Safety Assessment of Red Algae-Derived Ingredients as Used in Cosmetics

Status:	Draft Report for Panel Review
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The Expert Panel for Cosmetic Ingredient Safety members are: Chair, Wilma F. Bergfeld, M.D., F.A.C.P.; Donald V. Belsito, M.D.; Curtis D. Klaassen, Ph.D.; Daniel C. Liebler, Ph.D.; James G. Marks, Jr., M.D.; Lisa A. Peterson, Ph.D.; Ronald C. Shank, Ph.D.; Thomas J. Slaga, Ph.D.; and Paul W. Snyder, D.V.M., Ph.D. The Cosmetic Ingredient Review (CIR) Executive Director is Bart Heldreth, Ph.D. This safety assessment was prepared by Priya Cherian, Scientific Analyst/Writer, CIR.

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Memorandum

To: Expert Panel for Cosmetic Ingredient Safety Members and Liaisons
From: Priya Cherian, Scientific Writer/Analyst, CIR
Date: August 21, 2020
Subject: Safety Assessment of Red Algae-Derived Ingredients as Used in Cosmetics

Enclosed is the Draft Report of the Safety Assessment of Red Algae-Derived Ingredients as Used in Cosmetics (*redalg092020rep*). This is the first time the Panel is reviewing the safety assessment on these 59 ingredients that are from one or multiple species of red algae. Although this safety assessment includes 59 red algae-derived ingredients, it should be noted that several of these ingredients appear to be equivalent based on the accepted scientific name, as given in the definition by the wINCI *Dictionary*. Accordingly, the total number of distinct cosmetic ingredients in 56. Table 1 in the report includes all 59 ingredients, along with their respective synonymous names.

Since the issuing of the Scientific Literature Review on March 19, 2020, the Council has provided an ample amount of additional information regarding the red-algae derived ingredients. These data has been incorporated into the report. Several of these data supplements were submitted in French. English summaries of these submissions have been provided and notated as "translation" in the data file, as notated in the following table which provides an overview of the data received.

Data Point/Test Substance	Data	Data Source
Ahnfeltiopsis Concinna Extract		
Specifications of a trade name mixture containing 0.75% Ahnfeltiopsis Concinna Extract	< 0.05% Nitrogen, < 20 ppm heavy metals, < 2 ppm Arsenic	*redalg092020data3*
In Vitro dermal irritation study on a trade name mixture containing 0.75% Ahnfeltiopsis Concinna Extract	EpiDerm; 30 µL; undiluted test substance; 60 minute incubation; non-irritating	*redalg092020data3*
In Vitro ocular irritation study on a trade name mixture containing 0.75% Ahnfeltiopsis Concinna Extract	EpiOcular; 50 µL; undiluted test substance; 90 minute incubation; non-irritating	*redalg092020data3*
Asparagopsis Armata Extract		
Manufacturing data on an Asparagopsis Armata Extract	algae → grinding → extraction by water → stabilization with vegetable glycerin → filtration	*redalg092020data5*
Manufacturing data on an Asparagopsis Armata Extract	fresh seaweed → grinding → cold cellular extraction → filtration → concentration → freeze-drying under neutral atmosphere	*redalg092020data6*
Manufacturing data on an Asparagopsis Armata Extract	harvesting/identification → washing → grinding → extraction with solvents (propanediol and water) → filtration → quality control → packaging → quality control	*redalg092020data9*
Composition of a trade name mixture containing 0.42% Asparagopsis Armata Extract	50% glycerin, 49.18% water, 0.42% Asparagopsis Armata Extract, 0.15% potassium sorbate, 0.25% sodium benzoate	*redalg092020data4*
Genotoxicity assay on an Asparagopsis Armata Extract containing 8% dry algal matter	Ames assay; *S. typhimurium*; 52 – 5000 µg/plate with and without metabolic activation; non-mutagenic	*redalg092020data7* *(redalg092020data7translation)*
In Vitro skin tolerance assay on an Asparagopsis Armata Extract containing 4% dry algal matter	reconstructed human epidermis; 200 µL; diluted to 10%; 18 hour incubation; non-irritating	*redalg092020data7* *(redalg092020data7translation)*
Human dermal irritation assay on an Asparagopsis Armata Extract containing 4% dry algal matter	10 subjects; 20 µL; water used as vehicle; 48-hour patch test under occlusive conditions; non-irritating	*redalg092020data7* *(redalg092020data7translation)*

Human dermal irritation assay on an trade name mixture containing 0.5 –2% Asparagopsis Armata Extract, 56 – 62% water, and 38 – 42% propanediol	22 subjects; 25 µL; tested at a 3% dilution; 48-hour patch test under occlusive conditions; non-irritating	*redalg092020data9*
HRIPT on a trade name mixture containing 0.5 –2% Asparagopsis Armata Extract, 56 – 62% water, and 38 – 42% propanediol	104 subjects; 40 µL; tested at a 3% dilution; semi-occlusive conditions; non-irritating/non-sensitizing	*redalg092020data9*
HRIPT performed on a product containing 0.325% Asparagopsis Armata Extract	108 subjects; undiluted test substance; occlusive conditions; non-irritating/non-sensitizing	*redalg092020data8*
In vitro ocular tolerance assay on an Asparagopsis Armata Extract containing 4% dry algal matter	PREDISAFE method; undiluted test substance; slightly irritating	*redalg092020data7* *(redalg092020data7translation)*
Chondrus Crispus		
Human dermal irritation assay on an after-shave balm containing 0.8% Chondrus Crispus	30 subjects; 23 hour exposure per day for 14 days; undiluted test substance 0.2 mL; occlusive patches;	*redalg092020data10*
In vitro MatTek EpiOcular™ MTT assay performed on after-shave balm containing 0.8% Chondrus Crispus	undiluted test substance; non-irritating	*redalg092020data10*
Chondrus Crispus Extract		
Specifications of a trade name mixture containing 20% Chondrus Crispus Extract	< 20 ppm heavy metals; < 10 ppm lead; < 2 ppm arsenic, <1 ppm cadmium	*redalg092020data11*
Specifications of a trade name mixture containing 3.5% Chondrus Crispus Extract	< 20 ppm heavy metals; < 10 ppm lead; < 2 ppm arsenic, <1 ppm cadmium	*redalg092020data11*
In vitro MatTek EpiDerm™ MTT assay on a trade name mixture containing 3.5% Chondrus Crispus Extract	undiluted test substance; non-irritating	*redalg092020data11*
HRIPT performed on a product containing 0.49% Chondrus Crispus Extract	113 subjects; undiluted test substance; occlusive conditions; non-irritating/non-sensitizing	*redalg092020data8*
In vitro MatTek EpiOcular™ MTT assay on a trade name mixture containing 3.5% Chondrus Crispus Extract	undiluted test substance; non-irritating	*redalg092020data11*
Chondrus Crispus Powder		
Manufacturing of a Chondrus Crispus Powder	harvesting → naturally dried via sun exposure → grinding/sieving → packaging → sterilized via gamma ray treatment	*redalg092020data13*
Manufacturing of a Chondrus Crispus Powder	harvesting/identification → drying → cutting → ionization → quality control → packaging → quality control	*redalg092020data14*
Human dermal irritation assay on a Chondrus Crispus Powder	12 subjects; occlusive conditions; 24-hour; non-irritating	*redalg092020data14*
Corallina Officinalis Extract		
General information on the species	-calcified or calcareous algae reaching 5-12 cm height -varied in color; thallus appears to be dull purple when growing in deep water, becoming red yellow and finally white on exposure	*redalg092020data15*
Composition of a trade name mixture containing Corallina Officinalis Extract (1.5%)	50% glycerin; 30% water; 18.5 % undaria pinnatifida extract; 1.5% Corallina officinalis Extract	*redalg092020data22*
Method of manufacturing for a trade name mixture containing Corallina Officinalis Extract	dried grounded algae → extraction with water → testing → sifting → centrifugation → ultrafiltration → testing → homogenization → testing → sterile filtration	*redalg092020data23*
Metal and mineral analysis for a trade name mixture containing Corallina Officinalis Extract (1.5%)	Data in report	*redalg092020data24*
Human dermal irritation assay on a trade name mixture containing 1.5% Corallina officinalis Extract	10 subjects; 10% dilution; semi-occlusive conditions; 48-hourl; non-irritating	*redalg092020data25*
In vitro ocular irritation assay on a trade name mixture containing 1.5% Corallina officinalis Extract	HET-CAM assay; 10% dilution; non-irritating	*redalg092020data26*

Chondrus Crispus Extract and Gigartina Stellata Extract		
Manufacturing information on a trade name mixture containing Chondrus Crispus Extract and Gigartina Stellata Extract (98.10 – 98.95% total extract)	harvesting/identification → washing → condensation of cellular water by soft drying → filtration and UV treatment → quality control → addition of preservatives and pH adjustment → quality control → packaging → quality control	*redalg092020data12*
Composition of a trade name mixture containing Chondrus Crispus Extract and Gigartina Stellata Extract (98.10 – 98.95% total extract)	98.10 - 98.95% extract; 0.80 – 1.10% sodium benzoate; 0.25 – 0.35% potassium sorbate; 0 -0.30% lactic acid	*redalg092020data12*
Human dermal irritation assay on a trade name mixture containing Chondrus Crispus Extract and Gigartina Stellata Extract (98.10 – 98.95% total extract)	22 subjects; undiluted test substance; occlusive conditions; 48-hour; non-irritating	*redalg092020data12*
Corallina Officinalis Extract, Kappaphycus Alvarezii Extract, and Gigartina Stellata Extract		
Composition of trade name mixture containing Corallina Officinalis Extract, Kappaphycus Alvarezii Extract, and Gigartina Stellata Extract	water (45.7%), glycerin (40%), *Gigartina stellata* (4.43%), Kappaphycus Alvarezii Extract (5.9%), Corallina Officinalis Extract (3.97%)	*redalg092020data16*
Method of manufacture of a trade name mixture containing Corallina Officinalis Extract, Kappaphycus Alvarezii Extract, and Gigartina Stellata Extract	dried grounded algae → extraction with water → testing → sifting → centrifugation → ultrafiltration → testing → homogenization → testing → sterile filtration → testing → packing	*redalg092020data17*
Genotoxicity assay on a trade name mixture containing Corallina Officinalis Extract, Kappaphycus Alvarezii Extract, and Gigartina Stellata Extract	Ames assay; *S. typhimurium*; tested at up to 5000 µg/plate, with and without metabolic activation; non-mutagenic	*redalg092020data19*
Human dermal irritation assay on a trade name mixture containing Corallina Officinalis Extract, Kappaphycus Alvarezii Extract, and Gigartina Stellata Extract	25 subjects; 48-hour; 10% dilution; occlusive conditions; non-irritating	*redalg092020data20*
In vitro ocular irritation assay on a trade name mixture containing Corallina Officinalis Extract, Kappaphycus Alvarezii Extract, and Gigartina Stellata Extract	HET-CAM assay; 10% dilution	*redalg092020data21*
Gelidiella Acerosa Extract		
HRIPT performed on a product containing 0.0028% Gelidiella Acerosa Extract	105 subjects; undiluted test substance: occlusive conditions; non-irritating/non-sensitizing	*redalg092020data8*
Gelidium Cartilagineum Extract		
Manufacturing data on a trade name mixture containing Gelidium Cartilagineum Extract	harvesting/identification → drying → grinding → extraction with solvent (caprylic/capric triglyceride) → addition of sterol → filtration → quality control → packaging → quality control	*redalg092020data27*
Composition information on a trade name mixture containing Gelidium Cartilagineum Extract	>96% glycerides, mixed decanoyl and octanoyl; <2 % Gelidium Cartilagineum Extract; 1.5-2% 4-cholesten-3-one	*redalg092020datat27* *(redalg092020data27translation)*
Human dermal irritation assay on a trade name mixture consisting of >96% glycerides, mixed decanoyl and octanoyl; <2 % Gelidium Cartilagineum Extract; 1.5-2% 4-cholesten-3-one	10 subjects; 10% dilution; 24-hour; occlusive conditions; non-irritating	*redalg092020data27* *(redalg092020data27translation)*
HRIPT on a trade name mixture consisting of >96% glycerides, mixed decanoyl and octanoyl; <2 % Gelidium Cartilagineum Extract; 1.5-2% 4-cholesten-3-one	50 subjects; undiluted test substance; occlusive conditions; non-irritating/non-sensitizing	*redalg092020data27* *(redalg092020data27translation*)
Gelidium Sesquipedale Extract		
Composition of a trade name mixture containing Gelidium Sesquipedale Extract	48% water; 48% butylene glycol; 4% Gelidium Sesquipedale Extract	*redalg092020data29*
Mineral and metal analysis on a trade name mixture containing 4% Gelidium Sesquipedale Extract	Data in report	*redalg092020data30* *(redalg092020data30translation)*

General information on the species *Gelidium sesquipedale*	Composed of several erect aces, compressed and branched; thallus appears dark-red in color and can reach up to 25-30 cm long	*redalg092020data28*
Human dermal irritation assay on a trade name mixture consisting of 48% water; 48% butylene glycol; 4% Gelidium Sesquipedale Extract	10 subjects; 5% dilution; occlusive conditions; 48-hour; non-irritating	*redalg092020data31*
Gigartina Stellata Extract		
General species information for *Gigartina stellata*	Thallus bears dichotomously branched blades which arise form a basal discoid crust; purplish-brown in color	*redalg092020data32*
Hydrolyzed Corallina Officinalis Extract		
Manufacturing information on a trade name mixture containing Hydrolyzed Corallina Officinalis Extract	harvesting/identification → extraction with water → addition of sodium methylparaben or 2-phenoxyethanol→ filtration →quality control → packaging →quality control	*redalg092020data33*
Composition information on a trade name mixture containing Hydrolyzed Corallina Officinalis Extract	>96% water; 0.5-3% Hydrolyzed Corallina Officinalis Extract; 0.16-0.20% sodium methylparaben	*redalg092020data33*
Composition information on a trade name mixture containing Hydrolyzed Corallina Officinalis Extract	>96% water; 0.5-3% Hydrolyzed Corallina Officinalis Extract; 0.8-1.2% phenoxyethanol	*redalg092020data33*
>96% water; 0.5-3% Hydrolyzed Corallina Officinalis Extract; 0.16-0.20% sodium methylparaben	10 subjects; undiluted test substance; occlusive conditions; 24-hour; non-irritating	*redalg092020data33*
Human dermal irritation assay on a trade name mixture consisting of >96% water; 0.5-3% Hydrolyzed Corallina Officinalis Extract; 0.8-1.2% phenoxyethanol	11 subjects; undiluted test substance; occlusive conditions; 24-hour; non-irritating	*redalg092020data33*
HIRPT on a trade name mixture consisting of >96% water; 0.5-3% Hydrolyzed Corallina Officinalis Extract; 0.16-0.20% sodium methylparaben	51 subjects; undiluted test substance; occlusive conditions; non-sensitizing	*redalg092020data33*
Hypnea Musciformis Extract		
Manufacturing data on a trade name mixture containing Hypnea Musciformis Extract	harvesting/identification → drying → grinding → extraction with the solvent (water and butylene glycol) → addition of potassium gluconate and methylparaben → filtration →quality control → packaging → quality control	*redalg092020data34*
Manufacturing data on a Hypnea Musciformis Extract	solubilization of *Hypnea musciformis* in water → separation of soluble and insoluble phases → filtration → membrane sterilization	*redalg092020data35*
Composition of a trade name mixture containing Hypnea Musciformis Extract	72-77% water; 20-70% butylene glycol; 1-3% Hypnea Musciformis Extract; ≤1% potassium gluconate; 0.16-0.2% methylparaben	*redalg092020data34*
Composition on a Hypnea Musciformis Extract	o Sugars (mainly polysaccharides which average molecular weight is below to 700kDa) : 75% o Mineral ashes : 22% o Proteins : 3% o Absence of alkaloids and polyphenols	*redalg092020data35*
Impurities of a Hypnea Musciformis Extract	0.082 ppm arsenic, < 0.020 ppm cadmium, < 0.020 ppm cobalt, 0.052 ppm chromium, < 0.020 ppm mercury, 0.185 ppm nickel, < 0.020 ppm lead, < 0.020 ppm antimony, 0.031 ppm selenium, and 0.053 ppm vanadium; total aflatoxins did not exceed 0.4 µg/kg	*redalg092020data35*
Human dermal irritation assay on a trade name mixture consisting of 72-77% water; 20-70% butylene glycol; 1-3% Hypnea Musciformis Extract; ≤1% potassium gluconate; 0.16-0.2% methylparaben	12 subjects; undiluted test substance; occlusive conditions; 24-hour; slightly irritating at the 30-minute reading and non-irritating at the 24-hour reading	*redalg092020data34*
Human dermal irritation assay on a Palmaria Palmata Extract	11 subjects; 15% dilution in water (0.36% dry matter); 48-hour; occlusive conditions; non-irritating	*redalg092020data35*
HRIPT on a Palmaria Palmata Extract	100 subjects; 15% dilution in water (0.36% dry matter); non-irritating/non-sensitizing	*redalg092020data35*

Kappaphycus alvarezii		
General information on *Kappaphycus alvarezii*	the thallus shows a simple discoid hold-fast from which arises a main axis with irregular branches; thalli can reach up to 2m tall and their color is green or yellow	*redalg0920202data36*
Lithothamnion Calcareum Powder		
Manufacturing data on a trade name mixture containing Lithothamnion Calcareum Powder	harvesting → drying → grinding → micronization → ionization → mixture → addition of mannitol, zinc sulfate, and diatomaceous earth → packaging → quality control	*redalg092020data37*
Composition of a trade name mixture containing Lithothamnion Calcareum Powder	57-61% Lithothamnion Calcareum Powder. 26-31% mannitol, 9-11% diatomaceous earth, 0.7-1.5% zinc sulfate	*redalg092020data37*
Human dermal irritation assay on a trade name mixture consisting of 57-61% Lithothamnion Calcareum Powder. 26-31% mannitol, 9-11% diatomaceous earth, 0.7-1.5% zinc sulfate	11 subjects; undiluted test substance; 24-hour; occlusive conditions; non-irritating	*redalg092020data37*
In vitro ocular irritation assay on a trade name mixture consisting of 57-61% Lithothamnion Calcareum Powder. 26-31% mannitol, 9-11% diatomaceous earth, 0.7-1.5% zinc sulfate	HET-CAM assay; tested at dilutions of 2, 5, and 10% in water; moderately irritating at the 10% concentration; non-irritating at the 2 and 5% concentrations	*redalg092020data37*
Palmaria Palmata Extract		
Method of manufacturing information for a Palmaria Palmata Extract	solubilization of powder of *Palmaria palmata* in water → separation of soluble and insoluble phases → concentration of soluble phase → membrane sterilization	*redalg092020data35*
Composition information on a Palmaria Palmata Extract	o Sugars (mainly oligosaccharides which average molecular weight is between 540 and 2000 Da) : 73% o Mineral ashes : 24% o Proteins : 3% o Absence of alkaloids and polyphenols	*redalg092020data35*
Impurities of a Palmaria Palmata Extract	antimony (0.069 ppm); arsenic (1.480 ppm); chromium (0.046 ppm); nickel (0.433 ppm); vanadium (2.29); iodine (3.8 ppm); no aflatoxins detected	*redalg092020data35*
Human dermal irritation summary data on a Palmaria Palmata Extract	11 subjects; 10% dilution in water (0.75% dry matter); 48-hour; occlusive conditions; non-irritating	*redalg092020data35*
HRIPT on a Palmaria Palmata Extract	58 subjects; 25% dilution in water (1.87% dry matter); non-sensitizing	*redalg092020data35*
Polysiphonia Lanosa Extract		
Composition information on a trade name mixture containing Polysiphonia Lanosa Extract	67.5% water, 32% Polysiphonia Lanosa Extract	*redalg092020data38*
Human dermal irritation assay on a trade name mixture consisting of 67.5% water, 32% Polysiphonia Lanosa Extract	11 subjects; 5% dilution; occlusive conditions; 48-hour; non-irritating	*redalg092020data39* *(redalg092020data39translation)*
Porphyra Umbilicalis Extract		
General information on *Porphra umbilicalis*	-blades appear reddish brown, brownish, grey brown, or olive green in the field; in a dried state they are very thin and violet in color -blades constituted by a single cell layer can reach 60 cm in height	*redalg092020data40*
Method of manufacturing information on a trade name mixture containing Porphyra Umbilicalis Extract	circular flow extraction of 7.8% dry algae on dry algae → in-process control → maturation at room temperature → filtration of the supernatant → cationic exchange → filtration → cross flow filtration → encapsulation of the extract into liposomes → packaging → quality control	*redalg092020data41*
Method of manufacturing information on a trade name mixture containing Porphyra Umbilicalis Extract	dried grounded algae → extraction with water → testing → centrifugation → ultrafiltration → testing → sterile filtration → testing → packaging	*redalg092020data43*
Composition information on a trade name mixture containing Porphyra Umbilicalis Extract	1.25% Porphyra Umbilicalis Extract (dry matter), 3% lecithin, 0.16% sodium lactate, 0.8% phenoxyethanol, 15% ethanol, and 79.79% water	*redalg092020data41*
Composition information on a trade name mixture containing Porphyra Umbilicalis Extract	52% water, 48% Porphyra Umbilicalis Extract	*redalg092020data42*

Heavy metal impurities of a trade name mixture consisting of 52% water, 48% Porphyra Umbilicalis Extract	3679 µg/kg arsenic, < 10 µg/kg cadmium, < 10 µg/kg mercury, < 10 µg/kg lead	*redalg092020data44* *(redalg092020data44translation)*
Genotoxicity data on a trade name mixture consisting of 52% water and 48% Porphyra Umbilicalis Extract	chemiluminescent 3D assay; tested at 2, 10-25, 50, and 100%; genotoxic potential evaluated before and after UVB irradiation; non-genotoxic	*redalg092020data45*
HRIPT performed on a product containing 0.0004% Porphyra Umbilicalis Extract	103 subjects; undiluted test substance; occlusive conditions; non-irritating/non-sensitizing	*redalg092020data8*
In vitro phototoxicity assay on a trade name mixture consisting of 52% water and 48% Porphyra Umbilicalis Extract	cell monolayer (fibroblast Balb/c3T3 clone) incubation with test substance at 7 concentrations (concentrations not specified); non-phototoxic	*relag092020data45*
In vitro ocular irritation study on a trade name mixture consisting of 52% water and 48% Porphyra Umbilicalis Extract	HET-Cam assay; undiluted test substance; weakly irritating	*redalg092020data45*
Rhodymenia Palmata Extract		
Human dermal irritation assay on an eye cream containing 0.0375% Rhodymenia Palmata Extract	38 subjects; 7-day exposure; undiluted test substance; semi-occlusive conditions; non-irritating	*redalg092020data46*
In vitro ocular irritation assay on an eye cream containing 0.0375% Rhodymenia Palmata Extract	MatTek EpiOcular™ MTT assay; undiluted test substance; non-irritating	*redalg092020data46*

As it may help the Panel decide on a conclusion of safety for several of these red-algae derived ingredients, a table has been provided presenting each ingredient, as well as a notation of the presence or absence of systemic toxicity data (repeated dose studies or use in food/as a GRAS substance) and sensitization data. This table can be found in the packet as *redalg092020data1*.

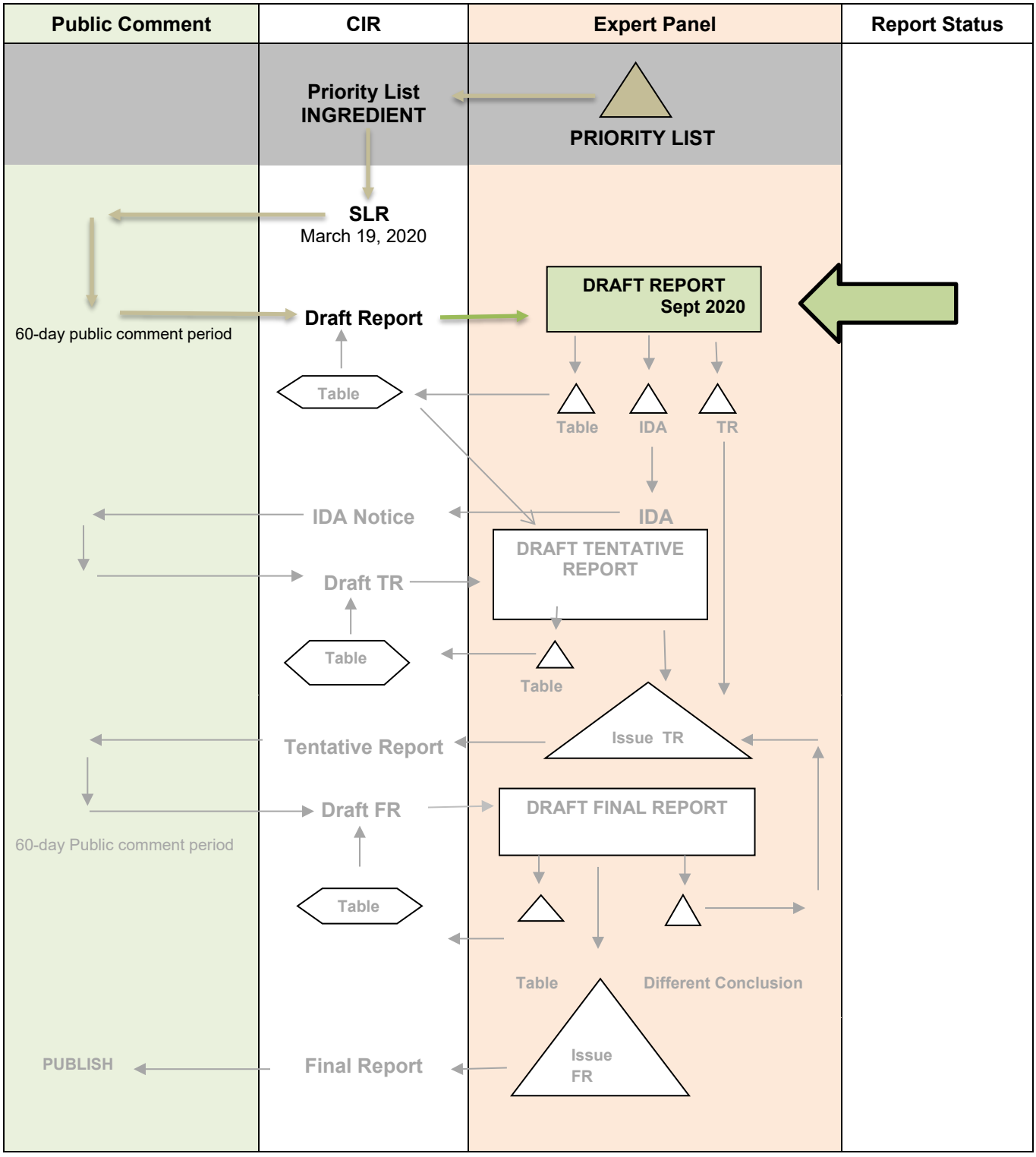
Comments provided by the Council after the SLR was issued have been addressed and included herein (*redalg092020pcpc*). The flow chart (*redalg092020flow*), data profile (*redalg092020prof*), 2020 VCRP data (*redalg092020FDA*), history (*redalg092020hist*), search strategy (*redalg092020strat*), and the presentation created for the Panel on Algal diversity and application given by Rex L. Lowe (*redalg092020data2*), have also been included in this packet.

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

SAFETY ASSESSMENT FLOW CHART

INGREDIENT/FAMILY Red-Algae Derived Ingredients

MEETING September 2020



Red Algae-Derived Ingredients History

March 2020

-SLR posted

April 2020

-concentration of use received

-Council comments on SLR received

-data received on the following:

-Chondrus Crispus Powder (manufacturing data)

-Asparagopsis Armata Extract (manufacturing data, sensitization data)

-*Corallina officinalis* (general information on the species)

-*Gelidium sesquipidale* (general information on the species)

-*Gigartina stellata* (general information on the species)

-*Kappaphycus alvarezii* (general information on the species)

-*Porphyra umbilicalis* (general information on the species)

-Gelidium Sesquipidale Extract (composition, physical and chemical properties, impurities, human dermal irritation)

-*Gigartina stellata*, Kappaphycus Alvarezii Extract, Corallina Officinalis Extract (physical and chemical properties, manufacturing, impurities, genotoxicity, human dermal irritation, in vitro ocular irritation)

-Porphyra Umbilicalis Extract (composition, physical and chemical properties, manufacturing, impurities, phototoxicity, photosensitization, genotoxicity, human sensitization, in vitro ocular irritation)

-Corallina Officinalis Extract and Undaria Pinnatifida Extract (composition, physical and chemical properties, method of manufacturing, impurities, human dermal irritation, in vitro ocular irritation)

-Polysiphonia Lanosa Extract (composition, physical and chemical properties, human dermal irritation

-Gelidiella Acerosa Extract (human sensitization)

-Chondrus Christpus Extract (human sensitization)

-Rhodymenia Palmata Extract (in vitro ocular irritation, human dermal irritation)

-Chondrus Crispus (in vitro ocular irritation, human dermal irritation

-Hypnea Musciformis Extract and Palmaria Palmata Extract (physical and chemical properties, manufacturing, impurities, human dermal irritation, human sensitization, use in food)

June 2020

-data received on the following:

-Chondrus Crispus Powder (manufacturing, human dermal irritation)

-Chondrus Crispus Extract and Gigartina Stellata Extract (manufacturing, composition, human dermal irritation)

-Gelidium Cartilagineum Extract (manufacturing, composition, human dermal irritation, human sensitization)

-Asparagopsis Armata Extract (manufacturing, composition, human dermal irritation, human sensitization)

-Hydrolyzed Corallina Officinalis Extract (manufacturing, composition, human irritation, human sensitization)

-Hypnea Musciformis Extract (manufacturing, composition, human dermal irritation, in vitro ocular irritation)

-Lithothamnion Calcareum Powder (manufacturing, composition, human dermal irritation, in vitro ocular irritation)

-Ahnfeltiopsis Concinna Extract (specifications, human dermal irritation, in vitro ocular irritation)

-Chondrus Crispus Extract (composition, specifications, human dermal irritation, in vitro ocular irritation)

September 2020

-Panel reviews Draft Report

Red Algae Profile – September 2020 – Writer, Priya Cherian

				Toxicokinetics			Acute Tox			Repeated Dose Tox			DART		Genotox		Carci		Dermal Irritation			Dermal Sensitization				Ocular Irritation		Clinical Studies	
	Reported Use	Method of Mfg	Impurities	log P	Dermal Penetration	ADME	Dermal	Oral	Inhalation	Dermal	Oral	Inhalation	Dermal	Oral	In Vitro	In Vivo	Dermal	Oral	In Vitro	Animal	Human	In Vitro	Animal	Human	Phototoxicity	In Vitro	Animal	Retrospective/ Multicenter	Case Reports
Ahnfeltiopsis Concinna Extract	x		x																x							x			
Asparagopsis Armata Extract	x	x													x				x		x			x		x			
Hydrolyzed Asparagopsis Armata Extract																													
Betaphycus Gelatinum Extract																													
Botryocladia Occidentalis Extract																													
Calliblepharis Ciliata Extract																													
Ceramium Kondoi Extract																													
Ceramium Rubrum Extract																													
Chondracanthus Teedei Powder																													
Chondrus Crispus	x		x																		x					x			
Chondrus Crispus Extract	x	x																	x		x			x		x			
Chondrus Crispus Powder	x	x																			x								
Hydrolyzed Chondrus Crispus Extract	x																												
Corallina Officinalis Extract	x	x	x												x						x					x			
Corallina Officinalis Powder																													
Corallina Officinalis Thallus Extract																													
Hydrolyzed Corallina Officinalis																								x					
Hydrolyzed Corallina Officinalis Extract	x	x																			x								
Cyanidium Caldarium Extract																													
Delesseria Sanguinea Extract	x																												
Digenea Simplex Extract	x	x																											
Dilsea Carnosa Extract																													
Furcellaria Lumbricalis Extract	x																												
Gelidiella Acerosa Extract	x	x												x	x									x					
Gelidium Amansii Extract		x																											
Gelidium Amansii Oligosaccharides																													
Gelidium Cartilagineum Extract	x	x																			x			x					
Gelidium Pulchrum Protein																													
Gelidium Sesquipedale Extract			x																		x								
Gigartina Skottsbergii Extract																													
Gigartina Stellata Extract	x	x													x						x								
Gloiopeltis Tenax Extract			x																										

Gloiopeltis Tenax Powder																								
Gracilaria Verrucosa Extract																								
Gracilariopsis Chorda Extract		x																						
Grateloupia Livida Powder							x																	
Hypnea Musciformis Extract	x	x	x															x			x			
Lithothamnion Calcareum Extract	x						x			x														
Lithothamnion Calcareum Powder	x	x																x					x	
Lithothamnion Corallioides Powder																								
Mesophyllum Lichenoides Extract																								
Palmaria Palmata Extract	x	x	x															x			x			
Palmaria Palmata Powder																								
Phymatolithon Calcareum Extract	x																							
Pikea Robusta Extract																								
Polysiphonia Lanosa Extract																		x						
Porphyra Linearis Powder																								
Porphyra Tenera Extract																								
Porphyra Tenera Sporophyte Extract																								
Porphyra Umbilicalis Extract	x	x	x										x								x	x	x	
Porphyra Umbilicalis Powder																								
Hydrolyzed Porphyra Yezoensis																								
Porphyra Yezoensis Extract	x																							
Porphyra Yezoensis Powder																								
Porphyridium Cruentum Culture Conditioned Media																								
Porphyridium Cruentum Extract	x																							
Porphyridium Purpureum Extratc																								
Rhodymenia Palmata Extract	x																	x					x	
Sarcodiotheca Gaudichaudii Extract																								

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

Red Algae Search Strategy

Ingredient	CAS #	InfoB	PubMed	TOXNET	FDA	EU	ECHA	IUCLID	SIDS	ECETOC	HPVIS	NICNAS	NTIS	NTP	WHO	FAO	NIOSH	FEMA	Web
Ahnfeltiopsis Concinna Extract		x				x													x
Asparagopsis Armata Extract		x	x			x													x
Hydrolyzed Asparagopsis Armata Extract		x				x													
Betaphycus Gelatinum Extract		x	x			x													x
Botryocladia Occidentalis Extract		x				x													x
Calliblepharis Ciliata Extract		x				x													x
Ceramium Kondoi Extract		x				x													x
Ceramium Rubrum Extract		x	x			x													x
Chondracanthus Teedei Powder		x	x			x													x
Chondrus Crispus		x	x			x													x
Chondrus Crispus Extract		x	x			x													x
Chondrus Crispus Powder		x				x													x
Hydrolyzed Chondrus Crispus Extract		x				x													x
Corallina Officinalis Extract		x	x			x													x
Corallina Officinalis Powder		x				x													
Corallina Officinalis Thallus Extract		x				x													
Hydrolyzed Corallina Officinalis		x				x													
Hydrolyzed Corallina Officinalis Extract		x				x													
Cyanidium Caldarium Extract		x	x			x													x
Delesseria Sanguinea Extract		x	x			x													x
Digenea Simplex Extract		x	x			x													x
Dilsea Carnosa Extract		x				x													x
Furcellaria Lumbricalis Extract		x				x													x

Ingredient	CAS #	InfoB	PubMed	TOXNET	FDA	EU	ECHA	IUCLID	SIDS	ECETOC	HPVIS	NICNAS	NTIS	NTP	WHO	FAO	NIOSH	FEMA	Web
Gelidiella Acerosa Extract		x	x			x													x
Gelidium Amansii Extract		x	x			x													x
Gelidium Amansii Oligosaccharides		x				x													
Gelidium Cartilagineum Extract	9495-01-4	x				x	x												x
Gelidium Pulchrum Protein		x				x													x
Gelidium Sesquipedale Extract		x	x			x													x
Gigartina Skottsbergii Extract		x	x			x													x
Gigartina Stellata Extract		x			x	x													x
Gloiopeltis Tenax Extract		x	x			x													x
Gloiopeltis Tenax Powder		x				x													
Gracilaria Verrucosa Extract		x	x			x													x
Gracilariopsis Chorda Extract		x	x			x													x
Grateloupia Livida Powder		x	x			x													x
Hypnea Musciformis Extract		x	x			x													x
Lithothamnion Calcareum Extract		x	x			x													x
Lithothamnion Calcareum Powder		x				x													
Lithothamnion Corallioides Powder		x				x													x
Mesophyllum Lichenoides Extract		x				x													x
Palmaria Palmata Extract		x	x			x													x
Palmaria Palmata Powder		x				x													
Phymatolithon Calcareum Extract		x				x													x
Pikea Robusta Extract		x				x													x
Polysiphonia Lanosa Extract		x				x													x
Porphyra Linearis Powder		x				x													x
Porphyra Tenera Extract		x	x		x	x													x

Ingredient	CAS #	InfoB	PubMed	TOXNET	FDA	EU	ECHA	IUCLID	SIDS	ECETOC	HPVIS	NICNAS	NTIS	NTP	WHO	FAO	NIOSH	FEMA	Web
Porphyra Tenera Sporophyte Extract		x																	
Porphyra Umbilicalis Extract		x	x																x
Porphyra Umbilicalis Powder		x																	
Hydrolyzed Porphyra Yezoensis		x																	
Porphyra Yezoensis Extract		x	x																x
Porphyra Yezoensis Powder		x																	
Porphyridium Cruentum Culture Conditioned Media		x																	
Porphyridium Cruentum Extract		x	x																x
Porphyridium Purpureum Extratc		x	x																x
Rhodymenia Palmata Extract		x	x		x														x
Sarcodiotheca Gaudichaudii Extract		x																	x

Typical Search Terms

- INCI names
- chemical/technical names
- genus names
- species names
- dermal
- irritation
- sensitization
- ocular
- metabolism
- ingestion
- food
- dietary
- cancer
- carcinogenicity
- genotoxicity
- mutagenicity
- synonymous genus/species names – accepted genus/species names

LINKS

Search Engines

- Pubmed (- http://www.ncbi.nlm.nih.gov/pubmed)
- Toxnet (https://toxnet.nlm.nih.gov/); (includes Toxline; HSDB; ChemIDPlus; DART; IRIS; CCRIS; CPDB; GENE-TOX)

appropriate qualifiers are used as necessary
search results are reviewed to identify relevant documents

Pertinent Websites

- wINCI - http://webdictionary.personalcarecouncil.org

- FDA databases http://www.ecfr.gov/cgi-bin/ECFR?page=browse
- FDA search databases: http://www.fda.gov/ForIndustry/FDABasicsforIndustry/ucm234631.htm;,
- EAFUS: http://www.accessdata.fda.gov/scripts/fcn/fcnnavigation.cfm?rpt=eafuslisting&displayall=true
- GRAS listing: http://www.fda.gov/food/ingredientspackaginglabeling/gras/default.htm
- SCOGS database: http://www.fda.gov/food/ingredientspackaginglabeling/gras/scogs/ucm2006852.htm
- Indirect Food Additives: http://www.accessdata.fda.gov/scripts/fdcc/?set=IndirectAdditives
- Drug Approvals and Database: http://www.fda.gov/Drugs/InformationOnDrugs/default.htm
- http://www.fda.gov/downloads/AboutFDA/CentersOffices/CDER/UCM135688.pdf
- FDA Orange Book: https://www.fda.gov/Drugs/InformationOnDrugs/ucm129662.htm
- OTC ingredient list: https://www.fda.gov/downloads/aboutfda/centersoffices/officeofmedicalproductsandtobacco/cder/ucm135688.pdf
- (inactive ingredients approved for drugs: http://www.accessdata.fda.gov/scripts/cder/iig/

- HPVIS (EPA High-Production Volume Info Systems) - https://ofmext.epa.gov/hpvis/HPVISlogon
- NIOSH (National Institute for Occupational Safety and Health) - http://www.cdc.gov/niosh/
- NTIS (National Technical Information Service) - http://www.ntis.gov/
- NTP (National Toxicology Program) - http://ntp.niehs.nih.gov/
- Office of Dietary Supplements https://ods.od.nih.gov/
- FEMA (Flavor & Extract Manufacturers Association) - http://www.femaflavor.org/search/apachesolr_search/

- EU CosIng database: http://ec.europa.eu/growth/tools-databases/cosing/
- ECHA (European Chemicals Agency – REACH dossiers) – http://echa.europa.eu/information-on-chemicals;jsessionid=A978100B4E4CC39C78C93A851EB3E3C7.live1
- ECETOC (European Centre for Ecotoxicology and Toxicology of Chemicals) - http://www.ecetoc.org
- European Medicines Agency (EMA) - http://www.ema.europa.eu/ema/
- IUCLID (International Uniform Chemical Information Database) - https://iuclid6.echa.europa.eu/search
- OECD SIDS (Organisation for Economic Co-operation and Development Screening Info Data Sets)- http://webnet.oecd.org/hpv/ui/Search.aspx
- SCCS (Scientific Committee for Consumer Safety) opinions: http://ec.europa.eu/health/scientific_committees/consumer_safety/opinions/index_en.htm
- NICNAS (Australian National Industrial Chemical Notification and Assessment Scheme)- https://www.nicnas.gov.au/

- International Programme on Chemical Safety http://www.inchem.org/
- FAO (Food and Agriculture Organization of the United Nations) - http://www.fao.org/food/food-safety-quality/scientific-advice/jecfa/jecfa-additives/en/
- WHO (World Health Organization) technical reports - http://www.who.int/biologicals/technical_report_series/en/

- www.google.com - a general Google search should be performed for additional background information, to identify references that are available, and for other general information

Botanical Websites, if applicable

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

Fragrance Websites, if applicable

- IFRA (International Fragrance Association) – http://www.ifraorg.org/
- Research Institute for Fragrance Materials (RIFM)

Safety Assessment of Red Algae-Derived Ingredients as Used in Cosmetics

Status: Draft Report for Panel Review
Release Date: August 21, 2020
Panel Meeting Date: September 14 – 15, 2020

The Expert Panel for Cosmetic Ingredient Safety members are: Chair, Wilma F. Bergfeld, M.D., F.A.C.P.; Donald V. Belsito, M.D.; Curtis D. Klaassen, Ph.D.; Daniel C. Liebler, Ph.D.; James G. Marks, Jr., M.D.; Lisa A. Peterson, Ph.D.; Ronald C. Shank, Ph.D.; Thomas J. Slaga, Ph.D.; and Paul W. Snyder, D.V.M., Ph.D. The Cosmetic Ingredient Review (CIR) Executive Director is Bart Heldreth, Ph.D. This safety assessment was prepared by Priya Cherian, Scientific Analyst/Writer, CIR.

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INTRODUCTION

The safety of the following 59 red algae ingredients, as used in cosmetics, is reviewed in this assessment.

Ahnfeltiopsis Concinna Extract
Asparagopsis Armata Extract
Betaphycus Gelatinum Extract
Botryocladia Occidentalis Extract
Calliblepharis Ciliata Extract
Ceramium Kondoi Extract
Ceramium Rubrum Extract
Chondracanthus Teedei Powder
Chondrus Crispus
Chondrus Crispus Extract
Chondrus Crispus Powder
Corallina Officinalis Extract
Corallina Officinalis Powder
Corallina Officinalis Thallus Extract
Cyanidium Caldarium Extract
Delesseria Sanguinea Extract
Digenea Simplex Extract
Dilsea Carnosa Extract
Furcellaria Lumbricalis Extract
Gelidiella Acerosa Extract
Gelidium Amansii Extract
Gelidium Amansii Oligosaccharides
Gelidium Cartilagineum Extract
Gelidium Pulchrum Protein
Gelidium Sesquipedale Extract
Gigartina Skottsbergii Extract
Gigartina Stellata Extract
Gloiopeltis Tenax Extract
Gloiopeltis Tenax Powder
Gracilaria Verrucosa Extract
Gracilariopsis Chorda Extract
Grateloupia Livida Powder
Hydrolyzed Asparagopsis Armata Extract
Hydrolyzed Chondrus Crispus Extract
Hydrolyzed Corallina Officinalis
Hydrolyzed Corallina Officinalis Extract
Hydrolyzed Porphyra Yezoensis
Hypnea Musciformis Extract
Lithothamnion Calcareum Extract
Lithothamnion Calcareum Powder
Lithothamnion Corallioides Powder
Mesophyllum Lichenoides Extract
Palmaria Palmata Extract
Palmaria Palmata Powder
Phymatolithon Calcareum Extract
Pikea Robusta Extract
Polysiphonia Lanosa Extract
Porphyra Linearis Powder
Porphyra Tenera Extract
Porphyra Tenera Sporophyte Extract
Porphyra Umbilicalis Extract
Porphyra Umbilicalis Powder
Porphyra Yezoensis Extract
Porphyra Yezoensis Powder
Porphyridium Cruentum Culture Conditioned Media
Porphyridium Cruentum Extract
Porphyridium Purpureum Extract
Rhodymenia Palmata Extract
Sarcodiotheca Gaudichaudii Extract

The majority of the ingredients in this review are extracts and powders derived from different species of red algae. Although a total of 59 International Nomenclature Cosmetic Ingredient (INCI) names identifying red-algae derived ingredients were found in the web-based *International Cosmetic Ingredient Dictionary and Handbook (*wINCI *Dictionary*) several ingredients appear to be equivalent based on the accepted scientific name, as given in the definition.[1] Accordingly, the total number of distinct cosmetic ingredients is 56.

According to the *Dictionary*, these red-algae derived ingredients are mostly reported to function as skin-conditioning agents (Table 1).[1] These ingredients are also reported to function as abrasives, antioxidants, exfoliants, skin protectants, skin bleaching agents, viscosity increasing agents, and anti-microbial agents.

The names of the ingredients in this report are written in accordance with the INCI naming conventions, i.e., capitalized without italics or abbreviations. When referring to the algae from which ingredients are derived, the standard taxonomic practice of using italics is followed (e.g., *Ahnfeltiopsis concinna*). It is often not known how the substance being tested in a study compares to the cosmetic ingredient. In the report text, if it is known that the material being tested is a cosmetic ingredient, the INCI naming convention will be used (e.g., Asparagopsis Armata Extract). However, if it is not known that the test substance is the same as the cosmetic ingredient, the taxonomic naming conventions (e.g., an *Asparagopsis armata* extract) will be used.

Several ingredients that are obtained from red algae, such as agar, carrageenan, hydrolyzed carrageenan, and hydrolyzed furcellaran, have been previously reviewed by the Expert Panel for Cosmetic Ingredient Safety (Panel).[2] In 2015, it was concluded that these ingredients were considered safe in the present practices of use and concentration as described in that safety assessment; however, available data were insufficient in determining the safety of hydrolyzed carrageenan in cosmetic products. The full report on these ingredients can be accessed on the Cosmetic Ingredient Review (CIR) website (https://www.cir-safety.org/ingredients).

This safety assessment includes relevant published and unpublished data that are available for each endpoint that is evaluated. Published data are identified by conducting an exhaustive search of the world's literature. A listing of the search engines and websites that are used and the sources that are typically explored, as well as the endpoints that the Panel typically

evaluates, is provided on the CIR website (https://www.cir-safety.org/supplementaldoc/preliminary-search-engines-and-websites; https://ewww.cir-safety.org/supplementaldoc/cir-report-format-outline). Unpublished data are provided by the cosmetics industry, as well as by other interested parties.

These red algae-derived ingredients may contain hundreds of constituents, some of which may have the potential to cause toxic effects. In this assessment, the Panel will review the potential toxicity of each of the red algae ingredients as a whole, complex mixture.

CHEMISTRY

Definition

The ingredients in this safety assessment are derived from various species of red algae. "Algae" is not a taxonomic group, but a functional group of convenience.[3] Not all algae should be considered to be plant-like (seaweed; macroalgae). While some algae are seaweed, some are protozoa, and some are unique and belong in other kingdoms. However, these aquatic and oxygenic organisms are all part of the eclectic group called "algae."

Algae Identification

There are several major groups of algae, commonly referred to as red algae (*Rhodophyta*), brown algae (*Phaeophyceae*), green algae (*Chlorophyta*), diatoms (*Bacillariophyceae*), chrysophytes (*Chrysophyta*), blue-green algae (*Cyanophyta*), dinoflagellates (*Pyrrhophyta*), and euglenoids (*Euglenophyta*). The various types of algae are arranged by storage products, pigmentation, and cell wall composition.[3] The corresponding subclass, order, family, and genus for each of the red-algae ingredients are presented in Table 2.

Red algae are of the kingdom Plantae, and are comprised of approximately 6100 species.[4] These algae lack flagella, and range in size from thin films to filamentous membranous forms of 1 m. The color of red algae results from the presence of the pigments phycoerythrin and phycocyanin. Red algae store floridean starch and floridoside, and the cells walls are made up of long-chain polysaccharide agars, carrageenans, and cellulose. General characteristics and the geographic distribution of several specific species of red algae that are included in this report are presented in Table 3.

Chemical Properties

No chemical properties of these red algae-derived ingredients were found in the published literature, and unpublished data were not submitted.

Method of Manufacture

Numerous methods of manufacture are provided in Table 4. General production of a red algae extract includes harvesting, washing to remove epiphytes/sand, drying, grinding, addition of a solvent and preservative, filtration, quality control, and packaging.[5-7] Typical solvents include water, caprylic/capric triglycerides, and butylene glycol.

Composition and Impurities

Red algae constituents comprise of approximately 50 - 75% carbohydrates, based on dry weight (DW), and the majority of such constituents are cellulose, xylan, mannan, or agar.[8] Red algae also contain proteins, polyphenols, polysaccharides, minerals, and amino acids. In addition, red algae may accumulate compounds like arsenic and antimony, and toxic metals such as cadmium, lead, mercury, tin, and aluminum.[9] The accumulation of these contaminants is influenced by environmental factors and structural features of the algae.

Ahnfeltiopsis Concinna Extract

A trade name mixture containing 0.75% Ahnfeltiopsis Concinna Extract was reported to have less than 20 ppm heavy metals and less than 2 ppm arsenic.[10] Similarly, two trade name mixture containing Chondrus Crispus Extract (20% and 3.5%) was reported to have < 20 ppm heavy metals, < 10 ppm lead, < 2 ppm arsenic, and < 1 ppm cadmium.[11]

Chondrus Crispus Extract

The composition of dried *Chondrus crispus* was reported to be 76.8% moisture, 27.7% ash, 4.58% potassium, 0.0736% iodine, 2.16% crude fiber, and 1.65% nitrogen.[12]

Cyanidium Caldarium Extract

The major lipids in algae samples of *Cyanidium caldarium* include monogalactosyl diglyceride, digalactosyl diglyceride, plant sulfolipid, lecithin, phosphatidyl glycerol, phosphatidyl inositol, and phosphatidyl ethanolamine.[13] The fatty acid composition is variable, but major fatty acids include palmitic acid, oleic acid, linoleic acid, and stearic acid.

Gelidiella Acerosa Extract

A phytochemical analysis was performed on several *Gelidiella acerosa* extracts extracted with solvents of varying polarity (hexane, dichloromethane, ethyl acetate, ethanol, and methanol).[14] Total polyphenols (61.2 µg/100 mg) and flavonoids (13 µg/100 mg) were highest in the ethyl acetate *Gelidiella acerosa* extract.

Gelidium Amansii Extract

The total polyphenolic and flavonoid content of a methanolic *Gelidium amansii* extract was reported to be 0.26 ± 0.08 mg/ml and 1.55 ± 0.16 mg/ml, respectively.[15]

Gelidium Sesquipedale Extract

A heavy metal and mineral analysis was performed on a trade name mixture containing 4% Gelidium Sesquipedale Extract; Table 5.[16] Ashes and iodine were detected in amounts of 0.4 g/100 g and 1.02 mg/kg, respectively. All other evaluated minerals and metals were present at 98.3 mg/100g or less.

Gloiopeltis Tenax Extract

The essential constituents of *Gloiopeltis tenax* were extracted by supercritical carbon dioxide extraction, and the constituents were identified and analyzed by gas chromatography-mass spectroscopy.[17] The identified constituents included six sesquiterpenes (14.39%), three ketones (5.02%), seven fatty acids and their esters (29.1%), two phenols (1.71%) and three sterols (12.81%). A list of 23 of the constituents identified is provided in Table 6.

Gracilaria Verrucosa Extract

Mycosporine-like amino acids (MAAs) were detected in a crude aqueous *Gracilariopsis longissima* extract (equivalent to *Gracilaria verrucosa* extract) via a high performance chromatography-photodiode array detector and electrospray ionization mass spectrometry.[18] The five MAAs detected include palythine (0.3 ± 0.1%), asterina-330 (42.9 ± 1.1%), shinorine (41.2 ± 2%), porphyra-334 (1.7 ± 0.1%), and palythinol (13.9 ± 0.5%) (percentages are in terms of the total amount of MAAs).

Gracilariopsis Chorda Extract

The amount of arachidonic acid in an ethanolic *Gracilariopsis chorda* extract and *Gracilariopsis chorda* powder was determined via reverse-phase high-pressure liquid chromatography.[19] The arachidonic acid content was calculated as 0.64% of the *Gracilariopsis chorda* extract, and 1.5 mg/100 DW of the *Gracilariopsis chorda* powder.

Grateloupia Livida Extract

The chemical composition of a petroleum ether fraction of *Grateloupia livida* was evaluated by gas chromatography-mass spectrometry.[20] The primary constituents detected were n-hexadecanoic acid (20.68%), mono-(2-ethylhexyl) phthalate (11.08%), cholesterol (9.16%), methyl eicosapentaenoate (6.98%), and heptadecane (6.68%).

Hypnea Musciformis Extract

The total phenolic content of a methanolic *Hypnea musciformis* extract was reported to be 6.9 mg gallic acid equivalent (GAE)/g.[21] According to a supplier, Hypnea Musciformis Extract is reported to be composed of 75% sugars (mainly polysaccharides which average molecular weight is below 700 kDa), 22% mineral ashes, and 3% proteins.[22] A heavy metal analysis performed on a Hypnea Musciformis Extract detected the following impurities: 0.082 ppm arsenic, < 0.020 ppm cadmium, < 0.020 ppm cobalt, 0.052 ppm chromium, < 0.020 ppm mercury, 0.185 ppm nickel, < 0.020 ppm lead, < 0.020 ppm antimony, 0.031 ppm selenium, and 0.053 ppm vanadium.[22] In addition, the sum of aflatoxins B1, B2, G1, and G2 in the Hypnea Musciformis Extract did not exceed 0.4 µg/kg.

Lithothamnion Calcareum Extract

A *Lithothamnion calcareum* extract was reported to contain 12% calcium, 1% magnesium, and measurable levels of 72 other trace minerals, including manganese, selenium, copper, and zinc.[23]

Palmaria Palmata Extract

The total protein content in *Palmaria palmata* has been reported to be in the range of 8 - 35%, and is variable based on geographical and seasonal variations.[24] The most abundant amino acids in this red algae species are alanine, aspartic acid, glutamic acid, and glycine. Samples of newly dried fresh, as well as stored dry, *Palmaria palmata* were analyzed for their contents of phylloquinone (vitamin K_1). The results indicated that the contents are fairly low (in the range of 2 - 7 µg/g). In addition, kainic acid has been reported to be present in *Palmaria palmata* and *Digenea simplex*. In the same study, levels of kainic acid in *Palmaria palmata* samples from Iceland ranged from 1 - 21 µg/g. The phenolic content in algae extracts are variable depending on extraction methods. The total phenolic content in *Palmaria palmata* extracted with distilled water, 80% methanol, 70% acetone, and 100% methanol was reported to be 31.8, 26.5, 25, and 10.7 mg GAE/g, respectively.[25] According to a manufacturer, Palmaria Palmata Extract is reported to be composed of 73% sugars (mainly oligosaccharides, which average molecular weight is between 540 and 2000 Da), 24% mineral ashes, and 3% proteins.[22]

Levels of iodine in *Palmaria palmata* can exhibit a wide range of value (10 - 100 µg/g) depending on location and time of harvest.[24] In one study, iodine levels from *Palmaria palmata* samples from several sources were reported to contain iodine in amounts of 5 µg/g or less. In a different study, the total iodine content of *Palmaria palmata* from Maine was reported to be 72 µg/g.[26] Arsenic content also varies widely based on location and age of the specimen. For example, *Palmaria palmata* (young, whole broad-leaf material) from Maine contained < 0.02 µg/g inorganic arsenic, whereas a granular product

produced from older *Palmaria palmata* was found to contain 0.3 µg/g. In the same study, the total amounts of arsenic in *Palmaria palmata* specimens from several locations range from 1 - 10 µg/g. Levels of cadmium and lead in *Palmaria palmata* from different sources are generally found to be below 1 µg/g.

According to a heavy metal analysis performed by a supplier, antimony, arsenic, chromium, nickel, and vanadium, were detected in a Palmaria Palmata Extract in amounts of 0.069, 1.480, 0.046, 0.433, and 2.29 ppm, respectively.[22] Approximately 3.8 ppm iodine was detected in the same extract. No aflatoxins were detected in this Palmaria Palmata Extract.

Porphyra Umbilicalis Extract

The heavy metal impurities of trade name mixture containing Porphyra Umbilicalis Extract was reported to be < 3.0 ppm arsenic, < 0.1 ppm cadmium, < 1.0 ppm lead, < 0.1 ppm mercury, < 0.5 antimony, < 1.0 chromium, < 1.0 nickel, and < 0.5 cobalt.[27] Due to manufacturing processes, traces of residual phenol (< 0.1 ppm) and ethylene oxide (< 0.02 ppm) may be present in this Porphyra Umbilicalis Extract. Heavy metals detected in a different Porphyra Umbilicalis Extract include 3679 µg/kg arsenic, < 10 µg/kg cadmium, < 10 µg/kg mercury, and < 10 µg/kg lead.[28]

Porphyra Tenera Extract, Porphyra Umbilicalis Extract, and Porphyra Yezoensis Extract

Dried *Porphyra* sp. contains numerous nutrients, including proteins, dietary fibers, polyunsaturated fatty acids, minerals, and vitamins.[29] The dried, raw *Porphyra* sp. contains approximately 40% proteins and 40% carbohydrates, which are mostly derived from the soluble dietary fiber, porphyran. Dried *Porphyra* sp. contains a small amount of lipids (approximately 4%), with eicosapentanoic acid (1200 mg/100 g) and palmitic acid (500 mg/100 g) being the predominant fatty acids. Vitamins and minerals, such as vitamin K (2600 µg/100 g), vitamin C (160 mg/100 g), folate (1200 µg/100 g), vitamin B_{12} (78 µg/100 g), potassium (3100 mg/100 g), and iodine (1400 µg/100 g) are found in dried *Porphyra* sp. A large amount of iron (11 mg/100 g) is also found in these species. *Porphyra* sp. also contain compounds such as polysaccharides (porphyrans; > 40% DW), phycobiliproteins (phycoerythrin and phycocyanin), peptides, MAAs, and phenolic compounds (phlorotannin and taurine).

Dried nori (*Porphyra* sp.) samples contained none or trace amounts of inorganic arsenic and total arsenic content.[29] However, dried, and toasted nori contain 2.1 – 21.6 mg of total arsenic/kg DW. In addition, Cadmium was reported to be present in dried *Porphyra* sp. products in concentrations varying from 0.58 – 11 mg/kg of DW.

Porphyra Tenera Extract, Porphyra Umbilicalis Extract, Porphyra Yezoensis Extract, Chondrus Crispus, Palmaria Palmata Extract, Gelidium Amansii Extract, Gelidium Cartilagineum Extrat, Gelidium Sesquipedale, Lithothamnion Calcareum Extract and Gracilaria Verrucosa Extract

Heavy metal and metalloid contents in several edible red algae species (*Porphyra* sp., *Chondrus crispus*, *Palmaria Palmata*, *Gracilaria* sp.) based on geographical location was evaluated.[30] Aluminum was present in *Gracilaria* species from Italy, *Palmaria palmata* from Spain, and *Porphyra* species from Spain in amounts of 8-120 mg/kg DW, 19-149 mg/kg, 62 mg/kg DW, and 15-1890 mg/kg DW, respectively. The concentration levels of 20 metals were analyzed by inductively coupled plasma atomic emission spectroscopy in various dehydrated red seaweed genera (*Chondrus, Gelidium, Palmaria, Porphyra,* and *Gracilaria*), from two origins (Asia and Europe).[31] The mean metal content in seaweed samples for the different genera of red algae is presented in Table 7. The highest levels of aluminum (32 mg/kg DW) was detected in Palmaria, and the highest content of lead (0.15 mg/kg DW) was detected in Porphyra.

Palmaria palmata, *Porphyra umbilicalis*, *Porphyra tenera*, *Porphyra yezoensis*, *Chondrus crispus*, *Gracilaria verrucosa*, and *Lithothamnion calcareum* are authorized as vegetables and condiments in France, with certain specifications.[9] Maximum allowed minerals and metals have been established by French legislature for these species when used in foods (inorganic arsenic, < 3 mg/kg DW); cadmium, < 0.5 mg/kg DW; mercury, < 0.1 mg/kg DW; lead, < 5 mg/kg DW; tin, < 5 mg/kg DW; and iodine, < 2000 mg/kg DW).

Gigartina Stellata Extract and Corallina Officinalis Extract

A mineral and heavy metal analysis was performed on a trade name mixture containing water (45.7%), glycerin (40%), *Gigartina stellata* (4.43%), *Kappaphycus alvarezii* extract (5.9%), and Corallina Officinalis Extract; Table 8.[32] Sodium, chlorides, and potassium were detected at levels of 419.9 mg/100g, 391 mg/100g, and 109.4 mg/100g, respectively. All other minerals and metals were detected in an amount of 11.9 mg/100g or less.

Undaria Pinnatifida Extract and Corallina Officinalis Extract

A mineral and heavy metal analysis was performed on a trade name mixture consisting of 50% glycerin, 30% water, 18.5% Undaria Pinnatifida Extract, and 1.5% Corallina Officinalis Extract; Table 9.[33] Iodine, arsenic, cadmium, mercury, and lead were present in amounts of 1.9 mg/l, 1383 µg/kg, 29 µg/kg, < 10 µg/kg, and 86 µg/kg, respectively.

USE

Cosmetic

The safety of the cosmetic ingredients addressed in this assessment is evaluated based on data received from the US Food and Drug Administration (FDA) and the cosmetics industry on the expected use of these ingredients in cosmetics. Use frequencies of individual ingredients in cosmetics are collected from manufacturers and reported by cosmetic product category in the FDA Voluntary Cosmetic Registration Program (VCRP) database. Use concentration data are submitted by the cosmetic industry in response to a survey, conducted by the Personal Care Products Council (Council), of maximum reported use concentrations by product category.

According to 2020 VCRP survey data, Chondrus Crispus Extract is reported to be used in 381 formulations (306 leave-on formulations, 74 rinse-off formulations, and 1 formulation diluted for bath; Table 10).[34] Hypnea Musciformis Extract is reported to be used in 141 formulations, Corallina Officinalis Extract is reported to be used in 96 formulations, and Palmaria Palmata Extract is reported to be used in 83 formulations. All other in-use ingredients are reported to be used in 55 formulations or less.

The results of the concentration of use survey conducted by Council in 2020 indicate Corallina Officinalis Extract has the highest reported maximum concentration of use; it is used at up to 2% in blushers, other makeup preparations, and face and neck products.[35] Chondrus Crispus is reported to be used at up to 1.4% in dentifrices. All other ingredients are reported to be used at 0.25% or less.

In some cases, reports of uses were received in the VCRP, but concentration of use data were not provided. For example, Ahnfeltiopsis Concinna Extract is reported to be used in 16 formulations, but no concentration of use data were reported. In other cases, no uses were reported in the VCRP, but concentration of use data were reported in the industry survey; Hydrolyzed Chondrus Crispus Extract had no reported uses in the VCRP, but a use concentration in eyeliners and other makeup preparations was provided in the industry survey. Therefore, it should be presumed there is at least one use in every category for which a concentration is reported. The ingredients not in use according to the VCRP and concentration of use data are listed in Table 11.

Several of these ingredients are used in formulations that are near the eye (e.g., Chondrus Crispus Extract in eyeshadows at up to 0.14%), could be incidentally ingested (e.g., Porphyridium Cruentum Extract in lipsticks at up to 0.00055%, and in formulations that come in contact with mucous membranes (e.g., Chondrus Crispus in bath oils, tablets, and salts and Chondrus Crispus Extract in bubble baths (concentrations not reported)).

Additionally, some red algae-derived ingredients are used in cosmetic sprays and could possibly be inhaled; for example, Chondrus Crispus is reported to be used at up to 0.08% in aerosol suntan products. In practice, 95% to 99% of the droplets/particles released from cosmetic sprays have aerodynamic equivalent diameters > 10 μm, with propellant sprays yielding a greater fraction of droplets/particles < 10 μm compared with pump sprays.[36,37] Therefore, most droplets/particles incidentally inhaled from cosmetic sprays would be deposited in the nasopharyngeal and thoracic regions of the respiratory tract and would not be respirable (i.e., they would not enter the lungs) to any appreciable amount.[38,39] Red-algae derived ingredients have also been reported to be used in face powders that could possibly be inhaled. Chondrus Crispus, Chondrus Crispus Extract, and Corallina Officinalis Extract were reported to be used in face powders at up to 0.15%. Conservative estimates of inhalation exposures to respirable particles during the use of loose powder cosmetic products are 400-fold to 1000-fold less than protective regulatory and guidance limits for inert airborne respirable particles in the workplace.[40-42]

None of the red algae-derived ingredients named in this report are restricted from use in any way under the rules governing cosmetic products in the European Union.[43]

Non-Cosmetic

Several species of red algae (e.g., *Palmaria palmata*) have become established as part of popular international cuisine.[44] According to the US FDA, several red algae species (*Gloiopeltis furcata*, *Porphyra crispata*, *Porphyra deutata*, *Porphyra perforata*, *Porphyra suborbiculata*, *Porphyra tenera*, and *Rhodymenia palmata*) are direct food substances that are generally recognized as safe (GRAS) for human consumption for use as flavor enhancers and flavor adjuvants, when the maximum level in food does not exceed the current good manufacturing practice (cGMP). [21CFR184.1121] Of these red algae species, two are relevant for the purposes of this report (*Porphyra tenera* and *Rhodymenia palmata*). Some red algae species are used in Hawaiian, Irish, or Asian cuisine (e.g., *Ahnfeltiopsis concinna*, *Chondrus crispus*, *Gracilaria verrucosa*, *Palmaria palmata*, *Porphyra* sp.) Other red algae species are used in jellies and as thickeners in food products (e.g., *Gelidiella* and *Gracilaria* sp).[45] A listing of red algae species that are frequently ingested by humans as foods is provided in Table 12.

In addition, red algae species have been used in historical folk medicine. Chinese and Japanese monks used preparations containing *Gelidium amansii* to treat sun stroke and fevers.[45] *Gloiopeltis tenax* has also been reported to be used in China to treat diarrhea and colitis.[17] In Japan and the Mediterranean area, *Gelidium cartilagineum* and *Chondrus Crispus* were used in diarrhea and urinary tract irritation treatment.[45] Extracts of the dried red algae, *Digenea simplex*, was sold by Asian apothecaries by the name of "helminol" to treat ascariasis and oxyuriasis.

Red algae species are still used in present-day holistic medicine for treatment and prevention of various ailments. Some red algae species (e.g., *Gigartina*) have been reported to be used in dietary supplements for immunity-boosting effects.[46] The red algae species, *Lithothamnion calcareum,* is marketed as a nutritional supplement for calcium and minerals in Brazil and other countries due to presence of calcium and magnesium carbonate precipitates in the cell wall.[47] This algae is also used in implants for bone surgery, animal nutrition, fertilizers, and soil treatments. *Gracilariopsis chorda* may be used as a medicinal food to prevent neurological disorders.[19] *Grateloupia livida* is also an edible and medicinal seaweed used to treat sore throat, stomachache, ascariasis, and dysentery.[48] Red algae species such as *Gelidium amansii*, *Gelidium cartilagineum*, and *Gigartina stellata* have been reported to be used in pharmaceutical and industrial preparations due to gelling, water-retention, emulsifying, and other physical properties.[21,45] *Corallina officinalis* extract is a popular ingredient in traditional Asian medicine used for the treatment of various ailments.[49] Several red algae species (e.g. *Chondrus crispus* (Irish moss) and *Gelidiella acerosa*) are widely used for the preparation of carrageenan, agar and for other industrial uses.[14,50]

TOXICOKINETIC STUDIES

No toxicokinetic studies on these ingredients were found in the published literature, and unpublished data were not submitted. In general, toxicokinetics data are not expected to be found on algal ingredients because each natural sourced ingredient is a complex mixture of constituents.

TOXICOLOGICAL STUDIES

Acute Toxicity Studies

Oral

Grateloupia Livida Extract

The acute oral toxicity of several *Grateloupia livida* extracts (petroleum ether, ethyl acetate, n-butyl alcohol, and aqueous) was evaluated in female Kumming mice (20/group).[20] Animals were dosed with 5, 30, 300, or 2000 mg/kg of the extracts. No mortality or severe toxic effects were seen with any extract or dose level. The median lethal dose (LD_{50}) values were expected to be greater than 2000 mg/kg.

Lithothamnion Calcareum Extract

A *Lithothamnion calcareum* aqueous suspension was evaluated for acute oral toxicity in groups of 5 female Wistar rats.[47] One group was treated with the aqueous vehicle and the other was treated with a single 2000 mg/kg dose of the *Lithothamnion calcareum* suspension. The method of oral administration was not stated. Clinical observation of the rats was conducted 5, 15, and 30 min, and each hour for 12 h. The rats were also examined twice a day for an additional 13 d. After 14 d, rats were euthanized and subjected to macroscopic and microscopic necropsy. No signs of toxicity were observed in any of the treated rats.

Subchronic Toxicity Studies

Oral

Lithothamnion Calcareum Extract

A *Lithothamnion calcareum* aqueous suspension was evaluated for oral toxicity in Wistar rats.[47] Rats were divided into five groups: a control group (10 rats/sex/group), two experimental groups (10 rats/sex/group), and two satellite test groups (5 rats/sex/group). The satellite control group received the aqueous vehicle alone while the satellite high-dose group received a dose of 2000 mg/kg (specific use of satellite groups not specified). A constant volume of *Lithothamnion calcareum* suspension (1000 or 2000 mg/kg) was administered to all test groups (including satellite groups), daily, via gavage, for 90 d. Following treatment, blood was collected and animals were euthanized. No significant abnormalities in mortality, feces, hair, or behavior were identified in any group. Food intake of groups receiving the test substance was statistically higher than in the control group. Serum creatine levels were increased in female rats treated with 1000 mg/kg of the test substance, and in male and female rats treated with 2000 mg/kg of the test substance. Total serum protein levels decreased in rats treated with 2000 mg/kg of the test substance, and an even greater decrease occurred in the high-dose satellite group. Decreased serum albumin levels were observed in male rats treated with 1000 mg/kg of the test substance and in high-dose male and female rats, with a greater decrease observed in the high-dose satellite group. Some differences were observed in the organ weights of the rats, although gross necropsy and histopathologic evaluation of the same organs revealed no abnormality or significant changes between treated and control groups.

DEVELOPMENTAL AND REPRODUCTIVE TOXICITY STUDIES

Gelidiella Acerosa Extract

The potential reproductive toxicity of a crude extract of *Gelidiella acerosa* was evaluated in albino rats.[51] In order to prepare the crude extract, *Gelidiella acerosa* was collected and extracted into a 1:1 methanol:methylene chloride solvent system and co-precipitated with polyvinylpyrrolidone (PVP). The co-precipitate was dissolved in distilled water to obtain the 1000 mg/kg dose in 1 ml aliquots. Pregnant rats (5/group) were orally administered (via gavage) either 1 ml vehicle (PVP in

distilled water) or 1 ml of the crude extract (PVP co-precipitate) in distilled water, daily, at different stages of gestation (on day 1 only, days 1 - 3, days 4 - 6, or days 7 - 8). On day 14 of gestation, animals were laparotomized, and the number of implantation sites, resorption sites, number of viable embryos, and the gross appearance and number of corpora lutea were observed. Administration of the crude extract did not cause significant ($p > 0.05$) change in any of the parameters evaluated in the animals treated during day 1, days 1 - 3, or days 4 - 6 of gestation. Administration of the crude extract on day 7 - 8 of gestation significantly ($p < 0.01$) reduced the total number of viable implantation sites (by 72%), and significantly ($p < 0.01$) increased the number of resorption sites and post-implantation loss (by 89%).

Within the same study, 12 rats were divided into two equal groups, and one received 1 ml of the vehicle/day, and the other 1 ml of the crude extract/day. Administration occurred on days 1 - 7 of gestation. On day 8 of pregnancy, animals were laparotomized and evaluated. After examination of the number of implantation sites, resorption sites, and viable embryos, animals were sutured, treated locally and subcutaneously with antibiotics, and allowed to recover. Apparent size and distribution of the embryos in the uterine horns were also noted. These animals were re-laparotomized on day 14 of gestation, and the above parameters were recorded. At first laparotomy, the size, appearance, and color of the implants in treated animals were similar to those of the control; however, a clumping of embryos towards the cervical end of the uterine horns was evident in crude extract-treated rats. At second laparotomy, control animals had the same number of viable implants on day 14 as on day 8 of pregnancy. All embryos in the treated group on day 14 of gestation were non-viable and resorbing. There was a 100% post-implantation loss in the treated group ($p < 0.001$).

GENOTOXICITY STUDIES

In Vitro

Asparagopsis Armata Extract

The mutagenic potential of an Asparagopsis Armata Extract containing 8% dry algal matter was evaluated in an Ames assay.[52] The test substance was evaluated with and without metabolic activation in *Salmonella typhimurium* strains (TA98, TA100, TA1537, and TA102) at concentrations of 52, 164, 512, 1600, and 5000 µg/plate. The test substance did not induce a mutagenic effect in the presence or absence of metabolic activation.

Corallina Officinalis Extract and Gigartina Stellata Extract

An Ames assay was performed using a trade name mixture consisting of water (45.7%), glycerin (40%), *Gigartina stellata* (4.43%), *Kappaphycus alvarezii* extract (5.9%), and Corallina Officinalis Extract (3.97%).[53] The assay was performed on *S. typhimurium* strains TA98, TA100, TA102, TA1535, and TA1537, with and without metabolic activation. The trade name mixture was tested at concentrations of 50, 160, 500, 1600, and 5000 µg/plate. No signs of mutagenicity were observed.

Gelidiella Acerosa Extract

An Ames assay was performed using *S. typhimurium* strains TA98, TA100, and TA1538 in order to evaluate the mutagenic potential of a benzene extract of *Gelidiella acerosa* (250, 500, 1000, 2000, and 4000 µg/plate).[54] Assays were performed with and without metabolic activation. No signs of mutagenicity were observed with or without metabolic activation.

Porphyra Umbilicalis Extract

A chemiluminescent 3D assay was performed on a test substance consisting of 52% water and 48% Porphyra Umbilicalis Extract.[55] Genotoxicity was measured by the reparation ratio of both irradiated and non-irradiated samples (with mid-wavelength ultraviolet light (UVB)). A positive control of chlorpromazine was used. The test substance was evaluated at concentrations of 2, 10, 25, 50, and 100%. No genotoxicity was observed directly or after UVB irradiation at any concentration.

CARCINOGENICITY STUDIES

No carcinogenicity studies on these red algae-derived ingredients were found in the published literature, and unpublished data were not submitted.

ANTI-CARCINOGENICITY STUDIES

Hypnea Musciformis Extract

The effect of an ethanolic *Hypnea musciformis* extract on anthracene-induced mammary carcinogenesis was evaluated in female Sprague-Dawley rats (8/group).[56] Rats in group 1 served as a control. Rats in group 2 and 3 received a single subcutaneous injection of 7,12-dimethylbenz[a]anthracene (DMBA) (25 mg/kg bw) in the mammary gland to develop a mammary carcinoma. Rats in group 3 were also orally administered 200 mg/kg bw/d of *Hypnea musciformis* extract for 16 weeks. Rats in group 4 received 200 mg/kg bw *Hypnea musciformis* extract alone, each day, orally, for 16 weeks. (The method of oral administration was not stated.) At the end of the treatment, animals in group 2 showed decreased weight gain compared to control rats ($p < 0.05$). This effect was not seen in animals in any other group. One hundred percent of animals treated with DMBA alone displayed tumors, however in animals treated with DMBA and *Hypnea musciformis* extract, the

incidence of mammary tumors was significantly lower (25%). No tumors were observed in control rats or rats treated with *Hypnea musciformis* extract alone.

Anti-Tumorigenicity

In Vitro

Asparagopsis Armata Extract and Gelidium Cartilagineum Extract

The antitumor potential of methanolic and dichloromethane extracts of *Asparagopsis armata* and *Plocamium cartilagineum* (equivalent to *Gelidium cartilagineum*) was evaluated in human liver cancer (HepG-2) cells via cell viability and cell proliferation studies.[57] For the cell viability and proliferation studies, extracts (1000 µg/ml) were incubated with HepG-2 cells for 24 h. Both methanolic and dichloromethane extracts of *Asparagopsis armata* presented high cytotoxicity with 11 ± 2.98 and 1.51 ± 0.38 % of HepG-2 live cells, respectively. Potent anti-proliferative activity was also induced by the dichloromethane extracts of *Asparagopsis armata* and *Plocamium cartilagineum*, with 98.56 ± 0.81 and 85.13 ± 1.04 % of cell's proliferation reduction, respectively.

Animal

Porphyra Tenera Powder

The effect of *Porphyra tenera* powder on intestinal tumor incidence was evaluated in Sprague-Dawley rats (10/group).[58] Tumors were induced in all experimental animals via a weekly subcutaneous injection of 1,2-dimethylhydrazine (DMH) for 12 weeks. Experimental animals were fed a dietary seaweed preparation containing 2% *Porphyra tenera* powder, and controls were fed a basic diet. Animals were necropsied 8 weeks after the cessation of the diet and DMH administrations. There was a significant decrease ($p < 0.01$) in the incidence of tumors in rats fed *Porphyra tenera* powder (2/10) versus control animals (8/10).

OTHER RELEVANT STUDIES

Cytotoxicity

Ceramium Virgatum Extract, Corallina Officinalis Extract, Furcellaria Lumbricalis Extract, Gelidium Cartilagineum Extract, Porphyra Linearis Extract, and Gelidium Cartilagineum Extract

The cytotoxic potential of *Ceramium virgatum* extract (equivalent to *Ceramium rubrum* extract), *Corallina officinalis* extract, *Furcellaria lumbricalis* extract, *Plocamium cartilagineum* extract (equivalent to *Gelidium cartilagineum* extract), *Porphyra linearis* extract, and *Mastocarpus stellata* extract (equivalent to *Gigartina stellata* extract), was evaluated using rat skeletal myoblasts (L6-cells).[59] Among all extracts tested, only *Corallina officinalis* showed some weak cytotoxic potential towards the mammalian cells (half maximal inhibitory concentration (IC_{50}) value of 88.6 µg/ml). The remaining extracts had no toxicity at the highest concentration.

Gracilariopsis Longissima Extract

The potential cytotoxicity of a crude aqueous *Gracilariopsis longissima* extract (equivalent to *Gracilaria verrucosa* extract) was evaluated by a 3-(4,5-dimethylthiazol-2yl)-diphenyl tetrazolium bromide (MTT) assay.[18] This assay was carried out in vitro in three cell lines: murine macrophages of the immune system (RAW264.7), gingival fibroblasts (HGF), and immortalized human keratinocytes (HaCaT). All cell lines were exposed to the extract at concentrations ranging from 0 - 10 mg/ml for 72 h. No cytotoxicity was observed in either human cell line (HGF or HaCaT) at any concentration; however, cytotoxicity was observed in murine tumor cells.

Photoprotective Effects

Porphyra Umbilicalis Extract

A study was performed to assess the photoprotective effects of cosmetic formulations containing *Porphyra umbilicalis*.[60] Four groups of four hairless mice were treated with topical formulations on the dorsum for 5 d as follows: group 1 – control (no treatment); group 2 – application of sunscreen formulation containing only ultraviolet light (UV) filters; group 3 – application of sunscreen formulation with 5% *Porphyra umbilicalis* extract; group 4 – application of the sunscreen formulation with 5% *Porphyra umbilicalis*, 1.5% *Ginkgo biloba*, and vitamins A, E, and C. After application, mice were immobilized and exposed to long-wavelength ultraviolet light (UVA)/ UVB radiation for 28 minutes, which resulted in a cumulative UVB dose of approximately 0.67 J/cm^2. Apoptosis and erythema were evaluated in each group. Immunohistochemical analysis showed that UV radiation caused an increase in the expression of tumor antigen p53 and apoptosis mediator caspase-3, confirming that the damage caused by UV radiation exposure led to apoptosis. Applications of the test material in groups 2, 3, and 4 resulted in a statistically significant reduction in the expression of p53 and caspase-3, with a more pronounced effect following treatment in group 3 (treatment of sunscreen formulation with *Porphyra umbilicalis* extract). Groups 3 and 4 displayed a statistically significant decrease in erythema values compared with the irradiated control ($p < 0.05$) group.

Anti-Allergic Activity of Porphyran

The effect of porphyran (a major component of *Porphyra tenera* and *Porphyra yezoensis*) on the contact hypersensitivity reaction in female Balb/c mice (10/group) was evaluated.[61] Control and treated groups were given a regular diet for 7 d. On day 7 and 8, mice were administered 2 topical applications of 50 µl of a 5% 2,4,6-trinitrochlorobenzene (TNCB) solution in acetone on shaved abdominal skin. The control and treated groups resumed regular diets, however, the porphyran-treated groups were administered either 0.5, 1, or 2% porphyran in drinking water for the remainder of the test period. The control group was given plain water only. Three days after administration of the TNCB solution, 20 µl of a 1% TNCB solution in acetone was applied to the right ear lobe of each mouse. Twenty-four hours later, the thickness of the ear lobe was measured. Oral administration of porphyran at 2% significantly suppressed ear edema induced by TNCB. In addition, it was found that porphyran suppressed the serum level of immunoglobin E and the production of interferon-γ in the challenged ear lobe.

DERMAL IRRITATION AND SENSITIZATION STUDIES

The dermal irritation and sensitization studies summarized below are presented in Table 13.

Irritation

In vitro dermal irritation assays were performed on a trade name mixture containing 0.75% Ahnfeltiopsis Concinna Extract (tested at 100%; other components of mixture not reported), an Asparagopsis Armata Extract containing 4% dry algal matter (tested at 10%; other components of extract not reported), and a trade name mixture containing 3.5% Chondrus Crispus Extract (tested at 100%; other component of mixture not reported).[52,62,63] All test substances were considered to be non-irritating.

Many human dermal irritation studies were provided using test substances containing a red algae ingredient, or combination of ingredients, along with other substances such as water, propanediol, glycerin, and butylene glycol. The majority of these studies yielded negative results; however, slight irritation was noted (at 30 min after patch removal) in a 24-h patch test assay in which the undiluted test substance (trade name mixture consisting of 72 - 77% water; 20 - 70% butylene glycol; 1 - 3% Hypnea Musciformis Extract; ≤ 1% potassium gluconate; 0.16 - 0.2% methylparaben) was applied to the skin of 12 subjects under occlusive conditions.[64]

Sensitization

All sensitization studies performed on humans, evaluating various red algae-derived ingredients (Asparagopsis Armata Extract (0.325% and 0.5 – 2%), Chondrus Crispus Extract (0.49%), Gelidiella Acerosa Extract (0.0028%), Gelidium Cartilagineum Extract (< 2%), Hydrolyzed Corallina Officinalis Extract (0.5 – 3%), Hypnea Musciformis Extract (0.36%), Palmaria Palmata Extract (1.87%), and Porphyra Umbilicalis Extract (0.0004%)), were negative.[22,65-70]

Phototoxicity

In Vitro

Porphyra Umbilicalis Extract

The phototoxic potential of a test substance consisting of 52% water and 48% Porphyra Umbilicalis Extract was evaluated.[55] Cytotoxicity was evaluated in a cell monolayer (fibroblast Balb/c3Tc clone) after incubation with the test substance at 7 concentrations (concentrations not specified), and irradiation with UVA. The test substance was considered to be non-cytotoxic.

OCULAR IRRITATION STUDIES

The in vitro ocular irritation studies summarized below are presented in Table 14.

An in vitro ocular irritation assay performed on reconstructed cornea epithelium using a trade name mixture containing 0.75% Ahnfeltiopsis Concinna Extract yielded negative results.[62] Slight irritation was noted in an in vitro ocular irritation assay performed using the PREDISAFE method on an Asparagopsis Armata Extract (4% dry algal matter).[52]

MatTek EpiOcular™ MTT viability assays were performed to evaluate the ocular irritation potential of three different test substances containing red algae-derived ingredients (an after-shave balm containing 0.8% Chondrus Crispus, a trade name mixture containing 3.5% Chondrus Crispus Extract, or an eye cream containing 0.0375% Rhodymenia Palmata Extract).[63,71,72] All test substances were considered to be non-irritating.

Several hen's egg test chorioallantoic membrane (HET-CAM) assays were performed on various red algae-derived ingredients (Corallina Officinalis Extract (0.15% and 0.397%), Lithothamnion Calcareum Powder (up to 5.7 – 6.1%), and Porphyra Umbilicalis Extract (48%)). Most assays reported slight or no irritation.[55,72-75] However, moderate irritation was noted when a trade name mixture consisting of 57 - 61% Lithothamnion Calcareum Powder, 26 - 31% mannitol, 9 - 11% diatomaceous earth, 0.7 - 1.5% zinc sulfate was used in a HET-CAM assay tested at 10%, but not at 2 and 5%.

SUMMARY

This is a safety assessment of 59 red algae-derived ingredients. However, several of these ingredients are equivalent according to accepted scientific names; accordingly, the number of distinct cosmetic ingredients is 56. The ingredients reviewed in this report are primarily extracts and powders derived from red algae species, and may be derived from the whole plant or a defined part of the plant. These ingredients are mostly reported to function in cosmetics as skin-conditioning agents.

According to 2020 VCRP survey data, Chondrus Crispus Extract had the highest amount of reported uses among the red-algae derived ingredients (381 formulations; 306 leave-on formulations). Hypnea Musciformis Extract, Corallina Officinalis Extract, and Palmaria Palmata Extract were reported to be used in 141, 96, and 83 formulations, respectively. All other in-use ingredients were reported to be used in 55 formulations or less. The results of the 2020 concentration of use survey conducted by Council indicate that Corallina Officinalis Extract has the highest reported maximum concentration of use; it is used at up to 2% in leave-on dermal products. All other in-use ingredients are reported to be used at 1.4% or less.

Several species of red algae have become established as part of popular international cuisine (e.g., *Ahnfeltiopsis concinna*, *Chondrus crispus*, *Gracilaria verrucosa*, *Palmaria palmata*, *Porphyra* sp.). According to the US FDA, *Porphyra tenera* and *Rhodymenia palmata* are direct food substances that are GRAS for human consumption for use as flavor enhancers and flavor adjuvants, when the maximum level in food does not exceed the cGMP. [21CFR184.1121] Several red algae species have historical and present-day use in holistic medicine. Red algae also have industrial uses due to their gelling and emulsifying properties.

The acute oral toxicity potential of multiple *Grateloupia livida* extracts were evaluated in female mice at up to 2000 mg/kg. No toxicity was observed with any extract or dose level. Similarly, no acute oral toxicity was observed in Wistar rats given a single 2000 mg/kg dose of an aqueous *Lithothamnion calcareum* suspension. The same test substance was used in a 90-d oral toxicity study in which Wistar rats were given either 1000 or 2000 mg/kg/d of the suspension. Serum creatine levels were increased in female rats given 1000 mg/kg of the test substance and in males and females treated with 2000 mg/kg of the test substance. Some differences were observed in the organ weights of the rats, although gross necropsy and histopathologic evaluation of the same organs revealed no abnormality or significant changes between treated and control groups.

The potential reproductive toxicity of a crude extract of *Gelidiella acerosa* (1000 mg/kg/d) was evaluated in female albino rats at different stages of gestation. Administration of the crude extract did not cause significant ($p > 0.05$) change in any of the parameters evaluated in the animals treated during most gestation periods. However, administration of the crude extract on day 7 - 8 of gestation significantly ($p < 0.01$) reduced the total number of viable implantation sites (by 72%), and significantly ($p < 0.01$) increased the number of resorption sites and post-implantation loss (by 89%). Within the same study, 12 rats were divided into two equal groups, and one received 1 ml of the vehicle/day, and the other 1 ml of the crude extract/day. Administration occurred on days 1 - 7 of gestation. Animals were first laparotomized on day 8 of gestation, and allowed to recover. Animals were then re-laparotomized and evaluated on day 14 of gestation. At first laparotomy, the size, appearance, and color of the implants in treated animals were similar to those of the control, however, a clumping of embryos towards the cervical end of uterine horns was evident in crude extract-treated rats. When rats were observed on day 14 of gestation, control animals had the same number of viable implants as on day 8 of pregnancy. All embryos in the treated group on day 14 of pregnancy were non-viable and resorbing. There was a 100% post-implantation loss in the treated group ($p < 0.001$).

Ames assays performed on an Asparagopsis Armata Extract (containing 8% dry algal matter), a trade name mixture containing Corallina Officinalis Extract (3.97%), *a Kappaphycus alvarezii* extract (5.9%), and *Gigartina stellata* (4.43%), and a *Gelidiella acerosa* extract, yielded negative results. A chemiluminescent 3D genotoxicity assay performed on a test substance containing 48% Porphyra Umbilicalis Extract also yielded negative results.

The effect of an ethanolic *Hypnea musciformis* extract on anthracene-induced mammary carcinogenesis was evaluated in female Sprague-Dawley rats. The test groups were given a subcutaneous injection of DMBA to induce carcinomas, along with 200 mg/kg bw/d of the algae extract, orally, for 16 weeks. One hundred percent of animals treated with DMBA alone displayed tumors, however in animals treated with DMBA and *Hypnea musciformis* extract, the incidence of mammary tumors was significantly lower (25%). No tumors were observed in control rats or rats treated with *Hypnea musciformis* extract alone.

The anti-tumorigenic potential of methanolic and dichloromethane extracts of *Asparagopsis armata* and *Plocamium cartilagineum* (equivalent to *Gelidium cartilagineum*) was evaluated in HepG-2 cells. Cells were incubated with 1000 µg/ml of the extracts and evaluated for cell viability and proliferation. Both methanolic and dichloromethane extracts of *Asparogopsis armata* presented high cytotoxicity with 11 ± 2.98 and 1.51 ± 0.38 % of HepG-2 live cells, respectively. Anti-proliferative activity of HepG-2 cells was observed in cells treated with dichloromethane extracts of both algae species. The effect of *Porphyra tenera* powder on intestinal tumor incidence was evaluated in Sprague-Dawley rats. Tumors were induced in animals via a weekly injection of DMH for 12 weeks, and algae-treated animals received a dietary seaweed

preparation containing 2% *Porphyra tenera* powder. Control animals were fed a regular diet. There was a significant decrease ($p < 0.01$) in the incidence of tumors in rats fed *Porphyra tenera* powder (2/10) versus control animals (8/10).

The cytotoxic potential of *Ceramium virgatum* extract (equivalent to *Ceramium rubrum* extract), *Corallina officinalis* extract, *Furcellaria lumbricalis* extract, *Plocamium cartilagineum* extract (equivalent to *Gelidium cartilagineum* extract), *Porphyra linearis* extract, and *Mastocarpus stellata* extract (equivalent to *Gigartina stellata* extract), was evaluated using L6-cells.[59] Among all extracts tested, only *Corallina officinalis* showed some weak cytotoxic potential towards the mammalian cells (half maximal inhibitory concentration (IC_{50}) value of 88.6 µg/ml). The remaining extracts had no toxicity at the highest concentration. An MTT assay was performed using human and tumor cells on a crude aqueous extract of *Gracilariopsis longissima* (equivalent to *Gracilaria verrucosa* extract) at up to 10 mg/ml for 72 h. No cytotoxicity was observed in either human cell line (HGF or HaCaT) at any concentration, however, significant cytotoxicity was observed in murine tumor cells.

The potential photoprotective effects of cosmetic formulations containing 5% *Porphyra umbilicalis* was evaluated in hairless mice (4 animals/group). After administration of the test substance, animals were exposed to radiation. A more pronounced reduction in the expression of p53 and caspase-3 and decreased erythema values were observed in groups treated with Porphyra umbilicalis compared to the control groups.

The effect of porphyran on the contact hypersensitivity reaction in female Balb/c mice was evaluated. Induced ear edema was evaluated after treatment with porphyran in the diet at up to 2%, for 7 days. Oral administration of porphyran at 2% significantly suppressed ear edema induced by TNCB. In addition, it was found that porphyran suppressed the serum level of immunoglobin E and the production of interferon-γ in the challenged ear lobe.

In vitro dermal irritation assays were performed on trade name mixture containing 0.75% Ahnfeltiopsis Concinna Extract (tested at 100%; other components of mixture not reported), an Asparagopsis Armata Extract containing 4% dry algal matter (tested at 10%; other components of extract not reported), and a trade name mixture containing 3.5% Chondrus Crispus Extract (tested at 100%; other component of mixture not reported). All test substances were considered to be non-irritating.

Many human dermal irritation studies were provided using test substances containing a red algae ingredient, or combination of ingredients, along with other substances such as water, propanediol, glycerin, and butylene glycol. The majority of these studies yielded negative results; however, slight irritation was noted (at 30 min after patch removal) in a 24-hour patch test assay on a trade name mixture containing 72 - 77% water; 20 - 70% butylene glycol; 1 - 3% Hypnea Musciformis Extract; ≤ 1% potassium gluconate; 0.16 - 0.2% methylparaben. All sensitization studies performed on humans, evaluating various red algae-derived ingredients (Asparagopsis Armata Extract (0.325% and 0.5 – 2%), Chondrus Crispus Extract (0.49%), Gelidiella Acerosa Extract (0.0028%), Gelidium Cartilagineum Extract (< 2%), Hydrolyzed Corallina Officinalis Extract (0.5 – 3%), Hypnea Musciformis Extract (0.36%), Palmaria Palmata Extract (1.87%), and Porphyra Umbilicalis Extract (0.0004%)), were negative.

No irritation was observed in vitro ocular irritation assay on a trade name mixture containing 0.75% Ahnfeltiopsis Concinna Extract. Slight irritation was noted in an in vitro ocular irritation assay performed using the PREDISAFE method on an Asparagopsis Armata Extract (4% dry algal matter). No irritation was observed in three MatTek EpiOcular™ MTT viability assays performed on an after-shave balm containing 0.8% Chondrus Crispus, a trade name mixture containing 3.5% Chondrus Crispus Extract, or an eye cream containing 0.0375% Rhodymenia Palmata Extract. Similarly, mild to no irritation was observed in HET-CAM assays performed on various red algae-derived ingredients (Corallina Officinalis Extract (1.5% and 3.97%), *Kappaphycus alvarezii* extract (5.9%), and Porphyra Umbilicalis Extract (48%). However, moderate irritation was noted when a trade name mixture consisting of 57 - 61% Lithothamnion Calcareum Powder, 26 - 31% mannitol, 9 - 11% diatomaceous earth, 0.7 - 1.5% zinc sulfate was used in a HET-CAM assay and tested at 10%, but not when tested at 2 and 5%.

