - $1{,}460_0$
Teneur en fucoxanthine *Fucoxanthine content*	MO PHY 084	≥ 5 mg/kg
Spectre IR *IR spectrum*	MO PHY 011	**Conforme au témoin** *Similar to the standard*
Solvant *Solvent*	**Mélange de triglycérides d'acides gras saturés (C8C10) d'origine végétale** *Mixture of triglycerides of saturated fatty acids (C8C10) of vegetal origin*	
Indice d'iode* *Iodine value**		≤ 1 g I_2/100g
Indice de saponification* *Saponification value**		320 – 350 mg KOH / g

* Indices du Caprylic/capric triglycérides (C8C10) garantis par notre fournisseur
* *Caprylic/capric triglycérides (C8C10) values guaranteed by our supplier*

BiotechMarine Z.I. – B.P. 72 – 22260 Pommeux – FRANCE Tél. +33 (0) 2 96 95 31 32

EPHEMER™

INCI NAME : Caprylic/Capric Triglyceride – Undaria Pinnatifida Extract
CAS N°: 73398-61-5 – 223751-81-3
EINECS N°: 277-452-2 –
Produit conforme Mass Balance (RSPO) BVC-RSPO-1-1972708497 / *Product Mass Balance compliant (Roumtable for Sustainable Palm Oil (RSPO)) BVC-RSPO-1-1972708497*

DONNEES PHYSICOCHIMIQUES
PHYSICO-CHEMICAL DATA

Numéro de référence / *Reference number* : Standard provisoire / *Temporary standard*

CARACTERISTIQUES *CHARACTERISTICS*	STANDARD PROVISOIRE *TEMPORARY STANDARD*
Métaux lourds (ppm)** *Heavy metals (ppm)***	
• **Arsenic mineral** *Mineral Arsenic*	< 2
• **Cadmium** *Cadmium*	< 3
• **Plomb** *Lead*	< 5
• **Nickel** *Nickel*	< 2
• **Argent** *Silver*	< 5
• **Mercure** *Mercury*	< 1

Iodine < 1 ppm

** Teneurs garanties sous contrôle statistique
** *Contents guaranteed under statistical control*

Date: vendredi 28 octobre 2016

SPECTRE IRFT

SPECTROPHOTOMETRE SPECTRUM 100 N° de série 77774 - PERKIN ELMER

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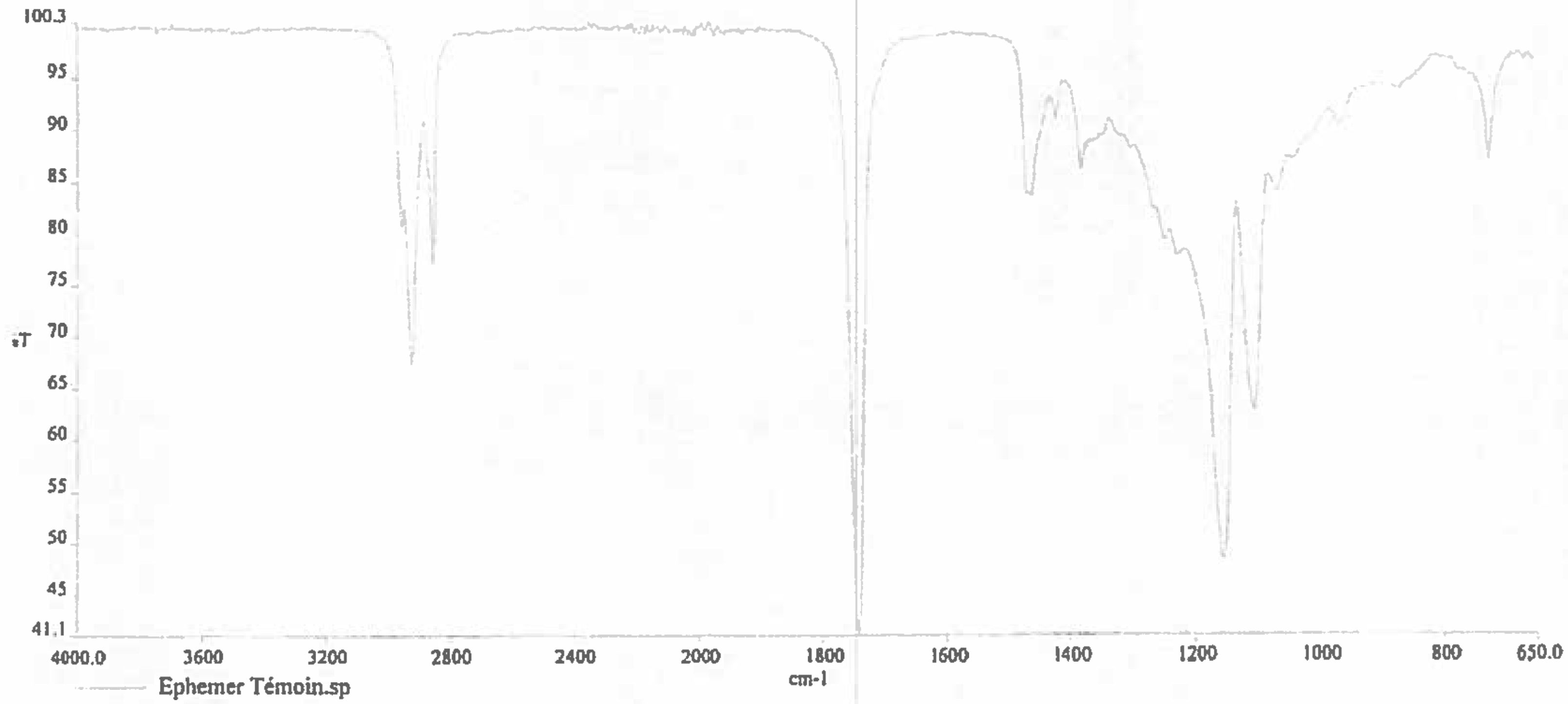

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INCI NAME : Caprylic/Capric Triglyceride – Undaria Pinnatifida Extract
CAS N°: 73398-61-5 – 223751-81-3
EINECS N°: 277-452-2 –
Produit conforme Mass Balance (RSPO) BVC-RSPO-1-1972708497 / *Product Mass Balance compliant (Roundtable for Sustainable Palm Oil (RSPO)) BVC-RSPO-1-1972708497*

DONNEES MICROBIOLOGIQUES
MICROBIOLOGICAL DATA

Numéro de référence / *Reference number* : Standard provisoire / *Temporary standard*

CARACTERISTIQUES *CHARACTERISTICS*	METHODES *METHODS*	STANDARD PROVISOIRE *TEMPORARY STANDARD*
Germes totaux*** *Total germs****	MO MIC 002 / NF EN ISO 21149	**< 100**
Germes Pathogènes *Pathogens*		
- *Staphylococcus aureus*	MO MIC 012 / NF EN ISO 22718	**Absence** *None*
- *Candida albicans*	MO MIC 010 / NF EN ISO 18416	**Absence** *None*
- *Pseudomonas aeruginosa*	MO MIC 011 / NF EN ISO 22717	**Absence** *None*
- *Escherichia coli*	MO MIC 025 / NF EN ISO 21150	**Absence** *None*
Levures / Moisissures*** *Yeasts / Moulds****	MO MIC 021 / NF EN ISO 16212	**< 100**

*** Les résultats sont indiqués en UFC/mL pour les liquides et en UFC/g pour les solides
*** *Results are indicated in CFU/mL for the liquids and in CFU/g for the solids*

CONFORME
CERTIFIED TRUE AND CORRECT
RESPONSABLE CONTROLE QUALITE: **P. SOUBIES**
QUALITY CONTROL MANAGER
01 DEC 2016

CONFORME
CERTIFIED TRUE AND CORRECT
ASSURANCE QUALITE : **A. HAMON**
QUALITY ASSURANCE
01 DEC 2016

- BiotechMarine Z.I. - B.P. 72 – 22260 Pontrieux - FRANCE Tel : +33 (0) 2 96 95 31 [illegible]

Groupe Dermscan

EVALUATION DU POTENTIEL SENSIBILISANT D'UN PRODUIT COSMETIQUE : TEST CLINIQUE FINAL DE SECURITE SOUS CONTRÔLE DERMATOLOGIQUE

ASSESSMENT OF THE SENSITIZING POTENTIAL OF A COSMETIC PRODUCT: FINAL CLINICAL SECURITY TEST UNDER DERMATOLOGICAL CONTROL

Rapport / *Report:*	**14E0898 (version 1)**
Référence etude / *Study reference:*	**DN-1344**
Produit / *Product* :	**LCA14027 - 14P0898-1** Ephemer Undaria Pinnat. F. da Extract in Caprylic / capric Triglyceride
Promoteur / *Sponsor* :	**SEPPIC** **Biotechmarine** **Z.I.** **22260 Pontrieux** **FRANCE**
C.R.O.	**DERMSCAN** Domaine Scientifique de la Doua 56, Boulevard Niels Bohr **69623 VILLEURBANNE Cedex - FRANCE**
Moniteur de l'étude / *Study Monitor*	**LISKIN - Dr. Bogdan WICHROWSKI** IMMEUBLE FONTENAY AFFAIRES 91, rue Boucicaut **92260 FONTENAY-AUX-ROSES - FRANCE**
Investigateur / *Investigator*	**PROCOS - Dr Marlena NOWAKOWSKA**

Lyon,16/07/2014

Groupe Dermscan

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Investigateur / *Investigator*	**PROCOS - Dr Marlena NOWAKOWSKA**

Lyon,16/07/2014

RAPPORT / *REPORT*

REFERENCE ETUDE / *STUDY REFERENCE*	**DN-1344/14E0898**
PRODUIT / *PRODUCT*	**«LCA14027 - 14P0898-1»**
NOMBRE DE SUJETS / *NUMBER OF SUBJECTS*	**100**
PROMOTEUR / *C.R.O*	**Groupe DERMSCAN**
MONITEUR / *MONITOR*	**LISKIN** **IMMEUBLE FONTENAY AFFAIRES** **91, rue Boucicaut** **92260 FONTENAY-AUX-ROSES** **FRANCE** **☎ : 33 (0)9 50 27 08 28** **🖷 : 33 (0)1 49 73 66 80**
INVESTIGATEUR / *INVESTIGATOR*	**Dr Marlena NOWAKOWSKA,** **Médecin Dermatologue / *Dermatologist***

Document comportant 28 pages / 28 pages document

SOMMAIRE / INDEX

RESUME DE L'ETUDE - *STUDY SUMMARY*

TITRE : TEST CLINIQUE FINAL DE SECURITE : ETUDE DU POUVOIR SENSIBILISANT D'UN PRODUIT, SELON LA METHODE DE MARZULLI-MAIBACH SUR 100 SUJETS PENDANT 6 SEMAINES.

TITLE : CLINICAL FINAL SECURITY TEST: *SENSITIZING POTENTIAL STUDY OF A PRODUCT ACCORDING TO MARZULLI-MAIBACH METHOD, ON 100 SUBJECTS DURING 6 WEEKS*

PRODUIT / PRODUCT: LCA14027 - 14P0898-1

REALISATION DE L'ETUDE : L'étude a été réalisée et les valeurs numériques saisies par l'Unité Clinique PROCOS, localisée en Pologne ; ul. Slowackiego 27/33 lok. 33/34 ; 01-592 Varsovie.
STUDY IMPLEMENTATION: The study was carried out and all test values recorded by the Clinical Unit PROCOS, localized in Poland, ul. Slowackiego 27/33 lok. 33/34, 01-592 Warsaw.

INVESTIGATEUR / INVESTIGATOR: Dr Marlena NOWAKOWSKA

PROTOCOLE : TEST DE MAXIMALISATION SELON MARZULLI-MAIBACH
PROTOCOL: SENSITIZATION TEST ACCORDING TO MARZULLI-MAIBACH METHOD.

BUT DE L'ETUDE : Evaluer sous contrôle dermatologique le potentiel irritant et sensibilisant d'un produit dans les conditions prévues par le promoteur de l'étude.
AIM OF THE STUDY: To evaluate the sensitizing potential of a product under dermatological control and under the conditions defined by study's sponsor.

SUJETS : 100 volontaires à peau normale correspondant aux critères d'inclusion et de non-inclusion déterminés par le Groupe DERMSCAN.
SUBJECTS: 100 healthy volunteers with normal skin corresponding to the inclusion and non-inclusion criteria defined by the DERMSCAN Group.

PERIODE DE L'ETUDE / STUDY DATE: 02/06/14 - 11/07/14 / *June 2nd to July 11th, 2014*

PLAN EXPERIMENTAL : Etude monocentrique en simple aveugle.
STUDY DESIGN: *Monocentric and simple blind study.*

PRINCIPAUX PARAMETRES DE TOLERANCE :

- Potentiel irritant (phase d'induction)
  Erythème, œdème, sécheresse, vésicules évalués par le dermatologue selon un score de 0 à 3
- Potentiel sensibilisant (phase de révélation)
  Réaction évaluée par le dermatologue selon un score de 0 à 3 établis par l'ICDRG (International Contact Dermatitis Research Group)

MAIN TOLERANCE PARAMETERS:

- *Irritation potential (Induction Phase)*
  Erythema, edema, desquamation, vesicles rated from 0 to 3 by the dermatologist
- *Sensitizing potential (Challenge Phase)*
  Reaction rated from 0 to 3 by the dermatologist according to ICDRG (International Contact Dermatitis Research Group)

RESULTATS - *RESULTS:*

Dénomination du produit - *Product name*	**POTENTIEL IRRITANT** *IRRITATION POTENTIAL*	**POTENTIEL SENSIBILISANT** *SENSITIZING POTENTIAL*
LCA14027 - 14P0898-1	non irritant *non-irritating*	Aucune réaction de type allergique *No allergic reaction*

CONCLUSION :
Dans les conditions de cette étude, le produit «LCA14027 - 14P0898-1» s'est avéré **non irritant** et **non sensibilisant.**

CONCLUSION :
*Under these study conditions, the product «LCA14027 - 14P0898-1» can be considered **non-irritating** and **non-sensitizing**.*

1. ASSURANCE QUALITE / *QUALITY ASSURANCE*

L'étude a été réalisée selon les règles des Bonnes Pratiques Cliniques définies par les ICH Topic E6 "Note for Guidance and good clinical practice" (CPMP/ICH/135/95), par la Déclaration d'Helsinki (1964, WMA) et ses mises à jours successives), par la CEE (Directives n° 91/507 et III 3976/88 EN du 11/07/1990) et par le Ministère de la Santé de la République Française.

The described study has been conducted according to the Good Clinical Practice defined by the ICH Topic E6 "Note for Guidance and good clinical practice" (CPMP/ICH/135/95), the Helsinki Declaration (1964, WMA) and its succesives updates, the EEC (Directives n° 91/507 and III 3976/88 of 11/07/1990) and to the Ministry of Health of the French Republic.

Il est de la responsabilité de l'industriel, fabricant du produit testé, de justifier qu'aucune substance constituant ce produit n'est sensibilisante.

The first evaluation of sensitization risks for all ingredients depends on the responsibility of the tested product manufacturer.

L'étude a été menée selon les Procédures Opératoires Standards et selon le protocole de l'étude défini par le promoteur.
Les cahiers d'observation et les journaux de suivi ont été vérifiés ainsi que l'exactitude des données.

The study has been conducted according to Standard Operating Procedures and to the study protocol defined by the sponsor. All study events recorded during the study are reported.

L'authenticité et la véracité des données expérimentales recueillies ont été confirmées par les personnes ayant participé à l'étude (ANNEXE II).

Controls on data veracity and conformity with the protocol have been performed and confirmed by persons participating in the study (APPENDIX II).

2. CERTIFICAT DE CONFORMITE / *CERTIFICATE OF CONFORMITY*

A ma connaissance, l'étude DN-1344/14E0898 a été conduite en accord avec l'**«Assurance qualité»** précitée.
I am aware that the study DN-1344/14E0898 *has been conducted according to the «**Quality Assurance**» described before.*

Il ne s'est pas produit d'événement susceptible d'affecter la qualité ou l'intégrité des données.
There was no event which may have affected the quality or integrity of the data.

Mme Charlotte OEHMICHEN
Directeur technique / Technical director

16/07/2014
date

3. METHODOLOGIE / *METHOD*

3.1 PRODUIT A L'ETUDE / *STUDY PRODUCT*

Le produit fourni par le Groupe DERMSCAN, présentait les caractéristiques suivantes :
The product supplied by Group DERMSCAN, had the following characteristics :

Dénomination du produit - *Product name*	Aspect du produit Product aspect	Code du produit *Product code*
LCA14027 - 14P0898-1	liquide jaune transparent *transparent yellow liquid*	KA

Le produit a été réceptionné le 26/05/2014.
The product was receipted on May 26th, 2014.

3.2 METHODES CLINIQUES / CLINICAL METHODS

3.2.1 Objectif de l'étude / Aim of the study

Evaluer le pouvoir irritant et sensibilisant du produit par la méthode de Marzulli-Maibach.
To evaluate irritating and the sensitizing potential of a product by Marzulli-Maibach method.

3.2.2 Plan expérimental / *Experimental design*

L'étude a été réalisée en ouvert.
This was an open study

3.2.3 Sujets de l'étude / *Study subjects*

Critères d'inclusion

- Volontaire sain d'origine caucasienne
- Age compris entre 18 et 70 ans
- Phototype II, III ou IV
- Personne ne présentant ni cicatrice, ni tatouage, ni tache pigmentaire d'aucune sorte, ni pilosité trop importante, ni lésion dermatologique, ni traces d'un maillot de bain au niveau du dos
- Personne ayant donné par écrit son consentement libre, éclairé et exprès
- Sujet coopérant, averti de la nécessité et de la durée des contrôles permettant d'espérer une parfaite adhésion au protocole mis en place par le Groupe DERMSCAN.

Inclusion criteria

- *Healthy volunteer of Caucasian origin*
- *Age between 18 and 70*
- *Phototype II, III or IV*
- *Volunteer without scars, active dermal lesions, tattoos, any pigmentary marks, excessive pilosity and uneven skin tones of the areas of the back to be tested.*
- *Subjects having given their informed, written consent*
- *Cooperative subjects, aware of the necessity and duration of controls so that perfect adhesion to the protocol established by the DERMSCAN Group could have been expected.*

Critères d'exclusion

- Femme enceinte ou qui allaite
- Exposition au soleil ou aux U.V. depuis 15 jours avant le début et pendant l'étude et /ou ayant reçu des photopatch-tests depuis moins de 2 mois
- Peau hyper irritable ou pathologie cutanée,
- Allergique ou sensibilité connues au sparadrap et /ou aux produits cosmétiques
- Pathologie cutanée, cicatrices, grains de beauté, tache de rousseur ou toute anomalie sur la zone d'expérience et/ou présentant une lucite
- Maladie grave ou évolutive
- Sujet suivant un traitement médicamenteux topique ou systémique :
  - anti-inflammatoires et/ou antihistaminiques pendant la semaine qui précède et durant l'étude
  - substances photosensibilisantes et /ou phototoxiques depuis moins d'un mois et pendant l'étude
  - immunosuppresseurs et /ou corticoïdes pendant les 4 semaines qui précèdent et durant l'étude
  - rétinoïdes pendant les 6 mois précédant l'étude et durant l'étude
- Troubles dus à l'absorption excessive d'alcool ou de substances toxiques.

Non-inclusion criteria

- *Pregnant or nursing women*
- *Sun exposure or UV exposure 15 days before or during the study and/or photopatch-tests from less than 2 months*
- *Hyperirritable skin or cutaneous pathology*
- *Known allergies or sensitivities to adhesive plaster and/or cosmetics products*
- *History of abnormal responses to sunlight or presence of active dermal lesions, Scars, beauty spots, freckle or any abnormality, on the back*
- *History of cancer or other important disease*
- *Volunteers undergoing a topical or systemic treatment:*
  - *anti-inflammatories and/or anti-histamines during the previous week and during the study*
  - *photo-allergic and/or phototoxic substances from less than 1 month and during the study*
  - *immuno-suppressors and/or corticoids during the four previous weeks and during the study*
  - *retinoids during the six previous months and during the study*
- *Excessive use of alcohol, tobacco and toxic substances.*

Inclusion

100 sujets volontaires ont été choisis en accord avec les critères d'inclusion et les critères d'exclusion, et 100 sujets ont réalisé la totalité de l'étude. Le tableau suivant regroupe les informations concernant la participation à l'étude de tous les sujets sélectionnés.

100 healthy volunteers were selected according to the inclusion and the non-inclusion criteria, and 100 subjects completed study. The table below presents the information concerning all the included volunteers.

	Non inclus *Non included*	Inclus *Included*	Arrêt en cours d'étude *Drop out*	Perdus de vue *Untraceable*
Nombre de sujets *Number of subjects*	0	100	0	0

Caractéristiques des sujets / *Subjects characteristics*

Le tableau récapitulatif ci-dessous présente une synthèse des observations concernant uniquement les volontaires inclus dans l'analyse des données.
The summary table below presents a synthesis of the observations concerning exclusively the volunteers taken into account for data analysis

Nombre de Volontaires *Number of subjects*	Sexe *Sex*	Age (moy±SEM) *Age (mean±SEM)*	Phototype	Evénements médicaux ou chirurgicaux et traitements médicaux *Medical or surgical events and medical treatments*	
				avant l'étude *Before the study*	pendant l'étude *During the study*
100	81 F 19 M	43 ± 1	II : 100 III : 0 IV : 0	cf. Tableaux en ANNEXE II *cf. Tables in the APPENDIX II*	

3.3 MATERIEL / MATERIAL

Les patch-tests utilisés sont des bandes de Webril ® ($4cm^2$) qui assure une semi occlusion. Après application du produit sur le patch, ce dernier est appliqué au niveau de la zone scapulaire des volontaires.

The semi-occlusive patch-tests used are Band of Webril® ($4cm^2$) ensuring a semi occlusion. The product is applied on the semi-occlusive patch test which is then placed on the volunteer's back.

4 APPLICATION DU PRODUIT / *PRODUCT APPLICATION*

Zones d'application	Zones scapulaires : homolatérale (zone d'induction) et controlatérale (zone de révélation)
Quantité et concentration appliquée	50 µl pur
Fréquence	Phase d'induction : 3 fois par semaine pendant 48 heures Phase de révélation : 1 fois pendant 48 heures
Durée	Phase d'induction : 3 semaines Phase de latence : 2 semaines Phase de révélation : 1 semaine
Conditions d'application	Avant application, la peau a été préalablement nettoyée et séchée. Le produit «LCA14027 - 14P0898-1» a été déposé dans un patch semi-occlusif (avec papier filtre), et appliqué sur le dos du volontaire. Un patch ne contenant aucun produit a été appliqué dans les mêmes conditions et a servi de témoin non traité. Durant toute la phase d'induction, la zone homolatérale n'a pas été mouillée. Les volontaires se sont douchés le dimanche après le retrait des patchs en faisant attention à ne pas mettre de produit détergent sur les sites. Lors de la Phase de Révélation, aucun lavage ni aucune application de quelconque produit n'ont été effectués sur la zone controlatérale.

Application area	*Scapular zones: homolateral (induction zone) and controlateral (challenge zone)*
Quantity and Concentration applied	*50 µl* *pure*
Frequency	*Induction Phase: 3 times a week during 48 hours* *Challenge Phase: once during 48 hours*
Contact time	*Induction Phase: 3 weeks* *Rest Phase: 2 weeks* *Challenge Phase: 1 week*
Application conditions	*Before application, the skin was cleaned and dried. The product «LCA14027 - 14P0898-1» was applied in a semi-occlusive patch with filter paper and applied to the volunteer's back.* *The patch containing no product was applied under the same conditions to serve as a non-treated control.* *During the whole induction phase, the homolateral zone was not wet.* *Volunteers took a shower on Sunday, after patches removing, and paid attention not to put a detergent product on all tested zones. During all the challenge phase, no washing and no product application took place on the controlateral zone.*

5 DEROULEMENT DE L'ETUDE / *STUDY SCHEDULE*

Phase d'induction - trois semaines (S1, S2, S3)
Induction phase – 3 weeks (W1, W2, W3)
S1 / *W1:*

Jour de la semaine *Day of the week*	Lu *Mo*	Ma *Tu*	Me *We*	Je *Th*	Ve *Fr*	Sa *Sa*	Di *Su*
Jour d'étude *Study day*	J1 *D1*	J2 *D2*	J3 *D3*	J4 *D4*	J5 *D5*	J6 *D6*	J7 *D7*
Application du produit *Product application*	↓		↓		↓		

S2 / *W2:*

Jour de la semaine *Day of the week*	Lu *Mo*	Ma *Tu*	Me *We*	Je *Th*	Ve *Fr*	Sa *Sa*	Di *Su*
Jour d'étude *Study day*	J8 *D8*	J9 *D9*	J10 *D10*	J11 *D11*	J12 *D12*	J13 *D13*	J14 *D14*
Application du produit *Product application*	↓		↓		↓		

S3 / *W3:*

Jour de la semaine *Day of the week*	Lu *Mo*	Ma *Tu*	Me *We*	Je *Th*	Ve *Fr*	Sa *Sa*	Di *Su*
Jour d'étude *Study day*	J15 *D15*	J16 *D16*	J17 *D17*	J18 *D18*	J19 *D19*	J20 *D20*	J21 *D21*
Application du produit *Product application*	↓		↓		↓		

Après enlèvement du dernier patch de la phase d'induction à leur domicile, il est demandé aux volontaires de se présenter à la clinique à J22 en cas d'apparition d'un nouveau signe (ou de dégradation d'un signe existant à J19)
After having removed the last patch of the induction phase at home, it was asked to the subjects, to come at the clinical unit D22 if a new sign appeared (or deterioration of an existing sign D19).