TABLES

Table 1. INCI names, definitions, and functions of the red algae-derived ingredients in this safety assessment[1]

Ingredient	Definition	Function
Ahnfeltiopsis Concinna Extract	Ahnfeltiopsis Concinna Extract is the extract of the alga, *Ahnfeltiopsis concinna*. The accepted scientific name for *Ahnfeltiopsis concinna* is *Gymnogongrus durvillei*.	Skin-Conditioning Agents - Emollient; Skin-Conditioning Agents - Miscellaneous
Asparagopsis Armata Extract	Asparagopsis Armata Extract is the extract of the red alga, *Asparagopsis armata*.	Skin-Conditioning Agents - Miscellaneous
Hydrolyzed Asparagopsis Armata Extract	Hydrolyzed Asparagopsis Armata Extract is the hydrolysate of Asparagopsis Armata Extract derived by acid, enzyme, or other method of hydrolysis.	Skin Protectants
Betaphycus Gelatinum Extract	Betaphycus Gelatinum Extract is the extract of the alga, *Betaphycus gelatinum.*	Skin Bleaching Agents
Botryocladia Occidentalis Extract	Botryocladia Occidentalis Extract is the extract of the alga, *Botryocladia occidentalis.*	Skin-Conditioning Agents - Miscellaneous
Calliblepharis Ciliata Extract	Calliblepharis Ciliata Extract is the extract of the algae, *Calliblepharis ciliate.*	Skin-Conditioning Agents - Miscellaneous
Ceramium Kondoi Extract	Ceramium Kondoi Extract is the extract of the algae, *Ceramium kondoi.*	Skin-Conditioning Agents - Humectant
Ceramium Rubrum Extract	Ceramium Rubrum Extract is the extract of the algae, *Ceramium rubrum.* The accepted scientific name for *Ceramium rubrum* is *Ceramium virgatum*.	Skin-Conditioning Agents – Emollient; Skin-Conditioning Agents - Humectant
Chondracanthus Teedei Powder	Chondracanthus Teedei Powder is the powder obtained from the dried, ground alga, *Chondracanthus teedei.*	Skin-Conditioning Agents - Miscellaneous
Chondrus Crispus	Chondrus Crispus is the material obtained from the whole alga, *Chondrus crispus.*	Exfoliants
Chondrus Crispus Extract	Chondrus Crispus Extract is the extract of the red alga, *Chondrus crispus.*	Humectants; Skin-Conditioning Agents - Miscellaneous
Chondrus Crispus Powder	Chondrus Crispus Powder is the powder obtained from the dried, ground alga, *Chondrus crispus.*	Abrasives
Hydrolyzed Chondrus Crispus Extract	Hydrolyzed Chondrus Crispus Extract is the hydrolysate of Chondrus Crispus Extract derived by acid, enzyme, or other method of hydrolysis	Skin-Conditioning Agents - Miscellaneous
Corallina Officinalis Extract	Corallina Officinalis Extract is the extract of the alga, *Corallina officinalis.*	Skin-Conditioning Agents - Miscellaneous
Corallina Officinalis Powder	Corallina Officinalis Powder is the powder obtained from the dried, ground alga, *Corallina officinalis*	Binders; Dispersing Agents – Nonsurfactant; Viscosity Increasing Agents - Nonaqeuous
Corallina Officinalis Thallus Extract	Corallina Officinalis Thallus Extract is the extract of the thallus of *Corallina officinalis.*	Skin-Conditioning Agents - Miscellaneous
Hydrolyzed Corallina Officinalis	Hydrolyzed Corallina Officinalis is the hydrolysate of the whole plant, *Corallina officinalis* derived by acid, enzyme, or other method of hydrolysis.	Skin-Conditioning Agents - Miscellaneous
Hydrolyzed Corallina Officinalis Extract	Hydrolyzed Corallina Officinalis Extract is the hydrolysate of the extract of the alga, *Corallina officinalis,* obtained by acid, enzyme, or other method of hydrolysis.	Not Reported
Cyanidium Caldarium Extract	Cyanidium Caldarium Extract is the extract of the alga, *Cyanidium caldarium.*	Skin-Conditioning Agents - Miscellaneous
Delesseria Sanguinea Extract	Delesseria Sanguinea Extract is the extract of the alga, *Delesseria sanguinea.*	Skin-Conditioning Agents - Miscellaneous
Digenea Simplex Extract	Digenea Simplex Extract is the extract of the alga, *Digenea simplex.*	Not Reported
Dilsea Carnosa Extract	Dilsea Carnosa Extract is the extract of the alga, *Dilsea carnosa.*	Skin Protectants
Furcellaria Lumbricalis Extract	Furcellaria Lumbricalis Extract is the extract of the alga, *Furcellaria lumbricalis.*	Skin-Conditioning Agents - Miscellaneous
Gelidiella Acerosa Extract	Gelidiella Acerosa Extract is the extract of the red alga, *Gelidiella acerosa.*	Skin-Conditioning Agents - Miscellaneous
Gelidium Amansii Extract	Gelidium Amansii Extract is the extract of the alga, *Gelidium amansii.*	Skin-Conditioning Agents - Miscellaneous
Gelidium Amansii Oligosaccharides	Gelidium Amansii Oligosaccharides are oligosaccharides produced by the enzymatic degradation of Agar that is obtained from *Gelidium amansii*.	Skin-Conditioning Agents - Humectant
Gelidium Cartilagineum Extract	Gelidium Cartilagineum Extract is the extract of the alga, *Gelidium cartilagineum.* The accepted scientific name for *Gelidium cartilagineum* is *Plocamium cartilagineum.*	Skin-Conditioning Agents - Miscellaneous
Gelidium Pulchrum Protein	Gelidium Pulchrum Protein is the protein fraction isolated from the alga, *Gelidium pulchrum.*	Skin-Conditioning Agents - Miscellaneous
Gelidium Sesquipedale Extract	Gelidium Sesquipedale Extract is the extract of the alga, *Gelidium sesquipedale*. The accepted scientific name for *Gelidium sesquipedale* is *Gelidium corneum.*	Skin Protectants
Gigartina Skottsbergii Extract	Gigartina Skottsbergii Extract is the extract of the alga, *Gigartina skottsbergii.*	Skin-Conditioning Agents - Miscellaneous
Gigartina Stellata Extract	Gigartina Stellata Extract is the extract of the thallus of the alga, *Gigartina stellata.* The accepted scientific name for *Gigartina stellata* is *Mastocarpus stellatus*	Humectants; Skin-Conditioning Agents - Miscellaneous
Gloiopeltis Tenax Extract	Gloiopeltis Tenax Extract is the extract of the alga, *Gloiopeltis tenax.*	Antifungal Agents; Antimicrobial Agents; Antioxidants
Gloiopeltis Tenax Powder	Gloiopeltis Tenax Powder is the powder obtained from the dried, ground alga, *Gloiopeltis tenax.*	Skin-Conditioning Agents - Miscellaneous

Table 1. INCI names, definitions, and functions of the red algae-derived ingredients in this safety assessment[1]

Ingredient	Definition	Function
Gracilaria Verrucosa Extract	Gracilaria Verrucosa Extract is the extract of the alga, *Gracilaria verrucosa.* The accepted scientific name for *Gracilaria verrucosa* is *Gracilariopsis longissima.*	Humectants; Skin-Protectants; Skin-Conditioning Agents - Humectant
Gracilariopsis Chorda Extract	Gracilariopsis Chorda Extract is the extract of the alga, *Gracilariopsis chorda.*	Skin-Conditioning Agents - Miscellaneous
Grateloupia Livida Powder	Grateloupia Livida Powder is the powder obtained from the dried, ground alga, *Grateloupia livida.*	Viscosity Increasing Agents - Aqueous
Hypnea Musciformis Extract	Hypnea Musciformis Extract is the extract of the red alga, *Hypnea musciformis*.	Skin-Conditioning Agents - Miscellaneous
Lithothamnion Calcareum Extract	Lithothamnion Calcareum Extract is the extract of the red alga, *Lithothamnion calcareum.* The accepted scientific name for *Lithothamnion calcareum* is *Phymatolithon calcareum.*	Skin-Conditioning Agents - Miscellaneous
Lithothamnion Calcareum Powder	*See Phymatolithon Calcareum Extract*	
Lithothamnion Corallioides Powder	Lithothamnion Corallioides Powder is the powder obtained from the dried, ground alga, *Lithothamnion corallioides.*	Abrasives
Mesophyllum Lichenoides Extract	Mesophyllum Lichenoides Extract is the extract of the alga, *Mesophyllum lichenoides.*	Skin-Conditioning Agents - Miscellaneous
Palmaria Palmata Extract	Palmaria Palmata Extract is the extract of the alga, *Palmaria palmata.*	Skin-Conditioning Agents - Miscellaneous
Rhodymenia Palmata Extract	Rhodymenia Palmata Extract is the extract of the alga, *Rhodymenia palmata.* The accepted scientific name for *Rhodymenia palmata* is *Palmaria palmata*	Antioxidants; Binders; Skin-Conditioning Agents - Emollient
Palmaria Palmata Powder	Palmaria Palmata Powder is the powder obtained from the dried, ground alga, *Palmaria palmata.*	Viscosity Increasing Agents - Aqueous
Phymatolithon Calcareum Extract	Phymatolithon Calcareum Extract is the extract of the alga, *Phymatolithon calcareum.*	Skin-Conditioning Agents - Miscellaneous
Lithothamnion Calcareum Powder	Lithothamnion Calcareum Powder is the powder obtained from the dried, ground red alga, *Lithothamnion calcareum.* The accepted scientific name for *Lithothamnion calcareum* is *Phymatolithon calcareum.*	Abrasives
Pikea Robusta Extract	Pikea Robusta Extract is the extract of the alga, *Pikea robusta.* The accepted scientific name for *Pikea robusta* is *Pikea pinnata.*	Antioxidants; Skin Protectants; Skin-Conditioning Agents - Miscellaneous
Polysiphonia Lanosa Extract	Polysiphonia Lanosa Extract is the extract of the alga, *Polysiphonia lanosa.* The accepted scientific name for *Polysiphonia lanosa* is *Vertebrata lanosa.*	Skin-Conditioning Agents - Miscellaneous
Porphyra Linearis Powder	Porphyra Linearis Powder is the powder obtained from the dried, ground alga, *Porphyra linearis.*	Exfoliants
Porphyra Tenera Extract	Porphyra Tenera Extract is the extract of the alga, *Porphyra tenera.* The accepted scientific name for *Porphyra tenera* is *Pyropia tenera.*	Skin-Conditioning Agents - Humectant
Porphyra Tenera Sporophyte Extract	Porphyra Tenera Sporophyte Extract is the extract of the sporophyte of the alga, *Porphyra tenera.* The accepted scientific name for *Porphyra tenera* is *Pyropia tenera.*	Antioxidants; Skin Protectants
Porphyra Umbilicalis Extract	Porphyra Umbilicalis Extract is the extract of the alga, *Porphyra umbilicalis.*	Skin-Conditioning Agents - Miscellaneous
Porphyra Umbilicalis Powder	Porphyra Umbilicalis Powder is the powder obtained from the dried, ground alga, *Porphyra umbilicalis.*	Abrasives; Absorbents; Binders; Colorants; Exfoliants; Viscosity Increasing Agents - Nonaqueous
Hydrolyzed Porphyra Yezoensis	Hydrolyzed Porphyra Yezoensis is the hydrolysate of the alga, *Porphyra yezoensis* derived by acid, enzyme, or other method of hydrolysis.	Hair Conditioning Agents; Skin-Conditioning Agents - Humectant
Porphyra Yezoensis Extract	Porphyra Yezoensis Extract is the extract of the alga, *Porphyra yezoensis.* The accepted scientific name for *Porphyra yezoensis* is *Pyropia yezoensis.*	Skin-Conditioning Agents - Miscellaneous
Porphyra Yezoensis Powder	Porphyra Yezoensis Extract is the extract of the alga, *Porphyra yezoensis.* The accepted scientific name for *Porphyra yezoensis* is *Pyropia yezoensis.*	Viscosity Increasing Agents - Aqueous
Porphyridium Cruentum Culture Conditioned Media	Porphyridium Cruentum Culture Conditioned Media is the growth media removed from cultures of the algae, *Porphyridium cruentum*, after several days of growth.	Antioxidants
Porphyridium Cruentum Extract	*See Porphyridium Purpureum Extract*	
Porphyridium Purpureum Extract	Porphyridium Purpureum Extract is the extract of the alga, *Porphyridium purpureum.*	Skin-Conditioning Agents – Miscellaneous
Porphyridium Cruentum Extract	Porphyridium Cruentum Extract is the extract of the alga, *Porphyridium cruentum.* The accepted scientific name for *Porphyridium cruentum* is *Porphyridium purpureum.*	Skin-Conditioning Agents - Miscellaneous
Rhodymenia Palmata Extract	*See Palmaria Palmata Extract*	
Sarcodiotheca Gaudichaudii Extract	Sarcodiotheca Gaudichaudii Extract is the extract of the alga, *Sarcodiotheca gaudichaudii.*	Antioxidants

Table 2. Taxonomy of red-algae derived ingredients based on currently accepted scientific name[76]

Subclass	Order	Family	Genus	Ingredient (INCI name)
Rhodymeniophycidae	Bonnemaisoniales	Bonnemaisoniaceae	Asparagopsis	Asparagopsis Armata Extract
Rhodymeniophycidae	Bonnemaisoniales	Bonnemaisoniaceae	Asparagopsis	Hydrolyzed Asparagopsis Armata Extract
Rhodymeniophycidae	Gigartinales	Solieriaceae	Betaphycus	Betaphycus Gelatinum Extract
Rhodymeniophycidae	Rhodymeniales	Rhodymeniaceae	Botryocladia	Botryocladia Occidentalis Extract
Rhodymeniophycidae	Gigartinales	Cystocloniaceae	Calliblepharis	Calliblepharis Ciliata Extract
Rhodymeniophycidae	Ceramiales	Ceramiaceae	Ceramium	Ceramium Kondoi Extract
Rhodymeniophycidae	Ceramiales	Ceramiaceae	Ceramium	Ceramium Rubrum Extract
Rhodymeniophycidae	Gigartinales	Gigartinaceae	Chondracanthus	Chondracanthus Teedei Powder
Rhodymeniophycidae	Gigartinales	Gigartinaceae	Chondrus	Chondrus Crispus
Rhodymeniophycidae	Gigartinales	Gigartinaceae	Chondrus	Chondrus Crispus Extract
Rhodymeniophycidae	Gigartinales	Gigartinaceae	Chondrus	Chondrus Crispus Powder
Rhodymeniophycidae	Gigartinales	Gigartinaceae	Chondrus	Hydrolyzed Chondrus Crispus Extract
Rhodymeniophycidae	Corallinales	Corallinaceae	Corallina	Corallina Officinalis Extract
Rhodymeniophycidae	Corallinales	Corallinaceae	Corallina	Corallina Officinalis Powder
Rhodymeniophycidae	Corallinales	Corallinaceae	Corallina	Corallina Officinalis Thallus Extract
Rhodymeniophycidae	Corallinales	Corallinaceae	Corallina	Hydrolyzed Corallina Officinalis Extract
Rhodymeniophycidae	Corallinales	Corallinaceae	Corallina	Hydrolyzed Corallina Officinalis Thallus Extract
Rhodymeniophycidae	Cyanidiales	Cyanidiaceae	Cyanidium	Cyanidium Caldarium Extract
Rhodymeniophycidae	Ceramiales	Delesseriaceae	Delesseria	Delesseria Sanguinea Extract
Rhodymeniophycidae	Ceramiales	Rhodomelaceae	Digenea	Digenea Simplex Extract
Rhodymeniophycidae	Gigartinales	Dumontiaceae	Dilsea	Dilsea Carnosa Extract
Rhodymeniophycidae	Gigartinales	Furcellariaceae	Furcellaria	Furcellaria Lumbricalis Extract
Rhodymeniophycidae	Gelidiales	Gelidiellaceae	Gelidiella	Gelidiella Acerosa Extract
Rhodymeniophycidae	Gelidiales	Gelidiaceae	Gelidium	Gelidium Amansii Extract
Rhodymeniophycidae	Gelidiales	Gelidiaceae	Gelidium	Gelidium Amansii Oligosaccharides
Rhodymeniophycidae	Gelidiales	Gelidiaceae	Gelidium	Gelidium Cartilagineum Extract
Rhodymeniophycidae	Gelidiales	Gelidiaceae	Gelidium	Gelidium Pulchrum Protein
Rhodymeniophycidae	Gelidiales	Gelidiaceae	Gelidium	Gelidium Sesquipedale Extract
Rhodymeniophycidae	Gigartinales	Gigartinaceae	Gigartina	Gigartina Skottsbergii Extract
Rhodymeniophycidae	Gigartinales	Gigartinaceae	Gigartina	Gigartina Stellata Extract
Rhodymeniophycidae	Gigartinales	Endocladiaceae	Gloiopeltis	Gloiopeltis Tenax Extract
Rhodymeniophycidae	Gigartinales	Endocladiaceae	Gloiopeltis	Gloiopeltis Tenax Powder
Rhodymeniophycidae	Gracilariales	Gracilariaceae	Gracilaria	Gracilaria Verrucosa Extract
Rhodymeniophycidae	Gracilariales	Gracilariaceae	Gracilariopsis	Gracilariopsis Chorda Extract
Rhodymeniophycidae	Halymeniales	Halymeniaceae	Grateloupia	Grateloupia Livida Powder
Rhodymeniophycidae	Gigartinales	Phyllophoraceae	Gymnogongrus	Ahnfeltiopsis Concinna Extract
Rhodymeniophycidae	Gigartinales	Cystocloniaceae	Hypnea	Hypnea Musciformis Extract
Corallinophycidae	Corallinales	Lithothamniaceae	Lithothamnion	Lithothamnion Corallioides Powder
Corallinophycidae	Hapalidiales	Mesophyllumaceae	Mesophyllum	Mesophyllum Lichenoides Extract
Nemaliophycidae	Palmariales	Palmariaceae	Palmaria	Palmaria Palmata Extract
Nemaliophycidae	Palmariales	Palmariaceae	Palmaria	Palmaria Palmata Powder
Corallinophycidae	Corallinales	Lithothamniaceae	Phymatolithon	Lithothamnion Calcareum Extract
Corallinophycidae	Corallinales	Lithothamniaceae	Phymatolithon	Lithothamnion Calcareum Powder
Corallinophycidae	Corallinales	Lithothamniaceae	Phymatolithon	Phymatolithon Calcareum Extract
Rhodymeniophycidae	Gigartinales	Dumontiaceae	Pikea	Pikea Robusta Extract
Rhodymeniophycidae	Ceramiales	Rhodomelaceae	Polysiphonia	Polysiphonia Lanosa Extract
Bangiophycidae	Bangiales	Bangiaceae	Porphyra	Porphyra Linearis Powder
Bangiophycidae	Bangiales	Bangiaceae	Porphyra	Porphyra Tenera Extract
Bangiophycidae	Bangiales	Bangiaceae	Porphyra	Porphyra Tenera Sporophyte Extract
Bangiophycidae	Bangiales	Bangiaceae	Porphyra	Porphyra Umbilicalis Extract
Bangiophycidae	Bangiales	Bangiaceae	Porphyra	Porphyra Umbilicalis Powder
Bangiophycidae	Bangiales	Bangiaceae	Porphyra	Hydrolyzed Porphyra Yezoensis
Bangiophycidae	Bangiales	Bangiaceae	Porphyra	Porphyra Yezoensis Extract
Bangiophycidae	Bangiales	Bangiaceae	Porphyra	Porphyra Yezoensis Powder
Porphyridiophyceae	Porphyridiales	Porphyridiaceae	Porphyridium	Porphyridium Cruentum Culture Conditioned Media
Porphyridiophyceae	Porphyridiales	Porphyridiaceae	Porphyridium	Porphyridium Cruentum Extract
Porphyridiophyceae	Porphyridiales	Porphyridiaceae	Porphyridium	Porphyridium Purpureum Extract
Rhodymeniophycidae	Rhodymeniales	Rhodymeniaceae	Rhodymenia	Rhodymenia Palmata Extract
Rhodymeniophycidae	Gigartinales	Solieriaceae	Sarcodiotheca	Sarcodiotheca Gaudichaudii Extract

Table 3. General characteristics and geographic distribution of several red algae species

Species	Description	Distribution/Habitat/Ecology	References
Asparagopsis armata	-pale purplish-red gametophytes, quickly degenerating when removed from water -fronds bushy with cylindrical axis (1mm wide and 200 mm long) -irregularly branched -harpoon-like barbs	-native to southern Australia and New Zealand; now found from the British Isles, the Canary, and Salvage Islands, to Senegal	76,77
Calliblepharis ciliata	-flattened, subcartilaginous, purple-red fronds -300 mm long and 20 -70 mm wide -irregularly pinnate -short, cylindrical stipe arises from creeping, branched holdfast	-common in South and West -larger lower intertidal pools and subtidal on stones, maerl, and shells -occasionally abundant on bedrock	76
Chondrus crispus	-thallus of cartilaginous consistency, perennial, erect, expanding gradually onto a flat, fan-like or curled -variable in form -blade is dichotomously branched in tufts from a discoid holdfast -color of fronds vary depending on time of year and depth of water (white to yellowing green in the summer and in shallow water; dark purplish-red in autumn and deeper water)	-mainly distributed on Atlantic coasts of Europe, East Africa, and North America -found in lower intertidal and shallow subtidal stages -on rocks and stones and also in tide pools	78
Corallina officinalis	-calcified or calcareous red marine algae reaching 5-12 cm in height -erect articulated thallus arising from a firmly attached crustose base up to 70 mm in diameter and bearing tufts of branches and articulated fronds up to 120 mm long -varied in color; thallus appears to be dull purple when growing in deep water, becoming red yellow and finally white on exposure	-widely distributed in temperate areas on rocks, mid tidal pools and drainage runnels	49
Delesseria sanguinea	-membranous, bright crimson fronds, with cartilaginous, cylindrical, branched stipe, from thickened discoid holdfast -up to 300 mm long -branches bear spirally arranged, leaf-like, ovate-lanceolate blades, each with short stipe and pinnately branched midrib	-on rocks, in deep shady lower intertidal pools and in the subtidal -generally distributed, common	76
Dilsea carnosa	-dark red, frequently becoming yellow -thickest of the foliose red algae in the North Atlantic -flattened cartilaginous fronds, arising in groups of small, medium, and large from a thick, discoid holdfast -up to 500 mm long, 250 mm wide	-on rocks in shady pools, lower intertidal on rock and shallow subtidal up to 25 m -usually on rock in kelp forests	76
Furcellaria lumbricalis	-cartilaginous, cylindrical, brownish-black fronds -repeatedly dichotomously branched -up to 2 mm diameter, 300 mm long, with acute apices	-on rocks, lower intertidal and shallow subtidal -in pools and runnels -in open situations, often on sandy and muddy shores -common, widespread	76
Gelidiella acerosa	-thallus yellow to dark red -cartilaginous with decumbent and erect terete axes up to 2 mm diameter -lateral branches, 1-3 mm long	-widespread in most warm seas, just below intertidal zone -attached to rock reefs at depths of 0-1 m	76
Gelidium sesquipedale	-composed of several erect axes, compressed and branched -axes bear secondary aces with ramuli short and pinnate -the thallus appears robust with a cartilaginous consistency, dark red in color -can reach up to 25-30 cm long	-develops on rocks in semi-exposed to exposed locations in the lower intertidal and shallow subtidal level	79
Gigartina stellata	-thallus bears dichotomously branches blades which arise form a basal discoid crust -stiff and cartilaginous -purplish-brown in color -10-20 cm high -stipe is narrow and compressed, expanding into strap-like blade, usually inrolled to form a channel	-found in large continuous mats on rocks, on exposed and semi-exposed sites in the low intertidal zone with some extension into the upper sublittoral	80

Table 3. General characteristics and geographic distribution of several red algae species

Species	Description	Distribution/Habitat/Ecology	References
Phymatolithon calcareum	-fragile, reddish-violet, branched, calcareous fronds -branches are 2-3 mm in diameter -variable in form	-free-living in clear, clean water, forming extensive beds of live and dead material, particularly where there are subtidal currents -widely distributed	76
Palmaria palmata	-reddish-brown, membranous or leathery, flattened fronds (50-300 mm long) -blade variable in shape, having broadly ovate to narrowly linear segments -palmate branching with finger-like extensions	-North Atlantic -on rock and mussels, intertidal and shallow subtidal -widely distributed	76
Polysiphonia lanosa	-cartilaginous, cylindrical, densely tufted, dark brown fronds up to 75 mm long -repeatedly pseudo dichotomous branches, apices pointed, widely forked	-hemiparasitic on *Ascophyllum nodosum*, more rarely on *Fucus vesiculosus* -never directly on rock -sheltered mid-tidal -generally distributed	76
Porphyra linearis	-delicate, linear, membranous, purple-brown fronds, 20-40 mm long and 5-10 mm broad -usually simple with short stipe with basal holdfast -orange patches when reproductive	-zone-forming on rock in the intertidal and splash zone of semi-exposed and exposed shores -generally distributed -winter occurrence	76
Porphyra umbilicalis	-blades appear reddish brown, brownish, grey brown, or olive green in the field; in a dried state they are very thin and violet in color -blades constituted by a single cell layer can reach 60 cm in height	-common and abundant everywhere on the rocky parts of coasts or on beach pebbles on the Atlantic coasts of Europe (from Scandinavia to Morocco) and North America -appears in the upper littoral zone singly or in dense colonies	81
Sarcodiotheca Gaudichaudii	-medium to large species with cylindrical, brittle fronds -color varies from straw yellow to deep red or reddish brown	-lower intertidal pools to upper subtidal -mainly on small stones and shells	76

Table 4. Methods of manufacture for brown algae-derived ingredients

Ingredient (characterization)	Method of Manufacture	Reference
Asparagopsis armata extract	fresh seaweed → wash → freeze → grind → extraction with 1:4 biomass:solvent ratio with methanol and dichloromethane	82
Asparagopsis Armata Extract	algae → grinding → extraction with water → stabilization with vegetable glycerin →filtration	83
Asparagopsis Armata Extract	fresh seaweed → grinding → cold cellular extraction → filtration → concentration → freeze-drying under neutral atmosphere	84
Asparagopsis Armata Extract	harvesting/identification → washing → grinding → extraction with solvents (propanediol and water) → filtration → quality control → packaging → quality control	85
Chondrus Crispus Extract and Gigartina Stellata Extract	harvesting/identification → washing → condensation of cellular water by soft drying → filtration and UV treatment → quality control → addition of preservatives and pH adjustment → quality control → packaging → quality control	86
Chondrus Crispus Powder	harvesting → naturally dried via sun exposure → grinding/sieving → packaging → sterilized via gamma ray treatment	87
Chondrus Crispus Powder	harvesting/identification → drying → cutting → ionization → quality control → packaging → quality control	88
Corallina Officinalis Extract and Gigartina Stellata Extract	dried grounded algae → extraction with water → testing → sifting → centrifugation → ultrafiltration → testing → homogenization → testing → sterile filtration → testing → packing	89
Corallina Officinalis Extract	dried grounded algae → extraction with water → testing → sifting → centrifugation → ultrafiltration → testing → homogenization → testing → sterile filtration → testing → packing	90
Digenea simplex extract	Dried algal powder (200 mg) extracted with 6 ml 80% methanol → ultrasonic bath → vortex → centrifuge → filtration → drying	91
Gelidiella acerosa extract	100 g seaweed packed in Soxhlet apparatus → addition of solvent (petroleum ether, hexane, benzene, dichloromethane, chloroform, ethyl acetate, acetone, methanol, or water) → re-distillation → filtration→ placed in desiccator	54
Gelidium amansii extract	algae collection → washing → dried at room temperature → grinding → powder extracted with 80% ethanol for 24 h → freeze-drying	15
Gelidium Cartilagineum Extract	harvesting/identification → drying → grinding → extraction with solvent (caprylic/capric triglyceride) → addition of sterol → filtration → quality control → packaging → quality control	6
Gracilariopsis chorda extract	seaweed collection → mechanical washing → drying in room temperature → pulverization → extraction with 95% ethanol→ mixture placed in orbital shaker at 200 rpm → centrifugation → filtration → concentration → drying under steam of nitrogen gas	19

Table 4. Methods of manufacture for brown algae-derived ingredients

Ingredient (characterization)	Method of Manufacture	Reference
Hydrolyzed Corallina Officinalis Extract	harvesting/identification → extraction with water → addition of sodium methylparaben or 2-phenoxyethanol → filtration →quality control → packaging →quality control	5,92
Hypnea Musciformis Extract	harvesting/identification → drying → grinding → extraction with the solvent (water and butylene glycol) → addition of potassium gluconate and methylparaben → filtration →quality control → packaging → quality control	7
Hypnea Musciformis Extract	solubilization of *Hypnea musciformis* in water → separation of soluble and insoluble phases → filtration → membrane sterilization	22
Lithothamnion Calcareum Powder	harvesting → drying → grinding → micronisation → ionization → mixture → addition of mannitol, zinc sulfate, and diatomaceous earth → packaging → quality control	93
Palmaria Palmata Extract	solubilization of powder of *Palmaria palmata* in water → separation of soluble and insoluble phases → concentration of soluble phase → membrane sterilization	22
Porphyra Umbilicalis Extract	circular flow extraction of 7.8% dry algae on dry algae → in-process control → maturation at room temperature → filtration of the supernatant → cationic exchange → filtration → cross flow filtration → encapsulation of the extract into liposomes → packaging → quality control	27
Porphyra Umbilicalis Extract	dried grounded algae → extraction with water → testing → centrifugation → ultrafiltration → testing → sterile filtration → testing → packaging	94

Table 5. Mineral and metal analysis of a trade name mixture containing 4% Gelidium Sesquipedale Extract[16]

Analysis	Results ± Uncertainties	Units
Ashes	0.4 ± 0.2	g/100 g
Calcium	<4.0	mg/100 g
Magnesium	14.0 ± 1.4	mg/100 g
Phosphorus	<2.0	mg/100 g
Potassium	82 ± 8.2	mg/100 g
Sodium	98.3 ± 9.8	mg/100 g
Copper	<0.3	mg/100 g
Iron	<0.2	mg/100 g
Manganese	<0.3	mg/100 g
Zinc	<0.3	mg/100 g
Arsenic	72	µg/kg
Cadmium	<10	µg/kg
Mercury	<5	µg/kg
Molybdenum	<51	µg/kg
Lead	<10	µg/kg
Selenium	<811	µg/kg
Iodine	1.02	mg/kg

Table 6. Chemical composition of a supercritical carbon dioxide extract of Gloiopeltis tenax[17]

Constituents	***%****
p-hydroxybenzaldehyde	0.57
(-) – thujopsene	4.68
α-curcumene	1.54
α-zingiberene	2.98
(+)-cuparene	0.28
(−)-β-bisabolene	1.00
cedrol	3.91
vanillylacetone	1.92
n-heptadecane	10.30
myristic acid	2.85
fitone	2.53
methhyl hexadecanoate	1.32
palmitic acid	21.21
linoleic acid	0.23
hexadeca-1,4-lactone	0.57
cis-9-octadecenoic acid	0.73
stearic acid	0.93
oleamide	0.24
2,2′-methylenebis(6-*tert*-butyl-4-methylphenol)	1.14
2-monopalmitin	1.83
cholesta-4,6-dien-3β-ol	6.62
cholesterol	5.74
cholesta-3,5-dien-7-one	0.45

*percentage of relative amount to total

Table 7. Mean metal content ± standard deviation in seaweed samples for different genera of red algae (mg/kg DW)[31]

	***Chondrus* (n = 2)**	***Gelidium* (n = 2)**	***Palmaria* (n = 4)**	***Porphyra* (n = 10)**	***Gracilaria* (n = 2)**
Sodium	6799 ± 84.6	1279 ± 0	3803 ± 463	2274 ± 675	-
Arsenic	-	-	-	-	15
Potassium	9901 ± 270	543 ± 53.2	8044 ± 0	6563 ± 854	-
Calcium	2028 ± 153	908 ± 7.01	459 ± 0.00	1793 ± 1211	-
Cadmium	-	-	-	-	0.04 – 0.4
Magnesium	3134 ± 45.7	452 ± 4.68	787 ± 87.6	3732 ± 5070	-
Boron	43.3 ± 6.60	4.50 ± 0.98	31.5 ± 6.45	5.10 ± 0.00	-
Barium	0.35 ± 0.08	0.30 ± 0.10	0.62 ± 0.28	3.19 ± 2.88	-
Cobalt	0.13 ± 0.01	0.008 ± 0.00	0.03 ± 0.01	0.12 ± 0.18	-
Chromium	0.15 ± 0.00	0.16 ± 0.001	0.15 ± 0.02	0.33 ± 0.14	-
Copper	0.79 ± 0.21	0.54 ± 0.02	1.03 ± 0.09	2.99 ± 0.68	-
Iron	22.3 ± 3.79	9.86 ± 0.24	34.7 ± 8.10	156 ± 239	-
Lithium	0.85 ± 0.01	0.93 ± 0.58	1.16 ± 0.45	1.41 ± 0.00	-
Manganese	9.78 ± 0.56	1.66 ± 0.01	1.62 ± 0.45	36.5 ± 56.9	-
Molybdenum	0.12 ± 0.01	0.008 ± 0.00	0.09 ± 0.01	0.22 ± 0.09	-
Nickel	5.08 ± 0.10	0.11 ± 0.001	0.05 ± 0.13	0.50 ± 0.87	-
Strontium	-	-	3.44 ± 0.36	2.22 ± 2.92	-
Vanadium	0.58 ± 0.47	-	25.5 ± 0.00	0.48 ± 0.41	-
Zinc	9.33 ± 2.57	2.21 ± 0.25	5.03 ± 1.06	13.6 ± 3.72	-
Aluminum	8.41 ± 2.85	8.21 ± 0.61	32 ± 5.18	28.9 ± 27.3	19 - 149
Cadmium	0.29 ± 0.03	0.008 ± 0.00	0.16 ± 0.11	0.58 ± 0.30	-
Lead	0.07 ± 0.00	0.05 ± 0.01	0.05 ± 0.02	0.15 ± 0.21	0.8 – 7

- = None reported

Table 8. Mineral and metal analysis of a trade name mixture containing water (45.7%), glycerin (40%), *Gigartina stellata* (4.43%), *Kappaphycus alvarezii* extract (5.9%), Corallina Officinalis Extract (3.97%)[32]

Determination	Results/Units
Sodium	419.9 mg/100 g
Calcium	4.8 mg/100 g
Phosphorus	<2 mg/100 g
Chlorides	391 mg/100 g
Magnesium	11.9 mg/100 g
Potassium	109.4 mg/100 g
Copper	<0.5 mg/100 g
Iron	<0.5 mg/100 g
Manganese	<0.5 mg/100 g
Zinc	<0.5 mg/100 g
Iodine	1.2 mg/kg
Arsenic, inorganic	<0.15 mg/kg
Arsenic	116 µg/kg
Cadmium	<10 µg/kg
Mercury	<10 µg/kg
Lead	<10 µg/kg
Selenium	<10 µg/kg

Table 9. Mineral and metal analysis of a trade name mixture consisting of 50% glycerin; 30% water; 18.5 % undaria pinnatifida extract; 1.5% Corallina officinalis Extract[33]

Determination	Results/Units
Sodium	420.4 mg/100 ml
Calcium	142.9 mg/100 ml
Phosphorus	8.9 mg/100 ml
Magnesium	60.7 mg/100 ml
Potassium	530.3 mg/100 ml
Copper	<0.5 mg/100 ml
Iron	<0.5 mg/100 ml
Manganese	0.0 mg/100 ml
Zinc	<0.5 mg/100 ml
Iodine	1.9 mg/l
Arsenic	1383 µg/kg
Cadmium	29 µg/kg
Mercury	<10 µg/kg
Lead	86 µg/kg
Selenium	<50 µg/kg
Silicon	0 mg/kg

Table 10. Frequency (2020) and concentration of use (2020) of red algae-derived ingredients[34,35]

	# of Uses	Max Conc of Use (%)	# of Uses	Max Conc of Use (%)	# of Uses	Max Conc of Use (%)
	Ahnfeltiopsis Concinna Extract		Asparagopsis Armata Extract		Chondrus Crispus	
Totals*	**16**	**NR**	**42**	**0.031 – 0.33**	**26**	**0.00004 – 1.4**
Duration of Use						
Leave-On	*15*	*NR*	*36*	*0.031 – 0.33*	20	*0.00004 – 0.8*
Rinse-Off	*1*	*NR*	*6*	*0.1*	4	*0.005 – 1.4*
Diluted for (Bath) Use	*NR*	*NR*	*NR*	*NR*	2	*NR*
Exposure Type						
Eye Area	1	NR	16	0.031	4	0.12
Incidental Ingestion	NR	NR	NR	NR	4	1.4
Incidental Inhalation-Spray	6[a]; 6[b]	NR	7[a]; 8[b]	NR	5[a]; 6[b]	0.018; 0.005[b]
Incidental Inhalation-Powder	6[a]	NR	7[a]	0.063[c]	1; 5[a]	0.13; 0.51[c]
Dermal Contact	16	NR	40	0.031 – 0.063	22	0.08 - 0.8
Deodorant (underarm)	NR	NR	NR	NR	NR	NR
Hair - Non-Coloring	NR	NR	2	0.1 – 0.33	NR	0.00004 – 0.005
Hair-Coloring	NR	NR	NR	NR	NR	NR
Nail	NR	NR	NR	NR	NR	NR
Mucous Membrane	NR	NR	1	NR	6	0.3 – 1.4
Baby Products	NR	NR	NR	NR	NR	NR

	Chondrus Crispus Extract		Chondrus Crispus Powder		Corallina Officinalis Extract	
Totals*	**381**	**0.000003 – 0.5**	**55**	**0.1**	**96**	**0.00013 – 2**
Duration of Use						
Leave-On	*306*	*0.000003 – 0.49*	49	*0.1*	76	*0.000013 – 2*
Rinse Off	*74*	*0.0018 – 0.5*	6	*NR*	20	*0.00014 – 0.11*
Diluted for (Bath) Use	*1*	*NR*	NR	*NR*	NR	*NR*
Exposure Type						
Eye Area	60	0.14 – 0.3	9	0.1	5	0.0004 – 0.01
Incidental Ingestion	14	NR	NR	NR	1	NR
Incidental Inhalation-Spray	107[a]; 69[b]	0.001[b]	1; 25[a]; 9[b]	NR	16[a]; 31[b]	NR
Incidental Inhalation-Powder	21; 107[a]	0.15; 0.0005 – 0.29[c]	25[a]	NR	2; 16[a]	2[c]
Dermal Contact	344	0.000003 – 0.5	53	0.1	81	0.00013 – 2
Deodorant (underarm)	NR	NR	NR	NR	NR	NR
Hair - Non-Coloring	21	0.001 – 0.0018	2	NR	1	NR
Hair-Coloring	1	0.01	NR	NR	NR	NR
Nail	NR	NR	NR	NR	13	0.099
Mucous Membrane	21	NR	1	NR	1	NR
Baby Products	NR	0.000003	NR	NR	NR	NR

Table 10. Frequency (2020) and concentration of use (2020) of red algae-derived ingredients[34,35]

	# of Uses	Max Conc of Use (%)	# of Uses	Max Conc of Use (%)	# of Uses	Max Conc of Use (%)
	Delesseria Sanguinea Extract		**Digenea Simplex Extract**		**Furcellaria Lumbricalis Extract**	
Totals*	**2**	**NR**	**1**	**NR**	**32**	**NR**
Duration of Use						
Leave-On	*2*	*NR*	*NR*	*NR*	*32*	*NR*
Rinse-Off	*NR*	*NR*	*1*	*NR*	*NR*	*NR*
Diluted for (Bath) Use	*NR*	*NR*	*NR*	*NR*	*NR*	*NR*
Exposure Type						
Eye Area	NR	NR	NR	NR	NR	NR
Incidental Ingestion	NR	NR	NR	NR	2	NR
Incidental Inhalation-Spray	1[a]; 1[b]	NR	NR	NR	9[a]; 11[b]	NR
Incidental Inhalation-Powder	1[a]	NR	NR	NR	9[a]	NR
Dermal Contact	2	NR	1	NR	30	NR
Deodorant (underarm)	NR	NR	NR	NR	NR	NR
Hair - Non-Coloring	NR	NR	NR	NR	NR	NR
Hair-Coloring	NR	NR	NR	NR	NR	NR
Nail	NR	NR	NR	NR	NR	NR
Mucous Membrane	NR	NR	NR	NR	2	NR
Baby Products	NR	NR	NR	NR	NR	NR

	Gelidium Cartilagineum Extract		**Gelidiella Acerosa Extract**		**Gigartina Stellata Extract**	
Totals*	**37**	**NR**	**19**	**0.0001 – 0.028**	**9**	**NR**
Duration of Use						
Leave-On	*35*	*NR*	*9*	*0.00065 - 0.028*	*3*	*NR*
Rinse-Off	*2*	*NR*	*10*	*0.0001 – 0.015*	*6*	*NR*
Diluted for (Bath) Use	*NR*	*NR*	*NR*	*NR*	*NR*	*NR*
Exposure Type						
Eye Area	3	NR	1	NR	1	NR
Incidental Ingestion	NR	NR	NR	NR	NR	NR
Incidental Inhalation-Spray	16[a]; 14[b]	NR	1[a]; 6[b]	NR	1[a]; 1[b]	NR
Incidental Inhalation-Powder	1; 16[a]	NR	1[a]	0.007 – 0.028[c]	NR	NR
Dermal Contact	37	NR	8	0.0001 – 0.028	3	NR
Deodorant (underarm)	NR	NR	NR	NR	NR	NR
Hair - Non-Coloring	NR	NR	7	0.0008	6	NR
Hair-Coloring	NR	NR	4	0.0045	NR	NR
Nail	NR	NR	NR	NR	NR	NR
Mucous Membrane	NR	NR	NR	0.015	NR	NR
Baby Products	1	NR	NR	NR	NR	NR

Table 10. Frequency (2020) and concentration of use (2020) of red algae-derived ingredients[34,35]

	# of Uses	Max Conc of Use (%)	# of Uses	Max Conc of Use (%)	# of Uses	Max Conc of Use (%)
	Hydrolyzed Chondrus Crispus Extract		Hydrolyzed Corallina Officinalis Extract		Hypnea Musciformis Extract	
Totals*	**NR**	**0.012 – 0.017**	**9**	**NR**	**141**	**0.0003 – 0.13**
Duration of Use						
Leave-On	*NR*	*0.012 – 0.017*	*6*	*NR*	*75*	*0.0003 – 0.08*
Rinse-Off	*NR*	*NR*	*3*	*NR*	*66*	*0.0004 – 0.13*
Diluted for (Bath) Use	*NR*	*NR*	*NR*	*NR*	*NR*	*NR*
Exposure Type						
Eye Area	NR	0.012 – 0.017	1	NR	16	NR
Incidental Ingestion	NR	NR	NR	NR	NR	NR
Incidental Inhalation-Spray	NR	NR	4[a]; 1[b]	NR	4; 7[a]; 27[b]	0.03
Incidental Inhalation-Powder	NR	NR	4[a]	NR	7[a]	0.02 – 0.08[c]
Dermal Contact	NR	0.012 – 0.017	9	NR	73	0.0003 – 0.13
Deodorant (underarm)	NR	NR	NR	NR	NR	NR
Hair - Non-Coloring	NR	NR	NR	NR	34	0.0045
Hair-Coloring	NR	NR	NR	NR	27	NR
Nail	NR	NR	NR	NR	5	NR
Mucous Membrane	NR	NR	1	NR	1	0.13
Baby Products	NR	NR	NR	NR	NR	NR

	Lithothamnion Calcareum Extract		Lithothamnion Calcareum Powder		Palmaria Palmata Extract	
Totals*	**22**	**0.0059 – 0.037**	**11**	**NR**	**83**	**0.0005 – 0.075**
Duration of Use						
Leave-On	*22*	*0.0059 – 0.037*	*2*	*NR*	*75*	*0.0005 – 0.075*
Rinse-Off	*NR*	*NR*	*9*	*NR*	*8*	*NR*
Diluted for (Bath) Use	*NR*	*NR*	*NR*	*NR*	*NR*	*NR*
Exposure Type						
Eye Area	4	0.012	NR	NR	8	NR
Incidental Ingestion	NR	NR	NR	NR	NR	NR
Incidental Inhalation-Spray	3[a]	NR	1[a]	NR	30[a]; 22[b]	0.0006
Incidental Inhalation-Powder	3[a]	0.0059[c]	1[a]	NR	30[a]	0.075[c]
Dermal Contact	7	0.0059 – 0.012	11	NR	83	0.0005 – 0.075
Deodorant (underarm)	NR	NR	NR	NR	NR	NR
Hair - Non-Coloring	NR	NR	NR	NR	NR	NR
Hair-Coloring	NR	NS	NR	NR	NR	NR
Nail	15	0.037	NR	NR	NR	0.0005
Mucous Membrane	NR	NR	NR	NR	7	NR
Baby Products	NR	NR	NR	NR	NR	NR

Table 10. Frequency (2020) and concentration of use (2020) of red algae-derived ingredients[34,35]

	# of Uses	Max Conc of Use (%)	# of Uses	Max Conc of Use (%)	# of Uses	Max Conc of Use (%)
	Phymatolithon Calcareum Extract		**Porphyra Umbilicalis Extract**		**Porphyra Yezoensis Extract**	
Totals*	**1**	**NR**	**42**	**0.0004 – 0.0035**	**10**	**NR**
Duration of Use						
Leave-On	*1*	*NR*	*35*	*0.0004*	*8*	*NR*
Rinse-Off	*NR*	*NR*	*7*	*0.0035*	*2*	*NR*
Diluted for (Bath) Use	*NR*	*NR*	*NR*	*NR*	*NR*	*NR*
Exposure Type						
Eye Area	1	NR	3	NR	1	NR
Incidental Ingestion	NR	NR	NR	NR	NR	NR
Incidental Inhalation-Spray	NR	NR	20[a]; 8[b]	NR	3[a]; 4[b]	NR
Incidental Inhalation-Powder	NR	NR	20[a]	NR	3[a]	NR
Dermal Contact	1	NR	40	0.0004 – 0.0035	10	NR
Deodorant (underarm)	NR	NR	NR	NR	NR	NR
Hair - Non-Coloring	NR	NR	2	NR	NR	NR
Hair-Coloring	NR	NR	NR	NR	NR	NR
Nail	NR	NR	NR	NR	NR	NR
Mucous Membrane	NR	NR	4	NR	NR	NR
Baby Products	NR	NR	NR	NR	NR	NR

	Porphyridium Cruentum Extract		**Rhodymenia Palmata Extract**	
Totals*	**47**	**0.00055 – 0.03**	**NR**	**0.038**
Duration of Use				
Leave-On	*40*	*0.00055 – 0.03*	*NR*	***0.038***
Rinse-Off	*7*	*0.00055 – 0.017*	*NR*	*NR*
Diluted for (Bath) Use	*NR*	*NR*	*NR*	*NR*
Exposure Type				
Eye Area	11	0.00055	NR	*0.038*
Incidental Ingestion	NR	0.00055	NR	NR
Incidental Inhalation-Spray	11[a]; 11[b]	0.00055[b]	NR	NR
Incidental Inhalation-Powder	11[a]	0.03[c]	NR	0.038[c]
Dermal Contact	47	0.00055 – 0.03	NR	0.038
Deodorant (underarm)	NR	NR	NR	NR
Hair - Non-Coloring	NR	0.00055	NR	NR
Hair-Coloring	NR	NR	NR	NR
Nail	NR	NR	NR	NR
Mucous Membrane	NR	0.00055	NR	NR
Baby Products	NR	NR	NR	NR

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

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

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

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

NR – no reported use

Table 11. Red algae-derived ingredients with no reported uses in the VCRP

Hydrolyzed Asparagopsis Armata Extract	Gracilaria Verrucosa Extract
Betaphycus Gelatinum Extract	Gracilariopsis Chorda Extract
Botryocladia Occidentalis Extract	Grateloupia Livida Powder
Calliblepharis Ciliata Extract	Lithothamnion Corallioides Powder
Ceramium Kondoi Extract	Mesophyllum Lichenoides Extract
Ceramium Rubrum Extract	Palmaria Palmata Powder
Chondracanthus Teedei Powder	Pikea Robusta Extract
Corallina Officinalis Powder	Polysiphonia Lanosa Extract
Corallina Officinalis Thallus Extract	Porphyra Linearis Powder
Hydrolyzed Corallina Officinalis	Porphyra Tenera Extract
Cyanidium Caldarium Extract	Porphyra Tenera Sporophyte Extract
Dilsea Carnosa Extract	Porphyra Umbilicalis Powder
Gelidium Amansii Extract	Hydrolyzed Porphyra Yezoensis
Gelidium Amansii Oligosaccharides	Porphyra Yezoensis Powder
Gelidium Pulchrum Protein	Porphyridium Cruentum Culture Conditioned Media
Gelidium Sesquipedale Extract	Porphyridium Purpureum Extract
Gigartina Skottsbergii Extract	(equivalent to Porphyridium Cruentum Extract)
Gloiopeltis Tenax Extract	Sarcodiotheca Gaudichaudii Extract
Gloiopeltis Tenax Powder	

Table 12. Red algae species ingested by humans as foods

Species	Methods of consumption	Reference
Ahnfeltiopsis concinna	Hawaiian cuisine; Eaten raw with limpets or baked with other foods	[95]
Chondrus crispus	Used as thickener/gelling agent; used in drinks; also known as Irish moss; eaten whole	[96]
Gelidiella sp.	Used in jellies	[45]
Gelidium amansii	Used in jellies	[15]
Gigartina stellata	Used interchangeably with Chondrus crispus; thickener/gelling agent	[45,76]
Gracilaria sp.	Used in jellies	[45]
Gracilaria verrucosa	Eaten whole, with salads	[96]
Lithothamnion calcareum	Used as vegetables and condiments in France	[9]
Palmaria palmata	Eaten fresh or dry; used in breads and cakes	[22,44]
Porphyra tenera	Typically, dried and used to make sushi; spices, seasoning, flavoring (GRAS)	21CFR184.1121, [29]
Porphyra umbilicalis	Typically, dried and used to make sushi	[81,96]
Porphyra yezoensis	Typically, dried and used to make sushi	[29,97]
Rhodymenia palmata	Spices, seasoning, flavoring (GRAS)	21CFR184.1121

Table 13. Dermal irritation and sensitization

Ingredient	Test Substance	Concentration/Dose of the test substance	Test Population	Procedure	Results	Reference
Irritation						
IN VITRO						
Ahnfeltiopsis Concinna Extract	Trade name mixture containing 0.75% Ahnfeltiopsis Concinna Extract (other components not reported)	100%; 30 µl (liquid) or 25 mg (solid)	3	Reconstructed human epidermal model; 3 tissues treated with test substance and incubated for 60 minutes	Non-irritating	[62]
Asparagopsis Armata Extract	An Asparagopsis Armata Extract containing 4% dry algal matter (other components not reported)	10%; 200 µl	2	Local tolerance evaluated in EPISKIN reconstructed human epidermis model; 18-hour incubation	Non-irritating	[52]
Chondrus Crispus Extract	Trade name mixture containing 3.5% Chondrus Crispus Extract (other components not reported)	100%; 20 µl	3	MatTek EpiDerm™ MTT Assay	Non-irritating	[63]
Human						
Asparagopsis Armata Extract	An Asparagopsis Armata Extract containing 4% dry algal matter in water	10%; 20 µl	10	48-hour patch test under occlusive conditions	Non-irritating	[52]
Asparagopsis Armata Extract	Trade name mixture containing 0.5 – 2% Asparagopsis Armata Extract, 56 – 62% water, and 38 – 42% propanediol	3%; 20 µl	22	48-hour patch test under occlusive conditions	Non-irritating	[98]
Chondrus Crispus	After-shave balm containing 0.8% Chondrus Crispus	100%; 0.2 ml	30	23-hour exposure per day for 14 d; occlusive conditions	Non-irritating	[99]
Chondrus Crispus Extract and Gigartina Stellata Extract	Trade name mixture containing Chondrus Crispus Extract and Gigartina Stellata Extract (98.10 – 98.95% extract, 0.80 – 1.10% sodium benzoate; 0.25 – 0.35% potassium sorbate; 0 -0.30% lactic acid)	100%; 25 µl	22	48-hour patch test; occlusive conditions	Non-irritating	[100]
Chondrus Crispus Powder	Chondrus Crispus Powder (100%)	100%; 0.02 ml	12	24-hour patch test; occlusive conditions	Non-irritating	[101]
Corallina Officinalis Extract and Gigartina Stellata Extract	Trade name mixture containing water (45.7%), glycerin (40%), *Gigartina stellata* (4.43%), *Kappaphycus alvarezii* extract (5.9%), Corallina Officinalis Extract (3.97%)	10%; 0.02 ml	25	48-hour patch test; occlusive conditions	Non-irritating	[102]
Corallina Officinalis Extract	Trade name mixture containing 50% glycerin; 30% water; 18.5 % undaria pinnatifida extract; 1.5% Corallina Officinalis Extract	10%; 160 µl	10	48-hour patch test; semi-occlusive conditions	Non-irritating	[103]
Gelidium Cartilagineum Extract	Trade name mixture containing >96% glycerides, mixed decanoyl and octanoyl; <2 % Gelidium Cartilagineum Extract; 1.5-2% 4-cholesten-3-one	10% dilution; 20 µl	10	24-hour patch test; occlusive conditions	Non-irritating	[104]
Gelidium Sesquipedale Extract	Trade name mixture containing 48% water; 48% butylene glycol; 4% Gelidium Sesquipedale Extract	5% dilution; 0.02 ml	10	48-hour patch test; occlusive conditions	Non-irritating	[105]

Table 13. Dermal irritation and sensitization

Ingredient	Test Substance	Concentration/Dose of the test substance	Test Population	Procedure	Results	Reference
Hydrolyzed Corallina Officinalis Extract	Trade name mixture containing >96% water; 0.5-3% Hydrolyzed Corallina Officinalis Extract; 0.16-0.20% sodium methylparaben	100%; 0.02 ml	11	24-hour patch test; occlusive conditions	Non-irritating	[106]
Hydrolyzed Corallina Officinalis Extract	Trade name mixture containing >96% water; 0.5-3% Hydrolyzed Corallina Officinalis Extract; 0.8-1.2% phenoxyethanol	100%; 20 µl	11	24-hour patch test; occlusive conditions	Non-irritating	[107]
Hypnea Musciformis Extract	Trade name mixture consisting of 72-77% water; 20-70% butylene glycol; 1-3% Hypnea Musciformis Extract; ≤1% potassium gluconate; 0.16-0.2% methylparaben	100%; 0.02 ml	12	24-hour patch test; occlusive conditions	Slightly irritating at the 30-minute reading (in 7/12 subjects) and non-irritating at the 24-hour reading	[64]
Hypnea Musciformis Extract	Hypnea Musciformis Extract in water (specific composition not reported)	15% (0.36% dry matter); dose not reported	11	48-hour patch test; occlusive conditions	Non-irritating	[22]
Lithothamnion Calcareum Powder	Trade name mixture consisting of 57-61% Lithothamnion Calcareum Powder. 26-31% mannitol, 9-11% diatomaceous earth, 0.7-1.5% zinc sulfate	100%; 0.02 ml	11	24-hour patch test; occlusive conditions	Non-irritating	[108]
Palmaria Palmata Extract	Palmaria Palmata Extract in water (specific composition not reported)	10% (0.75% dry matter); dose not reported	11	48-hour patch test; occlusive conditions	Non-irritating	[22]
Polysiphonia Lanosa Extract	Trade name mixture consisting of 67.5% water, 32% Polysiphonia Lanosa Extract	5%; 0.02 ml	11	48-hour patch test; occlusive conditions	Non-irritating	[109]
Rhodymenia Palmata Extract	Eye cream containing 0.0375% Rhodymenia Palmata Extract	100%; 0.2 g	38	7-day exposure; semi-occlusive conditions	Non-irritating	[110]
Sensitization						
Asparagopsis Armata Extract	Product containing 0.325% Asparagopsis Armata Extract	100%; dose not reported	108	HRIPT under occlusive conditions	Non-irritating; Non-sensitizing	[111]
Asparagopsis Armata Extract	Trade name mixture containing 0.5 – 2% Asparagopsis Armata Extract, 56 – 62% water, and 38 – 42% propanediol	3%; 40 µl	104	HRIPT under semi-occlusive conditions	Non-irritating; Non-sensitizing	[65]
Chondrus Crispus Extract	Product containing 0.49% Chondrus Crispus Extract	100%; dose not reported	113	HRIPT under occlusive conditions	Non-irritating; Non-sensitizing	[66]
Gelidiella Acerosa Extract	Product containing 0.0028% Gelidiella Acerosa Extract	100%; dose not reported	105	HRIPT under occlusive conditions	Non-irritating; Non-sensitizing	[67]
Gelidium Cartilagineum Extract	Trade name mixture consisting of >96% glycerides, mixed decanoyl and octanoyl; <2 % Gelidium Cartilagineum Extract; 1.5-2% 4-cholesten-3-one	100%; 25 µl	50	HRIPT under occlusive conditions	Non-irritating; Non-sensitizing	[68]
Hydrolyzed Corallina Officinalis Extract	>96% water; 0.5-3% Hydrolyzed Corallina Officinalis Extract; 0.16-0.20% sodium methylparaben	100%; 0.2 ml	51	HRIPT under occlusive conditions	Non-sensitizing	[69]

Table 13. Dermal irritation and sensitization

Ingredient	Test Substance	Concentration/Dose of the test substance	Test Population	Procedure	Results	Reference
Hypnea Musciformis Extract	Hypnea Musciformis Extract (specific composition not reported)	15% (0.36% dry matter); dose not reported	100	HRIPT (use of occlusion not reported)	Non-irritating; Non-sensitizing	22
Palmaria Palmata Extract	Palmaria Palmata Extract in water (specific composition not reported)	25% (1.87% dry matter); dose not reported	58	HRIPT (use of occlusion not reported)	Non-sensitizing	22
Porphyra Umbilicalis Extract	Product containing 0.0004% Porphyra Umbilicalis Extract	100%; dose not reported	103	HRIPT under occlusive conditions	Non-irritating; Non-sensitizing	70

HRIPT = Human Repeat Insult Patch Test; MTT = 3-(4,5-Dimethylthiazol-2-Yl)-2,5-Diphenyltetrazolium Bromide

Table 14. In Vitro Ocular Irritation Studies

Test Article	Concentration/Dose	Test Population	Procedure	Results	Reference
Trade name mixture containing 0.75% Ahnfeltiopsis Concinna Extract (other components not specified)	100%; 50 µl (liquid) or 50 mg (solid)	2	Test substance was applied to reconstructed cornea epithelium; after application, epithelia was incubated for 90 minutes	Non-irritating	62
An Asparagopsis Armata Extract containing 4% dry algal matter (other components not specified)	100%; dose not reported	NR	Cell viability assessed by using neutral red release assay (PREDISAFE) method	Slightly-irritating	52
After-shave balm containing 0.8% Chondrus Crispus (other components not specified)	100%; 100 µl	3	MatTek EpiOcular™ MTT assay	Non-irritating	71
Trade name mixture containing 3.5% Chondrus Crispus Extract (other components not specified)	100%; 50 µl (liquid) or 50mg (solid)	2	MatTek EpiOcular™ MTT assay	Non-irritating	63
Trade name mixture consisting of 50% glycerin; 30% water; 18.5 % undaria pinnatifida extract; 1.5% Corallina Officinalis Extract	10%; 5 ml	4	HET-CAM assay	Non-irritating	74
Trade name mixture consisting of water (45.7%), glycerin (40%), *Gigartina stellata* (4.43%), *Kappaphycus alvarezii* extract (5.9%), Corallina Officinalis Extract (3.97%)	10%; 5 ml	4	HET-CAM assay	Slightly-irritating	73
Trade name mixture consisting of 57-61% Lithothamnion Calcareum Powder, 26-31% mannitol, 9-11% diatomaceous earth, 0.7-1.5% zinc sulfate in water	2%, 5%, and 10%; 0.3 ml	4	HET-CAM assay	Moderately irritating at the 10% concentration; non-irritating at the 2 and 5% concentrations	75
Trade name mixture consisting of 52% water, 48% Porphyra Umbilicalis Extract	100%; dose not reported	6	HET-CAM assay	Weakly irritating	55
Eye cream containing 0.0375% Rhodymenia Palmata Extract	100%; 100 µl	8	MatTek EpiOcular™ MTT assay	Non-irritating	72

HET-CAM = hen's egg test chorioallantoic membrane; MTT = 3-(4,5-Dimethylthiazol-2-Yl)-2,5-Diphenyltetrazolium Bromide; NR = not reported

REFERENCES

1. Nikitakis J, Kowcz A. wINCI: *International Cosmetic Ingredient Dictionary and Handbook*. http://webdictionary.personalcarecouncil.org/jsp/Home.jsp. Washington, DC: Personal Care Products Council. Last Updated: 2020. Accessed: January 22, 2020.

2. Johnson W, Heldreth B, Bergfeld WF, et al. Safety Assessment of Polysaccharide Gums as Used in Cosmetics. 2015.

3. Lowe RL. 2015. Algal diversity and application. Washington, D.C.

4. Corino C, Modina SC, Giancamillo AD, Chiapparini S, Rossi R. Seaweeds in Pig Nutrition. *Animals (Basel).* 2019;9(12):1126.

5. Biotech Marine. 2016. Manufacturing Process Oligophycorail SPE (Hydrolyzed Corallina Officinalis Extract with 2-Phenoxyethanol as a preservative).

6. Biotech Marine. 2020. Manufacturing Process Rhodysterol™ S Sur Base Triglycerides (Gelidium Cartilagineum Extract).

7. Biotech Marine. 2012. Manufacturing process Biorestorer™ (Hypnea Musciformis Extract).

8. Lee Y, Oh H, Lee M. Anti-inflammatory effects of Agar free-*Gelidium amansii (GA)* extracts in high-fat diet-induced obese mice. *Nutrition Research and Practice.* 2018;12(6):479-485.

9. Centre d'Étude et de Valorisation des Algues (CEVA). 2014. Edible seaweed and French regulation http://www.cybercolloids.net/sites/default/files/seaweed%20and%20regulation2014.pdf. CEVA, ed.

10. Active Concepts. 2014. Product Specification ACB Cytoplasmic Extract J (contains 0.75% Ahnfeltiopsis Concinna Extract).

11. Active Concepts. 2017. Product Specification ABS Irish Moss Extract Sil (contains 20% Chondrus Crispus Extract).

12. Butler MR. Comparison of the chemical compostion of some marine algae. *Plant Physiol.* 1931;6(2):295-305.

13. Allen CF, Good P, Holton RW. Lipid Composition of *Cyanidium*. *Plant Physiol.* 1970;46(5):648-751.

14. Begum F, Chitra K, Joseph B, Sundrarajan R, Hemalatha S. Gelidiella acerosa inhibits lung cancer proliferation. *BMC Complement Altern Med.* 2018;18(1):104.

15. Kang J, Lee H, Kim H, Han J. Gelidium amansii extract ameliorates obesity by down-regulating adipogenic transcription factors in diet-induced obese mice. *Nutrition Research and Practice.* 2017;11(1):17-24.

16. Upscience. 2020. Mineral and metal analysis: GELYOL® GS45 (Gelidium Sesquipedale Extract).

17. Zheng J, Chen Y, Yao F, Weizhou C, Shi G. Chemical Composition and Antioxidant/Antimicrobial Activities in Supercritical Carbon Dioxide Fluid Extract of *Gloiopeltis tenax*. *Marine Drugs.* 2012;10(12):2634-2647.

18. Álvarez-Gómez F, Korbee N, Casas-Arrojo V, Abdala-Díaz RT, Figueroa FL. UV Photoprotection, Cytotoxicity and Immunology Capacity of Red Algae Extracts. *Molecules.* 2019;24(2):341.

19. Mohibbullah, Hannan A, Choi J, et al. The Edible Marine Alga *Gracilariopsis chorda* Alleviates Hypoxia/Reoxygenation-Induced Oxidative Stress in Cultured Hippocampal Neurons. *Journal of Medicinal Food.* 2015;18(9):960-971.

20. Jiang Z, Chen Y, Yao F, et al. Antioxidant, Antibacterial, and Antischistosomal Activites of Extracts from Grateloupia livida (Harv.) Yamada. *PLoS One.* 2013;8(11):e80413.

21. Chakraborty K, Joseph D, Praveen NK. Antioxidant activities and phenolic contents of three red seaweeds (Division: Rhodophyta) harvested from the Gulf of Mannar of Peninsular India. *J Food Sci Technol.* 2013;52(4):1924-2935.

22. Anonymous. 2020. Information Palmaria Palmata Extract and Hypnea Musciformis Extract.

23. Aslam MN, Bhaguvathula R, Paruchuri T, Hu X, Chakrabarty S, Varani J. Growth-inhibitory effects of a mineralized extract from the red marine algae, Lithothamnion calcareum, on Ca^{2+}-sensitive and Ca^{2+}-resistant human colon carcinoma cells. *Cancer Lett.* 2009;283(3):186-192.

24. Mouritsen OG, Vetter W, Dawczynski C, Jahreis G, Duelund L, Schröder M. On the human consumption of the red seaweed dulse (Palmaria palmata (L.) Weber and Mohr). *Journal of Applied Phycology.* 2013;25(6):1777-1791.

25. Machu L, Misurcova L, Ambrozova JV, et al. Phenolic Content and Antioxidant Capacity in Algal Food Products. *Molecules.* 2015;20(1):1118-1133.

26. Teas J, Pino S, Critchley A, Braverman LE. Variability of iodine content in common commercially available edible seaweeds. *Thyroid.* 2004;14(10):836-841.

27. Mibelle Group. 2020. Technical Data Sheet Helioguard™ 365 (trade name mixture containing 1.25% Porphyra Umbilicalis Extract).

28. Gelyma. 2020. Specification data sheet: HELIONORI® (Porphyra Umbilicalis Extract).

29. Bito T, Teng F, Watanabe F. Bioactive compounds of edible purple laver *Porphyra* sp. (Nori). *J Agric Food Chem.* 2017;65(49):10685-10692.

30. Circuncisão AR, Catarino MD, Cardoso SM, Silva AMS. Minerals from Macroalgae Origin: Health Benefits and Risks for Consumers. *Marine Drugs.* 2018;16(11):400.

31. Rubio C, Napoleone G, Luis-González G, et al. Metals in edible seaweed. *Chemosphere.* 2017;173(572-579).

32. Upscience. 2017. Mineral and metal analysis: ALGYL® (Gigartina Stellata/Kappaphycus Alvarezii Extracts and Corallina Officinalis Extract).

33. In Vivo Labs. 2016. Mineral and Metal analysis: PHYCO'DERM® (Undaria Pinnatifida Extract [brown algae] and Corallina Officinalis Extract [red algae]).

34. U.S. Food and Drug Administration Center for Food Safety & Applied Nutrition (CFSAN). 2020. Voluntary Cosmetic Registration Program - Frequency of Use of Cosmetic Ingredients. College Park, MD. ((Obtained under the Freedom of Information Act from CFSAN; requested as "Frequency of Use Data" January 6, 2020; received January 13, 2020).)

35. Personal Care Products Council. 2020. Concentration of Use by FDA Product Category: Red Algae-Derived Ingredients.

36. Johnsen M. The influence of particle size. *Spray Technol Marketing.* 2004;14(11):24-27.

37. Rothe H. Special Aspects of Cosmetic Spray Evaluation. 2011. Unpublished data presented at the 26 September 2011 Expert Panel meeting. Washington, D.C.

38. Bremmer HJ, Prud'homme de Lodder L, van Engelen J. Cosmetics Fact Sheet: To assess the risks for the consumer, Updated version for ConsExpo4. Bilthoven, Netherlands. 2006. http://www.rivm.nl/bibliotheek/rapporten/320104001.pdf. Accessed June 25, 2019. Pages 1-77.

39. Rothe H, Fautz R, Gerber, E, et al. Special aspects of cosmetic spray safety evaluations: Principles on inhalation risk assessment. Netherlands National Institute for Public Health and Environment; Bilthoven, Netherlands. *Toxicol Lett.* 2011;205(2):97-104.

40. CIR Science and Support Committee of the Personal Care Products Council (CIR SCC). 2015. (Nov 3rd) Cosmetic Powder Exposure. (Unpublished data submitted by the Personal Care Products Council on November 3, 2015.)

41. Aylott R, Byrne G, Middleton J, Roberts M. Normal use levels of respirable cosmetic talc: preliminary study. *Int J Cosmet Sci.* 1979;1(3):177-186.

42. Russell R, Merz R, Sherman W, Siverston J. The determination of respirable particles in talcum powder. *Food Cosmet Toxicol.* 1979;17(2):117-122.

43. European Commission. CosIng database; following Cosmetic Regulation No. 1223/2009. http://ec.europa.eu/growth/tools-databases/cosing/. Last Updated: 2016. Accessed: 07/12/2019.

44. Galland-Irmouli A, Fleurence J, Lamghari R, et al. Nutritional value of proteins from edible seaweed *Palmaria palmata* (Dulse). *The Journal of Nutritional Biochemistry.* 1999;10(6):353-359.

45. Anis M, Ahmed S, Hasan MM. Algae as nutrition, medicine, and cosmetic: The forgotten history, present status and future trends. *World Journal of Pharmaceutical Sciences.* 2017;6(6):1934-1959.

46. Joshi S, Kumari R, Upasani VN. Applications of Algae in Cosmetics: An Overview. *International Journal of Innovative Research in Science, Engineering, and Technology.* 2018;7(2):1269-1278.

47. Almeida F, Schiavo LV, Vieira AD, et al. Gastroprotective and toxicological evaluation of the *Lithothamnion calcareum* algae. *Food and Chemical Toxicology.* 2012;50:1399-1404.

48. Ye D, Jiang Z, Zheng F, et al. Optimized Extraction of Polysaccharides from Grateloupia livida (Harv.) Yamada and Biological Activities. *Molecules.* 2015;20(9):16817-16832.

49. Gelyma. 2018. *Corallina officinalis*: Algae synopsis.

50. Saito A, Idler DR. Sterols in irish moss (*Chondrus crispus*). *Canadian Journal of Biochemistry.* 1966;44(8):1195-1199.

51. Premakumara GAS, Ratnasooriya WD, Tillekeratne LMV. Studies on the post-coital contraceptive mechanisms of crude extract of Sri Lankan marine red algae, *Gelidiella acerosa*. *Contraception.* 1995;52(3):203-207.

52. Algues & Mer Cosmetics. 2020. Summary Toxicologie (studies done on Asparagopsis Armata Extract; in French).

53. Idea Lab. 2019. Bacterial reverse mutation assay: determination of the mutagenic activity of a test item (ALGYL®: Gigartina Stellata/Kappaphycus Alvarezii Extracts and Corallina Officinalis Extract) on Salmonella typhimurium (Ames test) according to the OECD 471.

54. Syad AN, Kasi PD. Assessment of Mutagenic Effect of G. acerosa and S. wightii in S. typhimurium (TA 98, TA 100, and TA 1538 strains) and Evaluation of Their Cytotoxic and Genotoxic Effect in Human Mononuclear Cells: A Non-Clinical Study. *Journal of Biomedicine and Biotechnology.* 2014;4.

55. Gelyma. 2020. HELIONORI® (Porphyra Umbilicalis Extract): Toxicological data.

56. Balamurugan M, Sivakumar K, Anand MAV, Suresh K. Modulating effect of Hypnea musciformis (red seaweed) on lipid peroxidation, antioxidants, and biotransforming enzymes in 7,12-dimethylbenz (a) anthracene induced mammary carcinogenesis in experimental animals. *Pharmacognosy Research (Epub ahead of print).* 2017;9(1):108-115.

57. Alves C, Pinteus S, Horta A, Pedrosa R. High cytotoxicity and anti-proliferative activity of algae extracts on an in vitro model of human hepatocellular carcinoma. *SpringerPlus.* 2016;5(1):1339.

58. Yamamoto I, Maruyama H. Effect of dietary seaweed preparations on 1,2-dimethylhydrazine-induced intestinal carcinogenesis in rats. *Cancer Letters.* 1985;26(3):241-251.

59. Allmendinger A, Spavieri J, Kaiser M, et al. Antiprotozoal, Antimycobacterial, and Cytotoxic Potential of Twenty-Three British and Irish Red Algae. *Phyotherapy Research.* 2010;24(7):1099-1103.

60. Mercurio DG, Wagemaker TAL, Alves VM, Benevenuto CG, Gaspar LR, Campos PMBGM. In vivo photoprotective effects of cosmetic formulations containing UV filters, vitamins, *Ginkgo biloba* and red algae extracts. *J Photochem Photobiol B.* 2015;153:121-126.

61. Ishihara K, Oyamada C, Matsushima R, Murata M, Muraoka T. Inhibitory effect of porphyran, prepared from dried "nori", on contact hypersensitivity in mice. *Biosci Biotechnol Biochem.* 2005;69(10):1824-1830.

62. Concepts A. 2015. Dermal and Ocular Irritation Tests ACB Cytoplasmic Extract J (contains 0.75% Ahnfeltiopsis Concinna Extract).

63. Active Concepts. 2018. Dermal and Ocular Irritation Tests Alg-MoistEAU (contains 3.5% Chondrus Crispus Extract).

64. palmer Research. 2004. Etude de la tolerance cutanee aigue d'une matiere premiere chez le volontaire adulte: Patch-test 24 heures occlusif sous controle dermatologique (Biorestorer™ contains 1-3% Hypnea Musciformis Extract).

65. Dermscan. Assessment of the sensitizing potential of a natural extract (Asparagopsis Armata Extract): Final clinical security test under dermatological control. 2018.

66. Eurofins CRL. 2019. Repeated insult patch test (product contains 0.49% Chondrus Crispus Extract).

67. Clinical Research Laboratories Inc. 2013. Repeated insult patch test (tested product contained 0.0028% Gelidiella Acerosa Extract).

68. Liskin. 2009. Etude du pouvoir sensibilasant d'un produit selon la methode de Marzulli-Maibach (Rhodysterol™ Sur Base Triglycerides (Gelidium Cartilagineum Extract)).

69. Palmer Research. 1995. Evaluation du potentiel allergisant apres applications epicutanees repetees sur 51 volontaires (Oligophycorail Hydrolyzed Corallina Officinalis Extract with Sodium Methylparaben as a preservative).

70. Clinical Research Laboratories Inc. 2018. Repeated insult patch test (product contains 0.0004% Porphyra Umbilicalis Extract).

71. Institute for In Vitro Sciences Inc. 2012. Tissue equivalent assay with Epiocular™ cultures (three after-shave balms with 0.8% Chondrus crispus).

72. Institute for In Vitro Sciences Inc. 2013. Tissue Equivalent Assay with Epiocular™ Cultures (Eye Cream with 0.0375% Rhodymenia Palmata Extract).

73. Eurofins. 2017. Assessment of the irritant potential of a test item (ALGYL®: Gigartina Stellata/Kappaphycus Alvarezii Extracts and Corallina Officinalis Extract) after application to the embryonic hen's egg chorioallantoic membrane - HET-CAM.

74. Eurofins. 2016. Assessment of the irritant potential of a test item after application to the embryonic hen's egg chorioallantoic membrane HET-CAM: PHYCO'DERM® (Undaria Pinnatifida Extract [brown algae] and Corallina Officinalis Extract [red algae]).

75. Seppic. 2001. Protocol. HET-CAM Test: Pycocorail® (contains 57-61% Lithothamnion Calcareum Powder).

76. Guiry MD. *AlgaeBase*. World-wide electronic publication. https://www.algaebase.org/. Galway, Ireland: national University of Ireland, Galway. Last Updated: 2020. Accessed: January 22, 2020.

77. Andreakis N, Kooistra W, Procaccini G. Asparagopsis taxiformis and Asparagopsis armata (Bonnemaisoniales, Rhodophyta): Genetic and morphological identification of Mediterranean populations. *European Journal of Phycology* 2004;39(3):273-283.

78. Food and Agriculture Organization of the United Nations (FAO) Fisheries and Aquaculture Department. Species Fact Sheets: *Chondrus crispus*. http://www.fao.org/fishery/species/2788/en. Last Updated: 2020. Accessed: August 5, 2020.

79. Gelyma. 2018. *Gelidium sesquipedale*: Algae synopsis.

80. Gelyma. 2018. *Gigartina stellata*: Algae synopsis.

81. Gelyma. 2018. *Porphyra umbilicalis*: Algae synopsis.

82. Pinteus S, Alves C, Monteiro H, Araújo E, Horta A, Pedrosa R. Asparagopsis armata and Sphaerococcus coronopifolius as a natural source of antimicrobial compounds. *World J Microbiol Biotechnol.* 2015;31(3):445-451.

83. Solabia Group. 2017. Manufacturing Process Glycerolat® of Neptune Harpoon (0.42% Asparagopsis Armata Extract).

84. Algues & Mer Cosmetics. 2019. Ysaline® 100 (Asparagopsis Armata Extract) Process flow.

85. Biotech Marine. 2020. Manufacturing Process Aspar'age™ (Asparagopsis Armata Extract).

86. Biotech Marine. 2020. Manufacturing Process Flakes of Hydralixir™ CC (Chondrus Crispus Extract and Gigartina Stellata Extract).

87. Anonymous. 2020. Production Process Chondrus Crispus Powder.

88. Biotech Marine. 2020. Manufacturing Process: Flakes of Chondrus Crispus.

89. Gelyma. 2020. Manufacturing flow chart: ALGYL® (Gigartina Stellata/Kappaphycus Alvarezii Extracts and Corallina Officinalis Extract).

90. Gelyma. 2020. Manufacturing flow chart: PHYCO'DERM® (Undaria Pinnatifida Extract [brown algae] and Corallina Officinalis Extract [red algae]).

91. Namjoyan F, Farasat M, Alishahi M, Jahangiri A, Mousavi H. The Anti-melanogenesis Activites of Some Seleced Red Macroalgae from Northern Coasts of the Persian Gulf. *Iranian Journal of Pharmaceutical Research.* 2019;18(1):383-390.

92. Biotech Marine. 2016. Manufacturing Process Oligophycorail (Hydrolyzed Corallina Officinalis Extract with Sodium Methylparaben as a preservative).

93. Biotech Marine. 2015. Manufacturing process Phycocorail™ (Lithothamnion Calcareum Powder).

94. Gelyma. 2020. Manufacturing flow chart: HELIONORI® (Porphyra Umbilicalis Extract).

95. Kelman D, Posner EK, McDermid KJ, Tabandera NK, Wright PR, Wright AD. Antioxidant Activity of Hawaiian Marine Algae. *Marine Drugs.* 2012;10(2):403-416.

96. Rouxel C, Daniel A, Jérôme M, Etienne M, Fleurence J. Species identification by SDS-PAGE of red algae used as seafood or a food ingredient. *Food Chemistry.* 2001;74:349-353.

97. Watanabe F, Takenaka S, Katsura H, et al. Characterization of a Vitamin B_{12} Compound in the Edible Purple Laver, *Porphyra yezoensis*. *Biosci Biotechnol Biochem.* 2000;64(12):2712-2715.

98. Dermscan. 2018. Evaluation of the acute cutaneous tolerance of a natural extract (Asparagopsis Armata Extract) on adult subjects.

99. Alba Science. 2011. A 14-day human cumulative irritation patch test (three aftershave balms, each containing 0.8% Chondrus crispus (CAS 9000-07-1)).

100. Dermscan. 2018. Evaluation of the acute cutaneous tolerance of a natural extract on adult subjects: single patch test (Hydralixir™ CC - Chondrus Crispus Extract and Gigartina Stellata Extract).

101. Palmer Research. 2004. Study of the acute tolerance of a raw material (flakes of *Chondrus crispus*) on adult volunteers: 24-hour occlusive patch test under dermatological control.

102. Eurofins. 2018. Assessment of the skin compatibility of a cosmetic raw material (ALGYL®: Gigartina Stellata/Kappaphycus Alvarezii Extracts and Corallina Officinalis Extract) under dermatological control after a single application under occluded patch during 48h on 20 subjects: patch test (study in French with an English summary).

103. Eurofins. 2016. Human patch test under dermatological control: PHYCO'DERM® (Undaria Pinnatifida Extract [brown algae] and Corallina Officinalis Extract [red algae]).

104. Laboratoire Coderma. 2015. Verification in humans of cutaneous compatability of a cosmeticproduct after a single application under patch (Rhodysterol™ Sur Base Triglycerides (Gelidium Cartilagineum Extract)).

105. Eurofins. 2020. Evaluation of the cutaneous tolerance of a cosmetic product after a single application under an occlusive patch during 48 hours: Patch test method GELYOL®GS45 (Gelidium Sesquipedale Extract).

106. Palmer Research. 2004. Etude de la tolerance cutanee aigue d'une matiere premiere chez le volontaire adulte: Patch test 24 heures occlusif sous controle dermatologique (Oligophycorail Hydrolyzed Corallina Officinalis Extract with Sodium Methylparaben as a preservative).

107. Cosderma Laboratoire. 2007. Verification chez l'homme de la compatibilite cutanee d'un produit cosmetique apres application unique sous pansement. Patch test 24 h (Hydrolyzed Corallina Officinalis Extract with 2-Phenoxyethanol as a preservative).

108. Palmer Research. 2003. Etude de la tolerance cutanee aigue d'un produit cosmetique chez le volontaire adulte: Patch-test 24 heures occlusif. Pycocorail (contains 57-61% Lithothamnion Calcareum Powder).

109. Gelyma. 2020. Patch test summary: SUN'YTOL®(Polysiphonia lanosa extract in water and phenoxyethanol).

110. TKL Research. 2013. Human Cummulative Irritation Patch Test (Eye Cream with 0.0375% Rhodymenia Palmata Extract).

111. Clinical Research Laboratories Inc. 2012. Repeated insult patch test (product contains 0.325% Asparagopsis Armata Extract).

Table 1. Data profile of red algae-derived ingredients

Ingredient	GRAS	Food	Tox	Sensitization data
Ahnfeltiopsis Concinna Extract		✓		
Asparagopsis Armata Extract				✓
Hydrolyzed Asparagopsis Armata Extract				✓
Chondrus Crispus		✓		✓
Chondrus Crispus Extract		✓		✓
Chondrus Crispus Powder		✓		✓
Hydrolyzed Chondrus Crispus Extract		✓		✓
Corallina Officinalis Extract				✓
Corallina Officinalis Powder				✓
Corallina Officinalis Thallus Extract				✓
Hydrolyzed Corallina Officinalis				✓
Hydrolyzed Corallina Officinalis Extract				✓
Gelidiella Acerosa Extract		✓		✓
Gelidium Amansii Extract		✓		
Gelidium Amansii Oligosaccharides		✓		
Gelidium Cartilagineum Extract				✓
Gigartina Stellata Extract		✓		
Gracilaria Verrucosa Extract		✓		
Hypnea Musciformis Extract				✓
Lithothamnion Calcareum Extract (synonymous with Phymatolithon Calcareum Extract)		✓	✓	
Lithothamnion Calcareum Powder		✓	✓	
Palmaria Palmata Extract (synonymous with Rhodymenia Palmata Extract)	✓	✓		✓
Palmaria Palmata Powder	✓	✓		✓
Phymatolithon Calcareum Extract (synonymous with Lithothamnion Calcareum Extract)		✓	✓	
Porphyra Tenera Extract	✓	✓		
Porphyra Tenera Sporophyte Extract	✓	✓		
Porphyra Umbilicalis Extract		✓		✓
Porphyra Umbilicalis Powder		✓		✓
Hydrolyzed Porphyra Yezoensis		✓		
Porphyra Yezoensis Extract		✓		
Porphyra Yezoensis Powder		✓		
Rhodymenia Palmata Extract (synonymous with Palmaria Palmata Extract)	✓	✓		✓

It should be noted that if data points were available for an ingredient of a given genus and species, then the same data points would be checked off for all other ingredient forms with the same genus and species. For example, since sensitization data was provided for Chondrus Crispus Extract, the sensitization data point is also checked off for Chondrus Crispus, Chondrus Crispus Extract, Chondrus Crispus Powder, and Hydrolyzed Chondrus Crispus Powder.

Ingredients with no GRAS/food data, systemic toxicity data, or sensitization data (27 ingredients)

Betaphycus Gelatinum Extract
Botryocladia Occidentalis Extract
Calliblepharis Ciliata Extract
Ceramium Kondoi Extract
Ceramium Rubrum Extract
Chondracanthus Teedei Powder
Cyanidium Caldarium Extract
Delesseria Sanguinea Extract
Digenea Simplex Extract
Dilsea Carnosa Extract
Furcellaria Lumbricalis Extract
Gelidium Pulchrum Protein
Gelidium Sesquipedale Extract
Gigartina Skottsbergii Extract
Gloiopeltis Tenax Extract
Gloiopeltis Tenax Powder
Gracilariopsis Chorda Extract
Grateloupia Livida Powder
Lithothamnion Corraloides Powder
Mesophyllum Lichenoides Extract
Pikea Robust Extract
Polysiphonia Lanosa Extract
Porphyra Linearis Powder
Porphyridium Cruentum Culture Conditioned Media
Porphyriidum Cruentum Extract (synonymous with Porphyridium Purpureum Extract)
Porphyridium Purpureum Extract (synonymous with Porphyridium Cruentum Extract)
Sardiotheca Gaudichaudii Extract

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: June 22, 2020

SUBJECT: Ahnfeltiopsis Concinna Extract

Active Concepts. 2014. Product Specification ACB Cytoplasmic Extract J (contains 0.75% Ahnfeltiopsis Concinna Extract).

Active Concepts. 2015. Dermal and Ocular Irritation Tests ACB Cytoplasmic Extract J (contains 0.75% Ahnfeltiopsis Concinna Extract).

Active Concepts

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Product Specification

Product Name: ACB Cytoplasmatic Extract J
Code Number: 20020
CAS #'s: 92128-82-0
EINECS #'s: 295-780-4
INCI Name: Algae Extract
Status: Approved

contains 0.75% Ahnfeltiopsis Concinna Extract

Specification	Parameter
Appearance	Clear Colorless Liquid
Odor	Characteristic
pH (direct)	6.0 – 7.0
NVM (1g-16hrs-105°C)	1.0% Maximum
Ash (800°C)	0.1% Maximum
Nitrogen	< 0.05%
Heavy Metals	< 20 ppm
Arsenic	< 2 ppm
Microbial Content	< 100 opg No pathogens

May Sediment upon Standing; Mix Well Prior to Use

Dermal and Ocular Irritation Tests

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Tradename: ACB Cytoplasmatic Extract J
contains 0.75% Ahnfeltiopsis Concinna Extract

Code: 20020

CAS #: 92128-82-0

Test Request Form #: 1746

Lot #: 42062P

Sponsor: *Active Concepts, LLC; 107 Technology Drive Lincolnton, NC 28092*
Study Director: *Erica Segura*
Principle Investigator: *Meghan Darley*

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 **ACB Cytoplasmatic Extract J** 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 a **non-irritant**. 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-irritating**. The negative and positive controls performed as anticipated.

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.

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Dermal and Ocular Irritation Tests

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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.

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.

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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™).

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.

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B. Tissue Viability Assay

The results are summarized in Figures 1 and 2. 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.

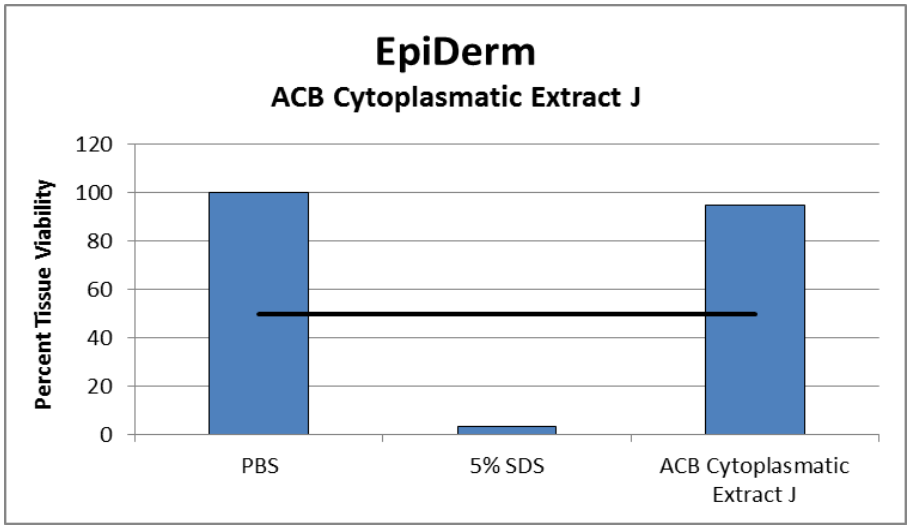

Figure 1: EpiDerm tissue viability

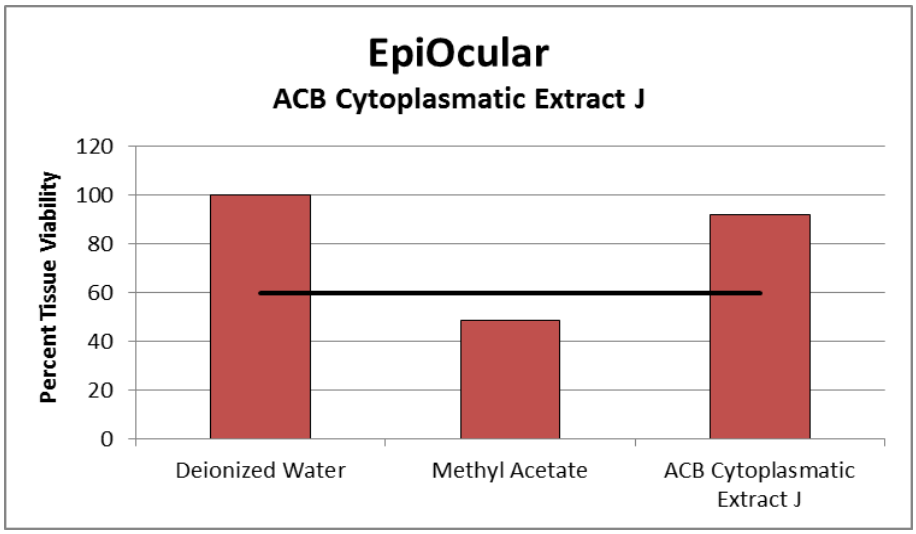

Figure 2: EpiOcular tissue viability

INGREDIENT BREAKDOWN
COMPOSITION CENTESIMALE

Glycerolat® of Neptune Harpoon
Glycérolat® de Harpon de Neptune

Ref. M0022

Glycerin ..... **50.00 %**

Water ..... **49.18 %**

Asparagopsis armata extract ..... **0.42 %**
2g, 16 hours at 105°C

Potassium sorbate ..... **0.15 %**

Sodium benzoate ..... **0.25 %**

Notes - *Remarques :*

- Because of the natural origin of the raw material, the centesimal composition is susceptible to slight variations.

  En raison de l'origine naturelle des matières premières, la composition centésimale est susceptible de subir une légère variation.

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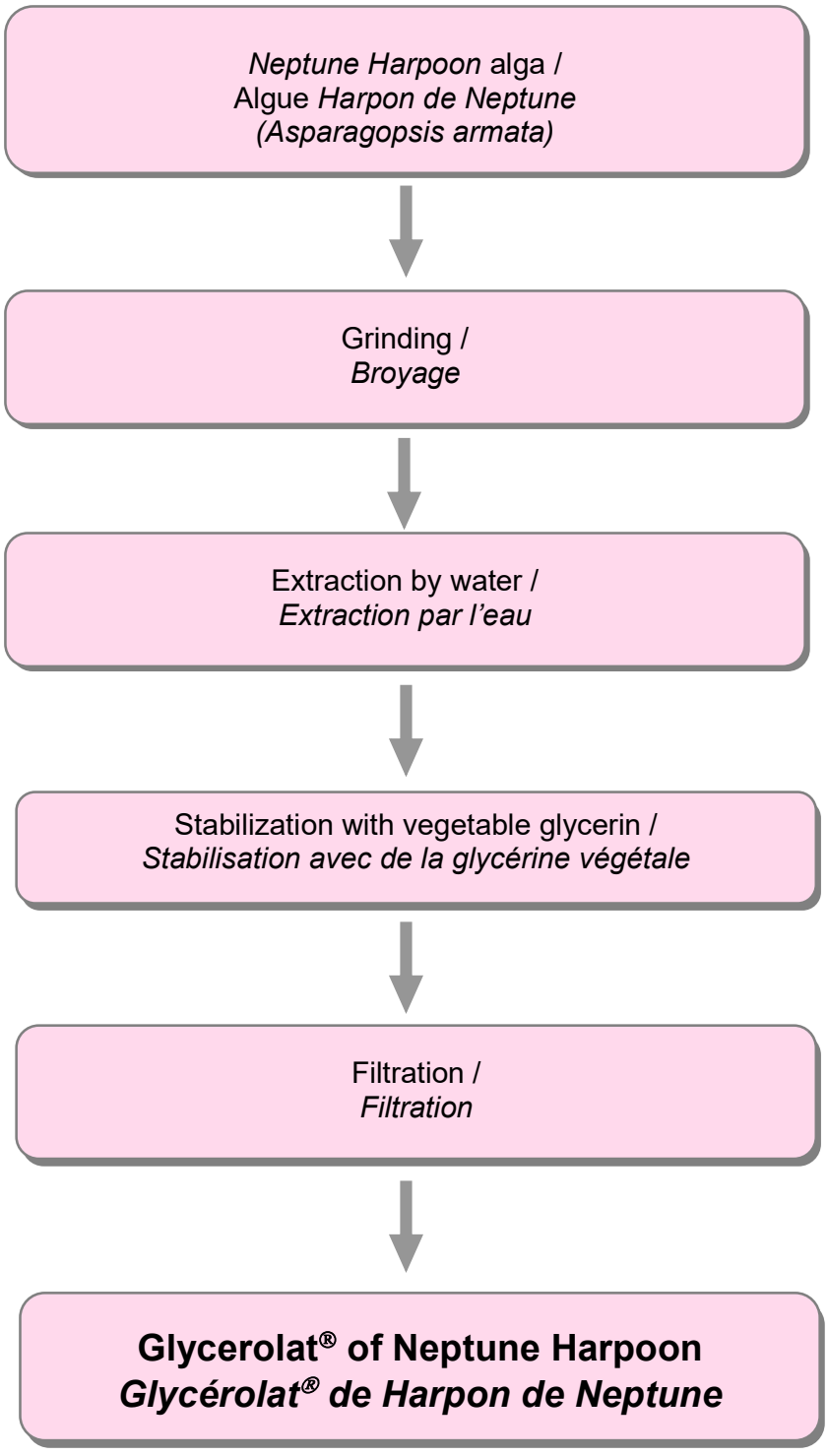
29 Rue Delizy – 93698 Pantin Cedex France
Tel 33 (0)1 48 10 19 40 – Fax 33 (0)1 48 91 18 77 - www.solabia.com

Glycerolat® of Neptune Harpoon
Glycérolat® de Harpon de Neptune

Ref. M0022



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algues & mer Cosmetics

Natural active ingredients extracted from seaweeds

Ysaline® 100 Process Flow

Ref. : M0010FB
Chemical name : *Asparagopsis armata* Extract

MANUFACTURING PROCESS

→ Fresh seaweeds,
→ Grinding,
→ Cold cellular extraction,
→ Filtrations,
→ Concentration,
→ Freeze-drying under neutral atmosphere.

RAW MATERIALS

→ *Asparagopsis armata* fresh seaweed,
→ Water.

ADDITIVES

None

PROCESSING AIDS

→ Perlite
→ Cellulose
→ Sodium hydroxide
→ Hydrochloric acid

TOXICOLOGIE

1. Données préalables à un essai clinique de la tolérance cutanée d'une solution à 4% d'Ysaline 100.

1.1. Objectif

La tolérance locale a été étudiée dans un modèle d'épiderme humain reconstruit EPISKIN. Elle a été évaluée par un examen histologique des effets du produit à l'essai sur l'architecture de l'épiderme. Les informations apportées par ce modèle concernent essentiellement la prévision du risque d'irritation

1) aiguë, puisque l'application répétée du produit à l'essai sur l'épiderme reconstruit n'a pas été faite
2) directe, puisque les autres composantes de toxicité, comme la composante immunitaire, ne sont pas modélisées
3) épidermique, puisque le modèle EPISKIN ne comporte pas de derme équivalent.

1.2. Réalisation expérimentale

* *Produit à l'essai* : **solution à 4% d'YSALINE 100** provenant de la société ALGUES ET MER.

* *Produit de référence* : laurylsulfate de sodium (LSS), agent tensioactif de référence, provenant de la société SIGMA.

* *Réactifs* : Tous les réactifs sont de qualité analytique. Leurs références (fournisseur, n° de catalogue, n° de lot) sont conservées dans le dossier d'essai au sein de BIOPREDIC.

L'eau provenait d'un système de purification de type MilliQ (Millipore).

La solution de rinçage des épidermes après incubation était le tampon PBS (n° de réactif BIOPREDIC 98B).

* *Système d'essai* : les épidermes reconstruits EPISKIN (lot 96-EPIS-006) ont été fournis par IMEDEX. Ils ont été utilisés dès réception. Les nacelles comportant les épidermes ont été directement placées dans des puits de culture qui contenaient le milieu d'incubation, correspondant au "milieu d'essai" (MIE), fourni par IMEDEX avec les épidermes et de composition non précisée.

* *Préparation du produit à l'essai et du produit de référence, incubation avec le système d'essai* :

Produit à l'essai	Concentration	Diluant	Volume déposé	Remarques
Sans	0	eau MilliQ	200 uL	Témoin
Sol. à 4% Ysaline 100	10 %	eau MilliQ	200 uL	Néant
LSS	1.5 mg/mL	eau MilliQ	200 uL	Néant

Les épidermes ont été incubés pendant 18 heures à 37°C dans une atmosphère contenant 5% de CO_2

Les essais ont été réalisés en duplicate.

* *Evaluation des effets* : à la fin de l'incubation, les épidermes ont été abondamment rincés avec du tampon PBS et découpés à l'aide d'un scalpel.
Les disques d'épidermes ainsi obtenus (diamètre 12 mm) ont été utilisés pour l'étude histologique.

* *Préparation des coupes histologiques* : les épidermes utilisés pour l'examen histologique ont été traités selon la technique suivante :

- fixation dans une solution de formol à 10% dans de l'eau pendant 12 heures à température ambiante
- déshydratation par passage dans des alcools de titre croissant, puis dans du toluène, et inclusion dans la paraffine
- réalisation des blocs de paraffine sur platine réfrigérante
- coupes des blocs (trois lames par bloc et deux plans de coupe par lame)
- colorations par l'hémalum / éosine / safran.

* *Examen microscopique* : tous les plans de coupe ont été examinés à six reprises, en totalité, à des jours différents et à un même grossissement. Les altérations morphologiques suivantes ont été recherchées par un médecin anatomo-pathologiste, expert en toxicologie :

- lésions superficielles, localisées dans la moitié supérieure de l'épiderme :
  - micro- ou macro-vésicules intercellulaires
  - clivage intraépidermique supra-basal
  - microvacuoles intracellulaires
  - nécroses cellulaires superficielles
- lésions profondes, localisées dans la moitié inférieure de l'épiderme :
  - décollement de l'épiderme de son support
  - clivage intraépidermique sous-cutané
  - nécroses cellulaires basales.

* *Traitement des données* : en fonction du type et du nombre d'altérations morphologiques observées, quatre scores sont attribués :

0	=>	pas d'altération
+	=>	altérations mineures
++	=>	altérations modérées
+++	=>	altérations majeures.

* *Qualité des données* : BIOPREDIC a un système d'assurance qualité (audité par l'Agence du Médicament pour les activités de test sur les nouveaux médicaments), qui permet de garantir la fiabilité des données obtenues. Toutes les données de l'essai sont archivées dans BIOPREDIC pendant dix ans après l'essai et sont à la disposition du commanditaire.

1.3. Synthèse des résultats - CONCLUSIONS

Produit à l'essai : solution à 4% d'YSALINE 100 (testée à 10%) - (p/v)
Système d'essai : épiderme reconstruit, EPISKIN, lot 96-EPSI-006

Observations histologiques des épidermes incubés pendant 18 heures avec les produits

Produit	Concentration testée	Altérations morphologiques
Sans	-	0
Produit de référence LSS	1.5 mg/mL	++
Produit à l'essai	10%	0

Dans les conditions expérimentales retenues, le produit Solution à 4% YSALINE 100 ne provoque pas d'altérations histologiques.

2. Etude prédictive de la tolérance oculaire de la solution à 4% d'YSALINE 100 par la méthode PREDISAFE

2.1. Introduction

Le 6ème amendement de la Directive CEE n°93/35 du 14 juin 1993, prévoit que l'expérimentation animale sera remplacée le 1er janvier 1998 "s'il y a eu des progrès suffisants dans la mise au point de méthodes pouvant se substituer de manière satisfaisante à l'expérimentation animale".

Des études de validation de ces méthodes alternatives sont nécessaires afin d'offrir au consommateur un degré de protection équivalent au niveau actuel en matière de tests de toxicité. Une corrélation satisfaisante avec les tests sur les animaux et des reproductibilités acceptables inter/intralaboratoires sont donc indispensables.

BIOPREDIC a développé une méthode *in vitro* pour l'évaluation de la tolérance oculaire appelée PREDISAFE. PREDISAFE est incluse dans plusieurs études interlaboratoires : celle du Laboratoire National de Santé (1993), qui a permis de classer PREDISAFE première parmi cinq autres méthodes *in vitro*, pour distinguer des produits corrosifs des non corrosifs, et celle du COLIPA (1994), qui a confirmé la fiabilité de la méthode.

Dans cette étude, il s'agissait d'apprécier la tolérance oculaire du produit : solution à 4% d'YSALINE 100 par la méthode PREDISAFE.

2.2. Matériels et méthode

* *Produit à l'essai* : **solution à 4% d'YSALINE 100** provenant de la société ALGUES ET MER.

* *Réalisation de l'essai* : le test PREDISAFE a été réalisé à l'aide d'un kit PREDISAFE, lot n° MW080698, fourni par BIOPREDIC International. Toutes les procédures indiquées par le fournisseur ont été suivies et les réactifs contenus dans ce kit ont été utilisés.

* *Traitement des données* : les données ont été traitées selon la méthode indiquée dans le manuel d'utilisation.

* *Validation de l'expérience* : différents contrôles qualités, décrits dans le manuel d'utilisation, sont réalisés pendant l'expérience afin de valider le test.

2.3. Résultats

Produit à l'essai : solution à 4% d'YSALINE 100 (testée pur)
Système d'essai : PREDISAFE kit, lot n° MW080696

Validation de l'expérience

- Rétention du rouge neutre	:	0.678 DO	OUI
- Coefficient de variation	:	0.009%	OUI

- Sensibilité des cellules aux produits de référence : classement sur une échelle de cytotoxicité de 0 à 50 (comparable à l'échelle de tolérance oculaire)

Tween 20	< 15	OUI
n-butanol	43	OUI
LSS	> 50	OUI

Classification du produit au point de vue cytotoxicité selon la méthode PREDISAFE

< 15 sur une échelle de 0 à >50

2.4. Discussion

La tolérance oculaire du produit solution à 4% d'YSALINE 100 a été évaluée selon la méthode in vitro PREDISAFE. Cette méthode a démontré un haut niveau de corrélation par rapport au test de Draize, et de reproductibilité.

La tolérance du produit solution à 4% d'YSALINE 100 était inférieure à 15 sur une échelle de tolérance oculaire arbitrairement graduée de 0 à 50 (comme celle utilisée dans le test de Draize pour la tolérance oculaire).

Ce score correspond à la classe I (0 < ou = à 15) "produits faiblement irritants".

Classification prédictive du produit à l'essai au point de vue de la tolérance oculaire

Classement sur une échelle de 0 à > 50

< 15

Classement : **Classe I**

faiblement irritant (slightly irritating)

"Benchmarking" :

Comparaison du produit à l'essai, en terme de tolérance oculaire, par rapport à des produits de mêmes caractéristiques physicochimiques ou de même usage.
Cette classification est basée sur des données collectées par BIOPREDIC.

Tolérance dans des conditions normales d'utilisation

	X	
Inférieure à la moyenne	**Moyenne**	Supérieure à la moyenne

3. Etude de la tolérance cutanée in vivo sur l'homme du produit solution à 4% d'YSALINE 100

3.1. Objectif

Dans cette étude, il s'agissait de déterminer la tolérance cutanée d'un ingrédient cosmétique *in vivo* chez des volontaires humains, dans un centre agréé.

3.2. Réalisation expérimentale

* *Ingrédient étudié* : **solution à 4% d'YSALINE 100**

***** *Matériel* : des patch-tests Finn Chambers on Scanpor ou chambres d'isolement qui assurent une bonne occlusion ont été utilisés. Il s'agit de cupules d'aluminium de 8 mm de diamètre, d'une capacité de 20 uL et couvrant une surface de 50 mm^2.

* *Sélection des volontaires* : dix volontaires sains ont été sélectionnés en fonction des critères d'inclusion et de non inclusion suivants :

Critères d'inclusion :

- Personne d'âge compris entre 18 et 60 ans
- Personne de sexe féminin ou masculin
- Personne ayant donné par écrit son consentement libre, éclairé et exprès
- Personne coopérante, avertie de la nécessité et de la durée des contrôles, ce qui permet d'espérer une parfaite adhésion au protocole mis en place
- Type caucasien
- Phototype : II - III - IV

Critère de non inclusion :

- Femme enceinte ou en période de lactation
- Personne présentant une peau "hyper-irritable"
- Personne allergique au sparadrap
- Pathologie cutanée sur la zone d'expérience
- Présence d'un traitement médicamenteux - immunosuppresseur, corticoïde, anti-histaminique ou anti-inflammatoire - pendant la semaine qui précède et pendant la période de l'étude
- Personne présentant une affection grave ou évolutive
- Utilisation de produits dermopharmaceutique ou cosmétique, sur les zones d'expérience, pendant la période de l'étude.

* *Réalisation des patch-tests* : le produit a été testé dilué à 10%. Il a été placé dans la cupule. Les patch-tests ont été appliqués sur la zone scapulaire gauche du dos des volontaires et recouverts d'adhésif de type "Micropore" pour parfaire l'adhérence. Des patch-tests sans produit ont été effectués en parallèle.

* *Evaluation de la tolérance cutanée* : quarante-huit heures après la pose des patch-tests, ceux-ci ont été décollés. Trente minutes plus tard, les signes éventuels d'irritation ont été observés par un médecin dermatologue et cotés sur une échelle de 0 à 3 selon la cotation suivante :

	Erythème	Oedème	Sécheresse	Vésicules
Absent	E0	O0	S0	V0
Léger	E1	O1	S1	V1
Net	E2	O2	S2	V2
Important	E3	O3	S3	V3

3.3. Résultats

Produit à l'essai : solution à 4% d'YSALINE 100 (testée diluée à 10%)

Volontaire	Age (ans)	Sexe	Antécédents médico-chirurgicaux pouvant influencer le déroulement de l'étude	Evénements survenus pendant la période de l'étude	Cotation de la tolérance sur une échelle de 0 à 3
1	22	F	néant	néant	0
2	21	F	néant	néant	0
3	20	F	néant	néant	0
4	21	F	néant	néant	0
5	54	M	néant	néant	0
6	21	F	néant	néant	0
7	23	F	néant	néant	0
8	25	F	néant	néant	0
9	23	F	néant	néant	0
10	23	F	néant	néant	0

3.4. Discussion

La tolérance cutanée in vivo chez l'homme du produit solution à 4% d'YSALINE 100 testé dilué à 10%, a été étudiée après patch occlusif de 48 heures sur dix volontaires.

Le produit solution à 4% d'YSALINE 100 n'a provoqué aucune réaction pour les 10 volontaires.

En conclusion, dans cette étude, avec les limites liées au protocole (c'est à dire inclusion du nombre limité de 10 volontaires), **le produit solution à 4% d'YSALINE 100 a montré qu'il n'était pas irritant chez l'homme.**

4. Etude de l'effet mutagène d'une solution à 8% YSALINE 100

4.1. Introduction

L'objectif de cette étude était d'évaluer le potentiel mutagène du produit solution à 8% YSALINE 100 au moyen de souches de *Salmonella typhimurium* 5 histidine-dépendante, en présence ou en l'absence d'un système d'activation métabolique.

Le protocole de l'étude (n° 935/080-D du 25/02/1999) s'inspire des guides OECD 471, FDA Redbook II, EEC guideline 92/69 et EPA section 798 chapitre 798.5265.

4.2. Informations sur le produit à l'essai, le contrôle négatif et le véhicule

* *Produit à l'essai*

- dénomination : solution à 8% d'YSALINE 100
- provenance : société ALGUES ET MER
- aspect : liquide incolore
- n° de lot : AW9805
- pureté : estimée à 100%
- densité : 0.96 (donnée prise en compte pour le calcul de la dose)
- stockage : à température ambiante et à l'abri de la lumière
- utilisation thérapeutique prévue : secteur cosmétique.

* *Véhicule et contrôle négatif*

- dénomination : eau pour injection
- provenance : BIOSEDRA
- n° de lot : LR82274, LR85139 et 3026
- dangers : aucun

* *Système d'activation métabolique*

- identification : fraction S9 lyophilisée de foie de rats traités avec un mélange de phénobarbital et méthylcholantrène.
- provenance : Iffa-Credo, France
- n° de lot : SL 63 (date d'expiration : 31 juillet 1999)
- solvant : eau pour injection (BIOSEDRA)

Toutes les préparations étaient utilisées le jour de leur formulation.

* *Contrôles positifs*

Réactifs	Souches	Dose (ug/boîte)
2-Nitrofluorène (2-NF)	TA98	5
t-Butyl hydroperoxide (t-BHP)	TA102	100
9-Aminoacridine (9-AA)	TA1537	50
Sodium azide (NaA)	TA100 et TA1535	10
2-Aminoanthracène (2-A)	toutes les souches ont un syst. d'activation métabolique.	5

Les références (fournisseur, n° de catalogue, n° de lot) sont conservées dans le dossier d'essai.

* *Préparation du produi à l'essai*

- Evaluation de la solubilité : le produit à l'essai donne une solution claire dans l'eau à 50 mg/mL, l'eau utilisé correspondant au véhicule sélectionné pour l'étude.

- Préparation : le produit à l'essai est préparé, par dilution en série à partir du stock initial, aux concentrations de 0.52, 1.64, 5.12, 16 et 50 mg/mL (étude préliminaire et expérimentation 1). Pour l'expérimentation 2, le produit à l'essai est préparé aux concentrations de 4.92, 8.78, 15.68, 28 et 50 mg/mL. Toutes les formulations ont été réalisées sous une hotte à flux laminaire.

- Stockage : toutes les préparations sont conservées à température ambiante et à l'abri de la lumière dans des récipients en verre fumé. Elles ont été utilisées approximativement 7 heures après leur formulation.

- Fréquence des préparation : les formulations sont réalisées à chaque jour de traitement.

4.3. Méthodes et modèle expérimental

Les méthodes et le modèle expérimental utilisé sont détaillés dans le protocole original n°935/080-D, disponible dans le dossier d'essai.

4.4. Résultats

* *Etude préliminaire*

L'étude préliminaire a été conduit utilisant la souche TA100, avec ou sans activation métabolique, afin d'évaluer la cytotoxicité du produit à l'essai et la présence de précipités dans les mélanges préparés sur une échelle de 52, 164, 512, 1 600 et 5 000 ug/boîte (progression d'un demi log).

- **évaluation d'un précipité** : après incorporation des préparations dans une couche d'agar (top agar), aucun précipité n'a été observé.

- **évaluation de la cytotoxicité** : aucun signe de cytotoxicité, tel qu'une réduction du tapis bactérien et/ou une diminution dose-dépendante du nombre de colonies initiales, n'est à noter.

Le nombre moyen de colonies initiales des boîtes de contrôle négatif est du même ordre de grandeur que les données de référence du contrôle négatif et la fréquence des colonies initiales augmente de façon significative dans les boîtes de contrôle positif. Aucune boîte de pétri n'a subi de contamination ou de détérioration due à une réaction parasite. Tous les critères de validation sont réunis. En l'absence de cytotoxicité, ces résultats sont utilisés en tant que données de potentiel mutagène pour la souche TA100 dans l'expérimentation 1.

* *Expérimentation 1*

Se basant sur les résultats obtenus au cours de l'étude préliminaire, le traitement est effectué avec 52, 164, 512, 1 600 et 5 000 ug/boîte soit des intervalles d'un demi log entre les doses.

Le nombre moyen de colonies initiales des boîtes de contrôle négatif est du même ordre de grandeur que les données de référence du contrôle négatif et la fréquence des colonies initiales augmente de façon significative dans les boîtes de contrôle positif. Aucune boîte de pétri n'a subi de contamination ou de détérioration due à une réaction parasite. Tous les critères de validation étant réunis, cette expérimentation est considérée comme valable et les données sont validées.

- **évaluation d'un précipité** : après incorporation des préparations dans une couche d'agar (top agar), aucun précipité n'a été observé.

- **évaluation de la cytotoxicité** : aucun signe de cytotoxicité, tel qu'une réduction du tapis bactérien et/ou une diminution dose-dépendante du nombre des colonies initiales, n'est à noter.

- **décompte des mutants** :

* **sans activation métabolique** (Table 1) : aucune augmentation statistiquement significative du nombre des colonies initiales, comparativement avec les données du contrôle négatif (véhicule), n'est observée avec les 5 souches utilisées (TA98, TA100, TA1535, TA1537 et TA102).

Toutes les valeurs moyennes étaient dans les mêmes ordres de grandeur que les données de référence du contrôle négatif.

* **avec activation métabolique** (Table 2) : aucune augmentation statistiquement significative du nombre des colonies initiales, comparativement avec les données du contrôle négatif (véhicule), n'est observée avec les souches TA98, TA100, TA1537 et TA102.

Une augmentation statistiquement significative ($p < 0.05$) du nombre de colonies initiales est notée avec la souche TA1535, avec une augmentation maximum de 1.46 fois plus par rapport au contrôle négatif pour le mélange 1 600 ug/boîte.

Toutes les valeurs moyennes étaient dans les mêmes ordres de grandeur que les données de référence du contrôle négatif.

* *Expérimentation 2*

Se basant sur les résultats de l'expérimentation 1, le second traitement, indépendant du premier, est effectué en utilisant une échelle plus serrée de concentration des mélanges (492, 878, 1 568, 2 800 et 5 000 ug/boîte) afin d'estimer, le plus précisément possible, quelles doses peuvent provoquer des effets mutagéniques.

Le nombre moyen de colonies initiales des boîtes de contrôle négatif est du même ordre de grandeur que les données de référence du contrôle négatif et la fréquence des colonies initiales augmente de façon significative dans les boîtes de contrôle positif. Aucune boîte de pétri n'a subi de contamination ou de détérioration due à une réaction parasite. Tous les critères de validation étant réunis, cette expérimentation est considérée comme valable et les données sont validées.

- **évaluation d'un précipité** : après incorporation des préparations dans une couche d'agar (top agar), aucun précipité n'a été observé.

- **évaluation de la cytotoxicité** : aucun signe de cytotoxicité, tel qu'une réduction du tapis bactérien et/ou une diminution dose-dépendante du nombre de colonies initiales, n'est à noter.

- **décompte des mutants** :

* **sans activation métabolique** (Table 3) : aucune augmentation statistiquement significative du nombre des colonies initiales, comparativement avec les données du contrôle négatif (véhicule), n'est observée avec les souches TA98, TA100, TA1537 et TA102.

Une augmentation statistiquement significative du nombre des colonies initiales est notée avec la souche TA1535 à 492 ug/mL ($p<0.05$) et à 5 000 ug/mL ($p<0.01$). Ces augmentations étaient de, respectivement, 1.51 et 1.74 fois supérieures aux valeurs des contrôles négatifs correspondants. A noter qu'aucun effet n'est observé aux concentrations intermédiaires.

Toutes les valeurs moyennes étaient dans les mêmes ordres de grandeur que les données de référence du contrôle négatif.

* **avec activation métabolique** (Table 4) : aucune augmentation statistiquement significative du nombre des colonies initiales, comparativement avec les données du contrôle

négatif (véhicule), n'est observée avec les 5 souches utilisées (TA98, TA100, TA1535, TA1537 et TA102).

Toutes les valeurs moyennes étaient dans les mêmes ordres de grandeur que les données de référence du contrôle négatif.

4.5. Discussion

Conformément aux guides, le potentiel mutagène a été évalué, jusqu'à la dose maximale exigée, dans une fourchette de 52 à 5 000 ug/boîte. Deux expérimentations indépendantes l'une de l'autre ont été réalisées avec ou sans activation métabolique.

Le nombre moyen de colonies initiales des boîtes de contrôle négatif était du même ordre de grandeur que les données de référence du contrôle négatif et la fréquence des colonies initiales augmentait de façon significative dans les boîtes de contrôle positif. Aucune boîte de pétri n'a subi de contamination ou de détérioration due à une réaction parasite. Tous les critères de validation étant réunis, cette étude est considérée comme valable et les données sont validées.

Aucun signe de cytotoxicité, tel qu'une réduction du tapis bactérien et/ou une diminution dose-dépendante du nombre de colonies initiales, n'est à noter. Aucun précipité n'a été observé et ce pour toutes les concentrations testées.

Les augmentations statistiquement significatives du nombre de colonies initiales ne sont signalées que pour la souche TA1535 en présence d'une activation métabolique (+S9) dans l'expérience 1 et en l'absence d'une activation métabolique (-S9) dans l'expérience 2.

L'augmentation maximale était de 1.74 fois supérieure au contrôle négatif correspondant, à 5 000 ug/boîte dans l'expérience 2 (-S9), mais ce phénomène n'apparaissait pas lors de l'expérience 1 (-S9) pourtant dans les mêmes conditions de concentration. Les deux autres augmentations statistiquement significatives ne sont pas dose-dépendantes puisqu' aucun effet biologique n'est signalé à des niveaux de concentrations supérieures. Toutes les valeurs moyennes étaient dans les mêmes ordres de grandeur que les données de références du contrôle négatif.

En conséquence, les résultats statistiquement significatifs apparaissant pour la souche TA1535 ne correspondent pas aux critères de définition d'un effet mutagène et ne sont donc pas considérés comme biologiquement pertinents.

Pour les souches TA98, RA100, TA1537 et TA102, aucune augmentation du nombre de colonies initiales, liée aux différents traitements, n'est notée, que ce soit en présence ou en l'absence d'une activation métabolique.

4.6. Conclusion

Testé jusqu'à la dose maximale exigée (5 000 ug/boîte), le produit à l'essai n'a induit aucune augmentation biologiquement pertinente du nombre de colonies initiales chez les 5 souches utilisées, ni en présence ni en l'absence d'activation métabolique.

Dans les conditions expérimentales de l'étude et selon les critères de la procédure, le produit **solution à 8% YSALINE 100 n'a pas induit d'effet mutagène lors du test AMES** en présence ou en l'absence d'activation métabolique.

English Translation 3F

Algues & Mer Received 2020

Summary Toxicologie (studies done on Asparagopsis Armata Extract) (concentrations stated in the study summaries represent the percent of dry algal matter)

1. Data prior to a clinical trial of skin tolerance to a 4% (concentration of dry algal matter) solution of Ysaline 100 (Asparagopsis Armata Extract)

1.1 Objective

Local tolerance was studied in a model of EPISKIN reconstructed human epidermis. It was evaluated by a histological examination of the effects of the test product on the architecture of the epidermis. The information provided by this model mainly concerns the prediction of the risk of irritation.

1) acute, since repeated application of the test product to the reconstructed epidermis has not been made
2) direct, since the other toxicity components, such as the immune component, are not modeled
3) epidermal, since the EPISKIN model does not have an equivalent dermis

1.2 Experimental Realization

* Test product: 4% solution of YSALINE 100 from the company ALGUES ET MER.
* Reference product: sodium lauryl sulfate (SLS), reference surfactant, from the company SIGMA.
* Reagents: All reagents are of analytical quality. Their references (supplier, catalog number, batch number) are kept in the test file within BIOPREDIC.
The water came from a MilliQ type purification system (Millipore).
The solution for rinsing the epidermis after incubation was PBS buffer (reagent no. BIOPREDIC 98B).
*Test system: EPISKIN reconstructed epidermis (lot 96-EPIS-006) was supplied by IMEDEX. They were used upon receipt. The nacelles comprising the epidermis were directly placed in culture wells which contained the incubation medium, corresponding to the "test medium" (MIE), supplied by IMEDEX with the epidermis and of composition not specified.
*Preparation of the test product and the reference product, incubation with the test system:

Test Product	**Concentration**	**Diluent**	**Volume Deposited**	**Remarks**
Without	0	Water MilliQ	200 µL	Witness
4% Ysaline 100	10%	Water MilliQ	200 µL	None
SLS	1.5 mg/L	Water MilliQ	200 µL	None

The epidermis was incubated for 18 hours at 37 ° C in an atmosphere containing 5% CO_2
The tests were carried out in duplicate.

*Effects evaluation: at the end of the incubation, the epidermis was thoroughly rinsed with PBS buffer and cut out using a scalpel.
The epidermal discs thus obtained (diameter 12 mm) were used for the histological study.

*Preparation of histological sections: the epidermis used for the histological examination were treated according to the following technique:
- fixation in a 10% formalin solution in water for 12 hours at room temperature
- dehydration by passage through alcohols of increasing titer, then in toluene, and inclusion in paraffin
- production of paraffin blocks on a cooling plate
- block cuts (three blades per block and two cutting planes per blade)
- staining with hematoxylin / eosin / saffron.

*Microscopic examination: all the section plans were examined six times, in total, on different days and at the same magnification. The following morphological alterations were investigated by an anatomical pathologist, expert in toxicology:
- superficial lesions, located in the upper half of the epidermis:
- intercellular micro- or macro-vesicles
- supra-basal intraepidermal cleavage
- intracellular microvacuoles
- superficial cellular necrosis
- deep lesions, located in the lower half of the epidermis:
- detachment of the epidermis from its support
- subcutaneous intraepidermal cleavage
- basal cell necrosis.

*Data processing: depending on the type and number of morphological alterations observed, four scores are assigned:
0 => no alteration
+ => minor alterations
++ => moderate alterations
+++ => major alterations.

*Data quality: BIOPREDIC has a quality assurance system (audited by the Medicines Agency for testing activities on new drugs), which guarantees the reliability of the data obtained. All test data are archived in BIOPREDIC for ten years after the test and are available to the sponsor.

1.3. Summary of results – CONCLUSIONS

Test product: 4% solution of YSALINE 100 (tested at 10%) - (w / v)

Test system: reconstructed epidermis, EPISKIN, lot 96-EPSI-006

Histological observations of the epidermis incubated for 18 hours with the products

Product	Concentration Tested	Morphologic Alterations
Without	-	0
SLS reference product	1.5 mg.ml	++
Test product	10%	0

Under the experimental conditions adopted, the 4% YSALINE 100 Solution product does not cause histological changes.

2. Predictive study of the ocular tolerance of the 4% solution of YSALINE 100 by the PREDISAFE method

   2.1 Introduction

   The 6th amendment to EEC Directive No. 93/35 of June 14, 1993 provides that animal testing will be replaced on January 1, 1998 "if there has been sufficient progress in developing methods that can replace satisfactorily to animal testing. "

   Validation studies of these alternative methods are necessary in order to offer the consumer a degree of protection equivalent to the current level in terms of toxicity tests. A satisfactory correlation with animal tests and acceptable inter / intralaboratory reproducibilities are therefore essential.

   BIOPREDIC has developed an in vitro method for the evaluation of ocular tolerance called PREDISAFE. PREDISAFE is included in several interlaboratory studies: that of the National Health Laboratory (1993), which made it possible to classify PREDISAFE first among five other in vitro methods, to distinguish between corrosive and non-corrosive products, and that of COLIPA (1994), which confirmed the reliability of the method.

   In this study, it was a question of assessing the ocular tolerance of the product: 4% solution of YSALINE 100 by the PREDISAFE method.

   2.2 Materials and Methods

   *Test product: 4% solution of YSALINE 100 from the company ALGUES ET MER.
   *Carrying out the test: the PREDISAFE test was carried out using a PREDISAFE kit, lot n ° MW080698, supplied by BIOPREDIC International. All the procedures indicated by the supplier were followed and the reagents contained in this kit were used.
   *Data processing: the data has been processed according to the method indicated in the user manual.
   * Validation of the experiment: various quality controls, described in the user manual, are carried out during the experiment in order to validate the test.

   2.3 Results

   Test product: 4% solution of YSALINE 100 (tested pure)

Test system: PREDISAFE kit, lot n ° MW080696

Validation of the experiment

Neutral red retention: 0.678 DO	YES
Coefficient of variation: 0.009%	YES

Sensitivity of cells to reference products: classification on a cytotoxicity scale from 0 to 50 (comparable to the ocular tolerance scale)

Tween 20	<15	Yes
n-butanol	43	Yes
SLS	>50	Yes

Classification of the product from the point of view of cytotoxicity according to the PREDISAFE method

<15 on a scale of 0 to >50

2.4 Discussion

The ocular tolerance of the 4% solution product of YSALINE 100 was evaluated according to the PREDISAFE in vitro method. This method demonstrated a high level of correlation compared to the Draize test, and of reproducibility.

The tolerance of the 4% solution product of YSALINE 100 was less than 15 on an eye tolerance scale arbitrarily graduated from 0 to 50 (like that used in the Draize test for eye tolerance).

This score corresponds to class I (0 <or = to 15) "slightly irritant products".

Predictive classification of the test product in terms of ocular tolerance
Ranking on a scale of 0 to> 50
<15
Classification: Class I slightly irritating

“Benchmarking”

Comparison of the product under test, in terms of ocular tolerance, compared to products with the same physicochemical characteristics or the same use.
This classification is based on data collected by BIOPREDIC.

Tolerance under normal conditions of use
Average

3. Study of the skin tolerance in vivo on humans of the product 4% solution of YSALINE 100

3.1 Objective

In this study, it was a question of determining the skin tolerance of a cosmetic ingredient in vivo in human volunteers, in an approved center.

3.2 Experiment Realization

*Ingredient studied: 4% solution of YSALINE 100

*Material: Finn Chambers on Scanpor patch tests or isolation chambers which ensure good occlusion were used. These are aluminum cups 8 mm in diameter, with a capacity of 20 µL and covering an area of 50 mm^2.

*Selection of volunteers: ten healthy volunteers were selected according to the following inclusion and non-inclusion criteria:

Inclusion criteria:

- Person between 18 and 60 years old

- Female or male

- Person having given free, informed and express consent in writing

- Cooperating person, warned of the need and duration of controls, which gives hope of perfect adherence to the protocol in place

- Caucasian type

- Phototype: II - III - IV

Non-inclusion criteria:

- Pregnant or lactating woman

- Person with "hyper-irritable" skin

- Person allergic to sticking plaster

- Skin pathology in the experimentarea

- Presence of drug treatment - immunosuppressant, corticosteroid, antihistamine or anti-inflammatory - during the week preceding and during the study period

- Person with a serious or progressive condition

- Use of dermopharmaceutical or cosmetic products, in the experimental areas, during the study period.

*Patch tests: the product has been tested diluted to 10%. It was placed in the cup. The patch tests were applied to the left shoulder area of the back of the volunteers and covered with

"Micropore" type adhesive to improve adhesion. Patch tests without product were carried out in parallel.

*Assessment of skin tolerance: forty-eight hours after the patch tests were applied, they were peeled off. Thirty minutes later, the possible signs of irritation were observed by a dermatologist and rated on a scale of 0 to 3 according to the following rating:

	Erythema	Edema	Dryness	Vesicles
Absent	E0	O0	S0	V0
Lightweight	E1	O1	S1	V1
Net	E2	O2	S2	V2
Important	E3	O3	S3	V3

3.3 Results

Test product: 4% solution of YSALINE 100 (tested diluted to 10%)

Volunteer	Age	Gender	Medico-history Past events Rating that may influence the study period during the tolerance period course of the study	Events that occurred during the period of the study	Quotation tolerance on a scale of 0 to 3
1	22	F	Nil	Nil	0
2	21	F	Nil	Nil	0
3	20	F	Nil	Nil	0
4	21	F	Nil	Nil	0
5	54	M	Nil	Nil	0
6	21	F	Nil	Nil	0
7	23	F	Nil	Nil	0
8	25	F	Nil	Nil	0
9	23	F	Nil	Nil	0
10	23	F	Nil	Nil	0

3.4 Discussion

The skin tolerance in vivo in humans of the 4% solution product of YSALINE 100 tested diluted to 10%, was studied after an 48-hour occlusive patch on ten volunteers.

The 4% solution product of YSALINE 100 did not cause any reaction for the 10 volunteers.

In conclusion, in this study, with the limits linked to the protocol (ie inclusion of the limited number of 10 volunteers), the 4% solution product of YSALINE 100 showed that it was not irritant in humans.

4. Study of the mutagenic effect of an 8% (concentration of dried algal matter) YSALINE 100 solution

4.1 Introduction

The objective of this study was to assess the mutagenic potential of the 8% YSALINE 100 solution product using 5 histidine-dependent strains of *Salmonella typhimurium*, in the presence or absence of a metabolic activation system.

The study protocol (n ° 935/080-D of 25/02/1999) is inspired by the guides OECD 471, FDA Redbook II, EEC guideline 92/69 and EPA section 798 chapter 798.5265.

4.2 Information on the product under test, the negative control and the vehicle

*Test product

- name: 8% solution of YSALINE 100
- provenance: ALGUES ET MER company
- appearance: colorless liquid
- lot number: AW9805
- purity: estimated at 100%
- density: 0.96 (data taken into account for calculating the dose)
- storage: at room temperature and protected from light
- intended therapeutic use: cosmetic sector.

*Vehicle and negative control

- name: water for injection
- provenance: BIOSEDRA
- lot number: LR82274, LR85139 and 3026
- dangers: none

*Metabolic activation system

- identification: freeze-dried S9 fraction from the liver of rats treated with a mixture of phenobarbital and methylcholanthrene.
- provenance: Iffa-Credo, France
- lot number: SL 63 (expiration date: July 31, 1999)
- solvent: water for injection (BIOSEDRA)

All preparations were used on the day of their formulation.

*Positive controls

Reagents	**Strains**	**Dose (µ/plate)**
2-Nitroflorene (2-NF)	TA98	5
t-Butyl hydroperoxide (t-BHP)	TA102	100
9-Aminoacridine (9-AA)	TA1537	50

Sodium azide (NaA)	TA100 and TA1535	10
2-Aminoanthracène (2-A)	all 5 strains have a syst. metabolic activation	5

The references (supplier, catalog number, batch number) are kept in the test file.

*Preparation of the product for testing

-Solubility evaluation: the test product gives a clear solution in water at 50 mg / mL, the water used corresponding to the vehicle selected for the study.
- Preparation: the test product is prepared, by serial dilution from the initial stock, at concentrations of 0.52, 1.64, 5.12, 16 and 50 mg / mL (preliminary study and experiment 1). For Experiment 2, the test product is prepared at the concentrations of 4.92, 8.78, 15.68, 28 and 50 mg / mL. All the formulations were carried out under a laminar flow hood.
- Storage: all preparations are stored at room temperature and protected from light in smoked glass containers. They were used approximately 7 hours after their formulation.
- Frequency of preparation: formulations are made on each treatment day.

4.3 Methods and experimental model

The methods and the experimental model used are detailed in the original protocol n ° 935/080-D, available in the test file.

4.4 Results

*Preliminary study

The preliminary study was conducted using the TA100 strain, with or without metabolic activation, in order to assess the cytotoxicity of the test product and the presence of precipitates in the mixtures prepared on a scale of 52, 164, 512, 1 600 and 5,000 ug / box (progression of half a log).
- evaluation of a precipitate: after incorporation of the preparations in a layer of agar (top agar), no precipitate was observed.
- evaluation of cytotoxicity: no sign of cytotoxicity, such as a reduction in the bacterial carpet and / or a dose-dependent reduction in the number of initial colonies, should be noted.

The average number of initial colonies in the negative control dishes is of the same order of magnitude as the reference data for the negative control and the frequency of initial colonies increases significantly in the positive control dishes. No petri dish was contaminated or deteriorated due to a parasitic reaction. All the validation criteria are met. In the absence of cytotoxicity, these results are used as mutagenic potential data for the TA100 strain in experiment 1.

*Experiment 1

Based on the results obtained during the preliminary study, the treatment is carried out with 52, 164, 512, 1600 and 5000 µg / plate, ie half log intervals between doses.

The mean number of initial colonies in the negative control dishes is of the same order of magnitude as the reference data for the negative control and the frequency of initial colonies increases significantly in the positive control dishes. No petri dish was contaminated or deteriorated due to a parasitic reaction. All the validation criteria being met, this experiment is considered valid and the data is validated.

- evaluation of a precipitate: after incorporation of the preparations in a layer of agar (top agar), no precipitate was observed.
- evaluation of cytotoxicity: no sign of cytotoxicity, such as a reduction in the bacterial carpet and / or a dose-dependent reduction in the number of initial colonies, should be noted.
- count of mutants:
* without metabolic activation (Table 1): no statistically significant increase in the number of initial colonies, compared with the negative control data (vehicle), is observed with the 5 strains used (TA98, TA100, TA1535, TA1537 and TA102) .
All the mean values were in the same orders of magnitude as the reference data of the negative control.
* with metabolic activation (Table 2): no statistically significant increase in the number of initial colonies, compared with data from the negative control (vehicle), is observed with strains TA98, TA100, TA1537 and TA102.
A statistically significant increase ($p < 0.05$) in the number of initial colonies is noted with the strain TA1535, with a maximum increase of 1.46 times more compared to the negative control for the mixture 1600 ug / dish.
All the mean values were in the same orders of magnitude as the reference data of the negative control.

*Experiment 2

Based on the results of Experiment 1, the second treatment, independent of the first, is carried out using a tighter scale of concentration of the mixtures (492, 878, 1,568, 2,800 and 5,000 ug / plate) in order to d estimate, as precisely as possible, which doses can cause mutagenic effects.

The mean number of initial colonies in the negative control dishes is of the same order of magnitude as the reference data for the negative control and the frequency of initial colonies increases significantly in the positive control dishes. No petri dish was contaminated or deteriorated due to a parasitic reaction. All the validation criteria being met, this experiment is considered valid and the data are validated.

- evaluation of a precipitate: after incorporation of the preparations in a layer of agar (top agar), no precipitate was observed.

- evaluation of cytotoxicity: no sign of cytotoxicity, such as a reduction in the bacterial carpet and / or a dose-dependent reduction in the number of initial colonies, should be noted.
- count of mutants:
* without metabolic activation (Table 3): no statistically significant increase in the number of initial colonies, compared with the negative control data (vehicle), is observed with strains TA98, TA100, TA1537 and TA102.

A statistically significant increase in the number of initial colonies is noted with the strain TA1535 at 492 µg / mL ($p < 0.05$) and at 5000 µg / mL ($p < 0.01$). These increases were 1.51 and 1.74 times, respectively, greater than the values of the corresponding negative controls. Note that no effect is observed at intermediate concentrations.

All the mean values were in the same orders of magnitude as the reference data of the negative control.

*with metabolic activation (Table 4): no statistically significant increase in the number of initial colonies, compared with the control data
negative (vehicle), is only observed with the 5 strains used (TA98, TA100, TA1535, TA1537 and TA102).
All the mean values were in the same orders of magnitude as the reference data of the negative control.

4.5 Discussion

In accordance with the guidelines, the mutagenic potential was assessed, up to the maximum dose required, in a range of 52 to 5,000 µg / plate. Two independent experiments were carried out with or without metabolic activation.

The mean number of initial colonies from the negative control dishes was of the same order of magnitude as the reference data from the negative control and the frequency of initial colonies increased significantly in the positive control dishes. No petri dish was contaminated or deteriorated due to a parasitic reaction. All the validation criteria being met, this study is considered valid and the data are validated.

No sign of cytotoxicity, such as a reduction in the bacterial carpet and / or a dose-dependent reduction in the number of initial colonies, is to be noted. No precipitate was observed for all the concentrations tested.

Statistically significant increases in the number of initial colonies are only reported for strain TA1535 in the presence of metabolic activation (+ S9) in experiment 1 and in the absence of metabolic activation (-S9) in experience 2.

The maximum increase was 1.74 times greater than the corresponding negative control, at

5,000 µg / plate in experiment 2 (-S9), but this phenomenon did not appear during experiment 1 (-S9), however, under the same concentration conditions. The other two statistically significant increases are not dose dependent since no biological effects are reported at higher concentration levels. All the mean values were in the same orders of magnitude as the reference data of the negative control.

Consequently, the statistically significant results appearing for the TA1535 strain do not correspond to the criteria for defining a mutagenic effect and are therefore not considered to be biologically relevant.

For strains TA98, TA100, TA1537 and TA102, no increase in the number of initial colonies, linked to the different treatments, is noted, whether in the presence or in the absence of metabolic activation.

4.6 Conclusion

Tested up to the maximum dose required (5,000 ug / box), the test product did not induce any biologically relevant increase in the number of initial colonies in the 5 strains used, neither in the presence or in the absence of metabolic activation.

Under the experimental conditions of the study and according to the criteria of the procedure, the 8% solution product YSALINE 100 did not induce a mutagenic effect during the AMES test in the presence or in the absence of metabolic activation.

Personal Care Products Council
Committed to Safety, Quality & Innovation

Memorandum

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

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

DATE: April 9, 2020

SUBJECT: Human Repeat Insult Patch Tests on Products Containing Red Algae-Derived Ingredients

Clinical Research Laboratories, Inc. 2013. Repeated insult patch test (tested product contained 0.0028% Gelidiella Acerosa Extract).

Eurofins CRL. 2019. Repeated insult patch test (product contains 0.49% Chondrus Crispus Extract).

Clinical Research Laboratories, Inc. 2012. Repeated insult patch test (product contains 0.325% Asparagopsis Armata Extract).

Clinical Research Laboratories, Inc. 2018. Repeated insult patch test (product contains 0.0004% Porphyra Umbilicalis Extract).

Clinical
Research
Laboratories, Inc.

Final Report

Repeated Insult Patch Test

Tested product contained 0.028%
Gelidiella Acerosa Extract

CLIENT: [redacted]

ATTENTION: [redacted]

TEST MATERIAL: [redacted]

CRL STUDY NUMBER: [redacted]

AUTHORIZED SIGNATURES:

Bruce E. Kanengiser, M.D.
President/Medical Director

Michael J. Muscatiello, Ph.D.
Executive Vice President/COO

Anita Lee Cham, M.D.
Dermatologist

REPORT DATE: March 11, 2013

371 Hoes Lane • Piscataway, NJ 08854 • (732) 981-1616 • FAX (732) 981-0520

Clinical Research Laboratories, Inc.

Good Clinical Practice
Quality Assurance Audit Statement

Clinical Study Number: [redacted]

Start Date: January 23, 2013

Completion Date: March 1, 2013

The clinical study listed above was conducted in accordance with Clinical Research Laboratories, Inc. Standard Operating Procedures, which incorporate the principles of Good Clinical Practice defined by applicable guidelines and regulations established by U.S. Regulatory Agencies. The conduct of the study was monitored for compliance, and the associated records, including source documents or raw data, were reviewed for documentation practices and accuracy by a Project Manager/Study Director and/or a Quality Assurance Representative. Standard Quality Assurance audit procedures for this final report and study related documents were conducted.

Karen Hayley
Signature of QA Auditor

March 11, 2013
Date

Clinical Research Laboratories, Inc.

FINAL REPORT

REPEATED INSULT PATCH TEST

PURPOSE

The purpose of this study was to determine the dermal irritation and sensitization potential of a test material.

INVESTIGATIVE SITE

Clinical Research Laboratories, Inc.
371 Hoes Lane, Suite 100
Piscataway, New Jersey 08854
732-981-1616

TEST MATERIAL

The following test material was provided by [redacted] and was received by Clinical Research Laboratories, Inc. on January 17, 2013:

Test Material	Test Condition	Patch Type
[redacted]	Test as Received	Occlusive*

The test material was coded with the following CRL identification number:

[redacted]

STUDY DATES

This study was initiated on January 23, 2013 and was completed on March 1, 2013.

* Occlusive Strip with Flexcon® (Strukmyer, LLC, Mesquite, TX or equivalent)

PANEL SELECTION

Each subject was assigned a permanent CRL identification number. All subjects signed an Informed Consent Form in compliance with 21 CFR Part 50: "Protection of Human Subjects" and a HIPAA Authorization Form in compliance with 45 CFR Parts 160 and 164. All subjects completed a Subject Profile/Medical History Form provided by Clinical Research Laboratories, Inc. prior to the study (Subject Demographics - Appendix I). Subjects who met the following Inclusion Criteria and none of the Exclusion Criteria were impaneled:

Inclusion Criteria

a. Male and female subjects between the ages of 18 and 70 years;

b. Subjects who do not exhibit any skin diseases which might be confused with a skin reaction from the test material;

c. Subjects who agree to avoid exposure of the test sites to the sun and to refrain from visits to tanning salons during the course of this study;

d. Subjects who agree to refrain from getting patches wet during the course of the study;

e. Subjects willing to sign an Informed Consent in conformance with 21CFR Part 50: "Protection of Human Subjects;"

f. Subjects who have completed a HIPAA Authorization Form in conformance with 45CFR Parts 160 and 164;

g. Subjects in generally good health who have a current Subject Profile/Medical History on file;

h. Subjects who are dependable and able to follow directions as outlined in the protocol.

Exclusion Criteria

a. Female subjects who are pregnant or nursing;

b. Subjects who are currently using any systemic or topical corticosteroids, anti-inflammatory drugs, or antihistamines on a regular basis;

c. Subjects who report allergies to cosmetics, toiletries or personal care products.

d. Subjects exhibiting any skin disorder, sunburn, scars, excessive tattoos, etc. in the test area.

TEST METHOD

Prior to the application of the patch, the test area was wiped with 70% isopropyl alcohol and allowed to dry. The test material, which was prepared as described in the Test Material section of the report, was applied to the upper back (between the scapulae) and was allowed to remain in direct skin contact for a period of 24 hours.

Patches were applied to the same site on Monday, Wednesday, and Friday for a total of 9 applications during the Induction Period. This schedule may have been modified to allow for missed visits or holidays. If a subject was unable to report on an assigned test date, the test material was applied on 2 consecutive days during the Induction Phase and/or a makeup day was added at the end of the Induction Phase.

The sites were graded by a CRL technician for dermal irritation 24 hours after removal of the patches by the subjects on Tuesday and Thursday and 48 hours after removal of the patches on Saturday, unless the patching schedule was altered as described above.

The sites were graded according to the following scoring system:

Dermal Scoring Scale

0 No visible skin reaction
± Barely perceptible erythema
1+ Mild erythema
2+ Well defined erythema
3+ Severe erythema and edema
4+ Erythema and edema with vesiculation

If a "2+" reaction or greater occurred, the test material was applied to an adjacent virgin site. If a "2+" reaction or greater occurred on the new site, the subject was not patched again during the Induction Phase but was challenged on the appropriate day of the study. At the discretion of the Study Director, patch sites with scores less than a "2+" may have been changed.

Following approximately a 2-week rest period, the challenge patches were applied to previously untreated test sites on the back. After 24 hours, the patches were removed by a CRL technician and the test sites were evaluated for dermal reactions. The test sites were re-evaluated at 48 and 72 hours. Subjects exhibiting reactions during the Challenge Phase of the study may have been asked to return for a 96-hour reading.

RESULTS

This study was initiated with 112 subjects. Six subjects discontinued study participation for reasons unrelated to the test material and one subject was disqualified for non - compliance. A total of 105 subjects completed the study.

Individual dermal scores recorded during the Induction and Challenge Phases appear in Table I.

CONCLUSION

Based on the test population of 105 subjects and under the conditions of this study, the test material identified as [redacted] did not demonstrate a potential for eliciting dermal irritation or sensitization.

RETENTION

Test materials and all original forms of this study will be retained by Clinical Research Laboratories, Inc. as specified in CRL Standard Operating Procedures 30.6 and 30.6C, unless designated otherwise by the Sponsor.

TABLE I

Summary of Dermal Scores

Test Material: [redacted]

Subject Number	Induction Scores									Challenge Scores		
	1	2	3	4	5	6	7	8	9	24 Hour	48 Hour	72 Hour
1	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
3	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
5	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
7	0	0	0	0	0	0	0	0	0	0	0	0
8	0	0	0	0	0	0	0	1+	±	±	1+	1+*
9	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
11	0	0	0	0	0	0	0	0	0	0	0	0
12	Discontinued											
13	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
15	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
17	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
19	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
21	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
23	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
25	0	0	0	0	0	0	0	0	0	0	0	0

*A reaction of ± was observed at the 96 hour evaluation

TABLE I
(Continued)

Summary of Dermal Scores

Test Material: [redacted]

Subject Number	Induction Scores									Challenge Scores		
	1	2	3	4	5	6	7	8	9	24 Hour	48 Hour	72 Hour
26	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
28	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
30	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
32	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
34	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
36	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
38	Discontinued											
39	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
41	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
43	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
45	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
47	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
49	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

Clinical Research Laboratories, Inc.

TABLE I
(Continued)

Summary of Dermal Scores

Test Material: [redacted]

Subject Number	Induction Scores									Challenge Scores		
	1	2	3	4	5	6	7	8	9	24 Hour	48 Hour	72 Hour
51	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
53	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
55	Discontinued											
56	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
58	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
60	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
62	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
64	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
66	0	0	0	0	0	0	0	0	0	0	0	0
67	0	0	0	0	0	0	0	0	0	0	0	X*
68	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
70	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
72	0	0	0	0	0	0	0	0	0	0	0	0
73	0	Discontinued										
74	0	0	0	0	0	0	0	0	0	0	0	0
75	0	0	0	0	0	0	0	0	0	0	X	0*

X= Subject Absent
*No reaction was observed at the 96 hour evaluation

TABLE I
(Continued)

Summary of Dermal Scores

Test Material: [redacted]

Subject Number	Induction Scores									Challenge Scores		
	1	2	3	4	5	6	7	8	9	24 Hour	48 Hour	72 Hour
76	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
78	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
80	0	0	0	0	0	0	Discontinued					
81	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
83	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
85	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
87	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
89	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
91	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
93	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
95	Disqualified											
96	0	0	0	0	0	0	0	0	0	0	0	0
97	0	Discontinued										
98	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
100	0	0	0	0	0	0	0	0	0	0	0	0

TABLE I
(Continued)

Summary of Dermal Scores

Test Material:	[redacted]

Subject Number	Induction Scores									Challenge Scores		
	1	2	3	4	5	6	7	8	9	24 Hour	48 Hour	72 Hour
101	0	0	0	0	0	0	0	0	0	0	X	0*
102	0	0	0	0	0	0	0	0	0	0	0	0
103	0	0	0	0	0	0	0	0	0	0	0	0
104	0	0	0	0	0	0	0	0	0	0	0	0
105	0	0	0	0	0	0	0	0	0	0	0	0
106	0	0	0	0	0	0	0	0	0	0	0	0
107	0	0	0	0	0	0	0	0	0	0	0	0
108	0	0	0	0	0	0	0	0	0	0	0	0
109	0	0	0	0	0	0	0	0	0	0	0	0
110	0	0	0	0	0	0	0	0	0	0	0	0
111	0	0	0	0	0	0	0	0	0	0	0	0
112	0	0	0	0	0	0	0	0	0	0	0	0

X = Subject Absent
*No reaction was observed at the 96 hour evaluation

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Appendix I

Subject Demographics

Subject Number	Subject Initials	CRL ID #	Age	Sex
1	JB	14759	61	F
2	JD	22089	34	M
3	MS	29053	40	F
4	VL	26981	48	F
5	BC	20385	47	F
6	LW	15333	43	F
7	JS	31190	43	M
8	DM	19176	51	F
9	JM	28964	28	F
10	SB	29429	40	F
11	AF	29064	47	F
12	DH	31154	52	F
13	EP	28178	35	F
14	JM	30980	65	M
15	KM	00302	59	F
16	WM	19523	55	F
17	BH	30605	50	F
18	RN	29323	56	F
19	RB	30093	67	F
20	DC	07247	51	F
21	RM	28735	56	F
22	VM	28951	64	F
23	SH	29051	46	F
24	JE	31060	48	F
25	AN	23619	45	M
26	CD	10733	52	F
27	DB	30672	61	F
28	LF	11640	42	F

Subject Number	Subject Initials	CRL ID #	Age	Sex
29	PC	05613	67	F
30	CD	22327	60	F
31	CS	17817	31	F
32	RD	21017	51	F
33	CJ	31053	51	F
34	EC	27743	32	F
35	JH	23464	66	F
36	RB	16803	53	M
37	AJ	23193	50	F
38	SB	23930	67	F
39	EA	30313	54	F
40	SR	30890	53	F
41	SS	25766	43	F
42	BS	25765	66	F
43	MR	18041	53	F
44	LB	29706	70	F
45	RA	23557	41	F
46	BS	05985	48	F
47	LG	27694	70	M
48	PG	27693	61	F
49	IA	29720	61	F
50	JL	31232	44	M
51	ML	20303	68	F
52	GA	31168	66	F
53	JA	05348	63	F
54	WP	03329	45	F
55	LG	19370	48	F
56	MP	30643	41	F

Appendix I

Subject Demographics (Continued)

Subject Number	Subject Initials	CRL ID #	Age	Sex
57	AV	27666	65	F
58	PB	27808	55	F
59	MR	10668	70	F
60	AG	21336	40	F
61	CJ	22452	19	M
62	DM	13440	47	F
63	CS	30893	45	F
64	MM	15953	20	M
65	SH	17712	53	M
66	CC	05998	57	F
67	ML	30397	47	F
68	DG	26155	47	F
69	KB	30245	54	F
70	TG	08029	50	F
71	RC	28317	44	F
72	IM	13453	70	F
73	KH	29028	33	F
74	AL	21695	50	F
75	LH	26840	65	M
76	NI	28247	24	F
77	IN	28003	31	F
78	KW	30192	62	F
79	FL	18275	56	M
80	SH	30473	18	F
81	JA	17685	47	F
82	TF	26326	48	F
83	UG	29837	54	F
84	LM	31052	65	F

Subject Number	Subject Initials	CRL ID #	Age	Sex
85	AK	06659	56	F
86	JL	19741	41	F
87	JH	31080	36	M
88	DB	31041	46	F
89	SG	28025	58	F
90	LD	30765	50	F
91	SD	30764	28	F
92	RR	10112	54	F
93	JV	25288	24	F
94	JP	24490	51	F
95	HR	30131	40	F
96	MB	29481	32	F
97	MJ	29483	44	M
98	LD	08929	55	F
99	MR	31230	40	M
100	BM	31234	50	F
101	GA	30182	61	M
102	NT	29337	24	F
103	LF	02384	50	F
104	LL	09586	54	M
105	NC	31225	55	F
106	FB	31074	23	M
107	NA	28912	51	M
108	KG	30932	29	F
109	DC	31189	26	M
110	TS	26793	56	M
111	CC	25222	43	F
112	RW	26017	59	M

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CLINICAL STUDY REPORT

Report Status:	Final
Report Date:	25 July 2019
CRL Study Number:	
CRL Protocol Number:	CL 1.0 2019
Study Title:	Repeated Insult Patch Test
Test Material:	Product Contains 0.4 9% Chondrus Crispus Extract
Sponsor:	
Sponsor Representative:	
Investigating Laboratory:	Eurofins | CRL, Inc. 371 Hoes Lane, Suite 100 Piscataway, New Jersey 08854 Telephone: (732) 981-1616 Fax: (732) 981-0520
Principal Investigator:	Winston Moy, MD Diplomate, American Board of Dermatology
Study Initiation Date:	31 May 2019
Study Completion Date:	12 July 2019

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PRINCIPAL INVESTIGATOR SIGNATURE

Study Title: Repeated Insult Patch Test

I have read Clinical Study Report _____ *confirm that to the best of my knowledge it accurately describes the conduct and results of the study.*

Winston Moy, M.D.

Digitally signed by Winston Moy, M.D.
DN: cn=Winston Moy, M.D., o=Eurofins CRL Inc, ou, email=winston.moy@crlresearchlabs.com, c=US
Date: 2019.07.29 08:06:47 -04'00'

Principal Investigator Signature/Date

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Quality Assurance Audit Statement

Clinical Study Number: [redacted]

Start Date: 31 May 2019

Completion Date: 12 July 2019

Eurofins | CRL, Inc. follows established, standardized procedures for clinical testing designed to ensure the well-being of clinical study subjects and the generation of reliable study data. The study was conducted in accordance with the study protocol and Eurofins | CRL, Inc. Standard Operating Procedures. In addition, the study was conducted following applicable ICH GCP standards to ensure reliability of data, subject safety and confidentiality. All data included in the report is accurately represented. The clinical study master file was reviewed by the Principal Investigator and the Quality Assurance representative.

Karen Hoyberg

Digitally signed by Karen Hoyberg
DN: cn=Karen Hoyberg, o=Eurofins CRL, Inc., ou=Quality Assurance, email=Karen.Hoyberg@crlresearchlabs.com, c=US
Date: 2019.07.25 11:05:25 -04'00'

Signature of QA Auditor and Date

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CLINICAL STUDY REPORT

Repeated Insult Patch Test (RIPT)

1.0 ETHICS

1.1. ETHICAL CONDUCT OF THE STUDY

Eurofins | CRL, Inc. (CRL) follows established, standardized procedures for clinical testing designed to ensure the well-being of clinical study subjects and the generation of reliable study data. It is the responsibility of the Study Sponsor to ensure the study complies with applicable Drug, Cosmetic or Medical Device regulations, which vary by product. The Study Sponsor is solely responsible for product marketing claims based on its interpretation of CRL studies.

1.2. PARTICIPANT INFORMATION AND INFORMED CONSENT

Each subject was given a copy of the Informed Consent Form (ICF) had the nature and the purpose of the study explained to them by CRL personnel. Prior to entry into the study, the subject gave voluntary written consent to participate by signing the ICF. The Principal Investigator retains the original signed Informed Consent Form in the subject's file and gave a copy of the Informed Consent Form to the subject.

1.3. SUBJECT CONFIDENTIALITY

The Principal Investigator ensures that the research subject's confidentiality was maintained. Subjects are identified by their study ID number only. Documents are kept in strict confidence by the Principal Investigator. Any use of personally identifiable data or private health information must be justified by the Principal Investigator.

2.0 OBJECTIVE

The objective of this study was to determine the potential of a test material to elicit dermal irritation and/or induce sensitization following repeated patch applications.

3.0 PRINCIPAL INVESTIGATOR AND INVESTIGATIVE SITE

Winston Moy, MD
Diplomate, American Board of Dermatology

Eurofins | CRL, Inc.
371 Hoes Lane, Suite 100
Piscataway, New Jersey 08854
(732)-981-1616

4.0 SPONSOR REPRESENTATIVE AND SPONSOR SITE

5.0 TEST MATERIALS AND RECORD RETENTION

The following test material was provided by and was received by Eurofins | CRL, Inc. on 26 April 2019.

Test Material	Test Condition	Patch Type
	Neat	Occlusive*

CRL Identification Number

The Sponsor assumed responsibility for the purity, stability, characterization, and adequate preservation of the test materials. The Sponsor provided assurance that the test materials submitted were determined to be safe for use in humans.

5.1. STORAGE AND RETENTION

Prior to study start, the test materials were stored at room temperature and humidity. All unused test materials will be retained by CRL for a minimum of 6 months, in accordance with CRL SOPs.

All original forms of this study will be retained by CRL as specified in CRL Standard Operating Procedures (SOPs).

* Occlusive Strip with Flexcon® (Strukmyer LLC, Mesquite,TX or equivalent)

6.0 RANDOMIZATION

No randomization was required for this study.

7.0 BLINDING

Subjects were not provided with information regarding the identity of the test material. The investigatory staff was not blinded. Test materials were labeled with unique CRL study identification and panel codes and subject numbers upon test material receipt by CRL.

8.0 STUDY DATES

This study was initiated on 31 May 2019 and was completed on 12 July 2019.

9.0 SUBJECT SELECTION

A total of 116 male and female subjects, ranging in age from 19 to 70 years who met all of the inclusion criteria and none of the exclusion criteria as outlined in the study protocol, were selected for study participation (Appendix I).

10.0 STUDY EVALUATIONS

The following Dermal Scoring System was used:

Dermal Score	Description
0	No visible skin reaction
±	Barely perceptible erythema
1+	Mild erythema
2+	Well defined erythema
3+	Severe erythema and edema
4+	Erythema and edema with vesiculation

Letter Codes

e = Edema
P = Peeling
S = Spreading of reaction beyond patch site.
Sc = Scabbing
d = Dryness/scaling
D = Oozing, crusting, and/or superficial erosions
I = Itching
F = Follicular irritation with or without pustule formation (folliculitis)
Hr = Hyperpigmentation
Ho = Hypopigmentation
X = Subject Absent
NP = No patching
Pa = Papules
C = Changed site
--- = No reading
B = Burning
SD = Site Discontinued
Ex = Excoriation

11.0 TEST METHOD

This study was conducted according to clinical study protocol CL 1.0 2019.

12.0 STUDY RESULTS

12.1. COMPLETED AND DISCONTINUED SUBJECTS

A total of 113 subjects completed the study. Discontinued subjects are listed below:

Subject Number	Reason for Discontinuation
58	Lost to follow up
83	Lost to follow up
93	Personal reasons

12.2. DERMAL EVALUATIONS

Individual dermal scores recorded during the Induction and Challenge Phases appear in Table I.

12.3. PROTOCOL DEVIATIONS

No protocol deviations occurred over the duration of the study.

12.4. PROTOCOL AMENDMENTS

There were no protocol amendments during this study.

12.5. ADVERSE EVENTS

No adverse events were reported during the study.

13.0 CONCLUSION

Based on the test population of 113 subjects and under the conditions of this study, the test material identified as did not demonstrate a potential for eliciting dermal irritation or inducing sensitization.

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Table I - Summary of Dermal Scores

Test Material:	

Subject Number	Induction Scores									Challenge Scores			
	1	2	3	4	5	6	7	8	9	24 Hour	48 Hour	72 Hour	96 Hour
1	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
3	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
5	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
7	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
9	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
11	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
13	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
15	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
17	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
19	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
21	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
23	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
25	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
27	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

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Table I - Summary of Dermal Scores (continued)

Test Material:	

Subject Number	Induction Scores									Challenge Scores			
	1	2	3	4	5	6	7	8	9	24 Hour	48 Hour	72 Hour	96 Hour
29	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
31	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
33	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
35	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
37	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
39	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
41	0	0	0	0	0	0	0	0	0	0	0	0	X
42	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
44	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
46	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
48	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
50	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
52	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
54	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
56	0	0	0	0	0	0	0	0	0	0	0	0	X

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Table I - Summary of Dermal Scores (continued)

Test Material:	

Subject Number	Induction Scores									Challenge Scores			
	1	2	3	4	5	6	7	8	9	24 Hour	48 Hour	72 Hour	96 Hour
57	0	0	0	0	0	0	0	0	0	0	0	0	0
58	0	0	Discontinued										
59	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
61	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	X
63	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
65	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
67	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
69	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
71	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
73	0	0	0	0	0	0	0	0	0	0	0	0	X
74	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
76	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
78	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
80	0	0	0	0	0	0	0	0	0	0	0	0	0
81	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
83	0	Discontinued											
84	0	0	0	0	0	0	0	0	0	0	0	0	0

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Table I - Summary of Dermal Scores (continued)

Test Material

Subject Number	Induction Scores									Challenge Scores			
	1	2	3	4	5	6	7	8	9	24 Hour	48 Hour	72 Hour	96 Hour
85	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
87	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
89	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
91	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
93	Discontinued												
94	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
96	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
98	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
100	0	0	0	0	0	0	0	0	0	0	0	0	0
101	0	0	0	0	0	0	0	0	0	0	0	0	0
102	0	0	0	0	0	0	0	0	0	0	0	0	0
103	0	0	0	0	0	0	0	0	0	0	0	0	0
104	0	0	0	0	0	0	0	0	0	0	0	0	0
105	0	0	0	0	0	0	0	0	0	0	0	0	0
106	0	0	0	0	0	0	0	0	0	0	0	0	0
107	0	0	0	0	0	0	0	0	0	0	0	0	0
108	0	0	0	0	0	0	0	0	0	0	0	0	0
109	0	0	0	0	0	0	0	0	0	0	0	0	0
110	0	0	0	0	0	0	0	0	0	0	0	0	0
111	0	0	0	0	0	0	0	0	0	0	0	0	0
112	0	0	0	0	0	0	0	0	0	0	0	0	0
113	0	0	0	0	0	0	0	0	0	0	0	0	X
114	0	0	0	0	0	0	0	0	0	0	0	0	0
115	0	0	0	0	0	0	0	0	0	0	0	0	0
116	0	0	0	0	0	0	0	0	0	0	0	0	0

Appendix I - Subject Demographics

Subject Number	Age	Sex
01	45	F
02	69	F
03	34	F
04	68	M
05	53	F
06	51	F
07	63	F
08	44	F
09	70	M
10	67	F
11	24	F
12	48	F
13	51	F
14	37	F
15	40	F
16	55	F
17	53	M
18	59	F
19	50	F
20	63	F
21	41	F
22	55	F
23	47	M
24	47	F
25	40	F
26	20	F
27	20	F
28	41	F
29	54	F

Subject Number	Age	Sex
30	58	F
31	39	M
32	69	F
33	21	F
34	24	M
35	58	F
36	41	F
37	68	F
38	65	M
39	56	F
40	59	F
41	36	M
42	61	F
43	51	F
44	67	M
45	49	F
46	56	F
47	48	F
48	60	F
49	37	F
50	61	F
51	65	F
52	37	F
53	53	M
54	64	F
55	44	F
56	57	F
57	28	F
58	44	F

Appendix I – Subject Demographics (continued)

Subject Number	Age	Sex
59	58	F
60	56	F
61	60	F
62	63	F
63	30	F
64	28	F
65	54	F
66	53	M
67	30	F
68	58	F
69	49	M
70	20	M
71	61	F
72	41	F
73	59	F
74	54	F
75	56	F
76	40	F
77	51	F
78	46	F
79	37	F
80	44	F
81	25	M
82	57	F
83	53	M
84	31	F
85	59	F
86	48	F
87	19	M

Subject Number	Age	Sex
88	61	F
89	70	M
90	58	M
91	47	F
92	67	F
93	66	F
94	51	F
95	58	F
96	60	M
97	68	F
98	19	F
99	65	F
100	65	F
101	39	F
102	37	F
103	55	F
104	66	M
105	47	F
106	58	M
107	57	F
108	52	F
109	31	M
110	59	F
111	25	F
112	30	M
113	34	F
114	67	M
115	38	F
116	45	F

Clinical Research Laboratories, Inc.

Final Report

Repeated Insult Patch Test

Product contains 0.325% Asparagopsis Armata Extract

CLIENT: [redacted]

ATTENTION: [redacted]

TEST MATERIAL: [redacted]

CRL STUDY NUMBER: [redacted]

AUTHORIZED SIGNATURES:

Bruce E. Kanengiser, M.D.
President/Medical Director

Michael J. Muscatiello, Ph.D.
Executive Vice President/COO

Anita Lee Cham, M.D.
Dermatologist

REPORT DATE: July 11, 2012

371 Hoes Lane, Suite 100 • Piscataway, NJ 08854 • (732) 981-1616 • FAX (732) 981-0520

Clinical Research Laboratories, Inc.

Good Clinical Practice
Quality Assurance Audit Statement

Clinical Study Number: [redacted]

Start Date: May 21, 2012

Completion Date: June 29, 2012

The clinical study listed above was conducted in accordance with Clinical Research Laboratories, Inc. Standard Operating Procedures, which incorporate the principles of Good Clinical Practice defined by applicable guidelines and regulations established by U.S. Regulatory Agencies. The conduct of the study was monitored for compliance, and the associated records, including source documents or raw data, were reviewed for documentation practices and accuracy by a Project Manager/Study Director and/or a Quality Assurance Representative. Standard Quality Assurance audit procedures for this final report and study related documents were conducted.

Karen Hayhey
Signature of QA Auditor

July 11, 2012
Date

FINAL REPORT

REPEATED INSULT PATCH TEST

PURPOSE

The purpose of this study was to determine the dermal irritation and sensitization potential of a test material.

INVESTIGATIVE SITE

Clinical Research Laboratories, Inc.
371 Hoes Lane, Suite 100
Piscataway, New Jersey 08854
732-981-1616

TEST MATERIAL

The following test material was provided by [redacted] and was received by Clinical Research Laboratories, Inc. on May 15, 2012:

Test Material	Test Condition	Patch Type
[redacted]	Test as Received	Occlusive*

The test material was coded with the following CRL identification number:

[redacted]

STUDY DATES

This study was initiated on May 21, 2012 and was completed on June 29, 2012.

* Occlusive Strip with Flexcon® (Brady Medical, Mesquite,TX)

PANEL SELECTION

Each subject was assigned a permanent CRL identification number. All subjects signed an Informed Consent Form in compliance with 21 CFR Part 50: "Protection of Human Subjects" and a HIPAA Authorization Form in compliance with 45 CFR Parts 160 and 164. All subjects completed a Subject Profile/Medical History Form provided by Clinical Research Laboratories, Inc. prior to the study (Subject Demographics - Appendix I). Subjects who met the following Inclusion Criteria and none of the Exclusion Criteria were impaneled:

Inclusion Criteria

a. Male and female subjects between the ages of 18 and 70 years;

b. Subjects who do not exhibit any skin diseases which might be confused with a skin reaction from the test material;

c. Subjects who agree to avoid exposure of the test sites to the sun and to refrain from visits to tanning salons during the course of this study;

d. Subjects who agree to refrain from getting patches wet during the course of the study;

e. Subjects willing to sign an Informed Consent in conformance with 21CFR Part 50: "Protection of Human Subjects;"

f. Subjects who have completed a HIPAA Authorization Form in conformance with 45CFR Parts 160 and 164;

g. Subjects in generally good health who have a current Subject Profile/Medical History on file;

h. Subjects who are dependable and able to follow directions as outlined in the protocol.

Exclusion Criteria

a. Female subjects who are pregnant or nursing;

b. Subjects who are currently using any systemic or topical corticosteroids, anti-inflammatory drugs, or antihistamines on a regular basis;

c. Subjects exhibiting any skin disorder, sunburn, scars, excessive tattoos, etc. in the test area.

TEST METHOD

Prior to the application of the patch, the test area was wiped with 70% isopropyl alcohol and allowed to dry. The test material, which was prepared as described in the Test Material section of the report, was applied to the upper back (between the scapulae) and was allowed to remain in direct skin contact for a period of 24 hours.

Patches were applied to the same site on Monday, Wednesday, and Friday for a total of 9 applications during the Induction Period. This schedule may have been modified to allow for missed visits or holidays. If a subject was unable to report on an assigned test date, the test material was applied on 2 consecutive days during the Induction Phase and/or a makeup day was added at the end of the Induction Phase.

The sites were graded by a CRL technician for dermal irritation 24 hours after removal of the patches by the subjects on Tuesday and Thursday and 48 hours after removal of the patches on Saturday, unless the patching schedule was altered as described above.

The sites were graded according to the following scoring system:

Dermal Scoring Scale

0 No visible skin reaction
± Barely perceptible erythema
1+ Mild erythema
2+ Well defined erythema
3+ Severe erythema and edema
4+ Erythema and edema with vesiculation

If a "2+" reaction or greater occurred, the test material was applied to an adjacent virgin site. If a "2+" reaction or greater occurred on the new site, the subject was not patched again during the Induction Phase but was challenged on the appropriate day of the study. At the discretion of the Study Director, patch sites with scores less than a "2+" may have been changed.

Following approximately a 2-week rest period, the challenge patches were applied to previously untreated test sites on the back. After 24 hours, the patches were removed by a CRL technician and the test sites were evaluated for dermal reactions. The test sites were re-evaluated at 48 and 72 hours. Subjects exhibiting reactions during the Challenge Phase of the study may have been asked to return for a 96-hour reading.

RESULTS

This study was initiated with 112 subjects. Four subjects discontinued study participation for reasons unrelated to the test material. A total of 108 subjects completed the study.

Individual dermal scores recorded during the Induction and Challenge Phases appear in Table I.

CONCLUSION

Based on the test population of 108 subjects and under the conditions of this study, the test material identified [redacted] did not demonstrate a potential for eliciting dermal irritation or sensitization.

RETENTION

Test materials and all original forms of this study will be retained by Clinical Research Laboratories, Inc. as specified in CRL Standard Operating Procedures 30.6 and 30.6C, unless designated otherwise by the Sponsor.

TABLE I

Summary of Dermal Scores

Test Material: [redacted]

Subject Number	Induction Scores									Challenge Scores		
	1	2	3	4	5	6	7	8	9	24 Hour	48 Hour	72 Hour
1	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
3	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
5	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
7	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
9	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
11	0	0	0	0	0	1+dPIC	0	0	0	0	0	0
12	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
14	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
16	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
18	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
20	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
22	0	0	0	0	0	0	0	0	0	Discontinued		
23	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
25	0	0	0	0	0	0	0	0	0	0	0	0

d = Dryness
P = Peeling
I = Itching
C = Changed Site

TABLE I
(Continued)

Summary of Dermal Scores

Test Material: [redacted]

Subject Number	Induction Scores									Challenge Scores		
	1	2	3	4	5	6	7	8	9	24 Hour	48 Hour	72 Hour
26	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
28	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
30	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
32	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
34	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
36	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
38	0	1+	0	0	0	0	0	0	0	0	0	0
39	0	0	0	0	0	0	0	0	0	0	0	0
40	0	0	0	0	Discontinued							
41	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
43	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
45	0	0	0	0	0	0	0	0	0	0	0	0
46	±	0	0	0	0	0	0	0	0	0	0	0
47	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
49	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

Clinical Research Laboratories, LLC

CLINICAL STUDY REPORT

Report Status:	Final Report
Report Date:	July 7, 2016
CRL Study Number:	
CRL Protocol Number:	CL 1.0 2016
Study Dates:	May 18, 2016 - June 24, 2016
Study Title:	Repeated Insult Patch Test (RIPT) –Shelanski Method
Test Material:	Product 0.0004% Porphyra Umbilicalis Extract
Sponsor:	
Sponsor Representative:	
Principal Investigator:	Anita Lee Cham, M.D. Dermatologist

APPROVAL SIGNATURES:

Anita Lee Cham, M.D.

Digitally signed by Anita Lee Cham, M.D.
DN: cn=Anita Lee Cham, M.D., o=Clinical Research Laboratories, LLC, ou, email=anita.cham@crlresearchlabs.com, c=US
Date: 2016.07.07 15:03:15 -04'00'

Principal Investigator Signature/Date

Clinical
Research
Laboratories, LLC

Good Clinical Practice
Quality Assurance Audit Statement

Clinical Study Number: [redacted]

Start Date: May 18, 2016

Completion Date: June 24, 2016

The clinical study listed above was conducted in accordance with Clinical Research Laboratories, LLC Standard Operating Procedures, which incorporate the principles of Good Clinical Practice defined by applicable guidelines and regulations established by U.S. Regulatory Agencies. The conduct of the study was monitored for compliance, and the associated records, including source documents and/or raw data, were reviewed for documentation practices and accuracy by the Principal Investigator and/or a Quality Assurance representative. Standard Quality Assurance audit procedures for this final report and study related documents were conducted.

Karen Hoyberg

Digitally signed by Karen Hoyberg
DN: cn=Karen Hoyberg, o=Clinical Research Laboratories, LLC, ou=Regulatory/Quality Assurance, email=karen.hoyberg@crlresearchlabs.com, c=US
Date: 2016.07.07 13:54:38 -04'00'

.....

Quality Assurance Auditor Signature/Date

Clinical Research Laboratories, LLC

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Clinical Research Laboratories, LLC

FINAL REPORT

Repeated Insult Patch Test (RIPT) - Shelanski Method

1.0 OBJECTIVE

The objectives of this study were to determine the potential of a test material to elicit dermal irritation or induce sensitization following repeated patch applications.

2.0 PRINCIPAL INVESTIGATOR/INVESTIGATIVE SITE

Anita Lee Cham, M.D.
Dermatologist

Clinical Research Laboratories, LLC
371 Hoes Lane, Suite 100
Piscataway, New Jersey 08854
732-981-1616

3.0 SPONSOR REPRESENTATIVE/SPONSOR

4.0 TEST MATERIAL

The following test material was provided by was received by Clinical Research Laboratories, LLC on May 5, 2016.

Test Material	Test Condition	Patch Type
	Neat	Occlusive*

The test material was coded with the following CRL identification number:

5.0 STUDY DATES

This study was initiated on May 18, 2016 and was completed on June 24, 2016.

* Occlusive Strip with Flexcon® (Strukmyer LLC, Mesquite,TX or equivalent)

Clinical Research Laboratories, LLC

6.0 PANEL SELECTION

Each subject was assigned a permanent CRL identification number. All subjects signed an Informed Consent Form in compliance with 21 CFR Part 50: "Protection of Human Subjects" and a HIPAA Authorization Form in compliance with 45 CFR Parts 160 and 164. All subjects completed a Subject Profile provided by Clinical Research Laboratories, LLC prior to the study (Subject Demographics - Appendix I). Subjects who met the following Inclusion Criteria and none of the Exclusion Criteria were impaneled:

6.1. INCLUSION CRITERIA

a. Subject is male or female between the ages of 18 and 70 years;

b. Subject does not exhibit any skin diseases which might be confused with a skin reaction from the test material;

c. Subject agrees to avoid exposure of the test sites to the sun and to refrain from visits to tanning salons during the course of this study;

d. Subject agrees to refrain from getting patches wet during the course of the study and from scrubbing or washing the test area with soap or applying powder, lotions or personal care products to the area during the course of the study;

e. Subject has signed an Informed Consent in conformance with 21CFR Part 50: "Protection of Human Subjects;"

f. Subject has completed a HIPAA Authorization Form in conformance with 45CFR Parts 160 and 164;

g. Subject is in generally good health and has a current Subject Profile/Medical History on file;

h. Subject is dependable and able to follow directions as outlined in the protocol.

6.2. EXCLUSION CRITERIA

a. Subject is pregnant, nursing, or planning to become pregnant;

b. Subject is currently using any systemic or topical corticosteroids, anti-inflammatory drugs, or antihistamines on a regular basis;

c. Subject reports allergies to cosmetics, toiletries, or personal care products;

d. Subject exhibits any skin disorders, sunburn, scars, excessive tattoos, etc. in the test area;

e. Subject has scheduled, or is planning to undergo, any medical or surgical procedures during the 6 week course of the study.

Clinical Research Laboratories, LLC

7.0 TEST METHOD SUMMARY

Prior to the application of the patch, the test area was wiped with 70% isopropyl alcohol and allowed to dry. The test material, which was prepared as described in the Test Material section of the report, was applied to the upper back (between the scapulae and the waist to either side of the spinal midline) and was allowed to remain in direct skin contact for a period of 24 hours.

Patches were applied to the same site on Monday, Wednesday, and Friday for a total of 9 applications during the Induction Period. This schedule may have been modified to allow for inclement weather, missed visits or holidays. If a subject was unable to report on an assigned test date, the test material was applied on 2 consecutive days during the Induction Phase and/or a makeup day was added at the end of the Induction Phase.

The sites were graded by a CRL technician for dermal irritation 24 hours after removal of the patches by the subjects on Tuesday and Thursday and 48 hours after removal of the patches on Saturday, unless the patching schedule was altered as described above.

The sites were graded according to the following scoring system:

Dermal Scoring Scale

0	No visible skin reaction
±	Barely perceptible erythema
1+	Mild erythema
2+	Well defined erythema
3+	Severe erythema and edema
4+	Erythema and edema with vesiculation

If a "2+" reaction or greater occurred, the test material was applied to an adjacent virgin site. If a "2+" reaction or greater occurred on the new site, the subject may not have been patched again during the Induction Phase but may have been challenged on the appropriate day of the study. At the discretion of the Study Director, patch sites with scores less than a "2+" may have been changed.

Approximately 10 to 21 days after the Induction Phase, the challenge patches were applied to previously untreated test sites on the back. After 24 hours, the patches were removed by a CRL technician and the test sites were evaluated for dermal reactions. The test sites were re-evaluated at 48 and 72 hours after application. Subjects exhibiting reactions during the Challenge Phase of the study may have been asked to return for a 96-hour reading.

Clinical Research Laboratories, LLC

8.0 PROTOCOL DEVIATIONS

The following protocol deviations occurred:

- Subjects #17 and #18 missed the 9th Induction evaluation but did receive 9 induction patches.

These deviations had no impact on the scientific validity or outcome of the study and did not affect the safety of the subjects.

Subjects were re-instructed in the importance of keeping all scheduled appointments.

9.0 RESULTS

This study was initiated with 113 subjects. Ten subjects discontinued study participation for reasons unrelated to the test material. A total of 103 subjects completed the study.

Individual dermal scores recorded during the Induction and Challenge Phases appear in Table I.

10.0 ADVERSE EVENTS

No adverse events were reported during the study.

11.0 CONCLUSION

Based on the test population of 103 subjects and under the conditions of this study, the test material identified as [redacted] did not demonstrate a potential for eliciting dermal irritation or inducing sensitization.

12.0 RETENTION

Test materials and all original forms of this study will be retained by Clinical Research Laboratories, LLC as specified in CRL Standard Operating Procedures 30.6 and 30.6C, unless designated otherwise by the Sponsor.

Clinical Research Laboratories, LLC

Table I - Summary of Dermal Scores

Test Material	[illegible]

Subject Number	Induction Scores									Challenge Scores		
	1	2	3	4	5	6	7	8	9	24 Hour	48 Hour	72 Hour
1	0	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0	0	X*
3	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
5	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
7	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
9	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
11	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
13	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
15	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
17	0	0	0	0	0	0	0	0	X	0	0	0
18	0	0	0	0	0	0	0	0	X	0	0	0
19	0	0	0	0	0	0	0	0	0	0	0	0
20	0	0	0	0	Discontinued							
21	0	0	0	0	0	0	0	0	0	0	0	0
22	0	0	0	Discontinued								
23	0	0	0	0	0	0	0	0	0	0	0	0
24	Discontinued											
25	0	0	0	0	0	0	0	0	0	0	0	0

X = Subject Absent

*No reaction was observed at the 96 hour evaluation.

Clinical Research Laboratories, LLC

Table I - Summary of Dermal Scores (continued)

Test Material:

Subject Number	Induction Scores									Challenge Scores		
	1	2	3	4	5	6	7	8	9	24 Hour	48 Hour	72 Hour
26	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
28	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
30	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
32	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
34	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
36	0	0	0	0	0	0	0	0	0	X	0	0*
37	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
39	0	0	0	0	0	0	0	0	0	Discontinued		
40	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
42	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
44	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
46	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
48	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
50	0	0	0	0	0	0	0	0	0	0	0	0

X = Subject Absent

*No reaction was observed at the 96 hour evaluation.

Clinical Research Laboratories, LLC

Table I - Summary of Dermal Scores (continued)

Test Material:

Subject Number	Induction Scores									Challenge Scores		
	1	2	3	4	5	6	7	8	9	24 Hour	48 Hour	72 Hour
51	0	0	0	0	0	0	0	0	0	0	0	0
52	0	0	0	0	0	0	0	0	0	X	0	0*
53	0	0	0	0	0	0	0	0	0	0	0	0
54	Discontinued											
55	0	0	0	0	0	Discontinued						
56	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
58	0	0	0	0	0	0	0	0	0	0	0	0
59	0	0	Discontinued									
60	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
62	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
64	0	Discontinued										
65	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
67	0	0	0	0	0	0	0	0	0	0	0	0
68	0	0	0	0	0	0	0	0	X	Discontinued		
69	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
71	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
73	0	0	0	0	0	0	0	0	0	0	0	0
74	0	0	0	0	0	0	0	±	±	0	0	0
75	0	0	0	0	0	0	0	0	0	0	0	0

X = Subject Absent

*No reaction was observed at the 96 hour evaluation.

Clinical Research Laboratories, LLC

Table I - Summary of Dermal Scores (continued)

Test Material												
Subject Number	Induction Scores									Challenge Scores		
	1	2	3	4	5	6	7	8	9	24 Hour	48 Hour	72 Hour
76	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
78	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
80	0	0	0	0	0	0	0	0	0	0	0	0
81	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
83	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
85	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
87	0	0	0	0	0	0	0	0	0	0	0	0
88	0	0	0	0	0	0	0	0	0	0	X	0*
89	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
91	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
93	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
95	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
97	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
99	0	0	0	0	0	0	0	0	0	0	0	0
100	0	0	Discontinued									

X = Subject Absent

*No reaction was observed at the 96 hour evaluation.

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Table I - Summary of Dermal Scores (continued)

Test Material:	

Subject Number	Induction Scores									Challenge Scores		
	1	2	3	4	5	6	7	8	9	24 Hour	48 Hour	72 Hour
101	0	0	0	0	0	0	0	0	0	0	0	0
102	0	0	0	0	0	0	0	0	0	0	0	0
103	0	0	0	0	0	0	0	0	0	0	0	0
104	0	0	0	0	0	0	0	0	0	0	0	0
105	0	0	0	0	0	0	0	0	0	0	0	0
106	0	0	0	0	0	0	0	0	0	0	0	0
107	0	0	0	0	0	0	0	0	0	0	0	0
108	0	0	0	0	0	0	0	0	0	0	0	0
109	0	0	0	0	0	0	0	0	0	0	0	0
110	0	0	0	0	0	0	0	0	0	0	0	0
111	0	0	0	0	0	0	0	0	0	0	0	0
112	0	0	0	0	0	0	0	0	0	0	0	0
113	0	0	0	0	0	0	0	0	0	0	0	0

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Appendix I - Subject Demographics

Subject Number	Subject Initials	Age	Sex
1	CC	68	F
2	AM	50	F
3	NT	46	F
4	AP	57	F
5	KH	43	F
6	DD	55	F
7	SP	39	F
8	CR	59	F
9	WA	58	F
10	CA	58	F
11	LP	55	M
12	BO	39	F
13	KM	65	F
14	JL	48	F
15	SM	42	F
16	AH	67	M
17	KP	55	M
18	NP	53	M
19	GP	47	F
20	PM	20	F
21	BC	33	M
22	IR	19	M
23	BS	22	F
24	MF	49	F
25	AS	18	F
26	CL	43	F
27	AD	65	F
28	RU	58	F

Subject Number	Subject Initials	Age	Sex
29	JM	19	M
30	KL	39	F
31	EJ	67	F
32	NF	47	F
33	TD	33	M
34	CD	63	F
35	PL	67	F
36	SC	57	M
37	HK	56	F
38	VF	56	M
39	PK	67	F
40	BF	43	F
41	SC	21	F
42	EG	58	F
43	NR	51	M
44	TM	48	F
45	ET	53	F
46	KT	38	F
47	MF	61	F
48	CB	67	F
49	CP	50	F
50	KT	65	F
51	JC	42	F
52	RP	19	M
53	MV	48	F
54	CT	26	M
55	CB	37	F
56	AT	55	F

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Appendix I - Subject Demographics (continued)

Subject Number	Subject Initials	Age	Sex
57	TH	53	F
58	LH	56	M
59	DP	68	F
60	WS	63	M
61	DF	59	F
62	QJ	21	F
63	BW	61	F
64	MR	47	M
65	CP	65	F
66	MR	42	F
67	AF	63	F
68	MW	43	M
69	FA	45	M
70	ME	49	F
71	PS	42	F
72	CR	59	F
73	MG	59	F
74	LC	41	F
75	JL	29	F
76	LC	69	F
77	SW	29	F
78	LT	69	F
79	SH	35	F
80	ME	55	M
81	JO	37	F
82	JW	62	F
83	SP	63	M
84	CS	44	F
85	LH	55	F

Subject Number	Subject Initials	Age	Sex
86	SH	47	F
87	JP	54	F
88	CB	18	M
89	DT	61	F
90	JT	38	F
91	CS	35	F
92	CS	64	F
93	MW	51	F
94	JL	52	M
95	SD	48	F
96	AH	42	F
97	MJ	52	F
98	GL	29	M
99	TL	34	F
100	MA	37	F
101	JM	66	F
102	DC	29	F
103	IN	34	F
104	ND	55	F
105	DP	39	F
106	NC	32	F
107	ET	40	M
108	TS	49	F
109	ZM	38	F
110	JB	22	F
111	CS	48	F
112	TW	44	F
113	OH	53	M

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: June 12, 2020

SUBJECT: Asparagopsis Armata Extract

Biotech Marine. 2020. Manufacturing Process Aspar'age™ (Asparagopsis Armata Extract).

Biotech Marine. 2018. Statement 18 292 01 Aspar'age™ Composition File (Asparagopsis Armata Extract).

Dermscan. 2018. Evaluation of the acute cutaneous tolerance of a natural extract (Asparagopsis Armata Extract) on adult subjects.

Dermscan. 2018. Assessment of the sensitizing potential of a natural extract (Asparagopsis Armata Extract): Final clinical security test under dermatological control.

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www.biotechmarine.com

FLOWCHART 18 291 01

MANUFACTURING PROCESS OF ASPAR'AGE™

HARVESTING / IDENTIFICATION *(Asparagopsis Armata)*

↓

WASHING

↓

GRINDING

↓

EXTRACTION WITH THE SOLVENTS
PROPANEDIOL & WATER

↓

FILTRATION

↓

QUALITY CONTROL

↓

PACKAGING

↓

QUALITY CONTROL

15/10/18

Operation Manager
Clément LANSALOT

BiotechMarine - Z.I. - B.P.72 - 22260 Pontrieux (FR)
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Statement 18 292 01 ASPAR'AGE™ Composition file gb

(In accordance with EN 45014 General criteria for supplier's declaration of conformity)

Composition of the product marketed by BiotechMarine:

ASPAR'AGE™

INCI Name: Aqua/Water - Propanediol - Asparagopsis Armata Extract

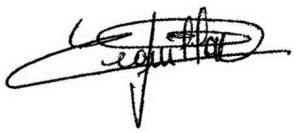
Composition

Components	Components usual Name	Function	% (Concentration range)	% (Typical Concentration*)
Aqua / Water	Water	Solvent	56.0 - 62.0	59.0
Propanediol	1,3-propanediol	Solvent	38.0 - 42.0	40.0
Asparagopsis Armata Extract	Asparagopsis armata, ext.	Active	0.5 - 2.0	1.0

* given as indicative value

The complete composition is provided. It includes the main components that are declared in the INCI name and the non-functional process additives and/or residual raw materials. In accordance with PCPC INCI naming rules, the process additives which do not give technical or functional properties to the ingredient are not included in the INCI name of the ingredient:
https://eservices.personalcarecouncil.org/BBK/Sci/INCI_Instructions.pdf

Document approved at Pontrieux, on October 15, 2018



By Laëtitia LE GUILLOU
Cosmetic Regulatory Technician from BIOTECHMARINE

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Asparagopsis Armata Extract (Asper'age)

SEPPIC
Mickael PUGINIER
127, chemin de la POUDRERIE
BP 228
81100 CASTRES Cedex
FRANCE

Gdansk, April 9, 2018

Study Report #18E0714 (version 1.0) /
Related to quote/ order #18D0714

ETUDE DE LA TOLERANCE CUTANEE AIGUE D'UN EXTRAIT NATUREL CHEZ LE VOLONTAIRE ADULTE : PATCH-TEST SIMPLE

EVALUATION OF THE ACUTE CUTANEOUS TOLERANCE OF A NATURAL EXTRACT ON ADULT SUBJECTS: SINGLE PATCH TEST

TOX18010

Nr Report: 18P0714-1PL

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comportant / *including* 14 pages

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SUMMARY OF THE STUDY REPORT #18E0714

ETUDE DE LA TOLERANCE CUTANEE AIGUE D'UN EXTRAIT NATUREL CHEZ LE VOLONTAIRE ADULTE : PATCH-TEST SIMPLE

EVALUATION OF THE ACUTE CUTANEOUS TOLERANCE OF A NATURAL EXTRACT ON ADULT SUBJECTS: SINGLE PATCH TEST

Objectif / *Objective*

Déterminer le potentiel irritant primaire d'un produit cosmétique après application unique sous patch-test / *To determine the acute irritating potential of a cosmetic product after single application under patch-test.*

Methodologie / *Methodology*

Etude monocentrique en simple aveugle / *Monocentric and simple blind study.*

Cinétique / *Kinetics*

	J0 / *D0*	J2 / *D2*	J2 / *D2*t30min	J3 / *D3*t24h
Recueil du consentement éclairé du volontaire / *Collection of the subject's informed consent*	●			
Vérificartion des critères d'inclusion et non-inclusion /*Verification of inclusion and non-inclusion criteria*	●			
Patch-test : application	●			
Patch-test : retrait / *removal*		●		
Scorage clinique / *clinical scoring*			●	●

Dates (JJ/MM/AAAA / *DD/MM/YYYY*)

Réception du produit / *Product reception:*	**Début d'étude /** *Study start:*	**Fin d'étude /** *Study end:*
19/03/2018	27/03/2018	06/04/2018

Produit / *Product*

Référence / *Reference:*	**Forme /** *Form:*	**Température de stockage /** *Storage temperature:*
TOX18010	Solution transparente / *Transparent solution*	Température ambiante / *Room temperature*

Application

Zone:	**Durée du patch /** *Patch duration:*	**Concentration**	**Type de patch /** *patch type:*
Dos (zone scapulaire) / *scapular part of the back*	48 heures / *48 hours*	Fourni / *Provided:* dilué 3% /*diluted 3%* Testé / *Tested :* PUR / *PURE*	Occlusif / *Occlusive*

Population étudiée / *Studied Population*

Critères principaux d'inclusion / *Main inclusion criteria*	**Age moyen /** *Average age*:	**Nombre de volontaires analysés /** *Number of subjects analysed:*
Age ≥18 ans / *Age ≥ 18 years old.* Phototype I à IV / *Phototype I to IV.*	47±3 ans/*years* (19 - 70)	22

Résultats / *Results*

Valeur d'I.I.C.M. / ***M.C.I.I. value :*** **0.01**

Conclusion : Non irritant / ***Non irritating***

Investigateur / *Investigator*

Nom et function / *Name and quality:*	**Date:**	**Signature:**
Dr. Agnieszka Cegielska Dermatologue / *Dermatologist*	09/04/2018	A Cegielska

1. PROTOCOLE EXPERIMENTAL / *EXPERIMENTAL PROTOCOL*

L'essai a été réalisé conformément aux procédures internes en vigueur.

The study was conducted according to the current internal procedures.

1.1. VOLONTAIRES / *SUBJECTS*

1.1.1. Caractéristiques des volontaires inclus / *Characteristics of included subjects*

- 22 volontaires ayant tout type de peau ont été inclus dans l'essai :

Sexe féminin	20
Sexe masculin	2
Age (moy±SEM)	19 à 70 ans (47±3)

- *22 subjects with every skin type were included in the study:*

Female subjects	*20*
Male subjects	*2*
Age (mean±SEM)	*19 to 70 years old (47±3)*

1.1.2. Critères d'inclusion / *Inclusion criteria*

- volontaire ayant donné son consentement libre et éclairé,
- aucun antécédent d'intolérance ou d'allergie à un produit de même type,
- phototype I à IV.

- *subjects having given their informed, written consent,*
- *no previous experience of intolerance or allergic reactions to this kind of product,*
- *phototype I to IV.*

1.1.3. Critères de non-inclusion / *Non-Inclusion criteria*

- femme enceinte ou qui allaite ou prévoyant un début de grossesse en cours d'étude,
- pathologie cutanée sur la zone d'étude (psoriasis, eczéma, vitiligo, pityriasis versicolore, acné, etc…),
- présence d'un traitement médicamenteux pouvant interférer avec l'évaluation du potentiel irritant, à l'apprécitation de l'investigateur,
- 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 tabac ou d'alcool.

- *pregnant or breast-feeding women or women planning to be pregnant during the study,*
- *cutaneous pathology on the study zone (psoriasis, eczema, vitiligo, pytiriasis versicolor, acne, etc…),*
- *subjects with medical treatments which may interfere with the acute skin tolerance evaluation, according to the investigator,*
- *exposure to the sun or to UV rays on the back during the previous month,*
- *subjects with very irritative skin,*
- *subjects presenting an important hairiness of the back, freckles, beauty spots or a tattoo on the back,*
- *subjects with a serious or progressive disease,*
- *excessive use of alcohol or tobacco.*

1.2. *PRODUIT A L'ETUDE / STUDY PRODUCT*

Le produit fourni par le promoteur, présente les caractéristiques suivantes :
The product supplied by the sponsor had the following specifications:

Référence du produit *Product reference*	Aspect du produit *Product aspect*	Date de réception à Dermscan Poland *Receipt date at Dermscan Poland*
TOX18010	Solution transparente *Transparent solution*	19/03/2018 *March 19, 2018*

1.3. *METHODOLOGIE / METHODOLOGY*

1.3.1. Matériel, dose, durée / *Instruments, dose, duration*

Le produit étudié a été appliqué dans les conditions suivantes :

The studied product was applied under the following conditions:

Zone / *Area:*	zone scapulaire / *scapular part of the back*
Type de Patch tests: *Patch tests type:*	Finn Chambers ® 8mm (50mm²) – occlusif *Finn Chamber® 8mm (50mm²) – occlusive*
Dose* :	25 µl
Conditions de l'application: *Application conditions:*	Fourni / *Provided:* dilué 3% /*diluted 3%* Testé / *Tested :* PUR / *PURE*
Durée de l'application: *Application duration:*	48 heures *48 hours*
Contrôle: *Control:*	patch sans produit *patch without product*

*Note: La dose est conditionnée par la capacité de la cupule, indiquée par le fabricant.

**Note:* *The quantity is determined according to the cupule capacity, indicated by the manufacturer.*

1.3.2. Lectures / *Readings*

Les examens macroscopiques cutanés ont été réalisés dans les mêmes conditions, en particulier au niveau de l'éclairage (lumière standardisée), 30 minutes après le retrait des patch-tests et 24 heures plus tard. Dans le cas d'une réaction cutanée > 1 à la lecture à 24 heures, le volontaire devait revenir au centre, des lectures étant effectuées jusqu'à réversibilité complète des réactions cutanées.

Les cotations des éventuelles réactions d'irritation, sur chaque site ayant reçu le produit étudié ainsi que sur la zone témoin, ont été réalisées selon les échelles numériques suivantes :

The macroscopic skin examinations were carried out under the same conditions, specifically the lighting (standardized light), 30 minutes after removal of the patch tests and 24 hours later. If the subject had a cutaneous reaction >1, he had to return to the centre and readings were done until complete reversibility of the cutaneous reactions.

The grading of the possible irritation reaction, on each zone that received the studied product and on the control zone, was done according to the following scale:

Score	Cotation *Quotation*	**CRITERES** / ***CRITERIA**:* description :	
		ERYTHEME «**E**» / *ERYTHEMA «**E**»*	OEDEME «**O**» / *OEDEMA «**O**»*
0	Absent	aspect normal / *no erythema*	aspect normal / *no oedema*
0,5	très léger / *very mild*	à peine perceptible : coloration rosée discrète d'une partie de la surface testée *fairly detectable: discreet pinkness of one part of the tested area*	palpable, à peine visible *palpable, barely visible*
1	Léger / *mild*	coloration rosée discrète de toute la surface testée ou coloration bien définie sur une partie de la surface testée *discreet pinkness of the complete tested area or rather visible on one part of the tested area*	palpable et visible *palpable, visible*
2	Modéré / *Moderate*	coloration rouge mate nette couvrant toute la surface testée *clearly distinguishable, dull red erythema covering the whole tested area*	œdème net (<1 mm d'épaisseur) avec ou sans présence de papule(s) ou vésicule(s) *obvious oedema (thickness < 1 mm) with or without papule(s) or vesicle(s)*
3	Sévère / *Severe*	coloration rouge feu ou rouge très foncé couvrant toute la surface testée ou érythème modéré diffusant en dehors de la surface testée *deep dark or fiery bright red color covering all the tested area or moderate erythema diffusing outside the tested area*	œdème important (≥1 mm d'épaisseur ou surface débordant la zone d'application) avec ou sans présence de vésicule(s) ou de bulle(s) *severe oedema (thickness ≥ 1 mm or diffusing outside the tested area) with or without vesicle(s) or blister(s)*

Une modification de structure cutanée (dessèchement, rugosité, épaississement, réflectivité) pouvant être liée à la nature même du produit étudié ou à l'un des ingrédients fait l'objet d'une description clinique dont l'intensité est appréciée selon le barème :

0,5 = douteux
1 = léger
2 = net
3 = important

A change in skin structure (dryness (D), roughness (R), thickness (T), reflectivity (Re)) that could be linked to the nature of the studied product or one of its components is clinically described and its intensity graded according to the following scale:

0.5 = doubtful
1 = slight
2 = obvious
3 = important.

1.3.3. Interprétation des résultats / *Results interpretation*

L'analyse et l'interprétation des résultats ont été réalisées en fonction des données obtenues dans les conditions expérimentales.
Elles sont descriptives et complétées par le calcul d'un indice d'irritation cumulative (I.I.C.) pour chaque volontaire, selon le rapport :

$$I.I.C. = \frac{\sum score(érythème + oedème)}{nombretotaldelecturesf}$$

Cet indice est ensuite moyenné par le nombre de sujets afin d'obtenir l'Indice d'Irritation Cumulative Moyen (I.I.C.M.) :

$$I.I.C.M. = \frac{\sum I.I.C.}{nombredesujets}$$

The analysis and the interpretation were carried out according to the results obtained in the experimental conditions.
They are descriptive and completed by the calculation of the cumulative irritation index (C.I.I.) for each subject according to the formula:

$$C.I.I. = \frac{\sum grades(erythema + edema)}{Totalnumberofreadings}$$

This index is then divided by the number of subjects in order to obtain the Mean Cumulative Irritation Index (M.C.I.I.):

$$M.C.I.I. = \frac{\sum C\,I.I.}{numberofsubjects}$$

L'indice ainsi obtenu (maximum 6), permet de classer arbitrairement le produit étudié selon le barème d'interprétation suivant :

I.I.C.M.	Classe
I.I.C.M. < 0,25	Non irritant (NI)
0,25 ≤ I.I.C.M. < 0,50	Très légèrement irritant (TLI)
0,5 ≤ I.I.C.M. < 1	Légèrement irritant (LI)
1 ≤ I.I.C.M. < 2	Moyennement irritant (MI)
I.I.C.M. ≥ 2	Irritant (I)

Les valeurs individuelles et la catégorie à laquelle appartient le produit étudié ont également été prises en compte pour une conclusion adaptée dans les conditions de l'essai.

The obtained index (maximum 6) allow to arbitrarily classify the studied product according to the following scale:

M.C.I.I.	*Class*
M.C.I.I. < 0.25	***Non irritating (NI)***
0.25 ≤ M.C.I.I. < 0.50	***Very slightly irritating (VSI)***
0.5 ≤ M.C.I.I. < 1	***Slightly irritating (SI)***
1 ≤ M.C.I.I. < 2	***Moderately irritating (MI)***
M.C.I.I. ≥ 2	***Irritating (I)***

Individual values and the product class were taken into account to write a suitable conclusion under the study conditions.

1.3.4. Bibliographie / *Bibliography*

1- COLIPA "Cosmetic product test guidelines for the assessment of human skin compatibility" 2nd edition – August 1997.
2- Patch-testing with the patient's own products - Peter J. FROSCH, Johannes GEIER, Wolfgang UTER, An GOOSENS - CONTACT DERMATITIS 4TH EDITION – 2006
3- Comparison of the cumulative irritation potential of Adapalene gel and cream with that of Erythromycin/Tretinoin solution and gel and Erythromycin/Tretinoin gel - Catherine QUEILLE-ROUSSEL, Michel PONCET, Stephane MESAROS, Alan CLUCAS, Michael BAKER and Andrew-Marc SOLOFF - CLINICAL THERAPEUTICS / VOL.23 N°2, 2001

2. RESULTATS – CONCLUSION / *RESULTS - CONCLUSION*

Les résultats individuels des lectures à chaque temps expérimental sont regroupés dans le tableau en ANNEXE I.
Les lectures effectuées 30 minutes et 24 heures après le retrait des patch-tests ont donné la valeur d'I.I.C.M. suivante :

The individual reading results at each experimental time are presented in the APPENDIX I.

The readings at 30 minutes and 24 hours after patch-tests removal gave of the following M.C.I.I value:

Référence produit *Product reference*	**Conditions** *Conditions*	**Durée de pose** *Patch duration*	**I.I.C.M.** *M.C.I.I.*	**Conclusion** *Conclusion*
TOX18010	**-Concentration /** ***Concentration:*** **Fourni** / *Provided:* **dilué 3%** /*diluted 3%* **Testé /** ***Tested :*** **PUR /** ***PURE*** **- Type de Patch tests:** *Patch tests type:* **Occlusif /** *Occlusive*	**48 heures /** *48 hours*	**0.01**	**Non irritant /** ***Non irritating***

ANNEXE I / *APPENDIX I*

CARACTERISTIQUES DES VOLONTAIRES / *SUBJECTS CHARACTERISTICS*

TABLEAU DES LECTURES / *TABLE OF READINGS*

CARACTERISTIQUES DES VOLONTAIRES / *SUBJECTS CHARACTERISTICS*

N° du sujet / *N° of subject*	Identification du sujet / *Identification of subject*	Age	Sexe / *Sex*	Phototype	Début de l'étude / Study Start	Fin de l'étude / Study end
1	JA/K	67	F	II	2018-03-27	2018-03-30
2	BI/R	32	M	III	2018-03-27	2018-03-30
3	BA/M	57	F	III	2018-03-27	2018-03-30
4	OR/B	65	F	III	2018-03-27	2018-03-30
5	NI/M	37	F	II	2018-03-27	2018-03-30
6	KA/K	40	F	II	2018-03-27	2018-03-30
7	CZ/A	36	F	II	2018-03-27	2018-03-30
8	KI/M	42	F	III	2018-03-27	2018-03-30
9	RO/Z	70	M	II	2018-03-27	2018-03-30
10	DU/N	29	F	II	2018-03-27	2018-03-30
11	MI/I	67	F	II	2018-03-27	2018-03-30
12	ST/E	56	F	II	2018-04-03	2018-04-06
13	BO/A	61	F	II	2018-04-03	2018-04-06
14	WI/A	42	F	II	2018-04-03	2018-04-06
15	CZ/O	19	F	II	2018-04-03	2018-04-06
16	KL/M	44	F	II	2018-04-03	2018-04-06
17	PR/K	60	F	III	2018-04-03	2018-04-06
18	BO/A	39	F	III	2018-04-03	2018-04-06
19	ST/H	59	F	II	2018-04-03	2018-04-06
20	KL/A	27	F	II	2018-04-03	2018-04-06
21	CZ/A	44	F	II	2018-04-03	2018-04-06
22	LA/K	38	F	III	2018-04-03	2018-04-06

Min	19	Nb F	phototype I
Max	70	20	0
Moy/Average	47	Nb M	phototype II
SEM	3	2	15
			phototype III
			7
			phototype IV
			0

TABLEAU DES LECTURES / *TABLE OF READINGS*

N° Volontaire / N° of subject	Lecture à 30 minutes / 30-minute reading				Lecture à 24 heures / 24-hour reading				IIC/CII	Modification de structure de la peau / Change in skin structure	
	T		P		T		P			Lecture 30 minutes / 30-minute reading	Lecture 24 heures / 24-hour reading
	E	O	E	O	E	O	E	O			
1	0	0	0	0	0	0	0	0	0	no change	no change
2	0	0	0	0	0	0	0	0	0	no change	no change
3	0	0	0	0	0	0	0	0	0	no change	no change
4	0	0	0	0	0	0	0	0	0	no change	no change
5	0	0	0	0	0	0	0	0	0	no change	no change
6	0	0	0	0	0	0	0	0	0	no change	no change
7	0	0	0	0	0	0	0	0	0	no change	no change
8	0	0	0	0	0	0	0	0	0	no change	no change
9	0	0	0	0	0	0	0	0	0	no change	no change
10	0	0	0	0	0	0	0	0	0	no change	no change
11	0	0	0	0	0	0	0	0	0	no change	no change
12	0	0	0	0	0	0	0	0	0	no change	no change
13	0	0	0	0	0	0	0	0	0	no change	no change
14	0	0	0	0	0	0	0	0	0	no change	no change
15	0	0	0	0	0	0	0	0	0	no change	no change
16	0	0	0	0	0	0	0	0	0	no change	no change
17	0	0	0	0	0	0	0	0	0	no change	no change
18	0	0	0	0	0	0	0,5	0	0,25	no change	no change
19	0	0	0	0	0	0	0	0	0	no change	no change
20	0	0	0	0	0	0	0	0	0	no change	no change
21	0	0	0	0	0	0	0	0	0	no change	no change
22	0	0	0	0	0	0	0	0	0	no change	no change
					I.I.C.M. / M.C.I.I.				**0,01**		

T: Témoin / Control
P: TOX18010
E: Erythème / Erythema
O: Oedème / Oedema

ANNEXE II / *APPENDIX II*

FEUILLE D'AUTHENTIFICATION DES RESULTATS /
AUTHENTIFICATION PAGE

ASSURANCE QUALITE / ***QUALITY ASSURANCE***
CERTIFICAT DE CONFORMITE / ***CERTIFICATE OF CONFORMITY***

FICHE D'AUTHENTIFICATION DES RESULTATS
AUTHENTIFICATION PAGE

A ma connaissance, l'étude n°18E0714
I am aware that the study #18E0714

a été conduite en accord avec le protocole et la fiche des paramètres d'étude.
has been conducted according to the PROTOCOL and THE STUDY PARAMETERS PAGE.

	Date	Signature
Agnieszka Cegielska Dermatologist	09/04/2018	
Beata Tylza Technician	09/04/2018	

ASSURANCE QUALITE	***QUALITY ASSURANCE***
L'étude a été réalisée dans l'esprit 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.	*The described study has been conducted in the spirit of 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 successive updates.*
L'étude a été menée selon les Procédures Opératoires Standards et selon le protocole de l'étude défini avec le promoteur. Les cahiers d'observation 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 with the sponsor.* *All the case report forms and the data were checked.*
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).*

Certificat de conformité / ***Certificate of conformity***

A ma connaissance, l'étude 18E0714 a été conduite en accord avec l'«**Assurance qualité**» précitée.
*I am aware that the study 18E0714 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.

Chef de projet / *Project manager*	Date	Signature
Aneta Orłowska	09/04/2018	

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Asparagopsis Armata Extract (Aspar'age)

Gdansk, July 20, 2018

Study Report #18E0715 (version 1.0) /
Related to quote/ order #18D0715

EVALUATION DU POTENTIEL SENSIBILISANT D'UN EXTRAIT NATUREL : TEST CLINIQUE FINAL DE SECURITE SOUS CONTRÔLE DERMATOLOGIQUE

ASSESSMENT OF THE SENSITIZING POTENTIAL OF A NATURAL EXTRACT: FINAL CLINICAL SECURITY TEST UNDER DERMATOLOGICAL CONTROL

TOX18010

Nr Report: 18P0714-1PL

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Investigator (dermatologist)
Dr.: Agnieszka Cegielska

Dermscan Poland est certifié ISO 9001 : 2008 / *Dermscan Poland is certified ISO 9001 : 2008*

comportant / *including* 26 pages

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RESUME DE L'ETUDE #18E0715 / *SUMMARY OF THE STUDY REPORT #18E0715*

EVALUATION DU POTENTIEL SENSIBILISANT D'UN EXTRAIT NATUREL :
TEST CLINIQUE FINAL DE SECURITE SOUS CONTRÔLE DERMATOLOGIQUE
ASSESSMENT OF THE SENSITIZING POTENTIAL OF A NATURAL EXTRACT:
FINAL CLINICAL SECURITY TEST UNDER DERMATOLOGICAL CONTROL

Objectif / *Objective*	Confirmer que l'application réitérée du produit dans des conditions d'utilisation maximalisées, chez des sujets sains, n'entraîne pas de réaction d'allergie de contact retardée / *To confirm that repeated applications of the product under maximized conditions, in healthy subjects, does not induce delayed contact allergy reactions.*		
Methodologie / *Methodology*	Etude monocentrique en simple aveugle / *Monocentric and simple blind study.*		
Critères d'évaluation / *Assessment criteria*	• Scorage érythème et œdème / *assessment of erythema and edema* • Irritation: Calcul de l'IICM (Indice d'Irritation Cumulative Moyen) / *Irritation: MCII (Mean Cumulative Irritation Index) calculation* • Sensibilisation: Cotation ICDRG / *Sensitization: ICDRG scoring*		
Cinétique / *Kinetics*	Induction : 3 semaines / *3 weeks* Repos / Rest : 2 semaines / *2 weeks* Challenge : 1 semaine / *1 week*		
Dates (JJ/MM/AAAA / *DD/MM/YYYY*)	**Réception du produit / *Product reception:***	**Début d'étude / *Study start:***	**Fin d'étude / *Study end:***
	19/03/2018	07/05/2018	13/07/2018
Produit / *Product*	**Référence / *Reference:***	**Forme / *Form:***	**Température de stockage / *Storage temperature:***
	TOX18010	Solution transparente / *Transparent solution*	Température ambiante / *Room temperature*
Application	**Zone:**	**Concentration**	**Type de patch / *patch type:***
	Dos (zone scapulaire) / *scapular part of the back*	Produit fourni par le client: dilué 3% / *Product provided by the customer: diluted 3%*	Semi-occlusif / *Semi-occlusive*
Population étudiée / *Studied Population*	Critères principaux d'inclusion / *Main inclusion criteria*	Age moyen / *Average age*:	Nombre de volontaires analysés / *Number of subjects analysed:*
	Age compris entre 18 et 70 ans / *Age between 18 et 70 years old*. Phototype I à III / *Phototype I to III.*	42±2 ans/*years* (18 - 70)	104
Résultats / *Results*	Potentiel irritant / *Irritating potential* - Phase d'induction / *Induction phase*: **Non irritant / *Non irritating*** Potentiel sensibilisant / *Sensitizing potential* - Phase de révélation / *Challenge phase:* **Non sensibilisant / *Non sensitizing***		
Investigateur / *Investigator*	**Nom et function / *Name and quality:***	**Date:**	**Signature:**
	Agnieszka CEGIELSKA Dermatologue / *Dermatologist*	20/07/2018	A Cegielska
Project Manager / *Chef de Projet*	**Aneta ORLOWSKA**	20/07/2018	Orłowska

1. PROTOCOLE / PROTOCOL

1.1. Objectif de l'étude / *Aim of the study*

L'objectif principal de cette étude est de confirmer que l'application réitérée d'un extrait naturel dans des conditions d'utilisation maximalisées, chez des sujets sains, n'entraîne pas de réaction d'allergie de contact retardée.

The main objective of this study is to confirm that repeated applications of a natural extract under maximized conditions, in healthy subjects, does not induce delayed contact allergy reactions.

Son objectif secondaire est de déterminer le potentiel irritant du produit après application unique d'une part et d'autre part, après utilisation réitérée.

Its secondary objective is to determine the irritating potential of the product first after a single application and second after repeated applications.

1.2. Plan expérimental / *Study design*

L'étude a été réalisée en ouvert, sans randomisation du site d'application.

This was an open study, without randomisation of the application site.

1.3. Sujets de l'étude / *Study subjects*

1.3.1. Critères d'inclusion / *Inclusion criteria*

- Sujet sain.
- Age compris entre 18 et 70 ans.
- Phototype I to III.
- Personne ne présentant ni cicatrice, ni tatouage, ni tache pigmentaire d'aucune sorte, ni pilosité trop importante, ni lésion dermatologique, ni traces irrégulières de bronzage 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 DERMSCAN.

- *Healthy subjects.*
- *Age between 18 and 70.*
- *Phototype I to III.*
- *Subjects without scars, tattoos, any pigmentary marks, excessive pilosity and uneven skin tone or active dermal lesions, on the concerned areas of the back.*
- *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 DERMSCAN could be expected.*

1.3.2. Critères de non-inclusion / *Non-Inclusion criteria*

- Femme enceinte ou qui allaite ou n'utilisant pas de contraception médicalement sûre (pour les femmes en âge de procréer).
- Exposition au soleil ou aux U.V. dans les 15 jours avant le début de l'étude.
- Sujet ayant participé à un essai de type patchs répétés ou ayant reçu des photopatch-tests depuis moins de 4 mois (avec un maximum de trois essais de ces types par an).
- Peau hyper irritable.
- Allergie ou sensibilité connues au sparadrap et/ou aux produits cosmetique ou antécédent d'allergie de contact retardée avérée.

- *Pregnant or nursing women or women without any medically efficient contraceptive method (for women of childbearing potential).*
- *Sun or UV exposure during the 15 days before the study.*
- *Subject having taken part to a repeated patch tests study or having received photopatch-tests from less than 4 months (with a maximum of three studies of this kind per year).*
- *Hyper irritable skin.*
- *Known allergy or sensitivity to adhesive plaster and/or cosmetic products or background of contact delayed allergy.*

- Pathologie cutanée, cicatrices, grains de beauté, taches de rousseur ou toute anomalie sur la zone d'expérience.
- Maladie grave ou évolutive (notamment antécédent de cancer cutané).
- Sujet suivant un traitement médicamenteux topique ou systémique:
  - anti-inflammatoires et/ou antihistaminiques pendant la semaine qui précède l'étude,
  - substances photosensibilisantes et / ou phototoxiques depuis moins d'un mois,
  - immunosuppresseurs et /ou corticoïdes pendant les 4 semaines qui précèdent,
  - rétinoïdes pendant les 6 mois précédant l'étude.
- Toute condition jugée, par l'investigateur, comme incompatible avec le protocole de l'étude.