Phase de latence - deux semaines (S4, S5) *Rest Phase - 2 weeks (W4, W5)*
Pas de lecture – *No reading*
S4/ *W4* :

Jour de la semaine *Day of the week*	Lu *Mo*	Ma *Tu*	Me *We*	Je *Th*	Ve *Fr*	Sa *Sa*	Di *Su*
Jour d'étude *Study day*	J22 *D22*	J23 *D23*	J24 *D24*	J25 *D25*	J26 *D26*	J27 *D27*	J28 *D28*

S5 / *W5:*

Jour de la semaine *Day of the week*	Lu *Mo*	Ma *Tu*	Me *We*	Je *Th*	Ve *Fr*	Sa *Sa*	Di *Su*
Jour d'étude *Study day*	J29 *D29*	J30 *D30*	J31 D31	J32 *D32*	J33 *D33*	J34 *D34*	J35 *D35*

Phase de révélation (double challenge test) - une semaine (S6)
Challenge Phase - 1 week (W6)
S6 / *W6* :

Jour de la semaine *Day of the week*	Lu *Mo*	Ma *Tu*	Me *We*	Je *Th*	Ve *Fr*
Jour d'étude *Study day*	J36 D36	J37 D37	J38 D38	J39 D39	J40 D40
Application du produit *Product application*	↓				
Jour d'étude *Study day*			L		L

6 CRITERES D'EVALUATION / *ASSESSMENT CRITERIA*

6.1 CRITERES CLINIQUES CONCERNANT LE POTENTIEL IRRITANT (PHASE D'INDUCTION)
CLINICAL CRITERIA REGARDING THE IRRITATING POTENTIAL (INDUCTION PHASE)

Après chaque application, le patch est enlevé et la lecture est effectuée 30 minutes plus tard pour éliminer l'effet de pression, d'occlusion et d'arrachement dû au matériel.
Le test est négatif si la peau garde un aspect normal.
Les quatre critères suivants sont évalués par le dermatologue selon une cotation de 0 à 3 :

After each application, the patch is removed and the clinical examination is performed by the investigator 30 minutes later in order to eliminate the pressure and the occlusion effects.
The result of examination is negative if the skin looks normal.
The clinical examination is made on the back using the following criteria and scale (Quotation 0 to 3):

Score	Quotation	CRITERES : description *CRITERIA : description*			
		ERYTHEME *ERYTHEMA*	OEDEME *EDEMA*	SECHERESSE *DRYNESS*	VESICULES *VESICLES*
0	absent	Aspect normal *Normal aspect*	Aspect normal *Normal aspect*	Aspect normal *Normal aspect*	Aspect normal *Normal aspect*
1	Léger *slight*	Coloration rosée discrète de toute la surface testée ou bien visible sur une partie de la surface testée *Discreet pink coloration of the whole tested area or rather visible on part of the tested area*	Plus palpable que visible *More palpable than visible*	Desquamation fine discrète, aspect dépoli *Discreet thin desquamation, tarnished aspect*	Vésicules plus palpables que visibles *More palpables than visible vesicles*
2	Net *obvious*	Erythème net couvrant toute la surface testée *Marked erythema covering the whole tested area*	Œdème visible *Visible edema*	Desquamation visible, aspect écailleux *Visible desquamation, flaky aspect*	Vésicules visibles *Visible vesicles*
3	Important *important*	Erythème intense couvrant toute la surface testée ou érythème diffusant en dehors de la surface testée *Severe erythema covering the whole tested area or erythema diffusing beyond the tested area*	Pouvant déborder de la surface testée *Edema diffusing beyond the tested area*	Desquamation importante, fissuration *Important desquamation, cracking*	Vésicules débordant de la zone testée ou bulles *Vesicles diffusing beyond the tested area or blisters.*

6.2 CRITERES CLINIQUES CONCERNANT LE POTENTIEL SENSIBILISANT (PHASE DE REVELATION)

CLINICAL CRITERIA REGARDING THE SENSITIZING POTENTIAL (CHALLENGE PHASE)

Les réactions allergiques ont été évaluées selon l'échelle suivante :
The allergic reactions were evaluated according to the following scale:

Critère - *Criterion*	Cotation ICDRG* *ICDRG (*)Quotation*	Cotation "notée" *Numeric score Quotation*
Absence de réaction *No reaction*	0	0
Réaction douteuse *Doubtful reaction*	?	?
Erythème et œdème *Erythema and edema*	+	1
Erythème, œdème et vésicules *Erythema, edema and vesicles*	++	2
Réaction forte avec présence de bulles ou d'ulcérations post-bulbeuses *Severe reaction with blisters*	+++	3

* (International Contact Dermatitis Research Group)

6.3 MODE D'EVALUATION / *ASSESSMENT METHOD*

6.3.1 Pouvoir irritant - Phase d'induction / *Irritating potential - Induction Phase*

A l'issue des 8 lectures de la phase d'induction, le score moyen de chaque volontaire a été calculé en additionnant les scores obtenus à chacune des lectures et en divisant cette somme par le nombre effectif de lectures effectuées à la clinique (une lecture n'était pas prise en compte s'il y avait réaction au témoin ou irritation globale).
Le pouvoir irritant du produit a été évalué lors de la phase d'induction, en faisant la moyenne des réactions survenues.
Le pouvoir irritant du produit a été déterminé selon la formule suivante :

At the conclusion of the 8 readings of the induction phase, the average score of every volunteer was calculated by adding the scores obtained for each of the readings and by dividing this sum by the actual number of readings made at the clinical unit (a reading was not taken into account if there was reaction of the control or global irritation).
The irritating power of the product was estimated, by calculating the mean of the reactions observed.
The irritating power of the product was determined according to the following formula

$$\text{Score moyen} = \frac{[(\sum \text{scores J1...J19 / nb de lectures) vol1} + ... + (\sum \text{scores J1...J19 / nb de lectures) volN}]}{\text{nb de volontaires (N)}}$$

$$\textit{Average score} = \frac{[(\sum \textit{scores D1...D19 / nb of readings) vol1} + ... + (\sum \textit{scores D1...D19 / nb of readings) volN}]}{\textit{nb of volunteers (N)}}$$

Score moyen *Average score*	Pouvoir irritant *Irritating Potential*
0,000 – 0,080	Non irritant *Non-irritating*
0,081 – 0,160	Très légèrement irritant *Very slightly-irritating*
0,161 – 0,560	Légèrement irritant *Slightly-irritating*
0,561 – 1,000	Modérément irritant *Moderately-irritating*
1,001 – 1,600	Fortement irritant *Strongly-irritating*
> 1,600	Très fortement irritant *Very strongly-irritating*

6.3.2 Pouvoir sensibilisant - Phase de révélation
Sensitizing potential - Challenge Phase

Une réaction allergique éventuelle au cours des Phases d'Induction ou de Révélation était notée de 0 à 3 selon les critéres de l'ICDRG (International Contact Dermatitis Research Group) – voir le tableau en paragraphe 6.2.
Lors de la révélation, une lecture sera faite 30 minutes après enlèvement des patch-tests puis 48h plus tard

The possible allergic reaction, during the Induction or Challenge Phase, was rated from 0 to 3 according to ICDRG (International Contact Dermatitis Research Group) – see the table paragraph 6.2.
During the Challenge Phase, the reading took place 30 minutes after patch-tests removal and 48 hours later.

Le pouvoir sensibilisant du produit a été évalué lors des lectures à J38 et J40 (phase de révélation) en fonction des critères suivants : réaction ++ (2) ou +++ (3) en l'absence de phénomène d'irritation surajouté.

The sensitizing potential of the product was assessed by the readings on D38 and D40 (Challenge Phase) according to the following criteria: reaction ++ (2) or +++ (3) in the absence of added irritation phenomenon.

La survenue d'un seul cas de sensibilisation active **(score supérieur ou égale à ++ (2))** du côté controlatérale conduit à la conclusion : « Produit potentiellement sensibilisant ».

*The presence of only one case of active sensitizing **(upper or equal score in ++ (2))** on controlateral side leads to the conclusion "Potentially sensitive product".*

7 ARRET PREMATURE / *PREMATURE STUDY TERMINATION*

Les sujets avaient le droit de sortir de l'essai à tout moment pour quelle que raison que ce soit.

The subjects had the right to leave the study at any time whatever the reason.

L'arrêt prématuré peut être du à des multiples raisons :
- non respect du calendrier des visites par le sujet
- événements indésirables (incluant les maladies intercurrentes)
- violations et déviations au protocole
- sorties après retrait du consentement du sujet.

The premature study termination could be due to multiple reasons:
- *non-compliance with the visits schedule,*
- *adverse events (including intercurrent diseases),*
- *protocol non-adherence/departures from protocol,*
- *withdrawal of subject consent*

Le médecin investigateur peut interrompre l'essai en cours, soit sur certains sujets, soit sur l'ensemble du panel, notamment, si le produit entraine des réactions cutanées importantes ou anormales, ou s'il juge que la poursuite de l'essai peut nuire à la santé du ou des sujets concernés.

The doctor investigator can interrupt the essay either on certain subjects or on the the whole panel, if the product induces important or abnormal cutaneous reactions or if he considers that the continuation of the essay can damage health of one or several concerned subjects.

8 AMENDEMENTS AU PROTOCOLE / *PROTOCOL AMENDMENT*

Néant / None.

9 RESULTATS / *RESULTS*

9.1 POUVOIR IRRITANT: PHASE D'INDUCTION / *IRRITATING POTENTIAL: INDUCTION PHASE*

Le TABLEAU DES LECTURES durant la phase d'induction est présenté en ANNEXE III.
The TABLE OF READINGS regarding the Induction Phase is presented in the APPENDIX III.

Ces lectures effectuées 30 minutes après le retrait des patch-tests ont montré les résultats suivants :
The readings done 30 minutes after having removed the patch-tests showed the following results:

Produit *Product*	score	J3 *D3*		J5 *D5*		J8 *D8*		J10 *D10*		J12 *D12*		J15 *D15*		J17 *D17*		J19 *D19*		Conclusion
		n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	
KA	T+	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	non irritant *non-irritating* (IRR = 0.000)
	0 :	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	
	1 :	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
	2 :	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
	3 :	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	

T+ = Témoin positif / *Positive control*
VM = valeur manquante / *missing value*
% = % of subjects / *% of subjects*
IRR = irritation globale / *global irritation*
n = nombre de sujet / *number of subjects*

Dans les conditions de cette étude, le produit «LCA14027 - 14P0898-1» a montré un score inférieur à 0,080.
Il peut donc être considéré comme non irritant.

Under these study conditions, the product «LCA14027 - 14P0898-1» showed a score lower than 0.080.
It can thus be considered as non-irritating.

9.2 POTENTIEL SENSIBILISANT : PHASE DE REVELATION
SENSITIZING POTENTIAL: CHALLENGE PHASE

Le TABLEAU DES LECTURES durant la phase de révélation est présenté en ANNEXE IV.
Les lectures effectuées 30 minutes et 48 heures après le retrait des patch-tests de révélation ont donné les résultats suivants :

The TABLE OF READING regarding the Challenge Phase is presented in APPENDIX IV.
These reading made 30 minutes and 48 hours after having removed the patch-tests showed the following results:

Code Produit : KA *Product Code : KA*	Zones	score	Jour de lecture *Day of the reading*				Résultat global *Global result*
			J38 / *D38*		J40 / *D40*		
			n	%	n	%	
LCA14027 - 14P0898-1	Lectures zone homolatérale *Homolateral zone readings*	T+ :	0	0	0	0	**non sensibilisant** *non-sensitizing*
		0 :	100	100	100	100	
		? :	0	0	0	0	
		1 :	0	0	0	0	
		2 :	0	0	0	0	
		3 :	0	0	0	0	
	Lectures zone controlatérale *Controlateral Zone readings*	T+ :	0	0	0	0	
		0 :	100	100	100	100	
		? :	0	0	0	0	
		1 :	0	0	0	0	
		2 :	0	0	0	0	
		3 :	0	0	0	0	

KA = LCA14027 - 14P0898-1

T+ = Témoin positif / Positive control
IRR = irritation globale / global irritation
VM = valeur manquante / missing value
n = nombre de sujet / *number of subjects*
% = % of subjects / *% of subjects*

Dans les conditions de cette étude, aucune réaction ++ (2) ou +++ (3) ont été constatées. Le produit «LCA14027 - 14P0898-1» peut donc être considéré comme non sensibilisant.

Under these study conditions no reaction ++ (2) nor +++ (3) were observed, so the product «LCA14027 - 14P0898-1» can be considered non-sensitizing.

10 CONCLUSION

Dans les conditions de cette étude, le produit «LCA14027 - 14P0898-1» s'est avéré non irritant et non sensibilisant.

Under these study conditions, the product «LCA14027 - 14P0898-1» can be considered non-irritating and non-sensitizing.

ANNEXE / *APPENDIX*

***ANNEXE I** / APPENDIX I*

FEUILLE D'AUTHENTIFICATION DES RESULTATS

AUTHENTIFICATION PAGE

IROCOS

KARTA AUTENTYCZNOŚCI REZULTATÓW
FICHE D'AUTHENTIFICATION DES RESULTATS
AUTHENTIFICATION PAGE

Według posiadanych przeze mnie informacji, badanie Nr :
A ma connaissance l'étude N° :
I am aware that the study N° :

DN – 1344

było przeprowadzone zgodnie PROTOKOŁEM oraz KARTĄ PARAMETRÓW TESTU.
a été conduite en accord avec le PROTOCOLE et la FICHE DES PARAMETRES D'ETUDE.
has been conducted according to the PROTOCOL and to the STUDY PARAMETERS PAGE.

Mgr inż. Barbara WAŁEJKO Odpowiedzialna za badania *Responsable d'unité* Unit head	podpis / *signature*	*11/07/2014* data *date*
Dr Marlena NOWAKOWSKA Lekarz dermatolog *Médecin dermatologue* Dermatologist	podpis / *signature*	*11/07/2014* data *date*
Mgr Magdalena KUREK Odpowiedzialna za jakość *Responsable qualité* Responsible for quality control	podpis / *signature*	*11/07/2014* data *date*
Barbara SZULC Asystentka *Assistante*	podpis / *signature*	*11/07/2014* data *date*

ANNEXE II / *APPENDIX II*

CARACTERISTIQUES DES VOLONTAIRES

SUBJECTS CHARACTERISTICS

CARACTERISTIQUES DES VOLONTAIRES
SUBJECTS CHARACTERISTICS

N° Volontaire / *Subject N°*	Identification du sujet / *Identification of subject*	Age *Age*	Sexe / *Sex*	Phototype *Phototype*	Nature de la peau / *Skin type*	Evénements médicaux ou chirurgicaux et traitements médicaux *Medical or surgical events and medical treatments*	
						avant l'étude *before the study*	pendant l'étude *during the study*
1	OLEIR	53	M	II	N	-	-
2	CHWKR	23	M	II	N	-	-
3	ZGOMA	36	F	II	N	-	-
4	ARKEL	40	F	II	N	-	-
5	GESLU	29	M	II	N	-	-
6	MIEAL	44	F	II	N	-	-
7	DLUKA	34	F	II	N	-	-
8	TABST	20	M	II	N	-	-
9	GILAN	47	F	II	N	-	-
10	SZARY	58	M	II	N	-	-
11	BRUMA	48	F	II	N	-	-
12	PTAPI	49	M	II	N	-	-
13	NIEIR	34	F	II	N	-	-
14	JAGJA	58	M	II	N	-	-
15	BRZKR	51	F	II	N	-	-
16	GLAJO	61	F	II	N	-	-
17	GORBO	31	M	II	N	-	-
18	SUSAN	60	M	II	N	-	-
19	ADARO	48	M	II	N	-	-
20	STEST	27	F	II	N	-	-
21	MARBA	53	F	II	N	-	-
22	WNUCZ	52	F	II	N	-	-
23	PIEDO	41	F	II	N	-	-
24	ABREM	27	F	II	N	-	-
25	KISDO	42	F	II	N	-	-
26	DABWE	21	F	II	N	-	-
27	OKRKR	34	M	II	N	-	-
28	KROMA	36	F	II	N	-	-
29	CZAJA	50	M	II	N	-	-
30	JANBO	41	F	II	N	-	-
31	MAJAN	23	F	II	N	-	-
32	WIKIW	55	F	II	N	-	-
33	KUTDA	27	F	II	N	-	-
34	CHOMA	51	F	II	N	-	-
35	ORKDO	49	F	II	N	-	-
36	MELUR	60	F	II	N	-	-
37	OLEDA	62	F	II	N	-	-
38	KOZAN	21	F	II	N	-	-
39	TRYED	37	F	II	N	-	-
40	WROAU	61	F	II	N	-	-

N : Normale / *normal*
S : Sensible / *sensitive*

CARACTERISTIQUES DES VOLONTAIRES
SUBJECTS CHARACTERISTICS

N° Volontaire / *Subject N°*	Identification du sujet / *Identification of subject*	Age *Age*	Sexe / *Sex*	Phototype *Phototype*	Nature de la peau / *Skin type*	Evénements médicaux ou chirurgicaux et traitements médicaux *Medical or surgical events and medical treatments*	
						avant l'étude *before the study*	pendant l'étude *dunng the study*
41	KROZB	57	M	II	N	-	-
42	GRUAN	29	F	II	N	-	-
43	JANJO	30	M	II	N	-	-
44	KALWI	47	F	II	N	-	-
45	KRUED	42	F	II	N	-	-
46	MAJJA	34	M	II	N	-	-
47	BAREW	47	F	II	N	-	-
48	KIEHE	38	F	II	N	-	-
49	BOCUR	41	F	II	N	-	-
50	GAJAN	22	F	II	N	-	-
51	CZOST	34	F	II	N	-	-
52	RUTBE	46	F	II	N	-	-
53	RUDMI	48	F	II	N	-	-
54	CEGHA	58	F	II	N	-	-
55	GRAIR	54	F	II	N	-	-
56	POPKA	29	F	II	N	-	-
57	PIEBA	63	F	II	N	-	-
58	DUDAG	36	F	II	N	-	-
59	SIEIW	34	F	II	N	-	-
60	KOWAL	20	F	II	N	-	-
61	SEPZO	54	F	II	N	-	-
62	PECBO	63	F	II	N	-	-
63	KIEEW	27	F	II	N	-	-
64	SIESA	58	F	II	N	-	-
65	PELBE	52	F	II	N	-	-
66	JANEM	43	F	II	N	-	-
67	ZOCSY	27	F	II	N	-	-
68	ZALGR	59	F	II	N	-	-
69	HACBO	55	F	II	N	-	-
70	HACMA	32	M	II	N	-	-
71	ZWIAP	45	F	II	N	-	-
72	GORMA	53	F	II	N	-	-
73	SKOBR	50	M	II	N	-	-
74	BAKAG	41	F	II	N	-	-
75	KURHE	49	F	II	N	-	-
76	JEDRE	53	F	II	N	-	-
77	ZIEDA	56	F	II	N	-	-
78	JANMA	52	F	II	N	-	-
79	ZAREW	28	F	II	N	-	-
80	GRAAN	49	F	II	N	-	-

N : Normale / *normal*
S : Sensible / *sensitive*

CARACTERISTIQUES DES VOLONTAIRES
SUBJECTS CHARACTERISTICS

N° Volontaire / *Subject N°*	Identification du sujet / *Identification of subject*	Age *Age*	Sexe / *Sex*	Phototype *Phototype*	Nature de la peau / *Skin type*	Evénements médicaux ou chirurgicaux et traitements médicaux *Medical or surgical events and medical treatments*	
						avant l'étude *before the study*	pendant l'étude *during the study*
81	RYLHA	38	F	II	N	-	-
82	POLJA	28	M	II	N	-	-
83	SZUEL	53	F	II	N	-	-
84	SENEL	56	F	II	N	-	-
85	RODKR	50	F	II	N	-	-
86	WAGAN	27	F	II	N	-	-
87	PASJA	29	F	II	N	-	-
88	PSZBE	23	F	II	N	-	-
89	DANEL	47	F	II	N	-	-
90	KURDA	40	F	II	N	-	-
91	KIEWA	52	F	II	N	-	-
92	MROMO	50	F	II	N	-	-
93	ZARBE	50	F	II	N	-	-
94	WYSSY	33	F	II	N	-	-
95	BULJO	42	F	II	N	-	-
96	KOBTO	44	M	II	N	-	-
97	LONMA	50	F	II	N	-	-
98	KANEW	54	F	II	N	-	-
99	CZAWI	27	F	II	N	-	-
100	BOGAG	38	F	II	N	-	-

N : Normale / *normal*
S : Sensible / *sensitive*

ANNEXE III / *APPENDIX III*

TABLEAUX DES LECTURES - PHASE D'INDUCTION

TABLES OF THE READINGS – INDUCTION PHASE

TABLEAUX DES LECTURES - PHASE D'INDUCTION
TABLES OF THE READINGS – INDUCTION PHASE

N° Volontaire / *Subject N°*	J3 *D3*		J5 *D5*		J8 *D8*		J10 *D10*		J12 *D12*		J15 *D15*		J17 *D17*		J19 *D19*	
	T	KA	T	KA	T	KA	T	KA	T	KA	T	KA	T	KA	T	KA
1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
8	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
9	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
10	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
11	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
12	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
13	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
14	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
15	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
16	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
17	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
18	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
19	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
20	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
21	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
22	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
23	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
24	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
25	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
26	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
27	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
28	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
29	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
30	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
31	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
32	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
33	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
34	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
35	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
36	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
37	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
38	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
39	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
40	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

P.V. = perdu de vue / *Untraceable*
T = témoin / control
KA = LCA14027 - 14P0898-1

TABLEAUX DES LECTURES - PHASE D'INDUCTION
TABLES OF THE READINGS – INDUCTION PHASE

N° Volontaire / *Subject N°*	J3 *D3*		J5 *D5*		J8 *D8*		J10 *D10*		J12 *D12*		J15 *D15*		J17 *D17*		J19 *D19*	
	T	KA	T	KA	T	KA	T	KA	T	KA	T	KA	T	KA	T	KA
41	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
42	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
43	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
44	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
45	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
46	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
47	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
48	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
49	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
50	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
51	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
52	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
53	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
54	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
55	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
56	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
57	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
58	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
59	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
60	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
61	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
62	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
63	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
64	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
65	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
66	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
67	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
68	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
69	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
70	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
71	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
72	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
73	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
74	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
75	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
76	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
77	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
78	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
79	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
80	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

P.V. = perdu de vue / *Untraceable*
T = témoin / *control*
KA = LCA14027 - 14P0898-1

TABLEAUX DES LECTURES - PHASE D'INDUCTION
TABLES OF THE READINGS – INDUCTION PHASE

N° Volontaire / *Subject N°*	J3 *D3*		J5 *D5*		J8 *D8*		J10 *D10*		J12 *D12*		J15 *D15*		J17 *D17*		J19 *D19*	
	T	KA	T	KA	T	KA	T	KA	T	KA	T	KA	T	KA	T	KA
81	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
82	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
83	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
84	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
85	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
86	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
87	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
88	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
89	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
90	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
91	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
92	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
93	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
94	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
95	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
96	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
97	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
98	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
99	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
100	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

P.V. = perdu de vue / *Untraceable*
T = témoin / control
KA = LCA14027 - 14P0898-1

ANNEXE IV / *APPENDIX IV*

TABLEAUX DES LECTURES - PHASE DE REVELATION

TABLES OF THE READINGS -- CHALLENGE PHASE

TABLEAUX DES LECTURES - PHASE DE REVELATION
TABLES OF THE READINGS – CHALLENGE PHASE

N° Volontaire / *Subject N°*	J38 zone homolatérale *D38 homolateral zone*		J38 zone controlatérale *D38 controlateral zone*		J40 zone homolatérale *D40 homolateral zone*		J40 zone controlatérale *D40 controlateral zone*	
	T	KA	T	KA	T	KA	T	KA
1	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0
4	0	0	0	0	0	0	0	0
5	0	0	0	0	0	0	0	0
6	0	0	0	0	0	0	0	0
7	0	0	0	0	0	0	0	0
8	0	0	0	0	0	0	0	0
9	0	0	0	0	0	0	0	0
10	0	0	0	0	0	0	0	0
11	0	0	0	0	0	0	0	0
12	0	0	0	0	0	0	0	0
13	0	0	0	0	0	0	0	0
14	0	0	0	0	0	0	0	0
15	0	0	0	0	0	0	0	0
16	0	0	0	0	0	0	0	0
17	0	0	0	0	0	0	0	0
18	0	0	0	0	0	0	0	0
19	0	0	0	0	0	0	0	0
20	0	0	0	0	0	0	0	0
21	0	0	0	0	0	0	0	0
22	0	0	0	0	0	0	0	0
23	0	0	0	0	0	0	0	0
24	0	0	0	0	0	0	0	0
25	0	0	0	0	0	0	0	0
26	0	0	0	0	0	0	0	0
27	0	0	0	0	0	0	0	0
28	0	0	0	0	0	0	0	0
29	0	0	0	0	0	0	0	0
30	0	0	0	0	0	0	0	0
31	0	0	0	0	0	0	0	0
32	0	0	0	0	0	0	0	0
33	0	0	0	0	0	0	0	0
34	0	0	0	0	0	0	0	0
35	0	0	0	0	0	0	0	0
36	0	0	0	0	0	0	0	0
37	0	0	0	0	0	0	0	0
38	0	0	0	0	0	0	0	0
39	0	0	0	0	0	0	0	0
40	0	0	0	0	0	0	0	0

T = témoin / control
KA = LCA14027 - 14P0898-1

TABLEAUX DES LECTURES - PHASE DE REVELATION
TABLES OF THE READINGS – CHALLENGE PHASE

N° Volontaire / *Subject N°*	J38 zone homolatérale *D38 homolateral zone*		J38 zone controlatérale *D38 controlateral zone*		J40 zone homolatérale *D40 homolateral zone*		J40 zone controlatérale *D40 controlateral zone*	
	T	KA	T	KA	T	KA	T	KA
41	0	0	0	0	0	0	0	0
42	0	0	0	0	0	0	0	0
43	0	0	0	0	0	0	0	0
44	0	0	0	0	0	0	0	0
45	0	0	0	0	0	0	0	0
46	0	0	0	0	0	0	0	0
47	0	0	0	0	0	0	0	0
48	0	0	0	0	0	0	0	0
49	0	0	0	0	0	0	0	0
50	0	0	0	0	0	0	0	0
51	0	0	0	0	0	0	0	0
52	0	0	0	0	0	0	0	0
53	0	0	0	0	0	0	0	0
54	0	0	0	0	0	0	0	0
55	0	0	0	0	0	0	0	0
56	0	0	0	0	0	0	0	0
57	0	0	0	0	0	0	0	0
58	0	0	0	0	0	0	0	0
59	0	0	0	0	0	0	0	0
60	0	0	0	0	0	0	0	0
61	0	0	0	0	0	0	0	0
62	0	0	0	0	0	0	0	0
63	0	0	0	0	0	0	0	0
64	0	0	0	0	0	0	0	0
65	0	0	0	0	0	0	0	0
66	0	0	0	0	0	0	0	0
67	0	0	0	0	0	0	0	0
68	0	0	0	0	0	0	0	0
69	0	0	0	0	0	0	0	0
70	0	0	0	0	0	0	0	0
71	0	0	0	0	0	0	0	0
72	0	0	0	0	0	0	0	0
73	0	0	0	0	0	0	0	0
74	0	0	0	0	0	0	0	0
75	0	0	0	0	0	0	0	0
76	0	0	0	0	0	0	0	0
77	0	0	0	0	0	0	0	0
78	0	0	0	0	0	0	0	0
79	0	0	0	0	0	0	0	0
80	0	0	0	0	0	0	0	0

T = témoin / control
KA = LCA14027 - 14P0898-1

TABLEAUX DES LECTURES - PHASE DE REVELATION
TABLES OF THE READINGS – CHALLENGE PHASE

N° Volontaire / *Subject N°*	J38 zone homolatérale *D38 homolateral zone*		J38 zone controlatérale *D38 controlateral zone*		J40 zone homolatérale *D40 homolateral zone*		J40 zone controlatérale *D40 controlateral zone*	
	T	KA	T	KA	T	KA	T	KA
81	0	0	0	0	0	0	0	0
82	0	0	0	0	0	0	0	0
83	0	0	0	0	0	0	0	0
84	0	0	0	0	0	0	0	0
85	0	0	0	0	0	0	0	0
86	0	0	0	0	0	0	0	0
87	0	0	0	0	0	0	0	0
88	0	0	0	0	0	0	0	0
89	0	0	0	0	0	0	0	0
90	0	0	0	0	0	0	0	0
91	0	0	0	0	0	0	0	0
92	0	0	0	0	0	0	0	0
93	0	0	0	0	0	0	0	0
94	0	0	0	0	0	0	0	0
95	0	0	0	0	0	0	0	0
96	0	0	0	0	0	0	0	0
97	0	0	0	0	0	0	0	0
98	0	0	0	0	0	0	0	0
99	0	0	0	0	0	0	0	0
100	0	0	0	0	0	0	0	0

T = témoin / control
KA = LCA14027 - 14P0898-1

Institut Pasteur de Lille

Fondation reconnue d'utilité publique

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Final Study Report

***IN VITRO* SKIN IRRITATION :**
RECONSTRUCTED HUMAN EPIDERMIS TEST METHOD

Study Number
FSR-IPL 140410

Study Completion
10 July 2014

Test Item Ephemer Undaria Pinnatifida Extract in Caprylic / Capric Triglyceride
LCA14026

Study Director
Mrs. Gwendoline DEWAELE

Sponsor
BiotechMarine

TEST FACILITY
INSTITUT PASTEUR DE LILLE
Genetic Toxicology Laboratory
1, rue du Professeur Calmette - BP. 245
59019 LILLE CEDEX

SPONSOR
BiotechMarine
Z.I.
22260 Pontrieux

SPONSOR REPRESENTATIVE
Mr. Mickaël PUGINIER
SEPPIC
127 Chemin de la poudrerie
81100 Castres

TABLE OF CONTENTS

STUDY INFORMATION

STUDY	***In Vitro* Skin Irritation: Reconstructed Human Epidermis Test Method**
TEST ITEM	**LCA14026**

I. TEST FACILITY INFORMATION:

Test facility	**Institut Pasteur de Lille**
Test facility address	Genetic Toxicology Laboratory 1, rue du Professeur Calmette - B.P. 245 59019 LILLE CEDEX France
Study director	**Mrs. Gwendoline DEWAELE** **Institut Pasteur de Lille** Genetic Toxicology Laboratory 1, rue du Professeur Calmette - B.P. 245 59019 LILLE CEDEX France
Deputy Study director	**Dr. Sophie SIMAR**
Quality Assurance	**Mrs. Frédérique LOBEZ**
Test facility management	**Dr. Fabrice NESSLANY** Head of Toxicology Department

II. SPONSOR INFORMATION:

Sponsor	**BiotechMarine**
Sponsor's address	Z.I. 22260 Pontrieux
Sponsor representative	**Mr. Mickaël PUGINIER** SEPPIC 127 Chemin de la poudrerie 81100 Castres

GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT AND REPORT AUTHENTICATION

* * *

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;
- Article Appendix II to the article D523-8 of the French environmental code;
- EC Commission Directive 2004/10/EC of 11th February 2004 (Official Journal No. L050).

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 to be tested sponsored by **BiotechMarine** is identical to the test item described in the Final Study Plan and in the Analytical Certificate.

NB : The certificate of analysis sponsored by the Sponsor was not compliant with GLP, GMP or ISO regulations.

The study was performed at the Toxicology Department of Institut Pasteur de Lille for irritation assay.

The following software, used during the study phase, Excel®, was not fully validated in accordance with GLP regulations. However, calculations were double checked within this study. Consequently, the use of the software was judged not to have impaired the integrity of the study.

Submitted by:

Study director **Mrs. Gwendoline DEWAELE** 10.07.2014 Date  Signature

STUDY	***In Vitro* Skin Irritation: Reconstructed Human Epidermis Test Method**
TEST ITEM	**LCA14026**
SPONSOR	**BiotechMarine**

This report was reviewed and approved by:

Test facility management

Dr. Fabrice NESSLANY
Head of Toxicology Department

10/09/2014
Date


Signature

Deputy Study director

Dr. Sophie SIMAR

QUALITY ASSURANCE STATEMENT

* * *

STUDY — ***In Vitro* Skin Irritation: Reconstructed Human Epidermis Test Method**

TEST ITEM — **LCA14026**

SPONSOR — **BiotechMarine**

TEST FACILITY — **INSTITUT PASTEUR DE LILLE**
Genetic Toxicology Laboratory
1, rue du Professeur Calmette
B.P. 245
59019 LILLE CEDEX FRANCE

This study was inspected by the Quality Assurance Unit, employing methods detailed in Standard Operating Procedures used at the Toxicology Department of Institut Pasteur de Lille.

- **STUDY PLAN AND AMENDMENT AUDITS**

Audit	Date of audit	Approved by the Study Director on	Approved by the Test Facility Management on
Study Plan	**22/05/2014**	**23/05/2014**	**27/05/2014**
Amendment No. 1	**30/06/2014**	**30/06/2014**	**30/06/2014**

The data presented in the report accurately reflect data collected during the conduct of the study. Any data supplied by or under the responsibility of the Sponsor were not reviewed.

- **IN STUDY AUDIT**

	Inspections / audits on critical phases of this study			Inspections / audits on critical phases associated with this type of study		
Phase audited	Dates of Inspection/ Audit	Approved by the Study director on	Approved by the Test facility Management on	Dates of Inspection/ Audit	Approved by the Study director on	Approved by the Test facility Management on
Receipt-preculture of epidermis	-	-	-	03/06/14	13/06/14	13/06/14
Treatment	04/06/14	13/06/14	13/06/14	-	-	-
MTT technique	-	-	-	06/06/14	13/06/14	13/06/14
Reading of OD	-	-	-	06/06/14	13/06/14	13/06/14

In addition, process and facility based audits 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	13/06/2014	19/06/2014	18/06/2014
Final report	10/07/2014	10/07/2014	10/07/2014

- **CONCLUSION**

Based on these audits, I declare that the data presented in this report accurately reflect the raw data collected during the current study.

le 10/07/2014

Mrs. Frédérique LOBEZ*
Quality Assurance Unit

** In absence of Mrs. Aurélie RIZZA, Mrs. Brigitte GOREZ has audited and signed the Amendment No. 1 to the Final Study Plan FSP-IPL140410 and Mrs. Frédérique LOBEZ has audited and signed the Final Study Report.*

ARCHIVE STATEMENT

* * *

Test Facility archives

The following study materials are retained in the archives of the Toxicology Laboratory of the Institut Pasteur de Lille (1, rue du Pr Calmette – BP 245 – 59019 Lille Cedex – France) for at least 10 years after the end of the study:

- Study plan and amendment,
- Raw data or authenticated copies thereof,
- Correspondence,
- Final report and possible amendments.

After the end of this period, they should 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 equipments 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 at the end of the study**.

After the finalisation of the whole package of studies, at Sponsor Representative's request and in accordance with the Final Study Plan, Institut Pasteur de Lille should destroy the remaining test item.

In Vitro Skin Irritation: Reconstructed Human *Epidermis* Test Method

SUMMARY

SPONSOR	:	**BiotechMarine**
TEST ITEM	:	**LCA14026**
BATCH NUMBER	:	**13.11.401**
STUDY LOCATION	:	**INSTITUT PASTEUR DE LILLE** Genetic Toxicology Laboratory 1, rue du Professeur Calmette - B.P. 245 59019 LILLE CEDEX FRANCE

THIS STUDY WAS CARRIED OUT IN COMPLIANCE WITH GOOD LABORATORY PRACTICE REGULATIONS

Study initiation date (date Study Director signed Study Plan):	27/05/2014
Experimental start date :	03/06/2014
Experimental completion date:	06/06/2014
Study completion:	10/07/2014

AIM OF THE STUDY

The skin irritant potential of **LCA14026 (batch 13.11.401)** sponsored by **BiotechMarine** was investigated by using the Episkin® culture system.

The *In Vitro* Skin Irritation using the Reconstructed Human *Epidermis* Test Method was designed to predict and classify the skin irritant potential of items according to chemical safety regulations, using the reconstructed human epidermis model Episkin™ small model. This method does not require the use of live animals or animal tissue for the assessment of skin irritant potential.
This test provided an *in vitro* procedure that may be used for the hazard identification of skin irritant chemicals (substances and mixtures) in accordance with OECD Guideline No. 439 and for the classification and Labelling of Chemicals according to the regulation United Nations Globally Harmonized System (2009).

METHOD

- Reconstructed human Epidermises	:	Human skin cells small model (Episkin® batch 14-EKIN-020)
- Culture media	:	Episkin® 'maintenance' culture medium (Episkin® batch 14-MAIN3-024) Episkin® 'assay' culture medium (Episkin® batch 14-ESSC-021)
- Negative control	:	phosphate Buffered Saline (PBS) (GIBCO batch 1376272)
- Positive control	:	sodium Dodecyl Sulfate 5% (SDS 5%, Biorad batch 210010817)

SEARCH FOR INTERFERENCE WITH THE TEST SYSTEM

- Possible direct MTT reduction	:	no interference
- coloring potential	:	no interference

IRRITATION ASSAY:

- Number of assays	:	1
- Number of replicates treated	:	3 for test item and for positive and negative controls
- Time exposure	:	15 ± 0.5 minutes
- Time of recovery period	:	42 hours ± 1 hour
- Dose tested	:	**10 µL of LCA14026 as supplied / epidermis**

RESULTS :

	Mean[1] of viability (%)	SD
Negative control	100	7.7
Test Item	106.1	8.6

SD : Standard deviation
[1] from 3 replicates

After 15 minutes treatment followed by 42 hours of recovery, the mean of cell viability was of 106.1%, when compared to the negative control, *i.e.* clearly higher than 50%.
Therefore, the test item **LCA14026** was considered as **not irritant** to skin under these experimental conditions.

CONCLUSION

The skin irritant potential of LCA14026 (batch 13.11.401) sponsored by BiotechMarine was investigated by using the *In Vitro* Skin Irritation: Reconstructed Human Epidermis Test Method in compliance with the OCDE Guideline No. 439.

The validity criteria for the assay were fulfilled. The current study is thus considered as valid.

Under these experimental conditions, LCA14026 is considered as not irritant to skin according to OECD Guideline No. 439 and have not to be classified according to UN GHS (2009).

In Vitro Skin Irritation: Reconstructed Human *Epidermis* Test Method

* * *

1. PURPOSE OF THE STUDY

Skin irritation refers to the production of reversible damage to the skin following the application of a test item.
The *In Vitro* Skin Irritation: Reconstructed Human *Epidermis* Test Method was designed to predict and classify the skin irritant potential of items according to chemical safety regulations, using the small model reconstructed human epidermis model Episkin™.
This test provided an *in vitro* procedure that may be used for the hazard identification of skin irritant chemicals (substances and mixtures) in accordance with OECD Guideline No. 439 and for the classification and Labelling of Chemicals according to the regulation United Nations Globally Harmonized System (UN GHS, 2009).

The current study was performed in accordance with the Final Study Plan FSP-IPL 140410 (see Appendix No. 5) and amendment (see Appendix No. 6).

Experimental phase:
Initiated – completed : 03/06/2014 – 06/06/2014

2. PRINCIPLE

The test item is applied topically to a three-dimensional RhE model, Episkin™. After a 15-minutes exposure time with the test item, epidermis are rinsed and then incubated for 42 additional hours.

Cell viability determination is based on cellular mitochondrial dehydrogenase activity, measured by MTT reduction and conversion into a blue formazan salt that is quantitatively measured after extraction from tissues (Faller C. *et al.*, 2002, Mosmann T., 1983). The reduction of cell viability in treated tissues is compared to negative controls and expressed as a percentage. The percentage reduction in viability is used to predict the irritation potential.

Irritant chemicals are identified by their ability to decrease cell viability below defined threshold levels (*i.e.* ≤ 50%). Chemicals that induce cell viabilities above the defined threshold level, may be considered non-irritant (*i.e.* > 50%).

3. MATERIALS AND METHOD

3.1. Reason of the choice of the reactive system

Human skin cells from Episkin™ is used for these different reasons:

- Episkin™ reconstitutes a model that is very close to human skin (histological structure, metabolic activity…).
- The production of Episkin™ is standardized and it is more reproducible than human skin.
- Test results on human skin cells are more pertinent in the assessment of possible human hazard.
- Cells are more easily isolated from Episkin™ than from human skin.
- Episkin® model is recommended by OECD Guideline No. 439.

3.2. Cells and culture media

Episkin® cells (batch 14-EKIN-020, see Appendix No. 2 for the Quality Certificate), which are 0.38 cm^2 skin equivalent and the culture media (batches 14-MAIN3-024 and 14-ESSC-021) were purchased from Episkin SNC (Lyon, France).

4. TEST ITEM AND VEHICLE INFORMATION

4.1. Test item

TEST ITEM	:	**LCA14026**
OTHER NAME / CODE	:	-
IPL REGISTRATION NUMBER	:	140410
BATCH NUMBER	:	13.11.401
EXPIRY DATE	:	10/2016
APPEARANCE	:	green liquid
PURITY	:	100% commercial product
COMPOSITION	:	9.5 mg/kg of fucoxanthine
WATER CONTENT	:	unknown
QUANTITY SUPPLIED	:	50 mL
STORAGE CONDITIONS	:	room temperature, protected from light
STABILITY UNDER STORAGE CONDITIONS	:	stable up to expiry date, *i.e.* 10/2016 for batch 13.11.401

Storage conditions: Immediately upon receipt, the test item was registered, then stored at room temperature, protected from light 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. 3, was tested in accordance with the Final Study Plan.

4.2. Check-method for possible direct MTT reduction with test item:

Prior to experiments the test item was put in contact with the MTT solution. A 12-well plate was filled with 2 mL of MTT solution (0.3 mg/mL). A volume of 10 µL of the test item was added and the plate was incubated for 2 hours and 30 minutes at 37°C protected from light. The MTT solution color became yellow, *i.e.* neither blue nor purple and it was concluded that the test item did not interact with MTT in a manner that it would have disturb the test system.

4.3. Check-method to detect the coloring potential of test item.

Prior to treatment, the test item was also evaluated for its intrinsic color or ability to become colored in contact with water). A volume of 100 µL of the test item was added to 900 µL of water, mixed and let for 15 minutes at room temperature. At the end of the period, the test item appeared as a layer above water, *i.e.* not miscible. Nevertheless, the Optical Densities (OD) at 550 nm were measured :

Item	OD at 550 nm	Mean of OD	ΔOD at 550 nm
Distilled water	0.036	0.035	-
	0.034		
Test item (100 µL+900 µL distilled water)	0.077	0.077	+ 0.042
	0.077		

The coloring potential of the test item did not interfere with the test system.

4.4. Formulation of the test item

A sufficient amount of test item should be applied to uniformly cover the epidermis surface while avoiding an infinite dose. As the test item **LCA14026** was a liquid, a volume of 10 µL was used (*i.e.* 26 µL/cm²), while a nylon mesh was used to improve the uniformity of treatment.

5. CONTROLS

Concurrent negative (PBS, GIBCO batch 1376272) and positive (SDS 5%, Biorad batch 210010817) controls were used in each run to demonstrate that viability, barrier function and resulting sensitivity of the tissues were within a defined historical acceptance range (see Appendix No. 4 for Historical Data).

6. PRECULTURE AND TREATMENTS

Upon receipt, the reconstituted skin inserts were transferred into 12-well plates containing 2 mL of Episkin® "maintenance" medium and placed in a cell incubator at 37°C under 5% CO_2 with 95% humidity, up to the treatment day within 18 to 24 hours.
Three replicates were used for test item and for positive and negative controls in each run.

After 15±0.5 minutes of exposure at room temperature, the epidermis were rinsed twice with bath and with 25 mL of pre-warmed PBS at 37°C.

The epidermis were then transferred into 12-well plates containing 2 mL of "maintenance" medium and incubated at +37°C for 42±1 h (under 5% CO_2, with 95% of humidity).

7. MTT TECHNIQUE

After incubation, the inserts were transferred into a 12-well plate containing 2 mL of MTT medium (0.3 mg/mL in "assay" medium). The plates were then incubated for 2 hours and 30 minutes to 3 hours in a CO_2 incubator at 37°C. After this contact time, the epidermises were dried and then placed in Eppendorf tubes containing 0.5 mL of acidified isopropanol. The Eppendorf tubes were then placed at room temperature, protected from light, for 4 hours. At the end of this period, the tubes were agitated, and 2 x 0.2 mL per well were transferred into a flat bottom plate. Optical densities (OD) were determinated using a spectophotometer at 550 nm.
For each epidermis, the mean optical densities values (OD) and the percentage of viability were calculated :

$$\textbf{Corrected OD} = OD_{treated} - OD_{blank^*}$$

** : extraction solvent alone (acidified isopropanol)*

The mean corrected OD of the 3 epidermis of negative control corresponds to 100 % of cell viability.

With the corrected $OD_{treated}$, the percentage of cell viability was calculated for each treated epidermis.

$$\textbf{\% cell viability} = (\textbf{Corrected } OD_{treated} / \textbf{ Mean corrected } OD_{control}) \times 100$$

The mean cell viability and the standard deviation was then determinated for the 3 epidermis and was used to determine the irritation potential.

8. ACCEPTANCE CRITERIA FOR THE RESULTS

The study was accepted as the following criteria were fulfilled :

- the optical density of the extraction solvent alone (OD_{blank}) was inferior to 0.1.
- the mean corrected $OD_{control}$ was within the acceptability range from 0.6 to 1.5 and the standard deviation was inferior or equals to 18.
- the mean viability of the positive control was below 40% and the standard deviation was inferior or equal to 18.

9. INTERPRETATION OF THE RESULTS

The test item is considered to be irritant to skin in accordance with OECD Guideline No. 439 if the tissue viability after exposure and post-treatment incubation is less than or equal (≤) to 50%. The test item is thus classified as irritant to skin Category 2 according to UN GHS (2009).
The test item may be considered as non-irritant to skin in accordance with OECD Guideline No. 439 if the tissue viability after exposure and post-treatment incubation is more than (>) 50%. The test item is thus not classified as irritant to skin according to UN GHS (2009).

10. RESULTS FOR THE IRRITATION ASSAY ON RECONSTRUCTED EPIDERMIS

The test results are given in Appendix No. 1 (Table 1).

No decrease of cells viability was noted after 15 minutes exposure followed by 42 hours recovery period, with percentage of relative survival of 106.1%, when compared to the solvent control.

Therefore, the test item **LCA14026** was considered as **not irritant** to skin under these experimental conditions.

11. STUDY PLAN ADHERENCE

11.1. Deviations

This study was performed in accordance with the Final Study Plan FSP-IPL 140410 and the Amendment No. 1.

There were no deviations from the Final Study Plan.

11.2. Notes

-

12. CONCLUSION

The skin irritant potential of LCA14026 (batch 13.11.401) sponsored by BiotechMarine was investigated by using the *In Vitro* Skin Irritation: Reconstructed Human Epidermis Test Method in compliance with the OCDE Guideline No. 439.

The validity criteria for the assay were fulfilled. The current study is thus considered as valid.

Under these experimental conditions, LCA14026 is considered as not irritant to skin according to OECD Guideline No. 439 and have not to be classified according to UN GHS (2009).

13. REFERENCES

EpiSkin™ SOP, Version 1.8 (February 2009), ECVAM Skin Irritation Validation Study: Validation of the EpiSkin™ test method 15 min - 42 hours for the prediction of acute skin irritation of chemicals.

Faller C., Bracher M., Dami N. and Roguet R. (2002) Predictive activity of reconstructed human epidermis equivalents for assessment of skin irritation of cosmetics.

Mosmann T. (1983). Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. *J. Immunological Methods* 65, 55-62.

O.E.C.D. Guideline for the Testing of Chemicals No. 439: *In Vitro* Skin Irritation: Reconstructed Human *Epidermis* Test Method, 26 July 2013.

UN (2009), United Nations Globally Harmonized System of Classification and Labelling of Chemicals (GHS), Third revised edition, UN New York and Geneva.