- *History of abnormal response to sunlight or presence of active dermal lesions, scars, beauty spots, freckles or any abnormality, on the back.*
- *Serious or progressive disease (mainly skin cancer background).*
- *Subjects undergoing a topical or systemic treatment:*
  - *anti-inflammatories and/or anti-histamines during the previous week,*
  - *photo-allergic and/or phototoxic substances from less than one month,*
  - *immuno-suppressors and/or corticoids during the four previous weeks,*
  - *retinoids during the six previous months.*
- *Any condition considered, by the investigator, as incompatible with the study protocol.*

1.3.3. Contraintes / *Restrictions*

- Ne pas s'exposer au soleil, ni aux U.V. pendant toute la durée de l'étude.
- Ne pas appliquer de produits ni de topiques au niveau des zones d'application. Pas de piscine, hammam… pendant toute la durée de l'étude.
- Ne pas débuter de traitement médicamenteux sans en informer l'investigateur.

- *No exposure to sun or UV rays during the whole study.*
- *Do not apply any products nor topic drugs to the application zones. No swimming pool, sauna… during the whole study.*
- *Do not start any medical treatment without informing the investigator.*

1.4. Produit à l'étude / *Study product*

Le produit fourni par le promoteur, présentait les caractéristiques suivantes :

The product supplied by the sponsor, had the following characteristics:

Référence du produit *Product reference*	Aspect du produit *Product aspect*	Date de réception à Dermscan Poland *Receipt date at Dermscan Poland*
TOX18010	Solution transparente *Transparent solution*	19/03/2018 *March 19, 2018*

1.5. Méthodologie / *Methodology*

1.5.1. Matériel, dose / *Instruments, dose*

Le produit étudié a été appliqué sur peau nettoyée (au sérum physiologique ou à l'eau distillée), et séchée dans les conditions suivantes :

The studied product was applied to a cleansed (with physiological saline or water) and dry skin under the following conditions:

Zones / *Areas:*	Zones scapulaires : homolatérale (zone d'induction) et controlatérale (zone de révélation) / *Scapular zones: homolateral (induction zone) and controlateral (challenge zone)*
Type de Patch tests / *Patch tests type:*	Webril® (1cm²) - semi-occlusif *Webril® (1cm²) - semi-occlusive*
Dose :	40 µl
Conditions de l'application: *Application conditions:*	Produit fourni par le client: dilué 3% *Product provided by the customer: diluted 3%*
Fréquence d'application / *Application frequency:*	Phase d'induction : 3 fois par semaine pendant 48 heures (72h pour le week-end). Phase de révélation : 1 fois pendant 48 heures. *Induction phase: 3 times a week during 48 hours (72h for the week-end).* *Challenge phase: once during 48 hours.*
Durée de l'étude / *Study design:*	Phase d'induction : 3 semaines. Phase de latence : 2 semaines. Phase de révélation : 1 semaine. *Induction phase: 3 weeks.* *Rest phase: 2 weeks.* *Challenge phase: 1 week*
Contrôle / *Control:*	Patch sans produit *Patch without product*

1.5.2. Evaluation clinique / *Clinical assessment*

1.5.2.1. Critères 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 (0 to 3 scale):

Score	Cotation *Quotation*	**CRITERES** / ***CRITERIA**:*	
		ERYTHEME «**E**» / *ERYTHEMA «**E**»*	OEDEME «**O**» / *EDEMA «**O**»*
0	Absent	aspect normal / *no erythema*	aspect normal / *no edema*
0,5	très léger / *very slight*	à peine perceptible : coloration rosée discrète d'une partie de la surface testée *barely perceptible: pinkish coloration of one part of the tested area*	palpable, à peine visible *palpable, barely visible*
1	Léger / *slight*	coloration rosée discrète de toute la surface testée ou bien visible sur une partie de la surface testée *pinkish coloration of the complete tested area or rather visible on one part of the tested area*	palpable et visible *palpable, visible*
2	Net / *obvious*	érythème net couvrant toute la surface testée *obvious erythema covering the whole tested area*	œdème net (<1 mm d'épaisseur) avec ou sans présence de papule(s) ou vésicule(s) *obvious edema (thickness < 1 mm) with or without blister(s) or vesicle(s)*
3	Important / *important*	érythème intense couvrant toute la surface testée ou érythème net diffusant en dehors de la surface testée *severe erythema covering all the tested area or obvious erythema diffusing outside the tested area*	œdème important (≥1 mm d'épaisseur ou surface débordant la zone d'application) avec ou sans présence de vésicule(s) ou de bulle(s) *severe edema (thickness ≥ 1 mm or diffusing outside the tested area) with or without vesicle(s) or blister(s)*

1.5.2.2. Critères cliniques concernant le potentiel sensibilisant (phase de révélation) / *Clinical criteria regarding the sensitizing potential (challenge phase)*

La survenue de réactions allergiques a été évaluée selon l'échelle suivante :

The occurrence of allergic reactions was assessed 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	?	0.5
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-bulleuses / *Severe reaction with blisters or post-blisters ulcerations*	+++	3

* (International Contact Dermatitis Research Group)
Un score ≥ 2 indique une réaction d'allergie de contact. / *A score ≥ 2 represents a contact allergy reaction.*

1.5.2.3. Conduite à tenir en cas de réactions particulières / *Behaviour in case of specific reactions*

En cas de réaction au support (patch), celui-ci est déplacé et positionné à proximité du site initial. Si une réaction est à nouveau observée, les applications sont stoppées définitivement.

In case of reaction to the support (patch), this one is moved and applied close to the initial site. If a reaction is observed again, applications are definitely stopped.

Au cours de la phase d'induction si l'investigateur observe, au niveau du site d'application du produit, dès la deuxième application une réaction de type :

- érythème ≥ 2 ou
- érythème ≥ 1 accompagné d'œdème ou d'infiltration discrète avec quelques papules,

les applications sont poursuivies sur un site adjacent avec poursuite de la lecture sur les deux sites jusqu'à la fin de l'induction.
Si une nouvelle réaction apparaît, les applications sont stoppées définitivement pour la phase d'induction. Le produit ne sera ré-appliqué que lors de la phase de révélation.

During the induction phase, if the investigator observes, at the level of the product application site, as soon as the second application, a reaction like:

- *erythema ≥ 2 or*
- *erythema ≥ 1 with edema or discreet infiltration with a few papules,*

applications continue to an adjacent site with readings on both sites until the end of induction.

If a new reaction occurs, applications are definitely stopped for the induction phase. The product will be applied again only during the challenge phase.

En cas de suspicion d'allergie, il est proposé une nouvelle application du produit pour confirmer la réaction et éventuellement identifier la(les) substance(s) en cause. Cette ré-exposition est réalisée au minimum trois semaines après disparition de la réaction initiale. Les conditions de ré-exposition sont à discuter entre l'investigateur et le promoteur. Le sujet est suivi jusqu'à disparition des manifestations.

In case of allergy suspicion, a new product application is proposed to confirm the reaction and possibly identify the concerned substance(s). This new exposure takes place at least three weeks after disappearance of the previous reaction. The new exposure conditions have to be discussed between the investigator and the sponsor. The subject is followed until total disappearance of the signs.

1.5.3. Arrêt prématuré / *Premature study termination*

Les sujets ont le droit de sortir de l'essai à tout moment pour quelle que raison que ce soit.

The subjects have the right to leave the study at any time whatever the reason.

L'arrêt prématuré peut être dû 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.*

1.5.4. Déroulement de l'essai / *Study schedule*

1.5.4.1. Phase d'induction / *Induction phase*

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*	J0 *D0*	J1 *D1*	J2 *D2*	J3 *D3*	J4 *D4*	J5 *D5*	J6 *D6*
Application du produit *Product application*	↓		↓		↓		
Retrait / Lecture *Removal / Assessment*			↓		↓		

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*	J7 *D7*	J8 *D8*	J9 *D9*	J10 *D10*	J11 *D11*	J12 *D12*	J13 *D13*
Application du produit *Product application*	↓		↓		↓		
Retrait / Lecture *Removal / Assessment*	↓		↓		↓		

S3 / *W3:*

Jour de la semaine *Day of the week*	Lu *Mo*	Ma *Tu*	Me *We*	Je *Th*	Ve *Fr*	Sa *Sa*	Di *Su*	Lu *Mo*
Jour d'étude *Study day*	J14 *D14*	J15 *D15*	J16 *D16*	J17 *D17*	J18 *D18*	J19 *D19*	J20 *D20*	J21 *D21*
Application du produit *Product application*	↓		↓		↓			
Retrait / Lecture *Removal / Assessment*	↓		↓		↓			↓ (L/*A*)

1.5.4.2. Phase de latence / *Rest phase*

S4 et S5 : Pas d'application, ni de lecture. | *W4 and W5: No application, nor assessment.*

1.5.4.3. Phase de révélation / *Challenge phase*

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*	J35 D35	J36 D36	J37 D37	J38 D38	J39 D39
Application du produit *Product application*	↓				
Retrait / Lecture *Removal / Assessment*			↓		↓ (L/*A*)

1.5.5. Interprétation des résultats / *Results interpretation*

1.5.5.1. Phase d'induction / *Induction phase*

A l'issue des 9 lectures de la phase d'induction, le score moyen de chaque sujet (Indice d'Irritation Cumulative (I.I.C.)) est calculé en additionnant les scores obtenus à chacune des lectures et en divisant cette somme par le nombre effectif de lectures (une lecture n'est pas prise en compte s'il y a une réaction au témoin ou une irritation globale).

$$I.I.C = \frac{\sum \text{score (érythème + œdème)}}{\text{nombre total de lectures}}$$

Le pouvoir irritant du produit est évalué lors de la phase d'induction, en faisant la moyenne des réactions survenues afin d'obtenir l'Indice d'Irritation Cumulative Moyen (IIM) :

IICM = ∑IIC / nb de sujets

L'indice ainsi obtenu (maximum 6), permet de classer arbitrairement le produit étudié selon le barème d'interprétation suivant :

I.I.C.M.	**Classe**
I.I.C.M. < 0,25	**Non irritant (NI)**
0,25 ≤ I.I.C.M. < 0,50	**Très légèrement irritant (TLI)**
0,5 ≤ I.I.C.M. < 1	**Légèrement irritant (LI)**
1 ≤ I.I.C.M. < 2	**Moyennement irritant (MI)**
I.I.C.M. ≥ 2	**Irritant (I)**

Les valeurs individuelles et la catégorie à laquelle appartient le produit étudié sont également prises en compte pour une conclusion adaptée dans les conditions de l'essai.

At the end of the 9 readings of the induction phase, the average score of each subject (Cumulative Irritation Index (C.I.I.)) is calculated by adding the scores obtained for each of the readings and by dividing this sum by the actual number of readings (a reading is not taken into account if there is a reaction on the control or a global irritation).

$$C.I.I. = \frac{\sum \text{of the grade (erythema + edema)}}{\text{Number of readings}}$$

The irritating potential of the product is estimated by calculating the mean of the reactions observed during the induction phase in order to obtain the Mean Cumulative Irritation Index (M.C.I.I.):

MCII = ∑CII / nb of subjects

The obtained index (maximum 6) allows to arbitrarily classify the studied product according to the following scale:

M.C.I.I.	***Classe***
M.C.I.I. < 0.25	***Non irritating (NI)***
0.25 ≤ M.C.I.I. < 0.50	***Very slightly irritating (VSI)***
0.5 ≤ M.C.I.I. < 1	***Slightly irritating (SI)***
1 ≤ M.C.I.I. < 2	***Moderately irritating (MI)***
M.C.I.I. ≥ 2	***Irritating (I)***

Individual values and the product class are also taken into account to write a suitable conclusion under the study conditions.

1.5.5.2. Phase de révélation / *Challenge phase*

Une réaction allergique éventuelle au cours des phases d'induction ou de révélation est notée de 0 à 3 selon les critères de l'ICDRG (International Contact Dermatitis Research Group) – voir le tableau en paragraphe 1.5.2.2.
Lors de la révélation, une lecture est faite 30 minutes après enlèvement des patch-tests puis 48 heures plus tard.

Le pouvoir sensibilisant du produit est évalué lors des lectures à J37 et J39 (phase de révélation) en fonction des critères suivants : réaction ++ (2) ou +++ (3) en l'absence de phénomène d'irritation surajouté.

La survenue d'un seul cas de sensibilisation active **(score supérieur ou égal à ++ (2))** du côté controlatéral conduit à la conclusion : « Produit potentiellement sensibilisant ».

A possible allergic reaction, during the Induction or Challenge Phase, is assessed from 0 to 3 according to ICDRG (International Contact Dermatitis Research Group) – see the table paragraph 1.5.2.2.
During the Challenge Phase, the reading is done 30 minutes after patch-tests removal and 48 hours later.

The sensitizing potential of the product is assessed by the readings on D37 and D39 (Challenge Phase) according to the following criteria: reaction ++ (2) or +++ (3) in the absence of added irritation phenomenon.

*The presence of only one case of active sensitization **(upper or equal score in ++ (2))** on contralateral side leads to the conclusion "Potentially sensitizing product"*

1.5.6. Bibliographie / *Bibliography*:

COLIPA "Cosmetic product test guidelines for the assessment of human skin compatibility" 2nd edition – August 1997.

AFSSAPS : “TEST CLINIQUE FINAL DE SECURITE D’UN PRODUIT COSMETIQUE EN VUE DE CONFIRMER SON ABSENCE DE POTENTIEL SENSIBILISANT CUTANE RETARDE : RECOMMANDATIONS AUX PROMOTEURS DE RECHERCHE ET AUX PRESTATAIRES DE SERVICE” - Recommandations TCFS – version finale de décembre 2008.

2. SUIVI DE L'ETUDE / *STUDY FOLLOW-UP*

2.1. Population étudiée / *Studied population*

2.1.1. Inclusion

110 sujets sains ont été sélectionnés en accord avec les critères d'inclusion et de non-inclusion, et 104 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.

110 healthy subjects were selected according to the inclusion and non-inclusion criteria, and 104 subjects completed study. The table below presents the information concerning all the included subjects.

	Non inclus *Non included*	Inclus *Included*	Arrêt en cours d'étude *Drop out*	Perdus de vue *Untraceable*
Nombre de sujets *Number of subjects*	0	110	6	0

2.1.2. Caractéristiques des sujets / *Subjects characteristics*

Le tableau récapitulatif ci-dessous présente une synthèse des observations concernant uniquement les sujets inclus dans l'analyse des données.

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

Nombre de Sujets *Number of subjects*	Sexe *Sex*	Age (moy±SEM) *Age (mean±SEM)*	Type de peau *Skin type*	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*
104	93 F 11 M	42±2	N: 85 S: 19	I: 3 II: 64 III: 37	cf. Tableaux en ANNEXE II *cf. Tables in the APPENDIX II*	

Legend:
F: femelle / female
M: mâle / male
N: normal
S: sèche / dry

2.2. Amendement et/ou déviation au protocole / *Protocol amendment and/or non-adherence*

Le tableau des déviations au protocole est présentée en Annexe II.

The table of protocol non-adherences is presented in the Appendix II.

3. RESULTATS / *RESULTS*

3.1. Potentiel 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	J2/D2		J4/D4		J7/D7		J9/D9		J11/D11	
		n	%	n	%	n	%	n	%	n	%
TOX18010	T:	0	0,0%	0	0,0%	0	0,0%	0	0,0%	0	0,0%
	0	100	100,0%	102	100,0%	103	100,0%	103	100,0%	103	100,0%
	0,5	0	0,0%	0	0,0%	0	0,0%	0	0,0%	0	0,0%
	1	0	0,0%	0	0,0%	0	0,0%	0	0,0%	0	0,0%
	1,5	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%
	2,5	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%
	4	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%
	6	0	0,0%	0	0,0%	0	0,0%	0	0,0%	0	0,0%

score	J14/D14		J16/D16		J18/D18		J21/D21		Conclusion
	n	%	n	%	n	%	n	%	
T:	0	0,0%	0	0,0%	0	0,0%	0	0,0%	IICM/MCII = 0,00 Non irritant (NI) / Non irritating (NI)
0	103	100,0%	104	100,0%	104	100,0%	103	100,0%	
0,5	0	0,0%	0	0,0%	0	0,0%	0	0,0%	
1	0	0,0%	0	0,0%	0	0,0%	0	0,0%	
1,5	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%	
2,5	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%	
4	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%	
6	0	0,0%	0	0,0%	0	0,0%	0	0,0%	

(IICM) / *(M.C.I.I.)* = l'Indice d'Irritation Cumulative Moyen / *Mean Cumulative Irritation Index*

n = nombre de sujets / *number of subjects*

% = % of subjects / *% of subjects*

Dans les conditions de cette étude, le produit «TOX18010» a montré un score 0,00. Il peut donc être considéré comme non irritant.

Under these study conditions, product "TOX18010" showed a score 0,00. It can thus be considered non irritating.

3.2. Potentiel sensibilisant : phase de révélation / *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 READINGS regarding the Challenge Phase is presented in APPENDIX IV.
These reading made 30 minutes and 48 hours after having removed the patch-test showed the following results:

Zones	score	J37/D37		J39/D39		Résultat global/ *Global result*
		n	%	n	%	
Lectures zone controlatérale / *Contralateral zone readings*	**T:**	0	0,0%	0	0,0%	**Non sensibilisant** ***Non-sensitizing***
	0	104	100,0%	104	100,0%	
	0,5	0	0,0%	0	0,0%	
	1	0	0,0%	0	0,0%	
	2	0	0,0%	0	0,0%	
	3	0	0,0%	0	0,0%	

n = nombre de sujets / *number of subjects*
% = % of subjects / *% of subjects*

Dans les conditions de cette étude, aucune réaction ++ (2) ou +++ (3) n'a été constatée. Le produit «TOX18010» peut donc être considéré comme non sensibilisant.

Under these study conditions no reaction ++ (2) nor +++ (3) was observed, so the product "TOX18010" can be considered non-sensitizing.

4. CONCLUSION / *CONCLUSION*

Dans les conditions de cette étude, le produit «TOX18010» s'est avéré non irritant et non sensibilisant.

Under these study conditions, the product "TOX18010" can be considered non irritating and non-sensitizing.

5. ANNEXES / *APPENDICES*

ANNEXE I / ***APPENDIX I***
FEUILLE D'AUTHENTIFICATION DES RESULTATS / *AUTHENTIFICATION PAGE*
ASSURANCE QUALITE / *QUALITY ASSURANCE*
CERTIFICAT DE CONFORMITE / *CERTIFICATE OF CONFORMITY*

ANNEXE II / ***APPENDIX II***
CARACTERISTIQUES DES SUJETS / *SUBJECTS CHARACTERISTICS*

ANNEXE III / ***APPENDIX III***
TABLEAUX DES LECTURES- PHASE D'INDUCTION /
TABLES OF THE READINGS – INDUCTION PHASE

ANNEXE IV / ***APPENDIX IV***
TABLEAUX DES LECTURES- PHASE DE REVELATION /
TABLES OF THE READINGS – CHALLENGE PHASE

***ANNEXE I** / APPENDIX I*

FEUILLE D'AUTHENTIFICATION DES RESULTATS

AUTHENTIFICATION PAGE

A ma connaissance, l'étude n°**18E0715**
I am aware that the study N°18E0715

a été conduite en accord avec le protocole d'étude.
has been conducted according to the STUDY PROTOCOL.

Agnieszka CEGIELSKA, MD	Date	Signature
Dermatologist	20/07/2018	

Beata TYLZA	Date	Signature
Technician	20/07/2018	

ASSURANCE QUALITE / ***QUALITY ASSURANCE***
CERTIFICAT DE CONFORMITE / ***CERTIFICATE OF CONFORMITY***

ASSURANCE QUALITE

L'étude a été réalisée dans l'esprit 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 jour successives.

Il est de la responsabilité de l'industriel (fabricant du produit testé), de justifier qu'aucune substance constituant ce produit n'est sensibilisante.

L'étude a été menée selon les Procédures Opératoires Standards et selon le protocole de l'étude défini par le promoteur. Tous les événements recueillis pendant l'étude sont reportés.

L'authenticité et la véracité des données expérimentales recueillies ont été confirmées par les personnes ayant participé à l'étude (ANNEXE I).

QUALITY ASSURANCE

The described study has been conducted in the spirit of 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 successive updates.

It is the responsibility of the product manufacturer to attest that no substance included in this product is sensitizing.

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.

Controls on data veracity and conformity with the protocol have been performed and confirmed by persons participating in the study (APPENDIX I).

Certificat de conformité / ***Certificate of conformity***

A ma connaissance, l'étude **18E0715** a été conduite en accord avec l'"**Assurance qualité**" précitée.
*I am aware that the study 18E0715 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.

Orłowska

20/07/2018

Chef de projet / *Project manager*
Aneta ORŁOWSKA

Date

ANNEXE II / *APPENDIX II*
CARACTERISTIQUES DES SUJETS
SUBJECTS CHARACTERISTICS

N° du sujet / N° of subject	Nom / Last name	Prenom / First name	Âge / Age	Sexe / Sex	Phototype	Type de peau / Skin type	Commentaires / Comments	Date d'inclusion / Inclusion date	Date de fin d'étude / End date
1	ZA	A	44	F	II	N	**Aucun** /None	2018-05-07	2018-06-15
2	ZD	M	22	F	II	N	**Aucun** /None	2018-05-07	2018-06-15
3	BA	E	45	F	II	N	**Aucun** /None	2018-05-07	2018-06-15
4	ZA	D	62	F	III	S	**Aucun** /None	2018-05-07	2018-06-15
5	ŁO	M	29	F	II	N	**Aucun** /None	2018-05-07	2018-06-15
6	WI	E	66	F	II	N	**Déviation au protocole /** Protocol non-adherence	2018-05-07	2018-06-15
7	AN	G	61	F	II	N	**Aucun** /None	2018-05-07	2018-06-15
8	RA	D	54	F	II	N	**Aucun** /None	2018-05-07	2018-06-15
9	RO	T	36	M	II	N	**Aucun** /None	2018-05-07	2018-06-15
10	KO	B	68	F	II	S	**Aucun** /None	2018-05-07	2018-06-15
11	WE	M	44	F	III	S	**Aucun** /None	2018-05-07	2018-06-15
12	SZ	B	64	F	II	N	**Aucun** /None	2018-05-07	2018-06-15
13	TO	K	26	F	III	N	**Aucun** /None	2018-05-07	2018-06-15
14	DU	J	26	M	III	N	**Aucun** /None	2018-05-07	2018-06-15
15	WA	T	62	F	III	N	**Aucun** /None	2018-05-07	2018-06-15
16	SO	K	47	F	II	N	**Aucun** /None	2018-05-07	2018-06-15
*(17)**	*(ZA)**	*(P)**	*(47)**	*(M)**	*(III)**	*(N)**	***(Déviation au protocole /** Protocol non-adherence)**	*(2018-05-07)**	*(2018-06-11)**
18	KO	L	59	F	II	N	**Aucun** /None	2018-05-07	2018-06-15
19	WE	J	62	F	II	N	**Aucun** /None	2018-05-07	2018-06-15
20	JA	I	61	F	II	S	**Aucun** /None	2018-05-07	2018-06-15
21	RO	Z	70	M	II	N	**Aucun** /None	2018-05-07	2018-06-15
22	DO	B	39	F	III	S	**Aucun** /None	2018-05-07	2018-06-15
23	JA	K	28	F	II	N	**Aucun** /None	2018-05-07	2018-06-15
24	AU	A	42	F	II	N	**Aucun** /None	2018-05-07	2018-06-15
25	MĄ	J	64	F	II	N	**Aucun** /None	2018-05-07	2018-06-15
26	RO	A	36	F	II	N	**Aucun** /None	2018-05-07	2018-06-15
27	RU	J	30	M	II	N	**Aucun** /None	2018-05-07	2018-06-15
28	BA	G	46	F	II	N	**Aucun** /None	2018-05-07	2018-06-15
29	ŻY	I	18	F	III	N	**Aucun** /None	2018-05-07	2018-06-15
30	DE	K	45	F	III	N	**Aucun** /None	2018-05-07	2018-06-15
31	ŻM	A	25	M	III	N	**Aucun** /None	2018-05-07	2018-06-15
32	PR	A	24	F	III	N	**Aucun** /None	2018-05-07	2018-06-15
33	BO	P	25	F	III	S	**Aucun** /None	2018-05-07	2018-06-15
34	PO	K	64	F	II	N	**Aucun** /None	2018-05-07	2018-06-15
35	ŁU	T	34	M	II	N	**Aucun** /None	2018-05-07	2018-06-15
36	SZ	N	30	F	II	N	**Aucun** /None	2018-05-07	2018-06-15
37	MA	E	44	F	II	N	**Aucun** /None	2018-05-07	2018-06-15
38	NE	D	21	F	II	N	**Déviation au protocole /** Protocol non-adherence	2018-05-07	2018-06-15
*(39)**	*(WI)**	*(T)**	*(54)**	*(F)**	*(III)**	*(N)**	***(Déviation au protocole /** Protocol non-adherence)**	*(2018-05-07)**	*(2018-06-11)**
*(40)**	*(OT)**	*(G)**	*(18)**	*(F)**	*(II)**	*(N)**	***(Déviation au protocole /** Protocol non-adherence)**	*(2018-05-07)**	*(2018-06-11)**
41	KR	K	62	F	II	N	**Aucun** /None	2018-05-07	2018-06-15
42	PR	I	61	F	II	S	**Aucun** /None	2018-05-07	2018-06-15
43	DU	A	22	F	II	N	**Aucun** /None	2018-05-07	2018-06-15
44	WO	B	49	F	II	N	**Aucun** /None	2018-05-07	2018-06-15
45	KL	R	63	F	II	N	**Aucun** /None	2018-05-07	2018-06-15
46	ZA	D	31	M	II	N	**Aucun** /None	2018-05-07	2018-06-15
47	ME	S	41	F	II	S	**Aucun** /None	2018-05-07	2018-06-15
48	PR	D	31	F	II	S	**Aucun** /None	2018-05-07	2018-06-15
49	GR	K	29	F	III	S	**Aucun** /None	2018-05-07	2018-06-15
50	GR	D	20	F	III	N	**Aucun** /None	2018-05-07	2018-06-15
51	ZA	K	38	F	II	S	**Aucun** /None	2018-05-07	2018-06-15
52	KO	K	28	F	II	N	**Déviation au protocole /** Protocol non-adherence	2018-05-07	2018-06-15
53	SI	A	54	F	III	N	**Déviation au protocole /** Protocol non-adherence	2018-05-07	2018-06-15
54	CZ	H	44	F	II	N	**Aucun** /None	2018-05-07	2018-06-15
55	PA	M	30	F	III	S	**Déviation au protocole /** Protocol non-adherence	2018-05-09	2018-06-15
56	CZ	A	36	F	II	N	**Aucun** /None	2018-06-04	2018-07-13
57	KU	P	27	F	III	N	**Déviation au protocole /** Protocol non-adherence	2018-06-04	2018-07-13
58	MA	P	32	F	III	N	**Aucun** /None	2018-06-04	2018-07-13
59	KA	M	57	F	II	N	**Aucun** /None	2018-06-04	2018-07-13
60	KU	M	29	F	III	N	**Aucun** /None	2018-06-04	2018-07-13

N° du sujet / N° of subject	Nom / Last name	Prenom / First name	Âge / Age	Sexe / Sex	Phototype	Type de peau / Skin type	Commentaires / Comments	Date d'inclusion / Inclusion date	Date de fin d'étude / End date
61	KR	Ż	24	F	I	N	**Aucun** /None	2018-06-04	2018-07-13
62	PI	P	21	F	III	N	**Aucun** /None	2018-06-04	2018-07-13
63	ST	M	26	F	II	N	**Aucun** /None	2018-06-04	2018-07-13
64	WO	D	70	F	I	S	**Aucun** /None	2018-06-04	2018-07-13
65	ŻU	K	37	F	II	S	**Aucun** /None	2018-06-04	2018-07-13
66	NA	M	41	M	III	N	**Aucun** /None	2018-06-04	2018-07-13
67	TH	A	36	F	III	N	**Aucun** /None	2018-06-04	2018-07-13
68	AN	Z	63	F	II	N	**Aucun** /None	2018-06-04	2018-07-13
69	GO	W	43	F	II	N	**Aucun** /None	2018-06-04	2018-07-13
*(70)**	*(SE)**	*(W)**	*(29)**	*(F)**	*(III)**	*(N)**	***(Déviation au protocole /** Protocol non-adherence)**	*(2018-06-04)**	*(2018-07-09)**
71	ZA	G	67	F	II	S	**Aucun** /None	2018-06-04	2018-07-13
72	GA	B	46	F	II	N	**Aucun** /None	2018-06-04	2018-07-13
73	RA	G	60	F	II	N	**Déviation au protocole /** Protocol non-adherence	2018-06-04	2018-07-13
74	BI	G	58	F	III	N	**Aucun** /None	2018-06-04	2018-07-13
75	RA	M	55	F	II	S	**Aucun** /None	2018-06-04	2018-07-13
76	CZ	L	65	M	II	N	**Aucun** /None	2018-06-04	2018-07-13
77	HU	W	62	F	II	S	**Aucun** /None	2018-06-04	2018-07-13
*(78)**	*(NO)**	*(M)**	*(34)**	*(F)**	*(III)**	*(N)**	***(Déviation au protocole /** Protocol non-adherence)**	*(2018-06-04)**	*(2018-06-15)**
79	KU	D	21	F	III	N	**Aucun** /None	2018-06-04	2018-07-13
80	DA	U	42	F	III	N	**Aucun** /None	2018-06-04	2018-07-13
81	RY	K	20	F	III	N	**Aucun** /None	2018-06-04	2018-07-13
82	NO	P	22	M	III	N	**Aucun** /None	2018-06-04	2018-07-13
83	KO	K	66	F	II	N	**Aucun** /None	2018-06-04	2018-07-13
84	SO	A	42	F	II	S	**Aucun** /None	2018-06-04	2018-07-13
85	TĘ	A	41	F	II	S	**Aucun** /None	2018-06-04	2018-07-13
86	HA	K	28	F	III	N	**Déviation au protocole /** Protocol non-adherence	2018-06-04	2018-07-13
87	ŁO	M	32	F	III	N	**Aucun** /None	2018-06-04	2018-07-13
88	LE	A	22	F	III	N	**Aucun** /None	2018-06-04	2018-07-13
89	KO	W	20	F	III	N	**Aucun** /None	2018-06-04	2018-07-13
90	WI	A	43	F	III	N	**Aucun** /None	2018-06-04	2018-07-13
91	GO	E	58	F	II	N	**Aucun** /None	2018-06-04	2018-07-13
92	BO	Z	65	F	III	N	**Aucun** /None	2018-06-04	2018-07-13
93	OK	J	67	F	II	N	**Aucun** /None	2018-06-04	2018-07-13
94	KO	A	19	F	II	N	**Aucun** /None	2018-06-04	2018-07-13
95	JA	D	18	M	II	N	**Aucun** /None	2018-06-04	2018-07-13
96	LE	A	45	F	II	N	**Aucun** /None	2018-06-04	2018-07-13
97	BR	J	24	F	I	N	**Aucun** /None	2018-06-04	2018-07-13
98	CY	K	21	F	III	N	**Aucun** /None	2018-06-04	2018-07-13
99	KA	M	27	F	II	N	**Aucun** /None	2018-06-04	2018-07-13
100	JA	J	43	F	III	N	**Déviation au protocole /** Protocol non-adherence	2018-06-04	2018-07-13
101	KW	E	40	F	II	N	**Aucun** /None	2018-06-04	2018-07-13
102	KU	K	22	F	II	N	**Aucun** /None	2018-06-04	2018-07-13
103	ŁU	M	31	F	II	N	**Aucun** /None	2018-06-04	2018-07-13
104	GO	M	29	F	II	N	**Aucun** /None	2018-06-04	2018-07-13
105	LO	K	33	F	III	N	**Déviation au protocole /** Protocol non-adherence	2018-06-05	2018-07-13
106	WI	E	69	F	II	N	**Déviation au protocole /** Protocol non-adherence	2018-06-05	2018-07-13
107	OK	G	68	F	III	N	**Déviation au protocole /** Protocol non-adherence	2018-06-05	2018-07-13
*(108)**	*(ME)**	*(M)**	*(28)**	*(F)**	*(II)**	*(N)**	***(Déviation au protocole /** Protocol non-adherence)**	*(2018-06-05)**	*(2018-07-02)**
109	RA	E	25	F	III	N	**Déviation au protocole /** Protocol non-adherence	2018-06-06	2018-07-13
110	RA	J	42	F	II	N	**Déviation au protocole /** Protocol non-adherence	2018-06-06	2018-07-13

Min	18	Nb F	phototype I	Normal
Max	70	**93**	**3**	**85**
Moy/ Average	**42**	Nb M	phototype II	Graisseux / Greasy
SEM	**2**	**11**	**64**	**0**
			phototype III	Sèche / Dry
			37	**19**

Legend : M: Mâle /Male
F: Femelle / Female
N: Normal
S: Sèche / Dry
()*: Valeurs non inclues dans l'analyse / Values not included in the analysis

CARACTERISTIQUES DES SUJETS
SUBJECTS CHARACTERISTICS

- NON-ADHERENCE / *NON-ADHERENCE*

Description de la non-adhérence	Type de non-adherence (mineure / majeure)	Données conservées dans l'analyse (oui / non)
Le volontaire n°6 n'est pas venu à J7.	mineure	oui
Le volontaire n°17 n'est pas venu à J11.	mineure	oui
Le volontaire n° 17 est sorti de l'essai à J35 suite à une déviation majeure au protocole : l'exposition au soleil pendant l'étude.	**majeure**	**non**
Le volontaire n°38 n'est pas venu à J2.	mineure	oui
Le volontaire n° 39 est sorti de l'essai à J35 suite à une déviation majeure au protocole : l'exposition au soleil pendant l'étude.	**majeure**	**non**
Le volontaire n° 40 est sorti de l'essai à J35 suite à une déviation majeure au protocole : l'exposition au soleil pendant l'étude.	**majeure**	**non**
Le volontaire n°52 n'est pas venu à J14.	mineure	oui
Le volontaire n°53 n'est pas venu à J4.	mineure	oui
Le volontaire n°55 a commencé l'étude à J2 au lieu de J0.	mineure	oui
Le volontaire n°57 n'est pas venu à J4.	mineure	oui
Le volontaire n°70 n'est sorti de l'étude à J35.	**majeure**	**non**
Le volontaire n°73 n'est pas venu à J9.	mineure	oui
Le volontaire n°78 n'est pas retourné au centre d'étude à J11.	**majeure**	**non**
Le volontaire n°86 n'est pas venu à J21.	mineure	oui
Le volontaire n°100 n'est pas venu à J11.	mineure	oui
Le volontaire n°105 a commencé l'étude à J1 au lieu de J0.	mineure	oui
Le volontaire n°106 a commencé l'étude à J1 au lieu de J0.	mineure	oui
Le volontaire n°107 a commencé l'étude à J1 au lieu de J0.	mineure	oui
Le volontaire n°108 n'est sorti de l'étude à J28.	**majeure**	**non**
Le volontaire n°109 a commencé l'étude à J2 au lieu de J0.	mineure	oui
Le volontaire n°110 a commencé l'étude à J2 au lieu de J0.	mineure	oui

Description of the non-adherence	Type of non-adherence (minor / major)	Data kept in the analysis (yes / no)
The subject #6 did not come on the visit on D7.	minor	yes
The subject #17 did not come on the visit on D11.	minor	yes
The subject #17 dropped out of the study on D35 because of a major protocol non-adherence: sun exposure during the study.	**major**	**no**
The subject #38 did not come on the visit on D2.	minor	yes
The subject #39 dropped out of the study on D35 because of a major protocol non-adherence: sun exposure during the study.	**major**	**no**
The subject #40 dropped out of the study on D35 because of a major protocol non-adherence: sun exposure during the study.	**major**	**no**
The subject #52 did not come on the visit on D14.	minor	yes
The subject #53 did not come on the visit on D4.	minor	yes
The subject #55 started the study on D2 instead of D0.	minor	yes
The subject #57 did not come on the visit on D4.	minor	yes
The subject #70 resigned from the study on D35.	**major**	**no**
The subject #73 did not come on the visit on D9.	minor	yes
The subject #78 did not return to the study centre on D11.	**major**	**no**
The subject #86 did not come on the visit on D21.	minor	yes
The subject #100 did not come on the visit on D11.	minor	yes
The subject #105 started the study on D1 instead of D0.	minor	yes
The subject #106 started the study on D1 instead of D0.	minor	yes
The subject #107 started the study on D1 instead of D0.	minor	yes
The subject #108 resigned from the study on D28.	**major**	**no**
The subject #109 started the study on D2 instead of D0.	minor	yes
The subject #110 started the study on D2 instead of D0.	minor	yes

- TRAITEMENTS CONCOMITANTS / *CURRENT TREATMENTS*

N° du sujet / *N° of subject*	**Médication** (nom commercial) / **Medication** (sales name)	**Indication**	**Début de prise** (par rapport à la cinétique) / **Beginning of treatment** (compared to the kinetics)	**Date de fin ou en cours** (par rapport à la cinétique) / **End date or ongoing of treatment** (compared to the kinetics)
10	Apap ®	**Céphalée /** Headache	**J25 /** D25	**J25/** D25
38	Taromentin ®	**Sinusite** / Sinusitis	**J3 /** D3	**J10/** D10
46	Paracetamol	**Céphalée /** Headache	**J35 /** D35	**J35/** D35
63	Nospa Maxl ®	**Douleur menstruelle /** Menstrual pain	**J13 /** D13	**J13 /** D13
65	Ibuprom ®	**Céphalée** / Headache	**J32 /** D32	**J32 /** D32
82	Ibuprom ®	**Céphalée** / Headache	**J37 /** D37	**J37 /** D37
86	Gripex Control ®	**Rhume /** Cold	**J3 /** D3	**J3 /** D3
	Apap ®	**Céphalée** / Headache	**J35 /** D35	**J35 /** D35
88	Ibuprom ®	**Céphalée** / Headache	**J12 /** D12	**J12 /** D12
		Douleur menstruelle / Menstrual pain	**J21 /** D21	**J21 /** D21
		Céphalée / Headache	**J29 /** D29	**J29 /** D29
89	Apap ®	**Douleur menstruelle /** Menstrual pain	**J28 /** D28	**J28 /** D28
107	Paracetamol	**Céphalée** / Headache	**J15 /** D15	**J15 /** D15
109	Ibuprom ®	**Céphalée** / Headache	**J20 /** D20	**J20 /** D20
			J34 / D34	**J34 /** D34

- EVENEMENT INDESIRABLE / *ADVERSE EVENTS*

# on AE form:				1	2	3	4	5	6	7	8	9
ID Sujet / Subject ID	**N° sujet /** Subject #	**Date signature consentement /** Consent date	**N° EI /** AE #	**Description de l'événement /** Event description	**Date de début de l'événement /** Event onset date	**Sévérité /** Severity	**Photo prise Oui/Non/NA /** Photo Yes/No/NA	**Action entreprise par rapport au produit étudié /** Action taken with the studied product	**Action vis à vis de l'événement /** Action taken due to the event	**EIG (Oui/Non) /** SAE (Yes/No)	**Délai entre l'événement et la dernière prise du produit étudié /** Time frame from last product administration	**Relation de causalité avec le produit étudié /** Causality assessment (studied product relationship)
Aucun / None	**Aucun** / None	**Aucun** / None	**Aucun** / None	**Aucun** / None	**Aucun** / None	**Aucun** / None	**Aucun** / None	**Aucun** / None	**Aucun** / None	**Aucun** / None	**Aucun** / None	**Aucun** / None

# on AE form:				10	11		**EI toujours en cours en fin d'étude /** On-going AE at study end			**Episodes supplémentaires /** Additional episodes			**Commentaires /** Comments
ID Sujet / Subject ID	**N° sujet /** Subject #	**Date signature consentement /** Consent date	**N° EI /** AE #	**Relation avec les procédures de l'étude /** Event relationship with the study methods	**Evolution (Non connue / En cours / Résolu) /** Outcome (unknown / ongoing / resolved)	**Durée /** Duration	**Date de guérison /** Event stop date	**Evolution**	**Réalisation d'un suivi après la fin de l'étude Oui/Non /** Follow-up Yes/No	**Nombre d'épisodes au cours de l'étude /** Number of episodes during study	**Date de l'épisode /** Episode date	**Durée de l'épisode /** Episode duration	
Aucun / None	**Aucun** / None	**Aucun** / None	**Aucun** / None	**Aucun** / None	**Aucun** / None	**Aucun** / None	**Aucun** / None	**Aucun** / None	**Aucun** / None	**Aucun** / None	**Aucun** / None	**Aucun** / None	**Aucun** / None

ANNEXE III / *APPENDIX III*
RESULTATS INDIVIDUELS – PHASE D'INDUCTION
INDIVIDUAL RESULTS – INDUCTION PHASE

N° du sujet / N° of subject	J2 / D2						J4 / D4						J7 / D7						J9 / D9						J11 / D11					
	Control			Product			Control			Product			Control			Product			Control			Product			Control			Product		
	E	O	E+O	E	O	E+O	E	O	E+O	E	O	E+O	E	O	E+O	E	O	E+O	E	O	E+O	E	O	E+O	E	O	E+O	E	O	E+O
1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	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	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	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	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	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	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	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	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	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	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	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	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	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	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	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	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)**	*(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	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	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	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	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	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	0	0	0	0	0	0	0	0	0	0	0	0	0	0
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27	0	0	0	0	0	0	0	0	0	0	0	0	0	0	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	0	0	0	0	0	0	0	0	0	0	0	0	0	0
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32	0	0	0	0	0	0	0	0	0	0	0	0	0	0	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	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	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	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	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	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	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)**	*(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)**	*(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	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	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	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	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	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	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	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	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	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	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	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	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	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	0	0	0	0	0	0	0	0	0	0	0	0	0	0
55	NA	NA	NA	NA	NA	NA	0	0	0	0	0	0	0	0	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	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	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	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	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	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	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	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	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	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	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	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	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	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	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)**	*(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	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	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	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	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	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	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	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)**	*(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	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	0	0	0	0	0	0	0	0	0	0	0	0	0	0
81	0	0	0	0	0	0	0	0	0	0	0	0	0	0	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	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	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	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	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	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	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	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	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	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	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	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	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	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	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	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	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	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	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	0	0	0	0	0	0	0	0	(-)	(-)	(-)	(-)	(-)	(-)
101	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
102	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
103	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
104	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
105	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
106	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
107	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
*(108)**	*(0)**	*(0)**	*(0)**	*(0)**	*(0)**	*(0)**	*(0)**	*(0)**	*(0)**	*(0)**	*(0)**	*(0)**	*(0)**	*(0)**	*(0)**	*(0)**	*(0)**	*(0)**	*(0)**	*(0)**	*(0)**	*(0)**	*(0)**	*(0)**	*(0)**	*(0)**	*(0)**	*(0)**	*(0)**	*(0)**
109	NA	NA	NA	NA	NA	NA	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
110	NA	NA	NA	NA	NA	NA	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Nb 0	100			100			102			102			103			103			103			103			103			103		
Nb 0,5	0			0			0			0			0			0			0			0			0			0		
Nb 1	0			0			0			0			0			0			0			0			0			0		
Nb 1,5	0			0			0			0			0			0			0			0			0			0		
Nb 2	0			0			0			0			0			0			0			0			0			0		
Nb 2,5	0			0			0			0			0			0			0			0			0			0		
Nb 3	0			0			0			0			0			0			0			0			0			0		
Nb 4	0			0			0			0			0			0			0			0			0			0		
Nb 5	0			0			0			0			0			0			0			0			0			0		
Nb 6	0			0			0			0			0			0			0			0			0			0		
Total	100			100			102			102			103			103			103			103			103			103		
	J2 / D2						J4 / D4						J7 / D7						J9 / D9						J11 / D11					
	Control			Product			Control			Product			Control			Product			Control			Product			Control			Product		

N° du sujet / N° of subject	J14 / D14						J16 / D16						J18 / D18						J21 / D21						
	Control			Product			Control			Product			Control			Product			Control			Product			
	E	O	E+O	E	O	E+O	E	O	E+O	E	O	E+O	E	O	E+O	E	O	E+O	E	O	E+O	E	O	E+O	IIC / CII
1	0	0	0	0	0	0	0	0	0	0	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	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	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	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	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	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	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	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	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	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	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	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	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	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	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	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)*	(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	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	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	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	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	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	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	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	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	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	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	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	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	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	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	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	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	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	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	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	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	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)*	(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)*	(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	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	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	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	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	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	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	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	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	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	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	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	0	0	0,0
53	0	0	0	0	0	0	0	0	0	0	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	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	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	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	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	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	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	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	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	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	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	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	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	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	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	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	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)*	(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	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	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	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	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	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	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	0	0	0	0	0	0	0	0	0,0
(78)*	(-)*	(-)*	(-)*	(-)*	(-)*	(-)*	(-)*	(-)*	(-)*	(-)*	(-)*	(-)*	(-)*	(-)*	(-)*	(-)*	(-)*	(-)*	(-)*	(-)*	(-)*	(-)*	(-)*	(-)*	(-)*
79	0	0	0	0	0	0	0	0	0	0	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	0	0	0	0	0	0	0	0	0,0
81	0	0	0	0	0	0	0	0	0	0	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	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	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	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	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	0	0	(-)	(-)	(-)	(-)	(-)	(-)	0,0
87	0	0	0	0	0	0	0	0	0	0	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	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	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	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	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	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	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	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	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	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	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	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	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	0	0	0	0	0	0	0	0	0,0
101	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0,0
102	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0,0
103	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0,0
104	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0,0
105	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0,0
106	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0,0
107	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0,0
(108)*	(0)*	(0)*	(0)*	(0)*	(0)*	(0)*	(0)*	(0)*	(0)*	(0)*	(0)*	(0)*	(0)*	(0)*	(0)*	(0)*	(0)*	(0)*	(0)*	(0)*	(0)*	(0)*	(0)*	(0)*	(0,0)*
109	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0,0
110	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0,0
			103			103			104			104			104			104			103			103	0,00 M.C.I.I.
			0			0			0			0			0			0			0			0	
			0			0			0			0			0			0			0			0	
			0			0			0			0			0			0			0			0	
			0			0			0			0			0			0			0			0	
			0			0			0			0			0			0			0			0	
			0			0			0			0			0			0			0			0	
			0			0			0			0			0			0			0			0	
			0			0			0			0			0			0			0			0	
			0			0			0			0			0			0			0			0	
			0			0			0			0			0			0			0			0	
			103			103			104			104			104			104			103			103	
	J14 / D14						J16 / D16						J18 / D18						J21 / D21						
	Control			Product			Control			Product			Control			Product			Control			Product			

Legend : E : erythème/Erythema

O : œdème/Edema

()* : valeurs non inclues dans l'analyse / values not included in the analysis

ANNEXE IV / *APPENDIX IV*
RESULTATS INDIVIDUELS – PHASE DE REVELATION
INDIVIDUAL RESULTS – CHALLENGE PHASE

N° du sujet / N° of subject	J37 D37		J39 / D39	
	T	P	T	P
1	0	0	0	0
2	0	0	0	0
3	0	0	0	0
4	0	0	0	0
5	0	0	0	0
6	0	0	0	0
7	0	0	0	0
8	0	0	0	0
9	0	0	0	0
10	0	0	0	0
11	0	0	0	0
12	0	0	0	0
13	0	0	0	0
14	0	0	0	0
15	0	0	0	0
16	0	0	0	0
*(17)**	*(-)**	*(-)**	*(-)**	*(-)**
18	0	0	0	0
19	0	0	0	0
20	0	0	0	0
21	0	0	0	0
22	0	0	0	0
23	0	0	0	0
24	0	0	0	0
25	0	0	0	0
26	0	0	0	0
27	0	0	0	0
28	0	0	0	0
29	0	0	0	0
30	0	0	0	0
31	0	0	0	0
32	0	0	0	0
33	0	0	0	0
34	0	0	0	0
35	0	0	0	0
36	0	0	0	0
37	0	0	0	0
38	0	0	0	0
*(39)**	*(-)**	*(-)**	*(-)**	*(-)**
*(40)**	*(-)**	*(-)**	*(-)**	*(-)**
41	0	0	0	0
42	0	0	0	0
43	0	0	0	0
44	0	0	0	0
45	0	0	0	0
46	0	0	0	0
47	0	0	0	0
48	0	0	0	0
49	0	0	0	0
50	0	0	0	0
51	0	0	0	0
52	0	0	0	0
53	0	0	0	0
54	0	0	0	0
55	0	0	0	0
56	0	0	0	0
57	0	0	0	0
58	0	0	0	0
59	0	0	0	0
60	0	0	0	0

N° du sujet / N° of subject	J37 D37		J39 / D39	
	T	P	T	P
61	0	0	0	0
62	0	0	0	0
63	0	0	0	0
64	0	0	0	0
65	0	0	0	0
66	0	0	0	0
67	0	0	0	0
68	0	0	0	0
69	0	0	0	0
*(70)**	*(-)**	*(-)**	*(-)**	*(-)**
71	0	0	0	0
72	0	0	0	0
73	0	0	0	0
74	0	0	0	0
75	0	0	0	0
76	0	0	0	0
77	0	0	0	0
*(78)**	*(-)**	*(-)**	*(-)**	*(-)**
79	0	0	0	0
80	0	0	0	0
81	0	0	0	0
82	0	0	0	0
83	0	0	0	0
84	0	0	0	0
85	0	0	0	0
86	0	0	0	0
87	0	0	0	0
88	0	0	0	0
89	0	0	0	0
90	0	0	0	0
91	0	0	0	0
92	0	0	0	0
93	0	0	0	0
94	0	0	0	0
95	0	0	0	0
96	0	0	0	0
97	0	0	0	0
98	0	0	0	0
99	0	0	0	0
100	0	0	0	0
101	0	0	0	0
102	0	0	0	0
103	0	0	0	0
104	0	0	0	0
105	0	0	0	0
106	0	0	0	0
107	0	0	0	0
*(108)**	*(-)**	*(-)**	*(-)**	*(-)**
109	0	0	0	0
110	0	0	0	0
Nb 0	104	104	104	104
Nb 0,5	0	0	0	0
Nb 1	0	0	0	0
Nb 2	0	0	0	0
Nb 3	0	0	0	0
Total	**104**	**104**	**104**	**104**

()* : valeurs non inclues dans l'analyse / values not included in the analysis

6. ANNEXES - EXIGENCES ETHIQUES / *APPENDICES - ETHICAL REQUIREMENTS*

6.1. Evenements indesirables / *Adverse event*

6.1.1. Evénement indésirable (EI) / *Adverse event (AE)*

Toute manifestation nocive survenant chez une personne qui se prête à une recherche biomédicale ; que cette manifestation soit liée ou non à la recherche ou au produit sur lequel porte cette recherche (ex : grippe, maux de tête, insomnie, examens biologiques anormaux,….).

Any noxious symptom, occurring in a subject taking part in a clinical trial, whether or not this symptom is related to the study or the studied product(s) (e.g. flu, headache, abnormal biological analysis…).

6.1.2. Evénement Indésirable Grave / Effet Indésirable Grave (EIG) / *Serious Adverse Event (SAE) / Serious Undesirable Effect (SUE)*

Tout événement qui :
- Entraîne la mort (note : la mort est l'issue et non l'évènement),
- Met en jeu le pronostic vital,
- Nécessite une hospitalisation (au moins une nuit) ou la prolongation de l'hospitalisation (n'inclut pas une hospitalisation déjà programmée avant l'inclusion),
- Entraine une incapacité fonctionnelle temporaire ou permanente ou un handicap,
- Se traduit par une anomalie congénitale,

Est jugé comme tel par l'investigateur

Any event that:
- *results in death (note: death is the outcome, not the event);*
- *is life threatening;*
- *requires in-patient hospitalization (at least one night) or prolongation of existing hospitalization (does not include hospitalization scheduled before the inclusion);*
- *results in temporary or permanent functional incapacity or disability;*
- *is a congenital anomaly;*
- *is considered like by the investigator.*

6.1.3. Documentation / *Documentation*

Tout traitement concomitant est noté dans le cahier d'observation et dans le rapport d'étude.

All concomitant treatments are reported in the CRF and the study report.

Si celui-ci nécessite l'arrêt d'utilisation (temporaire ou définitif) du produit étudié, la prise d'un traitement correcteur ou la sortie d'étude du sujet, un formulaire d'évènement indésirable est complété.

If it requires the temporary or definitive termination of the studied product, the need for a corrective treatment or the withdrawal of the subject, an Adverse Event form is completed.

Tout Evénement Indésirable Grave (EIG) est noté dans le cahier d'observation (CRF) et dans le rapport d'étude.

All SAE are reported in the CRF and the study report.

6.1.4. Notification / *Notification*

L'investigateur déclare au promoteur, par fax ou e-mail, la survenue des effets indésirables en fonction de leur sévérité et de leur caractère inattendu (à l'appréciation du médecin investigateur).

The investigator declares to the Sponsor, by e-mail, the occurrence of adverse reactions according to their severity and their unexpectedness (according to the investigator's advice).

Tout Evénement Indésirable Grave (EIG) est déclaré au promoteur sans délai par e-mail, au plus tard 24 heures après en avoir eu connaissance.

All SAE are transmitted by e-mail to the Sponsor without delay, at the latest 24 hours after knowledge of their occurrence.

Un formulaire de déclaration d'EIG avec signature d'un médecin est envoyé par e-mail avec accusé de réception dans les 48 heures.

A SAE declaration form signed by a physician is sent, within 48 hours, by e-mail with acknowledgement of receipt.

6.1.5. Suivi / *Follow-up*

Si un évènement indésirable susceptible d'être lié au produit de recherche ou au protocole persiste à la fin de l'étude, l'Investigateur suit le sujet jusqu'à la résolution de l'événement ou stabilisation des symptômes sans toutefois décharger le promoteur de ses obligations et responsabilités.

When an adverse event linked to the studied product or the protocol persists at the end of the study, the Investigator ensures that the subject is followed up until total resolution of the event or stabilization of the symptoms without releasing the Sponsor of any obligation or responsibility.

6.1.6. Survenue d'une grossesse / *Occurrence of pregnancy*

La survenue d'une grossesse (rapportée ou diagnostiquée) après l'inclusion dans l'essai est considérée comme un épisode intercurrent non lié au(x) produit(s) étudié(s) ou au protocole et entraîne la sortie d'essai immédiate du sujet.
Un suivi est réalisé selon les procédures internes en vigueur jusqu'au terme de la grossesse ou de son interruption.

The occurrence of a pregnancy (reported or diagnosed) after inclusion in the study is considered as an intercurrent event not related to the studied product(s) nor the protocol and induces the immediate dropping out of the subject.
A follow-up will be done according to the current internal procedures up to the end of the pregnancy or to its interruption.

Personal Care Products Council
Committed to Safety, Quality & Innovation

Memorandum

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

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

DATE: April 17, 2020

SUBJECT: Chondrus Crispus

Institute for In Vitro Sciences, Inc. 2012. Tissue equivalent assay with Epiocular™ cultures (three after-shave balms with 0.8% *Chondrus crispus*).

Alba Science. 2011. A 14 day human cumulative irritation patch test (three aftershave balms, each containing 0.8% Chondrus crispus (CAS 9000-07-1)).

FINAL REPORT

Study Title

TISSUE EQUIVALENT ASSAY WITH EPIOCULAR™ CULTURES

Test Articles

[redacted]

Three after-shave balms with 0.8% *Chondrus crispus.*

Authors

Greg Mun, B.A.
Awais Farooq, B.S.

Study Completion Date
8 March 2012

Performing Laboratory

Institute for In Vitro Sciences, Inc.
30 West Watkins Mill Road, Suite 100
Gaithersburg, MD 20878

Study Number

[redacted]

Laboratory Project Number

[redacted]

TISSUE EQUIVALENT ASSAY WITH EPIOCULAR™ CULTURES

SUMMARY

IIVS Test Article Number	Sponsor's Designation	Conc.	t_{50} (hours)		pH
			Preliminary (13 July 2011)	Trial 1 (20 July 2011)	
[redacted]					
	[redacted]	Neat	> 16	> 24	5.0
		Neat	> 16	> 24	5.0
		Neat	> 16	> 24	5.0-5.5
[redacted]					
Positive Control	0.3% Triton®-X-100	NA	36.7 minutes	20.2 minutes	NA

Three after-shave balms with 0.8% *Chondrus crispus*.

NA – Not Applicable

* - Extrapolated t_{50} value, the mean relative viability of all tissues exposed to the test article was less than 50%. The t_{50} was calculated between the shortest exposure time and the zero time point with 100% relative viability value.

^ - The tissues were rinsed with $Ca^{++}Mg^{++}$-Free DPBS at ~37°C and soaked in 5 mL of the assay medium at ~37°C for 10 to 20 minutes. These were modified procedures used to effectively remove all traces of the test article from the exposed tissues. A visual inspection of the tissues exposed to the test article following the rinsing and soaking procedure showed no presence of the test article.

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STATEMENT OF COMPLIANCE

The Tissue Equivalent Assay With EpiOcular™ Cultures of the test articles, [redacted] was conducted in compliance with the U.S. FDA Good Laboratory Practice Regulations as published in 21 CFR 58, and the principles presented in the OECD series on Good Laboratory Practice in all material aspects with the following exceptions:

The identity, strength, purity and composition or other characteristics to define the test articles have not been determined by the testing facility. The certificates of analysis were not provided by the sponsor.

The stability of the test articles under the storage conditions at the testing facility and under the actual test conditions has not been determined by the testing facility and is not included in the final report.

Greg Mun, B.A.
Study Director

8 March 2012
Date

QUALITY ASSURANCE STATEMENT

Study Title: Tissue Equivalent Assay with EpiOcular™ Cultures

Study Number: [redacted]

Study Director: Greg Mun, B.A.

This study was divided into a series of in-process phases. Using a random sampling approach, Quality Assurance monitored each of these phases over a series of studies. Procedures, documentation, equipment records, etc., were examined in order to assure that the study was performed in accordance with the U.S. FDA Good Laboratory Practice Regulations (21 CFR 58), the U.S. EPA GLP Standards (40 CFR 792 and 40 CFR 160) and the OECD Principles of Good Laboratory Practice and to assure that the study was conducted according to the protocol and relevant Standard Operating Procedures.

The following are the inspection dates, phases inspected and report dates of QA inspections of this study:

Phase Inspected	Audit Date(s)	Reported to Study Director	Reported to Management
Protocol and Initial Paperwork	12-Jul-11	12-Jul-11	15-Jul-11
Rinsing and Addition of MTT – 1 hour time point and positive control	13-Jul-11	13-Jul-11	15-Jul-11
Data and Draft Report	8, 11-12 Sep-11	12-Sep-11	14-Sep-11
Final Report	06-Mar-12	06-Mar-12	06-Mar-12

This report describes the methods and procedures used in the study and the reported results accurately reflect the raw data of the study.

Amanda K Ulrey, RQAP-GLP
Quality Assurance

08-March-2012
Date

SIGNATURE PAGE

TISSUE EQUIVALENT ASSAY WITH EPIOCULAR™ CULTURES

Initiation Date:	12 July 2011
Completion Date:	8 March 2012
Sponsor:	[redacted]
Sponsor's Representative:	[redacted]
Testing Facility and Study Director Address:	Institute for In Vitro Sciences, Inc. 30 West Watkins Mill Road, Suite 100 Gaithersburg, MD 20878
Archive Location:	[redacted]
Study Director:	8 March 2012 Greg Mun, B.A. Date
Laboratory Manager:	Nathan R. Wilt, B.S.
Laboratory Supervisor:	Allison Hilberer, M.S.

TEST ARTICLE RECEIPT

IIVS Test Article Number	Sponsor's Designation	Physical Description	Receipt Date	Storage Conditions*
[redacted]				
Three after-shave balms with 0.8% *Chondrus crispus.*	[redacted]	light brown cream	29 June 2011	room temperature
		light brown cream	29 June 2011	room temperature
		light brown cream	29 June 2011	room temperature
[redacted]				

* - Protected from exposure to light

TISSUE EQUIVALENT ASSAY WITH EPIOCULAR™ CULTURES

INTRODUCTION

The EpiOcular™ Human Cell Construct (MatTek Corporation) was used to assess the potential ocular irritancy of the test articles. The MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) conversion assay, which measures the NAD(P)H-dependent microsomal enzyme reduction of MTT (and to a lesser extent, the succinate dehydrogenase reduction of MTT) to a blue formazan precipitate, was used to assess cellular metabolism after exposure to a test article for various exposure times[1]. The duration of exposure resulting in a 50% decrease in MTT conversion in test article-treated EpiOcular™ human cell constructs, relative to control cultures, was determined (t_{50}).

The purpose of this study was to evaluate the potential toxicity of the test articles, supplied by [redacted] as measured by the conversion of MTT by EpiOcular™ human cell constructs after exposure to a test article for various exposure times. The laboratory phase of the study was conducted from 12 July 2011 to 21 July 2011 at the Institute for In Vitro Sciences, Inc. After a time range finding assay, the test articles were tested in a valid definitive assay to determine the time of exposure to a test article, which resulted in the t_{50} endpoint.

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1 Berridge, M.V., Tan, A.S., McCoy, K.D., Wang, R. (1996) The Biochemical and Cellular Basis of Cell Proliferation Assays That Use Tetrazolium Salts. **Biochemica** 4:14-19.

MATERIALS AND METHODS

Receipt of the EpiOcular™ Human Cell Construct Model

Upon receipt of the EpiOcular™ Human Cell Construct Kit (MatTek Corporation), the solutions were stored as indicated by the manufacturer. The EpiOcular™ human cell constructs were stored at 2-8°C until used. On the day of dosing an appropriate volume of EpiOcular™ human cell construct assay medium was removed and warmed to approximately 37°C. Nine hundred µL of assay medium were aliquoted into the wells of 6-well plates. The six-well plates were labeled to indicate test article and exposure time. The samples were inspected for air bubbles between the agarose gel and Millicell® insert prior to opening the sealed package. Cultures with air bubbles covering greater than 50% of the Millicell® area were not used. The 24-well shipping containers were removed from the plastic bag and their surfaces were disinfected with 70% ethanol. The EpiOcular™ human cell constructs were transferred aseptically into the 6-well plates. The EpiOcular™ human cell constructs were then incubated at 37±1°C in a humidified atmosphere of 5±1% CO_2 in air for at least one hour. The medium was then aspirated and nine hundred µL of fresh medium were added to each assay well below the EpiOcular™ human cell construct. The plates were returned to the incubator until treatment was initiated. Upon opening the shipping bag, any remaining unused tissues were briefly gassed with an atmosphere of 5% CO_2/95% air and placed back at 2-8°C for later use.

Test Article Preparation

As instructed by the Sponsor, each test article was administered to the test system without dilution.

Assessment of Direct Test Article Reduction of MTT

Each test article was added to a 1.0 mg/mL MTT (Sigma) solution in warm Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 2 mM L-glutamine (MTT Addition Medium) to assess its ability to directly reduce MTT. Approximately 100 µL of each test article were added to 1 mL of the MTT solution and the mixtures were incubated in the dark at 37°C for approximately one hour. If the MTT solution color turned blue/purple, the test article was presumed to have reduced the MTT. Water insoluble test materials may show direct reduction (darkening) only at the interface between the test article and the medium.

In cases where the test article was shown to reduce MTT, only those test articles that remained bound to the tissue after rinsing, resulting in a false MTT reduction signal, could present a problem. To evaluate whether residual test article was binding to the tissue and leading to a false MTT reduction signal, a functional check (using freeze-killed control tissue) was performed as described below in the section "Killed Controls (KC) for Assessment of Residual Test Article Reduction of MTT".

[redacted]

the three after-shave balms with 0.8% *Chondrus crispus*

The test articles, [redacted] were observed to reduce MTT directly in the absence of viable cells. A killed control experiment was performed

concurrently in the time range finding assay to determine the extent of the direct MTT reduction (if any) by the test articles alone.

pH Determination

The pH of each test article was measured using pH paper (EMD Chemicals Inc.). Initially, the test articles were added to pH paper with 0-14 pH range in 1.0 pH unit increments to approximate a narrow pH range. Next, the test articles were added to pH paper with a narrower range of 0-6 and/or 5-10 pH units with 0.5 pH unit increments, to obtain a more accurate pH value. The pH values obtained from the narrower range pH paper are presented in Table 1.

Time Range Finding Assay

A time range finding assay was performed to establish an appropriate exposure time range to be used in the definitive assay for each test article. Four exposure times of 1, 4, 8, and 16 hours were tested in the time range finding assay. One culture was treated per exposure time with 100 µL of the appropriate test article or control. [redacted] The negative control, 100 µL of sterile, deionized water (Quality Biological), was exposed for 16 hours. The positive control, 100 µL of 0.3% Triton®-X-100 (Fisher), was exposed for 15 and 45 minutes (one culture per exposure time). The exposed cultures were then incubated for the appropriate amount of time at 37±1°C in a humidified atmosphere of 5±1% CO_2 in air.

After the appropriate exposure time, the EpiOcular™ cultures were extensively rinsed with Calcium and Magnesium-Free Dulbecco's Phosphate Buffered Saline ($Ca^{++}Mg^{++}$-Free DPBS) and the wash medium was decanted. After rinsing, the tissue was transferred to 5 mL of Assay Medium for a 10 to 20 minute incubation at room temperature to remove any test article absorbed into the tissue. A 1.0 mg/mL solution of MTT in warm MTT Addition Medium was prepared no more than 2 hours before use. Three hundred µL of MTT solution were added to designated wells in a prelabeled 24-well plate. The EpiOcular™ constructs were transferred to the appropriate wells after rinsing with $Ca^{++}Mg^{++}$-Free DPBS. The trays were incubated at 37±1°C for approximately three hours in a humidified atmosphere of 5±1% CO_2 in air.

After the incubation period with MTT solution, the EpiOcular™ cultures were blotted on absorbent paper, cleared of excess liquid, and transferred to a prelabeled 24-well plate containing 2.0 mL of isopropanol in each designated well. The plates were sealed with parafilm and stored in the refrigerator (2-8°C) until the last exposure time was harvested. The plates were then shaken for at least two hours at room temperature.

At the end of the extraction period, the liquid within the Millicell® inserts was decanted into the well from which the Millicell® insert was taken. The extract solution was mixed and 200 µL were transferred to the appropriate wells of a 96-well plate. Two hundred µL of isopropanol were added to the two wells designated as the blanks. The absorbance at 550 nm (OD_{550}) of each well was measured with a Molecular Devices Vmax plate reader.

Killed Controls (KC) for Assessment of Residual Test Article Reduction of MTT

To evaluate whether residual test article was binding to the tissue and leading to a false MTT reduction signal, a functional check (using freeze-killed control tissue) was performed. Freeze killed tissues were stored in the freezer until use.

the three after-shave balms with 0.8% *Chondrus crispus*

For the test articles, [redacted] single killed tissues were treated with the test article in the normal fashion for 1 and 16 hours (the shortest and longest test article exposure time). The rinsing, MTT exposure, and solvent extraction procedures were performed exactly as described for the viable tissues. The negative control (100 µL of sterile, deionized water) was tested for 16 hours (the only negative control exposure time). A small amount of MTT reduction is expected from the residual NADH and associated enzymes within the killed tissue. This background reduction of MTT will be compared to the MTT reduction observed in the test article-treated killed-control tissues.

The raw OD_{550} value of the negative control-treated killed control was subtracted from the raw OD_{550} values for each of the test article-treated killed controls, to determine the net OD_{550} values of the test article-treated killed controls. The net OD_{550} values represent the amount of reduced MTT due to direct reduction by test article residues. For the test articles, [redacted] there was little or no direct MTT reduction in the test article-treated killed control compared to the negative control-treated killed controls, and the MTT reduction observed in the test article-treated viable tissue was ascribed to the viable cells.

the three after-shave balms

Definitive Assay

Based on the results of the time range finding assay, three to four exposure times were chosen for the definitive assay. [redacted] The exposure times for test articles, [redacted] were 16, 20, and 24 hours based on their non-cytotoxic responses in the preliminary assay. The exposure times for test articles, [redacted] were 8, 16, 20, and 24 hours. [redacted] The exposure times were chosen such that generally two exposure times were expected to result in survivals lower than 50% and two exposure times were expected to result in survivals greater than 50%. In general, the negative control exposure times were selected to fit the range of the test article or positive control exposure times. The negative control (100 µL of sterile, deionized water) was exposed for 0.25, 4, 8, and 24 hours. The positive control (100 µL of 0.3% Triton®-X-100) was exposed for 15 and 45 minutes. The procedures used to conduct the definitive assay were essentially the same as for the time range finding assay with the exception that at least duplicate cultures were dosed per exposure time. [redacted]

The first and third after-shave balms

the second after-shave balm

[redacted] All other cultures with greater than 3 minute exposure time were then incubated for the appropriate amount of time at 37±1°C in a humidified atmosphere of 5±1% CO_2 in air.

[redacted]

Presentation of Data

The raw absorbance values were captured. The mean OD_{550} value of the blank wells was calculated. The corrected mean OD_{550} value of the negative control was determined by subtracting the mean OD_{550} value of the blank wells from their mean OD_{550} values. The corrected OD_{550} value of the individual test article exposure times and the positive control exposure times was determined by subtracting the mean OD_{550} value of the blank control from their OD_{550} values. The individual % of Control values were averaged to get the mean % of Control value. All calculations were performed using an Excel spreadsheet. The following percent of control calculations were made:

$$\% \text{ of Control} = \frac{\text{Corrected } OD_{550} \text{ of Test Article or Positive Control Exposure Time}}{\text{Appropriate corrected mean } OD_{550} \text{ of Negative Control}} \text{ X } 100$$

Exposure time response curves were plotted with the % of Control on the ordinate and the test article or positive control exposure time on the abscissa. The t_{50} value was interpolated from each plot. To determine the t_{50}, the two consecutive points were selected, where one exposure time resulted in a relative survival greater than 50%, and one exposure time resulted in less than 50% survival. Two select points were used to determine the slope and the y-intercept for the equation $y=m(x) + b$. Finally, to determine the t_{50}, the equation was solved for y=50. If all of the exposure times show less than 50% survival, the t_{50} value is calculated based on 100% viability at zero exposure time and the shortest test article exposure time with less than 50% survival. If all of the exposure time points show greater than 50% survival, the t_{50} value is presented as greater than the longest test article exposure time.

Criteria for a Valid Test

The assay results were accepted if the positive control, 0.3% Triton®-X-100, caused a t_{50} value within two standard deviations of the historical mean. The corrected mean OD_{550} value for the minimum negative control exposure time should be within 20% of the corrected mean OD_{550} value for the maximum negative control exposure time (up to 4 hours).

RESULTS AND DISCUSSION

Time Range Finding Assay

A time range finding assay was performed, consisting of four exposure times 1, 4, 8, and 16 hours for the test articles supplied by [redacted] The exposure time response curves are included in Appendix B. Based upon the results of the time range finding assay, three to four exposure times were selected for each test article for the definitive assay (see Materials and Methods). The t_{50} results for the time range finding assay are reported in Table 1, under "Preliminary".

three after-shave balms with 0.8% *Chondrus crispus*

The test articles, [redacted], were determined to directly reduce MTT. Therefore, a killed-control experiment was performed concurrently in the exposure time range finding assay. The results of the killed control experiment showed that there was little or no direct MTT reduction in the test article-treated killed control compared to the negative control-treated killed controls; and the MTT reduction observed in the test article-treated viable tissue was ascribed to the viable cells. [redacted]

Definitive Assay

the first and third after-shave balm

[redacted] The exposure times for test articles, [redacted] were 16, 20, and 24 hours based on non-cytotoxic responses in the time range finding assay. The exposure times for test articles, [redacted], were 8, 16, 20, and 24 hours. [redacted] The negative control was also exposed in duplicate for 0.25, 4, 8 and 24 hours. Table 1 summarizes the t_{50} results of the definitive Tissue Equivalent Assay With EpiOcular™ Cultures for the test articles and the positive control, 0.3% Triton®-X-100, under "Trial 1". The exposure time response curves are included in Appendix B. Since the positive control fell within two standard deviations of the historical mean (15.8 – 38.9 minutes), and the corrected mean OD_{550} value for the minimum negative control exposure time (1.297) was within 20% of the corrected mean OD_{550} value for the maximum negative control exposure time (up to 4 hours) (1.366), the assay results were accepted.

the second after-shave balm

[redacted]

The test articles, [redacted] could not be completely removed from the exposed tissues following the rinsing and soaking process after 8, and 16 hour exposure times in the time range finding assay and all exposure times in the definitive assay. The test article, [redacted], could not be completely removed from the exposed tissues following the rinsing and soaking process after 1, 8, and 16 hour exposure times in the time range finding assay and after 16 hour exposure time in the definitive assay. The residual test article prolonged the exposure to the tissues, which may have influenced the toxic effect. For these test articles, the t_{50} results were not affected because the test article did not cause > 50% relative toxicity to the tissues up to the longest prescribed exposure time of 16 hours in the time range finding assay (t_{50} > 16 hours) and 24 hours in the definitive assay (t_{50} > 24 hours).

[redacted]

Table 1

IIVS Test Article Number	Sponsor's Designation	Conc.	t_{50} (hours)		pH
			Preliminary (13 July 2011)	Trial 1 (20 July 2011)	
[redacted]					
Three after-shave balms with 0.8% *Chondrus crispus.*	[redacted]	Neat	> 16	> 24	5.0
		Neat	> 16	> 24	5.0
		Neat	> 16	> 24	5.0-5.5
[redacted]					
Positive Control	0.3% Triton®-X-100	NA	36.7 minutes	20.2 minutes	NA

NA – Not Applicable

* - Extrapolated t_{50} value, the mean relative viability of all tissues exposed to the test article was less than 50%. The t_{50} was calculated between the shortest exposure time and the zero time point with 100% relative viability value.

^ - The tissues were rinsed with $Ca^{++}Mg^{++}$-Free DPBS at ~37ºC and soaked in 5 mL of the assay medium at ~37ºC for 10 to 20 minutes. These were modified procedures used to effectively remove all traces of the test article from the exposed tissues. A visual inspection of the tissues exposed to the test article following the rinsing and soaking procedure showed no presence of the test article.

EPIOCULAR™ BIOASSAY

EXPERIMENT DATE: 13-Jul-11
TEST MATERIAL: [redacted]
TEST ARTICLE: [redacted]

Study No. [redacted]

t_{50} = >16 Hours

PRELIMINARY
CONCENTRATION: 100%

TIME EXPOSURE (Hours)	PERCENT VIABLE
1	129.3
4	143.3
8	137.2
16	114.5

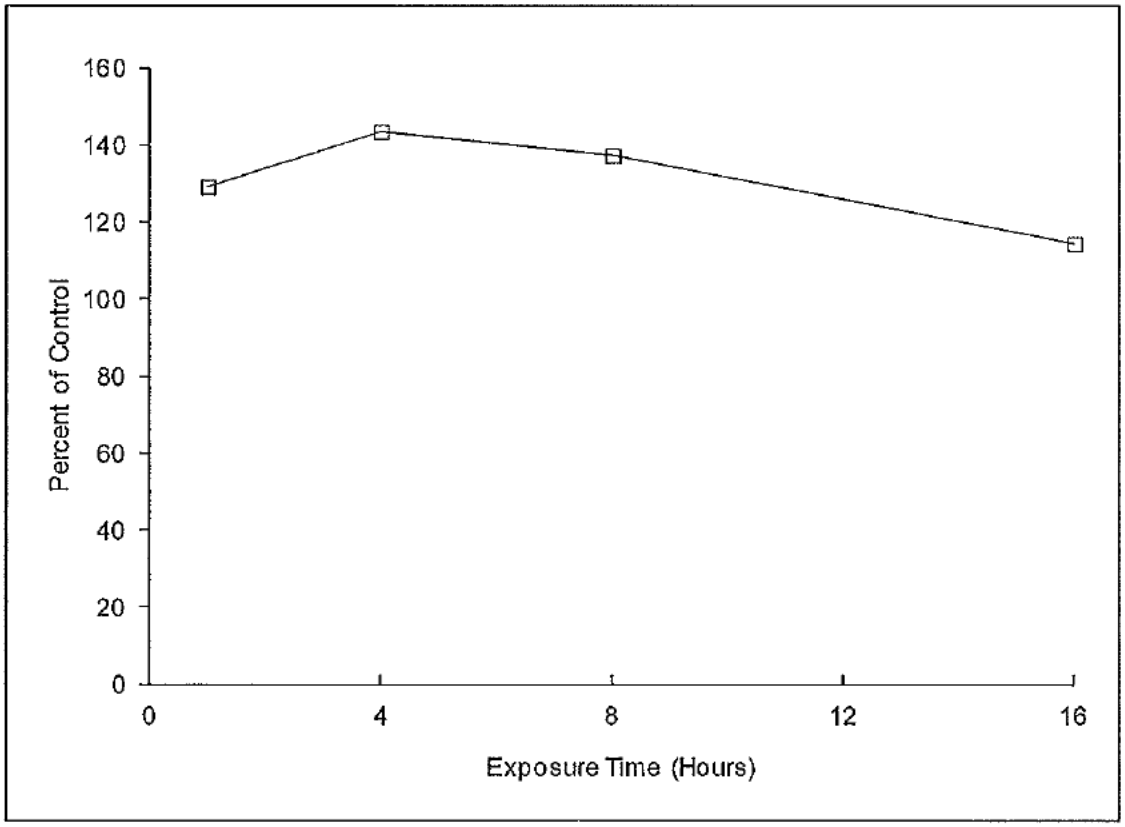
y = Percent Viable
x = Exposure Time
slope=rise/ run=(y1-y2)/ (x1-x2)
y intercept=y-(slope*x)

	X		Y
1	0.0	1	114.5
2	0.0	2	114.5
3	#DIV/0!	3	50
	slope =		#DIV/0!
	y intercept =		#DIV/0!

First after-shave balm with 0.8% *Chondrus crispus*.

[redacted]

CONCENTRATION: 100%
PRELIMINARY



EPIOCULAR™ BIOASSAY

EXPERIMENT DATE: 20-Jul-11
TEST MATERIAL: [redacted]
TEST ARTICLE: [redacted]

Study No. [redacted]

t_{50}= >24 Hours

Trial 1
CONCENTRATION: 100%

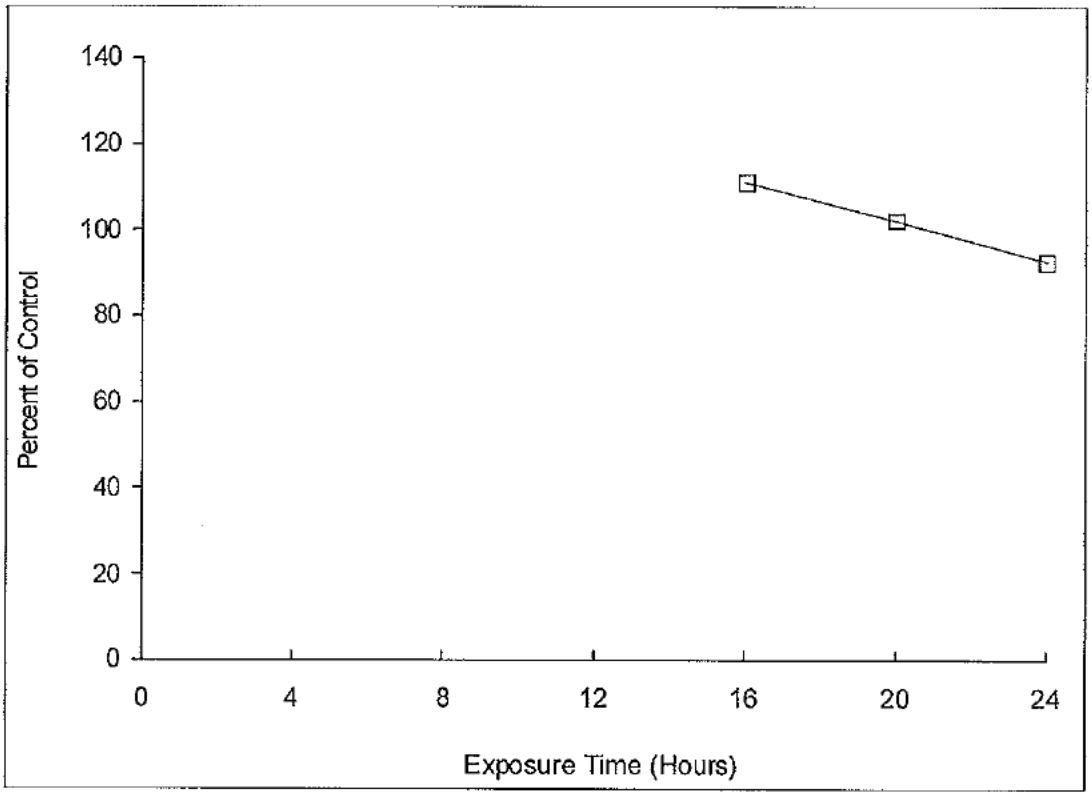
TIME EXPOSURE (Hours)	PERCENT VIABLE
16	111.2
20	102.4
24	92.7

y = Percent Viable
x = Exposure Time
slope=rise/run=(y1-y2)/(x1-x2)
y intercept=y-(slope*x)

	X		Y
1	0.0	1	92.7
2	0.0	2	92.7
3	#DIV/0!	3	50
slope =			#DIV/0!
y intercept =			#DIV/0!

First after-shave balm with 0.8% Chondrus crispus.

[redacted]
CONCENTRATION: 100%
Trial 1



EPIOCULAR™ BIOASSAY

EXPERIMENT DATE: 13-Jul-11
TEST MATERIAL: [redacted]
TEST ARTICLE:

Study No. [redacted]

t_{50} = >16 Hours

PRELIMINARY
CONCENTRATION: 100%

TIME EXPOSURE (Hours)	PERCENT VIABLE
1	157.0
4	141.6
8	129.0
16	79.1

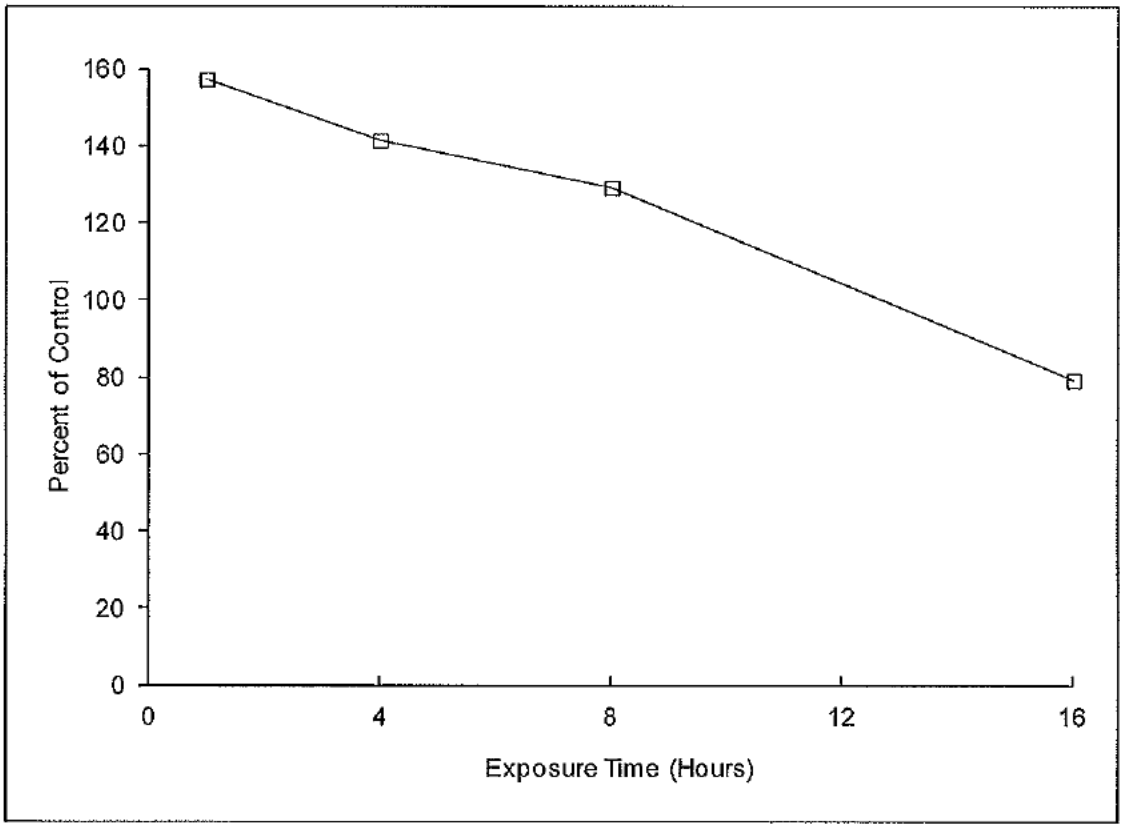
y = Percent Viable
x = Exposure Time
slope=rise/ run=(y1-y2)/ (x1-x2)
y intercept=y-(slope*x)

	X		Y
1	0.0	1	79.1
2	0.0	2	79.1
3	#DIV/0!	3	50
	slope =		#DIV/0!
	y intercept =		#DIV/0!

Second after-shave balm with 0.8% *Chondrus crispus.*

[redacted]

CONCENTRATION: 100%
PRELIMINARY



EPIOCULAR™ BIOASSAY

EXPERIMENT DATE: 20-Jul-11
TEST MATERIAL: [redacted]
TEST ARTICLE:

Study No. [redacted]

t_{50}= >24 Hours

Trial 1
CONCENTRATION: 100%

TIME EXPOSURE (Hours)	PERCENT VIABLE
8	106.9
16	94.6
20	86.8
24	75.5

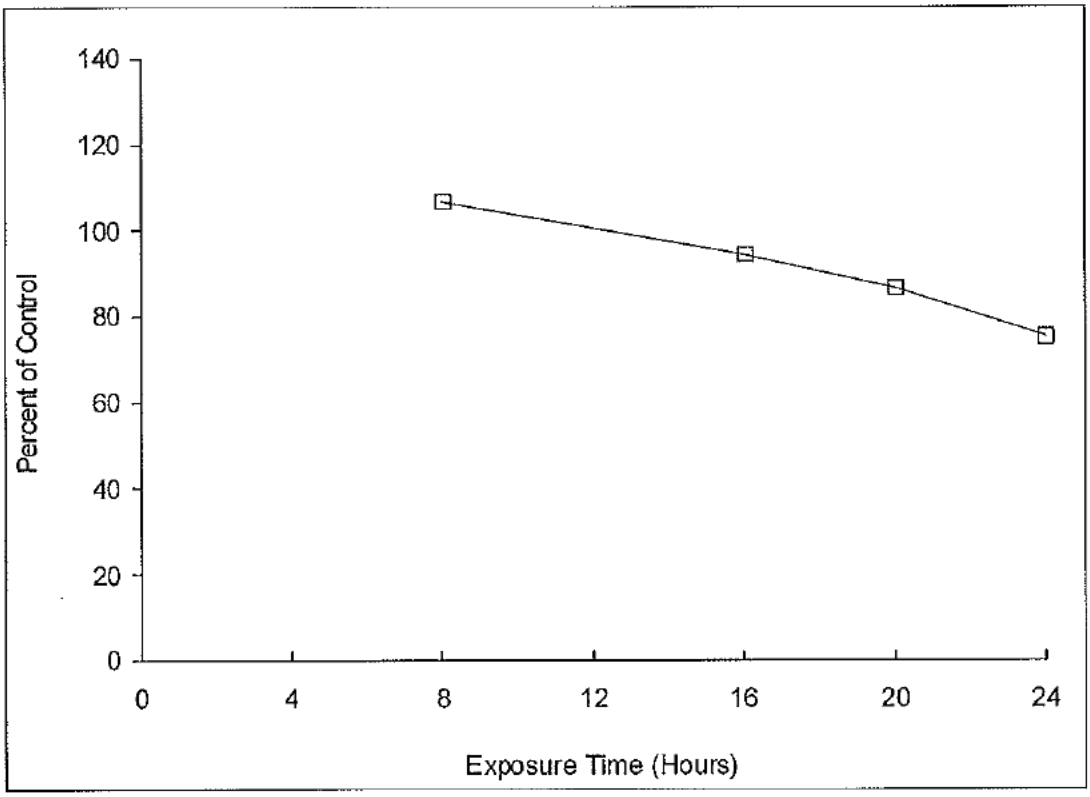
y = Percent Viable
x = Exposure Time
slope=rise/run=(y1-y2)/(x1-x2)
y intercept=y-(slope*x)

	X		Y
1	0.0	1	75.5
2	0.0	2	75.5
3	#DIV/0!	3	50
slope =			#DIV/0!
y intercept =			#DIV/0!

Second after-shave balm with 0.8% *Chondrus crispus*.

[redacted]

CONCENTRATION: 100%
Trial 1



EPIOCULAR™ BIOASSAY

EXPERIMENT DATE: 13-Jul-11
TEST MATERIAL: [redacted]
TEST ARTICLE:

Study No. [redacted]

t_{50} = >16 Hours

PRELIMINARY
CONCENTRATION: 100%

TIME EXPOSURE (Hours)	PERCENT VIABLE
1	136.8
4	138.0
8	125.9
16	121.0

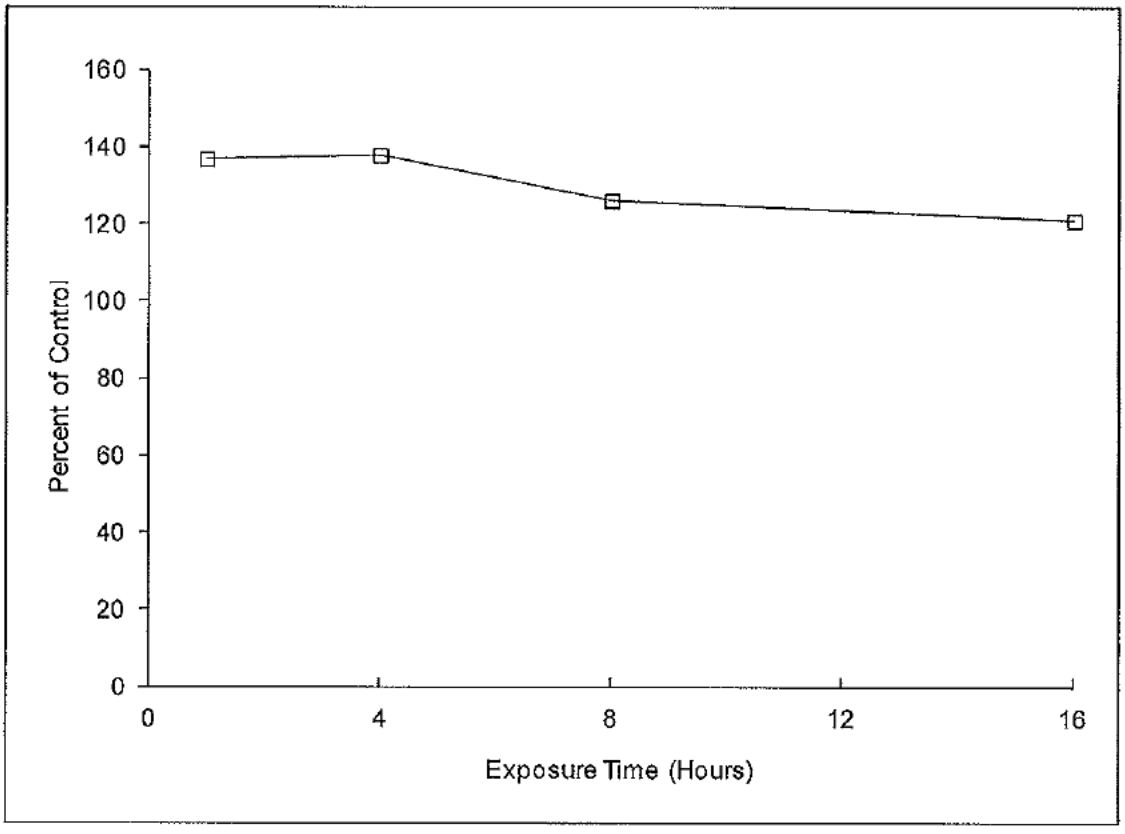
y = Percent Viable
x = Exposure Time
slope=rise/ run=(y1-y2)/ (x1-x2)
y intercept=y-(slope*x)

	X		Y
1	0.0	1	121
2	0.0	2	121
3	#DIV/0!	3	50
	slope =		#DIV/0!
	y intercept =		#DIV/0!

Third after-shave balm with 0.8% *Chondrus crispus*.

[redacted]

CONCENTRATION: 100%
PRELIMINARY



EPIOCULAR™ BIOASSAY

EXPERIMENT DATE: 20-Jul-11
TEST MATERIAL: [redacted]
TEST ARTICLE:

Study No. [redacted]

t_{50}= >24 Hours

Trial 1
CONCENTRATION: 100%

TIME EXPOSURE (Hours)	PERCENT VIABLE
16	134.7
20	114.4
24	104.1

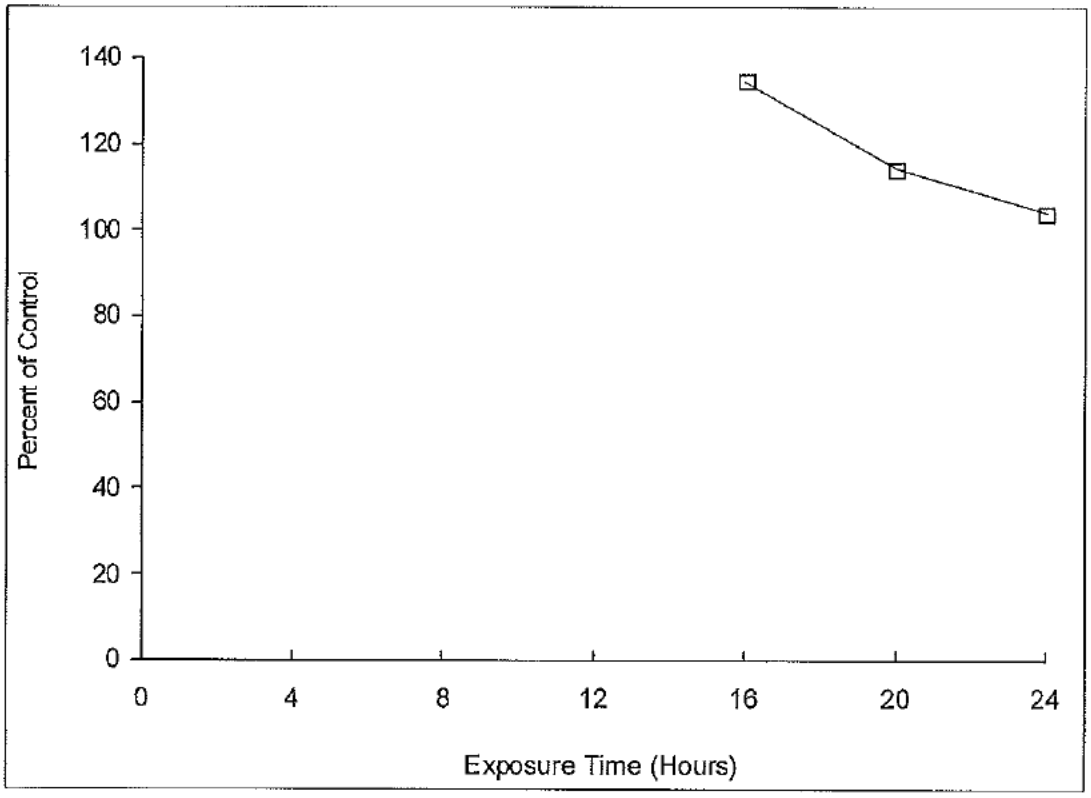
y = Percent Viable
x = Exposure Time
slope=rise/run=(y1-y2)/(x1-x2)
y intercept=y-(slope*x)

	X		Y
1	0.0	1	104.1
2	0.0	2	104.1
3	#DIV/0!	3	50
slope =			#DIV/0!
y intercept =			#DIV/0!

Third after-shave balm with 0.8% Chondrus crispus.

[redacted]

CONCENTRATION: 100%
Trial 1



Study No. [redacted]

EPIOCULAR™ BIOASSAY

EXPERIMENT DATE: 13-Jul-11

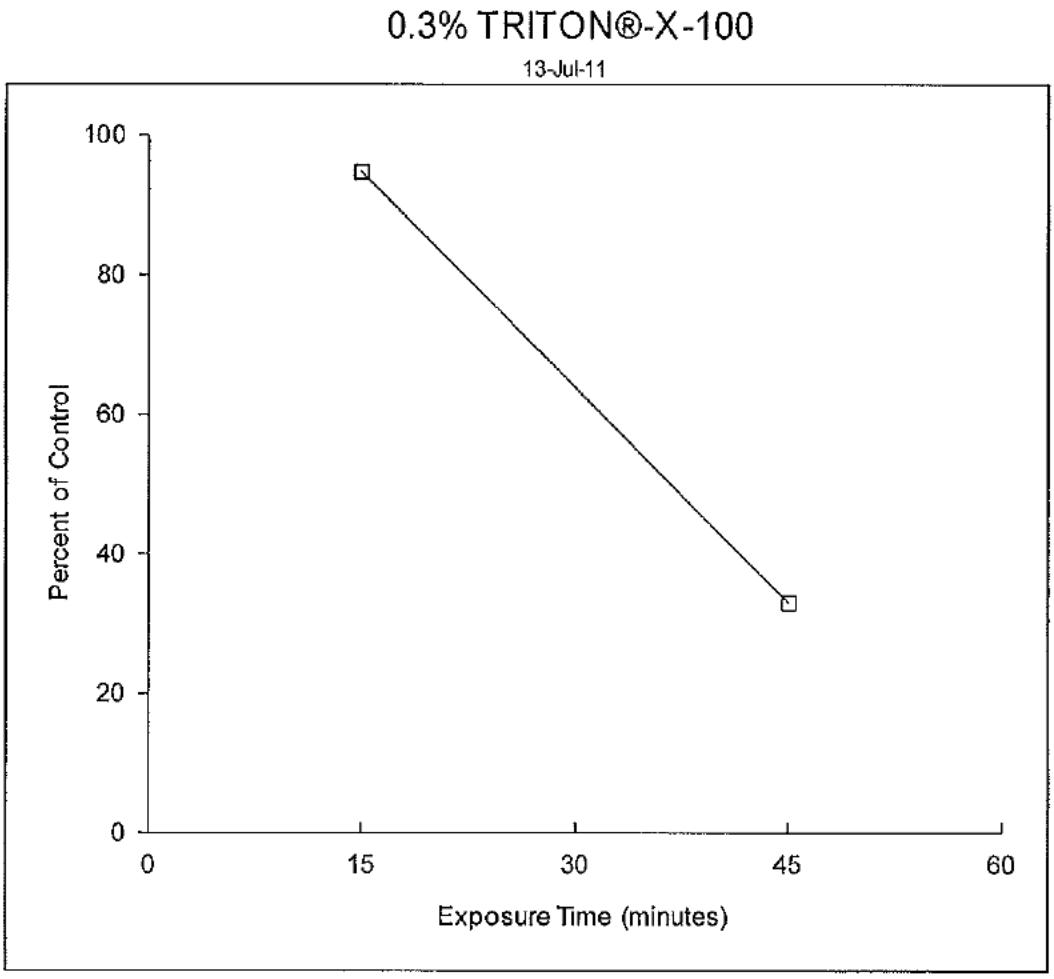
TEST MATERIAL: 0.3% TRITON® -X-100

t_{50}= 36.7 Minutes

TIME EXPOSURE (Minutes)	PERCENT VIABLE
15	94.7
45	32.9

y = Percent Viable
x = Exposure Time
slope=rise/ run=(y1-y2)/ (x1-x2)
y intercept=y-(slope*x)

	X		Y
1	15.0	1	94.7
2	45.0	2	32.9
3	36.699029	3	50
	slope =		-2.06
	y intercept =		125.6



Study No. [redacted]

EPIOCULAR™ BIOASSAY

EXPERIMENT DATE: 20-Jul-11

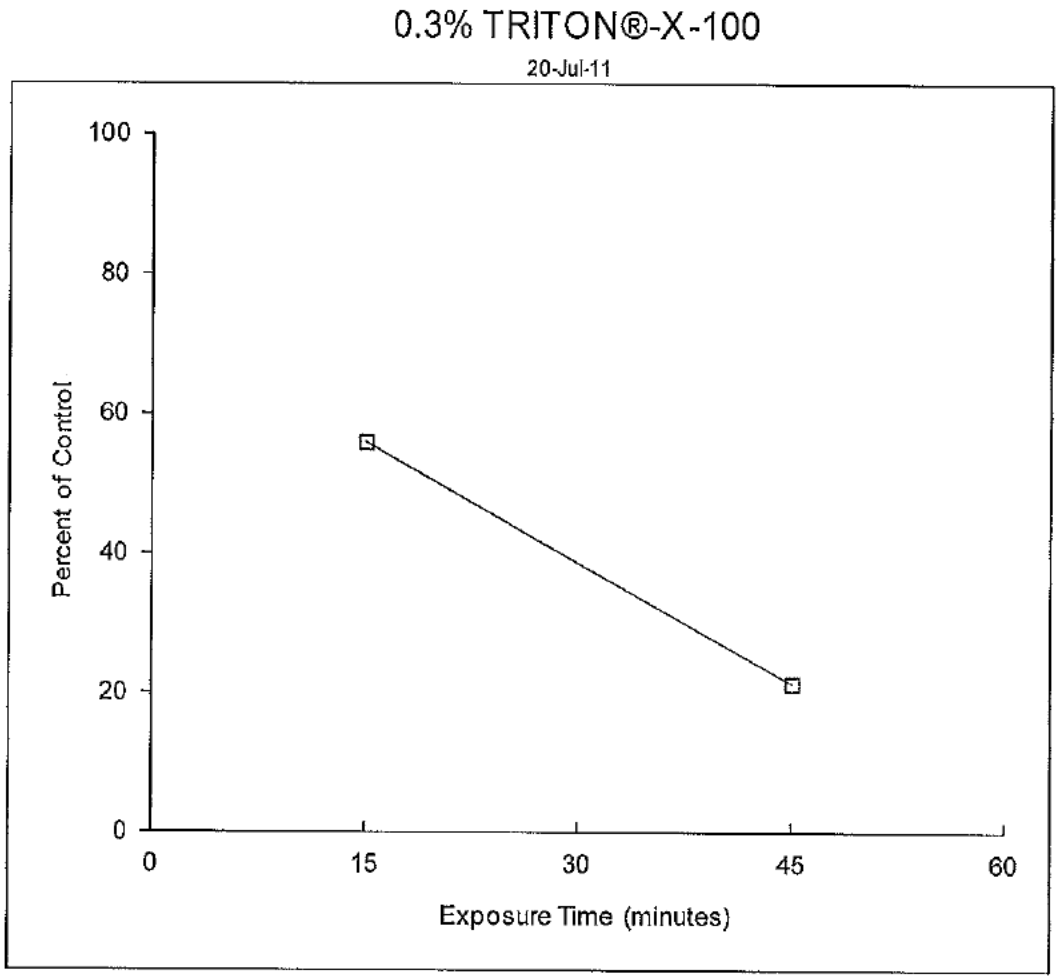
TEST MATERIAL: 0.3% TRITON® -X-100

t_{50}= 20.2 Minutes

TIME EXPOSURE (Minutes)	PERCENT VIABLE
15	56.0
45	21.3

y = Percent Viable
x = Exposure Time
slope=rise/ run=(y1-y2)/ (x1-x2)
y intercept=y-(slope*x)

	X		Y
1	15.0	1	56
2	45.0	2	21.3
3	20.18732	3	50
	slope =		-1.156667
	y intercept =		73.35



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Final Report

Study	
Sponsor Ref	
Sponsor	
Issued	23 Sep 2011
Version	

STUDY DETAILS

STUDY NO	[redacted]
SPONSOR STUDY NO	[redacted]
STUDY TITLE	A 14 Day Human Cumulative Irritation Patch Test
STUDY DATES	04 Jul 2011 - 18 Jul 2011
TEST MATERIALS	[redacted] Three aftershave balms, each containing 0.8% *Chondrus crispus* (CAS 9000-07-1).
CONTROL MATERIALS	Distilled Water Sodium Lauryl Sulfate
CRO NAME	Alba Science Ltd.
PROJECT MANAGER	Marie Reynolds BSc (Hons), SCS Dip.
CRO ADDRESS	Alba Science Ltd. 24 Broughton Street Edinburgh EH1 3RH United Kingdom
SPONSOR NAME	[redacted]
SPONSOR CO-ORDINATOR	[redacted]
SPONSOR ADDRESS	[redacted]

STUDY SUMMARY

STUDY NO	[redacted]
SPONSOR STUDY NO	[redacted]
STUDY TITLE	A 14 Day Human Cumulative Irritation Patch Test
REGULATORY STATUS	Cosmetic
STUDY DATES	04 Jul 2011 - 18 Jul 2011
STUDY OBJECTIVES	To assess the potential of test substances to elicit human skin irritation by repetitive topical application.
STUDY DESIGN	Single-centre, within-subject comparison, double blind, randomised
STUDY POPULATION	30 subjects
TEST MATERIALS	[redacted] Three after-shave balms with 0.8% *Chondrus crispus* (CAS 9000-07-1).
CONTROL MATERIALS	Distilled Water Sodium Lauryl Sulfate
ADMINISTRATION ROUTE	Topical. [redacted] and controls under fully occlusive patch conditions.
FREQUENCY & DURATION	14 applications (approximately 23h) over 15 Days
EVALUATION CRITERIA	Skin Assessment - Visual assessment of erythema, dryness and other signs.
ADVERSE EVENTS	Recorded throughout study period
STATISTICAL ANALYSIS	Statistical Analysis of Irritation.