Appendix No. 1: Results

TABLE 1 : Results

***In vitro* skin irritation : reconstructed human epidermis test method**

Reading of Optical Density at 550 nm and Viability calculation

Test item	LCA14026
Sponsor	BiotechMarine
MTT reduction	yellow solution
Coloring potential	non miscible
Beginning of the study	03/06/2014

Item	Epidermis No.	OD	Corrected OD*	Mean of corrected OD	Mean per item	Viability (%)	Mean of viability (%)	Standard deviation
Extraction solvent	-	*0.034*						
		0.032						
		0.034						
		0.035						
		0.034						
		0.035						
Negative Control (PBS)	1	*0.900*	0.866	0.863	0.803	107.5	100	7.7
		0.894	0.860					
	2	*0.827*	0.793	0.806		100.4		
		0.853	0.819					
	3	*0.767*	0.733	0.739		92.1		
		0.779	0.745					
Test Item	1	*0.900*	0.866	0.869	0.852	108.2	106.1	8.6
		0.905	0.871					
	2	*0.810*	0.776	0.776		96.7		
		0.810	0.776					
	3	*0.944*	0.910	0.911		113.5		
		0.946	0.912					
Positive control (SDS 5%)	1	*0.212*	0.178	0.157	0.146	19.5	18.2	4.0
		0.169	0.135					
	2	*0.190*	0.156	0.173		21.5		
		0.223	0.189					
	3	*0.149*	0.115	0.110		13.7		
		0.139	0.105					

OD : optical density

* with mean OD for extraction solvent = 0.034

% cell viability = (Corrected ODtreated / Mean corrected ODcontrol) x100

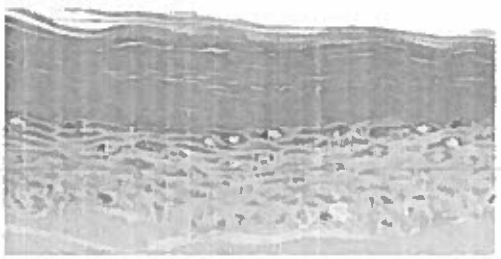
Mean cell viability (%) = (% cell viability epidermis 1 + % cell viability epidermis 2% +cell viability epidermis 3) / 3

Appendix No. 2: Quality Certificate of epidermis

SkinEthic
laboratories

TECHNICAL DATA, SAFETY SHEET AND CERTIFICATE OF ANALYSIS
RECONSTRUCTED HUMAN EPIDERMIS

CCL-037/14

Description:	**Episkin Small Model**		
	0.38 cm² reconstructed epidermis of normal human keratinocytes. Cells are grown on a collagen matrix, for 13 days.		
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% CO_2 with saturated humidity.		
Passage:	Second (Strains n° : 09-KERA-006, 09-KERA-003, 10-KERA-001, 11-KERA-001)		
Batch N°:	**14-EKIN-020**		
Origin:	Adult donors.		
Histology:	 **Control n° E140388**		
Quality Controls:	**Test**	**Specification**	**Result**
	Histology scoring (HES stained vertical paraffin sections, n = 6)	≥ 19.5	21.8 ± 0.5 (CV = 2.4 %)
		Well-differentiated epidermis consisting of a basal layer, several spinous and granular layers and a thick stratum corneum	
	IC 50 determination (SDS concentration, MTT test, n = 14)	≥ 1.5 mg/ml	2.2 mg/ml
	Statistical Analysis : → Histology : probability 0.95 that 100 % of the batch > 20 → IC 50 : probability 0.95 that IC 50 ≥ 2.2 mg/ml (threshold value)		
Biological safety:	On blood of the same donors, we have verified: . the absence of HIV1 and 2 antibodies (Architect Abbott) . the absence of hepatitis C antibodies (Architect Abbott) . the absence of hepatitis B antigen HBs (Architect Abbott) On epidermal cells of the same donors, we have verified: . the absence of bacteria, fungus and mycoplasma		
Expiration date	June 9, 2014.		

Lyon, June 3, 2014.
Certified and released by
Julie BIDOGGIA, Quality Control Manager

Manufactured in accordance to the ISO9001 quality system of Episkin.

EPISKIN
4, rue Alexander Fleming - 69366 Lyon Cedex 07 - France - Tél. (33) 04 37 28 22 00 - Fax (33) 04 37 28 22 01
au capital de 13 888 807 € - 412 127 585 R.C.S. Lyon - N° TVA intracommunautaire FR 46 412 127 585
Email : sales@skinethic.com

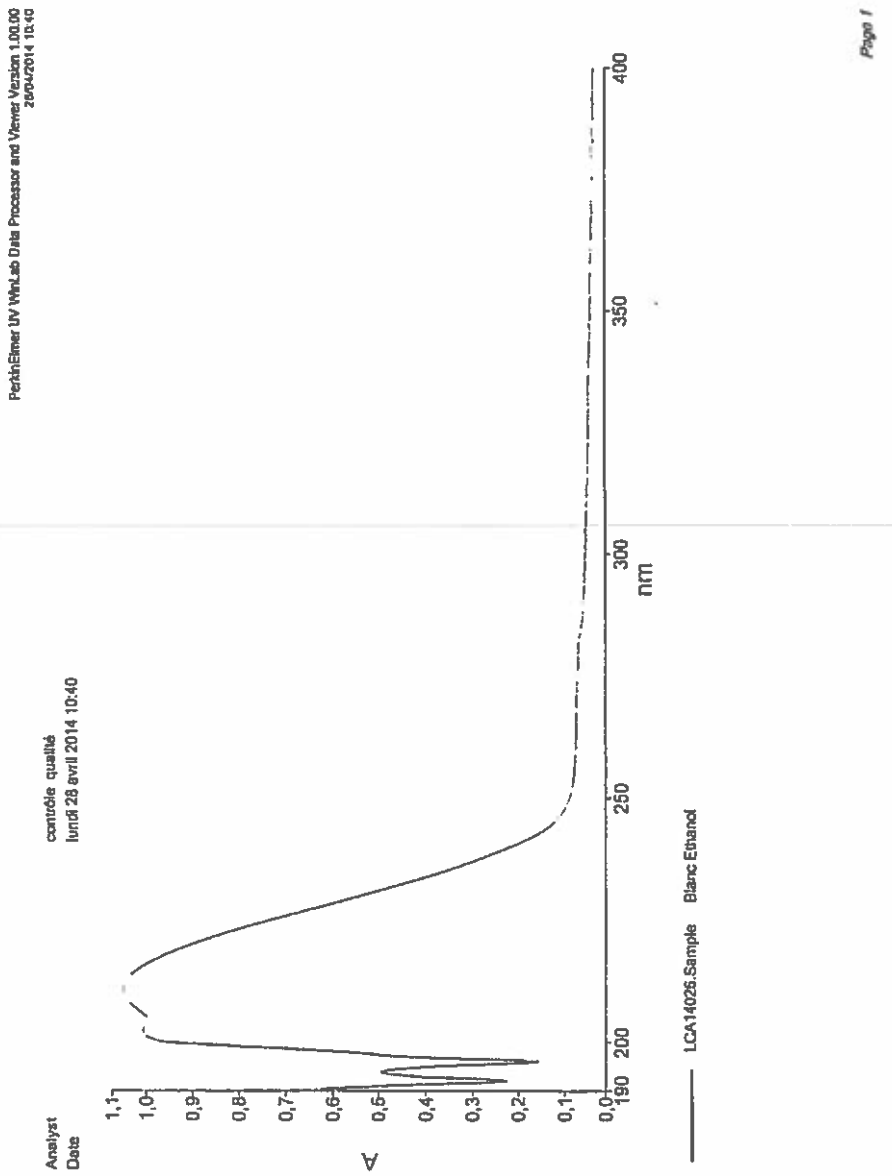
Appendix No. 3: Certificate of analysis of the test item

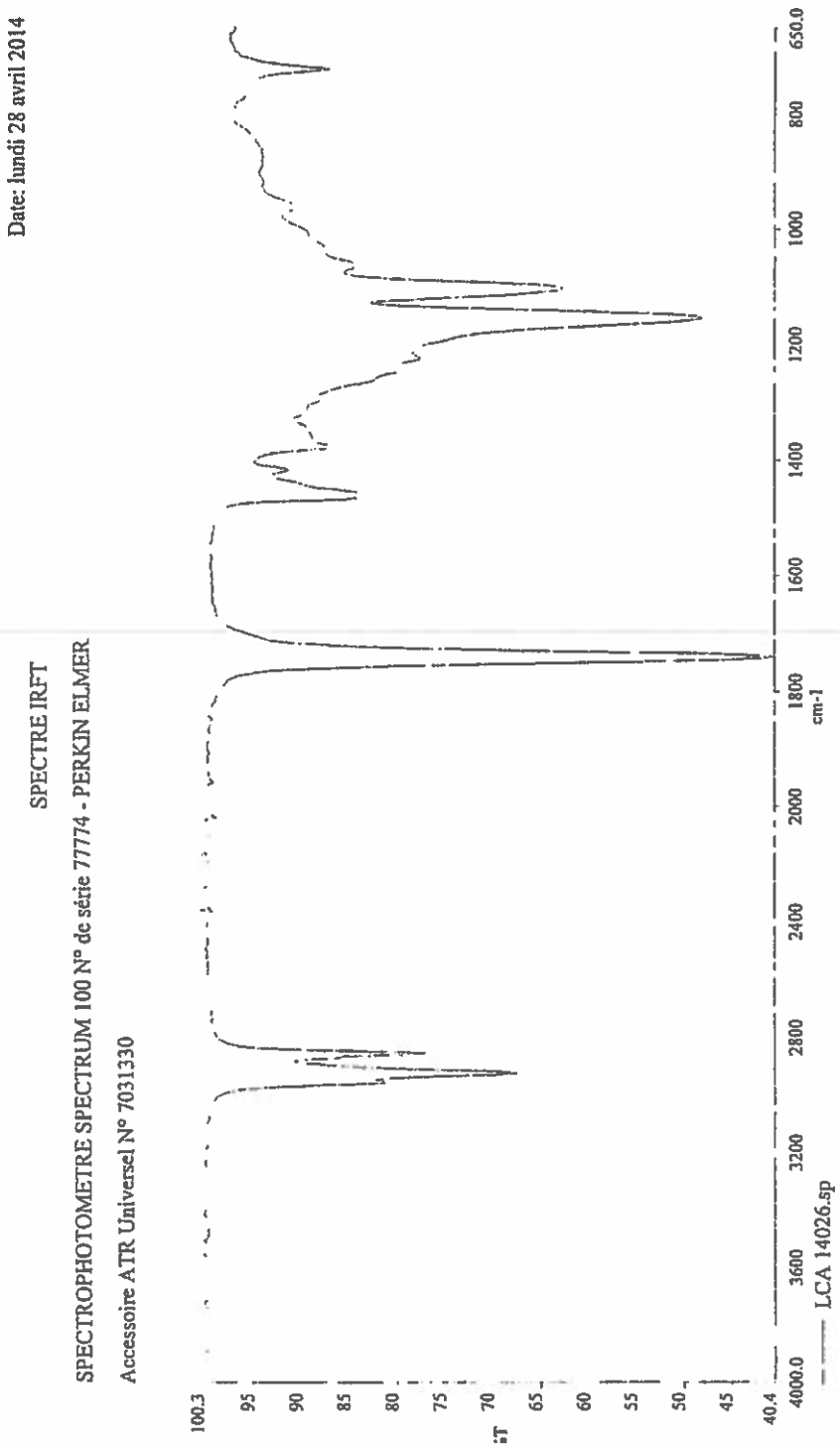
Biotech Marine

LCA14026

DONNEES PHYSICOCHIMIQUES
PHYSICO-CHEMICAL DATA
Numéro de référence (fabrication 10/2013-expiration 10/2016): 13.11.401
Reference number (manufacturing 10/2013-expiration 10/2016)

CARACTERISTIQUES *CHARACTERISTICS*	METHODES *METHODS*	RESULTATS *RESULTS*
Aspect *Aspect*	MO PHY 002	Liquide limpide *limpid liquid*
Couleur *Colour*	MO PHY 002	Vert *Green*
Odeur *Odour*	MO PHY 002	Faible *Slight*
Densité (20°C) *Density*	MO PHY 024	0,941
Indice de réfraction(20°C) *Refractive index*	MO PHY 008	1,450$_9$
Teneur en fucoxanthine *Fucoxanthine content*	MO PHY 034	9,5 mg/kg
Spectre UV *UV spectrum*	MO PHY 013	Enregistré *Registered*
Spectre IR *IR spectrum*	MO PHY 011	Enregistré *Registered*





LCA14026

DONNEES MICROBIOLOGIQUES
MICROBIOLOGICAL DATA
Numéro de référence (fabrication 10/2013-expiration 10/2016): 13.11.401
Reference number (manufacturing 10/2013-expiration 10/2016)

CARACTERISTIQUES *CHARACTERISTICS*	METHODES *METHODS*	STANDARD *STANDARD*
Germes totaux* *Total germs**	MO MIC 002	< 100
Germes Pathogènes *Pathogens*		
- *Staphylococcus aureus*	MO MIC 012	Absence *None*
- *Candida albicans*	MO MIC 010	Absence *None*
- *Pseudomonas aeruginosa*	MO MIC 011	Absence *None*
- *Enterobacteriaceae*	MO MIC 020	Absence *None*
Levures / Moisissures* *Yeasts / Moulds**	MO MIC 021	< 100

*Les résultats sont exprimés en UFC/ml pour les liquides et UFC/g pour les solides

CERTIFIE CONFORME
CERTIFIED TRUE AND CORRECT
COORDINATRICE ASSURANCE QUALITE : M. TANNIOU
QUALITY ASSURANCE COORDINATOR

Le 28/04/14

Appendix No. 4: Historical data

In vitro skin irritation : reconstructed human epidermidis test method

HISTORICAL DATA FROM FEBRUARY 2013 TO MAY 2013

Mean of corrected OD					
Negative control (PBS)			Positive control (SDS 5%)		
Mean ± *sd* (extreme deviations)			Mean ± *sd* (extreme deviations)		
0.855	**±**	0.137	**0.137**	**±**	0.115
0.765	-	1.013	0.054	-	0.269

Please note that the % of brown algae contained in each below mentioned EXTRACT ranges between 0.5 and 10%

INCI name PCPC	chemical characterization data	dermal toxicity data	dermal irritation and sensitization data -% test	description of the method of manufacture	solvent used to extract algae	presence of arsenic	presence of iodine
Water (and) **Cystoseira Baccata Extract**	/	acute cutaneous tolerance on the adult volunteer : Patch-test 24 hours The results obtained under the experimental conditions retained showed that the product applied pure and locally under an occlusive dressing during 24 hours, on the skin of 10 volunteers, was found to be no irritating.	Evaluation of the allergenic potential after repeated cutaneous applications over 50 volunteers. Results obtained under experimental conditions showed that the product was found to be no irritant with regard to the cutaneous tolerance and did not induce any significant skin reaction of contact allergy. It can be thus qualified as hypoallergenic. Concentration test : 100 %	extraction with water	water	arsenic mineral : 8,8 mg/kg (FCC V method), arsenic : 20 ppm (ICP-OES method)	/
caprylic/Capric Triglycerides (and) **Cystoseira Tamaricifolia Extract**	/	acute cutaneous tolerance on the adult volunteer : Patch-test 24 hours The results obtained under the experimental conditions retained showed that the product applied pure and locally under an occlusive dressing during 24 hours, on the skin of 10 volunteers, was found to be no irritating.	/	extraction with supercritical CO2	CO2		<1 mg/kg (colorimetry method)
Water (and) Dipropylene glycol (and) **Himanthalia elongata extract**	/	acute cutaneous tolerance on the adult volunteer : Patch-test 24 hours The results obtained under the experimental conditions retained showed that the product applied pure and locally under an occlusive dressing during 24 hours, on the skin of 10 volunteers, was found to be no irritating.	/	extraction with water and dipropylene glycol	water/dipropylene glycol		<9 mg/kg (alcaline mineralisation and potentiometric method)

Water (and) **Laminaria digitata extract** (and) Sea salt	/	Evaluation of the primary cutaneous tolerance on the rabbit, the product was found to be no irritant.	/	extraction with water	water	1,5 mg/kg (ICP-MS method)	62 mg/kg (alcaline mineralisation and potentiometric method)
Water (and) Glycerin (and) **Laminaria Digitata Extract**	see phycojuvenine						
Water (and) Dipropylene glycol (and) **Laminaria digitata extract**	/	Evaluation of the acute skin tolerance on the rabbit, the product was found to be no irritant.	/	extraction with water and dipropylene glycol	water/Dipropylene glycol	2,37 mg/kg (ICP-MS method)	87 mg/kg (alcaline mineralisation and potentiometric method), average : 110 ppm
Water (and) **Laminaria digitata extract**	/	acute cutaneous tolerance on the adult volunteer : Patch-test 24 hours. The results obtained under the experimental conditions retained showed that the product applied pure and locally under an occlusive dressing during 24 hours, on the skin of 10 volunteers with sensitivie skin, was found to be non irritating.	Evaluation of the allergic potential after repeated epicutaneous applications on 50 volunteers. The results obtained in the experimental conditions retained permitted to conclude that the product was found to be very well tolerated at the cutaneous level, showing no significant reaction of a contact allergy. It can thus be qualified as hypoallergenic. Concentration test : 100 %	extraction with water	water	contains less than 10 ppm	contains approximately 550 +/- 150 ppm of iodine (mean of 12 analysis performed on 12 different industrial batches produced between 2003 and 2006). Maximum value : 700 ppm
Water (and) **Laminaria digitata extract**	/	Evaluation of the primary cutaneous tolerance on the rabbit : the product was found to be slightly irritant.	/	extraction with water	water	yes, 19,06 mg/kg (ICP-MS method)	192 mg/kg (alcaline mineralisation and potentiometric method), average : 300 ppm
water (and) **phyllacantha fibrosa extract**	/	/	/	extraction with water	water	yes, 11,35 ppm (ICP-M	yes, 97 mg/l (method ionic chromatography), average :140 ppm
Water (and) Dipropylene glycol (and) **Sphacelaria scoparia extract**	/	acute cutaneous tolerance on the adult volunteer : Patch-test 24 hours. The results obtained under the experimental conditions retained showed that the product applied pure and locally under an occlusive dressing during 24 hours, on the skin of 11 volunteers, was found to be non irritating.	Evaluation of the allergic potential after repeated epicutaneous applications on 50 volunteers. The results obtained in the experimental conditions retained permitted to conclude that the product was found to be very well tolerated at the cutaneous level, showing no significant irritative reaction of a contact allergy reaction. It can thus be qualified as hypoallergenic. Concentration test : 100 %	extraction with water and dipropylene glycol	Water / Dipropylene glycol	yes, 0,73 mg/kg (ICP-MS method)	15 mg/kg (alcaline mineralisation and potentiometric method)

Water (and) Dipropylene glycol (and) **undaria pinnatifida extract**	/	acute cutaneous tolerance on the adult volunteer : Patch-test 24 hours The results obtained under the experimental conditions retained showed that the product applied pure and locally under an occlusive dressing during 24 hours, on the skin of 10 volunteers, was found to be no irritating.	/	extraction with water and dipropylene glycol	water/dipropylene glycol	/	<9 mg/kg (alcaline mineralisation and potentiometric method)

Brown Algae Data Profile for ***September*** **2018**. Writer – Priya Cherian																					
		ADME		Acute toxicity			Repeated dose toxicity			Irritation					Sensitization						
	Use	Log K_{ow}	Dermal Penetration	Oral	Dermal	Inhale	Oral	Dermal	Inhale	Ocular Animal	Ocular In Vitro	Dermal Animal	Dermal Human	Dermal In Vitro	Animal	Human	In Vitro	Repro/Devel	Genotoxicity	Carcinogenicity	Phototoxicity
1. Agarum Cribrosum Extract	x																				
2. Alaria Esculenta Extract	x												x			x					
3. Ascophyllum Nodosum							x														
4. Ascophyllum Nodosum Extract	x			x						x		x			x				x		
5. Ascophyllum Nodosum Powder	x	x																			
6. Cladosiphon Novae-Caledoniae Extract																					
7. Cladosiphon Okamuranus Extract	x																				
8. Cystoseira Amentacea/Caespitosa/ Branchycarpa Extract																					
9. Cystoseira Baccata Extract																					
10. Cystoseira Balearica Extract																					
11. Cystoseira Caespitosa Extract																					
12. Cystoseira Compressa Extract				x															x		
13. Cystoseira Compressa Powder																					
14. Cystoseira Tamariscifolia Extract																					
15. Dictyopteris Membranacea Extract (Retired)																					
16. Dictyopteris Polypodioides Extract	x																				
17. Dictyota Coriacea Extract																					
18. Durvillea Antarctica Extract	x																				
19. Ecklonia Cava Extract				x			x												x		
20. Ecklonia Cava Water							x														
21. Ecklonia Kurome Extract																					
22. Ecklonia Kurome Powder																					
23. Ecklonia/Laminaria Extract																					
24. Ecklonia Maxima Extract																					
25. Ecklonia Maxima Powder																					
26. Ecklonia Radiata Extract	x																				
27. Eisenia Arborea Extract																					
28. Fucus Serratus Extract	x																				
29. Fucus Spiralis Extract																					
30. Fucus Vesiculosus	x																				
31. Fucus Vesiculosus Extract	x			x			x												x		
32. Fucus Vesiculosus Powder	x																				
33. Halidrys Siliquosa Extract																					
34. Halopteris Scoparia Extract																					
35. Himanthalia Elongata Extract	x																				
36. Himanthalia Elongata Powder																					
37. Hizikia Fusiforme Extract																				x	
38. Hizikia Fusiformis Water																					
39. Hizikia Fusiformis Callus Culture Extract																					
40. Hydrolyzed Ecklonia Cava Extract																					

Brown Algae Data Profile for ***September*** **2018**. Writer – Priya Cherian																					
		ADME		Acute toxicity			Repeated dose toxicity			Irritation					Sensitization						
	Use	Log K_{ow}	Dermal Penetration	Oral	Dermal	Inhale	Oral	Dermal	Inhale	Ocular Animal	Ocular In Vitro	Dermal Animal	Dermal Human	Dermal In Vitro	Animal	Human	In Vitro	Repro/Devel	Genotoxicity	Carcinogenicity	Phototoxicity
41. Hydrolyzed Fucus Vesiculosus Extract																					
42. Hydrolyzed Fucus Vesiculosus Protein																					
43. Kappaphycus Alvarezii Extract																					
44. Laminaria Angustata Extract (Retired)																					
45. Laminaria Cloustoni Extract	x																				
46. Laminaria Diabolica Extract																					
47. Laminaria Digitata Extract	x																				
48. Laminaria Digitata Powder	x																				
49. Laminaria Hyperborea Extract	x																				
50. Laminaria Japonica Extract	x						x				x		x	x							
51. Laminaria Japonica Powder							x														
52. Laminaria Longissima Extract																					
53. Laminaria Ochotensis Extract (Retired)																					
54. Laminaria Ochroleuca Extract	x												x								
55. Laminaria Saccharina Extract													x			x			x		
56. Lessonia Nigrescens Extract	x																				
57. Lessonia Nigrescens Powder																					
58. Macrocystis Pyrifera (Kelp)	x																				
59. Macrocystis Pyrifera (Kelp) Blade/Pneumatocyst/Stipe Juice Extract																					
60. Macrocystis Pyrifera (Kelp) Extract	x										x			x							
61. Macrocystis Pyrifera (Kelp) Juice																					
62. Macrocystis Pyrifera (Kelp) Protein	x																				
63. Nereocystis Luetkeana Extract											x			x							
64. Pelvetia Canaliculata Extract	x																				
65. Pelvetia Siliquosa Extract																					
66. Phyllacantha Fibrosa Extract																					
67. Saccharina Angustata Extract																				x	
68. Saccharina Japonica Extract																					
69. Saccharina Longicruris Extract																					
70. Sargassum Filipendula Extract	x															x					
71. Sargassum Fulvellum Extract				x																	
72. Sargassum Fusiforme Extract																					

Brown Algae Data Profile for ***September*** **2018**. Writer – Priya Cherian																				
		ADME		Acute toxicity			Repeated dose toxicity			Irritation					Sensitization						
	Use	Log K_{ow}	Dermal Penetration	Oral	Dermal	Inhale	Oral	Dermal	Inhale	Ocular Animal	Ocular In Vitro	Dermal Animal	Dermal Human	Dermal In Vitro	Animal	Human	In Vitro	Repro/Devel	Genotoxicity	Carcinogenicity	Phototoxicity
73. Sargassum Glaucescens Extract													x								
74. Sargassum Horneri Extract																					
75. Sargassum Muticum Extract	x																				
76. Sargassum Pallidum Extract																				x	
77. Sargassum Siliquastrum Extract																					
78. Sargassum Thunbergii Extract																					
79. Sargassum Vulgare Extract	x																				
80. Sphacelaria Scoparia Extract	x																				
81. Undaria Peterseniana Extract																					
82. Undaria Pinnatifida Extract	x						x														
83. Undaria Pinnatifida Cell Culture Extract																					
84. Undaria Pinnatifida Leaf/Stem Extract																				x	
85. Undaria Pinnatifida Powder	x						x													x	
86. Undaria Pinnatifida Root Powder																					

Cosmetic Ingredient Review

Commitment & Credibility since 1976

Memorandum

To: CIR Expert Panel Members and Liaisons
From: Bart Heldreth, Executive Director and Jinqiu Zhu, Toxicologist
Date: September 14, 2018
Subject: Wave 2 Submission on Parabens

The submission described below (in *WVE092018submission* file) regarding parabens was received from Alexandra Scranton of Women's Voices for the Earth (WVE) and is submitted as an attachment to this memorandum.

There were five main issues raised.

1) Parabens exposure via vaginal products

WVE ask the Panel to consider two articles. The first is a study published in May 2018, claiming that parabens in vaginal products could potentially induce oxidative stress-associated DNA damage in human spermatozoa (Samarasinghe, S. V. A. C., et al., "Parabens generate reactive oxygen species in human spermatozoa," *Andrology* (2018)). The second study, published in 2009, claims paraben-induced gene expression using a yeast model (Mundy, Renee Domergue, and Brendan Cormack, "Expression of Candida glabrata adhesions after exposure to chemical preservatives," *The Journal of infectious diseases* 199.12 (2009): 1891-1898). The ostensible notion of relevance to cosmetic safety is that once such genes were stimulated following exposure to parabens, the adherence activity of *Candida glabrata* (a yeast strain) to vaginal epithelial cells can be induced.

2) Bioaccumulation

Here again, WVE suggest that Wang et al. (reference 91 in this report; "Accumulation of 19 environmental phenolic and xenobiotic heterocyclic aromatic compounds in human adipose tissue," *Environment International* 78 (2015): 45-50) demonstrates bioaccumulation in humans. However, the Panel discussed the merits of this paper at the March 2018 meeting and found the associations (the age-related differences of paraben levels) purported therein to be equivocal. Additionally, Barr et al. (reference 88 in this report) demonstrated that no correlations were found between paraben concentrations and age of patient (37-91 years), length of breast feeding (0-23 months), tumor location, or tumor estrogen receptor content.