SIGNATURES

STUDY NO	[redacted]
SPONSOR STUDY NO	[redacted]
STUDY TITLE	A 14 Day Human Cumulative Irritation Patch Test
PROJECT MANAGER	Marie Reynolds BSc (Hons), SCS Dip.

SIGNED Marie Reynolds DATE 23 Sep 2011

DOCUMENT INFORMATION

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ISSUED DATE	23 Sep 2011
AUTHOR	Lilian Fotheringham
STATUS	Final

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1 INTRODUCTION

This study was conducted on behalf of [redacted]. A 14 Day Cumulative Human Irritation Patch test was carried out with [redacted] After Shave Balms, undiluted, and 2 controls applied under fully occlusive patch conditions. The test materials were compared with the standard positive (0.1% SLS) and negative (Sterile water) controls. The study was completed in 33 healthy subjects.

2 STUDY OBJECTIVE

The purpose of this study is to assess the potential of test substances to elicit human skin irritation by repetitive topical application.

3 STUDY METHOD

Study Protocol

The study was performed in accordance with Alba Science Ltd. Study Protocol 210981.

A copy of the Study Protocol is included in Appendix A.

Design Description

A double-blind, within-subject comparison study was performed. There was 1 group of 33 subjects, each testing all of the test materials on each day of the study.

Primary Endpoints

The primary endpoint of the study was skin irritation (average skin grades and cumulative irritation index).

Secondary Endpoints

There was no secondary endpoint.

4 TEST MATERIALS

Supply of Test Materials

The following test materials were supplied for inclusion on the study by the Sponsor and were received by Alba Science on 22 Jun 2011.

Test Materials			
	Code	Name	Test Concentration
1	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]
6	[redacted]	Lemon After Shave Balm	100%
7	[redacted]	Lavender After Shave Balm	100%
8	[redacted]	Sandalwood After Shave Balm	100%

The following control materials were supplied for inclusion on the study by Alba Science.

Control Materials			
	Code	Name	Test Concentration
9	Positive Control	Sodium Lauryl Sulfate	0.1% w/v in Sterile Water
10	Negative Control	Sterile Water	100%

Test Material Receipt Procedure

Numbers were assigned to each of the test materials to facilitate the randomisation of the products during their application.

All containers were individually checked for integrity and to ensure that product / code numbers were correct as expected for the study. All containers were weighed and all details were entered into a controlled sample inventory and management computer system.

Test Material Safety

The test materials were formulated and tested to comply with current European regulations.

A safety assessment was conducted by an appropriately qualified individual on behalf of the Sponsor and the test materials were considered safe by that individual under reasonably foreseeable conditions of use associated with the study. The test materials were stable for the duration of the study.

Labelling

The test materials supplied were labelled with an Alba Science Test Material label, containing the Study Number and the assigned Test Material Number.

Storage

The test materials were stored in the dark at ambient temperature.

Preparation

The test materials were diluted to the concentrations described above prior to each daily application. They were applied directly to the Webril pads in 0.2 ml amounts no more than 5 min prior to the patch being applied to the skin.

5 LOCATION

The study was conducted at the premises of Alba Science Ltd., 24 Broughton Street, Edinburgh, EH1 3RH, United Kingdom.

6 TIMING

The study was performed from 04 Jul 2011 until 18 Jul 2011.

7 STUDY SUMMARY

Application and Exposure

Each subject was exposed to the test materials on one outer upper arm for 14 consecutive periods of approximately 23 hours. Prior to the first patch application the skin on the upper arms was wiped with isopropyl alcohol to remove excess oil.

[redacted] Test Materials coded 5 - 10 were applied to the skin by means of 2cm x 2cm squares of Webril backed with occlusive Blenderm adhesive tape. All test materials were applied in 0.2 ml amounts. Test

materials were applied to the patch no longer than 5 min prior to skin application. Skin markers were used to mark the skin at either end of the patch strips to allow exact relocation to the test areas at subsequent applications.

Approximately 23 hours (± 1 h) after application of the first patch scheme, the subjects returned to the test centre for patch removal. The test sites were wiped with water and patted dry to remove any residual test material. The test sites were assessed approximately 20 - 40 min later by a trained assessor following the scoring system detailed in section 13 of this document.

After assessment of reactions, an identical patch scheme was reapplied to the same area for a further period of approximately 23 hours. The test materials were reapplied to the same site as before following assessment. Any test material which elicited erythema or dryness with a score of 2 or more was not reapplied.

If a score of 2 or more was observed for any test material and consequently no longer applied, then a score of 2 will have been used for the remainder of the study for that site unless the score increased, in which case the higher score will have been recorded. The residual score will also have been recorded but not used for the calculations or statistical analysis.

Treatment was over 15 consecutive days commencing on Day 1 when the test materials were applied as described. The test materials were applied on Days 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13 and 14. Skin reactions were assessed on Days 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14 and 15. A Dermatologist also examined skin reactions on Day 15.

Application Areas

The application area for the test materials was the upper, outer arms. The 6 test materials applied under occlusive patch conditions were applied to the right arm

Treatment Period

The total study duration from screening to study completion was 15 days. The total treatment period for each subject was 14 days

8 SUBJECT PARTICIPATION

Participation Summary

43 subjects were recruited for the study, of which 37 were screened. One of these failed screening because of sunburn on his upper arm. 36 subjects participated in this study. 33 subjects completed the study. Three subjects were withdrawn for reasons unrelated to the study.

Information and Consent

Subjects were provided with a Volunteer Information document and were asked to provide signed consent to take part in the study. This was done by signing two copies of a Subject Consent Form. One copy is stored in the Project File and the other was retained by the subject.

Screening

Screening was based on the completion of a Medical and Dermatological History Questionnaire for each subject. Subjects were assessed for eligibility onto the main phase of the study based on the defined inclusion/exclusion criteria detailed in the Study Protocol.

9 RANDOMISATION

Test Materials were referred to throughout the study only by their test material number, as allocated during the test material receipt process outlined above.

A Randomised Product Application Schedule was generated by a computer system for each subject in order to randomly allocate each product to an application site. The Randomised Product Application Schedules were allocated to each subject prior to the application of any test materials. These are retained in the project file.

10 MEASURING EQUIPMENT

No special measuring equipment was used to aid skin assessments for this study.

11 ENVIRONMENTAL CONDITIONS

Standard Northlight lighting conditions were used throughout the study.

12 DATA COLLECTION AND PROCESSING

Recorded Data

The following data was recorded during the study.

- Skin Irritation Scores

Recording Methods

Data was recorded onto a 'Subject Assessment Sheet' at each visit to the test centre.

Data Checking

All data was 100% checked for accuracy and completeness by Alba Science personnel.

Data Storage

All data was entered into a computer system where it was subject to a further 100% check against source data.

Data Processing

For data processing, a computer system checked and prepared the raw study data to produce the processed data for the study. Where appropriate, this processing used any Randomised Product Application Schedules involved in the study. A Data Analysis Report was generated containing both raw and processed data, together with other information gathered throughout the study.

Data Analysis

The Data Analysis Report was made available for the purposes of formal independent Statistical Analysis and Quality Control inspection.

13 SCORING SYSTEM

The following [redacted] Laboratory Patch Test Grading Scale was used during the study.

[redacted] Laboratory Patch Test Grading Scale	
Grade	Description
0	No apparent cutaneous involvement.
0.5	Greater than 0, less than 1.
1	Faint but definite erythema, no eruptions or broken skin or no erythema but definite dryness; may have epidermal fissuring.
1.5	Greater than 1, less than 2.
2	Moderate erythema, may have a few papules or deep fissures, moderate-to-severe erythema in the cracks. Cut-off Grade - Patches are not reapplied
2.5	Greater than 2, less than 3.
3	Severe erythema (beet redness), may have generalized papules or moderate-to-severe erythema with slight oedema (edges well defined by raising).
3.5	Greater than 3, less than 4.
4	Generalized vesicles or eschar formations or moderate-to-severe erythema and/or oedema extending beyond the area of the patch.

NOTE: The degree of reaction expressed by such descriptive terms as "moderate" and "severe" is, in itself, subjective. Such terminology can be accurately understood only through experience. Any reaction of greater severity than Grade 4 should be described in detail. Unusual reactions not described by the scale should also be described.

Typical Examples of Half-Grade Scores	
Grade	Description
0.5	Faint, barely perceptible erythema or slight dryness (glazed appearance).
1.5	Well-defined erythema or faint erythema with definite dryness, may have epidermal fissuring.
2.5	Moderate erythema with barely perceptible oedema or severe erythema not involving a significant portion of the patch (halo effect around the edges), may have a few papules or moderate-to-severe erythema.
3.5	Moderate-to-severe erythema with moderate oedema (confined to patch area) or moderate-to-severe erythema with isolated eschar formations or vesicles.

14 ADVERSE REACTIONS / ADVERSE EVENTS

No adverse events/adverse reactions were noted during the study.

15 DISCUSSION AND CONCLUSION

All test materials were well tolerated under the conditions of the 14 Day Cumulative Human Skin Irritation Patch Test with significantly less irritation than the positive control.

16 QUALITY ASSURANCE

This draft report was reviewed by our Quality Assurance personnel and audited prior to this final report being issued.

All data was 100% checked for accuracy.

17 GOOD CLINICAL PRACTICE

No formal claim of GCP compliance has been made for this study, however the practices and procedures adopted during the conduct of this study have been consistent with the Principles of Good Clinical Practice (CPMP/ICH/135/95).

18 ETHICS COMMITTEE AND REGULATORY APPROVAL

As this was a cosmetic study, Ethics Committee and Regulatory approval for the study was not required. As the study involved human subjects, it was conducted in consideration of the requirements of the 1996 Declaration of Helsinki.

19 ARCHIVING

The Sponsor's study protocol states that appropriate documentation to permit complete reconstruction of the study will be retained by the Investigator (Alba Science Ltd).

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However, the sponsor's representative requires the Trial Master File to be returned to [redacted] upon the issue of the Final Report. Alba Science Ltd. will, therefore, not retain any study documentation pertaining to this study.

20 REFERENCES

None.

A TABLES OF MEAN IRRITATION AND CUMULATIVE IRRITATION INDEX

The following tables summarise the Mean Irritation scores for the 33 subjects that completed the study, together with the Overall Average Skin Grade and Cumulative Irritation Index for each test material on the study.

Mean Irritation and Cumulative Irritation Index										
No.	Code	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]		[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
6		0.03	0.06	0.05	0.08	0.12	0.09	0.09	0.11	0.23
7	[illegible]	0.05	0.05	0.05	0.05	0.06	0.09	0.08	0.11	0.26
8	[illegible]	0.03	0.12	0.05	0.11	0.05	0.11	0.11	0.15	0.21
9	NEG	0.05	0.06	0.11	0.11	0.08	0.06	0.09	0.23	0.26
10	POS	0.20	0.36	0.79	1.36	1.70	1.92	1.95	2.03	2.17

Mean Irritation and Cumulative Irritation Index								
No.	Code	Day 10	Day 11	Day 12	Day 13	Day 14	Grade	Index
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
6	[illegible]	0.27	0.24	0.21	0.21	0.12	0.14	0.03
7	[illegible]	0.24	0.20	0.21	0.12	0.11	0.12	0.03
8		0.23	0.21	0.23	0.18	0.18	0.14	0.03
9	NEG	0.32	0.33	0.26	0.29	0.33	0.18	0.05
10	POS	2.17	2.21	2.20	2.21	2.09	1.67	0.42

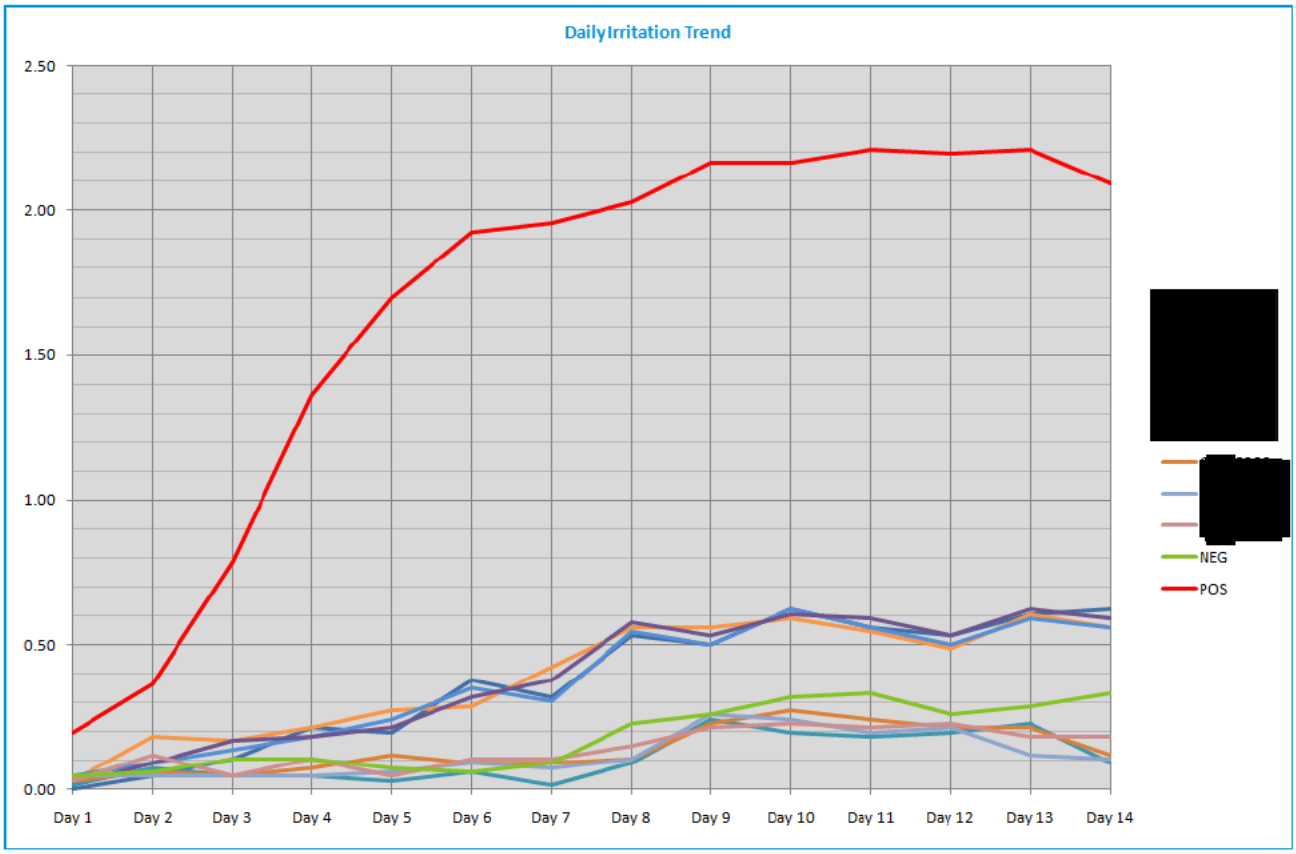
B GRAPHS OF RESULTS

Graphs of results follow on the next page.

The following graphs are included.

- Daily Irritation Trend
- Overall Average Skin Grades
- Cumulative Irritation Index

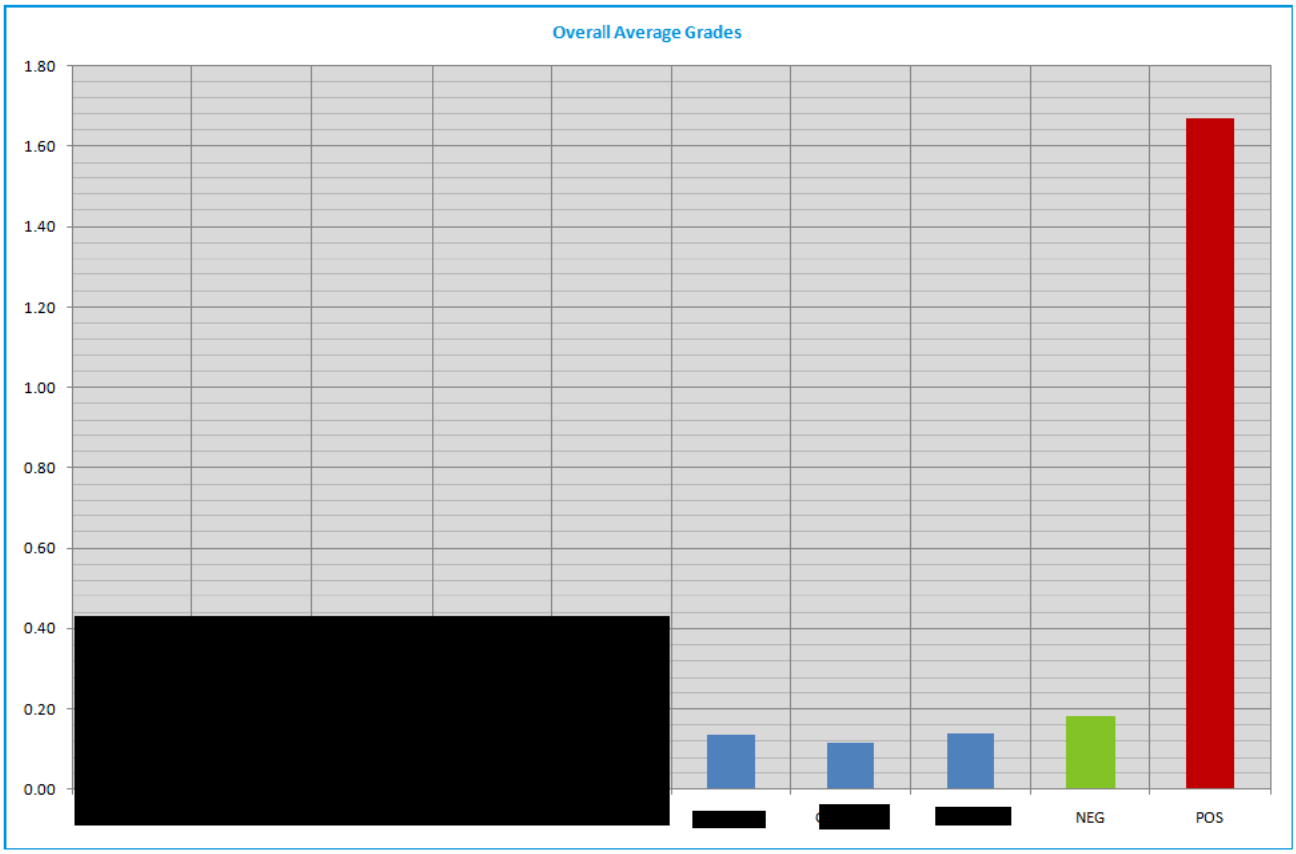
DAILY IRRITATION TREND



The above graph shows the Mean Irritation scores, calculated as defined in the Study Protocol for each of the test materials on the study.

This graph is based on the data presented in Appendix A above.

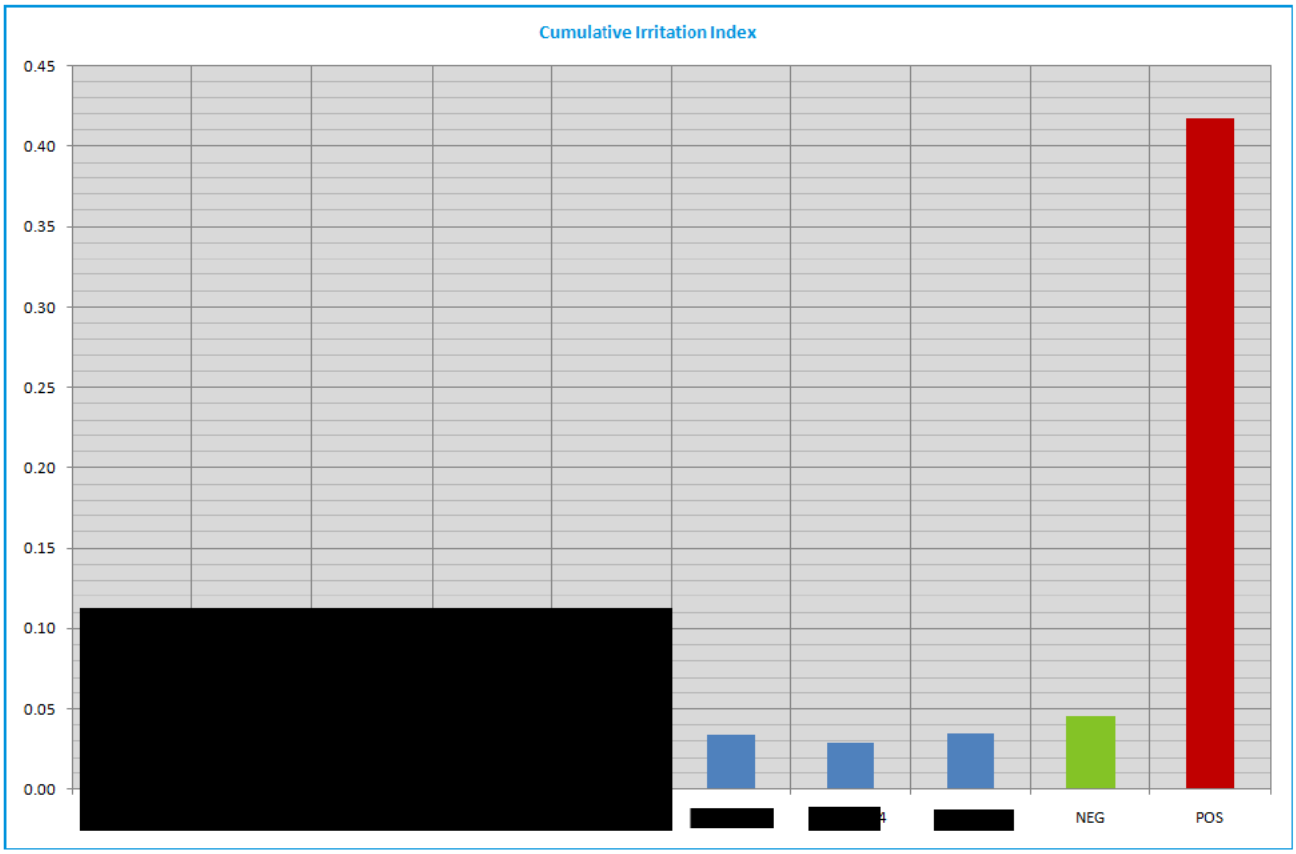
OVERALL AVERAGE SKIN GRADES



The above graph shows the Overall Average Skin Grades, calculated as defined in the Study Protocol for each of the test materials on the study.

This graph is based on the data presented in Appendix A above.

CUMULATIVE IRRITATION INDEX



The above graph shows the Cumulative Irritation Index for each of the test materials on the study, calculated as follows:

$$\text{Irritation Index} = \frac{\text{Total Irritation Score (add all scores on all days for all subjects)}}{\text{Highest possible score} \times \text{Number of subjects} \times \text{Number of study days}}$$

This graph is based on the data presented in Appendix A above.

Daily Grades	[redacted] - After Shave Balm (Lemon)													
Subject	1	2	3	4	5	6	7	8	9	10	11	12	13	14
110330	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
110331	0.0	0.0	0.0	0.0	0.5	0.0	0.0	0.0	0.0	0.5	0.0	0.0	0.5	0.0
110351	0.0	0.5	0.5	0.5	0.0	0.0	0.0	0.0	0.0	0.5	1.0	0.5	0.0	0.0
110381	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
110387	0.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.5	0.0	0.0	0.0
110399	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
110400	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
110413	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.5	0.5	0.5	0.5	0.5	0.5	0.5
110415	0.0	0.0	0.0	0.0	0.5	0.0	0.5	0.0	0.5	0.5	0.0	0.0	0.0	0.0
110525	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.5	0.5	0.0
110526	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.5	0.5	0.5	0.5	0.0	0.0
110561	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
111143	0.0	0.5	0.5	0.5	0.5	1.0	0.0	0.0	0.5	1.0	0.5	0.5	0.5	1.0
111191	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
111200	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.5	0.0	0.5	0.5	0.5	0.5	0.5
111339	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.5	0.0	0.5	0.5	0.0
111391	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.5	0.5	0.0	0.0	0.0	0.0
111820	0.0	0.0	0.5	0.0	0.5	0.0	0.0	0.0	0.5	0.5	0.5	0.5	0.5	0.0
111939	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
111943	0.0	0.0	0.0	0.0	0.0	0.5	0.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0
111955	0.0	0.0	0.0	0.0	0.0	0.0	0.5	0.0	0.5	0.5	0.5	0.0	0.5	0.0
112195	0.0	0.0	0.0	1.0	1.0	0.0	0.0	0.0	0.5	0.0	0.0	0.0	0.0	0.0
112366	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
112658	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.5	0.5
112674	0.0	0.5	0.0	0.0	0.5	0.0	0.0	0.5	0.5	0.0	0.5	0.5	1.0	0.0
113137	0.0	0.0	0.0	0.0	0.5	1.0	1.0	0.5	1.0	1.0	1.0	0.5	0.5	0.5
113573	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.5	0.0	0.0	0.5	1.0
114285	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.5	0.5	0.0	0.0	0.0	0.0	0.0
114339	0.0	0.0	0.0	0.0	0.0	0.0	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.0
115065	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.5	0.0	0.0	0.0
115444	0.0	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
115451	0.5	0.5	0.0	0.0	0.0	0.0	0.0	0.0	0.5	0.5	0.0	0.5	0.0	0.0
115460	0.0	0.0	0.0	0.5	0.0	0.5	0.0	0.5	0.0	0.5	1.0	1.0	0.0	0.0
Min	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Max	0.5	0.5	0.5	1.0	1.0	1.0	1.0	0.5	1.0	1.0	1.0	1.0	1.0	1.0
Total	1.0	2.0	1.5	2.5	4.0	3.0	3.0	3.5	7.5	9.0	8.0	7.0	7.0	4.0
SD	0.12	0.17	0.15	0.22	0.25	0.26	0.23	0.21	0.28	0.31	0.33	0.28	0.28	0.28
Daily Average	0.03	0.06	0.05	0.08	0.12	0.09	0.09	0.11	0.23	0.27	0.24	0.21	0.21	0.12
Overall Average Skin Grade														0.14
Cumulative Irritation Index														0.03

Daily Grades	[redacted] - After Shave Balm (Lavender)													
Subject	1	2	3	4	5	6	7	8	9	10	11	12	13	14
110330	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
110331	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
110351	0.0	0.0	0.0	0.0	0.0	0.0	1.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
110381	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.5
110387	0.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.5	0.0	0.5	0.0
110399	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
110400	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.5	0.0	0.0
110413	0.0	0.0	0.0	0.0	0.0	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
110415	0.0	0.0	0.0	0.0	0.0	0.5	0.0	0.0	0.5	0.5	0.0	0.5	0.0	0.0
110525	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.5	0.0	0.5	0.0	0.0
110526	0.0	0.0	0.5	0.0	0.0	0.0	0.0	0.0	0.5	0.0	0.5	0.0	0.0	0.0
110561	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
111143	0.0	0.0	0.5	0.0	0.0	0.0	0.0	0.5	0.5	0.5	0.0	0.0	0.0	0.5
111191	0.0	0.0	0.0	0.0	0.5	0.0	0.0	0.0	0.0	0.5	0.5	0.5	0.0	0.0
111200	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.5	0.5	0.0	0.0	0.0	0.0
111339	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.5	0.5	0.5	0.5	0.0	0.0
111391	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.5	0.0	0.0	0.0	0.0
111820	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.5	0.5	0.0	0.5
111939	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.5	0.0	0.0	0.5	0.0	0.0
111943	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
111955	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.5	0.0	0.5	0.0	0.0	0.0
112195	0.0	0.0	0.0	0.0	0.0	0.5	0.0	0.0	0.5	0.0	0.0	0.5	0.0	0.5
112366	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
112658	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.5
112674	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
113137	0.0	0.0	0.0	0.0	0.5	1.0	0.5	0.5	1.0	1.0	0.5	0.5	0.5	0.5
113573	0.0	0.0	0.0	0.5	0.5	0.0	0.0	0.0	0.0	0.5	0.0	0.0	0.5	0.0
114285	0.0	0.0	0.0	0.5	0.5	0.0	0.0	0.5	0.0	0.0	0.0	0.5	0.5	0.0
114339	0.0	0.0	0.0	0.0	0.0	0.5	0.0	0.5	0.5	1.0	0.5	0.5	0.5	0.0
115065	0.0	0.5	0.0	0.0	0.0	0.0	0.0	1.0	1.0	0.5	0.5	0.0	0.0	0.0
115444	0.0	0.5	0.0	0.0	0.0	0.0	0.0	0.0	0.5	0.5	0.5	0.5	0.5	0.0
115451	1.0	0.5	0.5	0.0	0.0	0.0	0.0	0.0	0.5	0.5	0.5	0.0	0.0	0.0
115460	0.0	0.0	0.0	0.5	0.0	0.0	0.0	0.0	0.5	0.0	0.5	0.5	0.5	0.0
Min	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Max	1.0	0.5	0.5	0.5	0.5	1.0	1.0	1.0	1.0	1.0	0.5	0.5	0.5	0.5
Total	1.5	1.5	1.5	1.5	2.0	3.0	2.5	3.5	8.5	8.0	6.5	7.0	4.0	3.5
SD	0.19	0.15	0.15	0.15	0.17	0.23	0.22	0.24	0.31	0.31	0.25	0.25	0.22	0.21
Daily Average	0.05	0.05	0.05	0.05	0.06	0.09	0.08	0.11	0.26	0.24	0.20	0.21	0.12	0.11
Overall Average Skin Grade														0.12
Cumulative Irritation Index														0.03

Daily Grades	[redacted] After Shave Balm (Sandalwood)													
Subject	1	2	3	4	5	6	7	8	9	10	11	12	13	14
110330	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
110331	0.0	0.0	0.0	1.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.5	0.0
110351	0.0	0.5	0.5	0.5	0.0	0.5	0.0	0.0	0.0	0.0	1.0	0.5	0.5	0.0
110381	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
110387	0.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
110399	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
110400	0.0	0.0	0.0	0.5	0.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
110413	0.0	0.0	0.0	0.0	0.0	0.0	1.0	0.5	1.0	1.0	1.0	1.0	1.0	1.0
110415	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.5	0.0	0.0	0.0	2.0
110525	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.5	0.0	0.0
110526	0.0	0.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.5	0.0	0.0	0.0
110561	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.5	0.0	0.0
111143	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.5	0.5	0.5	0.0	0.5	0.0	0.5
111191	0.0	0.0	0.0	0.0	0.5	0.0	0.0	0.0	0.0	0.5	0.0	0.5	0.5	0.0
111200	0.0	0.0	0.0	0.5	0.0	0.0	0.0	0.5	0.0	0.5	1.0	0.0	0.0	0.5
111339	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.5	0.0	0.5	0.5	0.0
111391	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
111820	0.0	0.0	0.0	0.0	0.0	0.0	0.5	0.5	0.5	0.5	0.0	0.5	0.5	0.0
111939	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.5	0.5	0.0	0.0	0.0	0.0	0.0
111943	0.0	0.0	0.0	0.0	0.0	0.5	0.5	0.5	0.0	0.0	0.5	0.0	0.0	0.0
111955	0.0	0.5	0.5	0.0	0.0	0.0	0.0	0.0	0.5	0.0	0.0	0.0	0.0	0.0
112195	0.0	0.0	0.0	1.0	0.0	0.0	0.5	0.5	0.0	0.0	0.0	0.5	0.0	0.5
112366	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
112658	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.5
112674	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
113137	0.0	0.0	0.0	0.0	0.0	1.0	0.5	0.5	1.0	1.0	0.5	0.5	0.5	0.5
113573	0.0	0.0	0.0	0.0	0.0	1.0	0.0	0.0	0.0	0.0	0.0	0.5	0.0	0.0
114285	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.5	0.5	0.5	0.0	0.0	0.0	0.0
114339	0.0	0.5	0.0	0.0	0.5	0.5	0.5	0.5	0.5	1.0	0.5	0.5	0.5	0.0
115065	0.0	0.5	0.0	0.0	0.0	0.0	0.0	0.0	0.5	0.0	0.5	0.0	0.0	0.0
115444	0.0	0.5	0.0	0.0	0.0	0.0	0.0	0.0	0.5	0.5	0.5	0.5	0.5	0.5
115451	0.5	0.5	0.5	0.0	0.0	0.0	0.0	0.0	0.5	0.5	0.5	0.0	0.5	0.0
115460	0.0	0.5	0.0	0.0	0.0	0.0	0.0	0.0	0.5	0.0	0.5	0.5	0.5	0.0
Min	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Max	0.5	0.5	0.5	1.0	0.5	1.0	1.0	0.5	1.0	1.0	1.0	1.0	1.0	2.0
Total	1.0	4.0	1.5	3.5	1.5	3.5	3.5	5.0	7.0	7.5	7.0	7.5	6.0	6.0
SD	0.12	0.22	0.15	0.27	0.15	0.27	0.24	0.23	0.31	0.33	0.33	0.28	0.27	0.41
Daily Average	0.03	0.12	0.05	0.11	0.05	0.11	0.11	0.15	0.21	0.23	0.21	0.23	0.18	0.18
Overall Average Skin Grade														0.14
Cumulative Irritation Index														0.03

Daily Grades	NEG - Distilled Water													
Subject	1	2	3	4	5	6	7	8	9	10	11	12	13	14
110330	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.5	0.0
110331	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.5	0.0	0.0	0.0	0.0
110351	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.5	0.5	0.0	0.0	0.0	0.5	0.0
110381	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
110387	0.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.5	0.5	0.5	0.5	0.5	0.5
110399	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
110400	0.0	0.0	0.0	0.5	0.5	0.0	0.0	0.0	0.0	0.0	0.5	0.0	0.5	0.5
110413	0.0	0.0	0.0	0.0	0.0	0.5	1.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0
110415	0.0	0.0	0.5	0.0	0.0	0.0	0.0	0.0	0.5	0.5	0.5	0.0	0.0	0.0
110525	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
110526	0.0	0.5	1.0	1.0	0.5	0.5	0.0	0.0	0.0	0.5	0.5	0.5	0.5	0.0
110561	0.0	0.0	0.0	0.0	1.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
111143	0.0	0.0	0.0	0.0	0.0	0.0	0.5	0.5	0.5	0.5	0.0	0.5	0.5	0.0
111191	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.5	0.5	0.0	0.0	0.0
111200	0.0	0.0	0.0	0.5	0.0	0.5	0.5	0.5	0.0	0.5	0.0	0.5	0.5	0.5
111339	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.5	0.0	0.5	0.5	0.0
111391	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.5	0.5	0.5	0.5	0.0	0.0	0.0
111820	0.5	0.0	0.5	0.0	0.5	0.0	0.5	0.5	0.0	0.5	0.0	0.5	0.0	0.5
111939	0.0	0.0	0.5	0.0	0.0	0.0	0.0	0.5	0.5	0.5	0.0	0.0	0.0	0.0
111943	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
111955	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.5	0.0	0.0	0.0
112195	0.0	0.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.5	0.5	0.0	0.0
112366	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.5	0.5	0.0	0.5	0.5	0.0	2.0
112658	0.5	0.0	0.0	0.0	0.0	0.0	0.5	0.5	0.5	0.5	1.0	1.0	0.5	0.5
112674	0.0	0.0	0.0	1.0	0.0	0.0	0.0	0.0	0.5	0.5	0.5	0.0	0.5	0.0
113137	0.0	0.5	0.0	0.0	0.0	0.0	0.0	0.5	1.0	1.0	0.5	0.5	0.5	1.0
113573	0.0	0.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.5	0.5	0.0	0.5	1.0
114285	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.5	0.0	0.0	0.5	0.5	0.5	0.5
114339	0.0	0.0	0.0	0.5	0.0	0.5	0.0	0.5	0.5	0.0	0.5	0.5	0.5	1.0
115065	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.5	0.0	0.5	0.0
115444	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.5	0.5	0.5	0.0	0.0	1.0
115451	0.0	0.0	1.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
115460	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Min	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Max	0.5	0.5	1.0	1.0	1.0	0.5	1.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0
Total	1.5	2.0	3.5	3.5	2.5	2.0	3.0	7.5	8.5	10.5	11.0	8.5	9.5	11.0
SD	0.15	0.17	0.27	0.27	0.22	0.17	0.23	0.40	0.42	0.41	0.41	0.42	0.40	0.55
Daily Average	**0.05**	**0.06**	**0.11**	**0.11**	**0.08**	**0.06**	**0.09**	**0.23**	**0.26**	**0.32**	**0.33**	**0.26**	**0.29**	**0.33**
Overall Average Skin Grade														**0.18**
Cumulative Irritation Index														**0.05**

Daily Grades	POS - Sodium Lauryl Sulfate													
Subject	1	2	3	4	5	6	7	8	9	10	11	12	13	14
110330	0.0	0.0	0.0	0.0	0.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
110331	0.0	0.0	0.0	0.0	1.5	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0
110351	0.0	0.0	0.5	1.5	2.5	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0
110381	0.0	0.0	1.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0
110387	1.5	0.5	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0
110399	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	0.0
110400	0.0	1.0	2.0	2.0	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
110413	0.0	0.0	0.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0
110415	0.0	0.5	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	0.0
110525	0.0	0.0	0.0	0.0	0.5	0.0	0.0	0.5	2.0	2.0	2.0	2.0	2.0	2.0
110526	0.0	1.0	1.0	1.0	0.5	1.5	1.5	2.0	2.0	2.0	2.0	2.0	2.0	2.0
110561	0.0	0.0	1.0	1.0	1.0	1.5	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0
111143	1.0	1.0	1.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
111191	0.0	1.0	2.0	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
111200	0.0	0.0	0.5	1.0	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
111339	0.0	0.0	0.0	1.0	1.5	1.5	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0
111391	0.0	1.0	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
111820	0.5	0.0	0.5	0.0	0.5	1.0	0.5	0.5	2.5	2.5	2.5	2.5	2.5	2.5
111939	1.0	1.0	1.0	2.5	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0
111943	0.0	0.5	0.0	0.0	1.5	1.5	1.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0
111955	0.5	0.5	1.0	1.5	1.5	1.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
112195	0.0	0.5	1.5	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0
112366	0.0	0.0	0.0	0.0	0.0	1.0	0.5	0.0	0.0	0.5	1.0	0.5	2.0	0.0
112658	0.0	0.0	0.0	1.5	1.0	1.5	1.5	2.0	2.0	2.0	2.0	2.0	2.0	2.0
112674	0.5	0.5	1.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
113137	0.0	0.0	0.0	0.5	0.5	1.0	1.5	2.0	2.5	2.5	2.5	2.5	2.5	2.5
113573	0.0	0.5	0.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
114285	0.5	0.5	0.5	1.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
114339	0.0	0.0	1.0	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
115065	0.0	0.5	0.5	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0
115444	0.0	0.0	0.0	1.5	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0
115451	1.0	1.0	1.0	1.0	1.5	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0
115460	0.0	0.5	1.0	0.5	0.5	0.5	0.5	0.5	1.0	0.5	1.5	1.5	0.5	0.5
Min	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Max	1.5	1.0	2.5	2.5	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0
Total	6.5	12.0	26.0	45.0	56.0	63.5	64.5	67.0	71.5	71.5	73.0	72.5	73.0	69.0
SD	0.39	0.40	0.75	0.92	0.87	0.81	0.85	0.82	0.69	0.68	0.59	0.62	0.61	0.81
Daily Average	**0.20**	**0.36**	**0.79**	**1.36**	**1.70**	**1.92**	**1.95**	**2.03**	**2.17**	**2.17**	**2.21**	**2.20**	**2.21**	**2.09**
Overall Average Skin Grade														**1.67**
Cumulative Irritation Index														**0.42**

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: June 22, 2020

SUBJECT: Chondrus Crispus Extract

Active Concepts. 2017. Product Specification ABS Irish Moss Extract Sil (contains 20% Chondrus Crispus Extract).

Active Concepts. 2019. Product Specification AC Alg-MoistEAU (contains 3.5% Chondrus Crispus Extract).

Active Concepts. 2018. Dermal and Ocular Irritation Tests Alg-MoistEAU (contains 3.5% Chondrus Crispus Extract).

Product Specification

info@activeconceptsllc.com • Phone: +1-704-276-7100 • Fax: +1-704-276-7101

Product Name: ABS Irish Moss Extract Sil contains 20% Chondrus Crispus Extract
Code Number: 10272
CAS #'s: 69430-24-6 & 244023-79-8
EINECS #'s: N/A & N/A
INCI Name: Cyclomethicone & Chondrus Crispus Extract
Status: Approved

Specification	Parameter
Appearance	Clear Liquid
Odor	Characteristic
Specific Gravity	0.930 – 0.980
Refractive Index	1.3950 – 1.4050
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.

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Product Specification

Product Name: AC Alg-MoistEAU contains 3.5% Chondrus Crispus Extract
Code Number: 20222
CAS #'s: 7732-18-5 & 244023-79-8 & 57-55-6
EINECS #'s: 231-791-2 & N/A & 200-338-0
INCI Name: Water & Chondrus Crispus Extract & Propylene Glycol
Status: Approved

Specification	Parameter
Appearance	Light Tan Viscous Liquid
Odor	Characteristic
pH	6.0 – 7.5
Non-Volatile Matter (1g-1hr-105^{0}C)	3.0 – 4.0%
Viscosity (Spindle LV4, 12 rpm)	15,000 – 60,000 cPs
Specific Gravity	1.010 – 1.025
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

If product is too viscous to pour from shipping container, it may be heated to 45 - 50˚C. This will lower the viscosity so that it is easily pourable and will not affect product performance.

Dermal and Ocular Irritation Tests

info@activeconceptsllc.com • Phone: +1-704-276-7100 • Fax: +1-704-276-7101

Tradename: AC Alg-MoistEAU contains 3.5% Chondrus Crispus Extract

Code: 20222

CAS #: 7732-18-5 & 244023-79-8 & 57-55-6

Test Request Form #: 5091

Lot #: 58730P

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 Alg-MoistEAU** 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.

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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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Dermal and Ocular Irritation Tests

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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.

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Dermal and Ocular Irritation Tests

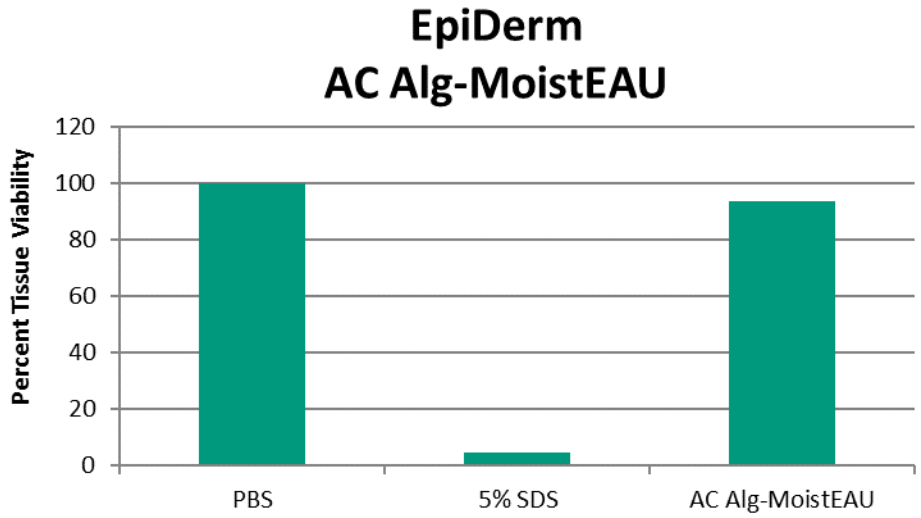

Figure 1: EpiDerm tissue viability

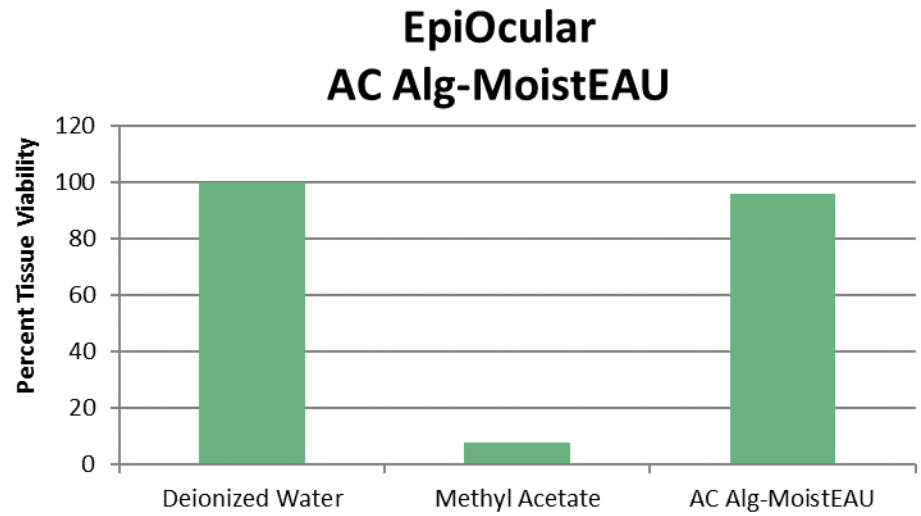

Figure 2: EpiOcular tissue viability

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Memorandum

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

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

DATE: June 11, 2020

SUBJECT: Chondrus Crispus Extract and Gigartina Stellata Extract

Biotech Marine. 2020. Manufacturing Process Flakes of Hydralixir™ CC (Chondrus Crispus Extract and Gigartina Stellata Extract).

Biotech Marine. 2019. Statement 18 383 03 HYDRALIXIR™ CC Composition file gb (Chondrus Crispus Extract and Gigartina Stellata Extract).

Dermscan. 2018. Evaluation of the acute cutaneous tolerance of a natural extract on adult subjects: single patch test (Hydralixir™ CC - Chondrus Crispus Extract and Gigartina Stellata Extract).

BiotechMarine - Z.I. - B.P.72 - 22260 Pontrieux (FR)
Tel.: +33 (0)2 96 95 31 32 - Fax: +33 (0)2 96 95 31 30
www.biotechmarine.com

FLOWCHART 18 365 01

MANUFACTURING PROCESS OF HYDRALIXIR™ CC

HARVESTING / IDENTIFICATION *(Chondrus Crispus and Gigartina Stellata)*

↓

WASHING

↓

CONDENSATION OF CELLULAR WATER BY SOFT DRYING

↓

FILTRATION AND UV TREATMENT

↓

QUALITY CONTROL

↓

ADDITION OF PRESERVATIVES AND pH ADJUSTMENT

↓

QUALITY CONTROL

↓

PACKAGING

↓

QUALITY CONTROL

Operation Manager
Clément LANSALOT

19/12/23

BiotechMarine - Z.I. - B.P.72 - 22260 Pontrieux (FR)
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Statement 18 383 03 HYDRALIXIR™ CC Composition file gb

(In accordance with EN 45014 General criteria for supplier's declaration of conformity)

Composition of the product marketed by BiotechMarine:

HYDRALIXIR™ CC

INCI Name*: Aqua / Water - Chondrus Crispus Extract - Gigartina Stellata extract

Composition

INCI names	Components	Function	% (Concentration range)	% (Typical Concentration**)
Aqua / Water - Chondrus Crispus Extract - Gigartina Stellata extract*	Cellular water issued from soft drying (condensation) of *Chondrus Crispus Extract* and *Gigartina Stellata*	Humectant	98.10 - 98.95	98.60
Sodium Benzoate	Sodium Benzoate	Preservative	0.80 - 1.10	0.95
Potassium Sorbate	Potassium (E,E)-hexa-2,4-dienoate	Preservative	0.25 - 0.35	0.30
Lactic acid	L-(+)-lactic acid	pH adjuster	0 - 0.30	0.15

* this INCI name is assigned by the PCPC
** given as indicative value

The complete composition is provided. It includes the main components that are declared in the INCI name and the non-functional process additives and/or residual raw materials. In accordance with PCPC INCI naming rules, the process additives which do not give technical or functional properties to the ingredient are not included in the INCI name of the ingredient:
https://eservices.personalcarecouncil.org/BBK/Sci/INCI_Instructions.pdf

Document approved at Castres, on October 23rd, 2019

By Sophie DINAND
Cosmetic Regulatory Manager

This certificate was established to the best of our knowledge and/or according to our suppliers' statements, at the date of this certificate. It is however your responsibility to abide by any clause of any regulations, which may apply to the product that you manufacture, or to the use you make of our product.

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Nota

The analytical specifications warranted are only those mentioned on the certificate of analysis supplied with each delivery of the product.

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Mickael PUGINIER
ZI BP72
22260 PONTRIEUX
FRANCE

Gdansk, December 3, 2018

Study Report #18E3486 (version 1.0) /
Related to quote/ order #18D3486

ETUDE DE LA TOLERANCE CUTANEE AIGUE D'UN EXTRAIT NATUREL CHEZ LE VOLONTAIRE ADULTE :
PATCH-TEST SIMPLE

Hydralixir™ CC (Chondrus Crispus Extract and Gigartina Stellata Extract)

EVALUATION OF THE ACUTE CUTANEOUS TOLERANCE OF A NATURAL EXTRACT ON ADULT SUBJECTS:
SINGLE PATCH TEST

TOX18056

Nr Report: 18P3486-1PL

Dermscan Project Manager
Aneta Orłowska: aor@dermscan.pl

Investigator (dermatologist)
Dr.: Agnieszka Cegielska

comportant / *including* 14 pages

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SUMMARY OF THE STUDY REPORT #18E3486

ETUDE DE LA TOLERANCE CUTANEE AIGUE D'UN EXTRAIT NATUREL CHEZ LE VOLONTAIRE ADULTE : PATCH-TEST SIMPLE

EVALUATION OF THE ACUTE CUTANEOUS TOLERANCE OF A NATURAL EXTRACT ON ADULT SUBJECTS: SINGLE PATCH TEST

Objectif / *Objective*

Déterminer le potentiel irritant primaire d'un produit cosmétique après application unique sous patch-test / *To determine the acute irritating potential of a cosmetic product after single application under patch-test.*

Methodologie / *Methodology*

Etude monocentrique en simple aveugle / *Monocentric and simple blind study.*

Cinétique / *Kinetics*

	J0 / *D0*	**J2 /** *D2*	**J2 /** *D2***t30min**	**J3 /** *D3***t24h**
Recueil du consentement éclairé du volontaire / *Collection of the subject's informed consent*	●			
Vérificartion des critères d'inclusion et non-inclusion /*Verification of inclusion and non-inclusion criteria*	●			
Patch-test : application	●			
Patch-test : retrait / *removal*		●		
Scorage clinique / *clinical scoring*			●	●

Dates (JJ/MM/AAAA / *DD/MM/YYYY*)

Réception du produit / *Product reception:*	**Début d'étude /** *Study start:*	**Fin d'étude /** *Study end:*
14/11/2018	20/11/2018	30/11/2018

Produit / *Product*

Référence / *Reference:*	**Forme /** *Form:*	**Température de stockage /** *Storage temperature:*
TOX18056	Solution transparente / *Transparent solution*	Température ambiante / *Room temperature*

Application

Zone:	**Durée du patch /** *Patch duration:*	**Concentration**	**Type de patch /** *patch type:*
Dos (zone scapulaire) / *scapular part of the back*	48 heures / *48 hours*	PUR / *PURE*	Occlusif / *Occlusive*

Population étudiée / *Studied Population*

Critères principaux d'inclusion / *Main inclusion criteria*	**Age moyen /** *Average age***:**	**Nombre de volontaires analysés /** *Number of subjects analysed:*
Age ≥18 ans / *Age ≥ 18 years old.* Phototype I à IV / *Phototype I to IV.*	51±3 ans/*years* (19 - 70)	22

Résultats / *Results*

Valeur d'I.I.C.M. / ***M.C.I.I. value :*** **0.01**

Conclusion : Non irritant / ***Non irritating***

Investigateur / *Investigator*

Nom et function / *Name and quality:*	**Date:**	**Signature:**
Dr. Agnieszka Cegielska Dermatologue / *Dermatologist*	03/12/2018	A Cegielska

1. PROTOCOLE EXPERIMENTAL / *EXPERIMENTAL PROTOCOL*

L'essai a été réalisé conformément aux procédures internes en vigueur.

The study was conducted according to the current internal procedures.

1.1. VOLONTAIRES / *SUBJECTS*

1.1.1. Caractéristiques des volontaires inclus / *Characteristics of included subjects*

- 22 volontaires ayant tout type de peau ont été inclus dans l'essai :

Sexe féminin	22
Sexe masculin	0
Age (moy±SEM)	19 à 70 ans (51±3)

- *22 subjects with every skin type were included in the study:*

Female subjects	*22*
Male subjects	*0*
Age (mean±SEM)	*19 to 70 years old (51±3)*

1.1.2. Critères d'inclusion / *Inclusion criteria*

- volontaire ayant donné son consentement libre et éclairé,
- aucun antécédent d'intolérance ou d'allergie à un produit de même type,
- phototype I à IV.

- *subjects having given their informed, written consent,*
- *no previous experience of intolerance or allergic reactions to this kind of product,*
- *phototype I to IV.*

1.1.3. Critères de non-inclusion / *Non-Inclusion criteria*

- femme enceinte ou qui allaite ou prévoyant un début de grossesse en cours d'étude,
- pathologie cutanée sur la zone d'étude (psoriasis, eczéma, vitiligo, pityriasis versicolore, acné, etc…),
- présence d'un traitement médicamenteux pouvant interférer avec l'évaluation du potentiel irritant, à l'apprécitation de l'investigateur,
- 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 tabac ou d'alcool.

- *pregnant or breast-feeding women or women planning to be pregnant during the study,*
- *cutaneous pathology on the study zone (psoriasis, eczema, vitiligo, pytiriasis versicolor, acne, etc…),*
- *subjects with medical treatments which may interfere with the acute skin tolerance evaluation, according to the investigator,*
- *exposure to the sun or to UV rays on the back during the previous month,*
- *subjects with very irritative skin,*
- *subjects presenting an important hairiness of the back, freckles, beauty spots or a tattoo on the back,*
- *subjects with a serious or progressive disease,*
- *excessive use of alcohol or tobacco.*

1.2. *PRODUIT A L'ETUDE / STUDY PRODUCT*

Le produit fourni par le promoteur, présente les caractéristiques suivantes :
The product supplied by the sponsor had the following specifications:

Référence du produit *Product reference*	Aspect du produit *Product aspect*	Date de réception à Dermscan Poland *Receipt date at Dermscan Poland*
TOX18056	Solution transparente *Transparent solution*	14/11/2018 *November 14, 2018*

1.3. *METHODOLOGIE / METHODOLOGY*

1.3.1. Matériel, dose, durée / *Instruments, dose, duration*

Le produit étudié a été appliqué dans les conditions suivantes :

The studied product was applied under the following conditions:

Zone / *Area:*	zone scapulaire / *scapular part of the back*
Type de Patch tests: *Patch tests type:*	Finn Chambers ® 8mm (50mm²) – occlusif *Finn Chamber® 8mm (50mm²) – occlusive*
Dose* :	25 µl
Conditions de l'application: *Application conditions:*	PUR *PURE*
Durée de l'application: *Application duration:*	48 heures *48 hours*
Contrôle: *Control:*	patch sans produit *patch without product*

*Note: La dose est conditionnée par la capacité de la cupule, indiquée par le fabricant.

**Note: The quantity is determined according to the cupule capacity, indicated by the manufacturer.*

1.3.2. Lectures / *Readings*

Les examens macroscopiques cutanés ont été réalisés dans les mêmes conditions, en particulier au niveau de l'éclairage (lumière standardisée), 30 minutes après le retrait des patch-tests et 24 heures plus tard. Dans le cas d'une réaction cutanée > 1 à la lecture à 24 heures, le volontaire devait revenir au centre, des lectures étant effectuées jusqu'à réversibilité complète des réactions cutanées.

Les cotations des éventuelles réactions d'irritation, sur chaque site ayant reçu le produit étudié ainsi que sur la zone témoin, ont été réalisées selon les échelles numériques suivantes :

The macroscopic skin examinations were carried out under the same conditions, specifically the lighting (standardized light), 30 minutes after removal of the patch tests and 24 hours later. If the subject had a cutaneous reaction >1, he had to return to the centre and readings were done until complete reversibility of the cutaneous reactions.

The grading of the possible irritation reaction, on each zone that received the studied product and on the control zone, was done according to the following scale:

Score	Cotation *Quotation*	**CRITERES** / ***CRITERIA**:* description :	
		ERYTHEME «**E**» / *ERYTHEMA «**E**»*	OEDEME «**O**» / *OEDEMA «**O**»*
0	Absent	aspect normal / *no erythema*	aspect normal / *no oedema*
0,5	très léger / *very mild*	à peine perceptible : coloration rosée discrète d'une partie de la surface testée *fairly detectable: discreet pinkness of one part of the tested area*	palpable, à peine visible *palpable, barely visible*
1	Léger / *mild*	coloration rosée discrète de toute la surface testée ou coloration bien définie sur une partie de la surface testée *discreet pinkness of the complete tested area or rather visible on one part of the tested area*	palpable et visible *palpable, visible*
2	Modéré / *Moderate*	coloration rouge mate nette couvrant toute la surface testée *clearly distinguishable, dull red erythema covering the whole tested area*	œdème net (<1 mm d'épaisseur) avec ou sans présence de papule(s) ou vésicule(s) *obvious oedema (thickness < 1 mm) with or without papule(s) or vesicle(s)*
3	Sévère / *Severe*	coloration rouge feu ou rouge très foncé couvrant toute la surface testée ou érythème modéré diffusant en dehors de la surface testée *deep dark or fiery bright red color covering all the tested area or moderate erythema diffusing outside the tested area*	œdème important (≥1 mm d'épaisseur ou surface débordant la zone d'application) avec ou sans présence de vésicule(s) ou de bulle(s) *severe oedema (thickness ≥ 1 mm or diffusing outside the tested area) with or without vesicle(s) or blister(s)*

Une modification de structure cutanée (dessèchement, rugosité, épaississement, réflectivité) pouvant être liée à la nature même du produit étudié ou à l'un des ingrédients fait l'objet d'une description clinique dont l'intensité est appréciée selon le barème :

0,5 = douteux
1 = léger
2 = net
3 = important

A change in skin structure (dryness (D), roughness (R), thickness (T), reflectivity (Re)) that could be linked to the nature of the studied product or one of its components is clinically described and its intensity graded according to the following scale:

0.5 = doubtful
1 = slight
2 = obvious
3 = important.

1.3.3. Interprétation des résultats / *Results interpretation*

L'analyse et l'interprétation des résultats ont été réalisées en fonction des données obtenues dans les conditions expérimentales.
Elles sont descriptives et complétées par le calcul d'un indice d'irritation cumulative (I.I.C.) pour chaque volontaire, selon le rapport :

$$I.I.C. = \frac{\sum score(érythème + oedème)}{nombretotaldelecturesf}$$

Cet indice est ensuite moyenné par le nombre de sujets afin d'obtenir l'Indice d'Irritation Cumulative Moyen (I.I.C.M.) :

$$I.I.C.M. = \frac{\sum I.I.C.}{nombredesujets}$$

The analysis and the interpretation were carried out according to the results obtained in the experimental conditions.
They are descriptive and completed by the calculation of the cumulative irritation index (C.I.I.) for each subject according to the formula:

$$C.I.I. = \frac{\sum grades(erythema + edema)}{Totalnumberofreadings}$$

This index is then divided by the number of subjects in order to obtain the Mean Cumulative Irritation Index (M.C.I.I.):

$$M.C.I.I. = \frac{\sum C\,I.I.}{numberofsubjects}$$

L'indice ainsi obtenu (maximum 6), permet de classer arbitrairement le produit étudié selon le barème d'interprétation suivant :

I.I.C.M.	Classe
I.I.C.M. < 0,25	Non irritant (NI)
0,25 ≤ I.I.C.M. < 0,50	Très légèrement irritant (TLI)
0,5 ≤ I.I.C.M. < 1	Légèrement irritant (LI)
1 ≤ I.I.C.M. < 2	Moyennement irritant (MI)
I.I.C.M. ≥ 2	Irritant (I)

Les valeurs individuelles et la catégorie à laquelle appartient le produit étudié ont également été prises en compte pour une conclusion adaptée dans les conditions de l'essai.

The obtained index (maximum 6) allow to arbitrarily classify the studied product according to the following scale:

M.C.I.I.	*Class*
M.C.I.I. < 0.25	***Non irritating (NI)***
0.25 ≤ M.C.I.I. < 0.50	***Very slightly irritating (VSI)***
0.5 ≤ M.C.I.I. < 1	***Slightly irritating (SI)***
1 ≤ M.C.I.I. < 2	***Moderately irritating (MI)***
M.C.I.I. ≥ 2	***Irritating (I)***

Individual values and the product class were taken into account to write a suitable conclusion under the study conditions.

1.3.4. Bibliographie / *Bibliography*

1- COLIPA "Cosmetic product test guidelines for the assessment of human skin compatibility" 2nd edition – August 1997.
2- Patch-testing with the patient's own products - Peter J. FROSCH, Johannes GEIER, Wolfgang UTER, An GOOSENS - CONTACT DERMATITIS 4TH EDITION – 2006
3- Comparison of the cumulative irritation potential of Adapalene gel and cream with that of Erythromycin/Tretinoin solution and gel and Erythromycin/Tretinoin gel - Catherine QUEILLE-ROUSSEL, Michel PONCET, Stephane MESAROS, Alan CLUCAS, Michael BAKER and Andrew-Marc SOLOFF - CLINICAL THERAPEUTICS / VOL.23 N°2, 2001

2. RESULTATS – CONCLUSION / *RESULTS - CONCLUSION*

Les résultats individuels des lectures à chaque temps expérimental sont regroupés dans le tableau en ANNEXE I.
Les lectures effectuées 30 minutes et 24 heures après le retrait des patch-tests ont donné la valeur d'I.I.C.M. suivante :

The individual reading results at each experimental time are presented in the APPENDIX I.

The readings at 30 minutes and 24 hours after patch-tests removal gave of the following M.C.I.I value:

Référence produit *Product reference*	**Conditions** *Conditions*	**Durée de pose** *Patch duration*	**I.I.C.M.** *M.C.I.I.*	**Conclusion** *Conclusion*
TOX18056	**-Concentration /** ***Concentration:*** **PUR /** ***PURE*** **- Type de Patch tests:** ***Patch tests type:*** **Occlusif /** ***Occlusive***	**48 heures /** ***48 hours***	**0.01**	**Non irritant /** ***Non irritating***

ANNEXE I / *APPENDIX I*

CARACTERISTIQUES DES VOLONTAIRES / *SUBJECTS CHARACTERISTICS*

TABLEAU DES LECTURES / *TABLE OF READINGS*

CARACTERISTIQUES DES VOLONTAIRES / *SUBJECTS CHARACTERISTICS*

N° du sujet / *N° of subject*	Identification du sujet / *Identification of subject*	Age	Sexe / *Sex*	Phototype	Début de l'étude / Study Start	Fin de l'étude / Study end
1	WI/P	23	F	II	2018-11-20	2018-11-23
2	CI/K	52	F	II	2018-11-20	2018-11-23
3	HA/I	64	F	III	2018-11-20	2018-11-23
4	KR/N	33	F	II	2018-11-20	2018-11-23
5	JA/S	55	F	I	2018-11-20	2018-11-23
6	DE/B	63	F	II	2018-11-20	2018-11-23
7	GŁ/K	63	F	II	2018-11-20	2018-11-23
8	BA/L	70	F	III	2018-11-20	2018-11-23
9	CZ/A	36	F	II	2018-11-20	2018-11-23
10	ZI/R	59	F	II	2018-11-20	2018-11-23
11	TU/B	45	F	III	2018-11-20	2018-11-23
12	KW/B	63	F	II	2018-11-27	2018-11-30
13	ŁU/B	59	F	II	2018-11-27	2018-11-30
14	PR/M	57	F	II	2018-11-27	2018-11-30
15	SI/Z	19	F	III	2018-11-27	2018-11-30
16	DY/S	34	F	III	2018-11-27	2018-11-30
17	RY/M	64	F	II	2018-11-27	2018-11-30
18	GR/B	67	F	III	2018-11-27	2018-11-30
19	GO/W	44	F	II	2018-11-27	2018-11-30
20	KL/M	45	F	II	2018-11-27	2018-11-30
21	PO/K	65	F	II	2018-11-27	2018-11-30
22	EH/T	44	F	III	2018-11-27	2018-11-30

	Min	19	Nb F	phototype I
	Max	70	22	1
	Moy/Average	51	Nb M	phototype II
	SEM	3	0	14
				phototype III
				7
				phototype IV
				0

TABLEAU DES LECTURES / *TABLE OF READINGS*

N° Volontaire / N° of subject	Lecture à 30 minutes / 30-minute reading				Lecture à 24 heures / 24-hour reading				IIC/CII	Modification de structure de la peau / Change in skin structure	
	T		P		T		P			Lecture 30 minutes / 30-minute reading	Lecture 24 heures / 24-hour reading
	E	O	E	O	E	O	E	O			
1	0	0	0	0	0	0	0	0	0	no change	no change
2	0	0	0	0	0	0	0	0	0	no change	no change
3	0	0	0	0	0	0	0,5	0	0,25	no change	no change
4	0	0	0	0	0	0	0	0	0	no change	no change
5	0	0	0	0	0	0	0	0	0	no change	no change
6	0	0	0	0	0	0	0	0	0	no change	no change
7	0	0	0	0	0	0	0	0	0	no change	no change
8	0	0	0	0	0	0	0	0	0	no change	no change
9	0	0	0	0	0	0	0	0	0	no change	no change
10	0	0	0	0	0	0	0	0	0	no change	no change
11	0	0	0	0	0	0	0	0	0	no change	no change
12	0	0	0	0	0	0	0	0	0	no change	no change
13	0	0	0	0	0	0	0	0	0	no change	no change
14	0	0	0	0	0	0	0	0	0	no change	no change
15	0	0	0	0	0	0	0	0	0	no change	no change
16	0	0	0	0	0	0	0	0	0	no change	no change
17	0	0	0	0	0	0	0	0	0	no change	no change
18	0	0	0	0	0	0	0	0	0	no change	no change
19	0	0	0	0	0	0	0	0	0	no change	no change
20	0	0	0	0	0	0	0	0	0	no change	no change
21	0	0	0	0	0	0	0	0	0	no change	no change
22	0	0	0	0	0	0	0	0	0	no change	no change
					I.I.C.M. / M.C.I.I.				**0,01**		

T: Témoin / Control
P: TOX18056
E: Erythème / Erythema
O: Oedème / Oedema

ANNEXE II / *APPENDIX II*

FEUILLE D'AUTHENTIFICATION DES RESULTATS /
AUTHENTIFICATION PAGE

ASSURANCE QUALITE / ***QUALITY ASSURANCE***
CERTIFICAT DE CONFORMITE / ***CERTIFICATE OF CONFORMITY***

FICHE D'AUTHENTIFICATION DES RESULTATS
AUTHENTIFICATION PAGE

A ma connaissance, l'étude n°18E3486
I am aware that the study #18E3486

a été conduite en accord avec le protocole et la fiche des paramètres d'étude.
has been conducted according to the PROTOCOL and THE STUDY PARAMETERS PAGE.

	Date	Signature
Agnieszka Cegielska Dermatologist	03/12/2018	
Beata Tylza Technician	03/12/2018	

ASSURANCE QUALITE

L'étude a été réalisée dans l'esprit 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.

L'étude a été menée selon les Procédures Opératoires Standards et selon le protocole de l'étude défini avec le promoteur.
Les cahiers d'observation ont été vérifiés ainsi que l'exactitude des données.

L'authenticité et la véracité des données expérimentales recueillies ont été confirmées par les personnes ayant participé à l'étude (ANNEXE II).

QUALITY ASSURANCE

The described study has been conducted in the spirit of 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 successive updates.

The study has been conducted according to Standard Operating Procedures and to the study protocol defined with the sponsor.
All the case report forms and the data were checked.

Controls on data veracity and conformity with the protocol have been performed and confirmed by persons participating in the study (APPENDIX II).

Certificat de conformité / ***Certificate of conformity***

A ma connaissance, l'étude 18E3486 a été conduite en accord avec l'«**Assurance qualité**» précitée.
*I am aware that the study 18E3486 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**.*

Chef de projet / *Project manager*	Date	Signature
Aneta Orłowska	03/12/2018	

Personal Care Products Council
Committed to Safety, Quality & Innovation

Memorandum

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

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

DATE: April 1, 2020

SUBJECT: Chondrus Crispus Powder

Anonymous. 2020. Production Process Chondrus Crispus Powder.

April 2020

Production Process Chondrus Crispus Powder

Chondrus crispus is harvested from wild.
It is dried naturally, exposed to sunlight.
Then it is ground and sieved.
It is packed
It is sterilized by gamma ray treatment.

This Chondrus Crispus Powder is used as an exfoliating ingredient.

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: June 11, 2020

SUBJECT: Chondrus Crispus Powder

Biotech Marine. 2020. Manufacturing Process Flakes of Chondrus Crispus.

Biotech Marine. 2017. Statement Flakes of Chondrus Crispus Composition File.

Palmer Research 2004. Study of the acute tolerance of a raw material (flakes of *Chondrus crispus*)on adult volunteers: 24-hour occlusive patch test under dermatological control

BiotechMarine - Z.I. - B.P. 72 - 22260 Pontrieux (FR)
Tel.: +33 (0)2 96 95 31 32 - Fax: +33 (0)2 96 95 31 30
ww.biotechmarine.com

MANUFACTURING PROCESS FLAKES OF CHONDRUS CRISPUS

HARVESTING / IDENTIFICATION *(Chondrus Crispus)*

↓

DRYING

↓

CUTTING

↓

IONIZATION

↓

QUALITY CONTROL

↓

PACKAGING

↓

QUALITY CONTROL

Production Manager
Jean-Marc CATROUX

BiotechMarine - Z.I. - B.P.72 - 22260 Pontrieux (FR)
Tel.: +33 (0)2 96 95 31 32 - Fax: +33 (0)2 96 95 31 30
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Statement FLAKES OF CHONDRUS CRISPUS COMPOSITION FILE

(In accordance with EN 45014 General criteria for supplier's declaration of conformity)

We declare, by the present one, that the following product supplied by BIOTECHMARINE:

FLAKES OF CHONDRUS CRISPUS

(INCI NAME (USA): Chondrus Crispus Powder

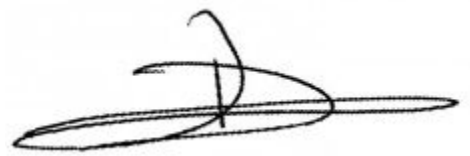
Composition

Components	Components usual Name	Function	% (Concentration range)
Chondrus Crispus Powder		Active	100

This certificate was established to the best of our knowledge and/or according to our suppliers' statements, at the date of this certificate. It is however your responsibility to abide by any clause of any regulations, which may apply to the product that you manufacture, or to the use you make of our product.

Document approved at Pontrieux, on Wednesday, March 22, 2017

By Delphine LE PEUCH
Regulatory & Documentary Affairs from BIOTECHMARINE



Nota
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PALMER RESEARCH

Study of the acute tolerance of a raw material on adult volunteers: 24-hour occlusive patch test under dermatological control

GROUPE DERMSCAN

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email : palmer@dermscan.com
internet : www.palmerresearch.com

Version No. 01/004 dated 07 January 2004

Study: 1030478PA

Raw material: FLAKES OF CHONDRUS CRISPUS
BATCH 3.06.174 (58332)

Sponsor: SECMA BIOTCHNOLOGIE MARINE
ZI – BP 65
22260 PONTRIEUX
FRANCE

Lyon, 07 January 2004

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STUDY SUMMARY REPORT

Sponsor: **SECMA BIOTECHNOLOGIE MARINE** **Address: ZI – BP 65** **22260 PONTRIEUX** **FRANCE**	**Raw material:** **FLAKES OF CHONDRUS CR IPSUS LOT 3.06.174** **PALMER Research code: 58332**

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 December 17 to December 19, 2003.	
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 **FLAKES OF CHONDRUS CR IPSUS LOT 3.06.174** is equal to **0.34 (slightly irritating)** at the 30-minute reading and to **0.04 (non irritating)** at the 24-hour reading.	
Dr Yvette WELTERT, ***Dermatologist***		

1- INTRODUCTION

At the request of the **SECMA BIOTECHNOLOGIE MARINE** Company, **ZI – BP 65, 22260 PONTRIEUX, FRANCE,** we investigated the acute cutaneous tolerance or irritant potential of the raw material:

FLAKE SOF CHONDRUS CRISPUS BATCH 3.06.174

following single application to back skin (scapular zone) in a 24-hour patch test.

This study was conducted according to an “open-label” design and patch test methodology.

In order to carry out this study, we received a sample of raw material on 5 December 2003, which we referenced under PALMER Research code **58332**.

The study began on 17 December and ended on 19 December 2003.

2 - CERTIFICATE OF AUTHENTICITY OF THE RESULTS

The study described in this report was conducted under my responsibility in accordance with the test protocol and the principles of Good Clinical Practice.

All of the observations and numerical data recorded during this study are reported in this document.

After reading the report and as the Investigator, I confirm that these data are a true reflection of the results obtained.
Doctor Yvette WELTERT, *Dermatologist*

Date: 02.02.04 Signature: (signed)

This report was audited by the Head of Quality Control.
It is deemed to be a true account of the data generated and current procedures implemented in accordance with the principles of Good Clinical Practice.

Date: 04.02.04 Name: BRUNET-DUNAND Séverine

Signature: (signed)

3 – TEST PROTOCOL

The test was carried out in accordance with the “Single Patch Test” method.

3.1 – Volunteers

3.1.1 – Characteristics of the subjects enrolled

√ 12 subjects were enrolled in the study

√ 9 females and 3 males

√ aged between 18 and 46 years (average age: 27 years).

All the subjects had to comply with the inclusion criteria and not present any non-inclusion criteria, especially:

3.1.2 – Inclusion criteria

√ no history of intolerance or allergy to the raw material

√ agreeing to sign the informed consent form to participate in the study

√ phototype I to III

3.1.3 – Non-inclusion criteria

√ pregnant or breast-feeding woman or woman intending to become pregnant during the study period

√ skin disease in the test zone (psoriasis, eczema, vitiligo, pytiriasis versicolor, acne, etc.)

√ receiving oral medication:

- antihistamines, anti-inflammatory drugs and/or antibiotics < 1 week
- anti-tussives and/or corticosteroids < 4 weeks
- immunosuppressants, retinoid and/or anti-cancer drugs < 6 months

√ beginning, discontinuation or change in hormone therapy (including the contraceptive pill)< 1.5 months

√ exposure of the back region to the sun or UV rays < 1 month

√ individual presenting with hyper-irritable skin

√ individual presenting with considerable body hair, freckles, beauty spots or a tattoo on the back

√ individual with a serious or progressive disease

√ immoderate use of alcohol or tobacco

3.2 – Methodology

3.2.1 – Material, dose, duration

The raw material was applied under the following conditions:

	FLAKES OF CHONDRUS CR IPSUS BATCH 3.06.174
Zone:	Scapular zone
Type of patch test:	Finn Chamber® 8 mm (50 mm^2) occlusive
Dose*:	Approximately 0.02 ml
Application conditions:	Pure, saturating a slightly moist filter paper disc
Duration of the application:	24 hours
Control:	Product-free patch

* N.B.: the choice of the dose is conditioned by the capacity of the well, indicated by the "Finn Chambers®" manufacturer.

3.2.2 – Readings

The skin was examined macroscopically under the same conditions, particularly in lit conditions ("daylight" lamp), 30 minutes after removing the patches. The test was stopped in the absence of any local skin reaction on reading the results 30 minutes after removing the patch. However, each volunteer was asked to check that no reaction was evident the next day. In the case of a visible reaction, the subject had to return to the centre and readings were taken until the skin reactions reversed.

The scores for any signs of irritation on each exposure site were compared to those recorded for the product-free sites, based on the following numerical scales:

Erythema "E":

E = 0	: no erythema
E = 0.5	: very slight erythema (barely perceptible: slight pinkish colouration of part of the exposure area)
E = 1	: slight erythema (slightly pinkish colouration over the entire exposure area)
E = 2	: moderate erythema (moderate erythema covering all of the exposure area)
E = 3	: severe erythema (intense erythema covering all of the exposure area or erythema diffusing beyond the exposure area)

Oedema "O":

O = 0	: no oedema
O = 0.5	: very slight oedema (palpable, barely visible)
O = 1	: slight oedema (palpable and visible)
O = 2	: moderate erythema with or without the presence of papule(s) or vesicle(s)
O = 3	: severe erythema (extending beyond the exposure area) with or without the presence of vesicle(s) or blister(s)

Changes in the skin structure (drying out, roughness, thickening, reflectivity) possibly associated with the nature of the test raw material or one of the ingredients were the subject of a clinical description. The intensity of each change was assessed according to the following score:

0.5 = doubtful

1 = slight

2 = moderate

3 = severe

3.2.3 – Interpretation of results

The results were analysed and interpreted based on the data obtained under the experimental conditions, with each reading.

They are descriptive and are completed by calculating a mean irritation index (M.I.I.) with each reading, based on the following equation:

$$M.I.I. = \frac{\text{E of the scores (erythema + oedema)}}{\text{Number of subjects}}$$

The index thus obtained (maximum of 12) can be used to arbitrarily classify the test raw material according to the following interpretation score:

M.I.I.	Class
M.I.I. < 0.20	Non-irritating (NI)
0.20 ≤ M.I.I. < 0.50	Slightly irritating (SI)
0.50 ≤ M.I.I. < 1	Moderately irritating (MI)
M.I.I. ≥ 1	Irritating (I)

The individual values and the category of raw materials to which the test raw material belongs were also taken into consideration to reach an appropriate conclusion under the test conditions (24-hour patch test).

* *References:*

- "Les essais cliniques en dermatologie", Thérapie, 1991, Volume 46, pages 183 to 187

- "Dermato-allergoloie de contact", G. DUCOMBS, Editions MASSON, 1988, pages 13 to 16, 36-37

- "Dermatotoxicolofy Methods: the laboratory worker's VADEMECUM"; N. MARXULLI – H. MAIBACH, ED. Taylor & Francis, 1998.

4 - RESULTS

The individual readings for each test time are grouped together in the following table.

FLAKES OF CHONDRUS CR IPSUS BATCH 3.06.174

(24-hour patch test – pure)

SUBJECTS					READINGS									
No.	Identification	Age	Sex	Skin type	Reading 30 minutes after patch removal					Reading 24 hours after patch removal				
					Control		Raw material		Change in structure	Control		Raw material		Change in structure
					E	O	E	O		E	O	E	O	-
1S51	FAU Fr	46	F	Normal	0.5	0	0.5	0	-	0	0	0	0	-
3S51	MES Fa	29	M	Normal	0.5	0	1	1	-	0	0	0	0	-
4S51	BEN Ec	22	F	Normal	0	0	0	0	-	0	0	0	0	-
5S51	BUA JY	28	M	Normal	0	0	0.5	0	-	0	0	0	0	-
6S51	GIC Ni	19	F	Normal	0	0	0.5	0	-	0	0	0	0	-
8S51	BER Ch	18	F	Normal	0	0	0	0	-	0	0	0	0	-
9S51	MON Ma	23	F	Normal	0	0	0	0	-	0	0	0	0	-
10S51	QUI Ce	21	F	Normal	0	0	2	0	-	0	0	0.5	0	-
11S51	DUB Li	24	F	Normal	0	0	0	0	-	0	0	0	0	-
12S51	GUI Vi	25	M	Normal	0	0	0.5	0	-	0	0	0	0	-
18S51	AGA Pa	45	F	Normal	0	0	0	0	-	0	0	0	0	-
19S51	CHE So	19	F	Normal	0	0	0	0	-	0	0	0	0	-
Average age		27	M.I.I.		0.08		0.42			0		0.04		-

M.I.I	0.34	0.04
Results	Slightly irritating	Non-irritating

(1): M = male

F = female

Comment: Volunteers No. 1S51 and No. 3S51 presented with a very slight erythematous reaction in the control well 30 minutes after removing the patches. These reactions disappeared after 24 hours and the subjects are included in the calculation.

M.I.I. is calculated from the difference between the product score and control score.

5. CONCLUSION

30 minutes after removing the occlusive patch, six volunteers (Nos. 1S51, 3S51, 5S51, 6S51, 10S51 and 12S51) presented with very slight to slight erythema accompanied by slight oedema in the case of subject No. 3S51.

The 24 hour reading confirmed the persistence of very slight erythema for subject No. 10S51.

The 4-day reading did not highlight any other reaction.

Furthermore, no side effect was observed.

Under these test conditions, it can therefore be concluded that the raw material, FLAKES OF CHONDRUS CR IPSUS BATCH 3.06.174, dermatologically controlled and administered as a pure, topical application beneath a 24-hour occlusive patch to the skin of 12 adult volunteers, is classed as **slightly** irritating according to the 30-minute reading and **non-**irritating based on the 24-hour reading and the M.I.I. score.

Dr. Yvette WELTERT

Dermatologist

(signed)

STUDY SUMMARY REPORT

Sponsor: **SECMA BIOTECHNOLOGIE MARINE** **Address: ZI – BP 65** **22260 PONTRIEUX** **FRANCE**	**Raw material:** **FLAKES OF CHONDRUS CR IPSUS LOT 3.06.174** **PALMER Research code: 58332**

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 December 17 to December 19, 2003.	
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 **FLAKES OF CHONDRUS CR IPSUS LOT 3.06.174** is equal to **0.34 (slightly irritating)** at the 30-minute reading and to **0.04 (non irritating)** at the 24-hour reading.	
Dr Yvette WELTERT, ***Dermatologist***		

Corallina officinalis

Algae synopsis

Red marine alga

Related actives: PHYCO'DERM® (*C.o.* combined with *Undaria pinnatifida*)
ALGYL® (*C.o.* combined with *Gigartina stellata* and *Kappaphycus alvarezii*)

V.1- 2018

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Taxonomy	2
Common names	2
Morphology	2
Biology	4
Ecology & Geographical distribution	4
Chemical composition	5
Bioactivities	6
Uses	6

These data don't pretend to be exhaustive.
They supply scientific pieces of information for conducing to a better understanding of the main characteristics and bioactivities of this algal species.

GELYMA Parc d'Affaires Marseille Sud (C4) – 1 Boulevard de l'Océan – 13009 Marseille – France.
Phone (33) 496 140 982 – Fax (33) 496 140 983 – E-mail : gelyma @ wanadoo.fr.

TAXONOMY

This alga belongs to:

Empire	*Eukaryota*
Kingdom	*Plantae*
Phylum	*Rhodophyta*
Class	*Florideophyceae*
Order	Corallinales
Family	*Corallinaceae*
Genus	*Corallina* Linnaeus 1758
Species	*officinalis* Linnaeus 1758.

Origin of the species name

from Greek "*corallion* " a coral and from Latin "*officina*" a shop.

Heterotypic Synonyms

Corallina calvadosii JV Lamouroux 1816
Corallina cretacea Postels & Ruprecht 1840
Amphiroa cretacea (Postels & Ruprecht) Endlicher 1843
Corallina compacta P. Crouan & H. Crouan 1867
Arthrocladia cretacea (Postels & Ruprecht) Weber-van-Bosse 1904
Pachyarthron cretaceum (Postels & Ruprecht) Manza 1937
Bossiella cretacea (Postels & Ruprecht) H.W. Johansen 1969.

cf. www. algaebase.org

COMMON NAMES

This alga is named in Britain "Sea Common Coral weed".

MORPHOLOGY

Corallina officinalis is a calcified or calcareous red marine alga reaching 5-12 cm high (Figs 1-2).

It shows an erect articulated (geniculate) thallus arising from a firmly attached crustose base up to 70 mm in diameter and bearing tufts of branched and articulated fronds up to 120 mm long.

The structure appears multiaxial combined with marked calcification. Numerous calcified segments are separated by uncalcified horny and flexible joints named genicula. These segmented fronds provide

flexibility in churning seawater. The branches are irregular, opposite and pinnate, resulting in a father-like appearance (Fig. 1).

The color of the alga can be widely varied. The thallus appears dull purple when growing in deep water, becoming red yellow and finally white on exposure.

Corallina officinalis has been the subject of many studies relative to:

- anatomy (Rosenvinge L.K. 1917- Kongelige Danske Videnskabernes Selskabs Skrifter, ser.7: 155-283; Suneson, 1937- Lunds Universitets Arsskrift 33 (2): 1-101)
- cytology, growth and development (Cabioch 1971 – Cahiers de Biologie Marine 12: 121-186, 1972- *ibid* 13: 137-288)
- ultrastructure (Giraud & Cabioch, 1976 – Phycologia 15: 405-414,1977 – Revue Algologique 12: 45-60; Borowitzka & Vesk 1978 – Marine Biology 46: 295-304)
- calcification (Pentecost 1978 – British Phycological Journal 13: 383-390), this alga making up calcium carbonate within its cells.

Fig. 1- Morphology of *Corallina officinalis*
Drawings from *HARVEY 1871*
Phycologia Britannica vol. II.

Fig.2- Morphology of *Corallina officinalis in* situ
(Photo GELYMA).

BIOLOGY

Corallina officinalis grows from 1-2 cm per year (Masaki *et al*. 1981- Proc. Int. Seaweed Symp. 10: 607-612).

This alga is usually exposed to high fluctuations of light intensity, light quality, temperature, and desiccation, all of which affect the temporal and spatial distribution as well as the morphology and the metabolism of this alga.

According to the study of Kim J-H. *et al.* 2013 (Algae 28 (4): 355-363), it exhibits a photo-acclimation strategy in which photosynthetic pigments are changed to keep photosynthesis at optimum efficiency. Exposure to high light increases the growth rate, but causes bleaching of the alga with the decrease in photosynthetic pigment contents. However, calcification is not affected by light intensity because the photosynthetic activity does not induce changes to the carbon chemical composition of seawater at the higher light levels.

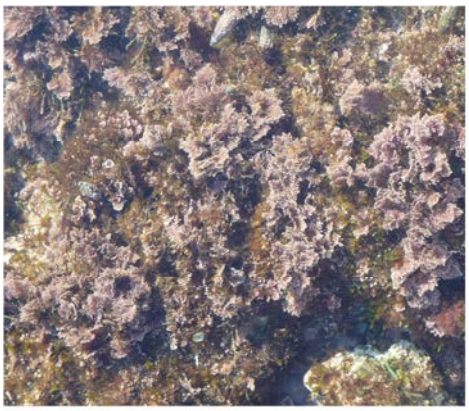
So, photo-acclimation strategies of *Corallina officinalis* may be regulated for optimal growth, with photosynthetic adaptation responding to different light intensities. In addition high photosynthetic activity causes a pH increase under higher light with the acceleration of calcification rate. The growth response pattern with high light inducing changes to calcification and photosynthesis, calcification being another important physiological process required by coralline algae for growth (*cf.* Borowitzka 1977- Mar. Biol. 15: 189-223)

Corallina officinalis is dioecious with male conceptacles elongated at the apex and female conceptacles, those bearing ovoid tetraspores.

ECOLOGY & GEOGRAPHICAL DISTRIBUTION

Corallina officinalis is present year around. It is widely distributed, particularly in temperate areas on rocks, mid tidal pools and drainage runnels (Figs 3-4). It can be found to 33 m deep.

It is a very common species which adapts to a wide range of habitats.



Figs 3-4 Populations of *Corallina officinalis* – Photos GELYMA.

Corallina officinalis is present in Atlantic Ocean from Norway to Morocco and from Greenland to Argentina. It is found in North America from Labrador to Maryland in the United States.

It is reported in W–Baltic, Mediterranean, Japan, China, Australia, South Africa and the Arctic Sea.

CHEMICAL COMPOSITION

The chemical composition of *Corallina officinalis* has been studied by different authors.

According to Marsham *et al*. (2007—Food Chemistry 100: 1331-1336), this red alga contains (% DW):

Ash	72.8 ± 1.2	Proteins	6.9 ± 0.1
Lipids	0.3 ± 0.2	Crude fibre	8.3 ± 3.2

Sebaaly C. *et al*. (2014 – J. Applied Pharmaceutical Sc. 4 (4): 30-37) report in thalli collected from Lebanese coast,

-high amounts of trace elements specially potassium = 3750 , sodium = 750 , calcium = 2000 and magnesium = 750) mg/100 g,

-lipids particularly saturated fatty acids = 70.81 % , monounsaturated fatty acids = 25.54 % and polyunsaturated fatty acid = 3.65% , the major fatty acids being saturated palmitic acid (= 52.13 %) and the monounsaturated cis-vaccenic acid (= 18.2 %).

Other compounds have been also characterized from this red alga:

- Peptides specially a pentapeptide (Haas P. & T.S. Hill, 1933 – Biochem. J. 27: 1802-1804),

- shinorine, a mycosporine like amino acid that would equal 0.292 ± 0.089 in mg/g DW (Karsten U. *et al*. 1998- Bot. Mar 41: 443-453),

- Pigments such as carotenoids and phycobiliproteins

  Carotenoids have been isolated among others beta-carotene, zeaxanthin, fucoxanthin, fucoxanthinol and the epimeric mutatoxanthins (Palermo J.A. *et al*. 1991- Phytochemistry 30(9): 2983-2986).

  It would exist in *Corallina officinalis* two phycoerythrins with different molecular weights and spectral properties (Van der Velde H.H. 1973 – Biochim. Biophys. Acta 303 (2): 246-257; Hildich N. *et al.* 1991- J. Appl. Phycol. 3 (4): 345-354).

- Polysaccharides

  The amount of cellulose reaches 5.1% (In Percival & Mc Dowell 1967- Chemistry & Enzymology of Marine Algal Polysaccharides –Acad. Press, London).

  Sulfated xylogalactans are well studied (Cases M.R. *et al.* 1994- Int. J. Biol Macromol. 16 (2): 93-97; Navarro D.A. & C.A. Stortz , 2002- Carbohydrate Polymers 49 (1): 57-62) showing large amounts of xylose and L-galactose (Cases M. R. *et al.* 1992- Phytochemistry 31 (11): 3897-3900).

- Heterosides specially floridoside (Haas P. & T.S. Hill, 1933 – Biochem. J. 27: 1802-1804; Wickberg B. – 1958 - Acta Chem. Scandinavia 12: 1183-1186; Majak *et al*. 1966 - Can. J. Bot. 44: 541-549; Craigie *et al*. 1968 - Can. J. Bot. 46: 605-611; Kirst G.O. 1980 - Phytochemistry 19 (6): 1107-1110).

- Sterols with a total amount of 16.7 mg/Kg DW, chlolesterol being the major one (Gibbons G.F. *et al*. 1967 - Phytochemistry 6: 617-683; A.S. Goldberg *et al.* 1982 - Bot. Mar. 25: 351-355).

- Several volatile compounds such as hydrocarbons (aliphatic & cyclic), terpenes, (mono- & diterpenes), aldehydes, ketones and esters (Borik R.M. 2014 - World Applied Sc. J. 30 (66): 741-746).

- Specific enzymes such as bromoperoxidases with important investigations relative to vanadium-dependent haloperoxidase (Yamada H. *et al.* 1985 - Agric. Biol. Chem., 49 (10): 2964-2967; Yu H. & J.W. Whitaker, 1989 - Biochem. Biophys. Res. Comm 160 (1): 87-92; Rush C. *et al.* 1995- FEBS Letters 359: 244-246; Littlechild J. & E. Garcia-Rodriguez 2003- Coordination Chemistry reviews 237 (1-2): 65-78).

BIOACTIVITIES

Various bioactivities have been emphasized in *Corallina officinalis* extract, among others:

- antibacterial activities against *Enterococcus foecalis*, *Enterobacter aerogenes* and *Escherichia coli* (Taskin E. *et al.* 2007 – African J. Biotechnology 6 (24): 2746-2751), activities recently confirmed by Sebaaly *et al*. 2014 (J. Applied Pharmaceutical Sc. 4 (4): 30-37)

- anticoagulant activities (Sebaaly *C. et al*. 2014 – J. Applied Pharmaceutical Sc. 4 (4): 30-37)

- antioxidant activities proved *in vitro* (Yang Y. *et al*. 2011 – Intern. J. Biol. Macromolecules 49(5): 1031-1037).

Corallina officinalis would offer weak anti-inflammatory activities relative to PLA2 and elastase (Oumaskour K. *et al.* 2013 – Int. J. Pharm. & Pharmaceutical Sc. 5 (3): 145-149).

Its lipoidal matters exhibit a sgignificant hypolipidaemic activity from alcohol and hexane extracts (Awad N.E. *et al.* 2003- Phytotherapy Research 17 (1): 19-25).

USES

The uses of *Corallina officinalis* concerns several domains of applications.

This alga would be appreciated by sheep in animal meals (in Guiry & Blunden, 1991- Seaweeds Resources in Europe, Wiley).

Corallina officinalis serves for agricultural purposes for example for improving the disease resistance of crop root systems against bacterial colonies (Patent CN 105777371 (A) 2916-07-20).

This alga is also added in antifungal composition (Patent KR 100852204 (B1) 2008-08-13) or in a mulberry root health tea (Patent CN 105194100 (A) 2015-12-30).

Corallina officinalis extract is known as a very popular ingredient for traditional Asian medicine.

Numerous Patents has been deposited, notably in Asia for treating *e.g.*

- hypothyroidism	CN 105749079 (A) 2016-07-13
- thyroid adenoma	CN 101515515 (A) 2015- 08-17 CN 105169226 (A) 2015-12-23
- dysmenorrhea	CN 104225150 (A) 2014-12-24
- acne	CN 104857078 (A) 2015-08-26
- transient synovitis	CN 104857224 (A) 2015-08-26
- vascular cognitive impairment	CN 104857079 (A) 2015-08-26
- chronic tracheatis	CN 86101200 (A)1987-01-24
- allergy	CN105168111 (A) 2015-12-23
- iron-deficiency anemia in children	CN 101912523 (A) 2010-12-15
- pig constipation	CN 103230550 (A) 2013-08-07

In Europe *Corallina sp.* served as vermifuge towards the end of the XVIII th century (in Chapman V.J. 1950 - Seaweeds and their uses, Methuen & Co Ltd, London).

This property would be linked to the presence of a peptide including kainic acid residues (Calaf R. *et al.* 1989 - J. Appl. Phycol. 1 (3): 257-266).

Several compounds present in *Corallina officinalis* show great industrial interest, particularly phycobiliproteins and haloperoxidases.

- Phycobiliproteins are economically important because they are used as colorants in food and cosmetics. They show therapeutic value by immunomodulating activity and anticancer activity. On account of their fluorescence properties, they take part in the development of phycofluor tags for immunodiagnostics and highly sensitive fluorescence techniques (*cf.* in Sinha R.P. *et al.* 2003 - Trends in Phytochemistry and Photobiology 10: 149-157).

  SIGMA-ALDRICH offers R-phycoerythrin from *Corallina officinalis* (ref. PO 159).

- Haloperoxidases catalyze the halogenation of various compounds *e.g*. iodine, bromine (Yamada H. *et al.* 1985 – Agric. Biol. Chem. 49(10): 2961-2967). The vanadium bromoperoxidase of *Corallina officinalis* presents considerable interest due to the exceptional stability for industrial catalysis in a variety of contexts (Sheffield *et al*. 1994 - Biotechnology Techniques 8: 579-582; Rush C. et al. 1995- FEBS Lett. 359: 244-246; Vreeland V. & E. Grotkopp Patent US 5 520 727 (A) 1996-05-28; Vreeland V. & Kwan L. Ng Patent US 2002/0035245 2002-03-21; Littechild J. & E. Garcia-Rodiguez 2003 - Coordination Chemistry Reviews 237 (1-2): 65-75). They have also medical applications *(cf.* in Vreeland V. & Kwan L. Ng Patent US 2002/0035245-2002).

  SIGMA-ALDRICH proposes bromoperoxidases from *Corallina officinalis* (refs B 2170; 17 965).

A Japanese Patent has been deposited for the production of organic halogen (JP S63196295 (A) 1988-08-15).

Corallina officinalis extract is also used for personal care.

It exists patented formulations using this alga

- in France

  -SECMA uses it for the preparation of compositions having infrared-screening, ultraviolet- screening, thermal insulation or absorbent activity (FR 2674126 (A1) 1992-09-25).

  -CODIF uses it as calcium supplier for slimming composition (FR 2892024 (A1) 2007-04-20).

- as well as in other countries, for example for shampoo (CN 103637956 (A) 2014-03-19) or improving sub-health condition of human body (CN 102935220 (A) 2013-02-30) or else removing chloesma and beautifying faces (CN 105362956 (A) 2016-03-02).

COTY BV has deposited a patent relative to a sun product combination included *Corallina officinalis* US 2009117060 (A1) 2009-05-07).

GELYMA offers two actives prepared from *Corallina officinalis*

- Phyco'Derm® in combination with an extract of *Undaria pinnatifida,* efficient eye contour care.

PHYCO'DERM®

Takes care of the delicate area
around the eyes
*
Stimulates the major defense systems
Improves the dermis properties
Minimizes the appearance of under-eye dark circles
Reduces the volume of under-eye bags

- ALGYL® in combination with *Gigartina stellata/ kappaphycus alvarezii* extract

ALGYL®

Marine guardian
of
skin barrier functionality
*
Multifaceted ways of action
for a complete skin barrier protection

SPECIFICATION DATA SHEET

Trade name: **ALGYL®**

Product: N° G-W-GSKA-CO Version: 1.0 - 2020

Specification: N° S.00 Print date: 01 - 2020

ALGYL® is an association of red seaweeds extracts in synergism with glycerin plant origin.

1 – Identification and composition of the preparation

Product	N° CAS	N°EINECS	Ingredients %
Water	7732-18-5	231-791-2	45.70
Glycerin	56-81-5	200-289-5	40.00
Gigartina stellata -------- *Kappaphycus alvarezii* extract	223751-69-7 / -	-	4.43 / 5.90 } 10.33
Corallina officinalis extract	89997-92-2	289-730-0	3.97
Preservative	None		

2 – Characteristics (standard)

Aspect: liquid with possible white small precipitates with the time

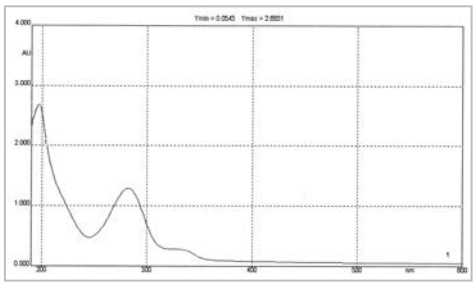
Colour: light yellow.

Odour: *sui generis.*

pH: 5.0 ± 1.0.

Density (20°C): 1.12 ± 0.10.

Spectrum UV (5% in water):



Microbiology:

Total germs (germs/ml):	< 100.
Pathogens:	absence.
Yeasts /moulds:	< 100.

Storage: 15°C < store < 25°C.
Validity date: 6 months.