WVE also suggest that the Panel should consider a newly published study (Artacho-Cordón, F., et al., "Environmental phenols and parabens in adipose tissue from hospitalized adults in Southern Spain," *Environment International* 119 (2018): 203-211) on the human adipose tissue concentrations of "non-persistent environmental pollutants." Therein, detection frequencies (n=144) and median concentrations were: Methylparaben (100.0%, 0.40 ng/g), Ethylparaben (20.1%, below level of detection), Isopropylparaben (0.0%, below limit of detection), Propylparaben (54.2%, 0.06 ng/g), Isobutylparaben (2.1%, below limit of detection), and Butylparaben (5.6%, below limit of detection). Additionally, WVE suggest that 2 papers by Xue et al., regarding paraben measurements in various tissues of various wildlife, are somehow relevant.

3) Source of exposure

WVE has proposed that the sources of exposure are mischaracterized in the CIR report. Most of the papers WVE cite in this section have already been covered in the CIR report, including Nassan et al. 2017, Ferguson et al. 2017, Tahan et al. 2016, and Fisher et al. 2017. One paper published in 2018 was not covered (Berger et al. 2018); however, similar data generated from the same HERMOSA study and from the same laboratory have already been included in the CIR report (reference 112 in the report, Harley et al. 2016). Nowhere does the CIR report suggest that cosmetics are not a source of

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exposure. Indeed, all statements of aggregate exposure sources in the CIR report are merely summaries of the above references.

4) Particle size of parabens

Additional particle size data, where relevant, will be added to the report. However, WVE is again confusing particle size of a raw material with particle size resulting from the use of final product formulations in sprays and powders. According to the ECHA dossier, 37.8% of particles in pure Propylparaben are smaller than 10 µm. However, the maximum use concentration of this ingredient in spray formulations is 0.13% and in powder formulations is 0.3%.

5) Margin of safety calculation

WVE point out the maximum reported concentration of use for Butylparaben is 0.5% (according to the most recent survey data), and so suggest using it instead of 0.4% for the MOS calculation. (In the 2008 report, we used the value of 0.4%.) If the Panel agrees, the MOS for Butylparaben can be updated from 270 to 216.

WOMEN'S VOICES FOR THE EARTH

OUR HEALTH. OUR FUTURE. TOXIC FREE.

September 12, 2018

To the CIR:

I am writing to provide additional comments to supplement the previous comments I have submitted on the CIR panel's assessment of parabens. Women's Voices for the Earth is very concerned about the potential health impacts of parabens, particularly on women's health. We appreciate the opportunity to comment and your careful consideration of the information provided.

There are several issues that should be addressed in the next draft of the safety assessment on parabens:

1) There is new research indicating significant hazards to sperm motility from the levels of parabens commonly found in vaginally applied cosmetic products. The research indicates that paraben-containing cosmetics applied vaginally prior to intercourse have the potential to adversely affect sperm. In addition, earlier research indicates that exposure to paraben-containing cosmetic products used vaginally, increased the adherence of Candida to vaginal epithelial cells, increasing the potential for yeast infections. It is important for the CIR to examine the safety of parabens used in vaginally applied cosmetic products, such as douches, vaginal moisturizers and vaginal deodorant suppositories in light of this research.
2) I previously commented that the CIR's prior claim that parabens do not accumulate in the body is outdated, and not supported by more recent research which finds parabens considerably more persistent and accumulative than previously believed. It is unclear if this previous language (which is currently included in the draft safety assessment but in italics) will remain in the final version or if it is just there for reference in the draft and will be removed. It is important not to include former language from previous assessments if the scientific understanding has changed, so as to avoid confusion.
3) The vast majority of the literature that has measured paraben exposure with respect to the use of cosmetic products has concluded that the use of cosmetics or personal care products is the most significant source of paraben exposure, much greater than the contributions from food or pharmaceuticals. The current draft assessment however, still includes language implying that non-cosmetic sources of parabens may be significant. This language should be corrected to reflect the most current information.
4) Table 4 on particle size ranges of parabens should include data on all the parabens included in the report, not just the four currently listed. This data is available from the ECHA database for all the parabens included in this report. Of note, the ECHA data indicate that for propylparaben, 37.8% of particles are smaller than 10 microns in diameter, the CIR's established level of concern for inhalation. This information contradicts the summary claim found in the report that 95-99% of particles have diameters greater than 10 microns and are thus unlikely to be inhaled deeply.

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5) The calculation used to derive a MOS for parabens needs to be updated to reflect more conservative product usage amounts and more conservative absorption rates that are consistent with data included in other sections of the assessment.

1.) **Parabens in vaginally applied cosmetics**

There are two recent studies on parabens used in vaginally applied cosmetic products that indicate hazards to human health.
The first is a 2018 study which examined the impacts of cosmetic products (vaginal moisturizers and lubricants) which contain parabens on sperm motility. The study found that the levels of parabens found in these consumer products led to oxidative stress and DNA damage that resulted in significant impacts to sperm motility and viability. The researchers concluded:

""The fact spermatozoa may spend several hours stored in cervical crypts during the early stages of sperm transport to the egg means that exposure to mM concentrations of parabens for several hours as a consequence of the topical application of vaginal lubricants is a realistic situation."
"Given that the permitted concentrations (SCCP, 2005) of methylparaben (0.4% 26 mM) and propylparaben (0.19% 10 mM) are well above the concentrations shown to be damaging to human spermatozoa in this study, the use of these preservatives in commercial products should be re-evaluated and couples should be made aware of their potential for harm in a reproductive context."
Samarasinghe SVAC et. al. (2018) Parabens generate reactive oxygen species in human spermatozoa. Andrology. 2018 May 2. doi: 10.1111/andr.12499.

The second study, from 2009, found that vaginal products containing parabens altered the expression of virulence-related genes in Candida glabrata, a vaginal yeast pathogen. Specifically, exposure to paraben-containing vaginal consumer products (including a test of Massengill douche for example) increased the adherence of Candida glabrata to human vaginal epithelial cells. Testing with vaginal products that did not contain parabens, did not show this effect.

Mundy RD and Cormack B (2009) Expression of Candida glabrata adhesins following exposure to chemical preservatives. J Infect Dis. 2009 June 15; 199(12): 1891–1898. doi:10.1086/599120.

Adherence of Candida to vaginal epithelial cells is a significant issue in women's health. As explained in the following study:
"'"Adherence has been shown to play a central role in the pathogenesis of many microbial infections. The adherence to various surfaces represents the first step in the mechanisms of pathogenesis and suggests means of controlling infection at an early stage."
El-Din A, Al-Basri H and El-Naggar M. (2012) Critical factors affecting the adherence of Candida albicans to the vaginal epithelium. Journal of Taibah University for Science. Vol. 6, pp 10-18. 2012.

Our research of currently available products indicates that in addition to vaginal moisturizers and lubricants, parabens can also found in other vaginally-applied cosmetics including douches, feminine washes and vaginal deodorant products.

We ask the CIR to address the specific health impacts of parabens in cosmetic products that are administered vaginally in the safety assessment.

2) <u>Bioaccumulation of parabens</u>

I previously commented that the CIR's prior claim that "parabens do not accumulate in the body" is outdated, and not supported by more recent research which finds parabens considerably more persistent and accumulative than previously believed. It is unclear if this previous language (which is currently included in the draft safety assessment but in italics) will remain in the final version or if it is just there for reference in the draft and will be removed. It is important not to include former language from previous assessments if the scientific understanding has changed, so as to avoid confusion.

Specifically the italicized sections of the draft assessment marked "Previous Discussions" contain most of the outdated language regarding bioaccumulation and excretion. These sections should be removed from the final draft so as not to cause confusion. Also, once removed, it would be helpful for the CIR safety assessment to include new summary language in the discussion of the ADME section which better reflects the CIR"s current understanding of the potential for parabens to be stored in the human body over time.

Currently the Discussion section states:

> "*The Panel adopted that the parabens are relatively lipid soluble compounds, they would tend to bioaccumulate in the lipid fraction of the biological tissues. Recent studies have showed the presence of parabens in breast, adipose, and placenta tissues. However, the metabolism, the excretion and the pharmacokinetics of the parabens made accumulation in the body not an issue*."

It is very unclear how the Panel was able to come to the conclusion from the data presented that "made accumulation in the body not an issue".

As I have commented before, parabens have long been understood both byb the CIR and others to be transient in the body, both metabolized and eliminated quickly. New research has found that this is not always the case, and that impacts on health should be considered from exposure to parabens which are retained in the body and which build up over time.

This change in thinking is best described by a 2018 study which assessed measured parabens in human adipose tissue. The researchers conclude:

> "*Urinary concentrations of non-persistent environmental pollutants (npEPs) are widely assessed in biomonitoring studies under the assumption that they are metabolised and eliminated in urine. <u>However, some of these chemicals are moderately lipophilic, and their presence in other biological matrices should also be evaluated to estimate mid/long-term exposure to npEPs and its impact on human health</u>."*
>
> and

> "*To the best of our knowledge, this study is among the very first to contribute evidence on the distribution and predictors of environmental phenols and parabens in adipose tissue from an adult cohort, showing the widespread presence of certain npEPs in the fat compartment. We consider these results of special interest to public health, given the increasing importance of adipose tissue as a biologically-active matrix, highly relevant in the development of chronic diseases.*"

(Source: Artacho-Cordón F et.al. (2018) Environmental phenols and parabens in adipose tissue from hospitalized adults in Southern Spain. Environ Int. 2018 Jun 29;119:203-211. doi: 10.1016/j.envint.2018.05.052.)

Similarly a 2015 study found high concentrations of parabens in human adipose tissue indicating bioaccumulation of parabens in humans can occur from chronic exposure over time. The researchers state:

> *"Environmental phenols and heterocyclic aromatic compounds are thought to be excreted completely from the body. However, relatively high concentrations of these chemicals found in human adipose tissues compared to the levels reported in urine suggest that,* ***bioaccumulation can occur from chronic daily exposures****. For some environmental phenols with endocrine disrupting activity, concentration as high as ~5000 ng/g (for BP-3) and 17,400 ng/g (for parabens including p-HB) were found in human adipose and these values were close to their effective concentration reported in in vitro experiments…"*

In addition, the researchers found a positive correlation between paraben levels in fat tissue and age of the subject indicating an accumulation over time.

> *"…a positive correlation between donor's age and CΣparabens (within the 75th percentile of adipose concentrations; n = 15) was observed (Fig. 2),* ***which suggests bioaccumulation in human adipose fat****."* (emphasis added)

(Source: Wang L, Asimakopoulos AG, Kannan K. (2015) Accumulation of 19 environmental phenolic and xenobiotic heterocyclic aromatic compounds in human adipose tissue. Environ Int. 2015 May;78:45-50. doi: 10.1016/j.envint.2015.02.015. Epub 2015 Mar 10.)

It is surprising that in direct contrast to the above mentioned study, the current draft assessment's discussion section states:

> "Some studies indicated that no correlations were found between parabens concentration in tissues and age groups of subjects, thereby suggests no bioaccumulation."

I was unable to find any references in the draft assessment to studies which measured both paraben concentration in tissues and age groups of subjects other than the Wang study mentioned above (which comes to the conclusion that correlations were found between paraben concentrations in tissues and

suggested that bioaccumulation can occur. This statement should be removed from the Discussion session unless multiple studies which back up this claim can be added to the assessment.

To further confirm this newer thinking on parabens, bioaccumulation and bioconcentration of parabens has also been noted in numerous recent wildlife studies, where animals highest on the food chain are commonly found to have the highest levels of parabens in the tissues sampled. For example:

"*In this study, accumulation profiles of six parabens and their metabolites were determined in 254 tissue (including liver, kidney, egg, and plasma) samples from 12 species of fish and seven species of birds collected from inland, coastal, and remote aquatic ecosystems. In addition, liver and kidney tissues from black bears were analyzed. Methyl paraben (MeP) was found in a majority of the tissues, with the highest concentration (796ng/g (wet weight [wet wt])) found in the liver of a bald eagle from Michigan. 4-Hydroxy benzoate (HB) was the major metabolite, found in 91% of the tissue samples analyzed at concentrations as high as 68,600ng/g, wet wt, which was found in the liver of a white-tailed sea eagle from the Baltic Sea coast.*"

> Xue J, Kannan K. (2016) Accumulation profiles of parabens and their metabolites in fish, black bear, and birds, including bald eagles and albatrosses. Environ Int. 2016 Sep;94:546-553. doi: 10.1016/j.envint.2016.06.015.
>
> "*The widespread exposure of humans to parabens present in personal care products is well-known. Nevertheless, little is known about the accumulation of parabens in marine organisms. In this study, six parabens and four common metabolites of parabens were measured in 121 tissue samples from eight species of marine mammals collected along the coastal waters of Florida, California, Washington, and Alaska. Methyl paraben (MeP) was the predominant compound found in the majority of the marine mammal tissues analyzed, and the highest concentration found was 865 ng/g (wet weight [wet wt]) in the livers of bottlenose dolphins from Sarasota Bay, FL. 4-Hydroxybenzoic acid (4-HB) was the predominant paraben metabolite found in all tissue samples. The measured concentrations of 4-HB were on the order of hundreds to thousands of ng/g tissue, and these values are some of the highest ever reported in the literature. MeP and 4-HB concentrations showed a significant positive correlation ($p < 0.05$), which suggested a common source of exposure to these compounds in marine mammals. Trace concentrations of MeP and 4-HB were found in the livers of polar bears from the Chuckchi Sea and Beaufort Sea, which suggested widespread distribution of MeP and 4-HB in the oceanic environment.*"

Source: Xue J, Sasaki N, Elangovan M, Diamond G, Kannan K. (2015) Elevated Accumulation of Parabens and their Metabolites in Marine Mammals from the United States Coastal Waters. Environ Sci Technol. 2015 Oct 20;49(20):12071-9. doi: 10.1021/acs.est.5b03601.

Similarly, the current safety assessment claims "*"Little or no unchanged paraben is excreted in the urine*." This claim is also outdated and is contradicted by newer data included in the current assessment which states:

"Free and conjugated parabens and their known, non-specific metabolites, p-hydroxybenzoic acid and p-hydroxyhippuric acid, were detected in the urine samples…17.4 %, 6.8 %, 5.6% of the doses of Methylparaben, Isobutylparaben and Butylparaben, respectively, were excreted in the urine; about 16% and 6% of Isobutylparaben and Butylparaben were excreted as 2OH-iso-butylparaben and 3OH-n-butylparaben, respectively; less than 1% was excreted as ring-hydroxylated metabolites "

Clearly when 17.4% of methylparaben is excreted as free parabens in the urine, the statement that "little or no unchanged paraben is excreted in the urine" is incorrect.

3.) **Personal care product use is the most significant contributor to paraben exposure**

The vast majority of the literature that has measured paraben exposure with respect to the use of cosmetic products has concluded that the use of cosmetics or personal care products is the most significant source of paraben exposure, much greater than the contributions from food or pharmaceuticals. The current draft assessment however, still includes language implying that non-cosmetic sources of parabens may be significant. This language should be corrected to reflect the most current information.

Specifically, the discussion section of the draft safety assessment currently states:

"*The high levels of Methylparaben and Propylparaben observed in tissues could be due to the fact that they are the most common compound used as preservative not only in cosmetics and hygiene products, but also in food, beverages, pharmaceuticals household pesticides, cleaning products, paints, pet supplies, and paper products."*

First, this statement is misleading, as it implies that parabens are the most common compound used as a preservative in food, beverages, pharmaceuticals, household pesticides, cleaning products, paints, pet supplies, and paper products. This is patently untrue, and should be deleted. Parabens are used as preservatives in these other products, but are certainly not the most common preservative used in any of these industries by any measure.

Secondly, this statement contradicts data included in other parts of the assessment. With respect to paraben exposure from food for example, the CIR safety assessment clearly states:

"…estimates for exposure to Methylparaben and Propylparaben via food are at last 25-fold lower than the estimates for aggregate exposure resulting from dermal exposure to cosmetic products."

Thus the contribution to body burden from foods is almost negligible compared to the contribution from cosmetic products, so the high levels of methylparaben and propylparaben observed in tissues are extraordinarily unlikely to be "due to the fact" that parabens are used as preservatives in food.

With respect to pharmaceuticals, the Estimate and Refinement of Aggregate Exposure section of the safety assessment states:

"*In addition to cosmetic and personal care products, parabens are also widely used in drugs and foods… The Dutch National Institute for Public Health and the Environment (RIVM) conducted an exposure assessment inconsideration of the aggregated exposure to parabens via three major sources: PCPs, foods, and medicinal products. For Methylparaben, adding exposures results in an aggregate exposure estimate of 3.0 mg/kg/day for both adults and children. The estimate for medicinal products contributes 70 - 74% of this value, while the contribution of food is less than 1%. For Propylparaben, adding the exposures results in an aggregate exposure estimate of 1.2 mg/kg/day for both children and adults; 64 - 72% of the exposure is from medicinal products, and less than 1% from food*."

Again these statements are highly misleading as they imply that the RIVM report claims that medicinal products contribute 64-74% of the aggregate exposure of parabens. What is missing from this section is the very important information that the RIVM report has very little confidence in the data on medicinal products (which were derived from a single study conducted in China.) In contrast the synopsis of the RIVM report states:

"*Exposure via personal care products has been examined in some detail and generally seems to be the greatest contributor to total exposure. Exposure via food appears to be negligible. Too little information is available for an acceptable estimate of exposure via medicines."*

Truly, scientists across the board, understand cosmetic products to be the most significant determinants of paraben exposure. This is confirmed in numerous studies showing the enormous increases in paraben levels in bodies of people who regularly use cosmetic products compared to those who do not – differences of up to 1000%. It is impossible to ignore the preponderance of data and continue to claim that a majority of paraben exposure could be due to other non-cosmetic factors.

Below is a summary of recent research indicating that personal care product use is the most significant predictor of paraben exposure:

"*Use of hair products, deodorants, face and hand creams were significantly associated with higher urinary levels of parabens…In the present study, we investigated both food consumption and use of PCPs as separate determinants of exposure. Use of body and face creams, deodorants and hair products were associated with higher concentrations of most parabens in both mothers and children… None of the environmental phenols showed a clear pattern with any of the food groups consumed in the present study… **Body and face creams, deodorants and hair products were the main determinants of urinary parabens** and BP-3."*

Sakhi AK, Sabaredzovic A, Papadopoulou E, Cequier E, Thomsen C. (2018) Levels, variability and determinants of environmental phenols in pairs of Norwegian mothers and children. Environ Int. 2018 May;114:242-251. doi: 10.1016/j.envint.2018.02.037

*"Overall, **use of 10 PCPs** within 6 h prior to collection explained at least 70% of the weighted score. These included cologne/perfume, deodorant, suntan/sunblock lotion, hand/body lotion, aftershave, other hair care products, mouthwash, conditioner/crème rinse, hairspray/hair gel, and liquid soap/body wash, which **explained at least a 254% and up to a 1,333% increase in MEP and the three parabens urinary concentrations.**"*

*"**For the three parabens, the strongest predictors were use of suntan/sunblock lotion (66–156% increase) and of hand/body lotion** (79–147% increase). Hairspray/hair gel, shaving cream, aftershave, mouthwash, and deodorant were moderate predictors for parabens. Liquid soap/body wash use was a strong predictor only for butylparaben (86%)."*

Nassan FL, Coull BA, Gaskins AJ, Williams MA, Skakkebaek NE, Ford JB, Ye X, Calafat AM, Braun JM, Hauser R. (2017) Personal Care Product Use in Men and Urinary Concentrations of Select Phthalate Metabolites and Parabens: Results from the Environment And Reproductive Health (EARTH) Study. Environ Health Perspect. 2017 Aug 18;125(8):087012. doi: 10.1289/EHP1374.

"*Girls who reported using makeup every day vs. rarely/never had higher urinary concentrations of monoethyl phthalate (MEP) (102.2 ng/mL vs. 52.4 ng/mL, P-value: 0.04), methyl paraben (MP) (120.5 ng/mL vs. 13.4 ng/mL, P-value < 0.01), and propyl paraben (PP) (60.4 ng/mL vs. 2.9 ng/mL, P-value < 0.01). Girls who reported recent use of specific makeup products, including foundation, blush, and mascara, had higher urinary concentrations of MEP, mono-n-butyl phthalate (MBP), MP, and PP… **Our findings suggest that personal care product use is associated with higher exposure to certain phthalates, parabens, and other phenols in urine**. This may be especially relevant in adolescent girls who have high use of personal care products during a period of important reproductive development*."

Berger KP, Kogut KR, Bradman A, She J, Gavin Q, Zahedi R, Parra KL, Harley KG. (2018) Personal care product use as a predictor of urinary concentrations of certain phthalates, parabens, and phenols in the HERMOSA study. J Expo Sci Environ Epidemiol. 2018 Jan 9. doi: 10.1038/s41370-017-0003-z.

*"…Women who used lotion had BP concentrations 111% higher (95% confidence interval (CI): 41%, 216%) than non-users, whereas their MBP concentrations were only 28% higher (CI: 2%, 62%)… **We observed a monotonic dose-response relationship between the total number of products used and urinary paraben** and phthalate metabolite concentrations."*

Braun JM, Just AC, Williams PL, Smith KW, Calafat AM, Hauser R. (2014) Personal care product use and urinary phthalate metabolite and paraben concentrations during pregnancy among women from a fertility clinic. J Expo Sci Environ Epidemiol. 2014 Sep-Oct;24(5):459-66. doi: 10.1038/jes.2013.69.

"*"Compared with individuals who reported "Never" using mouthwash, individuals who reported daily use had significantly elevated urinary concentrations of mono-ethyl phthalate, methyl and propyl parabens, and BP3 (28%, 30%, 39%, and 42% higher, respectively).* ***Individuals who reported "Always" using sunscreen had significantly higher urinary concentrations*** *of triclosan, methyl, ethyl, and propyl parabens, and BP3 (59%, 92%, 102%, 151%, and 510% higher, respectively)* ***compared with "Never" users of sunscreen****."*

Ferguson KK, Colacino JA, Lewis RC, Meeker JD. (2017) Personal care product use among adults in NHANES: associations between urinary phthalate metabolites and phenols and use of mouthwash and sunscreen. J Expo Sci Environ Epidemiol. 2017 May;27(3):326-332. doi: 10.1038/jes.2016.27

"A statistically significant difference was demonstrated between serum parabens in women who used lipstick containing these substances compared with those not using this cosmetic (p = 0.0005 and 0.0016, respectively), and a strong association was observed between serum parabens and lipstick use (Spearman correlation = 0.7202)."

Tahan GP, de Kássia Souza Santos N, Albuquerque AC, Martins I. (2016) Determination of parabens in serum by liquid chromatography-tandem mass spectrometry: Correlation with lipstick use. Regul Toxicol Pharmacol. 2016 Aug;79:42-8. doi: 10.1016/j.yrtph.2016.05.001. Epub 2016 May 3.

"The use of lotions in the previous 24 h was associated with 80−110% higher levels of parabens (BP, EP, MP, and PP). Users of shampoo, conditioner, and cosmetics (makeup and eye makeup) also had urinary BP concentrations 72−84% higher compared to nonusers. Soap use in the past 24 h was also significantly associated with higher PP and MP concentrations in urine. **Women who were categorized as "High Product Category Users" had between 100 and 200% higher parabens concentrations compared to "Low Product Category Users"**.

Fisher M et.al. (2017) Paraben Concentrations in Maternal Urine and Breast Milk and Its Association with Personal Care Product Use. Environ Sci Technol. 2017 Apr 4;51(7):4009-4017. doi: 10.1021/acs.est.6b04302

4) Particle Sizes of additional parabens should be included in Table 4

Table 4 on particle size ranges of parabens should include data on other parabens included in the report, not just the four parabens currently listed: Sodium Methylparaben; Ethylparaben; Sodium Ethylparaben; Sodium Propylparaben. Specifically:

For example, particle size data can also be found for:

Methylparaben

https://echa.europa.eu/registration-dossier/-/registered-dossier/14310/4/6

Propylparaben:

https://echa.europa.eu/registration-dossier/-/registered-dossier/13890/4/6

Butylparaben

https://echa.europa.eu/registration-dossier/-/registered-dossier/25335/4/6

Isopropylparaben

https://echa.europa.eu/registration-dossier/-/registered-dossier/19482/4/6

Isobutylparaben

https://echa.europa.eu/registration-dossier/-/registered-dossier/17752/4/6

4-hydroxtbenzoic acid:

https://echa.europa.eu/registration-dossier/-/registered-dossier/15944/4/6

Of note, the ECHA data indicate that for propylparaben, 37.8% of particles are smaller than 10 microns in diameter, the CIR's established level of concern for inhalation. This information contradicts the summary claim found in the report that 95-99% of particles have diameters greater than 10 microns and are thus unlikely to be inhaled deeply. This statement should be corrected.

5. **The Margin of Safety (MOS) Calculation should be more conservative.**

The calculation used to derive a MOS for parabens needs to be updated to reflect more conservative product usage amounts and more conservative absorption rates that are consistent with data included in other sections of the assessment.