Remark: *No responsibility or liability for any consequences arising from the use of this technical data sheet can be accepted, including possible infringement of any patent.*

GELYMA – Parc d'Affaires Marseille Sud (C4) – 1 Boulevard de l'Océan – 13009 Marseille – France.
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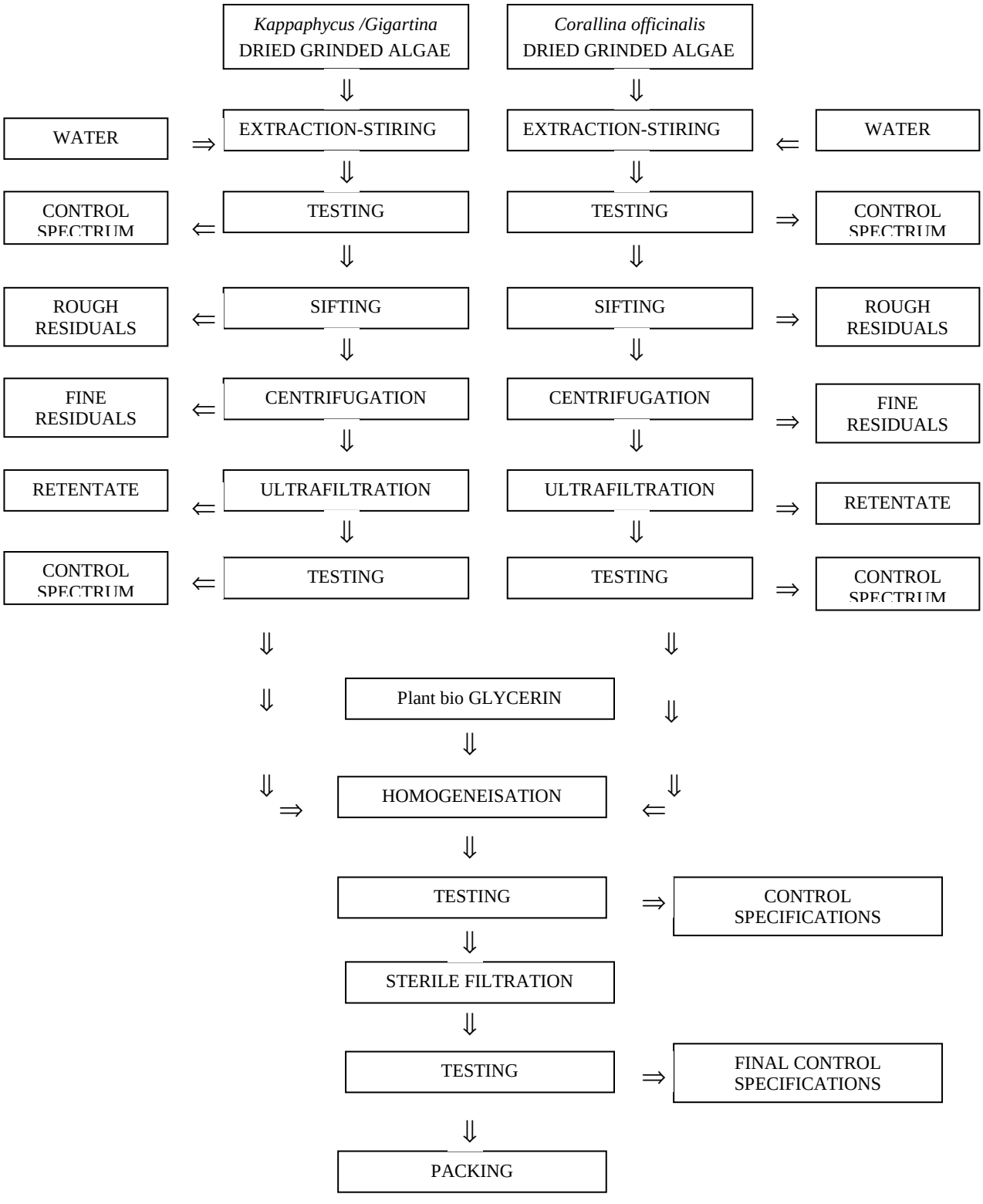
FLOW CHART FOR ALGYL®



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13009 Marseille
FRANCE

RAPPORT D'ESSAI FINAL

EXTRAIT D'ALGUES - ALGYL (EAU + GLYCERINE + ALGAE EXTRACT)

Date réception client :	
Date fabrication :	
N° lot client :	1707040
Fournisseur :	
N° lot fournisseur :	
Tonnage :	
DLUO :	

Demandeur :	Mme PELLEGRINI Liliane
N° commande :	
N° client :	
N° optim :	
N° étude :	
Réf. commerciale :	DS17CT004347
Tiers :	

Date réception labo : 03/10/2017

Masse brute (g):

Observations : DATE DE FABRICATION: 07/2017

Le code à 2 lettres indique le site Invivo Labs sur lequel a été réalisée l'analyse : CT = site de Chierry, SN = site de Saint-Nolff.

En cas de déclaration de conformité à la spécification, celle-ci ne prend pas en compte l'incertitude associée aux résultats.
Si ce rapport fait mention de résultats de mycotoxines, ils sont corrigés du taux de récupération. Ce rapport d'essai ne concerne que l'échantillon soumis à essai.
Si ce rapport fait mention de résultats de pesticides, ils ne sont pas corrigés du taux de récupération si celui-ci est compris entre 70 et 120 %.
« # » : analyse faite plusieurs fois

ANALYSES CHIMIQUES

Determination	Rés/brut	Rés/sec	Incertitude	Cible	Mini	Maxi	Conforme
SODIUM Méthode interne - ICP-H - CT	419,9 mg/100g		42,0 mg/100g				
CALCIUM Méthode interne - ICP-H - CT	74,8 mg/100g		7,5 mg/100g				
PHOSPHORE Méthode interne - ICP-H - CT	<2,0 mg/100g						
CHLORURES (exprimés en NaCl) Méthode interne CHL-H 14 adaptée du JORF : Arrêté du 24/08/83 - CT	391 mg/100g		50 mg/100g				
MAGNESIUM Méthode interne - ICP-H - CT	11,9 mg/100g		1,5 mg/100g				
POTASSIUM Méthode interne - ICP-H - CT	109,4 mg/100g		10,9 mg/100g				
CUIVRE Méthode interne - ICP-H - CT	<0,5 mg/100g						
FER Méthode interne - ICP-H - CT	<0,5 mg/100g						
MANGANESE Méthode interne - ICP-H - CT	<0,5 mg/100g						
ZINC Méthode interne - ICP-H - CT	<0,5 mg/100g						
IODE NF EN 15111 - CT	1,2 mg/kg		0,2 mg/kg				
MINERALISATION MICRO ONDES Méthode interne - ELTRACES-H - CT	Réalisée						
ARSENIC INORGANIQUE NF EN 15517 - avril 2008 - CT	<0,15 mg/kg						
ARSENIC Méthode interne - ELTRACES-H - CT	116 µg/kg		23 µg/kg				
CADMIUM Méthode interne - ELTRACES-H - CT	<10 µg/kg						
MERCURE Méthode interne - ELTRACES-H - CT	<10 µg/kg						
PLOMB Méthode interne - ELTRACES-H - CT	<10 µg/kg						
SELENIUM Méthode interne - ELTRACES-H - CT	<10 µg/kg						

Conclusion :

Validé le : 13-10-17

Melle BRU CAMILLE

Superviseur

Le code à 2 lettres indique le site Invivo Labs sur lequel a été réalisée l'analyse : CT = site de Chierry, SN = site de Saint-Nolff.

En cas de déclaration de conformité à la spécification, celle-ci ne prend pas en compte l'incertitude associée aux résultats.
Si ce rapport fait mention de résultats de mycotoxines, ils sont corrigés du taux de récupération. Ce rapport d'essai ne concerne que l'échantillon soumis à essai.
Si ce rapport fait mention de résultats de pesticides, ils ne sont pas corrigés du taux de récupération si celui-ci est compris entre 70 et 120 %.
« # » : analyse faite plusieurs fois

Translation 6C

Mineral and Metal Analysis Agyl (Gigartina Stellata/Kappaphycus Alvarezii Extracts and Corallina Officinalis Extract)

Determination	**Results/Units**	**Uncertainty**
Sodium Internal method ICP-H - CT	419.9 mg/100 g	42 mg/100 g
Calcium Internal method ICP-H - CT	74.8 mg/100 g	7.5 mg/100 g
Phosphorus Internal method ICP-H – CT	<2 mg/100 g	
Chlorides (expressed as NaCl) Internal method CHL-H 14 adapted from JORF: Decree of 08/24/83 - CT	391 mg/100 g	50 mg/100 g
Magnesium Internal method ICP-H - CT	11.9 mg/100 g	1.5 mg/100 g
Potassium Internal method ICP-H - CT	109.4 mg/100 g	10.9 mg/100 g
Copper Internal method ICP-H – CT	<0.5 mg/100 g	
Iron Internal method ICP-H – CT	<0.5 mg/100 g	
Manganese Internal method ICP-H – CT	<0.5 mg/100 g	
Zinc Internal method ICP-H – CT	<0.5 mg/100 g	
Iodine NF EN 15111 - CT	1.2 mg/kg	0.2 mg/kg
Microwave mineralization Internal method ELTRACES-H - CT	Completed	
Arsenic, inorganic NF EN 15517 - April 2008 - CT	<0.15 mg/kg	
Arsenic Internal method ELTRACES-H - CT	116 µg/kg	23 µg/kg
Cadmium Internal method ELTRACES-H - CT	<10 µg/kg	
Mercury Internal method ELTRACES-H - CT	<10 µg/kg	
Lead Internal method ELTRACES-H – CT	<10 µg/kg	
Selenium Internal method ELTRACES-H - CT	<10 µg/kg	

Conclusion: Validated on: 10/13/2017 Melle BRU CAMILLE
Supervisor

The 2-letter code indicates the In vivo Labs site on which the analysis was carried out: CT = site of Chierry, SN = site of Saint-Nolff.

In case of declaration of conformity to the specification, this does not take into account the uncertainty associated with the results.
If this report mentions mycotoxin results, they are corrected for the recovery rate. This test report only concerns the sample submitted for testing.
If this report mentions pesticide results, they are not corrected for the recovery rate if it is between 70 and 120%.

"#": Analysis made several times

Test item
ALGYL® - LOT : 19 06 280

Bacterial reverse mutation assay: determination of the mutagenic activity of a test item on *Salmonella typhimurium* (Ames test) according to the OECD #471

FINAL REPORT

Study Director : Francoise QUEFFURUS

Study # : 6.46_5S-52651-ID-19/07701

Sponsor	**Test Facility**
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GLP conformity statement

According to the Good Laboratory Practice (GLP) principles of France, the European Directive 2004/10/CE, the decree dated August 10th, 2004 from the JOFR, I state that:

- the study 6.46_5S-52651-ID-19/07701 was performed according to the GLP principles in IDEA Lab company laboratory, Brest location,
- the Study Plan and its modifications have been performed under my responsibility,
- all relevant SOPs have been followed,
- for confidentiality concerns, some characterisation data related to the test item composition may not be shown in this report. In this case, it is a deviation to GLP. However, this characterization had been provided by the Sponsor, brought to my attention, and then stored in a secure environment in accordance with the company procedures.
- raw data have been registered accurately,
- the study 6.46_5S-52651-ID-19/07701 is in conformity with the GLP principles despite of the following point which does not affect the reliability of results generated:
  the test item concentrations control in the different dilutions was not performed for the following reasons:
  - control of the test item preparation in its vehicle, particularly with micropipette and precision scales regularly controlled, calibrated and traceable with national or international standards of measurement,
  - the control of the homogeneity of the test item dilutions in the vehicle is performed using organoleptic criteria and is documented in the study log book,
  - test items dilutions are prepared extemporaneously,

This report accurately reflects the study carried out and the results obtained.
For these reasons, the Study Director acknowledges responsibility for the data validity of the study.

Date: 06 SEP. 2019

Francoise QUEFFURUS
Study Director
Microbiology Engineer

Quality Assurance statement

According to the Good Laboratory Practice, I state that:

- The General Study Plan was audited by the Quality Assurance and that the Specific Study Plan was verified before the beginning of the study.
- The different technical phases of the study 6.46_5S are regularly audited by the Quality Assurance. Facility audits are also carried out. The audit frequency is defined in the corresponding procedure.

  At the last technical audit (A-18/06), the following activities have been examined:
  Technical Phase preliminary cytotoxicity study
  - test item and controls preparation
  - S9-Mix preparation
  - test item and controls contact with the test system
  - plates incubation.
- The final report was audited by the Quality Assurance of IDEA Lab. It accurately reflects the raw data from the study and the application of the Standard Operating Procedures and the protocol.

Audit nature	Audits dates	Transmission dates of the audit report to the Study Director and the General Management
Technical phases of the study	From 25/06/2018 To 27/06/2018	03/07/2018
General Study Plan	21/12/2018	21/12/2018
Draft Report	04 SEP. 2019	04 SEP. 2019
Final Report	06 SEP. 2019	06 SEP. 2019

Date: 06 SEP. 2019

Quality Assurance
Delphine LEGEAIS

Study summary

The ability of the test item **ALGYL® - LOT : 19 06 280**, supplied by **GELYMA**, to induce mutation was assessed using the bacterial reverse mutation test (Ames test). The test was performed on five *Salmonella typhimurium* strains.

The test item dilutions were prepared in water for analysis.
A preliminary cytotoxicity test was performed on *S. typhimurium* TA100 strain.
The test has been performed at the concentrations 5000, 1600, 500, 160 and 50 µg/plate, with and without S9-Mix.

The preliminary study showed no cytotoxicity of the test item; therefore this concentrations range was used for the genotoxicity Test 1.
According to the result obtained for the Test 1, the Study Director decided to use the same dilution range for the test 2.

The revertant analysis shows that:

- No cytotoxic effect was observed,
- No concentration of the test item showed ratio R higher or equal at least to the double of the spontaneous rate of reversion for TA98, TA100 and TA102 and to the triple of the spontaneous rate of reversion for TA1535 and TA1537, with and without metabolic activation,
- No dose response was observed, whatever the test system or conditions of the test.

In the light of the results obtained during this study, we can conclude that the test item ALGYL® - LOT : 19 06 280 does not show any mutagenic nor pro-mutagenic activity, under the test conditions used.

Study presentation

1 Study objective

We have evaluated, using an *in vitro* test, the genotoxicity potential of a test item **ALGYL® - LOT : 19 06 280, ID-19/07701,** according to the general study plan 6.46_5S.
This study has been performed according to the OECD #471 Guideline (July 21st, 1997) and the Directive 2000/32/CE, method B13/14, dated June 8th 2000.

2 Test item

Reception of test item (including recording and verification) has been managed directly by **IDEA Lab, Brest Test Location**.

This activity is under Study Director's responsibility and performed according procedures in place within IDEA Lab, and is within the scope of installation audits performed by the Quality Assurance of the test facility.

Information linked to the identification, purity and stability of the test item are under responsibility of the Sponsor of the study. The technical data sheet of the test item was provided by the Sponsor of the study. In case of missing or incomplete data, results obtained during solubility and stability study performed during preliminary experiment will serve as evidences and are available in study book.

Name	: ALGYL® - LOT : 19 06 280
Internal code	: ID-19/07701
Batch number	: 19 06 280
Storage conditions	: Room temperature (20°C ± 5°C)
Test item nature	: Cosmetic ingredients mixture
Expiry date	: 28/06/2020
CAS number	: 7732-18-5 / 56-81-5 / 223751-69-7 / 89997-92-2

Physico-chemistry properties

Physical state at 20 °C	: Liquid		
Color	: Light yellow		
pH	: 5.01		
Density (for liquid)	: 1.11	☒ Actual	☐ Estimated
Homogeneity	: Yes		
Test item purity	: NA		

Solubility and stability

Solvent	: Water
Maximal concentration in the solvent	: 50 mg/ml
Stability in the solvent	: 8 hours

The certificate of analysis of the test item is shown at the end of the report.

3 Study principle

- Contact of the five mutant strains with several concentrations of the test item, with and without metabolic activation.
- Counting of revertant colonies with several concentrations of test item and comparison with the spontaneous revertant colonies.
- Validation of the test by positive controls (mutagenic substances) and negative controls.

4 Study course

4.1 Experimentation phases

Start of the experimental part of the study (cytotoxicity test): 08/08/2019.

End of the experimental part of the study (end of Test 2): 23/08/2019.

Study occurred in 3 main phases:

- A preliminary experiment performed in order to evaluate the cytotoxicity of the test item and to select the range of dose levels for the further experiments,
- A first experiment of genotoxicity (Test 1), with and without metabolic activation, with the direct plate incorporation method, on the range of concentrations defined by the preliminary study.
- A second experiment (Test 2), using the pre-incubation method, with and without metabolic activation, with dose levels defined by Study Director after analysis of results obtained from the first experiment. This second experiment has been performed in order to confirm or for complement results of the first one.

4.2 Material and reagents

4.2.1 Material

- Petri plates
- Plastic vials with screw cap for strains conservation (cryotubes)
- Automatic pipettes 1-2000 µl
- Test tubes and vials with appropriate volumes
- Autoclave
- Vortex
- pH-meter
- Analytical weights
- Spectrophotometer
- Bacteriological incubators
- Thermostatic agitators
- Laminar flow hood (PSM) with extraction system
- Freezer at -80°C (± 5°C)
- Freezer at -20°C (± 5°C)
- Refrigerators at 6°C (± 3°C)
- Colony counter and data processing system
  - Plates reader : Sorcerer, version 2.2 (Perceptive Instrument)
  - Transfer and raw data storage : Ames Study Manager, version 1.22 (Perceptive Instrument)
  - Result tables edition : Ames Report Generator, version 1 (Perceptive Instrument)

4.2.2 Reagents

- Nutrient broth for the strains culture
- Bottom agar
  - Vogel-Bonner medium E (concentrated 50 times)
  - Glucose solution at 400 g/l
- Top agar for *Salmonella typhimurium* TA
- Phosphate buffer 0.2 M, pH 7.4
- Reagents for preparation of S9-Mix
- Microsomal fraction of rat liver (S9)

4.3 Test system

4.3.1 Strains

The choice of strains was made according to OECD Guideline. It is five strains of *Salmonella typhimurium* LT2 for which the properties are summarised in the following table.

	Target mutation	Excision repear	Plasmid	Cell wall	Type of mutation
TA98	His D 3052	uvrB	pkM101	rfa	Frameshift
TA100	His G 46	uvrB	pkM101	rfa	Base-pair substitution
TA102	His G 428	intact excision	pkM101	rfa	Base-pair substitution
TA1535	His G 46	uvrB	/	rfa	Base-pair substitution
TA1537	His C 3076	uvrB	/	rfa	Frameshift

Table 1

Besides their histidin mutation, each strain has 1 or 2 further mutations which increase really their sensibility to mutagens:

- uvrB: excision of reparation system,
- rfa: the lipo-polysaccharidic wall is made permeable to big molecules.

Control of essential characteristics of strains is periodically performed according to OECD #471 Guidelines and instruction IL 11:

- Growing in presence of histidin
- Permeability of bacterial wall (rfa mutation)
- Ampicillin resistance
- Deletion of the DNA repair potential
- Spontaneous revertant rate

4.3.2 Media and growth conditions

The composition of media is summarized in annex to this document.

For each experiment, the test strains cultures were prepared in nutrient broth from frozen stocks and incubated at 37°C ± 2 °C on shaken platter to allow the culture to grow up to the late exponential or early stationary phase of growth (approximately 10^8-10^9 cells/ml). The optical density of each culture has been used to check the cell density.

Microbial suspension was put in contact with the test item or reference items, mixed with top agar and poured over minimal agar medium plate. After solidification, plates were incubated at 37°C ± 2°C during 48 to 72 hours. Positive and negative controls were included in the experiment.

4.3.3 Metabolic activation

Bacteria were exposed to the test item with and without a metabolic activation system. The system used is a cofactor enhanced post-mitochondrial fraction (S9), prepared from rat livers treated with an enzymatic inducer. The post-mitochondrial fraction (certificate given in annex) is used at 10% (v/v). The composition of the S9-Mix is described in annex. The acceptation criteria for the post-mitochondrial fraction are described in the working instruction IL REAC 01.

4.4 Reference items

Negative controls: the spontaneous revertant count with the solvent, with and without metabolic activation, was included in each experiment.

Negative control without treatment: the spontaneous revertant count without the solvent, with and without metabolic activation was included in each experiment for the control of absence of mutagen activity of vehicle.

Positive controls: known mutagens as defined in table 2.

Strain	Reference items used							
	-S9-Mix	Solvent	Dose (µg/plate)	Dose (µl)	+S9-Mix	Solvent	Dose (µg/plate)	Dose (µl)
TA98	2-NITROFLUORENE 0.1 mg/ml CAS No. (607-57-8)	DMSO	5	50	2-AMINOANTHRACENE 0.1 mg/ml CAS No. (613-13-8)	DMSO	5	50
TA100	SODIUM AZIDE 0.2 mg/ml CAS No. (26628-22-8)	Water	10	50	2-AMINOANTHRACENE 0.1 mg/ml CAS No. (613-13-8)		5	50
TA102	MITOMYCIN 0.01 mg/ml CAS No. (50-07-7)	Water	0.5	50	2-AMINOANTHRACENE 0.5 mg/ml CAS No. (613-13-8)		25	50
TA1535	SODIUM AZIDE 0.2 mg/ml CAS No. (26628-22-8)	Water	10	50	2-AMINOANTHRACENE 0.1 mg/ml CAS No. (613-13-8)		5	50
TA1537	9-AMINOACRIDINE 0.6 mg/ml CAS No. (90-45-9)	DMSO	30	50	2-AMINOANTHRACENE 0.1 mg/ml CAS No. (613-13-8)		5	50

Table 2

4.5 Solvent choice and test item preparation

The most commonly used solvents are deionized water for the analysis and dimethylsulfoxide (DMSO), or any appropriate solvent compatible with the test system and the test item or other solvent can be used at the request of the Sponsor if they are known or if it has been demonstrated that they are not cytotoxic nor genotoxic. The compatibility with the test item is therefore under the Sponsor responsibility.

A preliminary dissolution test was performed in order to define the most appropriate solvent as well as the maximal concentration tested.

A stock solution of the test item ALGYL® - LOT : 19 06 280 was prepared in deionized water for analyse.

The other tested solutions have been obtained by serial dilution from this stock solution in the same solvent. These solutions were prepared extemporaneously each day of manipulation.

4.6 Series definition

The solubility test showed no insolubility of the test item. Therefore, the maximal concentration retained was 5000 µg/plate.

According to OECD Guideline, 5 concentrations of test item have been studied with approximately half log (i.e. approximately √10) interval. These doses (rounded to the higher value) used for the preliminary cytotoxicity test were therefore the following: 5000, 1600, 500, 160 and 50 µg/plate.

As the preliminary experiment revealed no cytotoxicity of the test item, this range of concentrations has been conserved for the Test 1.

According to the results obtained in the Test 1, the Study Director decided to maintain range of concentrations for Test 2.

Each test item dilution and each reference item are tested on 3 Petri plates.

4.7 Test performance

4.7.1 Preliminary cytotoxicity study

The preliminary cytotoxicity study of the test item has been performed on the strain *S. typhimurium* TA100, in the same conditions as the Test 1 (cf. paragraph 4.7.3).

Results obtained are part of the Test 1 results if no cytotoxicity is observed. The test item has been dissolved in the suitable solvent.

The applied protocol was the following one:

- In 3 hemolysis tubes, introduce:
  - assay without metabolic activation:
    - 0.1 ml of the different test item concentrations,
    - 0.5 ml sterile phosphate buffer 0.2 M, pH 7.4,
    - 2 ml of top agar for *S. typhimurium*,
    - 0.1 ml of bacterial inoculum (TA100).
  - assay with metabolic activation:
    - 0.1 ml of the different test item concentrations,
    - 2 ml of top agar for *S. typhimurium*,
    - 0.1 ml of bacterial inoculum (TA100),
    - 0.5 ml of S9-Mix.
- Mix and pour on the surface of the bottom agar previously distributed in Petri dishes.
- Incubate at 37°C ± 2 °C for 48 to 72 hours.
- Count colonies. Results are interpreted according to the paragraph 5.

4.7.2 Control tests

These assays were performed for each test: preliminary cytotoxicity test, Test 1 and Test 2.
Non treated control, negative controls and positive controls made during pre-incubation method were incubated during 20-30 minutes at 37°C ± 2°C before pouring top agar, according described method paragraph 4.7.3.2.

4.7.2.1 Sterility control of the solvent used, the test item, the S9-Mix and the top agar

The applied protocol was the following one:

- In 4 fractions of 2 ml top agar for *S. typhimurium*, introduce:
  - 0.1 ml of phosphate buffer 0.2 M, pH 7.4,
  - 0.1 ml of solvent,
  - 0.1 ml of S9-Mix.
  - 0.1 ml of the test item preparation at the higher concentration,
- One fraction of 2 ml top agar for *S. typhimurium* is used to control its sterility.
- Mix and pour on the surface of the bottom agar previously distributed in Petri dishes.
- Incubate at 37°C ± 2°C for 48 to 72 hours.
- The test is performed in triplicate.
- No bacterial growth must be observed.

4.7.2.2 Non treated control and negative control (control of non mutagenic activity of the solvent used)

The applied protocol was the following one:

- For each strain, in 3 hemolysis tubes, introduce:
  - assay without metabolic activation:
    - 0.5 ml sterile phosphate buffer 0.2 M, pH 7.4,
    - 2 ml of top agar,
    - 0.1 ml of bacterial inoculum.
  - assay with metabolic activation:
    - 2 ml of top agar,
    - 0.1 ml of bacterial inoculum,
    - 0.5 ml of S9-Mix.
- For the negative control add 0.1 ml of the solvent used.
- Mix and pour on the surface of the bottom agar previously distributed in Petri dishes.
- Incubate at 37°C ± 2°C for 48 to 72 hours.
- Count colonies. Results are interpreted according to the paragraph 5.

4.7.2.3 Positive control

Each mutagenicity assay of a test item must include well-known mutagenic products, specific for each strain. Positive controls used are described in table 2.
The applied protocol was the following one:

- For each strain, in 3 hemolysis tubes, introduce:
  - assay without metabolic activation:
    - 0.5 ml sterile phosphate buffer 0.2 M, pH 7.4,
    - The quantity of positive control defined in table 2,
    - 2 ml of top agar,
    - 0.1 ml of bacterial inoculum.
  - assay with metabolic activation:
    - The quantity of positive control defined in table 2,
    - 2 ml of top agar,
    - 0.1 ml of bacterial inoculum,
    - 0.5 ml of S9-Mix.
- Mix and pour on the surface of the bottom agar previously distributed in Petri dishes.
- Incubate at 37°C ± 2°C for 48 to 72 hours.
- Count colonies. Results are interpreted according to the paragraph 5.

4.7.3 Test itself: Research of mutagenic activity

For at least 5 concentrations of the test item, a test without metabolic activation and a test with metabolic activation have been performed simultaneously as follow:

4.7.3.1 Test 1: direct method

- For each strain, in 3 hemolysis tubes, introduce:
  - assay without metabolic activation:
    - 0.1 ml of the different test item concentrations,
    - 0.5 ml sterile phosphate buffer 0.2 M, pH 7.4,
    - 2 ml of top agar,
    - 0.1 ml of bacterial inoculum.
  - assay with metabolic activation:
    - 0.1 ml of the different test item concentrations,
    - 2 ml of top agar,
    - 0.1 ml of bacterial inoculum,
    - 0.5 ml of S9-Mix.
- Mix and pour on the surface of the bottom agar previously distributed in Petri dishes.
- Incubate at 37°C ± 2°C for 48 to 72 hours.
- Count colonies. Results are interpreted according to the paragraph 5.

4.7.3.2 Test 2: method with pre-incubation

Pre-incubation method allows revealing more effectively mutagen activity of some compounds like aliphatic nitrosamines, bivalent metals, aldehydes, azoic coloring agent.
The applied protocol was the following one:

- For each strain, in 3 hemolysis tubes, introduce:
  - assay without metabolic activation:
    - 0.1 ml of the different test item concentrations,
    - 0.5 ml sterile phosphate buffer 0.2 M, pH 7.4,
    - 0.1 ml of bacterial inoculum.
  - assay with metabolic activation:
    - 0.1 ml of the different test item concentrations,
    - 0.1 ml of bacterial inoculum,
    - 0.5 ml of S9-Mix.

- Incubate at 37°C ± 2°C for 20 to 30 min.
- Add the 2 ml of top agar.
- Mix and pour on the surface of the bottom agar previously distributed in Petri dishes.
- Incubate at 37°C ± 2°C for 48 to 72 hours.
- Count colonies. Results are interpreted according to the paragraph 5.

4.8 Data evaluation

For each assay the following observations were performed and reported:

- Observation of the reagent mix before Petri plates pouring: reporting of any abnormal sign (precipitate, trouble, etc.),
- Petri plates observation and reporting of any cytotoxicity sign (bottom bacterial layer reduction). The cytotoxicity intensity on the bottom bacterial layer is evaluated qualitatively on each plate by naked eyes:
  - total destruction of the bottom bacterial layer (the revertants development does not occur in this case), this one is noted in tables of results as "A".
  - moderated destruction of the bottom bacterial layer. This one is noted in tables of results as "S".

Acquisition and storage of raw data were managed by the following electronic system:

- Reading of plates: Sorcerer, version 2.2.
- Transfer and storage of raw data: Ames Study Manager, version 1.22.

The result tables edition was managed by the Ames Report Generator, version 1.

5 Results expression and interpretation

5.1 Processing and presentation of the results

After 48 to 72 hours incubation at 37°C ± 2°C, revertants were counted on each plate. (If counting has not been performed at the end of incubation period, plates have been stored in fridge (6°C ± 3°C) and read within 72 hours, without affecting results of test).
Results are expressed in number of revertants (mean ± sd) per plate for each concentration of the test item.
The following ratio can be established:

$$R = \frac{\text{Number of revertants with test item}}{\text{Number of revertants with solvent without test item}}$$

Tables were set up where we can see all individual results obtained with the test item and the positive and negative controls.
Mean and standard deviation were calculated for each concentration of the test item.

The preliminary cytotoxicity test results are shown with the Test 1 results (cf. paragraph 7.2) if there is no cytotoxicity or if this one can be shown only at the maximum concentration. In case of the opposite, they are presented in paragraph 7.1 in a table in which bacterial layer aspect and R ratio values are shown for each concentration tested.

5.2 Acceptance criteria of data

The test is considered valid if the following criteria are fulfilled:

- The sterility tests are conform,
- The mean negative controls are within the historical data,
- The solvent used (negative control) must not show genotoxic nor cytotoxic activity,
- The revertants rate obtained for the positive controls must be in agreement with the historical data,

- The positive controls must show a revertants number equal at least to the double of the spontaneous rate of reversion for TA98, TA100 and TA102 ($R \geq 2$) and the triple of the spontaneous rate of reversion for TA1535 and TA1537 ($R \geq 3$),
- No more than 5% of the plates of the test are lost through contamination or any other unforeseen event,
- At least 3 concentrations are available for mutagenicity assessment.

5.3 Results interpretation

5.3.1 Preliminary test of cytotoxicity

We consider the test item as cytotoxic if the spontaneous revertant rate is lower than 0.7 ($R < 0.7$). The possible destruction of the bottom bacterial layer is also taken into account.

5.3.2 Mutagenicity test

- **The test item is considered as mutagenic** if at the end of the verifications steps, it has been obtained, in a reproducible way, a relation dose-effect on one or some of 5 strains with and/or without metabolic activation. The mutagenicity is taken into account for a given concentration only when the number of revertants is equal at least to the double of the spontaneous rate of reversion for TA98, TA100 and TA102 strains ($R \geq 2$) and the triple of the spontaneous rate of reversion for TA1535 and TA1537 strains ($R \geq 3$).
- **The test item is considered as non-mutagenic** if, in the outcome of the Test 1 and the Test 2, the rate of revertants always remained lower than the double of the rate of spontaneous reversion for all the concentrations of tested product, for TA98, TA100 and TA102 strains ($R < 2$) and lower than the triple of the spontaneous rate of reversion for TA1535 and TA1537 strains ($R < 3$), with and without metabolic activation, and on the condition of having made sure that the absence of mutagen effect is not bound to the toxicity of the tested concentrations.

The result validation was performed by the Study Director in agreement with the working instruction IL 04.

6 Study plan deviations and amendments

No deviation or amendment to the study plan has been observed during this study.

7 Results

7.1 Preliminary cytotoxicity

The preliminary cytotoxicity test performed on the TA100 strain showed no cytotoxicity of the test item ($R \geq 0.7$). Results are parts of the Test 1 (shown in table 3 and 4, paragraph 7.2).

7.2 Revertants analysis

Revertant analysis was performed on the 5 concentrations chosen following the cytotoxicity study. The results are shown in table 3, 4, 5 and 6.

- Test 1, without S9-Mix, direct assay: table 3
- Test 1, with S9-Mix, direct assay: table 4
- Test 2, without S9-Mix, with pre-incubation: table 5
- Test 2, with S9-Mix, with pre-incubation: table 6

Revertant analysis tables

Table 3: Test 1, without S9-Mix, direct assay

Study Name: 6.46-5S-52651-ID-19/07701
Experiment: Cytotoxicité. ID-19/07701
Assay Conditions: Plate incorporation assay

Study Code: ID-19/07701
Date Plated: 09/08/2019
Date Counted: 12/08/2019

Without metabolic activation

Strain	Compound	Dose level per plate	Mean revertants per plate	Standard Deviation	Ratio treated / solvent	Individual revertant colony counts
TA100	**ID-19/07701**	5000 µg	*175,7*	*36,7*	*1,2*	153, 156, 218
		1600 µg	*158,7*	*49,9*	*1,1*	117, 145, 214
		500 µg	*175,0*	*47,7*	*1,2*	145, 150, 230
		160 µg	*169,3*	*60,8*	*1,1*	117, 155, 236
		50 µg	*169,3*	*34,4*	*1,1*	149, 150, 209
	Eau / Water		*149,7*	*11,6*		142, 144, 163
	Untreated Control		*123,3*	*13,5*		110, 123, 137
TA100	**AZI**	10 µg	*1918,0*	*88,7*	*12,8*	1887, 2018, 1849

Key to Positive Controls

AZI SODIUM AZIDE

Study Name: 6.46-5S-52651-ID-19/07701
Experiment: Test 1. ID-19/07701
Assay Conditions: Plate incorporation assay

Study Code: ID-19/07701
Date Plated: 13/08/2019
Date Counted: 16/08/2019

Without metabolic activation

Strain	Compound	Dose level per plate	Mean revertants per plate	Standard Deviation	Ratio treated / solvent	Individual revertant colony counts
TA102	**ID-19/07701**	5000 µg	*429,3*	*24,8*	*1,2*	447, 401, 440
		1600 µg	*434,7*	*48,7*	*1,2*	395, 420, 489
		500 µg	*431,3*	*50,7*	*1,2*	378, 437, 479
		160 µg	*450,7*	*46,3*	*1,2*	398, 469, 485
		50 µg	*437,0*	*54,1*	*1,2*	385, 433, 493
	Eau / Water		*368,3*	*4,6*		371, 371, 363
	Untreated Control		*380,0*	*6,6*		373, 386, 381
TA1535	**ID-19/07701**	5000 µg	*17,0*	*8,2*	*0,9*	24, 8, 19
		1600 µg	*17,0*	*7,0*	*0,9*	14, 12, 25
		500 µg	*15,7*	*2,3*	*0,8*	17, 17, 13
		160 µg	*20,7*	*0,6*	*1,1*	21, 21, 20
		50 µg	*18,3*	*6,7*	*0,9*	15, 14, 26
	Eau / Water		*19,3*	*0,6*		20, 19, 19
	Untreated Control		*18,3*	*2,3*		21, 17, 17
TA1537	**ID-19/07701**	5000 µg	*16,3*	*2,9*	*1,0*	13, 18, 18
		1600 µg	*16,7*	*3,8*	*1,0*	14, 15, 21
		500 µg	*17,7*	*2,3*	*1,0*	15, 19, 19
		160 µg	*17,7*	*2,5*	*1,0*	15, 18, 20
		50 µg	*16,7*	*2,3*	*1,0*	18, 18, 14
	Eau / Water		*17,0*	*2,6*		14, 18, 19
	Untreated Control		*18,3*	*0,6*		19, 18, 18
TA98	**ID-19/07701**	5000 µg	*26,3*	*5,7*	*1,1*	31, 28, 20
		1600 µg	*25,3*	*2,3*	*1,0*	28, 24, 24
		500 µg	*22,7*	*4,6*	*0,9*	20, 20, 28
		160 µg	*26,3*	*0,6*	*1,1*	26, 27, 26
		50 µg	*22,3*	*6,4*	*0,9*	27, 25, 15
	Eau / Water		*25,0*	*9,5*		26, 34, 15
	Untreated Control		*23,0*	*4,6*		22, 28, 19
TA102	**MIT**	0.5 µg	*2538,3*	*58,5*	*6,9*	2523, 2603, 2489
TA1535	**AZI**	10 µg	*2024,0*	*57,5*	*104,7*	1985, 2090, 1997
TA1537	**9AA**	30 µg	*259,7*	*99,3*	*15,3*	238, 173, 368
TA98	**2NIT**	5 µg	*1287,0*	*32,4*	*51,5*	1323, 1260, 1278

Key to Positive Controls

MIT	MITOMYCIN
AZI	SODIUM AZIDE
9AA	9-AMINOACRIDINE
2NIT	2-NITROFLUORENE

Table 4: Test 1, with S9-Mix, direct assay

Study Name: 6.46-5S-52651-ID-19/07701
Experiment: Cytotoxicité. ID-19/07701
Assay Conditions: Plate incorporation assay

Study Code: ID-19/07701
Date Plated: 09/08/2019
Date Counted: 12/08/2019

With metabolic activation

Strain	Compound	Dose level per plate	Mean revertants per plate	Standard Deviation	Ratio treated / solvent	Individual revertant colony counts
TA100	**ID-19/07701**	5000 µg	*175,7*	*18,1*	*0,9*	189, 155, 183
		1600 µg	*200,0*	*5,6*	*1,1*	205, 201, 194
		500 µg	*165,0*	*14,0*	*0,9*	149, 175, 171
		160 µg	*176,3*	*8,1*	*0,9*	175, 169, 185
		50 µg	*172,3*	*12,3*	*0,9*	162, 169, 186
	Eau / Water		*186,3*	*2,3*		185, 189, 185
	Untreated Control		*173,7*	*9,3*		184, 171, 166
TA100	**A2A**	5 µg	*4159,3*	*270,2*	*22,3*	3867, 4400, 4211

Key to Positive Controls

A2A 2-AMINOANTHRACENE

Study Name: 6.46-5S-52651-ID-19/07701
Experiment: Test 1. ID-19/07701
Assay Conditions: Plate incorporation assay

Study Code: ID-19/07701
Date Plated: 13/08/2019
Date Counted: 16/08/2019

With metabolic activation

Strain	Compound	Dose level per plate	Mean revertants per plate	Standard Deviation	Ratio treated / solvent	Individual revertant colony counts
TA102	**ID-19/07701**	5000 µg	*443,3*	*26,0*	*1,0*	417, 444, 469
		1600 µg	*425,7*	*11,9*	*1,0*	412, 434, 431
		500 µg	*416,7*	*14,6*	*1,0*	400, 423, 427
		160 µg	*448,3*	*14,5*	*1,0*	439, 441, 465
		50 µg	*461,3*	*45,0*	*1,1*	412, 472, 500
	Eau / Water		*428,3*	*25,5*		408, 420, 457
	Untreated Control		*433,7*	*23,5*		410, 457, 434
TA1535	**ID-19/07701**	5000 µg	*19,0*	*0,0*	*1,1*	19, 19, 19
		1600 µg	*15,0*	*1,7*	*0,8*	14, 14, 17
		500 µg	*16,7*	*3,8*	*0,9*	21, 15, 14
		160 µg	*19,7*	*1,5*	*1,1*	21, 20, 18
		50 µg	*17,0*	*4,0*	*1,0*	17, 13, 21
	Eau / Water		*17,7*	*3,1*		21, 17, 15
	Untreated Control		*19,7*	*1,5*		20, 18, 21
TA1537	**ID-19/07701**	5000 µg	*22,3*	*1,5*	*1,1*	24, 22, 21
		1600 µg	*16,7*	*4,5*	*0,8*	21, 12, 17
		500 µg	*15,7*	*2,1*	*0,8*	15, 18, 14
		160 µg	*18,7*	*3,5*	*0,9*	22, 15, 19
		50 µg	*19,7*	*3,8*	*1,0*	17, 18, 24
	Eau / Water		*20,3*	*4,2*		19, 17, 25
	Untreated Control		*18,7*	*1,2*		18, 18, 20
TA98	**ID-19/07701**	5000 µg	*38,7*	*1,5*	*1,0*	37, 39, 40
		1600 µg	*34,7*	*3,5*	*0,9*	31, 35, 38
		500 µg	*42,0*	*4,4*	*1,1*	45, 37, 44
		160 µg	*36,7*	*5,9*	*0,9*	39, 30, 41
		50 µg	*38,3*	*2,3*	*1,0*	41, 37, 37
	Eau / Water		*39,7*	*2,1*		42, 39, 38
	Untreated Control		*37,0*	*7,0*		37, 44, 30
TA102	**A2A**	25 µg	*4071,3*	*477,6*	*9,5*	3676, 3936, 4602
TA1535	**A2A**	5 µg	*297,0*	*6,1*	*16,8*	290, 301, 300
TA1537	**A2A**	5 µg	*611,3*	*56,3*	*30,1*	552, 618, 664
TA98	**A2A**	5 µg	*2664,7*	*46,3*	*67,2*	2683, 2699, 2612

Key to Positive Controls

A2A 2-AMINOANTHRACENE

Table 5: Test 2, without S9-Mix, with pre-incubation

Study Name: 6.46-5S-52651-ID-19/07701
Experiment: Test 2. ID-19/07701
Assay Conditions: Pre-incubation assay

Study Code: ID-19/07701
Date Plated: 20/08/2019
Date Counted: 23/08/2019

Without metabolic activation

Strain	Compound	Dose level per plate	Mean revertants per plate	Standard Deviation	Ratio treated / solvent	Individual revertant colony counts
TA100	**ID-19/07701**	5000 µg	*203,0*	*31,5*	*1,1*	194, 177, 238
		1600 µg	*191,0*	*51,3*	*1,1*	143, 185, 245
		500 µg	*179,7*	*36,7*	*1,0*	156, 161, 222
		160 µg	*196,0*	*34,1*	*1,1*	168, 186, 234
		50 µg	*196,0*	*42,7*	*1,1*	151, 201, 236
	Eau / Water		*179,3*	*25,9*		161, 168, 209
	Untreated Control		*171,3*	*4,7*		173, 166, 175
TA102	**ID-19/07701**	5000 µg	*358,3*	*62,1*	*1,0*	320, 325, 430
		1600 µg	*379,3*	*17,5*	*1,1*	379, 362, 397
		500 µg	*373,0*	*27,2*	*1,1*	369, 402, 348
		160 µg	*357,0*	*12,3*	*1,0*	343, 362, 366
		50 µg	*379,7*	*46,9*	*1,1*	345, 361, 433
	Eau / Water		*353,3*	*3,8*		349, 356, 355
	Untreated Control		*373,0*	*13,0*		380, 358, 381
TA1535	**ID-19/07701**	5000 µg	*19,7*	*2,5*	*1,5*	22, 17, 20
		1600 µg	*15,0*	*4,6*	*1,2*	20, 11, 14
		500 µg	*17,3*	*4,0*	*1,3*	18, 13, 21
		160 µg	*13,7*	*5,7*	*1,1*	9, 20, 12
		50 µg	*17,0*	*5,2*	*1,3*	20, 20, 11
	Eau / Water		*13,0*	*5,0*		18, 8, 13
	Untreated Control		*12,0*	*1,0*		12, 13, 11
TA1537	**ID-19/07701**	5000 µg	*19,0*	*6,6*	*1,2*	13, 18, 26
		1600 µg	*18,7*	*2,9*	*1,2*	22, 17, 17
		500 µg	*18,0*	*5,2*	*1,1*	24, 15, 15
		160 µg	*14,7*	*5,9*	*0,9*	17, 8, 19
		50 µg	*16,0*	*3,6*	*1,0*	17, 12, 19
	Eau / Water		*16,0*	*2,6*		13, 18, 17
	Untreated Control		*17,3*	*2,1*		15, 18, 19
TA98	**ID-19/07701**	5000 µg	*28,7*	*3,2*	*1,3*	30, 31, 25
		1600 µg	*23,3*	*5,8*	*1,1*	20, 30, 20
		500 µg	*23,7*	*3,8*	*1,1*	28, 22, 21
		160 µg	*18,0*	*3,0*	*0,8*	21, 18, 15
		50 µg	*31,7*	*4,7*	*1,5*	37, 28, 30
	Eau / Water		*21,7*	*4,5*		22, 17, 26
	Untreated Control		*28,7*	*4,0*		25, 28, 33
TA100	**AZI**	10 µg	*1961,0*	*19,7*	*10,9*	1940, 1964, 1979
TA102	**MIT**	0.5 µg	*3713,3*	*367,0*	*10,5*	3295, 3864, 3981
TA1535	**AZI**	10 µg	*2202,3*	*118,2*	*169,4*	2324, 2195, 2088
TA1537	**9AA**	30 µg	*411,0*	*259,1*	*25,7*	225, 301, 707
TA98	**2NIT**	5 µg	*1422,0*	*51,8*	*65,6*	1481, 1384, 1401

Key to Positive Controls

AZI	SODIUM AZIDE
MIT	MITOMYCIN
9AA	9-AMINOACRIDINE
2NIT	2-NITROFLUORENE

Table 6: Test 2, with S9-Mix, with pre-incubation

Study Name: 6.46-5S-52651-ID-19/07701
Experiment: Test 2. ID-19/07701
Assay Conditions: Pre-incubation assay

Study Code: ID-19/07701
Date Plated: 20/08/2019
Date Counted: 23/08/2019

With metabolic activation

Strain	Compound	Dose level per plate	Mean revertants per plate	Standard Deviation	Ratio treated / solvent	Individual revertant colony counts
TA100	**ID-19/07701**	5000 µg	*177,3*	*37,2*	*0,8*	205, 135, 192
		1600 µg	*204,7*	*4,6*	*1,0*	202, 210, 202
		500 µg	*182,7*	*7,6*	*0,9*	176, 181, 191
		160 µg	*186,7*	*5,1*	*0,9*	191, 188, 181
		50 µg	*199,3*	*4,9*	*1,0*	205, 196, 197
	Eau / Water		*209,7*	*9,9*		221, 203, 205
	Untreated Control		*198,3*	*8,1*		191, 197, 207
TA102	**ID-19/07701**	5000 µg	*424,7*	*39,5*	*1,0*	385, 425, 464
		1600 µg	*426,3*	*22,2*	*1,0*	414, 413, 452
		500 µg	*420,3*	*34,6*	*1,0*	384, 424, 453
		160 µg	*427,0*	*12,5*	*1,0*	426, 440, 415
		50 µg	*434,0*	*25,7*	*1,0*	405, 443, 454
	Eau / Water		*420,0*	*23,1*		428, 438, 394
	Untreated Control		*448,7*	*32,4*		486, 428, 432
TA1535	**ID-19/07701**	5000 µg	*18,7*	*5,0*	*1,2*	18, 14, 24
		1600 µg	*11,7*	*3,1*	*0,8*	9, 15, 11
		500 µg	*14,7*	*5,5*	*1,0*	11, 12, 21
		160 µg	*18,0*	*4,0*	*1,2*	18, 22, 14
		50 µg	*13,3*	*1,5*	*0,9*	12, 13, 15
	Eau / Water		*15,0*	*4,6*		20, 11, 14
	Untreated Control		*19,0*	*3,6*		20, 15, 22
TA1537	**ID-19/07701**	5000 µg	*22,0*	*2,6*	*1,6*	25, 21, 20
		1600 µg	*17,7*	*3,2*	*1,3*	19, 14, 20
		500 µg	*16,3*	*6,4*	*1,2*	9, 21, 19
		160 µg	*20,0*	*1,7*	*1,5*	21, 21, 18
		50 µg	*15,7*	*8,3*	*1,1*	9, 25, 13
	Eau / Water		*13,7*	*1,2*		13, 15, 13
	Untreated Control		*16,7*	*2,9*		15, 20, 15
TA98	**ID-19/07701**	5000 µg	*40,7*	*6,7*	*1,2*	35, 48, 39
		1600 µg	*28,7*	*10,1*	*0,8*	34, 35, 17
		500 µg	*33,3*	*8,0*	*1,0*	25, 34, 41
		160 µg	*30,7*	*4,0*	*0,9*	33, 26, 33
		50 µg	*43,7*	*7,6*	*1,3*	42, 52, 37
	Eau / Water		*34,7*	*2,1*		37, 33, 34
	Untreated Control		*28,3*	*5,1*		24, 27, 34
TA100	**A2A**	5 µg	*2365,7*	*136,9*	*11,3*	2267, 2522, 2308
TA102	**A2A**	25 µg	*3024,0*	*251,1*	*7,2*	2737, 3132, 3203
TA1535	**A2A**	5 µg	*141,7*	*4,5*	*9,4*	142, 137, 146
TA1537	**A2A**	5 µg	*221,7*	*12,9*	*16,2*	231, 227, 207
TA98	**A2A**	5 µg	*2738,0*	*144,9*	*79,0*	2587, 2751, 2876

Key to Positive Controls

A2A	2-AMINOANTHRACENE

8 Discussion

Test validation

All the criteria defined in paragraph 5.2 are met. This allows to validate the test.

Test item

Revertant analysis tables show that:

- No cytotoxic effect was observed.
- No concentration of the test item showed ratio R higher or equal at least to the double of the spontaneous rate of reversion for TA98, TA100 and TA102 strains and to the triple of the spontaneous rate of reversion for TA1535 and TA1537 strains, with and without metabolic activation.
- No dose response was observed, whatever the test system or conditions of the test.
- In addition, no sign of precipitate was observed.

9 Conclusion

Based on the result of this study, the test item **ALGYL® - LOT : 19 06 280, ID-19/07701 was found to be non mutagenic and non pro-mutagenic** under the test conditions.

10 Archive

The study folder (study plan and any amendment, report, raw data) will be stored in the IDEA Lab archive room for 10 years.

According to the GLP principles for the short term studies, the test item won't be archived, but will be stored 2 months after the end of study, or until its expiry date, in the Ames study room. After this period of time, it will be destroyed or sent back to the Sponsor according to his choice.

The reference item samples will be stored 10 years, or until their expiry date, in the Ames study room, in the storage condition described in the quality form FL REAC 04.

Annex

Historical data

Positive and negative control follow-up:

HISTORIQUE MANIPULATIONS : NOMBRE DE REVERTANTS SPONTANES SANS S9 Mix / *Spontaneous revertants without S9 Mix*

TA98		TA100		TA102		TA1535		TA1537	
Nb de valeurs / *Nb of values*	525,0	Nb de valeurs / *Nb of values*	548,0	Nb de valeurs / *Nb of values*	514,0	Nb de valeurs / *Nb of values*	518,0	Nb de valeurs / *Nb of values*	525,0
Moyenne / *Mean*	25,7	Moyenne / *Mean*	154,7	Moyenne / *Mean*	372,8	Moyenne / *Mean*	17,3	Moyenne / *Mean*	16,6
Ecart-type / *Std deviation*	3,6	Ecart-type / *Std deviation*	33,9	Ecart-type / *Std deviation*	34,3	Ecart-type / *Std deviation*	4,9	Ecart-type / *Std deviation*	3,7
Nb mini / *Minimal value*	16,0	Nb mini / *Minimal value*	49,7	Nb mini / *Minimal value*	275,3	Nb mini / *Minimal value*	9,0	Nb mini / *Minimal value*	6,7
Nb maxi / *Maximal value*	42,0	Nb maxi / *Maximal value*	243,3	Nb maxi / *Maximal value*	587,7	Nb maxi / *Maximal value*	38,0	Nb maxi / *Maximal value*	30,3

HISTORIQUE MANIPULATIONS : NOMBRE DE REVERTANTS SPONTANES + S9 Mix/ *Spontaneous revertants with S9 Mix*

TA98		TA100		TA102		TA1535		TA1537	
Nb de valeurs / *Nb of values*	527,0	Nb de valeurs / *Nb of values*	535,0	Nb de valeurs / *Nb of values*	502,0	Nb de valeurs / *Nb of values*	511,0	Nb de valeurs / *Nb of values*	515,0
Moyenne / *Mean*	33,8	Moyenne / *Mean*	171,1	Moyenne / *Mean*	425,0	Moyenne / *Mean*	17,6	Moyenne / *Mean*	20,3
Ecart-type / *Std deviation*	4,5	Ecart-type / *Std deviation*	35,1	Ecart-type / *Std deviation*	38,3	Ecart-type / *Std deviation*	4,9	Ecart-type / *Std deviation*	4,8
Nb mini / *Minimal value*	23,3	Nb mini / *Minimal value*	58,3	Nb mini / *Minimal value*	310,0	Nb mini / *Minimal value*	10,0	Nb mini / *Minimal value*	10,0
Nb maxi / *Maximal value*	56,7	Nb maxi / *Maximal value*	264,0	Nb maxi / *Maximal value*	685,3	Nb maxi / *Maximal value*	38,3	Nb maxi / *Maximal value*	39,0

HISTORIQUE MANIPULATIONS : CONTROLES POSITIFS - S9 Mix/ Positive controls without S9 Mix

TA98		TA100		TA102		TA1535		TA1537	
Nb de valeurs / *Nb of values*	205,0	Nb de valeurs / *Nb of values*	214,0	Nb de valeurs / *Nb of values*	200,0	Nb de valeurs / *Nb of values*	202,0	Nb de valeurs / *Nb of values*	205,0
Moyenne / *Mean*	1202,2	Moyenne / *Mean*	2039,4	Moyenne / *Mean*	2695,7	Moyenne / *Mean*	2129,1	Moyenne / *Mean*	388,0
Ecart-type / *Std deviation*	220,6	Ecart-type / *Std deviation*	235,2	Ecart-type / *Std deviation*	537,1	Ecart-type / *Std deviation*	201,7	Ecart-type / *Std deviation*	222,5
Nb mini / *Minimal value*	746,3	Nb mini / *Minimal value*	1431,7	Nb mini / *Minimal value*	1737,7	Nb mini / *Minimal value*	1551,7	Nb mini / *Minimal value*	117,7
Nb maxi / *Maximal value*	2125,0	Nb maxi / *Maximal value*	3329,7	Nb maxi / *Maximal value*	4020,0	Nb maxi / *Maximal value*	2803,3	Nb maxi / *Maximal value*	1451,7

HISTORIQUE MANIPULATIONS : CONTROLES POSITIFS + S9 Mix/ Positive controls with S9 Mix

TA98		TA100		TA102		TA1535		TA1537	
Nb de valeurs / *Nb of values*	204,0	Nb de valeurs / *Nb of values*	205,0	Nb de valeurs / *Nb of values*	194,0	Nb de valeurs / *Nb of values*	197,0	Nb de valeurs / *Nb of values*	199,0
Moyenne / *Mean*	2663,4	Moyenne / *Mean*	3051,3	Moyenne / *Mean*	3703,0	Moyenne / *Mean*	237,1	Moyenne / *Mean*	350,3
Ecart-type / *Std deviation*	961,3	Ecart-type / *Std deviation*	1018,5	Ecart-type / *Std deviation*	767,3	Ecart-type / *Std deviation*	71,3	Ecart-type / *Std deviation*	134,2
Nb mini / *Minimal value*	931,0	Nb mini / *Minimal value*	923,3	Nb mini / *Minimal value*	1955,7	Nb mini / *Minimal value*	115,0	Nb mini / *Minimal value*	61,7
Nb maxi / *Maximal value*	4855,3	Nb maxi / *Maximal value*	5424,0	Nb maxi / *Maximal value*	6035,3	Nb maxi / *Maximal value*	433,7	Nb maxi / *Maximal value*	725,3

Mise à jour du : 26/08/2019

Annex

Culture media

- Nutrient broth for the strain culture

Beef extract	10 g
Peptone	10 g
Sodium chloride	5 g
Water for analysis	1 000 ml

- Bottom agar
    - Vogel-Bonner medium (concentrated 50 times)

Magnesium sulfate heptahydrated ($MgSO_4.7H_2O$)	10 g
Citric acid monohydrated ($C_6H_8O_7.H_2O$)	100 g
Potassium hydrogeno phosphate (K_2HPO_4)	500 g
Sodium and ammonium hydrogeno phosphate tetrahydrated ($NaNH_4HPO_4.4H_2O$)	175 g
Water for analysis	670 ml

    - Glucose solution 400 g/l
    - Completed medium

Powder agar	15 g
Water for analysis	930 ml
Vogel-Bonner medium (concentrated 50 times)	20 ml
Glucose solution 400 g/l	50 ml

- Top agar for *Salmonella typhimurium* TA

Powder agar	6 g
Sodium chloride	5 g
Water for analysis	1 000 ml
L-histidine and D-biotin mix solution at 0.5 mmol/l	10%

Reagents

- Metabolic activation S9-Mix

Reagents used for preparation of S9-Mix are prepared according to the working instruction IL REAC 04.

	Final concentration
$MgCl_2$ (0.4 M) + KCl (1.65 M)	8 mM + 33 mM
Glucose 6 Phosphate (0.2 M)	5 mM
NADP (0.1 M),	4 mM
Phosphate buffer for S9-Mix (pH 7.4 – 0.2 M),	0.1 M
S9 fraction	10%

Annex

Certificate of analysis

ID-19/07701

CERTIFICATE OF ANALYSIS

ALGYL®

1. IDENTIFICATION OF PRODUCT AND COMPANY

Company: GELYMA S.A.S.
Parc d'Affaires Marseille Sud – Bâtiment C4
1 Boulevard de l'Océan - 13009 MARSEILLE - FRANCE
Phone : 33 4 96 14 09 82
Fax : 33 4 96 14 09 83

Trade name ALGYL®

CTFA /INCI :

water	7732-18-5	231-791-2
glycerin	56-81-5	200-289-5
Kappaphycus alvarezii /Gigartina stellata extract	/223751-69-7	
Corallina officinalis extract	89997-92-2	289-730-0
Preservative	none	

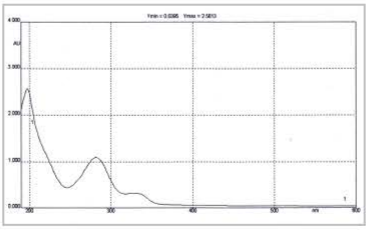
Batch product n°: 19 06 280 Manufact. Date :06-2019
Exp. date. 06-2020

2. ORIGIN OF PRODUCT

Origin of product association seaweeds extract and yeast extract.

2. SPECIFICATIONS

Aspect liquid
Colour yellow
Odour *sui generis*
pH 5.01
Relative density (20°C) 1.11
Dry residual (%) 41.42
Spectrum UV dilution 5% in water



Microbiology : Total germs < 100
Pathogens absence
Yeats moulds < 100
Preservatives none

Conservation temperature 15°C < store < 25°C

Remark: *To the best of our knowledge, the above data is correct. We can not accept liability for any errors in this document. The data on this certificate of analysis reflect the status at time of analysis.*

Annex

S9 certificate of analysis

TRINOVABIOCHEM

MOLTOX®
Molecular Toxicology, Inc.

POST MITOCHONDRIAL SUPERNATANT (S9)
QUALITY CONTROL & PRODUCTION CERTIFICATE

Animal Information
SPECIES: Rat
STRAIN: Sprague Dawley
SEX: Male
AGE: 5 – 6 weeks
WEIGHT: 175 – 199 g
TISSUE: Liver

Part Number Information
LOT NO.: 4008
PART NO.: 11-101
VOLUME: 1 & 5 mL
BUFFER: 0.15 M KCl
STORAGE: At or below -70°C

PREP: September 19, 2018
EXPIRY: September 19, 2020
INDUCING AGENT: Aroclor 1254, (Monsanto KL615), 500 mg/kg i.p.

REFERENCE: Maron, D & Ames, B., *Mutat Res*, **113**: 173, 1983. **For Research Purposes Only**

BIOCHEMISTRY:
- PROTEIN: 38.1 mg/ml — Assayed according to the method of Lowry et al., *JBC* **193**:265, 1951 using bovine serum albumin as the standard.

- ALKOXYRESORUFIN-0-DEALKYLASE ACTIVITIES

Activity	P450	Fold - Induction
BROD	2B1, 2B2	88.3
EROD	1A1, 1A2	151
MROD	1A1, 1A2	90.7
PROD	2B1, 2B2	40.1

Assays for ethoxyresorufin-0-deethylase (EROD), pentoxy-, benzyl- and methoxyresorufin-0-dealkylases (PROD, BROD, & MROD) were conducted using a modification of the methods of Burke, et al., *Biochem Pharm* **34**:3337, 1985. Fold-inductions were calculated as the ratio of the sample vs. uninduced specific activities (SA's). Control SA's (pmoles/min/mg protein) were 112.1, 93.2, 38.9, & 41.8 for BROD, EROD, MROD and PROD, respectively.

BIOASSAY:
- TEST FOR THE PRESENCE OF ADVENTITIOUS AGENTS
Samples of S-9 were assayed for the presence of contaminating microorganisms by plating 1.0 ml volumes on Nutrient Agar and Minimal Glucose (Vogel-Bonner E, supplemented with 0.05 mM L-histidine and D-biotin) media. Duplicate plates were read after 40 - 48 h incubation at 35 ± 2°C. The tested samples met acceptance criteria.

- PROMUTAGEN ACTIVATION

No. His+ Revertants	
TA98	TA1535
70	578

The ability of the sample to activate ethidium bromide (EtBr) and cyclophosphamide (CPA) to intermediates mutagenic to TA98 and TA1535, respectively, was determined according to Lesca, et al., *Mutation Res* **129**: 299, 1984. Data were expressed as revertants per µg EtBr or per mg CPA.

Dilutions of the sample S9, ranging from 0.2 – 10% in S9 mix, were tested for their ability to activate benzo(α)pyrene (BP) and 2-aminoanthracene (2-AA) to metabolites mutagenic to TA100. Assays were conducted as described by Maron & Ames, (*Mutat Res* **113**: 173, 1983).

	µl S9 per plate/number *his*+ revertants per plate					
Promutagen	0	1	5	10	20	50
BP (5 µg)	81	172	357	547	721	757
2-AA (2.5 µg)	89	464	1872	1943	1966	1938

Approved: Kathy Pickens 09/26/18

MOLECULAR TOXICOLOGY, INC. www.moltox.com (828) 264-9099

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B4002

TRINOVABIOCHEM

MOLTOX®
Molecular Toxicology, Inc.

POST MITOCHONDRIAL SUPERNATANT (S9)
QUALITY CONTROL & PRODUCTION CERTIFICATE

Animal Information
SPECIES: Rat
STRAIN: Sprague Dawley
SEX: Male
AGE: 5 – 6 weeks
WEIGHT: 175 – 199 g
TISSUE: Liver

Part Number Information
LOT NO.: 3974
PART NO.: 11-101
VOLUME: 1 & 2 mL
BUFFER: 0.15 M KCl
STORAGE: At or below -70°C

PREP: June 13, 2018
EXPIRY: June 13, 2020
INDUCING AGENT: Aroclor 1254, (Monsanto KL615), 500 mg/kg i.p.

REFERENCE: Maron, D & Ames, B., *Mutat Res*, **113**: 173, 1983. **For Research Purposes Only**

BIOCHEMISTRY:
- PROTEIN: 35.1 mg/ml

Assayed according to the method of Lowry et al., *JBC* **193**:265, 1951 using bovine serum albumin as the standard.

- ALKOXYRESORUFIN-0-DEALKYLASE ACTIVITIES

Activity	P450	Fold - Induction
BROD	2B1, 2B2	186.9
EROD	1A1, 1A2	386.9
MROD	1A1, 1A2	109.3
PROD	2B1, 2B2	116

Assays for ethoxyresorufin-0-deethylase (EROD), pentoxy-, benzyl- and methoxyresorufin-0-dealkylases (PROD, BROD, & MROD) were conducted using a modification of the methods of Burke, et al., *Biochem Pharm* **34**:3337, 1985. Fold-inductions were calculated as the ratio of the sample vs. uninduced specific activities (SA's). Control SA's (pmoles/min/mg protein) were 51.9, 30.4, 24.3, & 15.2 for BROD, EROD, MROD and PROD, respectively.

BIOASSAY:
- TEST FOR THE PRESENCE OF ADVENTITIOUS AGENTS

Samples of S-9 were assayed for the presence of contaminating microorganisms by plating 1.0 ml volumes on Nutrient Agar and Minimal Glucose (Vogel-Bonner E. supplemented with 0.05 mM L-histidine and D-biotin) media. Duplicate plates were read after 40 - 48 h incubation at 35 ± 2°C. The tested samples met acceptance criteria.

- PROMUTAGEN ACTIVATION

No. His+ Revertants	
TA98	TA1535
388	864

The ability of the sample to activate ethidium bromide (EtBr) and cyclophosphamide (CPA) to intermediates mutagenic to TA98 and TA1535, respectively, was determined according to Lesca, et al., *Mutation Res* **129**: 299, 1984. Data were expressed as revertants per µg EtBr or per mg CPA.

Dilutions of the sample S9, ranging from 0.2 – 10% in S9 mix, were tested for their ability to activate benzo(α)pyrene (BP) and 2-aminoanthracene (2-AA) to metabolites mutagenic to TA100. Assays were conducted as described by Maron & Ames, (*Mutat Res* **113**: 173, 1983).

	µl S9 per plate/number *his*⁺ revertants per plate					
Promutagen	0	1	5	10	20	50
BP (5 µg)	78	158	268	381	530	833
2-AA (2.5 µg)	94	383	1369	1781	1939	1674

Approved: Kathy Peterson 06/19/18

MOLECULAR TOXICOLOGY, INC. www.moltox.com (828) 264-9099

06 AOUT 2019

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RAPPORT D'ETUDE

Les résultats qui suivent ne s'appliquent qu'aux échantillons soumis au laboratoire et tels qu'ils sont définis dans le présent document. Les échantillons seront conservés dans nos locaux pendant une période de 2 mois à compter de la date figurant sur ce document. L'échantillon et les informations concernant l'échantillon ont été fournis par le client. Toutes les informations relatives à l'échantillon sont sous la responsabilité du client et n'ont pas été vérifiées par la société Eurofins ATS.

GELYMA
1 boulevard de l'Océan
Parc d'Affaires Marseille
Batiment C 4
13009 MARSEILLE

Le 10/01/2018

EVALUATION DE LA COMPATIBILITÉ CUTANEE D'UN INGREDIENT COSMETIQUE SOUS CONTRÔLE DERMATOLOGIQUE APRES APPLICATION UNIQUE SOUS PATCH OCCLUSIF PENDANT 48 HEURES SUR 20 SUJETS : *patch test*

Moniteur de l'étude : **Liliane PELLEGRINI, GELYMA**

N° du devis : **2017-51321-1**

Produit testé :

- Dénomination : ALGYL
- Référence client : WG GSCO / LOT 17 07 040
- N° d'échantillon : 639335
- Marque : -
- Type de produit : Ingrédient

Code des études : **028PT20V17 et 029PT20V17**

RESUME DE RAPPORT D'ETUDE

EVALUATION DE LA COMPATIBILITÉ CUTANEE D'UN INGREDIENT COSMETIQUE SOUS CONTRÔLE DERMATOLOGIQUE APRES APPLICATION UNIQUE SOUS PATCH OCCLUSIF PENDANT 48 HEURES SUR 20 SUJETS : *patch test*

- **Produit étudié :** ALGYL
- **Promoteur :** Liliane PELLEGRINI, GELYMA
- **Objectif de l'étude :** L'objectif de l'étude est d'apprécier la compatibilité locale épicutanée d'un ingrédient cosmétique, après application unique sur la peau du dos et sous patch occlusif, pendant 48h, chez des sujets adultes et sains.
- **Pertinence de l'étude :** Un patch test est pertinent pour évaluer la capacité du produit cosmétique à maintenir le corps humain en bon état.*
  **Conformément à la définition règlementaire européenne d'un produit cosmétique et au DECRET 2017-884 du 9 mai 2017.*
- **Investigateur :** Docteur Frédérique DURBEC, Dermatologue
  Docteur Géraldine TRIQUET, Dermatologue
- **Lieu de l'étude :** EUROFINS ATS
  505 rue Louis Berton
  CS 50550
  13594 Aix-en-Provence Cedex 3 - France
- **Dates de l'étude :** du 06/12/2017 au 08/12/2017 et du 18/12/2017 au 20/12/2017
- **Méthodologie :**

✓ ***Modalités d'application*** **:**

Zone d'application : dos
Quantité de produit : 0,02mL
Fréquence et durée : application unique pendant 48 heures.
Conditions d'application : ingrédient déposé dilué à 10% sous patch occlusif.

✓ ***Méthode d'évaluation*** **:**

L'observation clinique des effets provoqués est réalisée par le dermatologue investigateur, après le retrait du patch. La cotation clinique est donnée selon une échelle numérique déterminée, en fonction de l'intensité des phénomènes d'irritation observés (érythème, œdème, sécheresse, vésicule). Le score irritant moyen de l'ingrédient à l'essai est calculé en faisant la moyenne des cotations obtenues pour l'ensemble des sujets, permettant ainsi de classer l'ingrédient de « non irritant à très irritant ». L'évaluation se fait toujours par comparaison au témoin "négatif".

- **Population :** 25 sujets adultes, sains.
- **Résultat :** Le score irritant moyen de l'ingrédient est de 0,08.
- **Conclusion :**

Dans les conditions expérimentales de l'étude, l'ingrédient ALGYL référencé WG GSCO / LOT 17 07 040, peut être considéré comme non irritant du point de vue de sa compatibilité primaire cutanée.

AUTHENTICITE DES RESULTATS

L'étude faisant l'objet du présent rapport a été conduite sous ma responsabilité, en conformité avec le protocole expérimental, le plan qualité du laboratoire EUROFINS ATS, et dans le respect des bonnes pratiques cliniques.

Toutes les observations et données recueillies au cours de cet essai sont rapportées dans le présent rapport.

Je certifie avoir relu ce rapport et être en accord avec son contenu,

Chargée d'étude, Camille ZANKER

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1. OBJECTIF DE L'ETUDE

L'objectif de l'étude est d'apprécier la compatibilité locale épicutanée de l'ingrédient ALGYL sous contrôle dermatologique, après application unique sur la peau du dos et sous patch occlusif, pendant 48h, chez 20 sujets adultes.

2. PERTINENCE DE L'ETUDE

Un patch test est pertinent pour évaluer la capacité de l'ingrédient cosmétique à maintenir le corps humain en bon état.*

**Conformément à la définition règlementaire européenne d'un ingrédient cosmétique et au DECRET 2017-884 du 9 mai 2017.*

3. PRINCIPE

Le principe de l'étude est basé sur l'application unique de l'ingrédient à tester sur la peau du dos de sujets adultes. La quantité appliquée est :

- de 0,02 ml si le produit est liquide ou visqueux
- d'environ 0,02 g si le produit est solide,
- de 0,25 cm² (0,5 X 0,5 cm) si le produit est un tissu, papier, mouchoir.

Le produit est maintenu en contact avec la peau pendant 48 h sous patch occlusif.
Les produits sont testés purs ou dilués selon la catégorie à laquelle ils appartiennent ainsi que selon leurs modalités d'utilisation. En majorité, les produits sont testés purs. Les produits dits « rincés » sont testés dilués à 5%. Les produits détergents sont dilués à 1%.
Les produits hydrophiles sont dilués dans de l'eau déminéralisée, alors que les produits lipophiles sont dilués dans de l'huile minérale.
Les produits en poudre sont déposés purs dans la cupule du patch, puis une goutte d'huile minérale est ajoutée afin d'éviter une dispersion du produit lors de l'application.

L'observation clinique des effets provoqués est réalisée sous la supervision du dermatologue investigateur après le retrait du patch. L'évaluation est effectuée par comparaison avec un témoin "négatif", appliqué dans les mêmes conditions que le produit testé :

- si le produit est testé pur : patch seul vide,
- si le produit est testé dilué : patch contenant 0,02 ml du solvant utilisé (eau déminéralisée ou huile minérale).

La cotation clinique est donnée selon une échelle numérique déterminée, en fonction de l'intensité des phénomènes d'irritation observés (érythème, œdème, sécheresse/ desquamation, vésicule).
L'Indice d'Irritation Moyen (ou Irritation Primaire Cutanée) est calculé en faisant la moyenne des cotations obtenues pour l'ensemble des sujets.

4. REGLEMENTATION, CONFIDENTALITE et ARCHIVAGE

4.1. Références législatives et règlementaires

L'étude a été réalisée dans l'esprit des principes généraux d'éthique médicale en recherche clinique issus de la déclaration d'Helsinki (juin 1964) et ses amendements successifs, ainsi que les recommandations internationales relatives aux bonnes pratiques cliniques pour la conduite des essais cliniques pour les médicaments ICH E6(R2) du 09/11/2016 (CPMP/ICH/135/95).

L'étude a été conçue dans l'esprit des « lignes directrices pour l'évaluation de peau compatibilité of potentiellement Irritant Cosmetic Ingredients », COLIPA, édition de 1997 et les « lignes directrices pour l'évaluation de l'efficacité des produits cosmétiques », COLIPA, mai 2008.

Tel que décrit dans le Décret n° 2017-884 du 9 mai 2017, cette étude n'est pas considérée comme une « Recherche Impliquant la Personne Humaine » car elle n'a pas été réalisée en vue du développement des connaissances médicales ou biologiques. Elle a été réalisée dans le but d'évaluer un/plusieurs produits cosmétiques pour leur capacité à nettoyer, parfumer, changer l'aspect, protéger, maintenir en bon état le corps humain ou corriger les odeurs corporelles, conformément à la définition du produit cosmétique mentionnée à l'article L.5131-1 du règlement européen.

Les exigences éthiques, nécessaires dans le déroulement des études sur l'homme, sont respectées :

- Les sujets sont sélectionnés selon des critères d'inclusion et de non inclusion (voir chapitre 4)
- Tous les sujets sont informés du but et de la nature de l'étude, des risques prévisibles qu'ils prennent en participant à l'étude et donnent leur consentement, libre et éclairé, avant le début de l'étude.
- Avant que les sujets ne soient exposés aux produits à tester, des informations minimales concernant la sécurité des produits sont demandées au promoteur.
- Toutes les précautions sont prises pour éviter de causer des réactions cutanées excessives ou des effets néfastes sur la santé des sujets, durant l'étude.
- Des procédures de sécurité sont mises en place, dans le cas de réactions néfastes et inacceptables.
- Les sujets sont indemnisés, en compensation du temps passé, des inconvénients dus à l'étude.

4.2. Confidentialité

Les renseignements concernant l'état de santé des sujets, recueillis lors de leur admission définitive dans la base de données de sujets d'EUROFINS ATS et nécessaires au moment de leur recrutement et leur sélection dans le cadre des études, sont strictement confidentiels et sont soumis à la règle du secret médical suivant l'article 378 du Code Pénal et au Code de déontologie Médical (décret du 18 juin 1979, articles 11, 12 et 13). L'anonymat des sujets est respecté dans le cadre des études menées dans nos laboratoires. Cependant, chaque sujet participant à l'essai peut être facilement identifié, par le Médecin Investigateur et les personnes travaillant avec lui, grâce à son code personnel de sujet.
Conformément à l'article R. 5121-13 du code de la santé publique, la nature des produits étudiés, les essais, les personnes qui s'y prêtent et les résultats obtenus sont d'ordre strictement confidentiel et le secret est respecté par le Médecin Investigateur et par toutes les personnes appelées à travailler avec lui.
EUROFINS ATS s'engage à ne pas divulguer l'ensemble des données et résultats recueillis lors d'une étude.

4.3. Archivage

Toutes les données relatives à l'étude, sont conservées dans les archives d'EUROFINS ATS (505 rue Louis Berton – CS 50550 - 13594 Aix-en-Provence Cedex 3 - France), puis chez un prestataire, Société Générale d'Archives (ZI Les Estroublans - 49 boulevard de l'Europe – 13127 VITROLLES) pendant 10 ans. A la fin de la période indiquée, le moniteur de l'étude devra préciser si toutes les données liées à l'étude doivent être détruites ou lui être restituées. Une prolongation de la durée d'archivage peut être envisagée, à la charge du moniteur de l'étude.

5. POPULATION ETUDIEE

5.1. Nombre de sujets

L'ingrédient a été testé sur 27 sujets.

5.2. Caractéristiques de la population étudiée

Les sujets sont des personnes issues du panel de sujets d'Eurofins ATS. Tous les panélistes inscrits dans la base de données ont été recrutés selon les critères d'inclusion et d'exclusion présentés dans le paragraphe 5.4 et 5.5 et ont subi, avant leur admission définitive dans la base de données, un examen dermatologique avec le médecin recruteur de la société.

5.3. Recrutement, sélection et admission définitive des sujets pour une étude

A partir de la base de données de sujets, les panélistes répondant aux critères d'inclusion sont convoqués puis définitivement admis dans l'étude au terme d'un entretien préalable.
Lors de cet entretien préalable, les informations suivantes sont expliquées aux sujets : l'objectif, le protocole et le planning de l'étude, les modalités d'indemnité, les bénéfices éventuellement attendus, les contraintes liées à l'étude, les risques prévisibles et les conséquences en cas d'arrêt de l'essai avant son terme.
Les sujets doivent alors :

- lire et signer un formulaire de consentement libre, éclairé et exprès,
- remplir un auto-questionnaire médical de pré-étude, afin de s'assurer que les critères d'inclusion et de non inclusion sont bien respectés, avant leur admission définitive dans l'étude.

5.4. Critères d'inclusion

Dans cette étude, ont été inclus les sujets répondant aux critères suivants :

- Age : 18-70 ans,
- Genre : féminin et/ou masculin,
- Couverture sociale : les sujets doivent être affiliés à un régime de sécurité sociale.
- Indemnes de toutes lésions dermatologiques sur le site étudié,
- Volontaires pouvant justifier d'un domicile fixe,
- Compréhension de la langue française et sujets capables de comprendre les exigences de l'essai,
- Sujets répondant aux critères spécifiques de l'étude en cours (par exemple : peau sensible).

5.5. Critères de non inclusion

Les sujets présentant un des critères suivants n'ont pas été inclus dans cette étude :

- Sujets ne présentant pas les critères d'inclusion précités,
- Sujets en période d'exclusion entre deux essais,
- Les mineurs ou majeurs protégés par la loi et les personnes admises dans un établissement sanitaire ou social à d'autres fins que la recherche… (article L209-6)
- Les personnes privées de liberté par décision judiciaire ou administrative, malades en situation d'urgence (article L209-5),
- Femme enceinte et/ou allaitante,
- Sujets présentant une pathologie cutanée évolutive, une allergie de contact connue liée aux ingrédients du produit à tester,
- Sujets ayant refusé de donner leur accord et refusant de signer le consentement libre et éclairé,
- Sujets sous traitement antihistaminique, sous traitement anti-inflammatoire, sous corticoïdes, sous traitement désensibilisant et/ou tout traitement pouvant interférer avec le métabolisme cutané,
- Sujets présentant une peau récemment insolée ou ayant subi des séances de PUVA thérapie.

5.6. Interdictions et restrictions

Pendant toute la durée de l'étude, il est demandé aux sujets :

- De ne mettre aucun produit, y compris de l'eau sur la zone des patchs,
- De ne pas prendre de bain, ni de s'exposer aux UV,
- D'éviter une activité sportive trop intense qui augmente la sudation et qui risque de provoquer le décollement du patch,
- De ne pas prendre d'antihistaminiques, de corticoïdes, d'anti-inflammatoires et tout traitement réduisant ou inhibant les réactions inflammatoires ou allergiques ou interférant avec le métabolisme cutané.

5.7. Retrait des sujets

Un sujet peut être exclu de l'étude pour les raisons suivantes :

- Il ne suit plus les exigences et les contraintes de l'étude, expliquées lors de la signature du consentement.
- Il souffre d'une maladie développée pendant l'étude qui peut interférer avec les objectifs de l'étude,
- Il ne souhaite plus participer à l'étude.

6. INGREDIENT A L'ESSAI

- ✓ Dénomination de l'ingrédient : ALGYL
- ✓ Référence client : WG GSCO / LOT 17 07 040
- ✓ Numéro d'échantillon : 639335
- ✓ Type de produit : Ingrédient
- ✓ Conditions de stockage : à l'abri de la chaleur et de la lumière.

Un échantillon de l'ingrédient testé est conservé dans les laboratoires EUROFINS ATS, pendant 2 mois après la fin de l'étude. Passé cette date et sauf avis contraire du moniteur de l'étude, l'ingrédient sera détruit.

7. ETUDE CLINIQUE

7.1. Description du matériel utilisé

Matériel utilisé pour le patch occlusif : patch test IQ ULTRA, composé d'une cupule de 68 mm² en mousse plastique de polyéthylène avec un fond en papier filtre, destinée à recevoir le produit à l'essai, le tout fixé sur une bande adhésive en non tissé, hypoallergénique.

Matériel utilisé pour le patch semi-occlusif : patch test CURATEST, composé d'une cupule plastique de 0,64 cm² avec un fond de papier filtre destinée à recevoir le produit à l'essai, le tout fixé à une bande adhésive en non tissé.

7.2. Modalités d'application

Zones d'application : dos
Quantité de produit : 0,02mL
Fréquence et durée : application unique pendant 48 heures.
Conditions d'application : ingrédient déposé dilué à 10% sous patch occlusif.

La surface sur laquelle est déposé le patch est préalablement nettoyée avec de l'eau déminéralisée, puis séchée avec du papier en ouate de cellulose.

Les patchs sont déposés au niveau du dos. Un examen spécifique de la zone mise en contact avec le patch est réalisé juste avant le début de l'étude, afin d'appliquer le produit sur une surface exempte de traces macroscopiques d'irritation, de cicatrices ou toutes anomalies pouvant interférer avec la lecture des résultats.

Parallèlement à l'application du produit étudié, un patch témoin « négatif » est appliqué.

Les patchs ainsi préparés sont laissés en contact 48 heures.

7.3. Observation et examen clinique

Le retrait des patchs est réalisé par le technicien et la lecture est réalisée par le dermatologue investigateur 30 minutes après le retrait. L'analyse des cotations des réactions épidermiques est descriptive.

Les réactions cutanées éventuelles sont évaluées, pour chaque sujet, selon l'échelle suivante :

ERYTHEME :	
Absence	0
Erythème léger, à peine perceptible	1
Rougeur modérée et uniforme	2
Rougeur importante et uniforme	3
SECHERESSE / DESQUAMATION :	
Pas de sécheresse	0
Sec avec desquamation, aspect lisse et tendu, desquamation légère et fine	1
Desquamation modérée	2
Desquamation sévère avec de larges écailles	3
OEDEME	
Absence	0
Léger	1
Net	2
Important	3
VESICULE	
Absence	0
Léger	1
Net	2
Important	3

Les résultats obtenus pour le produit sont comparés à ceux obtenus sur la zone témoin. L'Indice d'Irritation Primaire est calculé en faisant la moyenne des cotations obtenues sur l'ensemble des panélistes, selon la formule suivante :

$$\frac{\left[\left(\sum \text{cotations T48h}\right) \text{ vol 1 à vol n}\right] / \text{nombre de lectures}}{\text{nombre de volontaires}}$$

7.4. Analyse des données et interprétation des résultats

L'interprétation des résultats se fait en se basant sur l'indice d'irritation obtenu, le nombre de sujets ayant réagi et l'importance des réactions, les conditions expérimentales adoptées et le type de produit étudié.

La classification du potentiel irritant est déterminée en fonction du score obtenu, selon le tableau 1.

Tableau 1 : classification du potentiel irritant

Score moyen	Classification
[0 – 0,08]	Non irritant
]0,08 – 0,16]	Très légèrement irritant
]0,16 – 0,56]	Légèrement irritant
]0,56 – 1]	Modérément irritant
]1 – 1,6]	Irritant
> 1,6	Très irritant

8. RESULTATS

8.1. Description de la population

Cette étude s'est déroulée du 06/12/2017 au 08/12/2017 et du 18/12/2017 au 20/12/2017 et inclut 27 sujets adultes, sains, dont les caractéristiques sont présentées dans le tableau 2.

Tableau 2 : caractéristiques des sujets

N° inclusion	Code vol	Genre	Age (ans)	Sensibilité de la peau du corps	Evènements survenus lors de l'étude
13	HAOGI	M	67	Non sensible	-
14	HAOAN	F	66	Non sensible	-
15	HANSA	F	29	Non sensible	-
16	HERVI1	F	32	Non sensible	Réaction témoin
17	LAFBE1	M	68	Non sensible	-
18	MAIAN	F	65	Non sensible	-
19	PISEL	F	67	Non sensible	-
20	ANGMA1	F	62	Non sensible	-
21	STOPA	F	67	Non sensible	-
22	STOJE	M	67	Non sensible	-
23	LEOAN1	M	65	Non sensible	-
24	CAURA	M	65	Non sensible	-
26	MOUVA	F	49	Non sensible	-
27	ROCME1	F	26	Non sensible	Réaction témoin
1’	DUBSY	F	50	Non sensible	-
2’	CARYV	F	68	Non sensible	-
3’	POISA	F	51	Non sensible	-
4’	CAMPA	F	44	Non sensible	-
5’	DIBVE	F	61	Non sensible	-
6’	NAPMI	F	58	Non sensible	-
7’	CARMA	F	59	Non sensible	-
8’	BAUMA3	F	65	Non sensible	-
9’	NOEPI	M	70	Non sensible	-
10’	BONCH1	F	67	Non sensible	-
11’	QUETH	M	52	Non sensible	-
12’	ROMLE	M	27	Non sensible	-
25’	LECJA	F	28	Non sensible	-
Moyenne			**55**		

Aucun des sujets sélectionnés ne prenait de traitement contre-indiqué avec l’étude.

8.2. Sorties d'étude

27 sujets ont été inclus dans cette étude.
Aucune sortie d'étude n'a été effectuée.

8.3. Analyses des résultats

Le tableau 3 présente les résultats obtenus pour chaque sujet ainsi que le score d'irritation correspondant.
Les sujets n°16 et n°27 ont réagi sur le site témoin. Leur résultats ne sont donc pas pris en compte.
Au final, les résultats sont rendus sur 25 sujets.

Tableau 3 : résultats

Code études : 028PT20V17 et 029PT20V17

Produits	**5 et 14**	**Nbre lecture :**	**1**
Code	**639335**	**Nbre de vol :**	**25**
VOL	**CODE VOL**	**Total lecture 48h**	**Total irritation / nbre lecture**
13	**HAOGI**	2	2
14	**HAOAN**	0	0
15	**HANSA**	0	0
17	**LAFBE1**	0	0
18	**MAIAN**	0	0
19	**PISEL**	0	0
20	**ANGMA1**	0	0
21	**STOPA**	0	0
22	**STOJE**	0	0
23	**LEOAN1**	0	0
24	**CAURA**	0	0
26	**MOUVA**	0	0
1	**DUBSY**	0	0
2	**CARYV**	0	0
3	**POISA**	0	0
4	**CAMPA**	0	0
5	**DIBVE**	0	0
7	**CARMA**	0	0
8	**BAUMA3**	0	0
9	**NOEPI**	0	0
10	**BONCH1**	0	0
11	**QUETH**	0	0
12	**ROMLE**	0	0
25	**LECJA**	0	0
SCORE D'IRRITATION			**0,08**
CLASSIFICATION			**très légèrement irritant**

Après 48 heures d'application, un érythème léger associé à un œdème léger sur un sujet a été noté par le dermatologue investigateur, sur le site traité par l'ingrédient ALGYL.

L'indice d'irritation moyen obtenu est égal à 0,08.

9. CONCLUSION

Dans les conditions expérimentales retenues, après application unique de 0,02mL d'ingrédient dilué à 10%, sous patch occlusif pendant 48 heures, chez 25 sujets adultes sains et selon le barème adopté pour l'interprétation des résultats, l'ingrédient ALGYL, référencé WG GSCO/ LOT 17 07 040, peut être considéré comme non irritant du point de vue de sa compatibilité primaire cutanée.

STUDY SUMMARY

ASSESSMENT OF THE SKIN COMPATIBILITY OF A COSMETIC RAW MATERIAL UNDER DERMATOLOGICAL CONTROL AFTER A SINGLE APPLICATION UNDER OCCLUDED PATCH DURING 48H ON 20 SUBJECTS: *patch test*

- **Product tested:** ALGYL
- **Promotor:** Liliane PELLEGRINI, GELYMA
- **Study objective:** Assessment of the skin local compatibility under dermatological control of the studied product after an epicutaneous test performed in occluded conditions, during 48 hours, on healthy adult subjects.
- **Study relevance:** A patch test is relevant to assess the ability of the product to keep the human body in good condition.*
  ** In accordance with the European regulatory definition of a cosmetic product and DECRET 2017-884 of May 9th, 2017.*
- **Investigators:** Frédérique DURBEC, M.D. Dermatologist
  Géraldine TRIQUET, M.D. Dermatologist
- **Place of the study:** EUROFINS ATS
  505 rue Louis Berton
  CS 50550
  13594 Aix-en-Provence Cedex 3 - France
- **Dates of study:** from 06/12/2017 to 08/12/2017 and from 18/12/2017 to 20/12/2017
- **Method:**

✓ ***Application:***

Area: on the back
Quantity of product: 0.02 mL
Frequency and duration: only one application during 48 hours
Conditions of application: diluted at 10% raw material under occluded patch.

✓ ***Assessment method:***

The clinical observation after the removal of the patch was performed by the dermatologist investigator. The quantification of the skin irritation is given through a numeric scale (erythema, oedema, dryness/desquamation, vesicle). The average irritant score of the product to be tested is calculated from the average of the quotations obtained for each subject, allowing to rank the product from "non irritant to very irritant". The assessment is always made by comparison with the "negative" control.

- **Panel:** 25 healthy adult subjects.
- **Result:** The average irritant score of the raw material is 0.08.
- **Conclusion:**

According to the experimental conditions of the study, the ALGYL raw material, referenced WG GSCO / BATCH 17 07 040, can be considered as non irritant regarding its primary skin compatibility.

ANNEXE 1 : Liste des personnes ayant participé à la réalisation de l'étude

Investigateurs :
Noms : Docteur Frédérique DURBEC, Dermatologue
Docteur Géraldine TRIQUET, Dermatologue
Adresse : 505 rue Louis Berton – CS 50550 - 13594 Aix-en-Provence Cedex 3 - France
Téléphone : +33 (0)4 42 39 30 92

Expérimentateur :
Noms : Aurélie PEREZ et Diane CAILLOZ
Adresse : 505 rue Louis Berton – CS 50550 - 13594 Aix-en-Provence Cedex 3 - France

Chargée d'étude :
Nom : Camille ZANKER
Adresse : 505 rue Louis Berton – CS 50550 - 13594 Aix-en-Provence Cedex 3 - France
Téléphone : +33 (0)4 42 37 14 25

ANNEXE 2 : Authenticité des résultats

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AUTHENTICITE DES RESULTATS

RESULTS AUTHENTICITY

L'étude **028PT20V17** a été conduite en conformité avec le protocole expérimental, le plan qualité du laboratoire EUROFINS ATS et dans le respect des bonnes pratiques cliniques.

The study **028PT20V17** *was carried out in accordance with the experimental protocol, the quality plan of EUROFINS ATS laboratory and follows the good clinical practices.*

MEDECIN INVESTIGATEUR / *DOCTOR*
Dermatologue / *Dermatologist*

Date, signature

Dr Frédérique DURBEC

08 DEC. 2017

TECHNICIEN / *TECHNICIAN*

Aurélie PEREZ

Date, signature

8/12/2017

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AUTHENTICITE DES RESULTATS

RESULTS AUTHENTICITY

L'étude **029PT20V17** a été conduite en conformité avec le protocole expérimental, le plan qualité du laboratoire EUROFINS ATS et dans le respect des bonnes pratiques cliniques.

The study **029PT20V17** *was carried out in accordance with the experimental protocol, the quality plan of EUROFINS ATS laboratory and follows the good clinical practices.*

MEDECIN INVESTIGATEUR / *DOCTOR*
Dermatologue / *Dermatologist*

Date, signature

Dr Geraldine TRIQUET

20/12/17

TECHNICIEN / *TECHNICIAN*

Date, signature
20/12/17

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RAPPORT D'ETUDE

Les résultats qui suivent ne s'appliquent qu'aux échantillons soumis au laboratoire et tels qu'ils sont définis dans le présent document. Les échantillons seront conservés pendant une période d'au moins 2 mois à compter de la date figurant sur ce document. L'échantillon et les informations concernant l'échantillon ont été fournis par le client. Toutes les informations relatives à l'échantillon sont sous la responsabilité du client et n'ont pas été vérifiées par la société Eurofins ATS.

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13009 MARSEILLE
FRANCE

RAPPORT D'ANALYSES

Promoteur de l'étude : Mme Liliane PELLEGRINI

N° de devis EUROFINS ATS : 2017 / 51321

Produit testé :

- Dénomination : ALGYL - Référence : WG GSCO - Lot : 17 07 040
- N° d'échantillon ATS : 639335
- Marque : -

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Il comporte 13 pages.

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EVIC france

REFERENCES ETUDE/ELEMENT D'ESSAI : **B17 1689 / 17-3330**

REFERENCE ETUDE EUROFINS ATS : **P879994 / E639335**

DONNEUR D'ORDRE : GELYMA
1 boulevard de l'Océan Parc d'Affaires
Marseille Batiment C 4
13009 MARSEILLE
FRANCE

ELEMENT D'ESSAI : ALGYL - Référence : WG GSCO - Lot : 17 07 040

EVALUATION DU POTENTIEL IRRITANT D'UN ELEMENT D'ESSAI PAR APPLICATION SUR LA MEMBRANE CHORIO-ALLANTOÏDIENNE DE L'ŒUF DE POULE EMBRYONNE

-HET-CAM-

Rapport Final

12 pages dans ce rapport

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EVALUATION DU POTENTIEL IRRITANT D'UN ELEMENT D'ESSAI PAR APPLICATION SUR LA MEMBRANE CHORIO-ALLANTOÏDIENNE DE L'ŒUF DE POULE EMBRYONNE - HET-CAM -

ASSESSMENT OF THE IRRITANT POTENTIAL OF A TEST ITEM AFTER APPLICATION TO THE EMBRYONIC HEN'S EGG CHORIOALLANTOIC MEMBRANE - HET-CAM -

RESUME/*SUMMARY*

- **PRINCIPE DE L'ETUDE/*PRINCIPLE OF THE STUDY***

L'étude a été basée sur l'observation, par une personne qualifiée, des effets irritants (hyperhémie, hémorragie, coagulation) pouvant survenir dans les cinq minutes suivant le dépôt d'un élément d'essai sur la membrane chorio-allantoïdienne (MCA) d'œufs de poule embryonnés au dixième jour d'incubation.
Le potentiel irritant a été scoré selon une échelle allant de 0 à 21. L'élément d'essai a été classé dans l'une des catégories définies en fonction du score moyen obtenu.

The study was based on the observation, by a trained person, of the irritant effects (hyperhemia, haemorrhage and coagulation) occurring during the five minutes after application of test item to the chorioallantoic membrane (CAM) of embryonic hen's eggs on the tenth day of incubation.
The irritant potential was scored according to a scale from 0 to 21. The test item was classified in one of the categories defined according to the mean score obtained.

Score moyen/*Mean Score* (Scm/*MSc*)	**Classification/*Classification***
Scm/*MSc* < 1	Pratiquement non irritant/*Practically non irritant*
1 ≤ Scm/*MSc* < 5	Faiblement irritant/ *Slightly irritant*
5 ≤ Scm/*MSc* < 9	Modérément irritant/*Moderately irritant*
Scm/*MSc* ≥ 9	Irritant/*Irritant*

- **DATE(S) DE DEBUT ET DE FIN D'EXPERIMENTATION / *EXPERIMENTAL STARTING DATE AND EXPERIMENTAL COMPLETION DATE*** : 04 décembre 2017 */ December 04, 2017*

- **RESULTATS/*RESULTS* :**

Elément d'essai *Test item*	**Concentration testée** *Tested concentration*	**Score moyen sur 4 œufs ± écart type** *Mean score on 4 eggs ±standard deviation*	**Classification** *Classification*	**Comparaison par rapport à des éléments d'essai appartenant à la même catégorie** *Comparison with test items belonging to the same category*
ALGYL - Référence : WG GSCO - Lot : 17 07 040	Dilué à 10% dans l'eau p.p.i. / *Diluted at 10% with water for injection*	1.3 ± 2.5	Faiblement irritant/ *Slightly irritant*	pas de comparaison disponible / *no available comparison*

I. OBJECTIF ET PRINCIPE DE L'ETUDE

Il a été demandé par le donneur d'ordre, l'évaluation semi-quantitative du potentiel irritant de l'élément d'essai, **ALGYL - Référence : WG GSCO - Lot : 17 07 040**, par une méthode alternative à l'expérimentation animale après application sur la membrane chorio-allantoïdienne de l'œuf de poule embryonné.

L'étude a été basée sur l'observation, par une personne qualifiée, des effets irritants (hyperhémie, hémorragie, coagulation) pouvant survenir dans les cinq minutes suivant le dépôt de l'élément d'essai sur la membrane chorio-allantoïdienne (MCA) d'œufs de poule embryonnés, au dixième jour d'incubation.

La technique utilisée a été adaptée de celle décrite par Luepke N.P. et Kemper F.H. (The Het-Cam test : « An alternative to the Draize eye test ». Food Chem. Toxicol. 1986, 24, n° 6/7, 495-496).
Cette méthode a suivi celle publiée au Journal Officiel de la République Française du 26 décembre 1996 à l'exception :
- du poids des œufs (entre 40 et 75 g au lieu de entre 50 et 65 g)
- de la destruction des embryons par refroidissement rapide (-20°C) au lieu d'une injection au pentobarbital.

Ces déviations, au texte du Journal Officiel de la République Française du 26/12/1996 n'ont pas eu d'impact sur la validité de l'étude.

Cette méthode est une alternative à l'expérimentation animale entrant dans une batterie de tests qui concourent à l'évaluation du potentiel irritant oculaire des éléments d'essai (notamment à base de tensioactifs).

II. INSTALLATION D'ESSAI ET EQUIPE TECHNIQUE

II.1. Installation d'Essai et équipe technique

EUROFINS- Evic France – Division Bio
122 rue Croix de Seguey
33000 Bordeaux

Tél : 05 56 95 59 95

Directeur de l'étude : Sarah JULIENNE

Techniciens responsables : Gaëlle BRINGAUD, Julie CHARMETANT, Sarah JULIENNE

II.2. Agréments de l'Installation d'Essai

L'étude a été entièrement réalisée dans les locaux de la Division Technique Bio de la Société EUROFINS- Evic France, à Bordeaux.
La vérification de la conformité aux Bonnes Pratiques de Laboratoire a été réalisée régulièrement par des autorités françaises de contrôle selon la nature de l'élément d'essai.

III. RECUEIL DES DONNEES

Toutes les données recueillies au cours de l'étude ont été consignées par le technicien responsable de l'étude, sur les documents réservés à cet effet.

Chaque page de ces documents a été paraphée et datée par le technicien responsable de l'étude.
Toute donnée manquante et toute correction ont été justifiées, paraphées et datées.

En fin d'étude, les documents de travail ont été archivés avec le rapport final et seront conservés pendant 10 ans dans une salle d'archives validée par le service Assurance Qualité de l'Installation d'Essai.
Cette salle d'archives est située chez un prestataire qui n'est pas soumis au champ d'application des BPL, mais qui garantit la qualité et l'intégrité des documents et des données de l'étude, et qui fait l'objet d'audits réguliers par le service Assurance Qualité.

A l'issue de cette période, l'Installation d'Essai définira avec le donneur d'ordre, la poursuite de l'archivage, la restitution des données ou leur destruction.

IV. ASSURANCE QUALITE

Le Service Assurance Qualité a vérifié par des audits réguliers, le respect du plan d'étude et des procédures de travail relatives à ce type d'étude.

Des audits portant sur l'Installation d'Essai ont été réalisés annuellement. Ces audits ont permis de vérifier que l'Installation d'Essai répondait aux exigences des autorités.

Des contrôles ont été effectués par les différentes personnes intervenant depuis la fin de l'expérimentation jusqu'à l'envoi du rapport final. Ces différents contrôles ont été formalisés à travers la documentation d'une fiche d'autocontrôle présente dans le dossier.
Toutes les données expérimentales et le rapport final ont fait l'objet d'un audit par le personnel du Service Qualité.

V. MODIFICATIONS AU PLAN D'ETUDE

Dans cette étude aucune modification au plan d'étude PEG.HC.06/17(12) n'a fait l'objet d'un amendement.

VI. ELEMENT D'ESSAI

VI.1. Référence de l'élément d'essai

Identification EUROFINS- Evic France	: 17-3330
Date de réception	: 29 novembre 2017
Identification	: ALGYL - Référence : WG GSCO - Lot : 17 07 040
Catégorie de l'élément d'essai	: ingrédient cosmétique
Description/Aspect	: liquide transparent jaune
Conditions de stockage	: à température ambiante et à l'abri de la lumière dans un local spécialement aménagé à cet effet
Particularité	: aucune
Quantité transmise par le donneur d'ordre	: 1 flacon en plastique de 30 ml
Date de péremption	: janvier 2018
Autre information fournie	: formule

Stable dans les conditions de stockage et d'essai.

VI.2. Stockage

L'élément d'essai ou le reste de l'élément d'essai a été stocké dans son conditionnement d'envoi, dans les locaux réservés à cet effet.

VII. SYSTEME D'ESSAI

Œufs de poule EOPS embryonnés de souche White Leghorn d'un poids supérieur ou égal à 40 g et inférieur ou égal à 75 g, fournis par : INRA Plate-forme d'infectiologie expérimentale (PFIE) - Zone volailles-lapins EOPS - 37380 NOUZILLY.

VII.1. Réception des œufs

A réception, les œufs ont été observés un à un. Les œufs fêlés ou cassés ont été éliminés.
Les oeufs ont été alors pesés. Les oeufs dont le poids n'était pas dans la norme fixée ont été éliminés.
Les œufs ont été identifiés par un numéro de lot (semaine/année) qui a été attribué aux œufs à réception.

Les œufs retenus ont été identifiés individuellement par le jour de mise en couveuse et ont été placés dans une enceinte thermostatée à 12 ± 1°C, à l'abri de la lumière, pendant au moins 24 heures avant la mise en couveuse.

VII.2. Mise en couveuse

Les œufs ont été placés dans une couveuse dans des conditions contrôlées de température (37,8 ± 1°C) et d'humidité relative (50 à 60 %).

Au cours de leur période d'incubation de 10 jours, les œufs ont été placés dans une couveuse à plateaux oscillants permettant un balancement automatique. Ils ont été placés en position verticale (poche d'air en haut).

Une déviation d'hygrométrie (49 % relevé au lieu de 50 % toléré) a été observée pendant l'incubation. Cette déviation a été jugée sans impact sur les résultats de l'étude, par le Directeur d'étude.

VIII. DATES DE L'ETUDE

Date de début d'étude (signature du complément spécifique au plan d'étude): 04 décembre 2017

Date de début d'expérimentation : 04 décembre 2017

Date de fin d'expérimentation : 04 décembre 2017

IX. MODE OPERATOIRE

IX.1. Préparation de l'élément d'essai

L'élément d'essai a été testé dilué à 10% dans l'eau p.p.i.

L'opération de dilution a été faite extemporanément en poids/poids dans l'eau pour préparation injectable (Cooper batch 19KM14GA) avec une balance de précision.

Aspect de l'élément d'essai après dilution : liquide incolore transparent

La dilution de l'élément d'essai a été amenée à 37 ± 1°C et a été homogénéisée à l'aide d'un vortex avant utilisation.

IX.2. Chronologie expérimentale

IX.2.1. Préparation des œufs

Les différentes étapes de l'étude ont été enchaînées rapidement sous un éclairage d'une intensité constante ne dégageant pas trop de chaleur pour ne pas dessécher la membrane chorio-allantoïdienne.

Au 10ème jour d'incubation, les œufs ont été sortis de l'incubateur et ont été mirés sous éclairage. Les œufs défectueux (image ne correspondant pas au stade de développement attendu) ont été éliminés et les œufs sélectionnés ont été posés sur le support « poche d'air » vers le haut.

La coquille de chaque œuf sélectionné a été percée (à l'aide d'une aiguille lancéolée), ouverte et découpée (à l'aide d'une pince et d'une paire de ciseaux à bouts ronds) au niveau de la poche d'air et jusqu'aux limites de la membrane coquillière.

Toute la surface de la membrane coquillière a été alors humidifiée avec une solution de chlorure de sodium à 0,9 %, tiédie à 37 ± 1°C (bain-marie). L'excès de solution de chlorure de sodium à 0,9 % a été ensuite éliminé par inclinaison de l'œuf et la membrane coquillière a été décollée délicatement avec une pince afin de découvrir la MCA sous-jacente.

Tout œuf dont la membrane chorio-allantoïdienne est apparue abîmée (déchirure, présence de traces d'hémorragie ou toute autre lésion) a été immédiatement rejeté.