Currently the calculations in the safety assessment are:

> *Systemic exposure dose (SED, Butylparaben) = 17.76 g/day of product x 0.4 % use concentration ÷ 60 kg person x 50 % absorption x 1000 mg/g conversion factor = 0.59 mg/kg/day*
>
> *MOS (adult, Butylparaben) = NOAEL/SED = 160 mg/kg/day / 0.59 mg/kg/day= 270*

Systemic exposure dose (SED, multiple parabens) = 17.76 g/day of product x 0.8 % use concentration ÷ 60 kg person x 50 % absorption x 1000 mg/g conversion factor = 1.18 mg/kg/day

MOS (adult, multiple paraben) = NOAEL/SED = 160 mg/kg/day / 1.18 mg/kg/day= 135

These calculations use an estimate of 0.4% use concentration of Butylparaben, and a 0.8% use concentration for multiple parabens. These numbers come from the regulatory limits applied in the EU in which no more than 0.4% butylparaben and no more than 0.8% cumulative parabens are allowed in products. Those regulatory limits however, do not apply in the United States. According to the VCRP data included in the safety assessment, the maximum reported use rate for butylparaben is 0.5% (not 0.4%). Therefore 0.5% (or higher) would be a more appropriate conservative use rate for exposure in the United States.

Similarly, for a use rate for cumulative parabens, 0.8% seems low. Unfortunately the VCRP data as reported does not provide data on levels of parabens used cumulatively in products. However, the data does show us that the maximum concentration of use of methylparaben is 0.9%, the maximum for propylparaben is 0.7%, the maximum for ethylparaben is 0.65% etc. We know that these parabens are used in combination in products, and cannot rule out that they would be used at these maximum concentrations together. The current use of 0.8% for combined parabens is not a conservative estimate for concentration of use and should be increased to get a more accurate margin of safety.

Secondly, the calculations currently use an estimated absorption rate of 50%. However, the safety assessment cites a risk assessment study published by Procter & Gamble researchers (Cowan-Ellsberry and Robison 2009) which supports the use of a conservative absorption rate of 80%. Specifically, the study states:

> *"The measured extent of dermal penetration and metabolism [of parabens] in these studies is somewhat variable ranging from 15% to 75% probably due to matrix effects, differences in animals used and other experimental artifacts. Therefore, we chose 80% as a conservative estimate of the amount of the parabens either as parent or metabolite penetrating the skin. This is consistent with the guidance that has recently been outlined for estimating exposure to materials which occur at low levels in cosmetic products."*

Source: Cowan-Ellsberry CE and Robison SH. Refining aggregate exposure: example using parabens. Regul Toxicol Pharmacol. 2009;55(3):321-329.

The CIR calculations should also reflect this more reasonable conservative estimate of absorption.

Lastly, the recent draft reflects a change in the calculations from using a NOAEL of 1,000 mg/kg/day to a NOAEL of 160 mg/kg/day. The recommendation to use this lower NOAEL came from George Daston , a Procter and Gamble researcher. While I am sure Dr. Daston is highly respected in his field, it cannot be ignored that his employer, Procter and Gamble would be significantly affected financially by a decision of the CIR that current usage of parabens does not result in an appropriate margin of safety. The conflict of interest is there. Given that, the draft assessment should be especially transparent on the

justification of the panel to use the NOAEL of 160 mg/kg/day rather than the more conservative NOAEL of 10 mg/kg/day form the Boberg study or even the NOEL of 2 mg/kg/day used by the European researchers. Currently the safety assessment briefly summarizes possible reasons for not using these other more conservative values, but doesn't give specifics. The wording used currently unfortunately gives the impression that Procter and Gamble has made the decision for the CIR to use a less protective level, rather than the panel themselves. Additional information specifying the individual reasons that the other two possible NOAEL/NOEL values were dismissed, would alleviate what currently appears to be a conflict of interest.

Thank you for the opportunity to comment on this safety assessment and appreciate the careful consideration of these comments by the Panel and staff of the CIR.

Sincerely,

Alexandra Scranton
Director of Science and Research
Women's Voices for the Earth

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Memorandum

To: CIR Expert Panel Members and Liaisons
From: Wilbur Johnson, Jr.
Senior Scientific Analyst
Date: September 14, 2018
Subject: Wave 2 Data on Polyol Phosphates

The data listed below (in *phytic092018data1 file*) on Sodium Mannose Phosphate were received from the Council and are being submitted as an attachment to this memorandum. A data summary document (*phytic092018wave2studysummaries*) is also attached for the Panel's review. The data that were received include:

- Method of manufacture
- Impurities data

These data will be added to the safety assessment after the Panel meeting.

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Wave 2 Data on Polyol Phosphates

CHEMISTRY

Method of Manufacture

Sodium Mannose Phosphate

Sodium Mannose Phosphate is manufactured by enzymatic reaction from pyrophosphate and mannose.[1] The reaction medium is then stabilized by denaturing the enzyme. This step is followed by purification of the medium.

Impurities

Sodium Mannose Phosphate

Possible impurities (0.1% to 0.5%) of Sodium Mannose Phosphate are: sodium phosphate, sodium pyrophosphate, sodium chloride, and magnesium and ammonium ions.[1]

References

1. Anonymous. 2018. Method of manufacture and impurities- Sodium Mannose Phosphate. Unpublished data submitted by Personal Care Products Council.

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: August 22, 2018

SUBJECT: Sodium Mannose Phosphate

Anonymous. 2018. Method of manufacture and impurities - Sodium Mannose Phosphate.

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August 2018

Method of Manufacture and Impurities - Sodium Mannose Phosphate

Sodium Mannose Phosphate is obtained by enzymatic reaction from pyrophosphate and mannose. The reaction medium is then stabilized by denaturing the enzyme. Then the stabilized synthesis medium is purified.

The possible impurities (0.1-0.5%) are the following :
- phosphate, sodium salt
- pyrophosphate, sodium salt
- sodium chloride
- magnesium and ammonium ions

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Memorandum

To: CIR Expert Panel Members and Liaisons
From: Wilbur Johnson, Jr.
Senior Scientific Analyst
Date: September 14, 2018
Subject: Wave 2 Data on Titanium Complexes

A summary of a publication on titanium sensitivity (*organo092018wave2studysummaries*) that was received from the Council is attached for the Panel's review. The data summarized are from a retrospective patient study, and more than 50% of the patients with positive reactions to titanium salts had a titanium-containing implant or reconstructive material. The patients were patch tested with five titanium salts, including Titanium Citrate.

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Wave 2 Data on Titanium Complexes

DERMAL IRRITATION AND SENSITIZATION STUDIES

Sensitization

Titanium Citrate

A retrospective chart review was conducted on 458 patients who underwent patch testing with Titanium Citrate and other titanium salts, over a 10-year period.[1] The patch testing of titanium salts was performed at a dermatology clinic in the Netherlands, using Van der Bend chambers that were applied to the back for 48 h. Reactions were scored on days 2, 3, and 7 according to International Contact Dermatitis Research Group (ICDRG)/European Society of Contact Dermatitis (ESCD) criteria. Reactions identified as +, ++, or +++ were classified as positive, whereas doubtful reactions (?+) were not. At least one positive reaction was observed in 26 (5.7%) of the 458 patients patch tested. Fifteen (57.7%) of these 26 patients had a proven titanium-containing implant or reconstructive material. Also, most of the titanium-positive patients had local symptoms, i.e., pain, erythema, dermatitis, pruritus, impaired wound healing, and swelling. For 16 (61.5%) of the 26 positive patients, complete or partial relevance of the positive result was determined. Overall, the percentage of positive reactions induced by each titanium salt was reported as follows: titanium (IV) oxalate hydrate (7.9%; 17 of 216 patients tested), titanium lactate (4.4%; 2 of 45 patients tested), titanium (IV) isopropoxide (2.9%; 8 of 272 patients tested), Titanium Citrate (2.2%; 1 of 45 patients tested), and titanium dioxide (0.9%; 3 of 329 patients tested).

Additional results presented relate to the fact that the 458 patients were divided into the following 3 groups: Group 1 (248 patients suspected of having titanium allergy), Group 2 (163 patients suspected of having metal allergy other than to titanium), and Group 3 (control group of 47 patients who were not exposed to titanium-containing medical devices and did not have a specific history of titanium allergy). The results (% positive reactions) were as follows. Group 1: titanium (IV) isopropoxide (0.01%: 0.44% positive, 1 of 224 patients tested; 1% concentration: 1.78% positive, 4 of 224 patients tested; 5% concentration: 0.46% positive, 1 of 224 patients tested; and 10% concentration: 0.44% positive, 2 of 224 patients tested); Titanium Citrate (0.16% concentration: 2.70% positive, 1 of 37 patients tested; and 0.32% concentration: 2.70% positive, 1 of 37 patients tested [it is not stated whether the same patient reacted to both concentrations]); titanium lactate (0.16% concentration: 5.41% positive, 2 of 37 patients tested); and titanium dioxide (as is: 0.72% positive, 1 of 139 patients tested); Group 2: titanium (IV) oxalate hydrate (5% concentration: 0% positive, 0 of 4 patients tested), titanium (IV) isopropoxide (up to 20% concentration: 0% positive, 0 of 4 patients tested); and titanium dioxide (as is: 1.26% positive, 2 of 159 patients tested); and Group 3: titanium (IV) oxalate hydrate (5% concentration: 5.26% positive, 2 of 38 patients tested), titanium (IV) isopropoxide (up to 20% concentration: 0% positive, 0 of 44 patients tested); Titanium Citrate (up to 0.32% concentration: 0% positive, 0 of 8 patients tested); titanium lactate (up to 0.24% concentration: 0% positive, 0 of 8 patients tested); and titanium dioxide (as is: 0% positive, 0 of 31 patients tested). Regarding these results, the authors stated that it should be noted that the patient groups tested with Titanium Citrate and titanium lactate were small when compared to the patient groups tested with the other salts.

In the conclusion for this study, the authors noted that, in evaluating titanium sensitivity, a single titanium salt cannot be used as a patch test preparation because patient-specific responses occur to different salts, their accuracy in diagnosing titanium sensitization is unknown, and determining the relevance of a positive result is still challenging.[1]

References

1. de Graaf N, Feilzer A, Kleverlaan C, et al. A retrospective study on titanium sensitivity: Patch test materials and manifestations. *Contact Dermatitis*. 2018;79:85-90.

A retrospective study on titanium sensitivity: Patch test materials and manifestations. de Graaf NPJ, Feilzer AJ, Kleverlaan CJ, Bontkes H, Gibbs S, Rustemeyer T. *Contact Dermatitis*. 2018 May 24 [Epub ahead of print]

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Memorandum

To: CIR Expert Panel Members and Liaisons
From: Wilbur Johnson, Jr.
Senior Scientific Analyst
Date: September 14, 2018
Subject: Wave 2 Data on Vinylpyrrolidone Polymers

The data listed below on Hydrolyzed Wheat Protein/PVP Crosspolymer (in *vinylp092018data4 file*) and on other vinylpyrrolidone polymers (in *vinylp092018data5 file)* were received from the Council and are submitted as an attachment to this memorandum. A data summary document (*vinylp092018wave2studysummaries*) is also attached for the Panel's review. Data on the other vinylpyrrolidone polymers were submitted in tabular form and are not included in the data summary document. The data that were received include:

Hydrolyzed Wheat Protein/PVP Crosspolymer

- Physical properties
- Method of production
- Impurities
- Skin irritation (*in vitro*)
- Ocular irritation (*in vitro*)

VP/Hexadecene Copolymer, VP/Eicosene Copolymer, Acrylic Acid/VP Crosspolymer, Butylated PVP, PVP, Styrene/VP Copolymer, Triacontanyl PVP, Vinyl Caprolactam/VP/Dimethylaminoethyl Methacrylate Copolymer, VP/Acrylates/Lauryl Methacrylate Copolymer, VP/Dimethylaminoethylmethacrylate Copolymer, VP/DMAPA Acrvlates Copolymer, VP/VA Copolymer, and VP/Vinyl Caprolactam/DMAPA Acrylates Copolymer

- Molecular weights
- Residual monomer content
- Other impurities

These data will be added to the safety assessment after the Panel meeting.

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Wave 2 Data on Vinylpyrrolidone Polymers

CHEMISTRY

Physical and Chemical Properties

Hydrolyzed Wheat Protein/PVP Crosspolymer

Using gel permeation chromatography, weight-average (M_w) molecular weight of Hydrolyzed Wheat Protein/PVP Crosspolymer was determined to be 41,020 Da.[1]

Method of Manufacture

Hydrolyzed Wheat Protein/PVP Crosspolymer

According to one manufacturer, the method of manufacture of Hydrolyzed Wheat Protein begins with a solution of protein, water, and enzyme.[2] The pH of the mixture is adjusted and additional enzyme is added. This is accompanied by addition of a denaturant, followed by filtration using activated carbon. Filtration is followed by purification, evaporation, and preservation. The next step is the copolymerization of vinylpyrrolidone in the presence of an initiator, and this is followed by pH adjustment and preservation. The reaction mixture is then diluted in accordance with established specifications, which is followed by filtration into packs.

Hydrolyzed Wheat Protein/PVP Crosspolymer

According to a chemical supplier, the main impurity in Hydrolyzed Wheat Protein/PVP Crosspolymer is ash, up to a maximum of 2%.[2] Furthermore, an internal specification of 0.2% maximum has been established for residual *N*-vinylpyrrolidone monomer.

DERMAL IRRITATION AND SENSITIZATION STUDIES

Irritation

In Vitro

Hydrolyzed Wheat Protein/PVP Crosspolymer

The skin irritation potential of Hydrolyzed Wheat Protein/PVP Crosspolymer (21% solids, i.e., concentration of Hydrolyzed Wheat Protein/PVP Crosspolymer) was evaluated using the Episkin™ reconstituted human epidermis model.[3] The principle of this assay is based on the measurement of cytotoxicity in epidermal cultures, following topical exposure to the test substance, using the colorimetric MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl-tertrazolium bromide) reduction assay. Cell viability is measured by enzymatic reduction of the yellow MTT tetrazolium salt to a blue formazan salt (within the mitochondria of viable cells) in test substance-treated cells relative to the negative controls. Dulbecco's phosphate buffered saline (PBS) with calcium and magnesium served as the negative control, and 5% w/v aqueous sodium dodecyl sulfate served as the positive control. Triplicate tissues were treated with the test substance (as supplied, 10 µl) for 15 min, followed by a post-exposure incubation period of 42 h. After 42 h, each tissue was obtained for MTT-loading. After loading, a total biopsy of each epidermis was made and formazan crystals were extracted out of the MTT-loaded tissues. At the end of extraction, optical density was measured at 540 nm. Data were presented in the form of % viability (i.e., MTT reduction in test substance-treated tissues relative to negative control tissues). The relative mean viability of tissues treated with Hydrolyzed Wheat Protein/PVP Crosspolymer for 15 min was 86.6%, and the test substance was classified as a non-irritant.

OCULAR IRRITATION STUDIES

In Vitro

Hydrolyzed Wheat Protein/PVP Crosspolymer

The ocular irritation potential of Hydrolyzed Wheat Protein/PVP Crosspolymer (21% solids, i.e., concentration of Hydrolyzed Wheat Protein/PVP Crosspolymer) was evaluated using the SkinEthic™ reconstituted human corneal epithelium model.[4] This test is based on the hypothesis that irritant chemicals are able to penetrate the corneal epithelial tissue and are sufficiently cytotoxic to cause cell death. The tissues were treated with 30 µl of the test substance for 10 min, and the study design consisted of a test for direct reduction of MTT. Triplicate tissues were also treated with 30 µl of a negative control solution (negative control) and 30 µl of 1% w/v sodium dodecyl sulfate (positive control). Following MTT loading, the reduced MTT was extracted from the tissues. After extraction, the absorbency of triplicate aliquots of the extracted MTT solution for each SkinEthic™ tissue was measured. The optical density was measured at 540 nm, and data were presented in the form of % viability (i.e., MTT conversion relative to negative controls). The % relative mean tissue viability of Hydrolyzed Wheat Protein/PVP Crosspolymer was ≥ 60%, classifying the test substance as a non-irritant.

References

1. Anonymous. Cirrus GPC sample injection report - Hydrolyzed Wheat Protein/PVP Crosspolymer. Unpublished data submitted by the Personal Care Products Council on 5-10-2018. 2013. pp.1-2.

2. Anonymous. Process flow diagram - Hydrolyzed Wheat Protein/PVP Crosspolymer. Unpublished data submitted by the Personal Care Products Council on 5-10-2018. 2018. pp.1

3. Harlan Laboratories, Ltd. Determination of skin irritation potential using the EpiskinTM reconstituted human epidermis model (Hydrolyzed Wheat protein/PVP Crosspolymer 21% solids). Unpublished data submitted by the Personal Care Products Council on 5-10-2018. 2009. pp.1-20.

4. Harlan Laboratories, Ltd. Assessment of ocular irritation potential using the Skinethic reconstituted human corneal epithelium model (Hydrolyzed Wheat Protein/PVP Crosspolymer 21% solids). Unpublished data submitted by the Personal Care Products Council on 5-10-2018. 2009. pp.1-20.

Personal Care Products Council
Committed to Safety, Quality & Innovation

Memorandum

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

FROM: Carol Eisenmann, Ph.D.
Personal Care Products Council

DATE: May 10, 2018

SUBJECT: Hydrolyzed Wheat Protein/PVP Crosspolymer

Anonymous. 2018. Process flow diagram - Hydrolyzed Wheat Protein/PVP Crosspolymer.

Anonymous. 2013. Cirrus GPC sample injection report - Hydrolyzed Wheat Protein/PVP Crosspolymer.

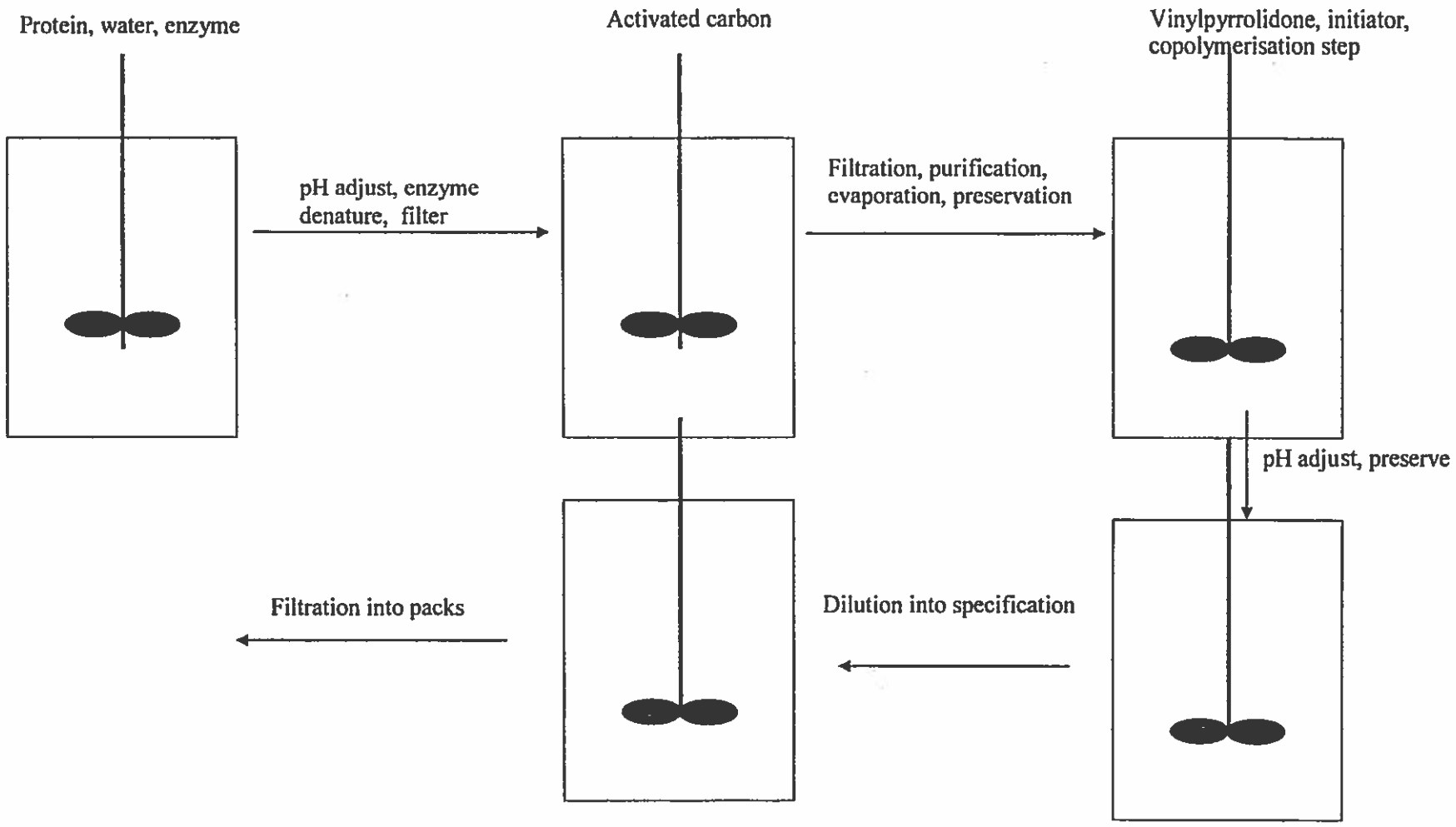
Harlan Laboratories, Ltd. 2009. Assessment of ocular irritation potential using the Skinethic reconstituted human corneal epithelium model (Hydrolyzed Wheat Protein/PVP Crosspolymer 21% solids).

Harlan Laboratories, Ltd. 2009. Determination of skin irritation potential using the Episkin™ reconstituted human epidermis model (Hydrolyzed Wheat Protein/PVP Crosspolymer 21% solids).

1620 L Street, N.W., Suite 1200 | Washington, D.C. 20036 | 202.331.1770 | 202.331.1969 (fax) | www.personalcarecouncil.org

May 2018

PROCESS FLOW DIAGRAM – Hydrolyzed Wheat Protein/PVP Crosspolymer



The main impurity in the product is ash (sodium chloride), up to a maximum of 2%.

We also have an internal specification for residual N-vinylpyrrolidone monomer of maximum 0.2%.

Cirrus GPC Sample Injection Report

18 March, 2013 9:51 AM

Sample Details Hydrolyzed Wheat Protein / PVP crosspolymer
Sample Name
Acquired: 18-Mar-13 9:43:58 AM
Batch Name: Imported

Wavelength=220 nm

Concentration: 1.00 mg/ml
Injection Volume: 5.0 ul
LIMS ID:

K of Sample: 14.1000
Alpha of Sample: 0.7000
Bottle ID:

Workbook Details
Eluent:
Column Set:
Detector:

Flow Rate: 1.00 ml/min
Column Set Length: 0 mm
Temperature:

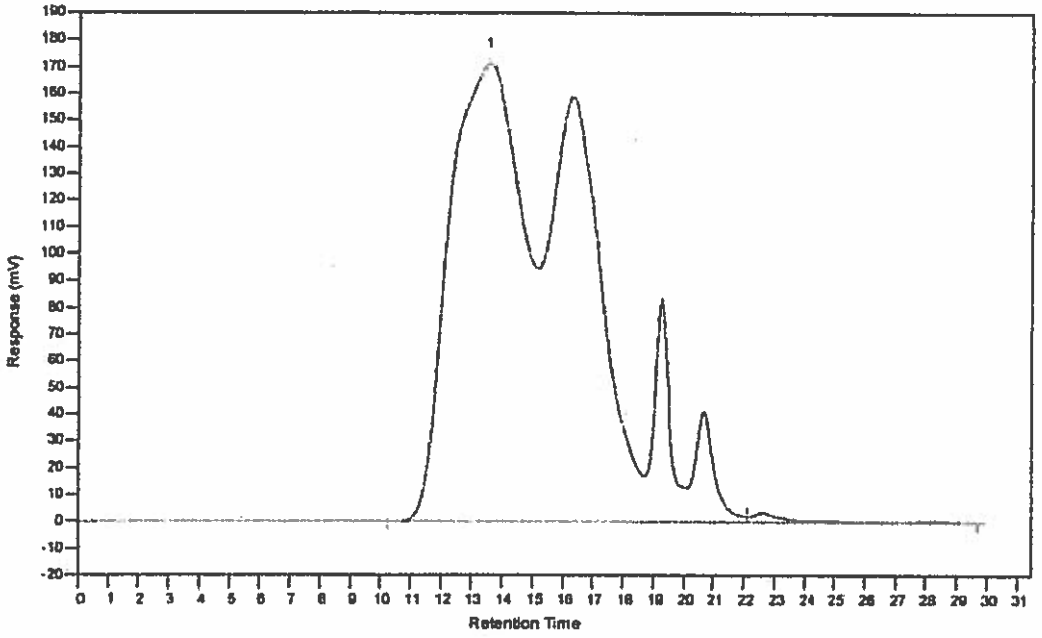
Analysis Using Method: Manual analysis
Comments: Standard integration

Calibration Used: 18-Mar-13 9:45:49 AM
Calibration Type: Narrow Standard
Calibration Curve: y = 12.004500 - 0.551028x^1

Curve Fit Used: 1

High Limit MW RT: 11.58 mins
High Limit MW: 421960
K: 14.1000
Alpha: 0.7000
FRCF: 1.0000

Low Limit MW RT: 15.49 mins
Low Limit MW: 2959
FRM Name:
Flow Marker RT: 0.00 mins



Sample Injection Report

MW Averages

Peak No	Mp	Mn	Mw	Mz	Mz+1	Mv	PD
1	35122	66	41020	190957	331846	27527	621.515

Processed Peaks

Peak No	Name	Start RT (mins)	Max RT (mins)	End RT (mins)	Pk Height (mV)	% Height	Area (mV.secs)	% Area
1		10.25	13.54	22.13	171.331	100	52992.6	100

Peak Detection

Peak No	Type	St Detect Code	End Detect Code	Is St Mod	Is Max Mod	Is End Mod
1	0	1	1	No	No	No

Baseline Detection

No	Start RT (mins)	End RT (mins)	Start Height	End Height	Is St Mod	Is End Mod
1	10.25	29.76	0.16	0.25	No	No

harlan™

Hydrolyzed Wheat Protein / PVP Cross polymer

ASSESSMENT OF OCULAR IRRITATION POTENTIAL USING THE SKINETHIC RECONSTITUTED HUMAN CORNEAL EPITHELIUM MODEL

AUTHOR: N Warren

STUDY SPONSOR:

TEST FACILITY:

Harlan Laboratories Ltd
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Shardlow
Derbyshire
DE72 2GD
UK

Telephone: +44 (0) 1332 792896

Facsimile: +44 (0) 1332 799018

QUALITY ASSURANCE REPORT

This study type is classed as short-term. The standard test method for this study type ("General Study Plan" in OECD terminology) was reviewed for compliance once only on initial production. Inspection of the routine and repetitive procedures that constitute the study is carried out as a continuous process designed to encompass the major phases at or about the time this study was in progress. In addition, inspection of general facilities not specifically related to this study are done monthly or annually in accordance with QA Standard Procedure.