IX.2.2. Application de la dilution de l'élément d'essai

La dilution de l'élément d'essai a été testée sur 4 oeufs.

300 µl ont été déposés sur la MCA avec une micropipette (P1000).

Aussitôt après application, le chronomètre a été déclenché.

IX.2.3. Lectures

Après 20 secondes de contact, la MCA a été rincée avec 5 ml de solution isotonique de chlorure de sodium (maintenu à 37 ± 1°C au bain-marie), à l'aide d'une seringue en évitant toute projection brutale.

Le liquide de rinçage a été éliminé par inclinaison de l'œuf.

Pendant un temps de 5 minutes, ont été observés, les éventuels phénomènes d'irritation selon le procédé décrit au paragraphe suivant. Le temps exact d'apparition de chaque phénomène a été relevé.

Les 20 secondes de contact ont été comprises dans les 5 minutes d'observation.

En fin d'essai, les embryons ont été détruits par refroidissement rapide (enceinte à -20°C).

IX.2.4. Procédé de lecture

Les observations prises en compte pour la notation de l'élément d'essai ont été réalisées à l'œil nu, sous la lampe.
Les phénomènes observés (hyperhémie, hémorragie, coagulation) n'ont pas été retenus en fonction de leur intensité mais en fonction de leur présence : réponse de type tout ou rien.

Le temps a été noté à l'apparition de chacun des phénomènes.

Les phénomènes observés se définissaient ainsi :

Hyperhémie

Phénomène observé : des capillaires non visibles avant l'ajout du produit deviennent visibles, alors que les capillaires visibles se dilatent et deviennent plus rouges. Ce phénomène peut également affecter les vaisseaux de diamètre supérieur.

Hémorragie

Phénomène observé : libération de sang s'échappant des vaisseaux et/ou des capillaires, pouvant se présenter sous différents aspects, et notamment en « chou-fleur », en nappe, en voile diffus, en piqueté (le sang s'échappe ponctuellement à différents endroits de la membrane).

Il est à noter que :
- l'hémorragie peut présenter un caractère éphémère ; elle doit néanmoins être prise en compte,
- l'observation, dans les 30 premières secondes, d'une hémorragie massive impose la prise en compte de l'hyperhémie masquée.

Coagulation (opacité et/ou thrombose)

Opacité

Phénomène observé : apparition sur tout ou partie de la membrane, soit d'un voile opalescent évoluant éventuellement vers une opacification, soit d'une opacification directe.
Il est nécessaire de vérifier que le phénomène n'est pas lié au comportement physico-chimique du produit en milieu aqueux (par exemple formation d'un colloïde, d'un précipité, ...).

Thrombose

Phénomène observé : rupture du flux sanguin dans les vaisseaux se traduisant par un aspect segmenté (alternance d'étranglements et de zones turgescentes plus ou moins sombres).

Il est à noter que les observations ne doivent pas prendre en compte les modifications intervenues au niveau des capillaires.

IX.3. Expression et interprétation des résultats

Les phénomènes observés ont été quantifiés selon le tableau ci-après, en fonction de leur délai d'apparition :

Phénomène	Temps		
	$t \leq 30$ s	**30 s $< t \leq$ 2 min**	**2 min $< t \leq$ 5 min**
Hyperhémie	5	3	1
Hémorragie	7	5	3
Coagulation	9	7	5

Chaque phénomène observé n'a été compté qu'une seule fois, au temps où il est apparu.

Le score pour chaque œuf a été la somme des notes d'hyperhémie, d'hémorragie et de coagulation. La notation de l'élément d'essai a été la moyenne arithmétique des scores obtenus sur 4 œufs, arrondie à une décimale (notation maximale = 21).

Le potentiel irritant sur la membrane chorio-allantoïdienne de l'élément d'essai a été donné par l'échelle suivante :

Score moyen (Sc m)	Classification
Sc m < 1	Pratiquement non irritant
1 ≤ Sc m < 5	Faiblement irritant
5 ≤ Sc m < 9	Modérément irritant
Sc m ≥ 9	Irritant

X. CONTROLE DU SYSTEME D'ESSAI, DES CONDITIONS OPERATOIRES ET DES EXPERIMENTATEURS

X.1. Contrôle négatif

Ce contrôle a été effectué au moyen d'une solution isotonique de NaCl à 0.9% (n° lot 13LDP203) préalablement chauffée à 37 ± 1°C avant l'utilisation des œufs.

Le contrôle a été jugé conforme s'il donnait un score compris entre 0.0 et 3.0.

X.2. Contrôle positif

Une vérification de la qualité du système d'essai, des conditions opératoires et des expérimentateurs a été réalisée à l'aide d'une référence.

Ce contrôle a été effectué en réalisant, en aveugle, une courbe étalon au moyen de solutions de lauryl sulfobétaïne préalablement chauffées à 37 ± 1°C (Sigma, lot 1421027V) à 0.05 %, 0.4 % et 3.2 % dans de l'eau pour préparations injectables (Cooper, lot 19KM14GA).

Le contrôle a été jugé conforme si:

- la concentration 0,05 % donnait un score compris entre 0.0 et 5.0
- la concentration 0,4 % donnait un score compris entre 10.5 et 12.5
- la concentration 3,2 % donnait un score compris entre 17.0 et 21.0

Les scores des contrôles positifs et négatifs sont indiqués dans le tableau ci-après.

X.3. Résultats des contrôles

Lot	Contrôle	Conc. (%)	Date	Scorage	Score moyen	±	Deviation Standard
Semaine 49/17	NaCl 0.9 %	Tel quel	04 décembre 2017	Sur 2 oeufs (Contrôle négatif)	0.0	±	0.0
04/17	Lauryl sulfobetaine	3.2 %	09 novembre 2017	Sur 4 oeufs	20.5	±	1.0
04/17	Lauryl sulfobetaine	0.4 %	09 novembre 2017	Sur 4 oeufs	12.0	±	0.0
04/17	Lauryl sulfobetaine	0.05 %	09 novembre 2017	Sur 4 oeufs	0.0	±	0.0

XI. RESULTATS

Œuf	**Injection** Cotation en fonction du temps ≤ 30 sec = 5 ≤ 2 min = 3 ≤ 5 min = 1			**Hémorragie** Cotation en fonction du temps ≤ 30 sec = 7 ≤ 2 min = 5 ≤ 5 min = 3		**Coagulation** Cotation en fonction du temps ≤ 30 sec = 9 ≤ 2 min = 7 ≤ 5 min = 5			**Score par œuf**
	Observée/ Masquée	temps (s)	note	temps (s)	note	Opacité/ Thrombose	temps (s)	note	
1	/	/	0	/	0	/	/	0	0
2	/	/	0	/	0	/	/	0	0
3	/	/	0	/	0	/	/	0	0
4	observée	24	5	/	0	/	/	0	5
Moyenne			1.3		0.0			0.0	**1.3**
Ecart-type			2.5		0.0			0.0	**2.5**

XII. CONCLUSION

Selon l'échelle de cotation définie, l'élément d'essai **ALGYL - Référence : WG GSCO - Lot : 17 07 040** testé dilué à 10% dans l'eau p.p.i, a été jugé **faiblement irritant** vis-à-vis de la membrane chorio-allantoïdienne de l'oeuf de poule embryonné.

Elément d'essai	Concentration testée	Score moyen obtenu sur 4 oeufs ± écart type	Classification
ALGYL - Référence : WG GSCO Lot : 17 07 040	Dilué à 10% dans l'eau p.p.i.	1.3 ± 2.5	faiblement irritant

Par manque de recul sur cette catégorie de produit (ingrédient cosmétique), la réponse obtenue pour cet élément d'essai ne peut être comparée à la base des données acquises au sein de l'Installation d'Essai..

XIII. ATTESTATION DU DIRECTEUR DE L'ETUDE

L'étude **B17 1689** était destinée à évaluer le potentiel irritant de l'élément d'essai **ALGYL - Référence : WG GSCO - Lot : 17 07 040**, consécutivement à son application sur la membrane chorio-allantoïdienne de l'œuf de poule embryonné (méthode Het-Cam).

La technique utilisée a été adaptée de celle décrite par Luepke N.P. et Kemper F.H. (The Het-Cam test : « An alternative to the Draize eye test ». Food Chem. Toxicol. 1986, 24, n° 6/7, 495-496).

La méthodologie a suivi celle publiée au Journal Officiel de la République Française du 26 décembre 1996.

Le donneur d'ordre a demandé que l'essai soit exécuté selon les principes des Bonnes Pratiques de Laboratoire en référence aux textes ci-après :

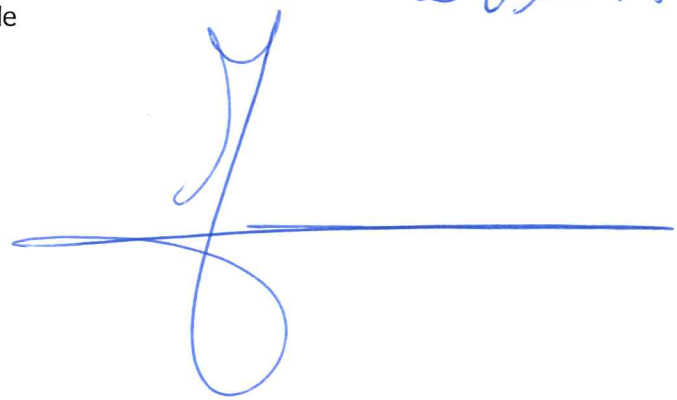
- Principes de l'OCDE de Bonnes Pratiques de Laboratoire (tels que révisés en 1997), ENV/MC/CHEM (98)17 et tous les documents de consensus ultérieurs.
- Directive 2004/10/CE du Parlement Européen et du Conseil du 11 février 2004 concernant le rapprochement des dispositions législatives, réglementaires et administratives relatives à l'application des principes de bonnes pratiques de laboratoire et au contrôle de leur application pour les essais sur les substances chimiques (JO n° L50 du 20/02/2004).
- Arrêté du 10 août 2004 pris pour l'application de l'article L. 5131-5 du code de la santé publique relatif aux Bonnes Pratiques de Laboratoire pour les produits cosmétiques (Journal Officiel n° 218 du 18 septembre 2004), Ministère de la santé et de la protection sociale.

Je soussignée, **Sarah JULIENNE,** engage ma responsabilité sur la validité des données de l'étude et déclare que l'étude est en conformité avec les textes des Bonnes Pratiques de Laboratoire cités ci-dessus.

Toutes informations relatives à l'élément d'essai sont sous la responsabilité du donneur d'ordre et n'ont pas été vérifiées par la société EUROFINS- Evic France.

Sarah JULIENNE — Date : 28/12/17

Directeur d'étude



XIV. ATTESTATION DE L'ASSURANCE QUALITE

Je soussignée, **Florence PROD'HOMME**, déclare que :

- le service Assurance Qualité s'est assuré du respect des procédures de travail relatives à ce type d'étude.

Des audits portant sur l'Installation d'Essai ont été réalisés annuellement. Ces audits ont permis de vérifier que l'Installation d'Essai répondait aux exigences des autorités.

Les audits d'étude (documentation et techniques appropriées à ce type d'étude) ont été réalisés ; le résultat des évaluations de l'Assurance Qualité a fait l'objet de comptes rendus qui ont été transmis au Directeur de l'étude et à la Direction générale comme suit :

Dates des audits	**Phase(s) auditée(s)**	**Dates de transmission au Directeur de l'étude et à la Direction**
04 décembre 2017	Préparation d'un élément d'essai	04 décembre 2017
28 décembre 2017	Rapport d'étude	28 décembre 2017

Le rapport final reflète de façon exacte et complète les procédures et les données brutes générées au cours de l'étude.

Seule la version papier du rapport final fait foi.

Florence PROD'HOMME
Assurance Qualité

Date : 28.12.17

SPECIFICATION DATA SHEET

[Distributed for Commercial Use] - Do Not Cite or Quote

Trade name: **PHYCO'DERM®**

Product: N° W-G-UNPI-COOF-01 Version: 1.0 – 2020

Specification: N° S.00 Print date: 01 - 2020

PHYCO'DERM® is an association of algae extracts in glycerin excipient (vegetal origin).

1 – Identification and composition of the substance

Product	N° CAS	N°EINECS	Ingredients %
Glycerin	56-81-5	200-289-5	50
Water	7732-18-5	231-791-2	30
Undaria pinnatifida extract	-	-	18.5
Corallina officinalis extract	89997-92-2	289-730-0	1.5
Preservative	None		

2 – **Characteristics** (standard)

Appearance: limpid liquid.

Color: yellow.

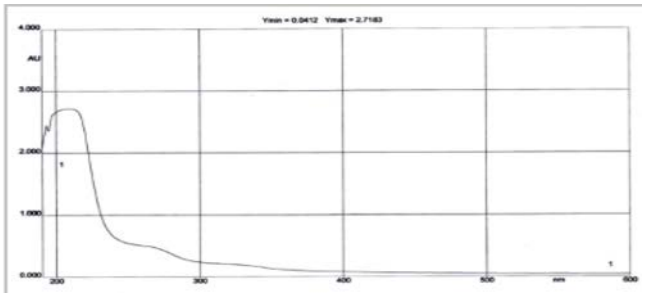
Odour: *sui generis*.

pH: 6.0 ± 1.0.

Relative density: 1.14 ± 0.02.

Dry residuals (%): 50 ± 5.

UV spectrum (1/8 in water):



Microbiological quality:

Total germs (germs/ml)	< 100.
Pathogens	absence.
Yeasts /moulds	< 100.

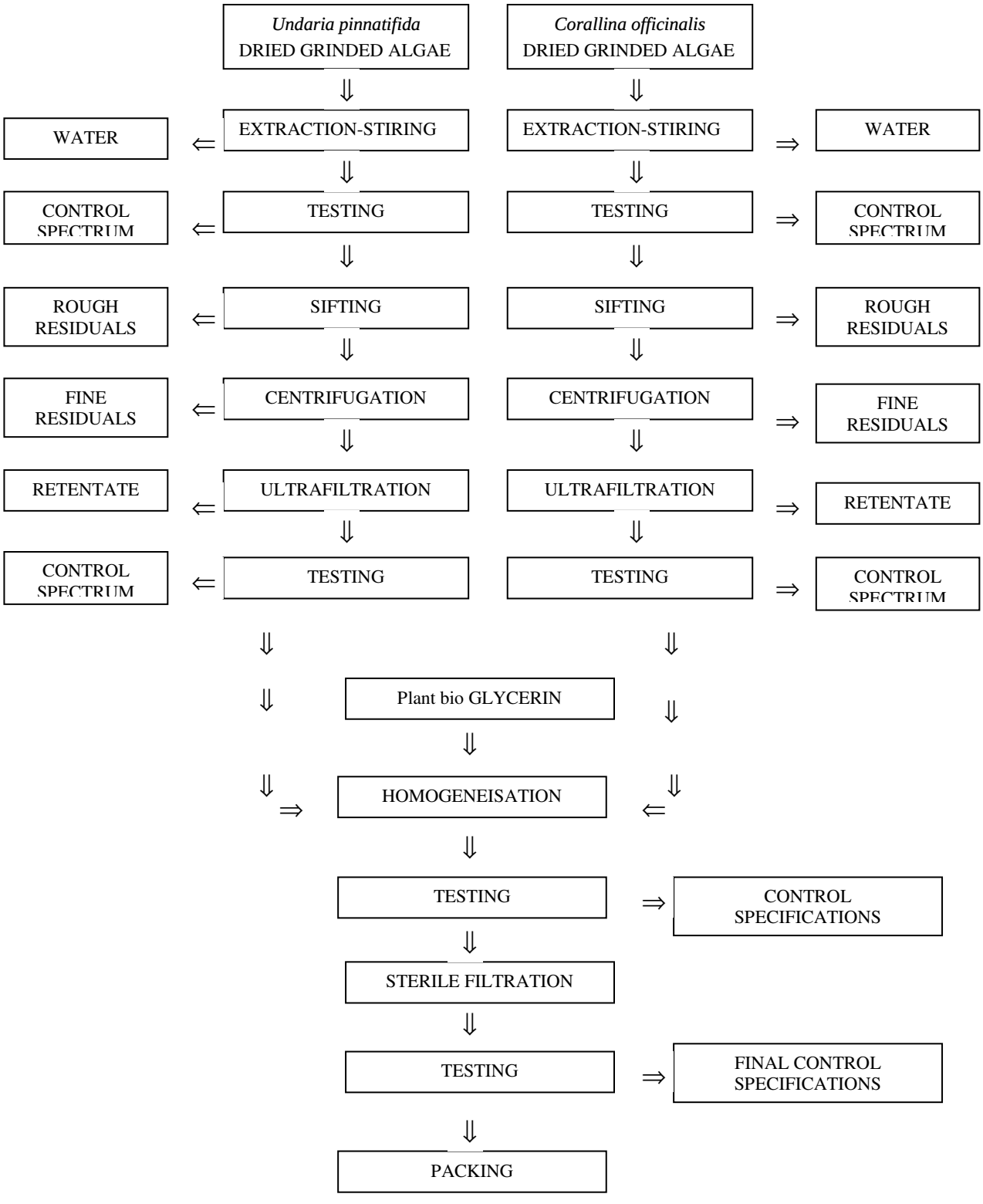
Storage: 15°C < store < 25°C.
Validity date: 6 months
Once opened, the whole drum must be used.

Remark:
No responsibility or liability for any consequences arising from the use of this technical data sheet can be accepted, including possible infringement of any patent.
Any addition of preservatives is at the request and under the responsibility of the applicant, the product being delivered in units of use (1-5 or 10 Kg).

GELYMA – Parc d'Affaires Marseille Sud (C4) – 1 Boulevard de l'Océan – 13009 Marseille – France.
Phone: (33) 496 140 982 – E-mail: gelyma @ wanadoo.fr – contact @ gelyma.com

Parc d'Affaires Marseille Sud
Bâtiment C4
1, Boulevard de l'Océan
13009 Marseille

FLOW CHART FOR PHYCO'DERM®



Siège social : Parc d'Affaires Marseille Sud, 1 Boulevard de l'Océan, Impasse du Paradou, 13009 Marseille
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1, Boulevardde l'Ocean

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RAPPORT D'ESSAI FINAL
LIQUIDE - PHYCO' DERM

Date réception client :	
Date fabrication :	
N° lot client :	16 06 070
Fournisseur :	
N° lot fournisseur :	
Tonnage :	
DLUO :	12/2017

Demandeur :	Mme PELLEGRINI Liliane
N° commande :	
N° client :	
N° optim :	
N° étude :	
Réf. commerciale :	DS16CT002822
Tiers :	

Date réception labo : 14/06/2016

Masse brute (g):

Observations :

Commentaires :

Le code à 2 lettres indique le site Invivo Labs sur lequel a été réalisée l'analyse : CT = site de Chierry, SN = site de Saint-Nolff.

En cas de déclaration de conformité à la spécification, celle-ci ne prend pas en compte l'incertitude associée aux résultats.
Si ce rapport fait mention de résultats de mycotoxines, ils sont corrigés du taux de récupération. Ce rapport d'essai ne concerne que l'échantillon soumis à essai.
Si ce rapport fait mention de résultats de pesticides, ils ne sont pas corrigés du taux de récupération si celui-ci est compris entre 70 et 110 %
« # » : analyse faite plusieurs fois

Dossier : CH-19916-00086 / Ech : 16CH024071 / V1

ANALYSES CHIMIQUES

Determination	Rés/brut	Rés/sec	Incertitude	Cible	Mini	Maxi	Conforme
SODIUM Méthode interne - ICP-H - CT	420,4 mg/100ml		42,0 mg/100ml				
CALCIUM Méthode interne - ICP-H - CT	142,9 mg/100ml		14,3 mg/100ml				
PHOSPHORE Méthode interne - ICP-H - CT	8,9 mg/100ml		1,5 mg/100ml				
MAGNESIUM Méthode interne - ICP-H - CT	60,7 mg/100ml		6,1 mg/100g				
POTASSIUM Méthode interne - ICP-H - CT	530,3 mg/100ml		53,0 mg/100ml				
CUIVRE Méthode interne - ICP-H - CT	<0,5 mg/100ml						
FER Méthode interne - ICP-H - CT	<0,5 mg/100ml						
MANGANESE Méthode interne - ICP-H - CT	0,0 mg/100ml						
ZINC Méthode interne - ICP-H - CT	<0,5 mg/100ml						
IODE NF EN 15111 - CT	1,9 mg/L		0,4 mg/L				
MINERALISATION MICRO ONDES Méthode interne - ELTRACES-H - CT	Réalisée						
ARSENIC Méthode interne - ELTRACES-H - CT	1383 µg/kg		277 µg/kg				
CADMIUM Méthode interne - ELTRACES-H - CT	29 µg/kg		6 µg/kg				
MERCURE Méthode interne - ELTRACES-H - CT	<10 µg/kg						
PLOMB Méthode interne - ELTRACES-H - CT	86 µg/kg		26 µg/kg				
SELENIUM Méthode interne - ELTRACES-H - CT	<50 µg/kg						
SILICIUM Analyse sous traitée	0 mg/kg						
DENSITE APPARENTE Méthode interne - DENSTASS-H - CT	1,13 -						
ANALYSE SOUS TRAITEE Analyse sous traitée	Fichier joint						

Conclusion :

Validé le : 05-07-16

Melle BRU CAMILLE

Superviseur

Le code à 2 lettres indique le site Invivo Labs sur lequel a été réalisée l'analyse : CT = site de Chierry, SN = site de Saint-Nolff.

En cas de déclaration de conformité à la spécification, celle-ci ne prend pas en compte l'incertitude associée aux résultats.
Si ce rapport fait mention de résultats de mycotoxines, ils sont corrigés du taux de récupération. Ce rapport d'essai ne concerne que l'échantillon soumis à essai.
Si ce rapport fait mention de résultats de pesticides, ils ne sont pas corrigés du taux de récupération si celui-ci est compris entre 70 et 110 %
« # » : analyse faite plusieurs fois

NANTES
Route de Gachet - BP 52703 - 44327 Nantes Cedex 3
Tél. 02 51 85 44 44 - Fax 02 51 85 44 50 - www.inovalys.fr

RAPPORT D'ANALYSE N° : **D160602281**

INVIVO LABS SITE DE CHIERRY

Rue de l'Eglise
BP 50019
02400 CHATEAU THIERRY

Tél. 03 23 84 80 21

Objet : ANALYSE ECH. 16CH024071 ET 24072 ENVOI 160615-007

Dossier enregistré le : 16/06/2016 Edité le : 05/07/2016

ECHANTILLON N° : E160606016 (Aliments composés pour animaux)
Réf Client : **16CH024071**
Descriptif : **LIQUIDE PHYCO'DERM**

Date début analyse échantillon : 04/07/2016

Analyses	Résultats	Références méthodes	LQ	Déb. analyse
Silicium n° CAS: 7440-21-3 n° Sandre : 5429	< 250 mg/kg	Selon ISO 14869-2 abrogée	250	04/07/2016
Silicium (en silice SiO2)	535,0 mg/kg	Selon ISO 14869-2 abrogée	500	04/07/2016

Approuvé le 05/07/2016 par Stephanie MENAGER , Resp. Labo. Chimie Agro-Alimentaire

Translation 8c - PHYCO'DERM® (Undaria Pinnatifida Extract [brown algae] and Corallina Officinalis Extract [red algae])

Chemical Analysis

Determination	Results/Units	Uncertainty
Sodium Internal method - ICP-H - CT	420.4 mg/100 ml	42.0 mg/100 ml
Calcium Internal method - ICP-H - CT	142.9 mg/100 ml	14.3 mg/100 ml
Phosphorus Internal method - ICP-H – CT	8.9 mg/100 ml	1.5 mg/100 ml
Magnesium Internal method - ICP-H – CT	60.7 mg/100 ml	6.1 mg/100 ml
Potassium Internal method - ICP-H – CT	530.3 mg/100 ml	53 mg/100 ml
Copper Internal method - ICP-H – CT	<0.5 mg/100 ml	
Iron Internal method - ICP-H – CT	<0.5 mg/100 ml	
Manganese Internal method - ICP-H – CT	0.0 mg/100 ml	
Zinc Internal method - ICP-H – CT	<0.5 mg/100 ml	
Iodine NF EN 15111 - CT	1.9 mg/L	0.4 mg/L
Microwave mineralization Internal method ELTRACES-H - CT	Completed	
Arsenic Internal method ELTRACES-H - CT	1383 µg/kg	277 µg/kg
Cadmium Internal method ELTRACES-H – CT	29 µg/kg	6 µg/kg
Mercury Internal method ELTRACES-H – CT	<10 µg/kg	
Lead Internal method ELTRACES-H – CT	86 µg/kg	26 µg/kg
Selenium Internal method ELTRACES-H – CT	<50 µg/kg	
Silicon Sub-contracted analysis	0 mg/kg	
Apparent density Internal method - DENSTASS-H - CT	1.13	
Sub-contracted analysis Sub-contracted analysis	Attached file	

Conclusion: Validated on: 07/05/2016 Melle BRU CAMILLE Supervisor

The 2-letter code indicates the In vivo Labs site on which the analysis was carried out: CT = site of Chierry, SN = site of Saint-Nolff.

In case of declaration of conformity to the specification, this does not take into account the uncertainty associated with the results.
If this report mentions mycotoxin results, they are corrected for the recovery rate. This test report only concerns the sample submitted for testing.
If this report mentions pesticide results, they are not corrected for the recovery rate if it is between 70 and 120%.

"#": Analysis made several times

Description: LIQUIDE PHYCO'DERM

Sample analysis start date: 07/04/2016

Analyses	Results	Method references	LQ Date of Analysis
Silicone n° CAS: 7440-21-3 n° Sandre : 5429	<250 mg/kg	According to ISO 14869-2 repealed	250 07/04/2016
Silicone (as silica SiO_2)	535.0 mg/kg	According to ISO 14869-2 repealed	500 07/04/2016

Approved on 07/05/2016 by Stephanie MENAGER, Resp. Labo. Chimie Agro-Alimentaire

This analysis report only concerns the products submitted for analysis. Reproduction of this analysis report is only authorized in its integral form. It has 3 page (s).

RAPPORT D'ETUDE

Les résultats qui suivent ne s'appliquent qu'aux échantillons soumis au laboratoire et tels qu'ils sont définis dans le présent document. Les échantillons seront conservés dans nos locaux pendant une période de 2 mois à compter de la date figurant sur ce document. L'échantillon et les informations concernant l'échantillon ont été fournis par le client. Toutes les informations relatives à l'échantillon sont sous la responsabilité du client et n'ont pas été vérifiées par la société Eurofins ATS.

GELYMA
1 boulevard de l'Océan
Parc d'Affaires Marseille
Batiment C 4
13009 MARSEILLE
FRANCE

Le 24 août 2016

PATCH TEST

Promoteur de l'étude : Mme PELLEGRINI Liliane - GELYMA

Référence EUROFINS ATS : 2016/46083/1

Suivi de l'étude : -

Produit testé :

- Dénomination : PHYCO'DERM
- Référence client : Gelyma 06 a-2016 - Lot 16 06 070
- Référence ATS : 573365
- Marque : -

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Il comporte 17 pages.

PATCH TEST CHEZ L'HOMME SOUS CONTRÔLE DERMATOLOGIQUE

Rapport d'étude – version n° 1 du 11/07/2016

REFERENCES ETUDE	**EUROFINS EVIC france – I16 0486** **E573365_P781564**

PRODUIT D'INVESTIGATION

Dénomination	**PHYCO'DERM**
Référence / Numéro de formule	**Gelyma 06 a-2016**
Numéro de lot	**16 06 070**
Catégorie cosmétique	**Ingrédient**
Forme galénique et caractères organoleptiques	**Liquide jaune orangé**

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MONITEUR D'ETUDE	**Liliane PELLEGRINI**
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afaq ISO 9001 Qualité AFNOR CERTIFICATION

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PATCH TEST CHEZ L'HOMME SOUS CONTRÔLE DERMATOLOGIQUE

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HUMAN PATCH TEST UNDER DERMATOLOGICAL CONTROL

Résumé en anglais / English synopsis

STUDY OBJECTIVE	To confirm the skin compatibility of the investigational product in a panel of healthy human subjects after single application under maximising and controlled experimental conditions.
TYPE OF THE STUDY	Monocentric randomised clinical study performed in single blind and defined as a non interventional clinical research according to the French law 2004-806 of 09/08/2004 relating to the policy of public health. The test subject was used as own control.
DATES OF STUDY PERFORMANCE	**Initiation date:** 05/07/2016
	Completion date: 07/07/2016
STUDY POPULATION	**Number of test subjects: 10** valid cases **Inclusion criteria: test subjects** • suitable to participate in the study 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 simultaneously in another clinical study which could interfere • 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 70 • female/male • with all types of skin on back • with a phototype (Fitzpatrick): I to IV • 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) **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 hospitalisation during the study • belonging to the staff of the investigating centre • being of age but protected by law • having received vaccination within the 3 weeks prior to the study or intending to be vaccinated during the course of the study • with personal history of adverse reactions to the same type of product as the investigational product • with personal history of adverse reaction to colophony, rubber, patch materials, adhesive plaster

Résumé en anglais (suite) / English synopsis (continuation)

STUDY POPULATION	• with documented history of contact allergy • exhibiting skin marks and/or moles and/or freckles in too great quantity and/or hyperpilosity on the experimental area able to interfere with the assessment of the possible skin reactions • 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 study results, • foreseeing, during the study, a treatment able to interfere with the interpretation of the study results (systemic or topical anti-acne medication, topical or systemic medication with anti-inflammatory or antihistamine, antibiotics, desensitisation treatment, ...) • having had a fever lasting more than 24 hours, within the 8 days prior to the study • having had any invasive aesthetic cares on chest and back (peeling, laser...) by a dermatologist within the 2 months prior to the study or foreseeing it for the duration of the study • having had any non invasive aesthetic cares on chest and back by an aesthetician within the month prior to the study or foreseeing it for the duration of the study • having received excessive or intensive exposure to sunlight (natural or artificial) within the month prior to the study or foreseeing UV exposures for the duration of the study • under treatment with PUVA or UVB within the month prior to the study • having participated in a human repeated insult patch test with challenge with or without sun exposure within the 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 maximum with or without challenge within the year prior to the study • foreseeing bath (in bathtub, sea or swimming pool), sauna or Turkish batch during the study period • regularly practicing intensive sport causing sweating and requiring frequent showers • 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
METHODOLOGY	**Definition and preparation of the experimental areas:** - Skin areas defined by the technician in charge of the study on the back of the test subjects, taking into account the skin appearance and avoiding the areas of friction with clothes - Before patching, wiping of the skin with a cotton pad **Application of the investigational product**, by the technician in charge of the study at the investigating centre: - once (on D1), - under maximising conditions of exposure (under Semi-occlusive patch - Trumed® : absorbent support in Webril® kept in position by a non woven medical adhesive (surface: 400 mm²) - quantity applied=160 µl; measured with a micropipette with disposable tip and put into the patch) - diluted at 10% in water for injection - during a defined time (48h ±4h) Application in parallel of water for injection (160 µl) to a skin area on back, under Semi-occlusive - Trumed® patch and during a defined time (48h ±4h) (control area, to take into account the possible effects not directly related to the investigational product but due to the patch material)

Résumé en anglais (suite) / English synopsis (continuation)

METHODOLOGY	**Checking of the skin compatibility** based on:
	• a skin examination of the treated and control areas, visually, by the same investigator with the appropriate experience, under standard "daylight" source, on:
	  ⊣ D1/T0 before application
	  ⊣ D3/T15-30 minutes after patches removal
	• the analysis of the sensations of discomfort reported directly by the test subjects to the investigator during the study
	Descriptive analysis – Percentage of reactive test subjects (erythema and other visible signs of reactivity)
	
	Expression of the results:
	• **Percentage of reactive test subjects:** calculated taking into account only the following signs of reactivity: erythema, dryness, œdema, papula, vesicle, bulla, scab, soap effect, pruritus
	Description of the other reactivity clinical signs or sensations of discomfort and calculation of the corresponding percentage of test subjects if justified by the appearance frequency
	• **Individual daily irritation score (IDIS)** calculated for each test subject :
	IDIS = sum of the marks obtained for all the signs observed
	• **Mean daily irritation score (MDIS)** calculated for the panel :
	MDIS = Σ (IDIS) / nb of valid cases
	Classification of the reaction according to ICDRG scale in case of reaction of allergy
	
	Descriptive analysis of the data

RESULTS

Characteristics of the included panel

Number of included subjects: 10
Number of exclusions: 0
Number of withdrawals: 0
Number of valid cases: 10

- Age: 24 to 69 (Mean= 51 years old)
- Sex: female
- Phototype: II to IV
- All types of skin on the back

Résumé en anglais (suite) / English synopsis (continuation)

Checking of the skin compatibility

No reaction was noted on the control site

For the investigational product:

Control time after patch removal	Type of reaction	Number of reactive test subjects	% of reactive test subjects	Mean daily irritation score MDIS	Skin compatibility of the product
T15-30 minutes (D3)	/	0	0 %	0	**Very good skin compatibility**

Legend: / = none

OVERALL CONCLUSION

Under the experimental conditions adopted:

single application of the product diluted at 10% in water for injection, under semi-occlusive patch, on a panel of 10 women, aged between 24 and 69 years old, with phototype II to IV and with all types of skin on back,

the product **PHYCO'DERM - Réf. Gelyma 06 a-2016 - Lot : 16 06 070** has a **very good** skin compatibility.

PATCH TEST CHEZ L'HOMME SOUS CONTRÔLE DERMATOLOGIQUE

Résumé en français

OBJECTIF DE L'ETUDE	Confirmer la compatibilité cutanée du produit d'investigation sur un panel de personnes saines après application unique dans des conditions expérimentales maximalisées et contrôlées.
TYPE DE L'ETUDE	Etude clinique monocentrique réalisée en simple aveugle, définie comme non interventionnelle conformément à la loi française 2004-806 du 09/08/2004 relative à la politique de santé publique. Chaque participant sera son propre témoin.
DATES DE REALISATION DE L'ETUDE	**Date de démarrage de l'étude :** 05/07/2016
	Date de fin de l'étude : 07/07/2016
POPULATION DE L'ETUDE	**Nombre de cas exploitables : 10** **Critères d'inclusion : participants** • aptes à participer à l'étude et répondant à la qualité de « sujet sain » • affiliés à la Sécurité Sociale • ayant signé le formulaire de consentement de participation à l'étude • certifiant ne pas participer simultanément à une autre étude clinique pouvant interférer • s'engageant sur la véracité des renseignements personnels fournis de façon déclarative à l'investigateur • fiables, capables de comprendre les instructions du protocole et d'en respecter les contraintes • disponibles pour assurer les visites au centre d'investigation • âgés de 18 à 70 ans • de sexe féminin/masculin • ayant tout type de peau au niveau du dos • ayant un phototype (Fitzpatrick) : I à IV • déclarant ne pas s'être exposées à un risque de grossesse au cours des 3 mois au moins précédant l'étude et s'engageant à utiliser un mode de contraception efficace pendant l'étude (pour les femmes en âge de procréer) **Critères de non inclusion : participants** • en période d'exclusion • privés de liberté par décision judiciaire ou administrative ou sous tutelle • ne pouvant être contactés en cas d'urgence • admis dans un établissement social • prévoyant une hospitalisation pendant l'étude • faisant partie du personnel du centre d'investigation • majeurs protégés par la loi • vaccinés dans les 3 semaines précédant le début de l'étude ou prévoyant d'être vaccinés durant le déroulement de l'étude • ayant des antécédents personnels de réactions indésirables à des produits similaires au produit d'investigation • ayant des antécédents personnels de réactions indésirables à la colophane, au caoutchouc, au matériel de patchage et/ou aux adhésifs • ayant des antécédents d'allergie de contact documentés

Résumé en français (suite)

POPULATION DE L'ETUDE	• présentant des marques cutanées et/ou grains de beauté et/ou taches de rousseur en trop grande quantité et/ou une hyperpilosité au niveau de la zone expérimentale pouvant interférer avec l'évaluation des potentielles réactions cutanées • présentant une réaction eczémateuse toujours visible, cicatrice, ou séquelles de tests antérieurs sur la zone expérimentale • sous traitement avant l'étude pouvant interférer avec les résultats de l'étude, • prévoyant pendant l'étude un traitement pouvant interférer avec l'interprétation des résultats (traitement systémique ou local anti-acnéique, traitement systémique ou local anti-inflammatoire, antihistaminique, antibiotique, désensibilisation • ayant présenté une affection fébrile pendant plus de 24 heures au cours des 8 jours précédant l'étude • ayant eu des soins esthétiques invasifs au niveau du buste et du dos (peeling, laser…) par un dermatologue dans les 2 mois précédant l'étude ou le prévoyant pendant la durée de l'étude • ayant eu des soins esthétiques non invasifs au niveau du buste et du dos (gommage, nettoyage de peau…) par une esthéticienne dans le mois précédant l'étude ou le prévoyant pendant la durée de l'étude • s'étant exposés intensivement au soleil ou en cabine UV dans le mois précédant l'étude ou prévoyant de s'exposer en cours d'étude • sous traitement PUVA ou UVB au cours du mois précédant l'étude • ayant participé à un patch test répété avec révélation avec ou sans exposition aux UV dans les 4 mois précédant l'étude • ayant participé à un test d'irritation cumulée dans les 2 mois précédant l'étude ou à un patch test simple dans le mois précédant l'étude • ayant déjà participé à 5 études cliniques avec patch dont au maximum 3 patch tests répétés avec révélation avec ou sans exposition aux UV au cours de l'année précédant l'étude • prévoyant de prendre des bains (en baignoire, mer ou piscine), sauna ou hammam pendant la durée de l'étude • pratiquant régulièrement un sport générant une transpiration abondante et nécessitant des douches fréquentes • allaitante, enceinte ou prévoyant une grossesse pendant l'étude (pour les femmes en âge de procréer) • ayant commencé ou changé de contraception orale oestroprogestative ou de traitement hormonal dans les 3 mois précédant l'étude ou le prévoyant pendant la durée de l'étude
METHODOLOGIE	**Définition et préparation des zones expérimentales :** - Définition des sites expérimentaux par le technicien en charge de l'étude au niveau du dos des participants, en tenant compte de l'aspect de la peau et en évitant les zones de frottement avec les vêtements. - Avant patchage, essuyage de la peau à l'aide d'un coton. **Application du produit d'investigation** par le technicien responsable au centre d'investigation : - une fois (à J1) - dans des conditions maximalisées d'exposition (sous pansement Semi-occlusif - Trumed® : support absorbant en Webril maintenu par un adhésif médical en non tissé (surface : 400 mm²) - quantité appliquée = 160 µl ; mesurés à l'aide d'une micropipette à embout jetable puis déposés dans chaque pansement) - dilué à 10% dans l'eau p.p.i - pendant un temps défini (48h ±4h) Application en parallèle d'eau p.p.i (160 µl) sur une zone cutanée définie (dos), sous patch Semi-occlusif - Trumed® et pendant un temps défini (48h ±4h) (site contrôle, pour s'affranchir des effets potentiels non liés directement au produit d'investigation mais possiblement liés au matériel de patchage)

Résumé en français (suite)

METHODOLOGIE	**Vérification de la compatibilité cutanée** basée sur :
	• un examen de la peau au niveau des sites traités et témoin, visuellement, par le même investigateur, sous éclairage standardisé type « lumière du jour », aux temps suivants :
	  J1/T0 avant application
	  J3/T15-30 minutes après enlèvement des pansements
	• l'analyse des sensations d'inconfort rapportées directement par les participants à l'investigateur en cours d'étude
	Analyse descriptive – pourcentage de participants réactifs (érythème et autres signes visibles de réactivité)
	
	Expression des résultats :
	• **Pourcentage de sujets réactifs** calculé en considérant uniquement les signes de réactivité suivants : érythème, sécheresse, œdème, papule, vésicule, bulle, croûtelle, effet savon, prurit
	Description des autres signes cliniques de réactivité ou des sensations d'inconfort et calcul du pourcentage de sujets correspondant si la fréquence d'apparition le justifie
	• **Score d'irritation journalier individuel (SijI)** calculé pour chaque sujet :
	(SijI) = somme des notes obtenues pour l'ensemble des signes observés
	• **Score d'irritation journalier moyen (SijM)** calculé pour le panel :
	SijM = Σ (SijI) / nombre de volontaires exploitables
	Classement de la réaction selon l'échelle ICDRG en cas de réaction allergique
	Analyse descriptive des données

RESULTATS

Caractéristiques du panel inclus :

Nombre de sujets inclus : 10
Nombre de sorties d'essai : 0
Nombre d'abandons : 0
Nombre de cas exploitables : 10

- Age : de 24 à 69 ans (Age moyen = 51 ans)
- Sexe : féminin
- Phototype : de II à IV
- Tout type de peau au niveau du dos

Résumé en français (suite)

Vérification de la compatibilité cutanée

Aucune réaction n'a été relevée sur le site contrôle

Pour le produit d'investigation :

Temps après retrait du patch	Type de réaction	Nombre de participants réactifs	% de participants réactifs	Score d'irritation journalier moyen SIJM	Compatibilité cutanée du produit
T15-30 minutes (J3)	/	0	0 %	0	Très bonne compatibilité cutanée

CONCLUSION GENERALE

Dans les conditions expérimentales adoptées :

application unique du produit dilué à 10% dans l'eau p.p.i, sous pansement semi-occlusif, sur un panel de 10 femmes, âgées de 24 à 69 ans, de phototype II à IV et ayant tout type de peau au niveau du dos,

le produit **PHYCO'DERM - Réf. Gelyma 06 a-2016 - Lot : 16 06 070** a une **très bonne** compatibilité cutanée.

PATCH TEST CHEZ L'HOMME SOUS CONTRÔLE DERMATOLOGIQUE

Signatures et dates

Investigateur : Dr MAGNE Françoise (Dermatologue)

Je soussignée, Dr MAGNE Françoise (Dermatologue), déclare que l'ensemble de l'étude s'est déroulée sous ma responsabilité conformément au protocole et aux procédures internes du centre d'investigation et dans l'esprit des principes des Bonnes Pratiques Cliniques (recommandations internationales ICH E6(R1) du 10/06/1996 et de la Directive du Parlement européen et du Conseil 2001/20/CE.
J'assume la responsabilité de la validité des données brutes obtenues au cours de l'étude qui sont reportées dans le présent rapport d'étude.

pp Dr HAVET Violette (Dermatologue)

Date : 18-07-16
Signature :

Assurance qualité : PROD'HOMME Florence

Je soussignée, PROD'HOMME Florence, déclare que :

- ce type d'étude a été audité selon les procédures du centre d'investigation :

Référence du "Patch test" audité	Phase d'audit	Date de réalisation de l'audit	Date de transmission du rapport d'audit	
			à l'investigateur	à la Direction du centre d'investigation
I16 0425	J1	5/07/16	5/07/16	5/07/16

- le rapport final a été audité le 18 juillet 2016,
- les résultats consignés reflètent de façon exacte et complète les données brutes de l'étude.

Date : 18.07.16
Signature :

ANNEXES

Annexe 1

CARACTERISTIQUES TYPOLOGIQUES DES PARTICIPANTS

Participants		âge *(ans)*	sexe *F = féminin M = masculin*	type de peau au niveau du dos[1]	phototype[2]
Réf.	Code *Identifiant unique du panel général*				
1	23338	63	F	S	III
2	23245	49	F	N	III
3	24599	68	F	S	III
4	23396	69	F	N	II
5	12662	58	F	N	II
6	24215	24	F	N	II
7	22155	40	F	N	IV
8	14583	54	F	N	IV
9	21811	26	F	N	III
10	19274	62	F	N	II

Légende :

*[1] **type de peau au niveau du dos : N** = normale, **S** = sèche, **G** = grasse*
*[2] **phototype : Type I =** Brûle toujours facilement, ne bronze jamais, **Type II =** Brûle toujours facilement, bronze légèrement, **Type III =** Brûle modérément, bronze progressivement, **Type IV =** Brûle faiblement, bronze toujours facilement, **Type V =** Brûle rarement, bronze intensément, **Type VI =** Ne brûle jamais, fortement pigmenté*

Annexe 2

INFORMATIONS SPECIFIQUES CONCERNANT LES PARTICIPANTS

Participants		Réactivité cutanée orthoergique	Antécédents d'atopie	Traitement médicamenteux en cours		Contraception
Réf.	Code *Identifiant unique du panel général*			Si oui *(préciser la dénomination commerciale)*		Si oui *Type à préciser*
				A l'inclusion	Pendant l'étude	
1	23338	x	/	/	/	NC
2	23245	x	/	/	/	Préservatif
3	24599	/	/	Inexium®	Inexium®	NC
4	23396	/	/	/	/	NC
5	12662	x	/	/	/	NC
6	24215	/	/	/	/	Préservatif
7	22155	/	/	/	/	Pilule
8	14583	/	/	/	/	Préservatif
9	21811	/	x	/	/	Implant
10	19274	/	/	/	/	NC

Légende: / = non x = oui NC = Non concerné

Annexe 3/1

COMPATIBILITE CUTANEE – EXAMEN CUTANE ET INTERROGATOIRE PRODUIT D'INVESTIGATION

REACTIONS : ***Signes cliniques*** ***E :*** *Erythème :* ***E1*** *= très léger,* ***E2*** *= léger,* ***E3*** *= modéré,* ***E4*** *= sévère* ***D*** *= Sècheresse :* ***D1*** *= très légère,* ***D2*** *= légère,* ***D3*** *= modérée,* ***D4*** *= sévère* ***Oe*** *= Œdème / infiltration homogène* ***Pa*** *= Papules :* ***Pa1*** *= présence en petit nombre (≤3),* ***Pa2*** *= présence en grand nombre (>3)* ***V*** *= Vésicules :* ***V1*** *= présence en petit nombre (≤3),* ***V2*** *= présence en grand nombre (>3)* ***Bu*** *= Bulles* ***Cr*** *= Croûtelle – Exsudation et/ou incrustation de surface* ***S*** *= Effet savon (peau brillante avec rides éventuelles)* ***Hypo*** *= Hypo-pigmentation* ***Hyper*** *= Hyperpigmentation* ***C*** *= coloration cutanée* ***Sensations d'inconfort*** ***Pr*** *= Prurit :* ***Pr1*** *= très léger,* ***Pr2*** *= léger,* ***Pr3*** *= modéré,* ***Pr4*** *= sévère* ***Br*** *= Brûlure*	***d*** *: diffus* ***p*** *: ponctué* ***péri*** *: périphérique* ***/: pas de réaction***

Participants		**Temps expérimentaux**	
		J3	
Réf.	**Code**	Réactions + intensité	SijI
1	23338	/	0
2	23245	/	0
3	24599	/	0
4	23396	/	0
5	12662	/	0
6	24215	/	0
7	22155	/	0
8	14583	/	0
9	21811	/	0
10	19274	/	0
SijM		0	

Commentaires : aucun

Annexe 3/2

COMPATIBILITE CUTANEE – EXAMEN CUTANE ET INTERROGATOIRE
SITE TEMOIN

REACTIONS : ***Signes cliniques*** ***E :*** *Erythème :* ***E1*** *= très léger,* ***E2*** *= léger,* ***E3*** *= modéré,* ***E4*** *= sévère* ***D*** *= Sècheresse :* ***D1*** *= très légère,* ***D2*** *= légère,* ***D3*** *= modérée,* ***D4*** *= sévère* ***Oe*** *= Œdème / infiltration homogène* ***Pa*** *= Papules :* ***Pa1*** *= présence en petit nombre (≤3),* ***Pa2*** *= présence en grand nombre (>3)* ***V*** *= Vésicules :* ***V1*** *= présence en petit nombre (≤3),* ***V2*** *= présence en grand nombre (>3)* ***Bu*** *= Bulles* ***Cr*** *= Croûtelle – Exsudation et/ou incrustation de surface* ***S*** *= Effet savon (peau brillante avec rides éventuelles)* ***Hypo*** *= Hypo-pigmentation* ***Hyper*** *= Hyperpigmentation* ***C*** *= coloration cutanée* ***Sensations d'inconfort*** ***Pr*** *= Prurit :* ***Pr1*** *= très léger,* ***Pr2*** *= léger,* ***Pr3*** *= modéré,* ***Pr4*** *= sévère* ***Br*** *= Brûlure*	***d*** *: diffus* ***p*** *: ponctué* ***péri*** *: périphérique* ***/: pas de réaction***

Participants		**Temps expérimentaux**	
		J3	
Réf.	**Code**	Réactions + intensité	SijI
1	23338	/	0
2	23245	/	0
3	24599	/	0
4	23396	/	0
5	12662	/	0
6	24215	/	0
7	22155	/	0
8	14583	/	0
9	21811	/	0
10	19274	/	0
SijM		0	

Commentaires : aucun

RAPPORT D'ETUDE

Les résultats qui suivent ne s'appliquent qu'aux échantillons soumis au laboratoire et tels qu'ils sont définis dans le présent document. Les échantillons seront conservés dans nos locaux pendant une période de 2 mois à compter de la date figurant sur ce document. L'échantillon et les informations concernant l'échantillon ont été fournis par le client. Toutes les informations relatives à l'échantillon sont sous la responsabilité du client et n'ont pas été vérifiées par la société Eurofins ATS.

GELYMA
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FRANCE

Le 1er Août 2016

RAPPORT D'ANALYSES

Promoteur de l'étude : GELYMA

Référence Client : Evaluation de la tolérance

Référence EUROFINS ATS : 2016-46083/1

Suivi de l'étude : Nadine GOBERT

Produit testé :

- Dénomination : PHYCO'DERM
- Référence client : Gelyma 06 a-2016 Lot 16 06 070
- Référence ATS : 573365
- Marque : -

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LABORATOIRES DE RECHERCHE ET D'EXPERIMENTATION

REFERENCES ETUDE/ELEMENT D'ESSAI : **B16 0622 / 16-1324**

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Batiment C 4
13009 Marseille

ELEMENT D'ESSAI : PHYCO'DERM - Réf. Gelyma 06 a-2016
Lot : 16 06 070

EVALUATION DU POTENTIEL IRRITANT D'UN ELEMENT D'ESSAI PAR APPLICATION SUR LA MEMBRANE CHORIO-ALLANTOÏDIENNE DE L'ŒUF DE POULE EMBRYONNE

-HET-CAM-

Rapport Final

Bordeaux, le 19 juillet 2016

11 pages dans ce rapport

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EVALUATION DU POTENTIEL IRRITANT D'UN ELEMENT D'ESSAI PAR APPLICATION SUR LA MEMBRANE CHORIO-ALLANTOÏDIENNE DE L'ŒUF DE POULE EMBRYONNE - HET-CAM -

ASSESSMENT OF THE IRRITANT POTENTIAL OF A TEST ITEM AFTER APPLICATION TO THE EMBRYONIC HEN'S EGG CHORIOALLANTOIC MEMBRANE - HET-CAM -

RESUME/*SUMMARY*

- **PRINCIPE DE L'ETUDE/*PRINCIPLE OF THE STUDY***

L'étude a été basée sur l'observation, par une personne qualifiée, des effets irritants (hyperhémie, hémorragie, coagulation) pouvant survenir dans les cinq minutes suivant le dépôt d'un élément d'essai sur la membrane chorio-allantoïdienne (MCA) d'œufs de poule embryonnés au dixième jour d'incubation.
Le potentiel irritant a été scoré selon une échelle allant de 0 à 21. L'élément d'essai a été classé dans l'une des catégories définies en fonction du score moyen obtenu.

The study was based on the observation, by a trained person, of the irritant effects (hyperhemia, haemorrhage and coagulation) occurring during the five minutes after application of test item to the chorioallantoic membrane (CAM) of embryonic hen's eggs on the tenth day of incubation.
The irritant potential was scored according to a scale from 0 to 21. The test item was classified in one of the categories defined according to the mean score obtained.

Score moyen/*Mean Score* (Scm/*MSc*)	**Classification/*Classification***
Scm/*MSc* < 1	Pratiquement non irritant/*Practically non irritant*
1 ≤ Scm/*MSc* < 5	Faiblement irritant/ *Slightly irritant*
5 ≤ Scm/*MSc* < 9	Modérément irritant/*Moderately irritant*
Scm/*MSc* ≥ 9	Irritant/*Irritant*

- **DATE(S) DE DEBUT ET DE FIN D'EXPERIMENTATION / *EXPERIMENTAL STARTING DATE AND EXPERIMENTAL COMPLETION DATE*** : 27 et 30 juin 2016 */ June 27 and 30, 2016*

- **RESULTATS/*RESULTS* :**

Elément d'essai *Test item*	**Concentration testée** *Tested concentration*	**Score moyen sur 4 œufs ± écart type** *Mean score on 4 eggs ±standard deviation*	**Classification** *Classification*	**Comparaison par rapport à des éléments d'essai appartenant à la même catégorie** *Comparison with test items belonging to the same category*
PHYCO'DERM Réf. Gelyma 06 a-2016 - Lot : 16 06 070	Dilué à 10% dans l'eau p.p.i. / *Diluted at 10% with water for injection*	0.8 ± 1.5	pratiquement non irritant / *practically non irritant*	pas de comparaison disponible / *no available comparison*

I. OBJECTIF ET PRINCIPE DE L'ETUDE

Il a été demandé par le donneur d'ordre, l'évaluation semi-quantitative du potentiel irritant de l'élément d'essai, **PHYCO'DERM - Réf. Gelyma 06 a-2016 - Lot : 16 06 070**, par une méthode alternative à l'expérimentation animale après application sur la membrane chorio-allantoïdienne de l'œuf de poule embryonné.

L'étude a été basée sur l'observation, par une personne qualifiée, des effets irritants (hyperhémie, hémorragie, coagulation) pouvant survenir dans les cinq minutes suivant le dépôt de l'élément d'essai sur la membrane chorio-allantoïdienne (MCA) d'œufs de poule embryonnés, au dixième jour d'incubation.

La technique utilisée a été adaptée de celle décrite par Luepke N.P. et Kemper F.H. (The Het-Cam test : « An alternative to the Draize eye test ». Food Chem. Toxicol. 1986, 24, n° 6/7, 495-496).
Cette méthode a suivi celle publiée au Journal Officiel de la République Française du 26 décembre 1996 à l'exception :
- du poids des œufs (entre 40 et 75 g au lieu de entre 50 et 65 g)
- de la destruction des embryons par refroidissement rapide (-20°C) au lieu d'une injection au pentobarbital.

Ces déviations, au texte du Journal Officiel de la République Française du 26/12/1996 n'ont pas eu d'impact sur la validité de l'étude.

Cette méthode est une alternative à l'expérimentation animale entrant dans une batterie de tests qui concourent à l'évaluation du potentiel irritant oculaire des éléments d'essai (notamment à base de tensioactifs).

II. INSTALLATION D'ESSAI ET EQUIPE TECHNIQUE

II.1. Installation d'Essai et équipe technique

Eurofins-Evic France – Division Bio
122 rue Croix de Seguey
33000 Bordeaux

Tél : 05 56 95 59 95

Directeur de l'étude : Julie CHARMETANT

Techniciens responsables : Julie CHARMETANT, Sarah JULIENNE

II.2. Agréments de l'Installation d'Essai

L'étude a été entièrement réalisée dans les locaux de la Division Technique Bio de la Société Eurofins-Evic France, à Bordeaux.
L'Installation d' Essai Evic-Bio a été reconnue conforme aux Bonnes Pratiques de Laboratoire par l'ANSM (arrêté du 10 août 2004 publié au Journal Officiel de la République française (JORF) du 18 septembre 2004 et l'arrêté du 14 mars 2000 publié au JORF du 23 mars 2000) et le GIPC (décret n° 2006-1523 du 4 décembre 2006 publié au Journal Officiel de la République française du 6 décembre 2006).

II.3. Centre Coordinateur

EUROFINS ATS
ZI Les Milles – Actimart
1140, rue Ampère
13851 Aix en Provence Cedex 3
Tel: + 33 442371418
Fax : + 33 442397781
Coordinateur : Nadine GOBERT

III. RECUEIL DES DONNEES

Toutes les données recueillies au cours de l'étude ont été consignées par le technicien responsable de l'étude, sur les documents réservés à cet effet.

Chaque page de ces documents a été paraphée et datée par le technicien responsable de l'étude.
Toute donnée manquante et toute correction ont été justifiées, paraphées et datées.

En fin d'étude, les documents de travail ont été archivés avec le rapport final et seront conservés pendant 3 ans dans une salle d'archives validée par le service Assurance Qualité de l'Installation d'Essai.
Cette salle d'archives est située chez un prestataire qui n'est pas soumis au champ d'application des BPL, mais qui garantit la qualité et l'intégrité des documents et des données de l'étude, et qui fait l'objet d'audits réguliers par le service Assurance Qualité.

A l'issue de cette période, l'Installation d'Essai définira avec le donneur d'ordre, la poursuite de l'archivage, la restitution des données ou leur destruction.

IV. MODIFICATIONS AU PLAN D'ETUDE

Dans cette étude aucune modification au plan d'étude PEG.HC.01/13(11)-ANSM n'a fait l'objet d'un amendement.

V. ELEMENT D'ESSAI

V.1. Référence de l'élément d'essai

Identification Eurofins-Evic France	: 16-1324
Date de réception	: 21 juin 2016
Identification	: PHYCO'DERM - Réf. Gelyma 06 a-2016 Lot : 16 06 070
Catégorie de l'élément d'essai	: ingrédient cosmétique
Description/Aspect	: liquide jaune orangé
Conditions de stockage	: à température ambiante et à l'abri de la lumière dans un local spécialement aménagé à cet effet
Particularité	: aucune
Quantité transmise par le donneur d'ordre	: 1 flacon en plastique de 30 ml
Date de péremption	: 01 décembre 2017
Autre information fournie	: formule

Stable dans les conditions de stockage et d'essai.

VI.2. Archivage

L'élément d'essai ou le reste de l'élément d'essai a été archivé dans l'échantillothèque de l'Installation d'Essai et sera conservé pendant une période de 6 mois.

VI. SYSTEME D'ESSAI

Œufs de poule EOPS embryonnés de souche White Leghorn d'un poids supérieur ou égal à 40 g et inférieur ou égal à 75 g, fournis par : INRA Plate-forme d'infectiologie expérimentale (PFIE) - Zone volailles-lapins EOPS - 37380 NOUZILLY.

VI.1. Réception des œufs

A réception, les œufs ont été observés un à un. Les œufs fêlés ou cassés ont été éliminés.
Les oeufs ont été alors pesés. Les oeufs dont le poids n'était pas dans la norme fixée ont été éliminés.
Les œufs ont été identifiés par un numéro de lot (semaine/année) qui a été attribué aux œufs à réception.

Les œufs retenus ont été identifiés individuellement par le jour de mise en couveuse et ont été placés dans une enceinte thermostatée à 12 ± 1°C, à l'abri de la lumière, pendant au moins 24 heures avant la mise en couveuse.

VI.2. Mise en couveuse

Les œufs ont été placés dans une couveuse dans des conditions contrôlées de température (37,8 ± 1°C) et d'humidité relative (50 à 60 %).

Au cours de leur période d'incubation de 10 jours, les œufs ont été placés dans une couveuse à plateaux oscillants permettant un balancement automatique. Ils ont été placés en position verticale (poche d'air en haut).

VII. DATES DE L'ETUDE

Date de début d'étude (signature du complément spécifique au plan d'étude): 27 juin 2016

Date de début d'expérimentation : 27 juin 2016

Date de fin d'expérimentation : 30 juin 2016

Date de fin d'étude (signature du rapport final) : 19 juillet 2016

VIII. MODE OPERATOIRE

VIII.1. Préparation de l'élément d'essai

L'élément d'essai a été testé dilué à 10% dans l'eau p.p.i.

L'opération de dilution a été faite extemporanément en poids/poids dans l'eau pour préparation injectable (Cooper batch 19IF09GA) avec une balance de précision.

Aspect de l'élément d'essai après dilution : liquide transparent incolore.

La dilution de l'élément d'essai a été amenée à 37 ± 1°C avant utilisation.

VIII.2. Chronologie expérimentale

VIII.2.1. Préparation des œufs

Les différentes étapes de l'étude ont été enchaînées rapidement sous un éclairage d'une intensité constante ne dégageant pas trop de chaleur pour ne pas dessécher la membrane chorio-allantoïdienne.

Au 10ème jour d'incubation, les œufs ont été sortis de l'incubateur et ont été mirés sous éclairage. Les œufs défectueux (image ne correspondant pas au stade de développement attendu) ont été éliminés et les œufs sélectionnés ont été posés sur le support « poche d'air » vers le haut.

La coquille de chaque œuf sélectionné a été percée (à l'aide d'une aiguille lancéolée), ouverte et découpée (à l'aide d'une pince et d'une paire de ciseaux à bouts ronds) au niveau de la poche d'air et jusqu'aux limites de la membrane coquillière.

Toute la surface de la membrane coquillière a été alors humidifiée avec une solution de chlorure de sodium à 0,9 %, tiédie à 37 ± 1°C (bain-marie). L'excès de solution de chlorure de sodium à 0,9 % a été ensuite éliminé par inclinaison de l'œuf et la membrane coquillière a été décollée délicatement avec une pince afin de découvrir la MCA sous-jacente.

Tout œuf dont la membrane chorio-allantoïdienne est apparue abîmée (déchirure, présence de traces d'hémorragie ou toute autre lésion) a été immédiatement rejeté.

VIII.2.2. Application de la dilution de l'élément d'essai

La dilution de l'élément d'essai a été testée sur 4 oeufs.

300 µl ont été déposés sur la MCA avec une micropipette (P1000).

Aussitôt après application, le chronomètre a été déclenché.

VIII.2.3. Lectures

Après 20 secondes de contact, la MCA a été rincée avec 5 ml de solution isotonique de chlorure de sodium (maintenu à 37 ± 1°C au bain-marie), à l'aide d'une seringue en évitant toute projection brutale.

Le liquide de rinçage a été éliminé par inclinaison de l'œuf.

Pendant un temps de 5 minutes, ont été observés, les éventuels phénomènes d'irritation selon le procédé décrit au paragraphe suivant. Le temps exact d'apparition de chaque phénomène a été relevé.

Les 20 secondes de contact ont été comprises dans les 5 minutes d'observation.

En fin d'essai, les embryons ont été détruits par refroidissement rapide (enceinte à -20°C).

VIII.2.4. Procédé de lecture

Les observations prises en compte pour la notation de l'élément d'essai ont été réalisées à l'œil nu, sous la lampe.
Les phénomènes observés (hyperhémie, hémorragie, coagulation) n'ont pas été retenus en fonction de leur intensité mais en fonction de leur présence : réponse de type tout ou rien.

Le temps a été noté à l'apparition de chacun des phénomènes.

Les phénomènes observés se définissaient ainsi :

Hyperhémie

Phénomène observé : des capillaires non visibles avant l'ajout du produit deviennent visibles, alors que les capillaires visibles se dilatent et deviennent plus rouges. Ce phénomène peut également affecter les vaisseaux de diamètre supérieur.

Hémorragie

Phénomène observé : libération de sang s'échappant des vaisseaux et/ou des capillaires, pouvant se présenter sous différents aspects, et notamment en « chou-fleur », en nappe, en voile diffus, en piqueté (le sang s'échappe ponctuellement à différents endroits de la membrane).

Il est à noter que :
- l'hémorragie peut présenter un caractère éphémère ; elle doit néanmoins être prise en compte,
- l'observation, dans les 30 premières secondes, d'une hémorragie massive impose la prise en compte de l'hyperhémie masquée.

Coagulation (opacité et/ou thrombose)

Opacité

Phénomène observé : apparition sur tout ou partie de la membrane, soit d'un voile opalescent évoluant éventuellement vers une opacification, soit d'une opacification directe.
Il est nécessaire de vérifier que le phénomène n'est pas lié au comportement physico-chimique du produit en milieu aqueux (par exemple formation d'un colloïde, d'un précipité, ...).

Thrombose

Phénomène observé : rupture du flux sanguin dans les vaisseaux se traduisant par un aspect segmenté (alternance d'étranglements et de zones turgescentes plus ou moins sombres).

Il est à noter que les observations ne doivent pas prendre en compte les modifications intervenues au niveau des capillaires.

VIII.3. Expression et interprétation des résultats

Les phénomènes observés ont été quantifiés selon le tableau ci-après, en fonction de leur délai d'apparition :

Phénomène	Temps		
	$t \leq 30$ s	30 s $< t \leq$ 2 min	2 min $< t \leq$ 5 min
Hyperhémie	5	3	1
Hémorragie	7	5	3
Coagulation	9	7	5

Chaque phénomène observé n'a été compté qu'une seule fois, au temps où il est apparu.

Le score pour chaque œuf a été la somme des notes d'hyperhémie, d'hémorragie et de coagulation. La notation de l'élément d'essai a été la moyenne arithmétique des scores obtenus sur 4 œufs, arrondie à une décimale (notation maximale = 21).

Le potentiel irritant sur la membrane chorio-allantoïdienne de l'élément d'essai a été donné par l'échelle suivante :

Score moyen (Sc m)	Classification
Sc m < 1	Pratiquement non irritant
1 ≤ Sc m < 5	Faiblement irritant
5 ≤ Sc m < 9	Modérément irritant
Sc m ≥ 9	Irritant

IX. CONTROLE DU SYSTEME D'ESSAI, DES CONDITIONS OPERATOIRES ET DES EXPERIMENTATEURS

IX.1. Contrôle négatif

Ce contrôle a été effectué au moyen d'une solution isotonique de NaCl à 0.9% (n° lot 19ID10GA) préalablement chauffée à 37 ± 1°C avant l'utilisation des œufs.

Le contrôle a été jugé conforme s'il donnait un score compris entre 0.0 et 3.0.

IX.2. Contrôle positif

Une vérification de la qualité du système d'essai, des conditions opératoires et des expérimentateurs a été réalisée à l'aide d'une référence.

Ce contrôle a été effectué en réalisant, en aveugle, une courbe étalon au moyen de solutions de lauryl sulfobétaïne préalablement chauffées à 37 ± 1°C (Sigma, lot 1421027V) à 0.05 %, 0.4 % et 3.2 % dans de l'eau pour préparations injectables (Cooper, lot 19IF09GA).

Le contrôle a été jugé conforme si:

- la concentration 0,05 % donnait un score compris entre 0.0 et 5.0
- la concentration 0,4 % donnait un score compris entre 10.5 et 12.5
- la concentration 3,2 % donnait un score compris entre 17.0 et 21.0

Les scores des contrôles positifs et négatifs sont indiqués dans le tableau ci-après.

IX.3. Résultats des contrôles

Lot	Contrôle	Conc. (%)	Date	Scorage	Score moyen	±	Deviation Standard
Semaine 26/16	NaCl 0.9 %	Tel quel	27 juin 2016	Sur 2 oeufs (Contrôle négatif)	0.0	±	0.0
02/16	Lauryl sulfobetaine	3.2 %	06 juin 2016	Sur 4 oeufs	20.5	±	0.0
02/16	Lauryl sulfobetaine	0.4 %	06 juin 2016	Sur 4 oeufs	12.0	±	0.0
02/16	Lauryl sulfobetaine	0.05 %	06 juin 2016	Sur 4 oeufs	0.0	±	0.0

X. RESULTATS

Œuf	**Injection** Cotation en fonction du temps ≤ 30 sec = 5 ≤ 2 min = 3 ≤ 5 min = 1			**Hémorragie** Cotation en fonction du temps ≤ 30 sec = 7 ≤ 2 min = 5 ≤ 5 min = 3		**Coagulation** Cotation en fonction du temps ≤ 30 sec = 9 ≤ 2 min = 7 ≤ 5 min = 5			**Score par œuf**
	Observée/ Masquée	temps (s)	note	temps (s)	note	Opacité/ Thrombose	temps (s)	note	
1	/	/	0	/	0	/	/	0	0
2	/	/	0	/	0	/	/	0	0
3	/	/	0	/	0	/	/	0	0
4	observée	102	3	/	0	/	/	0	3
Moyenne			0.8		0.0			0.0	**0.8**
Ecart-type			1.5		0.0			0.0	**1.5**

XI. CONCLUSION

Selon l'échelle de cotation définie, l'élément d'essai **PHYCO'DERM - Réf. Gelyma 06 a-2016 - Lot : 16 06 070** testé dilué à 10% dans l'eau p.p.i, a été jugé **pratiquement non irritant** vis-à-vis de la membrane chorio-allantoïdienne de l'oeuf de poule embryonné.

Elément d'essai	Concentration testée	Score moyen obtenu sur 4 oeufs ± écart type	Classification
PHYCO'DERM - Réf. Gelyma 06 a-2016 - Lot : 16 06 070	Dilué à 10% dans l'eau p.p.i.	0.8 ± 1.5	pratiquement non irritant

Par manque de recul sur cette catégorie de produit (ingrédient cosmétique), la réponse obtenue pour cet élément d'essai ne peut être comparée à la base des données acquises au sein de l'Installation d'Essai..

XII. ATTESTATION DU DIRECTEUR DE L'ETUDE

L'étude **B16 0622** était destinée à évaluer le potentiel irritant de l'élément d'essai **PHYCO'DERM - Réf. Gelyma 06 a-2016 - Lot : 16 06 070**, consécutivement à son application sur la membrane chorio-allantoïdienne de l'œuf de poule embryonné (méthode Het-Cam).

La technique utilisée a été adaptée de celle décrite par Luepke N.P. et Kemper F.H. (The Het-Cam test : « An alternative to the Draize eye test ». Food Chem. Toxicol. 1986, 24, n° 6/7, 495-496).

La méthodologie a suivi celle publiée au Journal Officiel de la République Française du 26 décembre 1996.

Je soussignée, **Julie CHARMETANT**, déclare que l'étude s'est déroulée sous ma responsabilité.

Le rapport final reflète de façon exacte et complète les procédures et les données générées au cours de l'étude.

Julie CHARMETANT

Directeur d'étude

Date : 19.07.2016

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: June 11, 2020

SUBJECT: Gelidium Cartilagineum Extract

Biotech Marine. 2020. Manufacturing Process Rhodysterol™ S Sur Base Triglycerides (Gelidium Cartilagineum Extract).

Biotech Marine. 2016. Statement Rhodysterol™ S Sur Base Triglycerides (Gelidium Cartilagineum Extract) Composition file.

Laboratoire Cosderma. 2005. Verification in humans of cutaneous compatability of a cosmetic product after a single application under patch (Rhodysterol™ Sur Base Triglycerides (Gelidium Cartilagineum Extract)).

Liskin. 2009. Etude du pouvoir sensibilasant d'un produit selon la methode de Marzulli-Maibach (Rhodysterol™ Sur Base Triglycerides (Gelidium Cartilagineum Extract)).

MANUFACTURING PROCESS

RHODYSTEROL™ S SUR BASE TRIGLYCERIDES

HARVESTING / IDENTIFICATION *(Gelidium Cartilagineum)*

↓

DRYING

↓

GRINDING

↓

EXTRACTION WITH THE SOLVENT CAPRYLIC/CAPRIC TRIGLYCERIDE ← Addition of sterol

↓

FILTRATION

↓

QUALITY CONTROL

↓

PACKAGING

↓

QUALITY CONTROL

Production Manager
Jean-Marc CATROUX

11/11/16

BiotechMarine - Z.I. - B.P.72 - 22260 Pontrieux (FR)
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www.biotechmarine.com

Statement RHODYSTEROL™ S SUR BASE TRIGLYCERIDES COMPOSITION FILE

(In accordance with EN 45014 General criteria for supplier's declaration of conformity)

We declare, by the present one, that the following product supplied by BIOTECHMARINE:

RHODYSTEROL™ S SUR BASE TRIGLYCERIDES

(INCI NAME (USA): Caprylic Capric / Triglyceride - Gelidium Cartilagineum Extract - Phytosterols)

Composition

Components	Components usual Name / family name	Function	% (Concentration range)
Caprylic / Capric Triglyceride	Glycerides, mixed decanoyl and octanoyl	Solvent	> 96.0
Gelidium Cartilagineum Extract	Algae extract	Active	< 2.0
4-cholesten-3-one	Phytosterols	Active	1.5 - 2.0

This certificate was established to the best of our knowledge and/or according to our suppliers' statements, at the date of this certificate. It is however your responsibility to abide by any clause of any regulations, which may apply to the product that you manufacture, or to the use you make of our product.

Pontrieux, on 01/11/2016

Guénolé LE CALVEZ.
Managing Director

Nota

The analytical specifications warranted are only those mentioned on the certificate of analysis supplied with each delivery of the product.
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REPORT

VERIFICATION IN HUMANS OF CUTANEOUS COMPATIBILITY OF A COSMETIC PRODUCT AFTER SINGLE APPLICATION UNDER PATCH

Patch test

Study product

RHODYSTEROL SUR BASE TRIGLYCERIDES
BATCH 5.05.154

Sponsor: **BIOTECHMARINE**
ZI – BP 72
2260 PONTRIEUX

For the attention of Ms. Nicole MEKIDECHE

Bordeaux, 27 juin 2005

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Laboratoire COSDERMA - Service de dermatologie Pr. Taïeb - Groupe Hospitalier Saint André -1 rue Jean Burguet • BP 50057 - 33023 Bordeaux Cedex Tel: +33 (0)5 56 94 75 40 - Fax: +33 (0)5 56 79 49 75 email: laboratoire@cosderma.com

1- STUDY OBJECTIVE

The objective of this study was to evaluate the cutaneous compatibility of a product "**RHODYSTEROL SUR BASE TRIGLYCERIDES BATCH 5.05.154"** after a single application on the skin for 24h in 10 volunteers.

The cutaneous compatibility of the product was verified by a visual examination of the experimental zone carried out by the investigator or the technician under his/her authority.

Given that the study aims at obtaining greater knowledge on the cutaneous compatibility of the test product and the predictable risk incurred by the patients who participated in the studies was minimal, the relation between the study objective and the potential risks was adequate.

2- METHODOLOGY

2.1 Experimental design

This study was without direct individual benefit, each participating volunteer was his/her own control. It was an open monocentre study. The duration of the study was 24h and included 2 visits to the Institute (D0, T24h).

D0	T24h
Inclusion	Patch removal
Application of patch	Visual examination

2.2 Investigator centre

Laboratoire COSDERMA

Dermatology department headed by 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 site

Dermatology department headed by Pr Taïeb
Groupe Hospitalier Saint André
1 rue Jean Burguet
33000 Bordeaux

2.4 Technical team

Investigator:	Jérôme Asserin
Technician	Nadège Durand

3- DATE OF PERFORMANCE OF THE STUDY

Start: 15 June 2005

End: 16 June 2006

4- STUDY PRODUCT

4.1 Information

The information transmitted by the sponsor accompanying the samples was the commitment letter concerning in particular the compliance of the formula with the current regulations and its safety.

4.2 Identification

Name	Ref.	Quantity for the study	Packaging n°.
RHODYSTEROL SUR BASE TRIGLYCERIDES	BATCH 5.05.154	5ml	1x5ml

4.3 Normal use conditions

Name	Instructions for use	
RHODYSTEROL SUR BASE TRIGLYCERIDES BATCH 5.05.154	site	face
	Application once daily on the face	

4.4 Conditions of use during the study

Product name	Type of dressing	Application conditions	Contact time	Quantity applied	Control time after patch removal
RHODYSTEROL SUR BASE TRIGLYCERIDES BATCH 5.05.154	Occlusive (Finn chambers®)	Diluted at 10% in water	24h	**20 µl**	**D1/T15**

Finn Chambers®: Occlusive dressing composed of an aluminium cup, 8 mm in diameter (area 50 mm^2) on which 20 µl (20 mg) of product are deposited.

A control patch, corresponding to the type of dressing used, containing an ad hoc quantity of water for injection, was applied in parallel.

The patch removal was performed by the investigator or the technician under his responsibility.

The amount of product was measured using a disposable syringe.

5- VOLUNTEERS

5-1 Panel

The panel of volunteers participating in the study was representative of the population likely to use the product. All the volunteers selected complied with the inclusion and non-inclusion criteria.

5-2 Number of subjects

The number of volunteers participating in the study was of **10**.

The number of volunteers for which the data are presented is of **10**.

5-3 Inclusion criteria

The inclusion criteria were the following:

- age: 18 to 65 years
- sex: female and/or male
- phototype (Fitzpatrick): I to III
- all skin types

All the volunteers satisfied the inclusion criteria. The typological characteristics of the volunteers are presented in **Appendix 1.**

5-4 Non-inclusion criteria

The non-inclusion criteria were the following:

- Skin marks in the experimental zone that could interfere with the evaluation of the skin reactions (pigmentation disorders, scars, excess hair, too many freckles or naevi, sunburn, etc.)
- Eczema type reaction that has not completely disappeared, scar or pigment sequels from previous tests at the level of the experimental zone
- Rosin and nickel allergy
- Allergy or reactivity to the same category of products
- Cutaneous hyper-reactivity
- Reactivity to ethanol, adhesive plaster
- Participation in more than 5 tests using maximisation including 3 or more for hypoallergenicity tests in the 12 months preceding the study.
- Intense sun exposure in the month preceding the study
- Expected intense sun exposure (to natural sun or UVA cubicle) for the duration of the study

- Intention to have baths in a bathtub, in the sea or a swimming pool, to have saunas or Turkish baths during the test period
- Intensive or regular practice of one or several sports for which a temporary interruption could be problematical
- Having stopped a treatment based on vitamin A acid or its derivatives since less than 3 months before the start of the study
- Having stopped a topical corticosteroid treatment on the experimental zone less than 8 days before the study
- Having stopped a PUVA or UVB treatment based since less than 1 month before the study
- Scheduled vaccination during the test period, last vaccination in the 3 weeks before the study

No volunteer corresponding to these criteria was included.

5-5 Study constraints

The study constraints were the following:

- No application of products other than those tested on the experimental zone
- No wearing of too tight clothing or resulting in contention at the level of the experimental zone, likely to cause rubbing and detachment of the patch
- No baths in a bathtub, in the sea or a swimming pool, no saunas or Turkish baths during the study
- Protection of the experimental zone during shower, no violent projection of water and no soaping of this zone to avoid the detachment of the patch or appearance of intercurrent phenomena and wipe very gently if necessary,
- No excessive perspiration and no intensive physical activity likely to result in the detachment of the dressing
- No intense sun exposure (to natural sun or UVA cubicle) for the duration of the study, especially once the dressing is removed
- Maintenance of face and boy hygiene habits,
- Maintenance of make-up habits,
- No anti-allergy, anti-inflammatory (systemic or topical corticosteroid therapy) treatment or with vitamin A acid or its derivatives based proprietary medicinal products the day of the study (in case of therapeutic need: withdrawal from study envisaged)

All the study constraints were respected by the volunteers.

5-6 Control of compliance with protocol procedures

The investigator verified if the constraints had been respected.

The summary of the answers to the different questions asked is attached in Appendices 2-1 and 2-2.

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

The volunteers complied with all the constraints of the study, defined in the protocol.

6- EVALUATION

6-1 Schedule

	START	CONTACT TIME
Day	D0	T24h + 2h
Selection of volunteers	X	
Attribution no. of volunteers	X	
Volunteer information	X	
Signed informed consent *	X	
Application of dressings by technician	X	
Patch removal by technician		X
Evaluation criteria (15 min after patch removal)		X

The fact that the application of the product as well as the clinical examinations had been perfectly controlled, the number of volunteers and the duration of the study allowed the verification of the cutaneous compatibility of the test product and assess any irritation phenomena.

A copy of the participation consent was given to the volunteers on the day of the study inclusion visit. The original is kept by the investigator.

6-2 Evaluation of cutaneous compatibility

- Principle and bibliography

Cutaneous compatibility is verified via the application of dressings on the skin which create a certain occlusion of the product and favour their penetration. Under maximising experimental conditions, the irritant potential of products may be more easily revealed.

The methodology has been discusses in numerous publications, such as:

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 model. Seifen-öle-fette-wachse, Matthies W, 1991, 117, 42-43

The Duhrino 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 are placed on the dressings, extemporaneously, using a 1 ml syringe. The dressings are applied afterwards on the skin as quickly as possible carefully avoiding zones exposed to rubbing or compression. The investigator or technician under his authority will verify that the skin zone concerned is free of the presence of moles, scares and skin accidents. The type of dressing, the maximum number of possible test products, the amount of product to apply, the application methodology and the removal of the dressings and the visual clinical examination comply with the reference laboratory procedures for this type of study. The application site of the products chosen is the back.

- Environmental conditions

The environmental conditions imposed to the volunteers are the following:

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

Clinical examination

- -Sites

The investigator or the technician under his authority carries out a visual control of each experimental zone under a "daylight" type standardised lightning.

- Frequencies

The visual examination is performed at T24h ± 2h, 15 minutes after the patch removal (or more if redness appeared on removal of the patch).

- Evaluation criteria

  - Clinical signs

Description	Laboratory code	Intensity	Appearance	Score
Erythema	E	- ordinal 3 point scale: • mild • moderate • severe	- erythema: • diffuse • punctuated • peripheral	• mild = 1 • moderate = 2 • severe = 3 • diffuse = d • punctuated = p • peripheral = peri
Oedema	Oe			
Dryness	D			
Colour	C			
Comedone, microcyst	Co, Mi	counted		
Vesicle, papulla	V, Pa	ordinal 2 point scale: • 1 to 2 vesicles • vesicles in number > 2		• 1 to 2 = 1 • n° >2 =2
Bulla, scrab	Bu, Cr	described		if described = 2

The investigator or the technician under his authority recorded any clinical sign, its location, intensity, evolution, and any medical treatment undertaken. He establishes the usual or unusual character of the clinical sign, questioning the volunteer on what he/she observes in daily life, with the use of similar products.

- Sensations of discomfort

DESCRIPTION	LABORATORY CODE	INTENSITY	SCORE
Heating	H	ordinal 3 point scale: • mild • moderate • severe	• mild = 1 • moderate = 2 • severe = 3
Stinging	St		
Pruritus (itching)	Pr		
Tightness	Ti		
Burning	Br		

- Expression of results

All the volunteers who underwent the visit D0 were taken into account for the evaluation of the cutaneous compatibility. The expression of the results of the cutaneous examination and the interrogation complied with the reference laboratory procedure for this type of study.

The individual results are expressed:

- **in percentage of reactive volunteers** taking into account for this calculation only the visually detectable clinical signs such as erythema, oedema, vesicle, bulla, papulla and scab.
- **in a descriptive manner** for the other visually detectable signs or the discomfort sensations, the percentage of volunteers for whom they were observed, could be calculated if the frequency of appearance of these signs justifies it.
- **in cutaneous irritation score** calculated from the "scores" attributed to the visually detectable clinical signs.

For each volunteer and at each observation time, an individual daily irritation score (IdiS), which is sum of notes obtained for the signs observed was calculated.

For the panel and at each observation time, a mean daily irritation score (MdiS) was calculated which corresponds to the following formula:

$$MdiS = \Sigma (IdiS)/\text{Number of volunteers taken into account}$$

The maximum mean irritation score (MiSMax), defined as the highest mean irritation score among the scores obtained at the different experimental times was recorded.

- Interpretation of results

The investigator reached a conclusion in terms of the **very good, good, average or bad** cutaneous compatibility in an absolute manner. The interpretation of the results of the cutaneous examination and the interrogation complied with the reference laboratory procedure for this type of study.

7- RESULTS AND DISCUSSION

The individual data of the cutaneous examination and the questioning of the volunteers are attached **in Appendix 3**.

In summary:

Product name	Control time after patch removal	Number of reactive volunteers	Types of reaction	Mean daily irritation score MdiS	% of reactive volunteers
RHODYSTEROL SUR BASE TRIGLYCERIDES BATCH 5.05.154	T15	0	/	0	0%

8- CONCLUSION

Under the experimental conditions adopted, the product "RHODYSTEROL SUR BASE TRIGLYCERIDES BATCH 5.05.154" has a very good cutaneous compatibility.

Signatures and dates:

Pierre Boussault (Dermatologist)
Investigator

(Original signed)

Nadège Durand
Clinical Assistant

(Original signed)

Nicole MEKIDECHE
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APPENDICES

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APPENDIX 1

TYPOLOGICAL CHARACTERISTICS OF THE VOLUNTEERS

Volunteers		Age (years)	Sex F = female	Phototype *	Healthy skin on the back
Ref.	Surname / First name				
1	LARR/S	22	F	III	X
2	GUER/A	26	F	III	X
3	FENE/S	19	F	II	X
4	RABO/S	22	F	II	X
5	GIAC/C	27	F	II	X
6	PRIE/E	27	F	II	X
7	PARC/S	45	F	III	X
8	COLI/M	24	F	III	X
9	TASS/C	22	F	III	X
10	RATI/N	23	F	III	X

Legends: / = n *x = yes*

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

TYPE	HAIR	SKIN	FRECKLES	SUNBURN
I	Red	milky	+++	Constant / no tan
II	Blond	light	++	Frequent / light tan
III	Light / brown	light	+	Inconstant / light to dark tan
IV	Brown	dark	0	None / dark tan
v	Black and curly	black	0	0

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APPENDIX 2-1

CONTROL OF THE COMPLIANCE

Constraints

Constraints (10 results exploited)	Number of volunteers who respected the constraints	Percentage of volunteers who respected the constraints
No application of products (other than those tested) on the experimental zone Deviation: none	10	100%
No wearing of too tight clothing or resulting in contention at the level of the experimental zone, likely to cause rubbing and detachment of the patch Deviation: none	10	100%
No baths (in a bathtub, in the sea or a swimming pool) and no saunas or Turkish baths during the study Deviation: none	10	100%
In case of shower, protection of the experimental zone, or no violent projection of water and no soaping of this zone to avoid the detachment of the patch or appearance of intercurrent phenomena and wipe very gently if necessary, Deviation: none	10	100%
No excessive perspiration and no intensive physical activity likely to result in the detachment of the dressing Deviation: none	10	100%

APPENDIX 2-2

CONTROL OF THE COMPLIANCE

CONSTRAINTS

Constraints (10 results exploited)	Number of volunteers who respected the constraints	Percentage of volunteers who respected the constraints
No intense sun exposure (to natural sun or UVA cubicle) for the duration of the study, especially once the dressing is removed Deviation: none	10	100%
No anti-allergy, anti-inflammatory (systemic or topical corticosteroid therapy) treatment or with vitamin A acid or its derivatives based proprietary medicinal products during the study – no medicine that may interfere with the study Deviation: none	10	100%
No vaccination during the study Deviation: none	10	100%

APPENDIX 3-1

VERIFICATION OF CUTANEOUS COMPATIBILITY
PRODUCT: "RHODYSTEROL SUR BASE TRIGLYCERIDES BATCH 5.05.154"
(FINN CHAMBERS)

Volunteers		T15	
Ref.	**Surname/first name**	**Cutaneous examination**	**IdiS**
1	LARR/S	/	/
2	GUER/A	/	/
3	FENE/S	/	/
4	RABO/S	/	/
5	GIAC/C	/	/
6	PRIE/E	/	/
7	PARC/S	/	/
8	COLI/M	/	/
9	TASS/C	/	/
10	RATI/N	/	/
MdiS		0	

Legends: / = no clinical sign

APPENDIX 3-2

VERIFICATION OF CUTANEOUS COMPATIBILITY
PRODUCT N°2 : CONTROL (FINN CHAMBERS)

Volunteers		T15	
Ref.	**Surname/first name**	**Cutaneous examination**	**IdiS**
1	LARR/S	/	/
2	GUER/A	/	/
3	FENE/S	/	/
4	RABO/S	/	/
5	GIAC/C	/	/
6	PRIE/E	/	/
7	PARC/S	/	/
8	COLI/M	/	/
9	TASS/C	/	/
10	RATI/N	/	/
MdiS		0	

Legends: / = no clinical sign

LISKIN

ETUDE DU POUVOIR SENSIBILISANT D'UN PRODUIT SELON LA METHODE DE MARZULLI-MAIBACH

RAPPORT

ETUDE REF.	**ET-374**
PRODUIT	**«RHODYSTEROL BASE TRIGLYCERIDES LOT 8.06.174»**
NOMBRE DE SUJETS	**50**
PROMOTEUR	**BIOTECHMARINE**
COMMANDITAIRE	**EUROTEST**
MONITEUR	**M. 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**
INVESTIGATEUR	**Dr Marlena Nowakowska, médecin dermatologue**

DOCUMENT CONFIDENTIEL - PROPRIETE DU PROMOTEUR

Document comportant 23 pages

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RESUME DE L'ETUDE

TITRE : ETUDE DU POUVOIR SENSIBILISANT D'UN PRODUIT «RHODYSTEROL BASE TRIGLYCERIDES LOT 8.06.174», SELON LA METHODE DE MARZULLI-MAIBACH SUR 50 SUJETS PENDANT 6 SEMAINES.

REFERENCE DE L'ETUDE : ET-374

PRODUIT : RHODYSTEROL BASE TRIGLYCERIDES LOT 8.06.174

REALISATEUR DE L'ETUDE : L'étude a été réalisée et les valeurs numériques saisies par l'Unité Clinique PROCOS, localisée en Pologne ; ul. Słowackiego 27/33 lok. 33/34 ; 01-592 Varsovie.

INVESTIGATEUR : Dr Marlena NOWAKOWSKA

MONITEUR DE L'ETUDE : Dr ing. Bogdan WICHROWSKI

PROTOCOLE : TEST DE MAXIMALISATION SELON MARZULLI-MAIBACH POUR UN PRODUIT

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.

SUJETS : 50 volontaires à peau normale correspondant aux critères d'inclusion et de non-inclusion déterminés par LISKIN.

PERIODE DE L'ETUDE : 06/04/09 - 15/05/09

PLAN EXPERIMENTAL : Etude monocentrique en simple aveugle.

PRINCIPAUX PARAMETRES DE TOLERANCE :

- <u>Potentiel irritant (phase d'induction)</u>
  Erythème, œdème, sécheresse, vésicules, évalués par le dermatologue selon un score de 0 à 3
- <u>Potentiel sensibilisant (phase de révélation)</u>
  Réaction évaluée par le dermatologue selon un score de 0 à 3 établis par l'ICDRG (International Contact Dermatitis Research Group)

RESULTATS :

Dénomination du produit	Potentiel irritant	Potentiel sensibilisant
RHODYSTEROL BASE TRIGLYCERIDES LOT 8.06.174	Score moyen de 0,000 = non irritant	Aucune réaction de type allergique

CONCLUSION :

Dans les conditions de cette étude, le produit «RHODYSTEROL BASE TRIGLYCERIDES LOT 8.06.174» s'est avéré non irritant et non sensibilisant.

1. ASSURANCE QUALITE

L'étude a été réalisée selon les règles de Bonnes Pratiques Cliniques définies par la FDA (FR du 8/08/1978. Part V - Décret n° 77N-0278), 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.
L'étude a été menée selon les procédures opératoires standards et selon le protocole de l'étude défini par le promoteur de l'étude. Les cahiers d'observation et les journaux de suivi ont été vérifiés ainsi que l'exactitude des données.

L'authenticité et la véracité des données expérimentales recueillies ont été confirmées par les personnes ayant participé à l'étude. Voir ANNEXE I.

2. CERTIFICAT DE CONFORMITE

A ma connaissance, l'étude ET-374 a été conduite en accord avec l'«**Assurance qualité**» précitée.
Il ne s'est pas produit d'événement susceptible d'affecter la qualité ou l'intégrité des données.

Dr ing. B WICHROWSKI
Moniteur

26 Mai 2009
date

3. METHODOLOGIE

3.1. DESCRIPTION DE L'ETUDE

3.1.1. Produit à l'étude

Le produit fourni par EUROTEST, présente les caractéristiques suivantes :

Dénomination du produit QI	Nature de produit	Code du produit dans l'étude
RHODYSTEROL BASE TRIGLYCERIDES LOT 8.06.174	huile incolore transparente	QI

Le produit a été réceptionné le 30/03/2009.

3.2. METHODES CLINIQUES

3.2.1. Objectifs de l'étude

Apprécier le pouvoir irritant et sensibilisant du produit par la méthode de sensibilisation de Marzulli-Maibach.

3.2.2. Plan expérimental

L'étude a été réalisée en ouvert.

3.2.3. Sujets de l'étude

Critères d'inclusion

- Volontaire sain d'origine caucasienne,
- Age compris entre 18 et 65 ans,
- Phototype II, III et IV,
- Peau normale,
- 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 LISKIN.

Critères d'exclusion

- Femme enceinte ou qui allaite,
- Personne s'étant exposée au soleil ou aux U.V. depuis moins d'un mois et /ou ayant reçu des photo patch tests depuis moins de deux mois,
- Sujet présentant une peau hyper irritable,
- Sujet allergique au sparadrap et /ou aux produits cosmétiques,
- Sujet ayant une pathologie cutanée sur la zone d'expérience,
- Sujet atteint d'une maladie grave ou évolutive,
- Sujet suivant un traitement médicamenteux topique ou systémique :
  - anti-inflammatoires, anti-histaminiques, immunosuppresseurs, corticoïdes et rétinoïdes.

Inclusion

Cinquante sujets volontaires ont été choisis en accord avec les critères d'inclusion et les critères d'exclusion, et 50 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.

	Non inclus	Inclus	Arrêt en cours d'étude	Perdus de vue
Nombre de sujets	0	50	0	0

Caractéristiques des sujets

Le tableau récapitulatif ci-dessous présente une synthèse des observations concernant uniquement les volontaires inclus dans l'analyse des données.

Nombre de volontaires	Sexe	Age (moy±SEM)	Phototype	Evénements médicaux ou chirurgicaux et traitements médicaux	
				avant l'étude	pendant l'étude
50	46 F 4 M	41 ± 2	I : 0 II : 50 III : 0	cf. Tableaux en ANNEXE II	

3.3. MATERIEL

Les patch-tests utilisés sont les FINN CHAMBERS ON SCANPOR® large qui assurent une bonne occlusion.

Le FINN CHAMBER constitue une cupule d'isolement qui assure une bonne occlusion.

Après application du produit sur le patch, ce dernier est appliqué au niveau de la zone scapulaire des volontaires.

4. APPLICATION DES PRODUITS

Le produit est appliqué sur le dos.

Zones d'application	Zones scapulaires : homolatérale (zone d'induction) et controlatérale (zone de révélation)
Quantité appliquée Concentration	25 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	Le produit «RHODYSTEROL BASE TRIGLYCERIDES LOT 8.06.174» a été déposé dans une cupule 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.

5. DEROULEMENT DE L'ETUDE

L'étude a été réalisée selon le schéma suivant :

Phase d'induction - trois semaines (S1, S2, S3)

S1 :

Jour de la semaine	Lu	Ma	Me	Je	Ve	Sa	Di
Jour d'étude	J1	J2	J3	J4	J5	J6	J7
Application du produit	↓		↓		↓		

S2 :

Jour de la semaine	Lu	Ma	Me	Je	Ve	Sa	Di
Jour d'étude	J8	J9	J10	J11	J12	J13	J14
Application du produit	↓		↓		↓		

S3 :

Jour de la semaine	Lu	Ma	Me	Je	Ve	Sa	Di
Jour d'étude	J15	J16	J17	J18	J19	J20	J21
Application du produit	↓		↓		↓		

Phase de latence - deux semaines (S4, S5)

S4 :

Jour de la semaine	Lu	Ma	Me	Je	Ve	Sa	Di
Jour d'étude	J22	J23	J24	J25	J26	J27	J28

S5 :

Jour de la semaine	Lu	Ma	Me	Je	Ve	Sa	Di
Jour d'étude	J29	J30	J31	J32	J33	J34	J35

Phase de révélation (double challenge test) - une semaine (S6)

S6 :

Jour de la semaine	Lu	Ma	Me	Je	Ve
Jour d'étude	J36	J37	J38	J39	J40
Application du produit	↓				
Lectures			L		L

6. CRITERES D'EVALUATION

6.1. CRITERES CLINIQUES CONCERNANT LE POTENTIEL IRRITANT (PHASE D'INDUCTION)

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 :

Score	Cotation	**CRITERES** : description			
		ERYTHEME	OEDEME	SECHERESSE	VESICULES
0	absent	aspect normal	aspect normal	aspect normal	aspect normal
1	léger	Coloration rosée discrète de toute la surface testée ou bien visible sur une partie de la surface	Plus palpable que visible	Desquamation fine discrète, aspect dépoli	Vésicules plus palpables que visibles
2	net	Erythème net couvrant toute la surface testée	Œdème visible	Desquamation visible, aspect écailleux	Vésicules visibles
3	important	Erythème intense couvrant toute la surface testée ou érythème diffusant en dehors de la surface	Pouvant déborder de la surface testée	Desquamation importante, fissuration	Vésicules débordant de la zone testée ou bulles

6.2. CRITERES CLINIQUES CONCERNANT LE POTENTIEL SENSIBILISANT (PHASE DE REVELATION)

En cas de survenue de réaction allergique au cours des phases d'induction ou de révélation, celle-ci sera cotée en fonction de critères de l'ICDRG (International Contact Dermatitis Research Group) :

Critère	Cotation ICDRG	Cotation "notée"
Absence de réaction	0	0
Réaction douteuse	?	?
Erythème et œdème	+	1
Erythème, œdème et vésicules	++	2
Réaction forte avec présence de bulles ou d'ulcérations post-bulbeuses	+++	3

6.3. MODE D'EVALUATION

6.3.1. Pouvoir irritant - Phase d'induction

A l'issue des 8 lectures de la phase d'induction, le score moyen de chaque volontaire est calculé en additionnant les scores obtenus à chacune des lectures et en divisant cette somme par le nombre effectif de lectures (une lecture ne sera pas prise en compte s'il y a réaction au témoin ou irritation globale).
Le pouvoir irritant du produit sera évalué lors de la phase d'induction, en faisant la moyenne des réactions survenues.

Le pouvoir irritant du produit est déterminé selon la formule suivante :

$$\text{Score moyen} = \frac{[(\sum \text{scores J1...J19/ nb de lectures) vol1} + ... + (\sum \text{scores J1...J19/ nb de lectures) volN}]}{\text{nb de volontaires (N)}}$$

Score moyen	Pouvoir irritant
0 – 0,08	Non irritant
0,081 – 0,16	Très légèrement irritant
0,161 – 0,56	Légèrement irritant
0,561 - 1	Modérément irritant
1,001 – 1,6	Fortement irritant
> 1,6	Très fortement irritant

6.3.2. Pouvoir sensibilisant - Phase de révélation

Une réaction allergique éventuelle au cours des phases d'induction ou de révélation sera notée de 0 à 3 selon les critères 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.

Le pouvoir sensibilisant du produit sera évalué lors des lectures à J38 et J40 (phase de révélation) en fonction des critères suivants : réaction ++ (2) ou +++ (3).

La survenue d'un seul cas de sensibilisation active côté controlatérale conduit à la conclusion : « Produit potentiellement sensibilisant ».

6.4. ARRET PREMATURE

Les sujets ont le droit de sortir de l'essai à tout moment pour quelle que raison que ce soit.
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.

6.5. AMENDEMENTS AU PROTOCOLE

Néant

7. RESULTATS

7.1. POUVOIR IRRITANT : PHASE D'INDUCTION

Le TABLEAU DES LECTURES durant la phase d'induction est présenté en ANNEXE III.

Ces lectures effectuées 30 min après le retrait des patch-tests ont montré les résultats suivants :

Produit QI	J3	J5	J8	J10	J12	J15	J17	J19	Conclusion
RHODYSTEROL BASE TRIGLYCERIDES LOT 8.06.174	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+ = Témoin positif IRR = irritation globale VM = valeur manquante

Compte tenu de l'ensemble des résultats, le produit «RHODYSTEROL BASE TRIGLYCERIDES LOT 8.06.174» peut donc être considéré comme <u>non irritant</u> dans les conditions de cette étude (score inférieur à 0,080).

7.2. POTENTIEL SENSIBILISANT : PHASE DE REVELATION

Le TABLEAU DES LECTURES durant la phase de révélation est présenté en ANNEXE IV.

Les lectures effectuées 30 min et 48h après le retrait des patch-tests de révélation ont donné les résultats suivants :

Produit QI	Zone	Jour de lecture		Résultat global
		J38	J40	
RHODYSTEROL BASE TRIGLYCERIDES LOT 8.06.174	Lectures zone homolatérale	T+ : 0 QI : 0 : 50 ? : 0 1 : 0 2 : 0 3 : 0	T+ : 0 QI : 0 : 50 ? : 0 1 : 0 2 : 0 3 : 0	non sensibilisant
	Lectures zone controlatérale	T+ : 0 QI : 0 : 50 ? : 0 1 : 0 2 : 0 3 : 0	T+ : 0 QI : 0 : 50 ? : 0 1 : 0 2 : 0 3 : 0	

T+ = Témoin positif
QI = RHODYSTEROL BASE TRIGLYCERIDES LOT 8.06.174
IRR = irritation globale
VM = valeur manquante

Le produit «RHODYSTEROL BASE TRIGLYCERIDES LOT 8.06.174» peut donc être considéré comme non sensibilisant dans les conditions de cette étude.

8. CONCLUSION

Dans les conditions de cette étude, le produit «RHODYSTEROL BASE TRIGLYCERIDES LOT 8.06.174» s'est avéré non irritant et non sensibilisant.