This report has been audited by the Quality Assurance Unit, and is considered to be an accurate account of the data generated and of the procedures followed.

In each case, the outcome of QA evaluation is reported to the Study Director and Management on the day of evaluation. Audits of study documentation, and process inspections appropriate to the type and schedule of this study were as follows:

	19 November 2008	Standard Test Method Compliance Audit
	13 May 2009	Test Material Preparation
	19 May 2009	Test System Preparation
	13 May 2009	Exposure
	21 May 2009	Assessment of Response
§	11 June 2009	Draft Report Audit
§	Date of QA Signature	Final Report Audit

§ Evaluation specific to this study

..... DATE: 23 JUN 2009

For the Quality Assurance Unit*

*Authorised QA Signatures:

Manager, Quality Assurance:	J G Riley BSc (Hons) MRQA
Deputy Head of Department:	JM Crowther MIScT MRQA
Senior Audit Staff:	G Wren ONC MRQA

GLP COMPLIANCE STATEMENT

The work described was performed in compliance with UK GLP standards (Schedule 1, Good Laboratory Practice Regulations 1999 (SI 1999/3106 as amended by SI 2004/0994)). These Regulations are in accordance with GLP standards published as OECD Principles on Good Laboratory Practice (revised 1997, ENV/MC/CHEM(98)17); and are in accordance with, and implement, the requirements of Directives 2004/9/EC and 2004/10/EC.

This report fully and accurately reflects the procedures used and data generated.

..... DATE: 22/6/2009

N Warren MIAT
Study Director

CONTENTS

ASSESSMENT OF OCULAR IRRITATION POTENTIAL USING THE SKINETHIC RECONSTITUTED HUMAN CORNEAL EPITHELIUM MODEL

SUMMARY

Introduction. The purpose of this study was to determine the eye irritation potential of the test material using the SkinEthic Reconstituted Human Corneal model (HCE, SkinEthic Laboratories, Nice, France) after a treatment period of 10 minutes. The test is based on the hypothesis that irritant chemicals are able to penetrate the corneal epithelial tissue and are sufficiently cytotoxic to cause cell death.

Methods. The experimental design of the study consists of a test for direct reduction of MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl-tetrazolium bromide) by the test material followed by the main test.

For the main test, triplicate SkinEthic tissues were treated with 30 µl of the test material for 10 minutes. Triplicate tissues treated with 30 µl of Solution A served as the negative control and triplicate tissues treated with 30 µl of 1% w/v Sodium Dodecyl Sulphate served as the positive control.

At the end of the exposure period each SkinEthic tissue was rinsed. The rinsed tissues (two per group) were taken for MTT loading. The remaining tissues were retained for possible histopathology. Following MTT loading the reduced MTT was extracted from the tissues.

After extraction the absorbency of triplicate aliquots of the extracted MTT solution for each SkinEthic tissue was measured. The optical density was measured at 540 nm (OD_{540}). Data are presented in the form of % viability (MTT conversion relative to negative controls).

The test material was classified according to the following criteria:

i) If the % relative mean tissue viability was ≥ 60% the test material was considered to be non-irritant.

ii) If the % relative mean tissue viability was < 60% the test material was considered to be an irritant.

Results. The relative mean viability of the test material treated tissues after a 10 minute exposure was 108.0%.

It was considered unnecessary to proceed with tissue histopathology.

Quality criteria. The quality criteria required for acceptance of results in the test were satisfied.

Conclusion. According to the protocol followed the test material was considered to be a Non-Irritant (NI).

ASSESSMENT OF OCULAR IRRITATION POTENTIAL USING THE SKINETHIC RECONSTITUTED HUMAN CORNEAL EPITHELIUM MODEL

1. INTRODUCTION

The purpose of this study was to determine the eye irritation potential of the test material using the SkinEthic Reconstituted Human Corneal model (HCE, SkinEthic Laboratories, Nice, France) after a treatment period of 10 minutes [1, 2].

The SkinEthic RHC model consists of transformed human keratinocytes of the cell line HCE (LSU EYE Center, New Orleans, USA) that form a corneal epithelial tissue (mucosa), devoid of stratum corneum, resembling, histologically, the mucosa of the human eye. Test materials are applied directly to the culture surface, at the air interface, so that undiluted and/or end use dilutions can be tested directly. The model consists of an airlifted, living, corneal tissue construct, produced in polycarbonate inserts in serum-free and chemically defined medium.

The test is based on the hypothesis that irritant chemicals are able to penetrate the stratum corneum of the SkinEthic RHC model and are sufficiently cytotoxic to cause cell death in the underlying cell layers.

Cytotoxicity was determined by the reduction of MTT to formazan by viable cells in the test material treated tissues (quantitative measurement of tissue viability) relative to the negative control.

One tissue for each treatment group was retained for possible tissue histopathology.

The control groups served as common controls with Harlan Laboratories Ltd Project number 2724/0005.

The study was performed between 26 May 2009 and 28 May 2009.

2. TEST MATERIAL

2.1 Description, Identification and Storage Conditions

Sponsor's identification	:	Hydrolyzed Wheat Protein / PVP Cross Polymer
Description	:	amber coloured liquid
Batch number	:	
Date received	:	11 December 2008
Storage conditions	:	room temperature in the dark

The integrity of supplied data relating to the identity, purity and stability of the test material is the responsibility of the Sponsor.

A Certificate of Analysis supplied by the Sponsor is given in Appendix 2.

2.2 Preparation of Test Material

The test material was used as supplied.

3. NEGATIVE AND POSITIVE CONTROLS

The negative control material, Solution A, was used as supplied (composition of Solution A included as Appendix 1).

The positive control material, Sodium Dodecyl Sulphate (SDS), was prepared as a 1% w/v solution in sterile distilled water.

4. SKINETHIC HCE MODEL

Supplier	:	SkinEthic Laboratories, Nice, France
Date Received	:	26 May 2009

5. METHODS

5.1 Pre-Test

5.1.1 Assessment of Direct Test Material Reduction of MTT

One limitation of the assay is possible interference of the test material with MTT. A test material may directly reduce MTT to formazan, thus mimicking dehydrogenase activity of the cellular mitochondria of viable cells. This property of the test material is only a problem, if at the time of the MTT test (after the chemical has been rinsed off) there are still sufficient amounts of the test material on or in the tissues. To identify this possible interference, the test material was checked for its ability to reduce MTT directly.

30 µl of test material was added to a 0.5 mg/ml MTT solution and incubated at room temperature in the dark for 60 minutes. Untreated MTT solution was used as a control. If the MTT solution turned black/purple, the test material was presumed to have reduced the MTT.

5.1.2 Receipt of Tissues

On arrival, the SkinEthic HCE tissues (Day 6 cultures), were stored at room temperature prior to transferring into 24-well plates designated 'arrival plates' containing 300 µl of maintenance medium. It was important to ensure that there were no air bubbles present under the tissue inserts. The tissues were incubated overnight at 37°C, 5% CO_2 in air.

5.1.3 Preparation of Tissues

Using sterile techniques, 1 ml of maintenance medium at room temperature, was dispensed into the appropriate number of wells of 6-well plates designated 'treatment plates'. Each well was labelled with details of the treatment and the appropriate exposure time. Separate treatment plates were used for the test material and negative and positive controls to avoid the possibility of cross contamination occurring. Before treatment, the 7 day old tissues were transferred from the 'arrival plates' into the wells of the 'treatment plates' containing the maintenance medium.

5.2 Main Test

Triplicate tissues were treated with 30 µl of the test material for 10 minutes. The tissues were dosed at regular timed intervals to allow for the period taken to rinse each insert following exposure and to ensure each tissue received an equal exposure time. Triplicate tissues were treated with 30 µl of solution A to serve as negative controls and triplicate tissues were treated with 30 µl of 1 % w/v SDS to serve as positive controls. The plates were incubated at 37°C, 5% CO_2 in air during the exposure time.

At the end of the relevant exposure period, each tissue insert was removed from the well using forceps and rinsed using a wash bottle containing Dulbecco's Phosphate Buffered Saline (DPBS). Rinsing was achieved by filling and emptying each tissue insert using a constant soft stream of DPBS to gently remove any residual test material. Excess DPBS was removed by blotting the bottom of the insert with absorbent paper. Each tissue was placed into a pre-labelled 24-well plate designated 'holding plate' containing 300 µl of maintenance medium (at room temperature) until all the tissues were rinsed. Following rinsing, the tissues (two per group) were transferred to a pre-labelled 24-well plate designated 'MTT Loading plate' containing 300 µl of a 0.5 mg/ml MTT solution freshly prepared in maintenance medium. The MTT loading plate was placed into an incubator for approximately three hours at 37°C, 5% CO_2 in air.

At the end of the incubation period, the tissues were visually examined and the degree of MTT staining evaluated (qualitative evaluation of tissue viability). The inserts were blotted on absorbent paper to remove residual MTT solution and transferred to a pre-labelled 24-well plate designated 'MTT extraction plate' containing 0.75 ml of Isopropanol in each of a sufficient number of wells. An extra 0.75 ml of Isopropanol was added onto each tissue and the plate sealed to prevent Isopropanol evaporation. The plate was wrapped in aluminium foil (to protect from light) and allowed to stand overnight at room temperature to extract the formazan crystals out of the tissue.

At the end of the extraction period, each tissue insert was pierced with a pipette fitted with a 1000 µl tip and the extraction solution forced vigorously up and down through the tissue insert until a homogeneous solution was obtained. The empty inserts were discarded. For each tissue triplicate 200 µl samples were transferred to the appropriate wells of a pre-labelled 96-well plate. 200 µl of Isopropanol alone was added to three wells designated as 'blanks'. The optical density was measured (quantitative measurement of tissue viability) at 540nm (OD_{540}) using the Anthos 2001 microplate reader.

5.2.1 Tissue Histology

One tissue for each treatment group was retained for possible tissue histopathology.

The tissues were carefully cut out of the polycarbonate inserts with a sharp scalpel. The tissues were carefully cut in half. Both halves were placed into a pre-labelled 1.5 ml Eppendorf tube containing 1 ml of 10% Formalin and stored at room temperature.

6. INTERPRETATION OF RESULTS

The mean OD_{540} values of the duplicate tissues were calculated. Each of these OD_{540} values had already been corrected for blanks by the microplate reader.

The relative mean tissue viabilities (% of the negative control) were calculated as follows:

$$\text{Relative mean tissue viability} = \frac{\text{mean } OD_{540} \text{ of test material}}{\text{mean } OD_{540} \text{ of negative control}} \times 100$$

The mean tissue viabilities for the test material was compared to the respective untreated negative control and classified according to the following table:

Relative Mean tissue viability (% negative control)	Prediction
Tissue viability <60	Irritant (I)
Tissue viability ≥60	Non-Irritant (NI)

7. ASSAY ACCEPTANCE CRITERIA

The results of the assay are considered acceptable if the following assay acceptance criterion was achieved:

Assay Acceptance Criterion: Positive Control

The assay meets the acceptance criterion for an acceptable test if a decrease in relative tissue viability is observed following the 10-minute exposure period.

8. ARCHIVES

Unless instructed otherwise by the Sponsor, all original data and the final report will be retained in the Harlan Laboratories Ltd, Shardlow, UK archives for five years, after which instructions will be sought as to further retention or disposal.

9. RESULTS

9.1 Assessment of Direct Test Material Reduction of MTT

The test material was not able to directly reduce MTT.

9.2 Assessment of Eye Irritation Potential

The mean OD_{540} values and mean viabilities for each treatment group are given in Table 1.

The relative mean viability of the test material treated tissues after a 10 minute exposure was 108.0%.

It was considered unnecessary to proceed with tissue histopathology.

9.3 Qualitative Evaluation of Tissue Viability (MTT Uptake Visual Assessment)

The qualitative evaluation of tissue viability is presented in Table 2.

The test material and negative control material treated tissues appeared blue which was considered to be indicative of viable tissue. The positive control material treated tissues appeared blue/white which was considered to be indicative of semi-viable tissue.

9.4 Assay Acceptance Criterion

The quality criterion required for the acceptance of results in the test was satisfied.

10. CONCLUSION

According to the protocol followed the test material was considered to be a Non-Irritant (NI).

11. REFERENCES

1. Van Goethem F., Adriaens E., Alépée N., Straube F., De Wever B., Cappadoro M., Catoire S., Hansen E., Wolf A. and Vanparys P. Prevalidation of a new *in vitro* reconstituted human cornea model to assess the eye irritating potential of chemicals. Toxicology *in vitro* 20 (2006), pp.1-17.

2. Nguyen D. H., Beuerman R. W., De Wever B. and Rosdy M. Three-dimensional construct of the human corneal epithelium for *in vitro* toxicology. In: H. Salem and S.A. Katz, Editors, Alternative Toxicological Methods, CRC Press (2003), pp. 147-159.

Hydrolyzed Wheat Protein/PVP Cross Polymer

ASSESSMENT OF OCULAR IRRITATION POTENTIAL USING THE SKINETHIC RECONSTITUTED HUMAN CORNEAL EPITHELIUM MODEL

Table 1 Assessment of Eye Irritation Potential – Viability of RHC Tissues

Material	Mean Tissue Viability	Mean OD_{540}	% Viability
Negative Control⊕	0.960 0.857	0.909	100•
Positive Control⊕	0.704 0.660	0.682	75.0
Test Material	0.964 0.999	0.982	108.0

• = The mean viability of the negative control tissues is set at 100%
⊕ = Control group shared with Harlan Laboratories Ltd Project number 2724/0005

Hydrolyzed Wheat Protein / PVP Cross Polymer

ASSESSMENT OF OCULAR IRRITATION POTENTIAL USING THE SKINETHIC RECONSTITUTED HUMAN CORNEAL EPITHELIUM MODEL

Table 2 Qualitative Evaluation of Tissue Viability (MTT uptake visual evaluation)

Material	Score	
	Tissue 1	Tissue 2
Negative Control⊕	-	-
Positive Control⊕	+	+
Test Material	-	-

MTT Visual Scoring Scheme of SkinEthic Tissues

- \- = Blue tissue (viable)
- \+ = Blue/White tissue (semi viable)
- ++ = Tissue completely white (dead)

⊕ = Control group shared with Harlan Laboratories Ltd Project number 2724/0005

Hydrolyzed Wheat Protein/PVP Cross Polymer

ASSESSMENT OF OCULAR IRRITATION POTENTIAL USING THE SKINETHIC RECONSTITUTED HUMAN CORNEAL EPITHELIUM MODEL

Appendix 1 Solution A Composition for 1 Litre

- Na_2HPO_4 0.142 g/l
- Glucose 1.802 g/l
- HEPES 7.149 g/l
- KCl 0.224 g/l
- NaCl 7.597 g/l

Appendix 2 Certificate of Analysis

Certificate of Analysis

A quality management system registered to the international standard ISO 9001 was used to manufacture and test this material.
The name printed at the end of this document is an electronic signature.

Customer details

Customer Ref.
Inspection Lot 090000083934
C of A Printed. 28.11.2008

Quantity. 0.000
QA Contact.
Fax No.

Batch Details
Product Name:
Product Code:
Cust. Product Name:
Cust. Product Code:
Batch No.
Date of Test: 05.11.2008
Specification: REVIEWED 23-JUN-2004
Date of Manufacture: 09.04.2008
Date of Expiry: 09.04.2010

Quality Control Results

Analytical Test Method No.	Characteristic	Specification Limit Lower	Upper	Value	Unit	Status
	Addendum 00	Pass or Fail		Pass	-	P
	DP17/1B APPEARANCE (COLOUR)	YELLOW		Pass	-	P
	DP17/1B APPEARANCE (CLARITY)	CLEAR		Pass	-	P
	DP17/1B APPEARANCE (FORM)	VISCOUS LIQUID		Pass	-	P
	DP17/3 ODOUR	CHARACTERISTIC,FREE OF OBJECTIONAL ODOUR		Pass	-	P
	DP2/1B ASH	0.0	2.0	1.3	%	P
	DP14/5 COLOUR	0.0	7.0	4.2	Gardner	P
	DP6/5 PH	4.5	5.5	5.5		P
	DP1/3B TOTAL SOLIDS	20.0	24.0	21.0	%	P
	DC2/2 NITROGEN	2.2	3.4	2.8	%	P
	DM1/2S TOTAL COUNT @30°C	100 CFU/G MAX		Pass	-	P
	DM2/1S YEASTS & MOULDS @ 25°C	100 CFU/G MAX		Pass	-	P
	DM16/1S GRAM	ABSENT		Pass	-	P

Appendix 2 (continued) Certificate of Analysis

Certificate of Analysis

A quality management system registered to the international standard ISO 9001 was used to manufacture and test this material.
The name printed at the end of this document is an electronic signature.

Customer details

Customer Ref.	
Inspection Lot	0900000063034
C of A Printed.	28.11.2008
Quantity.	0.000
QA Contact.	
Fax No.	

NEGATIVE BACILLI 1G DM11/2S STAPHLAUREUS 1G	ABSENT	Pass	P

It is recommended that this product be used within 6 months of delivery

Batch Status: Pass

Confirmed by

Stephen Hughes

Appendix 3 Statement of GLP Compliance in Accordance with Directive 2004/9/EC

DH Department of Health

THE DEPARTMENT OF HEALTH OF THE GOVERNMENT OF THE UNITED KINGDOM

GOOD LABORATORY PRACTICE

STATEMENT OF COMPLIANCE IN ACCORDANCE WITH DIRECTIVE 2004/9/EC

TEST FACILITY	TEST TYPE
Harlan Laboratories Ltd. Shardlow Business Park London Road Shardlow Derby DE72 2GD	Analytical/Clinical Chemistry Environmental Fate Environmental Toxicology Mutagenicity Phys/Chem Toxicology

DATE OF INSPECTION

19th August 2008

A general inspection for compliance with the Principles of Good Laboratory Practice was carried out at the above test facility as part of the UK GLP Compliance Programme.

At the time of inspection no deviations were found of sufficient magnitude to affect the validity of non-clinical studies performed at these facilities.

4/3/09

Dr. Andrew J. Gray
Head, UK GLP Monitoring Authority

MHRA

harlan™

Hydrolyzed Linear protein/ PVP Cross Polymer

DETERMINATION OF SKIN IRRITATION POTENTIAL USING THE EPISKIN™ RECONSTITUTED HUMAN EPIDERMIS MODEL

PROJECT NUMBER:

AUTHOR: N Warren

STUDY SPONSOR:

TEST FACILITY:

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Shardlow Business Park
Shardlow
Derbyshire
DE72 2GD
UK

Telephone: +44 (0) 1332 792896

Facsimile: +44 (0) 1332 799018

QUALITY ASSURANCE REPORT

This study type is classed as short-term. The standard test method for this study type ("General Study Plan" in OECD terminology) was reviewed for compliance once only on initial production. Inspection of the routine and repetitive procedures that constitute the study is carried out as a continuous process designed to encompass the major phases at or about the time this study was in progress. In addition, inspection of general facilities not specifically related to this study are done monthly or annually in accordance with QA Standard Procedure.

This report has been audited by the Quality Assurance Unit, and is considered to be an accurate account of the data generated and of the procedures followed.

In each case, the outcome of QA evaluation is reported to the Study Director and Management on the day of evaluation. Audits of study documentation, and process inspections appropriate to the type and schedule of this study were as follows:

	19 November 2008	Standard Test Method Compliance Audit
	16 April 2009	Test Material Preparation
	15 April 2009	Test System Preparation
	16 April 2009	Exposure
	21 April 2009	Assessment of Response
§	26 May 2009	Draft Report Audit
§	Date of QA Signature	Final Report Audit

§ Evaluation specific to this study

..... G. Wren ..... DATE: 09 JUN 2009
For the Quality Assurance Unit*

*Authorised QA Signatures:
Manager, Quality Assurance: J G Riley BSc (Hons) MRQA
Deputy Head of Department: JM Crowther MIScT MRQA
Senior Audit Staff: G Wren ONC MRQA

GLP COMPLIANCE STATEMENT

The work described was performed in compliance with UK GLP standards (Schedule 1, Good Laboratory Practice Regulations 1999 (SI 1999/3106 as amended by SI 2004/0994)). These Regulations are in accordance with GLP standards published as OECD Principles on Good Laboratory Practice (revised 1997, ENV/MC/CHEM(98)17); and are in accordance with, and implement, the requirements of Directives 2004/9/EC and 2004/10/EC.

This report fully and accurately reflects the procedures used and data generated.

..... N. Warren ..... DATE: ..... 4/6/2009 .....

N Warren MIAT
Study Director

CONTENTS

SolPerForm 100:

DETERMINATION OF SKIN IRRITATION POTENTIAL USING THE EPISKIN™ RECONSTITUTED HUMAN EPIDERMIS MODEL

SUMMARY

Introduction. The purpose of this test was to evaluate the skin irritation potential of the test material using the EPISKIN™ reconstituted human epidermis model after a treatment period of 15 minutes followed by a post-exposure incubation period of 42 hours. The principle of the assay was based on the measurement of cytotoxicity in reconstituted human epidermal cultures following topical exposure to the test material by means of the colourimetric MTT reduction assay. Cell viability is measured by enzymatic reduction of the yellow MTT tetrazolium salt (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl-tetrazolium bromide) to a blue formazan salt (within the mitochondria of viable cells) in the test material treated tissues relative to the negative controls. The concentration of the inflammatory mediator IL-1α in the culture medium retained following the 42 hour post-exposure incubation period is also determined for test materials which are found to be borderline non-irritant based upon the MTT reduction endpoint. This complimentary end-point will be used to either confirm a non-irritant result or will be used to override the non-irritant result.

Triplicate tissues were treated with the test material for an exposure period of 15 minutes. At the end of the exposure period each tissue was rinsed before incubating for approximately 42 hours. At the end of the post-exposure incubation period each tissue was taken for MTT-loading. The maintenance medium from beneath each tissue was transferred to pre-labelled micro tubes and stored in a freezer for possible inflammatory mediator determination. After MTT loading a total biopsy of each epidermis was made and placed into micro tubes containing acidified isopropanol for extraction of formazan crystals out of the MTT-loaded tissues.

At the end of the formazan extraction period each tube was mixed thoroughly and duplicate 200 µl samples were transferred to the appropriate wells of a pre-labelled 96-well plate. The optical density was measured at 540 nm.

Data are presented in the form of % viability (MTT reduction in the test material treated tissues relative to negative control tissues).

Results: The relative mean viability of the test material treated tissues was 86.6% after a 15-minute exposure.

Quality criteria: The quality criteria required for acceptance of results in the test were satisfied.

Conclusion: The test material was considered to be Non-Irritant.

DETERMINATION OF SKIN IRRITATION POTENTIAL USING THE EPISKIN™ RECONSTITUTED HUMAN EPIDERMIS MODEL

1. INTRODUCTION

The purpose of this test was to evaluate the skin irritation potential of the test material using the EPISKIN™ reconstituted human epidermis model after a treatment period of 15 minutes followed by a post-exposure incubation period of 42 hours [1, 2, 3, 4]. The principle of the assay is based on the measurement of cytotoxicity in reconstituted human epidermal cultures following topical exposure to the test material by means of the colourimetric MTT reduction assay. Cell viability is measured by enzymatic reduction of the yellow MTT tetrazolium salt (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl-tetrazolium bromide) to a blue formazan salt (within the mitochondria of viable cells) in the test material treated tissues relative to the negative controls. The concentration of the inflammatory mediator IL-1α in the culture medium retained following the 42 hour post-exposure incubation period is also determined for test materials which are found to be borderline non-irritant based upon the MTT reduction endpoint. This complimentary end-point will be used to either confirm a non-irritant result or will be used to override the non-irritant result.

The EPISKIN™ model is a three-dimensional reconstituted human epidermis model consisting of adult human-derived epidermal keratinocytes seeded on a dermal substitute consisting of a collagen type I matrix coated with type IV collagen. A highly differentiated and stratified epidermis model is obtained after 13 day culture period comprising the main basal, supra basal, spinous and granular layers and a functional stratum corneum.

The control group served as a common control with Harlan Laboratories Ltd Project number 2724/0007.

The study was performed between 22 April 2009 and 28 April 2009.

2. TEST MATERIAL

2.1 Description, Identification and Storage Conditions

Sponsor's identification	:	Hydrolyzed Wheat Protein/PVP Cross Polymer
Description	:	amber coloured liquid
Batch number	:	
Date received	:	11 December 2008
Storage conditions	:	room temperature in the dark

The integrity of supplied data relating to the identity, purity and stability of the test material is the responsibility of the Sponsor.

A Certificate of Analysis supplied by the Sponsor is given in Appendix 1.

2.2 Preparation of Test Material

The test material was used as supplied.

3. NEGATIVE AND POSITIVE CONTROL MATERIALS

Dulbecco's Phosphate Buffered Saline (PBS) with Ca^{++} and Mg^{++} was used as the negative control.

Sodium Dodecyl Sulphate (SDS) was used as the positive control.

3.1 Preparation of Negative and Positive Control Materials, MTT and Acidified Isopropanol

The negative control material was used as supplied.

The positive control material was prepared as a 5% w/v aqueous dilution.

A 3 mg/ml MTT stock solution was prepared in PBS. The stock solution was diluted to 0.3 mg/ml with assay medium when required.

A 0.04 N concentration of hydrochloric acid in Isopropanol was prepared when required.

4. EPISKIN™ Model Kit

Date received : 21 April 2009

5. PROCEDURE

5.1 Pre-Test

5.1.1 Assessment of Direct Test Material Reduction of MTT

5.1.1.1 MTT dye metabolism, cell viability assay

The MTT assay, a colourimetric method of determining cell viability, is based on reduction of the yellow tetrazolium salt (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl-tetrazolium bromide) to a purple formazan dye by mitochondrial succinate dehydrogenase in viable cells.

5.1.1.2 Assessment of Direct Test Material Reduction of MTT

One limitation of the assay is possible interference of the test substance with MTT. A test substance may directly reduce MTT, thus mimicking dehydrogenase activity of the cellular mitochondria. This property of the test substance is only a problem, if at the time of the MTT test (after rinsing) there is still sufficient amounts of the test substance present on or in the tissues. In this case, the true metabolic MTT reduction and the false direct MTT reduction can be differentiated and quantified by the procedure described below.

5.1.1.3 Test for Direct MTT Reduction

As specified, a test substance may interfere with the MTT endpoint, if it is able to directly reduce MTT and at the same time is present on or in the tissues when the MTT viability test is performed. To identify this possible interference, each test substance is checked for the ability to directly reduce MTT according to the following procedure:

10 µl of the test material was added to 2 ml of a 0.3 mg/ml MTT solution freshly prepared in assay medium. The solution was incubated in the dark at 37°C, 5% CO_2 in air for 3 hours. Untreated MTT solution was used as a control.

If the MTT solution containing the test substance turns blue/purple, the test substance is presumed to have reduced the MTT.

5.1.2 Pre-Incubation (Day 0: tissue arrival)

2 ml of maintenance medium, warmed to approximately 37°C, was pipetted into the first column of 3 wells of a pre-labelled 12-well plate. Each epidermis unit was transferred into the maintenance medium filled wells (3 units per plate). A different 12-well plate was used for the test material and each control material. The tissues were incubated at 37°C, 5% CO_2 in air for at least 24 hours.

5.2 Main Test

5.2.1 Application of Test Material and Rinsing (Day 1)

2 ml of maintenance medium, warmed to approximately 37°C, was pipetted into the second column of 3 wells of the 12-well plate.

Triplicate tissues were treated with the test material for an exposure period of 15 minutes. The test material was applied topically to the corresponding tissues ensuring uniform covering. 10 µl of the test material was applied to the epidermis surface. Triplicate tissues, treated with 10 µl of PBS, were used to serve as negative controls. Triplicate tissues, treated with 10 µl of SDS 5% w/v, were used to serve as positive controls. To ensure satisfactory contact with the positive control material the SDS solution was spread over the entire surface of the epidermis using a pipette tip (taking particular care to cover the centre). After 7 minutes contact time the SDS solution was re-spread with a pipette tip to maintain the distribution of the SDS for the remainder of the contact period. The plate(s) were kept in the biological safety cabinet at room temperature for 15 ± 0.5 minutes.

At the end of the exposure period, each tissue was removed from the well using forceps and rinsed using a wash bottle containing PBS with Ca^{++} and Mg^{++}. Rinsing was achieved by filling and emptying each tissue insert for approximately 40 seconds using a constant soft stream of PBS to gently remove any residual test material. The rinsed tissues were transferred to the second column of 3 wells containing 2 ml of maintenance medium in each well. The rinsed tissues were incubated at 37°C, 5% CO_2 in air for approximately 42 hours.

5.2.2 MTT Loading/Formazan Extraction (Day 3)

Following the 42-hour post-exposure incubation period each 12-well plate was placed onto a plate shaker for 15 ± 2 minutes to homogenise the released mediators in the maintenance medium. 1.6 ml of the maintenance medium from beneath each tissue was transferred to pre-labelled micro tubes and store in a freezer at -14 to -30°C for possible inflammatory mediator determination.

2 ml of a 0.3 mg/ml MTT solution, freshly prepared in assay medium, was pipetted into the third column of 3 wells of the 12 well plate(s). The tissues were transferred to the MTT filled wells, being careful to remove any excess maintenance medium from the bottom of the tissue insert by blotting on absorbent paper. The tissues were incubated for 3 hours at 37°C, 5% CO_2 in air. At the end of the 3 hour incubation period each tissue was placed onto absorbent paper to dry. The tissues were examined and the degree of MTT staining evaluated (qualitative evaluation of cell viability) using the MTT Visual Scoring scheme. Following qualitative evaluation of tissue viability a total biopsy of the epidermis was made using the EPISKIN™ biopsy punch. The epidermis was carefully separated from the collagen matrix using forceps and both parts (epidermis and collagen matrix) placed into labelled 1.5 ml micro tubes containing 500 µl of acidified isopropanol, ensuring that both the epidermis and collagen matrix were fully immersed. Each tube was plugged to prevent evaporation and mixed thoroughly on a vortex mixer. The tubes were refrigerated at 1 to 10°C until Day 6 of the experiment, allowing the extraction of formazan crystals out of the MTT-loaded tissues.

5.2.3 Absorbance/Optical Density Measurements (Day 6)

At the end of the formazan extraction period each tube was mixed thoroughly on a vortex mixer to produce a homogenous coloured solution.

For each tissue, duplicate 200 µl samples were transferred to the appropriate wells of a pre-labelled 96-well plate. 200 µl of acidified isopropanol alone was added to the two wells designated as 'blanks'. The optical density was measured (quantitative viability analysis) at 540 nm (without a reference filter) using the Anthos 2001 microplate reader.

6. INTERPRETATION OF RESULTS

6.1 Quantitative MTT Assessment (percentage tissue viability)

For the test material the relative mean tissue viabilities obtained after the 15 minute treatment followed by the 42 hour post-exposure incubation period were compared to the mean of the negative control treated tissues (n=3). The relative mean viabilities were calculated in the following way:

$$\% \text{ Relative viability} = \frac{\text{mean } OD_{540} \text{ of test material}}{\text{mean } OD_{540} \text{ of negative control}} \times 100$$

Classification of irritation potential is based upon relative tissue viability following the 15 minute exposure period followed by the 42 hour post-exposure incubation period according to the following table:

Criteria for *in vitro* interpretation	Classification
Mean tissue viability is ≤50%	Irritant (I) R38
Mean tissue viability is >50%	Non-Irritant (NI)•

6.2 Quality Criteria

The results of the assay are considered acceptable if the following assay acceptance criterion is achieved:

Positive Control

The assay establishes the acceptance criterion for an acceptable test if the relative mean tissue viability for the positive control treated tissues was ≤40% relative to the negative control treated tissues, and the Standard Deviation (SD) value of the % viability is ≤20%.

• = The concentration of the inflammatory mediator IL-1α in the culture medium retained following the 42 hour post-exposure incubation will be determined for test materials which are found to be borderline non-irritant based upon the MTT cell viability endpoint (mean tissue viability 51 - 60%). This complimentary end-point will be used to either confirm a non-irritant result or will be used to override the non-irritant result.

Negative Control

The assay establishes the acceptance criterion for an acceptable test if the mean OD_{540} for the negative control treated tissues was ≥0.6, and the SD value of the % viability is ≤20%.

7. ARCHIVES

Unless instructed otherwise by the Sponsor, all original data and the final report will be retained in the Harlan Laboratories Ltd, Shardlow, UK archives for five years, after which instructions will be sought as to further retention or disposal.

8. RESULTS

8.1 Direct MTT Reduction

The MTT solution containing the test material did not turn blue/purple which indicated that the test material did not directly reduce MTT.

8.2 Test Material, Positive Control Material and Negative Control Material

The individual and mean OD_{540} values, standard deviations and tissue viabilities for the test material, negative control material and positive control material are given in Table 1. The mean viabilities and standard deviations of the test material and positive control, relative to the negative control are also given in Table 1.

The relative mean viability of the test material treated tissues was 86.6% after a 15-minute exposure.

The qualitative evaluation of tissue viability is given in Table 2.

Following the 15-minute exposure the test material treated tissues appeared blue which was considered indicative of viable tissue.

8.3 Quality Criteria

The relative mean tissue viability for the positive control treated tissues was ≤40% relative to the negative control treated tissues and the Standard Deviation (SD) value of the % viability was ≤20%. The positive control acceptance criterion was therefore satisfied.

The mean OD_{540} for the negative control treated tissues was ≥0.6 and the SD value of the % viability was ≤20%. The negative control acceptance criterion was therefore satisfied.

9. CONCLUSION

The test material was considered to be Non-Irritant.

10. REFERENCES

1) Fentem, J.H., Briggs, D., Chesne, C., Elliott, G.R., Harbell, J.W., Heylings, J.R., Portes, P., van de Sandt, J.J.M. and P.A. Botham (2001) A prevalidation study on *in vitro* tests for acute Skin Irritation: results and evaluation by the Management Team. Toxicology *in vitro* **15**, pp.57-93.

2) Zuang, V., Balls, M., Botham, P.A., Coquette, 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. and A.P Worth (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**, pp. 109-129.

3) Cotovio, J., Grandidier, M-H., Portes, P., Roguet, R. and G. Rubinsteen (2005) The *in vitro* acute Skin Irritation of chemicals: Optimisation of the EPISKIN Prediction Model within the Framework of the ECVAM Validation Process. ATLA **33**, pp.329-349.

4) Hartung. T. (2007) ESAC statement on the validity of *in vitro* tests for skin irritation. European Commission, Directorate General, Joint Research Centre, Institute for Health and Consumer Protection, European Centre for the Validation of Alternative Methods (ECVAM). Ispra. Website: http://ecvam.jrc.it/index.htm.

Hydrolyzed Wheat Protein/PVP Cross Polymer

: DETERMINATION OF SKIN IRRITATION POTENTIAL USING THE EPISKIN™ RECONSTITUTED HUMAN EPIDERMIS MODEL

Table 1 Mean OD_{540} Values and % Viabilities for the Negative Control Material, Positive Control Material and Test Material

Material	OD_{540} of tissues	Mean OD_{540} of triplicate tissues	± SD of OD_{540}	Relative individual tissue viability %	Relative mean % viability	± SD of % viability
Negative Control Material⊕	0.927	0.951	0.04	97.5	100*	na
	0.932			98.0		
	0.995			104.6		
Positive Control Material⊕	0.079	0.042	0.04	8.3	4.4	4.1
	0.045			4.7		
	0.001			0.1		
Test Material	0.814	0.823	0.04	85.6	86.6	3.7
	0.793			83.4		
	0.863			90.7		

* = The mean viability of the negative control tissues is set at 100%
na = Not applicable
⊕ = Control group shared with Harlan Laboratories Ltd Project number 2724/0007

Hydrolyzed Wheat Protein/PVP Cross Polymer

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Table 2 Qualitative Evaluation of Tissue Viability (MTT uptake visual evaluation)

Material	Tissue 1	Tissue 2	Tissue 3
Negative Control Material⊕	-	-	-
Positive Control Material⊕	++	++	++
Test Material	-	-	-

MTT visual scoring scheme

- = blue tissue (viable)
+ = blue/white tissue (semi-viable)
++ = tissue is completely white (dead)

⊕ = Control group shared with Harlan Laboratories Ltd Project number 2724/0007

Appendix 1 Certificate of Analysis

Certificate of Analysis

A quality management system registered to the international standard ISO 9001 was used to manufacture and test this material.
The name printed at the end of this document is an electronic signature.

Customer details

Customer Ref.
Inspection Lot 090000083934
C of A Printed. 28.11.2008

Quantity. 0.000
QA Contact.
Fax No.

Batch Details
Product Name: Hydrolyzed Wheat Protein/PVP Cross Polymer
Product Code:
Cust. Product Name:
Cust. Product Code:
Batch No.
Date of Test: 05.11.2008
Specification: REVIEWED 23-JUN-2004
Date of Manufacture: 09.04.2008
Date of Expiry: 09.04.2010

Quality Control Results

Analytical Test Method No.	Characteristic	Specification Limit Lower	Upper	Value	Unit	Status
	Addendum 00	Pass or Fail		Pass	-	P
	DP17/1B APPEARANCE (COLOUR)	YELLOW		Pass	-	P
	DP17/1B APPEARANCE (CLARITY)	CLEAR		Pass	-	P
	DP17/1B APPEARANCE (FORM)	VISCOUS LIQUID		Pass	-	P
	DP17/3 ODOUR	CHARACTERISTIC,FREE OF OBJECTIONAL ODOUR		Pass	-	P
	DP2/1B ASH	0.0	2.0	1.3	%	P
	DP14/5 COLOUR	0.0	7.0	4.2	Gardner	P
	DP6/5 PH	4.5	5.5	5.5		P
	DP1/3B TOTAL SOLIDS	20.0	24.0	21.0	%	P
	DC2/2 NITROGEN	2.2	3.4	2.8	%	P
	DM1/2B TOTAL COUNT @30°C	100 CFU/G MAX		Pass	-	P
	DM2/1B YEASTS & MOULDS @ 25°C	100 CFU/G MAX		Pass	-	P
	DM16/1B GRAM	ABSENT		Pass	-	P

Appendix 1 (continued) Certificate of Analysis

Certificate of Analysis

A quality management system registered to the international standard ISO 9001 was used to manufacture and test this material.
The name printed at the end of this document is an electronic signature.

Customer details

Customer Ref.	
Inspection Lot	0900000063934
C of A Printed	28.11.2008
Quantity.	0.000
QA Contact.	
Fax No.	

NEGATIVE BACILLI 1G DM11/2S STAPH.AUREUS 1G	ABSENT	Pass	P

It is recommended that this product be used within 6 months of delivery

Batch Status: Pass

Confirmed by

Stephen Hughes

Appendix 2 Statement of GLP Compliance in Accordance with Directive 2004/9/EC

Department of Health

THE DEPARTMENT OF HEALTH OF THE GOVERNMENT OF THE UNITED KINGDOM

GOOD LABORATORY PRACTICE

STATEMENT OF COMPLIANCE IN ACCORDANCE WITH DIRECTIVE 2004/9/EC

TEST FACILITY	TEST TYPE
Harlan Laboratories Ltd. Shardlow Business Park London Road Shardlow Derby DE72 2GD	Analytical/Clinical Chemistry Environmental Fate Environmental Toxicology Mutagenicity Phys/Chem Toxicology

DATE OF INSPECTION

19th August 2008

A general inspection for compliance with the Principles of Good Laboratory Practice was carried out at the above test facility as part of the UK GLP Compliance Programme.

At the time of inspection no deviations were found of sufficient magnitude to affect the validity of non-clinical studies performed at these facilities.

4/3/09

Dr. Andrew J. Gray
Head, UK GLP Monitoring Authority

MHRA

Personal Care Products Council
Committed to Safety, Quality & Innovation

Memorandum

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

FROM: Carol Eisenmann, Ph.D.
Personal Care Products Council

DATE: September 13, 2018

SUBJECT: VP Polymers

Anonymous. 2018. Summary information - molecular weight and impurities VP Polymers.

1620 L Street, N.W., Suite 1200 | Washington, D.C. 20036 | 202.331.1770 | 202.331.1969 (fax) | www.personalcarecouncil.org

Sept. 2018

S.No.	Ingredients	Average MW (number or weight) [or range of averages]	Fraction less than 1000 daltons	Residual monomer levels	Any other impurities
1	VP/Hexadecene Copolmer	60000 - 125000	14% 17%	Vinyl Pyrrolidone <= 100 ppm	t-Butanol < 0.5%, Isopropanol < 0.5%
2	VP/Eicosene Copolymer	116000	12%	Vinyl Pyrrolidone <=20 ppm	t-Butanol < 0.5%, Isopropanol < 0.5%
3	Acrylic Acid/VP Crosspolymer	23200	11%	Acrylic Acid <= 500 ppm Vinyl Pyrrolidone <= 100 ppm	Heptane < 0.99%
4	Butylated PVP	37300	1%	Vinyl Pyrrolidone <= 100 ppm	-
6	PVP	PVP K-15 - 6000 - 15000 PVP K-30- 40000 - 80000 PVP K-60- 240000 - 450000 PVP K-90- 1000000 - 1700000	K15: 13% K30: 1% K60: 0% K90: 0%	Vinyl Pyrrolidone <=1000ppm	-
7	Styrene/VP Copolymer	2.4 million	No detectable fraction	Styrene < 0.2% N-Vinylpyrrolidone < 10 ppm	-
8	Triacontanyl PVP	129000	9%	Vinyl Pyrrolidone <=20 ppm	Hexanol <= 0.05%
9	Vinyl Caprolactm/VP/Dimethylaminoethyl Methacrylate Copolymer	Mw 53000	5.0%	Vinylpyrrolidone <0.1% Vinylcaprolactum<=1%	
10	VP/Acrylates/Lauryl Methacrylate Copolymer	~185000	0% 1%	Lauryl methacrylate <= 1000ppm Vinyl Pyrrolidone <= 1000ppm Acrylic Acid <= 2000ppm	Heptane <1%
11	VP/Dimethylaminoethylmethacrylate Copolymer	Copolymer 845: Mw 1110000 Copolymer 937: Mw 1190000 Copolymer 958: Mw 102000	0% 0% 0%	Vinylpyrrolidone <= 1000ppm	-
12	VP/DMAPA Acrylates Copolymer	2390000	0%	Vinyl Pyrrolidone <= 100ppm	-
13	VP/VA Copolymer	E335: Mw 26700 E535: Mw 31000 E635: Mw 40000 E735: Mw 45800 I335: Mw 12900 I535: Mw 15800 I735: Mw 22700 S630: Mw 22600 W735: Mw 26000	3% 2% 2% 2% 6% 5% 4% 3% 3%	Vinyl pyrrolidone <= 1000ppm Vinyl Acetate <= 1000ppm	-
14	VP/Vinyl Caprolactam/DMAPA Acrylates Copolymer	29800	1%	Vinyl Pyrrolidone <= 100 ppm Vinyl Caprolactum <= 100 ppm	Water

Memorandum

To: CIR Expert Panel Members and Liaisons
From: Priya Cherian, Scientific Writer/Analyst
Date: September 14, 2018
Subject: Wave 2 – Xanthine Alkaloids

The Council has provided contact sensitization data on a body care product containing 6% Caffeine, tested neat (*xanalk092018data_wave2*). An (human repeated insult patch test (HRIPT) performed on 105 test subject revealed no irritation during the induction period, and no allergic reactions during the challenge phase.

1620 L Street, NW, Suite 1200 ♢ Washington, DC 20036-4702
(Main) 202-331-0651 (Fax) 202-331-0088
(Email) cirinfo@cir-safety.org (Website) www.cir-safety.org

Personal Care Products Council
Committed to Safety, Quality & Innovation

Memorandum

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

FROM: Carol Eisenmann, Ph.D.
Personal Care Products Council

DATE: September 7, 2018

SUBJECT: Caffeine

Anonymous. 2013. English synopsis: Human repeated insult patch test with challenge body care product containing 6% Caffeine.

1620 L Street, N.W., Suite 1200 | Washington, D.C. 20036 | 202.331.1770 | 202.331.1969 (fax) | www.personalcarecouncil.org

[redacted]

HUMAN REPEATED INSULT PATCH TEST WITH CHALLENGE

Study report – version n°1 (09/05/2013)

STUDY REFERENCES [redacted]

INVESTIGATIONAL PRODUCT

Denomination **BODY CARE** Contains 6% Caffeine

Reference / Formula number [redacted]

Batch number **F1 du 12/feb/2013**

SPONSOR	[redacted]
STUDY MONITOR	[redacted]
COORDINATING CENTRE	[redacted]
INVESTIGATING CENTRE	[redacted]
MAIN INVESTIGATOR	[redacted]
CO-INVESTIGATOR	[redacted]

Initiation date of study performance	**18/03/2013**
Completion date of study performance	**26/04/2013**

Date of the study report: 09/05/2013

HUMAN REPEATED INSULT PATCH TEST WITH CHALLENGE

Table of contents

[redacted]

HUMAN REPEATED INSULT PATCH TEST WITH CHALLENGE

English synopsis

STUDY OBJECTIVES	Mainly, to confirm, in a panel of healthy human adult subjects, that the application of the investigational product, under maximizing conditions of exposure, did not induce delayed contact sensitization Secondarily, to assess the skin compatibility of the investigational product during the induction phase of the study
SPONSOR	[redacted]
COORDINATING CENTRE	[redacted]
INVESTIGATING CENTRE	[redacted]
MAIN INVESTIGATOR	[redacted]
CO-INVESTIGATOR	[redacted]
TYPE OF THE STUDY	Monocentric randomized study performed in simple blind Study project previously approved by an independent ethics committee
DATES OF STUDY PERFORMANCE	From March 18th to April 26th 2013
INVESTIGATIONAL PRODUCT	[redacted] **Modalities of use in the study:** As it is - 20 µl under occlusive patch (Finn Chamber Standard®) *Switch to semi-occlusive patch if reactions ≥ 2 occur

[redacted]

English synopsis (continuation)

STUDY POPULATION	**Number of test subjects: 100 valid cases** **Population characteristics: test subjects** • suitable to participate in the study (after the clinical examination and questioning) and corresponding to the quality of "healthy subject" • declaring to have a health coverage • signing an "informed consent form" for this study • certifying not to take part in another clinical study in another investigating centre • certifying the truth of the personal information declared to the investigator • capable of following directions and reliable to respect the constraints of the protocol • free to ensure the visits to the investigating centre • aged from 18 to 65 (the 60-65 age bracket not exceeding 10% of the total number of subjects) • female and/or male • with a phototype (Fitzpatrick): I - V • 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) • with all types of skin on body **Non inclusion criteria: test 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 hospitalization 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 personal history of adverse reaction to colophony, rubber, nickel, aluminium, patch materials, adhesive plaster, • with documented history of contact allergy • with orthoergic skin reactivity on body • with family or personal history of atopy • 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 • foreseeing, during the study, a treatment able to interfere with the interpretation of the study results • 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

English synopsis (continuation)

STUDY POPULATION	• 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 4 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 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
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 maximizing conditions of exposure (under 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 phase) After a minimal 2-week 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 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 phase and the application of the challenge and after each patch removal by the same investigator / technician, supervised by the investigator Reporting of the sensations of discomfort directly by the test subjects to the investigator / 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 and the induction phase

English synopsis (continuation)

RESULTS

Characteristics of the included panel

Number of included subjects: 108
Number of exclusions (reason): None
Number of withdrawals (reason): 3 (ref. 08a, 17a and 29b) for personal reasons independent of the study
Number of valid cases: 105

- Age: 18 to 64 (Mean: 43)
- Sex: F/M
- Phototype: II to IV
- Skin type on the application site: ATS (100 %; n=108)

Checking of the skin compatibility

No reaction was noted on the control site

For the investigational product:

Induction phase			
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%	
A: ICDRG scale	None	0 / 0%	

Challenge			
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, [redacted] under occlusive patch on a panel of 105 test subjects, with all types of skin on body, induced no reaction or irritation.

Based on these results, the product has a very good skin compatibility.

- During the challenge phase, the repeated applications induced no allergic reaction.

[redacted]

HUMAN REPEATED INSULT PATCH TEST WITH CHALLENGE

Signatures and dates

Investigator: [redacted]

I the undersigned [redacted] 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(R1) of 10/06/1996, Directive of the European Parliament and Council 2001/20/EC – OJ/EC of 01/05/2001).
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: 24/05/2013
Signature: [redacted]

Quality Assurance Personnel: [redacted] **(person in charge of the quality control)**

I the undersigned, [redacted] declare that:

- this study was audited according to the procedure of the investigating centre:

Reference of the audited "HRIPT"	Audited phase	Date of audit performance	Date of audit report transmission to	
			the investigator	the management of the investigating centre
ER 13/018	Technical execution and raw data	18/03/2013	20/03/2013	20/03/2013

- the draft of the report was audited on May 15th 2013
- the final report was audited, on May 24th 2013
- the reported results accurately and completely reflected the raw data of the study.

Date: 24/05/2013
Signature: [redacted]