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ANNEXE I

FEUILLE D'AUTHENTIFICATION DES RESULTATS

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 – 374

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*	*15/05/2009* data */date*
Dr Marlena NOWAKOWSKA Lekarz dermatolog *Médecin dermatologue* Dermatologist	Nowakowska podpis / *signature*	*15/05/2009* data */date*
Anna MAREK Technik *Technicienne* Technician	A. Marek podpis / *signature*	*15/05/2009* data */date*
Anna MIŚKIEWICZ Asystentka medyczna *Assistante médicale* Medical assistant	A. Miśkiewicz podpis / *signature*	*15/05/2009* data */date*

ANNEXE II

CARACTERISTIQUES DES VOLONTAIRES

CARACTERISTIQUES DES VOLONTAIRES

N° du sujet	Code du sujet	Age	Sexe F ou M	Phototype	Nature de la peau (Normale ou Sensible)	Evénements médicaux ou chirurgicaux et traitements médicaux	
						avant l'étude	pendant l'étude
1	PYRBA	59	F	II	N	-	-
2	GRAAN	22	F	II	N	-	-
3	KOLEW	51	F	II	N	-	-
4	ZUCKA	22	F	II	N	-	-
5	KAZHE	55	F	II	N	-	-
6	STEBE	21	F	II	N	-	-
7	GORMA	48	F	II	N	-	-
8	PECKA	51	F	II	N	-	-
9	ZYSEL	60	F	II	N	-	-
10	BIZMA	54	F	II	N	-	-
11	ROGIR	23	F	II	N	-	-
12	SZUWL	56	M	II	N	-	-
13	JAGWA	59	F	II	N	-	-
14	RADDO	31	F	II	N	-	-
15	RYZWI	22	F	II	N	-	-
16	STAEL	49	F	II	N	-	-
17	URBNA	34	F	II	N	-	-
18	MIEAL	65	F	II	N	-	-
19	GIEMI	63	F	II	N	-	-
20	KALWI	43	F	II	N	-	-
21	ZIMIR	31	F	II	N	-	-
22	KOWMA	49	F	II	N	-	-
23	GALFE	58	F	II	N	-	-
24	SKABR	65	F	II	N	-	-
25	SCIKR	60	F	II	N	-	-
26	DYMBA	27	F	II	N	-	-
27	LESKA	22	F	II	N	-	-
28	JATJA	47	M	II	N	-	-
29	LUKMA	25	M	II	N	-	-
30	GUSMA	19	F	II	N	-	-

P.V. = perdu de vue

CARACTERISTIQUES DES VOLONTAIRES – (suite)

N° du sujet	Code du sujet	Age	Sexe F ou M	Phototype	Nature de la peau (Normale ou Sensible)	Evénements médicaux ou chirurgicaux et traitements médicaux	
						avant l'étude	pendant l'étude
31	PERWI	29	F	II	N	-	-
32	WOJKR	60	F	II	N	-	-
33	CHODA	41	F	II	N	-	-
34	CACJO	48	F	II	N	-	-
35	AMBBA	55	F	II	N	-	-
36	SOTKR	22	M	II	N	-	-
37	KOTLI	63	F	II	N	-	-
38	BOGBE	21	F	II	N	-	-
39	SOBAG	28	F	II	N	-	-
40	GUSWI	24	F	II	N	-	-
41	KORAG	36	F	II	N	-	-
42	SCIJA	33	F	II	N	-	-
43	MARBE	45	F	II	N	-	-
44	ZULMA	25	F	II	N	-	-
45	KOWDA	54	F	II	N	-	-
46	BORJO	60	F	II	N	-	-
47	KALAN	44	F	II	N	-	-
48	MUSKA	29	F	II	N	-	-
49	ZIEBE	35	F	II	N	-	-
50	ZARMA	24	F	II	N	-	-

P.V. = perdu de vue

ANNEXE III

TABLEAU DES LECTURES

PHASE D'INDUCTION

TABLEAU DES LECTURES - phase d'induction

N° volontaire	J3		J5		J8		J10		J12		J15		J17		J19	
	T	QI	T	QI	T	QI	T	QI	T	QI	T	QI	T	QI	T	QI
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

P.V. = perdu de vue
T = témoin
QI = RHODYSTEROL BASE TRIGLYCERIDES LOT 8.06.174

TABLEAU DES LECTURES - phase d'induction (suite)

N° volontaire	J3		J5		J8		J10		J12		J15		J17		J19	
	T	QI	T	QI	T	QI	T	QI	T	QI	T	QI	T	QI	T	QI
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

P.V. = perdu de vue
T = témoin
QI = RHODYSTEROL BASE TRIGLYCERIDES LOT 8.06.174

ANNEXE IV

TABLEAU DES LECTURES

PHASE DE REVELATION

TABLEAU DES LECTURES - phase de révélation

N° Volontaire	J38 zone homolatérale		J38 zone controlatérale		J40 zone homolatérale		J40 zone controlatérale	
	T	QI	T	QI	T	QI	T	QI
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

P.V. = perdu de vue
T = témoin
QI = RHODYSTEROL BASE TRIGLYCERIDES LOT 8.06.174

TABLEAU DES LECTURES - phase de révélation (suite)

N° Volontaire	J38 zone homolatérale		J38 zone controlatérale		J40 zone homolatérale		J40 zone controlatérale	
	T	QI	T	QI	T	QI	T	QI
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

P.V. = perdu de vue
T = témoin
QI = RHODYSTEROL BASE TRIGLYCERIDES LOT 8.06.174

Translation of the Summary of the last study with memo 18 - Gelidium Cartilagineum Extract

Summary of Study

TITLE: STUDY OF THE SENSITIZING POWER OF A "RHODYSTEROL BASE TRIGLYCERIDES LOT 8.06.174" PRODUCT, ACCORDING TO THE MARZULLI-MAIBACH METHOD ON 50 SUBJECTS FOR 6 WEEKS.

STUDY REFERENCE: ET-374

PRODUCT: RHODYSTEROL BASE TRIGLYCERIDES LOT 8.06.174

DIRECTOR OF THE STUDY: The study was carried out and the numerical values entered by the PROCOS Clinical Unit, located in Poland; ul. Stowackiego 27/33 lok. 33/34; 01-592 Warsaw

INVESTIGATOR: Dr Marlena NOWAKOWSKA

STUDY MONITOR: Dr ing. Bogdan WICHROWSKI

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

PURPOSE OF THE STUDY: To evaluate under dermatological control the irritant and sensitizing potential of a product under the conditions provided by the promoter of the study.

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

STUDY PERIOD: 06/04/09 - 05/15/09

EXPERIMENTAL PLAN: Single-blind single-center study

MAIN PARAMETERS OF TOLERANCE

Irritant potential (induction phase)

Erythema, edema, dryness, vesicles, evaluated by the dermatologist according to a score of 0 to 3

- Sensitizing potential (revelation phase)

Reaction evaluated by the dermatologist according to a score of 0 to 3 established by the ICDRG (International Contact Dermatitis Research Group)

RESULTS:

Product Name	Irritant Potential	Sensitizing Potential
RHODYSTEROL BASE TRIGLYCERIDES LOT 8.06.174	Average score of 0.000 = non-irritating	No allergic type reaction

CONCLUSION:

Under the conditions of this study, the product "RHODYSTEROL BASE TRIGLYCERIDES LOT 8.06.174" was found to be non-irritant and non-sensitizing.

Additional details found in the study report but not included in the Summary

The patch tests used are the FINN CHAMBERS ON SCANPOR® large which ensure good occlusion.

The FINN CHAMBER constitutes an isolation cup which ensures good occlusion.

After applying the product to the patch, it is applied to the scapular area of the volunteers

Application zones: Scapular zones: homolateral (induction zone) and contralateral (revelation zone)

Applied quantity/Concentration 25 µl/as supplied

Application conditions: The product "RHODYSTEROL BASE TRIGLYCERIDES LOT 8.06.174 ”was placed in a well with filter paper and applied to the back of the volunteer. A patch containing no product was applied under the same conditions and served as an untreated witness. Throughout the induction phase, the homolateral zone has not been wet. Volunteers showered on Sunday after removing the patches being careful to not put detergent on the sites. When during the revelation phase, no washing or application of any product was carried out on the contralateral area.

Gelidium sesquipedale

Algae synopsis

Red marine alga

Related extract: GELYOL® GS 45

V.1- 2018

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Uses	6

These data don't pretend to be exhaustive.
They supply scientific pieces of information for conducing to a better understanding of the main characteristics and bioactivities of this algal species.

TAXONOMY

This alga belongs to

Empire	*Eukaryota*
Kingdom	*Plantae*
Phylum	*Rhodophyta*
Class	*Florideophyceae*
Order	Gelidiales
Family	*Gelidiaceae*
Genus	*Gelidium* J.V. Lamouroux 1813
Species	*sesquipedale* (Clemente) Thuret in Bornet & Thuret 1876.

Origin of genus name

From Greek “gelidus” congealed.

Status of name

This name is currently regarded as a synonym of *Gelidium corneum* (Hudson) J.V.Lamouroux.

Basionym

Fucus corneus var. *sesquipedalis* Clemente.

cf. www. algaebase.org

COMMON NAMES

This alga is named in

-England	vegetable isinglass
-China	yang ts’ai.

MORPHOLOGY

Gelidium sesquipedale is composed of several erect axes, compressed and branched (Figs 1-2). Axes bear secondary axes with ramuli short and pinnate.

The thallus appears robust with a cartilaginous consistency, dark-red in colour. It can reach up 25-30 cm long.

Figs 1-2 – Morphology of *Gelidium sesquipedale* collected in Atlantic Ocean
Photos GELYMA.

BIOLOGY

Gelidium sesquipedale is perennial with an apical growth of axes.

Along the Basque coast, frond elongation rates equal from 6.1 to 7.9 cm yr^{-1} (measures recorded during four consecutive years).
In winter elongation rates of fronds were rather low. Elongation did not seem to be affected by frond length, the self-shading effect of the canopy, or changes in depth (Gorostiaga J.M. 1994- Mar. Biol. 120 (2): 311-322).

The life cycle shows the triphasic *Polysiphonia*-type with the isomorphic tetrasporophyte and gametophyte phases plus the small carposporophyte developing directly on the female thallus.

ECOLOGY & GEOGRAPHICAL DISTRIBUTION

This alga develops on rocks in semi-exposed to exposed locations in the lower intertidal and shallow subtidal level.

Gelidium sesquipedale forms extensive sublittoral stands in the Atlantic waters of Biscaye Bay, Northern Spain, Portugal and Morocco. A homogenous vegetation of extensive beds exits along the eastern Basque coast, extending from 0 to 10-15m water depth.

Gelidium sesquipedale is also present in Azores and found in the Mediterranean along the seashores of Italy, Spain and Corsica.

CHEMICAL COMPOSITION

Gelidium sesquipedale is an agarophyte alga. It contains between 40%-45% DW agar that includes a high 3,6-anhydrogalactose content (40-45 mol%) and low amounts of 6-O-methylgalactose (around 1 mol%) and sulfate (1.0 – 1.6% DW). The agar synthesis and quality is affected by the reproductive status of the alga (Mouradi-Givernaud *et al.* 1999 – Hydrobiologia 398/399: 391-395).

Phenolics would be also present, the quantities varying according to determination methods from 3.49 $\pm$ 0.51 to 101.05 $\pm$ 1.30 (in mg gallic acid eq/g DW) (Metidji H. *et al*. 2015- J. Mater. Environ. Sci. 6(11): 3184-3196).

A new polyhalogenated monocyclic monoterpene has been identified in this red alga (Aazizi M.A. *et al.* 1989 – J. Natural products 52 (4): 829-831).

Different mycosporine-like amino acids have been found there: palythinol, porphyra 334, shinorine, palythine and asterina (Karsten U. *et al*. 1998- Bot. Mar. 41: 4439453; Volkmann M. & A.A. Gorbushina 2006- FEMS Microbiol Lett. 255: 286-295).

According to Karsten U. *et al*. 1998 (*ibid*), the different concentrations would be as follows:

Shinorine	0.178 $\pm$ 0.044
Porphyra	334: 0.004 $\pm$ 0.001
Palythine	0.584 $\pm$ 0.040
Asterina	2.168 $\pm$ 0.124
Palythinol	0.004 $\pm$ 0.001

with MAAs total amount = 2.939 $\pm$ 0.208 mg.g DW.

The researchers of the University of Malaga (Spain) deposited a Patent (WO 2007/026037 (A2) 2007-03-08) relative to the content in mycosporine like amino acids (palythine and Asterine 330) in *Gelidium sesquipedale* related to anti-oxidant and UV protectrice properties.

As a matter of interest

Agar is known as “kanten” in Japan.

The ancestor of agar is called « tokoroten », popular snack in the city of Edo (now Tokyo).

Tokoroten is said originally come from China and introduced in Japan during Nera era (710-794).

Kanten “The Japanese product of chance”

On the course of the Edo period (1603-1868), feudal lords travelled between the capital of Edo and their domains. During one of these travellings in 1658, Lord Shimazu of the Satsuma clan stayed at an inn called Minoya in Fushimi (now Kyoto) where “tokoroten” was served.

The innkeeper, Tarozaemon, left the leftovers outside. The “tokoroten” froze overnight, defrosted and dehydrated by the next afternoon. In several day’s time the mass had gone thoroughly dry.

Intrigued, Tarozaemon tried boiling the desiccated mass. It melted and as it cooled down, it resolidified into a white translucent jelly without the characteristic smell of seaweed.

So “tokoroten” became freeze dried, thus named “kanten” (*i.e* freezed tokoroten).

At Shimizu-mura in Japan a monument commemorates the first commercial manufacture of “kanten” by a relative of Tarozaemon , Miyta Hanbei of AzaShiroyama.

Fig.3 – Different actual uses of “Kanten” in Asian countries.

BIOACTIVITIES

Gelidium sesquipedale offers sevral biological properties:

- anti-oxidant properties (Bengueddour Y. *et al*. 2014 Nature & Technologie 10: 29-33; Metidji H. *et al*. 2015- J. Mater. Environ. Sci. 6 (11): 3184-3196) probably related to the presence phenolic compounds, ascorbic acid and functions thiols

- anti-microbial activities, phenolics being probably responsible for these biological activities. (Metidji H. *et al*. 2015- J. Mater. Environ. Sci. 6 (11): 3184-3196)

  These anti-microbial activities have been previously found by other authors (Younes F. *et al*. 2009- J. Appl. Biosciences 24:1543-1552; Bouhlal R. *et al*. 2010- African J. Biotechnol. 9: 7968-7975 ; Farid Y. 2010- Thesis Uni. Chouaib Doukkali).

- anti-inflammation properties (Chatter Riathi R. *et al.* 2011- Archs Inst. Pasteur Tunis 88 (1-4): 19-28).

The potential of the agar obtained from this alga could serve to prepare biodegradable films that appear homogenous, transparent and flexible (Guerrero P. *et al*. 2014- Carbohydrate Polymers 99: 491-498).

USES

The genus *Gelidium* is the major source of agar that has been manufactured firstly in Japan since around 1760.

Agar has been used for human consumption many years ago (*cf.* page 5).

Gelidium sesquipedale is prized for its high-quality agar, particularly for the preparation of microbiological media. Its industrial exploitation is an important part of the economy of Morocco (Fig. 4), Spain and France (Biscaye Bay) (Figs 5-6).

Fig.4 – Accumulation of *Gelidium sesquipedale* along the seashores of Morocco.

Figs 5-6 – Preparation for transport to Spanish factories of *Gelidium sesquipedale* collected along the French seashores of Basque Bay - Photos GELYMA.

Gelidium sesquipedale also is used for cosmetic purposes.

Laboratoires de Biarritz include this alga in preparations for its content in mycosporine like amino acids (Patent FR 3025426 (A) 2011-08-10).

GELYMA developed GELYOL® GS 45 from *Gelidium sesquipedale* for imporving skin health..

GELYOL ® GS 45

*

Re-mineralizing & revitalizing properties

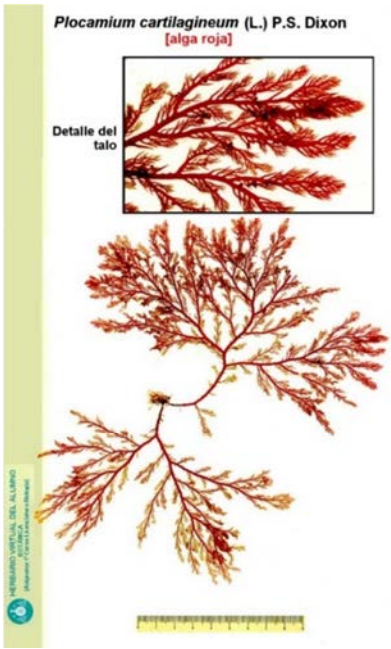
A probable problem of taxonomic denomination

In Brittany *Geliidium sesquipedale* is not present in abundance. It is mixed with the other red alga named *Plocamium cartilagineum.* So seaweed collectors are in the habit of denominating the mixed algae "*Gelidium cartilagineum ".*

According to algaebase, *Gelidium cartilagineum* (Linnaeus) Gaillon 1828 is currently regarded as a synonym of *Plocamium cartilagineum* (Linnaeus) P.S.Dixon.

So it is clear that extracts prepared from the mixed thalli collected in Brittany as *Gelidium cartilagineum* might contain different algal species.

The pictures from herbarium sheets show large morphological similitudes between these two red algae that can easily explain species confusion (Figs 7-8).



Figs 7-8 -Herbarium sheet from www.unioviedo.es –Herbario virtual

SPECIFICATION DATA SHEET

Trade name: GELYOL® GS45

Product: N° BG-GESE-00 Version: 1.0 – 2020

Specification: N° S.00 Print date: 01 - 2020

GELYOL® GS 45 is a standardized and concentrated hydroglycolic extract, selectively prepared from the red alga *Gelidium sesquipedale* (also named incorrectly *Gelidium cartilagineum*).

1 – Identification and composition of the substance

Product	N° CAS	N°EINECS	Ingredients %
Water	7732-18-5	231-791-2	48
Butylene glycol	107-88-0	203-529-7	48
Gelidium sesquipedale extract	-	-	4
Preservative	None		

2 – Characteristics (standard)

Appearance: limpid liquid.

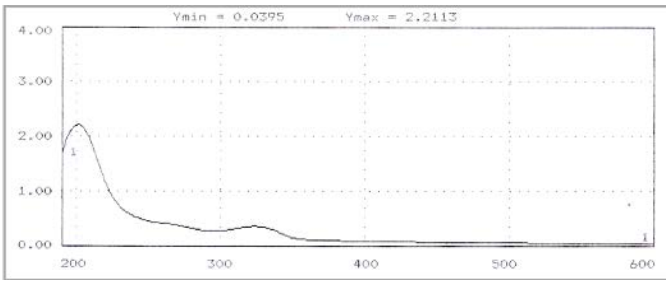
Color: greenish yellow.

Odour: *sui generis.*

pH: 5.5 ± 1.0.

Relative density: 1.030 ± 0.020.

UV spectrum (5% in water):



Microbiological quality:

Total germs (germs/ml)	< 100.
Pathogens	absence.
Yeasts /moulds	< 100.

Storage: 15°C < store < 25°C.
Validity date: 6 months
Once opened, the whole drum must be used.

Remark:
No responsibility or liability for any consequences arising from the use of this technical data sheet can be accepted, including possible infringement of any patent.
Any addition of preservatives is at the request and under the responsibility of the applicant, the product being delivered in units of use (1-5 or 10 Kg).

GELYMA – Parc d'Affaires Marseille Sud (C4) – 1 Boulevard de l'Océan – 13009 Marseille – France.
Phone: (33) 496 140 982 – E-mail: gelyma @ wanadoo.fr – contact @ gelyma.com

Rapport d'essai n° 191141175-003 V1

Edité le 09/03/2020

N° de contrat : CNO-D1751-1019
N° bon de commande : CNO-D1751-1019

GELYMA SARL
LILIANE PELLEGRINI
PARC D'AFFAIRES MARSEILLE SUD C4
1 BOULEVARDDE L'OCEAN
13009 MARSEILLE

UPSCIENCE SAINT NOLFF
TALHOUET
56250 SAINT NOLFF
www.upscience-labs.com/my-account

Données fournies par le client

N° échantillon client : -
Descriptif: GELYOL GS 45
Date fabrication/réception client : -
N° étude : -
Données complémentaires : -

DLUO : -
Fournisseur : -
N° Lot : 19 08 280
N° OPTIM : -

Données de réception renseignées par le laboratoire

Classification de l'échantillon : ALGUES HUMIDES / ALGUES
Date de réception échantillon : 25/11/2019
Etat : Conforme à la réception

Poids à la réception : 120,00 mL

Commentaire : -

Rapport d'essai n° 191141175-003 V1

ANALYSE	RESULTATS ± INCERTITUDES		UNITES	CIBLE / SPECIFICATIONS	CONFORMITE
CENDRES (pour dosage minéraux)					
Méthode interne CEND-H 13					
CENDRES BRUTES	0,4	± 0.2	g/100g	-	-
MINERAUX ICP-AES (SN)					
Méthode interne - MINEROL 15					
CALCIUM	< 4,0		mg/100g	-	-
MAGNESIUM	14,0	± 1.4	mg/100g	-	-
PHOSPHORE	< 2,0		mg/100g	-	-
POTASSIUM	82,1	± 8.2	mg/100g	-	-
SODIUM	98,3	± 9.8	mg/100g	-	-
CUIVRE	< 0,3		mg/100g	-	-
FER	< 0,2		mg/100g	-	-
MANGANESE	< 0,3		mg/100g	-	-
ZINC	< 0,3		mg/100g	-	-
MINERALISATION					
Analyse sous-traitée					
MINERALISATION	FAIT			-	-
ELEMENTS TRACE ET METAUX LOURDS (ICP-MS)					
Analyse sous-traitée					
ARSENIC	72		µg/kg	-	-
CADMIUM	< 10		µg/kg	-	-
MERCURE	< 5		µg/kg	-	-
MOLYBDENE	< 51		µg/kg	-	-
PLOMB	< 10		µg/kg	-	-
SELENIUM	< 811		µg/kg	-	-
IODE					
Analyse sous-traitée					
IODE	1,02		mg/kg	-	-

MAHE Magali
Gestionnaire de compte
Validé le 09/03/2020

Ce rapport d'essai ne concerne que l'échantillon soumis à l'essai. Sa reproduction n'est autorisée que sous sa forme intégrale.
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L'accréditation de la section « Laboratoires » du Cofrac atteste de la compétence du laboratoire sur les seules analyses couvertes par l'accréditation.
Chimie : < dans la colonne Résultats = limite de quantification (LQ) / ND = Non détecté / D = Détecté / NA = Non Analysé
Les informations règlementaires associées aux résultats des analyses microbiologie sont gérées sur le bulletin du sous-traitant.
Si ce rapport fait mention de résultats d'acides gras : ND : pic non présent sur le chromatogramme
La somme des esters méthyliques d'acides gras correspond à la somme des esters méthyliques d'acides gras identifiés.
La quantification des esters méthyliques d'acides gras est déterminée par étalonnage interne.
Un facteur de correction est utilisé pour le calcul des esters méthyliques d'acides gras de C4 à C10.
Les esters méthyliques d'acides gras de C4 à C7 ne sont pas dans le domaine d'application de la norme. Ils sont toutefois inclus dans la somme des Acides Gras Saturés (AGS).
Fichier joint = document joint au présent rapport

English Translation:

5b Mineral and metal analysis: GELYOL® GS45 (contains 4% Gelidium Sesquipedale Extract)

Analysis	Results ± Uncertainties	Units
Ashes (for mineral dosing) Internal method CENDH-13 Raw ashes	0.4 ± 0.2	g/100 g
Minerals ICP-AES Internal method – MINEROL-15		
Calcium	<4.0	mg/100 g
Magnesium	14.0 ± 1.4	mg/100 g
Phosphorus	<2.0	mg/100 g
Potassium	82 ± 8.2	mg/100 g
Sodium	98.3 ± 9.8	mg/100 g
Copper	<0.3	mg/100 g
Iron	<0.2	mg/100 g
Manganese	<0.3	mg/100 g
Zinc	<0.3	Mg/100 g
Trace elements and heavy metals (ICP-MS) Sub-contracted analysis		
Arsenic	72	µg/kg
Cadmium	<10	µg/kg
Mercury	<5	µg/kg
Molybdenum	<51	µg/kg
Lead	<10	µg/kg
Selenium	<811	µg/kg
Iodine Subcontracted analysis		
Iodine	1.02	mg/kg

MAHE Magali
Account manager
Validated 03/09/2020

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The accreditation of the "Laboratories" section of Cofrac attests to the competence of the laboratory only on the analyzes covered by the accreditation.
Chemistry: <in the Results column = limit of quantification (LQ) / ND = Not detected / D = Detected / NA = Not Analyzed
The regulatory information associated with the results of microbiology analyzes is managed in the subcontractor's bulletin.
If this report mentions fatty acid results: ND: peak not present on the chromatogram.
The sum of the methyl esters of fatty acids corresponds to the sum of the methyl esters of fatty acids identified.
The quantification of the fatty acid methyl esters is determined by internal calibration.
A correction factor is used for the calculation of the fatty acid methyl esters from C4 to C10.
The fatty acid methyl esters of C4 to C7 are outside the scope of the standard. They are however included in the sum of Saturated Fatty Acids (AGS).
Attached file = document attached to this report.

Les résultats qui suivent ne s'appliquent qu'aux échantillons soumis au laboratoire et tels qu'ils sont définis dans le présent document.
Les échantillons seront conservés dans nos locaux pendant une période de 2 mois à compter de la date figurant sur ce document.

RAPPORT D'ETUDE

GELYMA
1 boulevard de l'Océan
Parc d'Affaires Marseille
Bâtiment C4
13009 Marseille

Le 04/04/2020

EVALUATION DE LA TOLERANCE CUTANEE D'UN PRODUIT COSMETIQUE APRES APPLICATION UNIQUE SOUS PANSEMENT OCCLUSIF PENDANT 48 HEURES : *Méthode des patchs tests*

Moniteur de l'étude : **Mme Liliane PELLEGRINI**

Référence Client :

Référence EUROFINS ATS : 9677

Responsable de l'essai : Cosette STEGLE

Produits testés :

- Dénomination : GELYOL G.S.
- Référence client : Lot 05 11 100
- Référence ATS : 133870
- Type de produit: Matière première

RESUME DE RAPPORT D'ETUDE

EVALUATION DE LA TOLERANCE CUTANEE D'UN PRODUIT COSMETIQUE APRES APPLICATION UNIQUE SOUS PANSEMENT OCCLUSIF PENDANT 48 HEURES :
Méthode des patchs tests

- **Produit étudié** : GELYOL G.S. Lot 05 11 100
- **Promoteur** : GELYMA
- **Objectif de l'étude** : L'objectif de l'étude est d'apprécier la tolérance locale épicutanée d'un produit cosmétique, après application unique sur la peau du dos et sous patch occlusif, pendant 48h, chez des volontaires adultes, sains.
- **Investigateur :** Docteur Mary CREST
- **Lieu de l'étude :** EUROFINS SCIENTIFIC TEST CENTER
  3 allée des Ingénieurs
  1140 rue André Ampère
  13851 AIX EN PROVENCE cedex 3
- **Dates de l'étude :** du 20/12/05 au 22/12/05
- **Méthodologie** :
  - ✓ ***Modalités d'application*** **:**

Zones d'application : dos
Quantité de produit : 0.02 ml
Fréquence et durée : application unique pendant 48 heures.
Conditions d'application : produit déposé dilué à 5%, sous patch occlusif.

  - ✓ ***Méthode d'évaluation*** **:**

L'observation clinique des effets provoqués est réalisée, par un dermatologue, après le retrait du patch. La cotation clinique est donnée selon une échelle numérique déterminée, en fonction de l'intensité des phénomènes d'irritation observés (érythème, œdème, sécheresse, vésicule). Le score irritant moyen du produit à l'essai est calculé en faisant la moyenne des cotations obtenues pour l'ensemble des volontaires, permettant ainsi de classer le produit de « non irritant à très irritant ». L'évaluation se fait toujours par comparaison au témoin "négatif" : patch contenant de l'eau déminéralisée.

- **Population :** 10 volontaires adultes, sains.
- **Résultats :** Le score irritant moyen du produit est de 0,0.
- **Conclusion :**

Dans les conditions expérimentales retenues, après application unique de 0.02 ml de produit, sous patch occlusif pendant 48 heures, chez 10 volontaires adultes sains et selon le barème adopté pour l'interprétation des résultats, **la matière première GELYOL G.S. référencée Lot 05 11 100,** peut être considérée comme **non irritante du point de vue de sa tolérance primaire cutanée.**

AUTHENTICITE DES RESULTATS

L'étude faisant l'objet du présent rapport a été conduite sous ma responsabilité, en conformité avec le protocole expérimental, le plan qualité du laboratoire EUROFINS ATS, et dans le respect des bonnes pratiques cliniques.
Toutes les observations et données recueillies au cours de cet essai sont rapportées dans le présent rapport.
Chargé d'étude, Cosette STEGLE

Après relecture, je certifie ces données conformes à la réalité des résultats obtenus,

Investigateur, Docteur Mary CREST

Je certifie avoir relu ce rapport et être en accord avec son contenu,

Responsable contrôle qualité, Cécile COLOMBAN

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STUDY SUMMARY 15

1 OBJECTIF

L'objectif de l'étude est d'apprécier la tolérance locale épicutanée de la matière première GELYOL G.S. Lot 05 11 100, après application unique sur la peau du dos et sous patch occlusif, pendant 48h, chez des volontaires adultes.

2 PRINCIPE

Le principe de l'étude est basé sur l'application unique de 0.02ml, de produit à l'essai, sur la peau du dos de volontaires adultes. Le produit a été maintenu en contact avec la peau pendant 48h sous patch occlusif.

L'observation clinique des effets provoqués a été réalisée par un dermatologue, après le retrait du patch. L'évaluation a été faite par comparaison à un témoin "négatif": patch contenant de l'eau déminéralisée, appliqué parallèlement et dans les mêmes conditions que le produit à tester.

La cotation clinique est donnée selon une échelle numérique déterminée, en fonction de l'intensité des phénomènes d'irritation observés (érythème, œdème, sécheresse, vésicule).
L'Indice d'Irritation Moyen (ou Irritation Primaire Cutanée) a été calculé en faisant la moyenne des cotations obtenues pour l'ensemble des volontaires.

3 TYPE D'ETUDE

Les recherches sont menées en accord avec la Déclaration d'HELSINKI (1964) et suivent les « guidelines for assessment of human skin tolerance of potentially irritant cosmetic ingredient », COLIPA, 1997. Les locaux, le matériel, et le personnel sont en conformité avec les réglementations en vigueur et suivent les BPC.

Les exigences éthiques, nécessaires dans le déroulement des études sur l'homme, sont respectées :

- ✓ Les volontaires sont sélectionnés selon des critères d'inclusion et de non inclusion (voir chapitre volontaires)
- ✓ Tous les volontaires sont informés du but et de la nature de l'étude, des risques prévisibles qu'ils prennent en participant à l'étude et donnent leur consentement, libre et éclairé, avant le début de l'étude.
- ✓ Avant que les volontaires soient exposés aux produits à tester, des informations minimums concernant la sécurité des produits sont demandées au promoteur.
- ✓ Toutes les précautions sont prises pour éviter de causer des réactions cutanées excessives ou des effets néfastes sur la santé des volontaires, durant l'étude.
- ✓ Des procédures de sécurité sont mises en place, dans le cas de réactions néfastes et inacceptables, incluant un matériel de sécurité médical.
- ✓ Les volontaires sont indemnisés, en compensation du temps passé, des inconvénients dus à l'étude.

4 POPULATION ETUDIEE

4.1 Nombre de sujets

Le produit a été testé sur 10 volontaires.

4.2 Caractéristiques de la population étudiée

Les volontaires sont des personnes issues du panel général « volontaires » de la société. Tous les panélistes inscrits dans la base de données ont été recrutés selon les critères d'inclusion et d'exclusion ci dessous et ont subi, avant leur admission définitive dans la base de données, un examen médical (certificat médical) et un examen dermatologique avec le médecin recruteur de la société.

4.3 Recrutement, sélection et admission définitive des volontaires pour une étude

A partir de la base de données « volontaires », les panélistes répondant aux critères d'inclusion sont convoqués puis définitivement admis dans l'étude au terme d'un entretien préalable.
Lors de cet entretien préalable, l'objectif et le protocole de la recherche, le planning de l'étude, les modalités d'indemnité, ainsi que les bénéfices éventuellement attendus, les contraintes liées à l'étude et les risques prévisibles, y compris en cas d'arrêt de l'essai avant son terme, leur sont expliqués.
Les panélistes doivent alors lire et signer un formulaire de consentement libre, éclairé et exprès.

Les volontaires doivent également remplir un auto questionnaire médical de pré-étude, afin de s'assurer que les critères d'inclusion et de non inclusion sont bien respectés, avant leur admission définitive dans l'étude.

4.4 Critères d'inclusion

Dans cette étude, a été inclus, les volontaires répondant aux critères suivants :

- ✓ Age : 18-70 ans,
- ✓ Sexe : féminin et/ou masculin,
- ✓ Couverture sociale : les volontaires doivent être affiliés à un régime de sécurité sociale,
- ✓ Indemnes de toutes lésions dermatologiques sur le site étudié,
- ✓ Volontaires pouvant justifier d'un domicile fixe,
- ✓ Compréhension de la langue française et capable de comprendre les exigences de l'essai,

4.5 Critères de non inclusion

- ✓ Volontaires ne présentant pas les critères d'inclusion précités,
- ✓ Volontaires en période d'exclusion entre deux essais,
- ✓ Les mineurs ou majeurs protégés par la loi et les personnes admises dans un établissement sanitaire ou social à d'autres fins que la recherche… (article L209-6)
- ✓ Les personnes privées de liberté par décision judiciaire ou administrative, malades en situation d'urgence (article L209-5),
- ✓ Volontaires présentant une pathologie cutanée évolutive, une allergie de contact connue liée aux ingrédients du produit à tester,
- ✓ Volontaires ayant refusé de donner leur accord et refusant de signer le consentement libre et éclairé,
- ✓ Volontaires sous traitement antihistaminique, corticoïdes, désensibilisant et/ou tout traitement pouvant interférer avec le métabolisme cutané,
- ✓ Volontaires présentant une peau récemment insolée ou ayant subi des séances de PUVA thérapie.

4.6 Interdictions et restrictions

Pendant toute la durée de l'étude, il est demandé aux volontaires :

- ✓ De ne mettre aucun produit, y compris de l'eau sur la zone des patchs,
- ✓ De ne pas prendre de bain, ni de s'exposer aux UV,
- ✓ D'éviter une activité sportive trop intense qui augmente la sudation et qui risque de provoquer le décollement du pansement,
- ✓ De ne pas prendre d'aspirine, d'anti-histaminiques, de corticoïdes, d'anti-inflammatoires, et tout traitement réduisant ou inhibant les réactions inflammatoires ou allergiques ou interférent avec le métabolisme cutané.

4.7 Retrait des volontaires

Un volontaire peut être exclu de l'étude pour les raisons suivantes :

- ✓ Il ne suit plus les exigences et les contraintes de l'étude, expliquées lors de la signature du consentement.
- ✓ Il souffre d'une maladie développée pendant l'étude qui peut interférer avec les objectifs de l'étude,
- ✓ Il ne souhaite plus participer à l'étude.

5 PRODUIT A L'ESSAI

- ✓ Dénomination du produit : GELYOL G.S.
- ✓ Référence : Lot 05 11 100
- ✓ Code identification pour l'étude : 133870
- ✓ Présentation (forme galénique, couleur) : Liquide transparent jaune
- ✓ Conditionnement : Flacon 60 ml
- ✓ Nombre d'échantillons reçus : 2
- ✓ Date de péremption : /
- ✓ Conditions de stockage : T°C ambiante et à l'abri de la lumière.

Un échantillon du produit testé est conservé dans les laboratoires EUROFINS ATS, pendant 2 mois après la fin de l'étude. Passé cette date et sauf avis contraire du moniteur de l'étude, le produit sera détruit.

- ✓ Véhicule (si nécessaire) : /

Eau déminéralisée

6 ETUDE CLINIQUE

6.1 Description du matériel utilisé

Le matériel utilisé est le FINN CHAMBER TEST, qui se compose d'une cupule en aluminium, de 8mm de diamètre, fixée sur une bande adhésive, et destinée à recevoir le produit à l'essai.

6.2 Modalités d'application

La surface sur laquelle est déposé le patch est préalablement nettoyée avec de l'eau déminéralisée, puis séchée avec du papier en ouate de cellulose.

Les patchs sont déposés au niveau du dos. Un examen spécifique de la zone mise en contact avec le patch est réalisé juste avant le début de l'étude, afin d'appliquer le produit sur une surface exempte de traces macroscopiques d'irritation, de cicatrices ou toutes anomalies pouvant interférer avec la lecture des résultats.

Parallèlement à l'application du produit étudié, un patch contenant de l'eau déminéralisée est apposé, dans les mêmes conditions, pour servir de témoin « négatif ».

Les patchs ainsi préparés sont laissés en contact 48 heures.

6.3 Observation et examen clinique

Le retrait des patchs et la lecture sont réalisés par le médecin Dermatologue. L'analyse des cotations des réactions épidermiques est descriptive.
Les réactions cutanées éventuelles sont évaluées, pour chaque volontaire, selon l'échelle suivante :

ERYTHEME :	
Absence	0
Erythème léger, à peine perceptible	1
Rougeur modérée et uniforme	2
Rougeur importante et uniforme	3
SECHERESSE / DESQUAMATION :	
Pas de sécheresse	0
Sec avec desquamation, aspect lisse et tendu, desquamation légère et fine	1
Desquamation modérée	2
Desquamation sévère avec de larges écailles	3
OEDEME	
Absence	0
Léger	1
Net	2
Important	3
VESICULE	
Absence	0
Léger	1
Net	2
Important	3

Les résultats obtenus sont comparés à ceux obtenus sur la zone témoin. L'indice d'irritation primaire est calculé en faisant la moyenne des cotations obtenues sur l'ensemble des panélistes, selon la formule suivante :

$$\frac{[(\Sigma \text{cotations T48}) \text{ vol 1 à vol n}]/ \text{ nombre de lectures}}{\text{nombre de volontaires}}$$

6.4 Analyse des données et interprétation des résultats

L'interprétation des résultats se fait en se basant sur l'indice d'irritation obtenu, le nombre de volontaires ayant réagi et l'importance des réactions, les conditions expérimentales adoptées et le type de produit étudié.

La classification du potentiel irritant est déterminée en fonction du score obtenu, selon le tableau ci dessous :

Score moyen	Classification
[0 - 0.08]	Non irritant
]0.08 - 0.16]	Très légèrement irritant
]0.16 – 0.56]	Légèrement irritant
]0.56 – 1]	Modérément irritant
]1 – 1.6]	Irritant
> 1.6	Très irritant

7 CONFIDENTIALITES, REGLEMENTATION ET ARCHIVAGE

7.1 Confidentialité

Tous les renseignements concernant l'état de santé des panélistes, recueillis lors de leur admission définitive dans la base de données volontaires d'EUROFINS et nécessaires au moment de leur recrutement et leur sélection dans le cadre des études, sont d'ordre strictement confidentiel et sont soumis à la règle du secret médical suivant l'article 378 du Code Pénal et au Code de déontologie Médical (décret du 18 juin 1979, articles 11, 12 et 13).
L'anonymat des volontaires est respecté dans le cadre des études. Cependant, chaque sujet participant à l'essai peut être facilement identifié par le Médecin Investigateur grâce à son numéro personnel de volontaire.
Conformément à l'article R5120 du Décret n°90-872 du 27.09.90 de la loi n°88-1138 du 20.12.88 modifiée, relative à la protection des personnes qui se prêtent à des recherches biomédicales, la nature des produits étudiés, les essais, les résultats des tests sont d'ordre strictement confidentiel, et le secret est respecté par le Médecin Investigateur et par toutes les personnes appelées à travailler avec lui.

7.2 Réglementation

Cette étude, bien que ne rentrant pas dans le champ d'application de la loi 88-1138 du 20 décembre 1988, modifiée par les lois 90-86 du 23 janvier 1990 et 94-630 du 25 juillet 1994, comme cela indiqué dans le guide des textes législatifs et réglementaires (BOMS 91/12-13bis), concernant la protection des personnes qui se prêtent à des recherches biomédicales, sera menée dans l'esprit de cette loi. Elle sera réalisée conformément aux recommandations les plus récentes de l'Association Médicale Mondiale (Déclaration d'Helsinki 1964, 48^{e} Assemblée générale Somerest West, octobre 1996).
Aucune information ne sera communiquée au fichier national des personnes qui se prêtent à des recherches biomédicales et l'avis du Comité Consultatif ne sera pas sollicité.

7.3 Archivage

Le cahier de laboratoire contenant toutes les informations (données brutes) concernant l'étude, et les rapports d'étude sont conservés dans les archives d'EUROFINS (Pôle d'activité d'Aix les Milles - ACTIMART – 3 allée des ingénieurs, 1140 rue André Ampère – 13851 AIX EN PROVENCE), pendant 10 ans.

8 RESULTATS

8.1 Description de la population

Cette étude a été réalisée du 20/12/05 au 22/12/05 et inclut 10 volontaires adultes, sains, dont les caractéristiques sont présentées dans le tableau 1 ci-dessous :

N° inclusion	Code vol	Sexe	Age (ans)	Poids	Evènements survenus
1	PERNA1	F	35	69	-
2	ALLEL1	F	40	92	-
3	PAYJE	F	39	65	-
4	BEACH	H	26	70	-
5	GOROL	H	25	90	-
6	DUCDA	H	28	75	-
7	PRINA	F	40	48	-
8	GOBSA	F	26	62	-
9	DEBE	H	58	75	-
10	LORIS	F	25	95	-
MOYENNE			34	74	-

Tableau 1 : caractéristiques des volontaires

Aucun des sujets sélectionnés ne prenait de traitement contre indiqué avec l'étude.

8.2 Sorties d'étude

100% des volontaires ont terminé l'étude.

8.3 Analyses des résultats

Le tableau ci-dessous présente les résultats obtenus pour chaque volontaire ainsi que le score d'irritation correspondant.
Aucune réaction cutanée n'a été notée par le dermatologue, sur le site témoin.

Code étude : **037PT10V05**

Produit	6	Nbre lecture :	1
Code	133 870	Nbre de vol :	10
VOL	CODE VOL	Total lecture 48h	Total irritation / nbre lecture
1	PERNA1	0	0
2	ALLEL1	0	0
3	PAYJE	0	0
4	BEACH	0	0
5	GOROL	0	0
6	DUCDA	0	0
7	PRINA	0	0
8	GOBSA	0	0
9	DEBE	0	0
10	LORIS	0	0
SCORE D'IRRITATION			0,00

Tableau 2 : résultats produit 133870, méthode des patchs tests

Après 48 heures d'application, aucune manifestation d'intolérance n'a été notée par le Dermatologue, sur le site traité par la matière première GELYOL G.S. Lot 05 11 100.

L'indice d'irritation moyen obtenu est égal à 0,0.

9 DISCUSSION ET CONCLUSION

Dans les conditions expérimentales retenues, après application unique de 0.02 ml de produit, sous patch occlusif pendant 48 heures, chez 10 volontaires adultes sains et selon le barème adopté pour l'interprétation des résultats, **la matière première GELYOL G.S. Lot 05 11 100,** peut être considérée comme **non irritante du point de vue de sa tolérance primaire cutanée.**

ANNEXE 1

Liste des personnes ayant participées à la réalisation de l'étude

Investigateur principal, responsable de l'étude :
Nom : Mary CREST, Docteur en Dermatologie
Adresse : ACTIMART
3 allée des Ingénieurs
1140 rue André Ampère
13851 AIX EN PROVENCE cedex 3
Téléphone : 04 42 39 30 92

Médecin recruteur :
Nom : Emmanuel CLYTI, Docteur en Dermatologie
Adresse : ACTIMART
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1140 rue André Ampère
13851 AIX EN PROVENCE cedex 3
Téléphone : 04 42 39 30 92

Chargé d'étude:
Nom : Cosette STEGLE, Licence Chimie
Adresse : ACTIMART
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1140 rue André Ampère
13851 AIX EN PROVENCE cedex 3
Téléphone : 04 42 37 14 26

Responsable contrôle qualité :
Nom : Cécile COLOMBAN, DEA d'immunologie
Adresse : ACTIMART
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13851 AIX EN PROVENCE cedex 3
Téléphone : 04 42 37 16 25

Technicienne de laboratoire, assistante de production :
Nom : Amandine SAGE, B.E.A.T.E.P. Gestion de projets institutionnels
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13851 AIX EN PROVENCE cedex 3
Téléphone : 04 42 39 30 92

STUDY SUMMARY

EVALUATION OF THE CUTANEOUS TOLERANCE OF A COSMETIC PRODUCT AFTER A SINGLE APPLICATION UNDER OCCLUSIVE PATCH DURING 48 HOURS:
Patch test method

- **Product tested** : GELYOL G.S. Lot 05 11 100
- **Promoter** : GELYMA
- **Monitor** : L. PELLEGRINI, General Manager
- **Objective** : Assessment of the cutaneous local tolerance of the studied product after an epicutaneous test performed in occlusive conditions, during 48 hours.
- **Place of the study:** EUROFINS SCIENTIFIC TEST CENTER,
  ACTIMART
  3 allée des Ingénieurs
  1140 rue André Ampère
  13851 AIX EN PROVENCE cedex 3
- **Investigator** : Docteur Mary CREST
- **Date of study:** from 20/12/05 to 22/12/05
- **Methodology:**
  - ✓ ***Application modes:***

Area of application : on the back
Quantity of product : 0.02 ml
Frequency and duration : only one application during 48 hours
Conditions of application : product applied at 5%, under occlusive patch.

  - ✓ ***Assessment method:***

A dermatologist makes the clinical observation, after the removal of the patches. The quantification of the cutaneous irritation is given according to a numeric scale (rash, oedema, dryness, blister). The average irritant score of the product to be tested is measured with the average of the quotations obtained for the whole volunteers, allowing ranking the product from "not irritant to very irritant". The assessment is always made by comparison with the "negative" control: patch with demineralised water.

- **Population:** 10 healthy adult volunteers.
- **Results:** The average irritant score of the product is 0,0.
- **Conclusion:**

According to the experimental conditions taken into account, after only one application of 0.02 ml of product, under occlusive patch and during 48 hours, on 10 healthy adult volunteers, and according to the scale used for the interpretation of the results, the raw material **"GELYOL G.S." batch 05 11 100, can be considered as not irritant regarding its primary cutaneous tolerance**.

Gigartina stellata

Algae synopsis

Red marine alga

Related actives: ALGYL® (*G.s*. combined with *Kappahycus alvarezii* and *Corallina officinalis*)

V.1- 2018

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These data don't pretend to be exhaustive.
They supply scientific pieces of information for conducing to a better understanding of the main characteristics and bioactivities of this algal species.

GELYMA Parc d'Affaires Marseille Sud (C4) – 1 Boulevard de l'Océan – 13009 Marseille – France.
Phone (33) 496 140 982 – Fax (33) 496 140 983 – E-mail : gelyma @ wanadoo.fr.

TAXONOMY

This alga belongs to

Empire	*Eukaryota*
Kingdom	*Plantae*
Phylum	*Rhodophyta*
Class	*Florideophyceae*
Order	Gigartinales
Family	*Gigartinacea*
Genus	*Gigartina* Stackhouse 1809
Species	*stellata* (Stackhouse) Batters 1902.

Origin of the genus name

Adjective (Latin), stellate, with narrow divisions radiating from the center like the rays of a star.

Basionym

Fucus stellatus Stackhouse.

Status of name

This name is currently regarded as a synonym of *Mastocarpus stellatus* (Stackhouse) Guiry.

Mastocarpus stellatus

Homotypic Synonyms

Fucus stellatus Stackhouse 1796
Chondrus stellatus (Stackhouse) Stackhhouse 1797
Sphaerococcus crispus var. *stellatus* (Stackhouse) C.Agardh 1817
Chondrus crispus var. *stellatus* (Stackhouse) Lyngbye 1819
Gigartina stellata (Stackhouse) Batters 1902.

Heterotypic Synonyms

Fucus coronopifolius Zoega 1772
Fucus mamillosus Goodenough & Woodward 1797
Chondrus mammillosus (Goodenough & Woodward) Stackhouse 1797
Fucus echinatus Stackhouse 1797
Chondrus echinatus (Stackhouse) Stackhouse 1797
Fucus alveolatus Esper 1799
Fucus mamillosus var. *acutus* Turner 1802
Fucus mamillosus var. *incurvus* Turner 1802
Fucus degener Esper 1804
Mammillaria expansa Stackhouse 1809
Mammillaria echinata Stackhouse 1809

Fucus crispus var. *mamillosus* (Goodenough & Woodward) Stackhouse 1816
Sphaerococcus mamillosus (Goodenough & Woodward) C.Agardh 1817
Fucus mamillosus var. *prolifer* Turner 1819
Mastocarpus mamillosus (Goodenough & Woodward) Kützing 1843
Phyllophora mamillosa (Goodenough & Woodward) Fries 1845
Rhodymenia mamillosa (Goodenough & Woodward) Areschoug 1847
Gigartina mamillosa (Goodenough & Woodward) J.Agardh 1851
Petrocelis cruenta J.Agardh 1851
Gigartina stellata f. *acuta* (Turner) Batters 1902
Gigartina stellata f. *prolifera* (Turner) Batters 1902
Gigartina stellata f. *incurvata* (Turner) Batters 1902
Gigartina cornopifolia (Zoega) P.C.Silva 1952.

cf. www. algaebase.org

COMMON NAMES

This alga is named as *Gigartina stellata* in

-England	Irish moss, Carrageen
-Finland	Punalevà-suku
-Ireland	Carraigin fiadhain, Clüimhin cait
-Portugal	Musgos, Botelhas.

It is named as *Mastocarpus stellatus* in

-England	Grape Pip Weed
-Danemark	Vortetang
-Portugal	Corninho , Crespo, Alface miùda, Botelha.

Several Trade names, often for edible algae are used in

-England	Irish moss, carraigin, False Irish moss, Carrageen moss
-Germany	nadeltang.

MORPHOLOGY

The thallus of *Mastocarpus stellata* bears dichotomously branched blades which arise from a basal discoid crust.
It is stiff and cartilaginous, purplish brown in color, 10-20 cm high ‘Figs 1-2). Stipe is narrow and compressed, expanding into strap-like blade, usually inrolled to form a channel.

This alga, often named “False Irish moss”, resembles to *Chondrus crispus* but is easily distinguishable by the distinctive channelling on one side. Its internal structure is multiaxial like that of *Chondrus crispus*.

Fig. 1- Morphology of *Gigartina stellata* Drawings from *HARVEY 1871 as Gigartina mamillosa* - Phycologia Britannica vol. III.

Fig. 2- Morphology of *Gigartina stellata* *In situ* – Photo GELYMA.

BIOLOGY

The life history of *Gigartina stellata* has given rise to several studies (Chen L.C.M. *et al.* 1974- Phycologia 13: 287-294; Edelstein T *et al*. 1974- Phycologia 13: 99-107).

According to Guiry M.D. & M.M. Coleman (1982- Br. Phycol. J. 17 (2): 232), it would exist two types of *Gigartina stellata* in North Atlatic populations: a dioecious and a monoecicous entities which forms further monoecious blades directly.

Dioecious plant presents a strongly heteromorphic life cycle in which the gametophytes are the most obvious stage. The tetrasporophytes are rather thick, mucilaginous crusts formerly known as *Petrocelis* (Guiry M.D. *et al.* 1984- Taxon 33 (1):53-63).

The upper part of frond of female plants bears papillae to 10 mm or more long on surfaces and margins. Male plants lack papillae. They are generally rare.

The tetrasporophyte is a purplish-black crust *Petrocelis*-phase, formed of vertical filaments of cells fixed on basal discs (West J.A. & A.R. Polanshek 1977- Br. Phycol. J. 12: 45-53) (Figs 3-4).

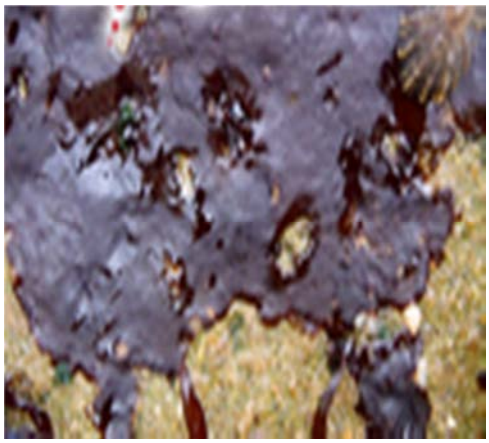


Fig 3-4 - Morphology of *Petrocelis cruenta,* the tetrasporophyte of *Mastocarpus stellatus* Photos Algae base.

Tetraspores are formed under special conditions, only in short days and low temperatures(*e.g.* with 8 h light per 24 h but not with 16h light and at 10°C but not 15°C). This corresponds to winter and spring in nature (Guiry M.D. & J.A. West 1983 - J. Phycol. 19: 474-494).

Some studies of photoperiodic responses (GuiryM.D. 1984 – Helgolaänder Meeresunters 38: 335-347; Guiry M.D. & Cunningham 1984 – Phycologia 23: 357-367) have identified reproductive "windows" and suggested explanations for the increasingly exclusive vegetative propagation, as opposed to gametangial and tetrasporangial production, in high-latitude populations of a widespread European species. Genetic determination of branching patterns and upper limits of temperature tolerance have been established for several species by Guiry M.D. *et al*. (1987 - Helgoländer Meeresnumters 41: 283-295).

It is important to note that *Mastocarpus* and *Chondrus* which seem to be closely related, show quite different types of life cycle: isomorphic in *Chondrus* but heteromorphic in *Mastocarpus.*

According to Burns R.L. & A.C. Mathieson (1972 - J. exp. Mar. biol. ecol. 9 (1): 77-95), the period of fastest growth coincides with increasing summer temperatures while maximum carpospores release occurs during the coldest temperature in populations settled in New Hampshire.

ECOLOGY & GEOGRAPHICAL DISTRIBUTION

Mastocarpus stellatus is a common perennial red alga found, often in large continuous mats on rocks, on exposed and semi-exposed sites in the low intertidal zone with some extension into the upper sublittoral. It is very rare in subtidal.

This alga is widely distributed along the seashores on the eastern and western coasts of the North Atlantic. In Europe it exists from Northern Norway to Portugal. Its southern limit is in Morocco. It has been found in Iceland, Baltic Sea, North America (Connecticut, New Hampshire, Maine, North Carolina, Nova Scotia), Canary Island, Antarctic and sub-Antarctic Islands and also in Asia (Taiwan, Japan).

Fig. 5- Populations of *Gigartina stellata* in Brittany
Photo GELYMA.

It is also present in the Mediterranean basin, notably in Greece and Sicily.

Mastocarpus stellatus and *Chondrus crispus* live in the same kinds of habitat.

However, *Mastocarpus stellatus* shows a higher stress tolerance than *Chondrus. crispus* (Davison I.R. *et al.* 1989- Mar. Ecol.Prog. Ser. 58:123-131 Dudgeon S.R. *et al*. **–** Mar. Biol. 101:107-114 ; 1995 – Mar. Ecol. Prog. Ser. 117:193-206; Bischof K. *et al.* 2000 – Helgol. Mar. Res. 54:47-52).

These stress tolerance particularities would be linked:

-either to the reactive oxygen metabolism (Collin J. & I.R. Davison 1999 - Plant Cell Environ. 22: 1143-1151),

-or to the chemical composition, notably in fatty acids (Koch K. 2016 -Thesis Univ. Bremen),

that might explain why this species is more competitive in the highly variable upper intertidal compared to *Chondrus crispus*.

The salinity would be a dominant factor influencing the local distribution and growth of *Gigartina stellata* (Burns R.L. & A.C. Mathieson 1972 - J. exp. Mar. boil. Ecol. 9 (1): 77-95).

Mastocarpus stellatus also shows a very low herbivore susceptibility (Lubchenco J. - 1978- Am. Nat. 112: 23-39; Slocum C.J. 1980 - J. exp. Mar. Biol. Ecol. 46: 99-110; Dudgeon S.R. 1992- Thesis – Univ. Maine).

CHEMICAL COMPOSITION

According to Gomez-Ordonez E. *et al*. 2010 (- Food Res. Int. 43 : 2289–2294), *Mastocarpus stellatus* contains (in % DW)

Ash	24.99 ± 0.12	protein	21.30 ± 0.18	lipid	0.39 ± 0.002
Soluble fibre	22.85 ± 0.19	insoluble fibre	8.85 ± 0.67		
Total dietary fibre	31.70 ± 0.23				

Neutral sugars 5.18 ± 0.24 with abundance of galactose (3.72) and glucose (1.08)

These data are in agreement with the study of Marsham S. *et al.* 2007 (- Food Chem. 100: 1331-1336).

The composition in amino acids has been studied by Munda I.M. & F. Gubensek (1976 - Bot. Mar. 19: 85-92). The major amino acids (in mg/g DW) are: aspartic acid (13.2), glutamic acid (12.6), lysine (9.1), arginine (9.0), alanine (7.0) with total nitrogen equaling to 2.38 g/100 g DW.

Mastocarpus stellatus contains a typical hybrid kappa/iota/mu/nu-carrageenan (Gomez-Ordonez E. & P. Ruperez – 2011 - Food Hydrocolloids 25: 1514-1520). However the extraction conditions for carrageenans showed differences in MW of soluble fractions (Gomez-Ordonez E. *et al.* 2012 – Talanta)

According to the studies of Hilliou L. *et al.* (-2006- J. Agric. Food Chem. 54, 7870-7878) the mechanical properties of gels prepared from *Mastocarpus stellatus* seem to be correlated with polysaccharides chemical parameters such as the degree of sulfate groups, the molecular weight distribution, and the relative content in *é*-carrageenan monomers. They also depends on the extraction parameters (L. Hilliou *et al.* 2006 - Biomolecular Engineering 23: 201–208),

Garcia-Tasende M. *et al*. (2013- J. Applied Phycol. 25: 587-596) founded qualitative and quantitative differences in carrageenan composition of gametophytes of *Mastocarpus stellatus*. Carrageenans in gametophytes belong to the kappa family (κ-, ι-, ν-, μ-carrageenan). The dominant fractions were κ- and ι-carrageenan (more than 80 % of the total carrageenans). Mean total carrageenan content in gametophytes was of 37.32 ± 1.21 % DW.

Spatial and seasonal variations were observed, mainly related to changes on environmental and oceanographic factors and the role of carrageenans in adapting the fronds to these changes. These spatial differences in carrageenan content would be due to interactions of different factors, rather than the effect of a single factor

whereas seasonal variations in carrageenan content resulted to be more related to other factors directly correlated with the input of energy in the ecosystems (irradiance, sunshine hours and insolation). Thus, carrageenan content began to increase in early spring when the number of sunlight hours increased. Maximum values were reached in late spring or early summer, just before maximum values of irradiance and air temperature were achieved.

According to these analyses, *Mastocarpus stellatus* would be a more interesting species to obtain κ- and ι-carrageenan than *Chondrus crispus*, because carrageenan yield is more predictable. In addition, *Mastocarpus stellatus* is located higher in the intertidal zone, which makes the access by foot to the beds easy and only gametophytes are harvested. The harvest season of *Mastocarpus stellatus* should be different from *Chondrus crispus*, since maximum production period takes place only during late spring or early summer in the former species, and two maximum periods in the latter species were observed.

Floridoside is a neutral heteroside found in many red algae, notably *Rhodymenia palmata* and *Mastocarpus stellatus*.

Produced directly from photosynthesis, floridoside constitutes an important soluble carbon reserve readily available according to cellular needs (Simon-Colin C. *et al.* 2002- Carbohydr. Res. 337 (3), 279-80; Simon-Colin C. *et al*. 2003- Carbohydr. Res. 338 (22): 2413-2416).

The molecular structure of floridoside was first established by Putman E.W. & W.Z. Hassid in 1954 (- Biochem. J., 79, 7-12). It is characterised as a 2-O-α-D- galactopyranosylglycerol.

The amount of floridoside undergoes seasonal variation (in mg.g-1 DW):

Winter	29.3 ± 3.8
Spring	28.7 ± 3.0
Summer	29.3 ± 2.6
Autumn	34.0 ± 3.2

(*cf.* Kerjean V. *et al.* 2007 - Bot. Mar. 50: 59-64). The procedure of extraction has been optimized with a purity criterion required for biomedical applications, notably for applications in the pharmaceutical industry. In aqueous solution, it behaves as an osmoregulator involved in the resistance of the algal cell wall to changes in external salinity.

The fatty acid composition of *Mastocarpus* has been compared in thallus collected in different levels of lower intertidal, with an abundance of 16:0, 20:4 (n-6) and 20:5 (n-3):

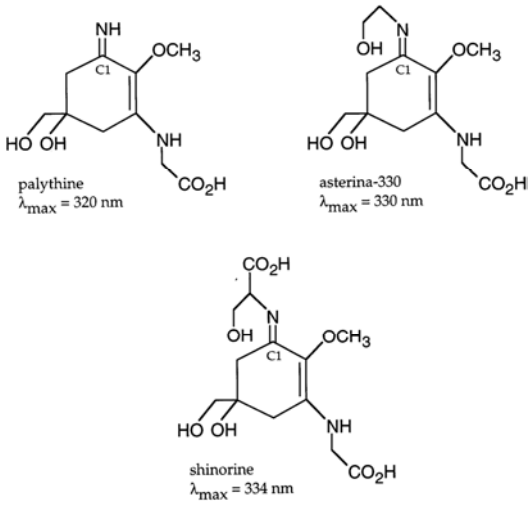
Major Fatty acids	*Mastocarpus* In higher levels	*Mastocarpus* in deeper levels
16:0	32.0 ± 0.7	31.2 ± 0.6
20:4 (n-6)	25.0 ± 0.4	23.7 ± 0.4
20:5 (n-3)	25.8 ± 0.4	26.0 ± 0.7

(*cf.* Kock K. – 2016- Thesis, Univ. Bremen; Koch K. *et al*. 2017- Helgol. Mar. Res. 71:15).

Mastocarpus stellatus shows a remarkable diversity in carotenoid composition (Esteban R. *et al.* 2009- Eur. J. Phycol. 44:2, 221-230,) with (%)

α carotene	10.2 ± 1.5	β carotene	28.5 ± 0.4
Zeaxanthina	2.6 ± 1	antheraxanthin	0.7 ± 0.4
Lutein	28.5 ± 0.4		

Mastocarpus stellatus also contains high amount of shinorine, a mycosporine like amino acids (2.139 ± 0.711 mg/g DW) (Karsten U. *et al*. 1998- Bot. Mar. 41 : 443-453).



Palythine and asterina-330 would be also present in trace quantities (Mason D.S. *et al*. 1998- Comp. Biochem. Physiol. Part A 120: 587-598).

Assays have been performed on diets including this algal species for

-aquacultural fishes (Mason D.S. *et al*. 1998- *Ibid*)

- sea urchins (Carroll A.K. & J.M. Shick 1996- Mar. Biol. 124: 561-569)

They confirm the dietary acquisition of MAAs for a possible protection against UV-induced damage.

Such results offer interesting effects for the aquaculture of fishes and invertebrates.

Hordenine (N-N-dimethyltyrenine) has been found in *Mastocarpus stellatus* but not in *Chondrus crispus* (Barwell C.J. & G. Blunden 1981- J. Nat. Prod. Lloydia 44 (4): 500-502; Barwell C.J. *et al.* 1982 – Br. Phycol J. 17 (2): 229; Barwell C.J. *et al.* 1989- Phytotherapy Res. 3: 67-69).

BIOACTIVITIES & USES

Like *Chondrus crispus, Mastocarpus stellatus* is of significant ecological and economic importance, providing food and habitat to associated invertebrates (McLachlan J.L. 1991 - In: Mauchline J & T. Nemoto (eds) Marine biology, its accomplishments and future prospects. Hokusen-sha Publ Co, Tokyo: 221-237, Kornmann P. & T-H. Sahling 1994 - Helgol Wiss. Meeresunters 48: 365-406).

It represents an important source of carrageenan, which is used in the food, cosmetic and pharmaceutical industries (Gómez-Ordóñez *et al*. 2010 – Food Res. Int. 43: 2289-2294).

Carrageenan may be used as polymer matrix for the production of tablets and al. for pharmaceutical applications (Li L.R. *et al.* 2013 – Carbohydrate Polymers 103: 1-11).

The biopolymers extracted from *Mastocarpus stellatus* have been successful used for the production of biodegradable films and coatings for food applications (Larotonda F.D.S. 2012- Depart. Engenharia Quimica 1: 275-281), notably for antioxidant films (Aleman A. *et al*. 2016- Food Hydrocolloids 56 : 277-284).

The carrageenan extracted from *Mastocarpus stellatus* shows important antioxidant properties (Jimenez-Escrig A. *et al*. 2011- J Appl Phycol. DOI 10.1007/s10811-011-9742-8) depending of the extraction process. The reduction power would be linked to sulfation of polysaccharides.

These antioxidant properties have been confirmed by other studies (Gomez-Ordonez E. *et al*. 2014- Bioactive Carbohydrates & Dietary fibre 3 (1): 29-40; Koch K. 2016 – Thesis Univ. Bremen; Maehuenda J. *et al*. 2016 - Agro Food Ind. Hi tech 27 (2) :57-59).

Anti-coagulant activity of this carrageenan has been also quoted (Gomez-Ordonez E. *et al.* 2014 – *Ibid*).

Some biological activities have been demonstrated on various extracts prepared from *Mastocarpus stellatus* or *Gigartina stellata.*

They concern:

- antiviral properties (Patent US 20110189221-A1 – 2011-01-14),
- antimicrobial activities useful to fight aquacultural losses (Dubber D. & H. Tilman 2008- Aquaculture 274 (2-4): 196-200).

Therapeutic applications have been also drawn attention.

- A mix including ionic aqueous solution and sulfated polysaccharides from *Gigartina stellata* would be interesting to treat respiratory mucosal-related condition, notably rhinosinusitis or allergic rhinitis – Patent US 2017 0000817 A1 – 2014-11-28).
- *Mastocarpus stellatus* shows beneficial effect on lipid metabolism with a significant reduction of triglycerides and total cholesterol in serum of rats. That might be useful for the prevention of hyperlipidemia and thrombosis due to lipid metabolism, antioxidant status and anticoagulant capacity (Gomez-Ordonez E. *et al.* 2012- Food Chemistry 135: 806-811).
- Additionally, this alga would be of commercial interest due to its high content of polyunsaturated fatty acids with 20 carbon atoms such as 20:4 (n-6) (arachidonic acid) and 20:5 (n-3) (eicosapentaeonic acid) (Mabeau S. & J. Fleurence 1993 - Trends Food Sci. Tech. 4: 103-107).

  Arachidonic acid has medical significance as precursor of prostaglandins, whereas eicosapentaeonic acid is an essential constituent in the feed of several mariculture species and is suggested to reduce the risk of thrombosis, artherosclerosis and heart disease in humans (Flore to EAT. *et al*. 1993 **–** Bot. Mar 36: 217-222, Ortiz J. *et al.* 2009 **–** Eur. J. Lipid Sci. Technol. 111: 320-327).

Several components present in *Mastocarpus stellatus* / *Gigartina stellata* offer great potential, notably floridoside and mycosporine like amino acids.

- Courtois *et al* (2008- Mar. Drugs 6: 407-417) showed that the floridoside extracted from *Mastocarpus stellatus* activates a complement cascade *via* the classical complement pathway, through the recruitment and activation of natural IgM. This algal molecule could represent an important step in the development of a potent new anti-complementary agent for use in therapeutic complement depletion.

  An European Patent (EP 1743628 A1 – 2006-07-13) concerns the association of acid isethionique with floridoside extracted from red algae, notably from *Mastocarpus stellatus* for skin hydration and anti-aging.

- According to Athukorala Y. *et al*. 2016 (Molecules 21(1): 119) some extracts rich in some mycosporine like amino acids, notably in polar palythine and asterina-330 and those prepared from *Mastocarpus stellatus* show antiproliferative as inducers of apoptosis in HeLa cells.

Other Patents concern the use of *Gigartina stellata* for

- Preparation of agar (GB 577533 (A) – 1946-05-22)
- Preparation of a dessert gel (GB 1212813 (A) – 1970-11-18)
- Use of extracts made notably from *Gigartina stellata* using saline hot water sources offers multi-faceted effects for pharmaceutical and cosmetic purposes (US 2010028376 (A1)-2010-02-04)
- Use of natural antioxidants from seaweed hydrolysates, notably from *Gigartina stellata* for numerous applications *e.g*. food, animal feed, fish feed, fertilizer, pharmaceutical preparations, cosmetics (US 201527491 (A1)- 2015-10-01).

Gigartina stellata has been rarely used for cosmetic applications as:

- as moisturizinng agent in butylenes glycol : HYDRANE BG (PERSPERSE)
- as anti-aging agent in butylene glycol associated with other algal species : PHYTLENE COMPLEX EGX 772 BG (GREENTECH).

GELYMA offers ALGYL® prepared from *Gigartina stellata* in combination with an extract of *Corallina officinalis* for efficient protection of skin barrier functions.

ALGYL®

Marine innovative approach

for a smart global epidermal barrier protection

based on

multifaceted intelligent ways of action

for strengthening the different barrier functions

(outside-in ∗ inside-out ∗ immune∗ anti-oxidant functions)

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: June 12, 2020

SUBJECT: Hydrolyzed Corallina Officinalis Extract

Biotech Marine. 2016. Manufacturing Process Oligophycorail (Hydrolyzed Corallina Officinalis Extract with Sodium Methylparaben as a preservative).

Biotech Marine. 2016. Manufacturing Process Oligophycorail SPE (Hydrolyzed Corallina Officinalis Extract with 2-Phenoxyethanol as a preservative).

Biotech Marine. 2016. Statement Oligophycorail Composition File (Hydrolyzed Corallina Officinalis Extract with Sodium Methylparaben as a preservative).

Biotech Marine. 2016. Statement Oligophycorail SPE Composition File (Hydrolyzed Corallina Officinalis Extract with 2-Phenoxyethanol as a preservative).

Palmer Research. 2004. Etude de la tolerance cutanee aigue d'une matiere premiere chez le volontaire adulte: Patch test 24 heures occlusif sous controle dermatologique (Oligophycorail Hydrolyzed Corallina Officinalis Extract with Sodium Methylparaben as a preservative).

Cosderma Laboratoire. 2007. Verification chez l'homme de la compatibilite cutanee d'un produit cosmetique apres application unique sous pansement. Patch test 24 h (Hydrolyzed Corallina Officinalis Extract with 2-Phenoxyethanol as a preservative).

Palmer Reserach. 1995. Evaluation du potentiel allergisant apres applications epicutanees repetees sur 51 volontaires (Oligophycorail Hydrolyzed Corallina Officinalis Extract with Sodium Methylparaben as a preservative).

Confidential

MANUFACTURING PROCESS
OLIGOPHYCOCORAIL

HARVESTING / IDENTIFICATION *(Corallina Officinalis)*

↓

Addition of Sodium Methylparaben → EXTRACTION WITH WATER

↓

FILTRATION

↓

QUALITY CONTROL

↓

PACKAGING

↓

QUALITY CONTROL

Managing Director
Guénolé LE CALVEZ

BIOTECHMARINE (6/24/2016)

Confidential

MANUFACTURING PROCESS
OLIGOPHYCOCORAIL SPE

HARVESTING / IDENTIFICATION *(Corallina Officinalis)*

↓

Addition of 2-Phenoxyethanol → EXTRACTION WITH WATER

↓

FILTRATION

↓

QUALITY CONTROL

↓

PACKAGING

↓

QUALITY CONTROL

Managing Director
Guénolé LE CALVEZ

BIOTECHMARINE (6/24/2016)

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www.biotechmarine.com

Statement OLIGOPHYCOCORAIL COMPOSITION FILE

(In accordance with EN 45014 General criteria for supplier's declaration of conformity)

We declare, by the present one, that the following product supplied by BIOTECHMARINE:

OLIGOPHYCOCORAIL
(INCI NAME (USA): **Aqua / Water - Hydrolyzed Corallina Officinalis Extract**

Composition

Components	Components usual Name	Function	% (Concentration range)
Aqua / Water		Solvent	> 96
Hydrolyzed Corallina Officinalis Extract		Active	0,5 - 3,0
Sodium Methylparaben	Methyl 4-hydroxybenzoate sodium salt	Preservative	0,16 - 0,20

This certificate was established to the best of our knowledge and/or according to our suppliers' statements, at the date of this certificate. It is however your responsibility to abide by any clause of any regulations, which may apply to the product that you manufacture, or to the use you make of our product.

Pontrieux, on 7/1/2016

Guénolé LE CALVEZ.
Managing Director

Nota

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Statement OLIGOPHYCOCORAIL SPE COMPOSITION FILE

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OLIGOPHYCOCORAIL SPE

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Composition

Components	Components usual Name	Function	% (Concentration range)
Aqua / Water		Solvent	> 96
Hydrolyzed Corallina Officinalis Extract		Active	0,5 - 3,0
Phenoxyethanol	2-Phenoxyethanol	Preservative	0,80 - 1,20

This certificate was established to the best of our knowledge and/or according to our suppliers' statements, at the date of this certificate. It is however your responsibility to abide by any clause of any regulations, which may apply to the product that you manufacture, or to the use you make of our product.

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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 06 janvier 2004

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Etude : 1030478PA

Matière première : OLIGOPHYCOCORAIL
LOT 3.05.132 (58344)

Promoteur: **SECMA BIOTECHNOLOGIE MARINE**
ZI – BP 65
22260 PONTRIEUX
FRANCE

Lyon, le 06 janvier 2004

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RESUME DU RAPPORT D'ETUDE

Promoteur : **SECMA BIOTECHNOLOGIE MARINE** **Adresse : ZI – BP 65** **22260 PONTRIEUX** **FRANCE**	**Matière première:** **OLIGOPHYCOCORAIL LOT 3.05.132** **Code PALMER Research : 58344**

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 17 au 19 décembre 2003.	
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 : 10.
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 : $I.I.M = \frac{\text{score total des réactions (érythème + œdème)}}{\text{nombre total de volontaires}}$ 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 **OLIGOPHYCOCORAIL LOT 3.05.132** est égal à **0** à la lecture 30 minutes et à **0** à la lecture 24 heures. Dans les conditions expérimentales retenues, la matière première est classée **non irritante**.	
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 10 volontaires adultes, la tolérance cutanée aiguë ou potentiel irritant de la matière première:

OLIGOPHYCOCORAIL LOT 3.05.132

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 **58344**.

L'essai a commencé le 17 décembre pour s'achever le 19 décembre 2003.

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 : 04.02.04 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

- ✓ 10 sujets ont été inclus dans l'essai,
- ✓ dont sept de sexe féminin et trois de sexe masculin,
- ✓ âgés de 18 à 53 ans (moyenne d'âge: 30 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 :

	OLIGOPHYCOCORAIL LOT 3.05.132
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 :

$$\textbf{I.I.M} = \frac{\textbf{Σ des cotations (érythème + œdème)}}{\textbf{Nombre de sujets}}$$

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 pagez 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.

OLIGOPHYCOCORAIL LOT 3.05.132
(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	
13S51	SEM Ou	19	F	Normale	0	0	0	0	-	0	0	0	0	-
14S51	ABD Ra	30	F	Normale	0	0	0	0	-	0	0	0	0	-
15S51	MAZ Pa	45	F	Normale	0	0	0	0	-	0	0	0	0	-
16S51	MIC Be	21	M	Normale	0	0	0	0	-	0	0	0	0	-
21S51	BEN Fa	39	F	Normale	0	0	0	0	-	0	0	0	0	-
22S51	MAU Je	53	M	Normale	0	0	0	0	-	0	0	0	0	-
23S51	BER Ar	23	F	Normale	0	0	0	0	-	0	0	0	0	-
24S51	VIC An	18	F	Normale	0	0	0	0	-	0	0	0	0	-
26S51	MIC Tr	24	M	Normale	0	0	0	0	-	0	0	0	0	-
28S51	PON Vi	24	F	Normale	0	0	0	0	-	0	0	0	0	-
Age moyen		30	I.I.M		0		0		-	0		0		-

I.I.M	**0**	**0**
Résultats	**non irritant**	**non irritant**

(1) : M = masculin
F = féminin

5 - CONCLUSION

30 minutes et 24 heures après l'enlèvement du patch occlusif, aucun volontaire n'a présenté de réaction d'irritation significative d'une réaction d'intolérance cutanée.

Par ailleurs, aucun effet secondaire n'a été observé.

Dans les conditions expérimentales retenues, on peut donc conclure que la matière première **OLIGOPHYCOCORAIL LOT 3.05.132** testée sous contrôle dermatologique, et appliquée pure et localement sous pansement occlusif pendant 24 heures, sur la peau de 10 volontaires adultes, est classée **non irritante**.

Dr Yvette WELTERT
Dermatologue

STUDY SUMMARY REPORT

Sponsor: **SECMA BIOTECHNOLOGIE MARINE** **Address: ZI – BP 65** **22260 PONTRIEUX** **FRANCE**	**Raw material:** **OLIGOPHYCOCORAIL LOT 3.05.132** **PALMER Research code: 58344**

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 December 17 to December 19, 2003.	
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: 10.
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 $0.20 \leq M.I.I. < 0.50$: Slightly irritating if $0.50 \leq M.I.I. < 1$: Moderately irritating if $M.I.I. \geq 1$: Irritating	
Conclusion:	The irritation index of the raw material **OLIGOPHYCOCORAIL LOT 3.05.132** is equal to **0** at the 30-minute reading and to **0** at the 24-hour reading. Under these study conditions, the raw material is classified **non irritating.**	
Dr Yvette WELTERT, ***Dermatologist***		

RAPPORT

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

Produit testé :

OLIGOPHYCOCORAIL SPE REF 7.11.299

Promoteur

BIOTECHMARINE
ZI – BP 65
22260 PONTRIEUX

Bordeaux, le 28/12/2007

JA/AG

LABORATOIRE COSDERMA
SERVICE DE DERMATOLOGIE DU Pr. ALAIN TAÏEB
GROUPE HOSPITALIER SAINT ANDRÉ
1 RUE JEAN BURGUET BP50057 33023 BORDEAUX CEDEX
laboratoire@cosderma.com
TEL : 05 56 94 75 40
FAX : 05 56 79 49 75

1- But de l'étude

Le but de cette étude était d'évaluer la compatibilité cutanée du produit **«OLIGOPHYCOCORAIL SPE REF 7.11.299»,** après application unique sur la peau pendant 24h (J1) dans des conditions maximalisantes, chez **10** volontaires.

La compatibilité cutanée du produit a été vérifiée à J1, 15 minutes après dépatchage, par un examen visuel de la zone expérimentale réalisé par l'investigateur ou la technicienne responsable sous son autorité. En cas de réaction(s) clinique(s), un examen a été réalisé à J2 et J3, jusqu'à disparition complète de celle(s)-ci.

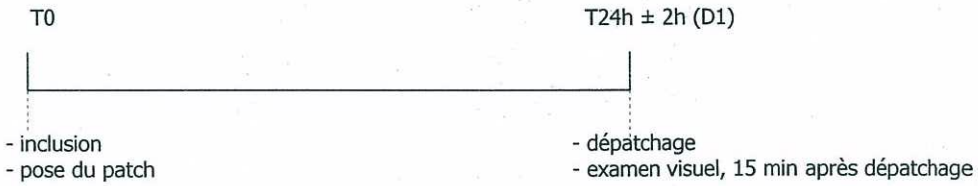
La compatibilité du produit avec la peau, après utilisation dans les conditions normales d'emploi, a été appréciée par extrapolation des résultats obtenus dans ces conditions particulières.

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 2 visites au laboratoire (T0, T24h).



2-2. Centre investigateur

Laboratoire COSDERMA
Service de dermatologie du Pr Taïeb – Unité 5
Groupe Hospitalier Saint André
1 rue Jean Burguet - BP 50057
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email : laboratoire@cosderma.com

2-3. Lieu de l'investigation

Service de dermatologie du Pr Taïeb - Unité 5
Groupe Hospitalier Saint André
1 rue Jean Burguet
33000 Bordeaux

2-4. Equipe technique

Investigateur :	Pr Alain Taïeb
Technicienne :	Aurélie Germain

3- Dates de réalisation de l'étude

Début le :	4/12/2007
Fin le :	5/12/2007

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 du produit	Quantité pour l'étude	Nb conditionnement
OLIGOPHYCOCORAIL SPE REF 7.11.299	5 ml	1 x 5 ml

4.3 Conditions normales d'emploi

Nom du produit	Site et mode d'emploi usuel
OLIGOPHYCOCORAIL SPE REF 7.11.299	Application 1 fois par jour sur le corps

4.4 Conditions d'utilisation pendant l'étude

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

Nom du produit	Type de pansement	Conditions d'application	Quantité appliquée	Temps de contact	Temps de contrôle à J1
OLIGOPHYCOCORAIL SPE REF 7.11.299	Occlusif (Finn chambers®)	Pur	20 µl	24h	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.

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 **11**.
Le nombre de volontaires dont les données sont présentées est de **11**.

5-3 Critères d'inclusion

Les critères d'inclusion étaient les suivants :

- origine : caucasienne,
- 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),
- Pas de vaccination pendant l'étude.

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 (J1)
Sélection des volontaires	X	
Attribution n° des volontaires	X	
Information volontaire et consentement participation (IVCP*)	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 de l'IVCP a été remis aux volontaires le jour de la visite d'inclusion pour l'étude. L'original a été 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. Un patch témoin, correspondant au type de pansement utilisé, contenant une quantité ad hoc d'eau pour préparation injectable, est appliqué parallèlement.

- *Conditions environnementales*

Les conditions environnementales imposées aux volontaires ont été 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 a effectué un contrôle visuel de chaque zone expérimentale sous un éclairage standardisé type « lumière du jour ».

- Fréquences

L'examen visuel a été réalisé à T24h ± 2h (J1), 15 minutes après dépatchage (ou plus si des rougeurs sont apparues à l'enlèvement du patch).
En cas de réaction(s) clinique(s), un examen a été réalisé à J2 et J3, jusqu'à disparition complète de celle(s)-ci.

- *Critères d'évaluation*

- *signes cliniques*

Description	Code laboratoire	intensité	aspect	note
Erythème	**E**	- échelle ordinale en 5 points : • absent • très léger • léger • modéré • sévère	- érythème : • diffus • ponctué • périphérique	• Absent = 0 • Très léger = 0,5 • Léger = 1 • Modéré = 2 • sévère = 3 • diffus = d • ponctué = p • périphérique = peri
Oedème	**Oe**			
Sécheresse	**S**			
Desquamation	**Dq**			
Dessèchement	**De**			
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, a 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 5 points : • absent • très léger • léger • modéré • sévère	• Absent = 0 • Très léger = 0,5 • Léger = 1 • Modéré = 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 à J1 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 indice d'irritation cutanée** calculé à partir des « notes » attribuées uniquement aux signes cliniques décelables visuellement à type d'érythème, œdème.

° **de façon descriptive** pour les autres signes décelables visuellement ou pour les sensations d'inconfort. Dans ce cas, le pourcentage de volontaires chez qui ils ont été observés, est éventuellement calculé si la fréquence d'apparition de ces signes le justifiait.

Pour chaque volontaire à J1 (à J2 et J3 dans le cas de suivi de réactions), un indice d'irritation journalier individuel (IijI) a été calculé. Il représente la somme des notes obtenues pour les signes observés.

Pour le panel à J1 (à J2 et J3 dans le cas de suivi de réactions), un indice d'irritation journalier moyen (IijM) a été calculé. Il correspond à la formule :

IijM= $\sum$ (IijI) (érythème + oedème) / Nombre de volontaires pris en compte

◆ *Interprétation des résultats*

L'indice d'irritation obtenu permet de classer arbitrairement le produit cosmétique étudié selon le barème suivant:

IijM	Classe
< 0,20	Non irritant (NI)
0,20 $\leq$ IijM < 0,50	Légèrement irritant (LI)
0,50 $\leq$ IijM < 1	Modérément irritant (MI)
IijM $\geq$ 1	Irritant (I)

Annexe 1

CARACTERISTIQUES TYPOLOGIQUES DES VOLONTAIRES

Volontaires		Age (ans)	Sexe	Phototype *	Type de peau au niveau du corps
Réf.	Nom /prén				
1	GUIN/M	59	F	II	Normale
2	LACH/V	31	F	II	Normale
3	SCHA/N	46	F	II	Sensible
4	CARR/E	55	F	III	Normale
5	ACCA/H	64	F	II	Normale
6	GIAC/C	43	F	II	Normale
7	DEMO/J	60	F	II	Normale
8	EMER/B	48	F	II	Sensible
9	GAZA/F	45	F	II	Normale
10	GUIL/N	52	F	II	Normale
11	DUPU/I	42	F	II	Normale

légendes : *sexe : F= féminin / M= masculin*

***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

Annexe 2

CONTROLE DE L'OBSERVANCE
Contraintes

Contraintes (11 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	11	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	11	100 %
Pas de bain (en baignoire ou en piscine ou en mer) et pas de hammam ou de sauna pendant l'étude Déviation : aucune	11	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	11	100 %
Pas de sudation excessive et de sport intensif, susceptibles d'entraîner le décollement du pansement Déviation : aucune	11	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	11	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	11	100 %
Pas de vaccination pendant l'étude Déviation : aucune	11	100 %

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Annexe 3-1

VERIFICATION DE LA COMPATIBILITE CUTANEE

Produit N°1: **«OLIGOPHYCOCORAIL SPE REF 7.11.299»** (Finn chambers®)

Temps de contrôle		**15 minutes après dépatchage (J1)**	
Réf	**Nom/Prén**	**Examen cutané**	**IijI**
1	GUIN/M	/	0
2	LACH/V	/	0
3	SCHA/N	/	0
4	CARR/E	/	0
5	ACCA/H	/	0
6	GIAC/C	/	0
7	DEMO/J	/	0
8	EMER/B	/	0
9	GAZA/F	/	0
10	GUIL/N	/	0
11	DUPU/I	/	0
IijM		0	

Légende : / = aucun signe clinique

Laboratoire COSDERMA - Hôpital St André - Service Dermatologie Pr. Taieb - 1, rue Jean Burguet - BP 50057 - 33023 Bordeaux cedex
Tel : 05.56.94.75.40 - Fax : 08.26.71.50.03 – *email : laboratoire@cosderma.com*

Annexe 3-2

VERIFICATION DE LA COMPATIBILITE CUTANEE

Produit N°2: Témoin (Finn chambers®)

Temps de contrôle		15 minutes après dépatchage (J1)	
Réf	**Nom/Prén**	**Examen cutané**	**IijI**
1	GUIN/M	/	0
2	LACH/V	/	0
3	SCHA/N	/	0
4	CARR/E	/	0
5	ACCA/H	/	0
6	GIAC/C	/	0
7	DEMO/J	/	0
8	EMER/B	/	0
9	GAZA/F	/	0
10	GUIL/N	/	0
11	DUPU/I	/	0
IijM		0	

Légendes : / = aucun signe clinique

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Tel : 05.56.94.75.40 - Fax : 08.26.71.50.03 – email : laboratoire@cosderma.com

L'investigateur a conclu en terme de caractère **non irritant, légèrement irritant, modérément irritant ou irritant** du produit 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 :

Nom du produit	Temps de contrôle	Nombre de volontaires réactifs	Types de réaction	Indice d'irritation journalier moyen (IijM)	Résultats
OLIGOPHYCOCORAIL SPE REF 7.11.299	**15 minutes après dépatchage (J1)**	**0**	**/**	**0**	**Non irritant**

8- Conclusion

Dans les conditions expérimentales adoptées, l'indice d'irritation journalier moyen (IijM) du produit «OLIGOPHYCOCORAIL SPE REF 7.11.299» appliqué sous pansement occlusif pendant 24h, est égal à 0, 15 minutes après dépatchage (J1), et classifie le produit comme non irritant.

Signatures et dates :

Pr Alain Taïeb (Dermatologue)

Investigateur

8-1-2008

Aurélie Germain

Assistante Clinique

03-01-08

Jérôme Asserin

Directeur d'étude

8/01/08

Aurélie Cherchouly

Responsable qualité

03/01/08

Laboratoire COSDERMA - Hôpital St André - Service Dermatologie Pr. Taieb - 1, rue Jean Burguet - BP 50057 - 33023 Bordeaux cedex
Tel : 05.56.94.75.40 - Fax : 08.26.71.50.03 – *email : laboratoire@cosderma.com*

PALMER Research

PRODUIT : OLIGOPHYCOCORAIL lot 4 05 051

SOCIETE : SECMA Biotechnologies Marines
BP 65
22260 PONTRIEUX

EVALUATION DU POTENTIEL ALLERGISANT APRES APPLICATIONS EPICUTANEES REPETEES SUR 51 VOLONTAIRES

Mérignac, Janvier 1995

SOCIÉTÉ DE CONSEIL–EXPERTISE PHARMACEUTIQUE & COSMÉTOLOGIQUE

3, RUE DU GOLF - PARC INNOLIN - 33700 MÉRIGNAC - TÉL : 56 34 75 55 – FAX : 56 47 64 70
S.A.R.L. AU CAPITAL DE 120.000 FRANCS – APE 8301 – SIRET 384 324 141 00017

Je, soussigné, **Dominique SABOUREAU**, Docteur en Pharmacie, gérant de la Société **PALMER RESEARCH** (sis : 3 rue du Golf - 33700 MERIGNAC), atteste que le produit **OLIPHYCOCORAIL Lot 4 05 051** a été confié à la Société HARRISON Research Laboratoires, INC (sis HRL : 1264 Springfield Avenue, MAPLEWOOD - NJ 07040), afin d'évaluer le potentiel allergisant de cette préparation , par applications épicutanées, sur 60 volontaires (51 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 #94-140 (7).

Cette étude a été réalisée selon le schéma expérimental suivant :

METHODOLOGIE

- *Volontaires inclus*

51 volontaires sains de type caucasien et des deux sexes (19 hommes et 32 femmes), sans affection cutanée ni antécédent médical empêchant l'application topique de substances, et âgés de 14 à 64 ans.

- *Traitements*

Phase d'induction :

9 applications successives (lundi, mercredi et vendredi, pendant 3 semaines) du produit tel quel, à raison de 0,2ml 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.

- ***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.

- ***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 **OLIPHYCOCORAIL Lot 4 05 051** n'a induit aucune réaction d'intolérance cutanée locale au cours de la phase d'induction et de la période de repos.

Après la dernière application, phase déclenchante, il n'a été noté aucun érythème, aucun oedème, avec ou sans papules et/ou légère vésiculisation.

CONCLUSION

On peut conclure que le produit **OLIPHYCOCORAIL Lot 4 05 051** s'est révélé très bien toléré au niveau cutané, n'entraînant aucune réaction d'allergie de contact significative. Il peut donc être qualifié d'**HYPOALLERGENIQUE**.

Mérignac, le 11 janvier 1995

Dominique SABOUREAU,
Docteur en Pharmacie.

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: June 16, 2020

SUBJECT: Hypnea Musciformis Extract

Biotech Marine. 2012. Manufacturing process Biorestorer™ (Hypnea Musciformis Extract).

Biotech Marine. 2017. Statement Biorestorer™ Composition File (Hypnea Musciformis Extract).

Palmer Research. 2004. Etude de la tolerance cutanee aigue d'une matiere premiere chez le volontaire adulte: Patch-test 24 heures occlusif sous controle dermatologique (Biorestorer™ contains 1-3% Hypnea Musciformis Extract).

BiotechMarine - Z.I. - B.P.72 - 22260 Pontrieux (FR)
Tél. : 33 (0)2 96 95 31 32 Fax : 33 (0)2 96 95 31 30
ww.biotechmarine.com

MANUFACTURING PROCESS BIORESTORER™

HARVESTING / IDENTIFICATION *(Hypnea Musciformis)*

↓

DRYING

↓

GRINDING

↓

EXTRACTION WITH THE SOLVENT WATER & BUTYLENE GLYCOL ← ***Addition of :*** ***- Potassium Gluconate*** ***-Methylparaben***

↓

FILTRATION

↓

QUALITY CONTROL

↓

PACKAGING

↓

QUALITY CONTROL

Deputy Production Manager
Yann QUERREC
le 10/2/17

BiotechMarine - Z.I. - B.P.72 - 22260 Pontrieux (FR)
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Statement BIORESTORER™ COMPOSITION FILE

(In accordance with EN 45014 General criteria for supplier's declaration of conformity)

We declare, by the present one, that the following product supplied by BIOTECHMARINE:

BIORESTORER™

(INCI NAME (USA): Aqua / Water - Butylene Glycol – Hypnea Musciformis Extract

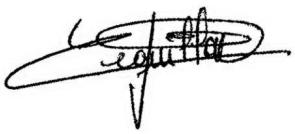
Composition

Components	Components usual Name	Function	% (Concentration range)
Aqua / Water		Solvent	72,0 – 77,0
Butylene Glycol	Butane-1,3-diol	Solvent	20,0 – 27,0
Hypnea Musciformis Extract		Active	1,0 – 3,0
Potassium Gluconate	D-Gluconic Acid Potassium Salt	Additive	≤1
Methylparaben	Methyl 4-Hydroxybenzoate	Preservative	0,16 - 0,20

This certificate was established to the best of our knowledge and/or according to our suppliers' statements, at the date of this certificate. It is however your responsibility to abide by any clause of any regulations, which may apply to the product that you manufacture, or to the use you make of our product.

Document approved at Pontrieux, on February 07, 2017

By Laëtitia LE GUILLOU
Regulatory & Documentary Affairs from BIOTECHMARINE



Nota
The analytical specifications warranted are only those mentioned on the certificate of analysis supplied with each delivery of the product.
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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 07 janvier 2004

GROUPE DERMSCAN

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Internet : www.palmer-research.com

Etude : 1030478PA

Matière première : BIOSTRUCTURER LOT 3.05.129 (58331)

BIORESTORER

contains 1-3% Hypnea Musciformis Extract

Promoteur: **SECMA BIOTECHNOLOGIE MARINE**
ZI – BP 65
22260 PONTRIEUX
FRANCE

Lyon, le 07 janvier 2004

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RESUME DU RAPPORT D'ETUDE

Promoteur : **SECMA BIOTECHNOLOGIE MARINE** **Adresse : ZI – BP 65** **22260 PONTRIEUX** **FRANCE**	**Matière première:** **BIOSTRUCTURER LOT 3.05.129** **Code PALMER Research : 58331**

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 17 au 19 décembre 2003.	
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 : $I.I.M = \frac{\text{score total des réactions (érythème + œdème)}}{\text{nombre total de volontaires}}$ 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 **BIOSTRUCTURER LOT 3.05.129** est égal à **0.25 (légèrement irritante)** à la lecture 30 minutes et à **0.04 (non 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:

BIOSTRUCTURER LOT 3.05.129

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 **58331**.

L'essai a commencé le 17 décembre pour s'achever le 19 décembre 2003.

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.

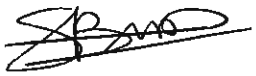
Après relecture et en tant qu'Investigateur, je certifie ces données conformes
à la réalité des résultats obtenus.
Docteur Yvette WELTERT, *Dermatologue.*

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 : 04.02.04 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 neuf de sexe féminin et trois de sexe masculin,
- ✓ âgés de 18 à 46 ans (moyenne d'âge: 27 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 :

	BIOSTRUCTURER LOT 3.05.129
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 :

$$\text{I.I.M} = \frac{\Sigma \text{ des cotations (érythème + œdème)}}{\text{Nombre de sujets}}$$

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 pagez 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.

BIOSTRUCTURER LOT 3.05.129
(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	
1S51	FAU Fr	46	F	Normale	0.5	0	0.5	0	-	0	0	0	0	-
3S51	MED Fa	29	M	Normale	0.5	0	0.5	0	-	0	0	0	0	-
4S51	BEN Ec	22	F	Normale	0	0	0	0	-	0	0	0	0	-
5S51	BUA JY	28	M	Normale	0	0	0	0	-	0	0	0	0	-
6S51	GIC Ni	19	F	Normale	0	0	0.5	0	-	0	0	0	0	-
8S51	BER Ch	18	F	Normale	0	0	0.5	0	-	0	0	0	0	-
9S51	MON Ma	23	F	Normale	0	0	0.5	0	-	0	0	0	0	-
10S51	QUI Ce	21	F	Normale	0	0	1	0	-	0	0	0.5	0	-
11S51	DUB Li	24	F	Normale	0	0	0.5	0	-	0	0	0	0	-
12S51	GUI Vi	25	M	Normale	0	0	0	0	-	0	0	0	0	-
18S51	AGA Pa	45	F	Normale	0	0	0	0	-	0	0	0	0	-
19S51	CHE So	19	F	Normale	0	0	0	0	-	0	0	0	0	-
Age moyen		27	I.I.M		0.08		0.33		-	0		0.04		-

I.I.M	0.25	0.04
Résultats	**légèrement irritante**	**non irritante**

(1) : M = masculin
F = féminin

Remarque : Les volontaires n°1S51 et n°3S51 ont présenté une très légère réaction érythémateuse au niveau de la cupule témoin 30 minutes après le retrait des patchs. Ces réactions ayant disparu à 24 heures, les sujets sont inclus dans le calcul.
Le calcul de l'I.I.M est effectué par différence entre le score produit et le score témoin.

5 - CONCLUSION

30 minutes après l'enlèvement du patch occlusif, sept volontaires (n°1S51, 3S51, n°6S51, n°8S51, n°9S51, n°10S51 et n°11S51) ont présenté un très léger à léger érythème.
A la lecture 24 heures, un très léger érythème était toujours observé chez le sujet n°10S51.
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 **BIOSTRUCTURER LOT 3.05.129** testée sous contrôle dermatologique, et appliquée pure et localement sous pansement occlusif pendant 24 heures, sur la peau de 12 volontaires adultes, est classée **légèrement irritante** à la lecture 30 minutes et **non 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: BIOSTRUCTURER LOT 3.05.129** **PALMER Research code: 58331**

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 December 17 to December 19, 2003.	
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 **BIOSTRUCTURER LOT 3.05.129** is equal to **0.25 (slightly irritating)** at the 30-minute reading and to **0.04 (non** irritating) at the 24-hour reading.	
Dr Yvette WELTERT, *Dermatologist*		

Personal Care Products Council
Committed to Safety, Quality & Innovation

Memorandum

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

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

DATE: April 30, 2020

SUBJECT: Palmaria Palmata Extract and Hypnea Musciformis Extract

Anonymous. 2020. Information Palmaria Palmata Extract and Hypnea Musciformis Extract.

Date : 30 April of 2020

Information Palmaria Palmata Extract and Hypnea Musciformis Extract

Palmaria Palmata Extract:

- **Physico-chemical properties**
  - Aspect : limpid liquid
  - Odor : characteristic
  - Color : yellow

- **Main steps of the manufacturing process :**
  - Solubilization of powder of *Palmaria palmata* in water
  - Separation of soluble and insoluble phases
  - Concentration of soluble phase
  - Membrane sterilization

- **Composition :**
  - Sugars (mainly oligosaccharides which average molecular weight is between 540 and 2000 Da) : 73%
  - Mineral ashes : 24%
  - Proteins : 3%
  - Absence of alkaloids and polyphenols

- **Impurities:**

The toxicological profile of our cosmetic active was determined:

- Heavy metals:

Compounds	**Quantity (ppm)**	**Acceptability Threshold (ppm)**
Antimony	0.069	0.5
Arsenic	1.480	2.0
Cadmium	NQ	0.5
Chromium	0.046	0.5
Cobalt	NQ	0.5
Mercury	NQ	0.5
Nickel	0.433	1.0
Lead	NQ	0.5
Vanadium	2.29	3.0

- **Aflatoxins**

The determination and quantification of 4 aflatoxins (B1, B2, G1 and G2) are performed by high performance liquid chromatography (HPLC).

NAME	Results	LQ (ppb)
Aflatoxin B1	ND	< 0.3
Aflatoxin B2	ND	< 0.3
Aflatoxin G1	ND	< 0.3
Aflatoxin G2	ND	< 0.3

- **Iodine**

Quantification of iodine is carried out by inductively coupled plasma mass spectrometry technique (ICP-MS).
The concentration of iodine in Palmaria palmata extract is quantified at 3.8 ppm.

- **Toxicological data:**
  - Dermal irritation/sensitization data :
    - Evaluation of the skin safety of a cosmetic product after a single application of an occlusive bandage to 11 healthy volunteers for 48 hours – 10% of active in water meaning tested at 0,75% of dry matter (January 2007): **non irritant**
    - Evaluation of the sensitizing capacity in 58 adult volunteers with normal skin for 6 weeks (Marzulli-Maibach) – 25% of active in water meaning tested at 1,87% of dry matter: **Non-sensitizing**

Other important data: *Palmaria palmata* is an edible seaweed used in different regions of the world. It is used in the composition of breads, cakes etc... It is often called "dulse" in organic and soul-söll shops in Norway. On the Icelandic coast, domestic livestock also consume this seaweed.
Palmaria palmata is one of the plants whose use is authorized in food supplements according to the French Decree of June 27, 2017 establishing the list of plants, other than mushrooms, authorized in food supplements.
According to the French Decree, all parts of *Palmaria palmata* can be used without any restriction of use and no particular substance is to be monitored.

Reference:
https://www.legifrance.gouv.fr/affichTexte.do?cidTexte=JORFTEXT000029254516&dateTexte=20200421

Hypnea Musciformis Extract

- **Physico-chemical properties**
  - Aspect : limpid liquid
  - Odor : characteristic
  - Color : very clear yellow

- **Main steps of the manufacturing process :**
  - Solubilization of Hypnea musciformis in water
  - Separation of soluble and insoluble phases
  - Filtration
  - Membrane sterilization

- **Composition :**
  - Sugars (mainly polysaccharides which average molecular weight is below to 700kDa) : 75%
  - Mineral ashes : 22%
  - Proteins : 3%
  - Absence of alkaloids and polyphenols

- **Impurities:**

The toxicological profile of our cosmetic active was determined:

- **Heavy metals**:

Identification of heavy metals is carried out by inductively coupled plasma-optical emission spectrometer (ICP-OES). Quantification is performed thanks to a calibration curve of standards.

Compounds	RATE (ppm)	Acceptability threshold (ppm)
Arsenic	0.082	0.5
Cadmium	<0.020	0.5
Cobalt	<0.020	0.5
Chromium	0.052	0.5
Mercury	<0.020	0.5
Nickel	0.185	1.0
Lead	<0.020	0.5
Antimony	<0.020	0.5
Selenium	0.031	0.5
Vanadium	0.053	0.5

- **Aflatoxines**

The determination and quantification of 4 aflatoxins (B1, B2, G1 and G2) are performed by high performance liquid chromatography (HPLC).

The sum of aflatoxins B1, B2, G1 and G2 in Hypnea Musciformis Extract does not exceed 0.4 µg/kg.

- **Toxicological data:**
  - Dermal irritation/sensitization data :
    - Evaluation of the skin safety of a cosmetic product after a single application of an occlusive bandage to 11 healthy volunteers for 48 hours – 15% of active in water meaning tested at 0.36% of dry matter (November 2013) : compatible
    - Evaluation of the sensitizing capacity in 100 adult volunteers with normal skin (Marzulli-Maibach) – 15% of active in water meaning tested at 0.36% of dry matter (January 2014): Non-irritant, non-sensitizing

Kappaphycus alvarezii

Algae synopsis

Red marine alga

Related actives: SEA MOIST COMPLEX®
ALGYL® (*C.o.* combined with *Gigartina stellata* and *coralline officinalis)*

V.1- 2018

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Common names	3
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These data don't pretend to be exhaustive.
They supply scientific pieces of information for conducing to a better understanding of the main characteristics and bioactivities of this algal species.

TAXONOMY

This alga belongs to

Empire	*Eukaryota*
Kingdom	*Plantae*
Phylum	*Rhodophyta*
Class	*Florideophyceae*
Subclass	*Rhodymeniophycidae*
Order	Gigartinales
Family	*Solieriaceae*
Genus	*Kappaphycus* Doty 1988
Species	*alvarezii* (Doty) Doty ex P.C. Silva 1996.

Basionym *Eucheuma alvarezii* Doty.

The genus *Kappaphycus* is become independent from the genus *Eucheuma* in 1988 according to the works of Professor Maxwell S. Doty (University of Hawaii) on the basis of morphological, anatomical and biochemical characters (*cf*. In: Taxonomy of economic seaweeds with reference to some Pacific and Caribbean species, La Jolla vol.2: 159-207).

It is an entity currently accepted taxonomically.

Eponomy The specific name *« alvarezii »* as applied to *Kapppaphycus alvarezii* by Doty commemorates Vicente B. Alvarez, a pioneer of the Bureau of Fisheries and Aquatic Resources in the cultivation methods of *Eucheuma* in the Philippines.

Vicente Alvarez became the first Manager of Marine Colloids in the Philippines.

Homotypic Synonym

*Eucheuma alvarezi*i Doty 1985.

Heterotypic Synonyms

*Eucheuma cottoni*i var. *erecta* No authority known
Eucheuma cottonii Weber-van Bosse 1913.

cf. www. algaebase.org

COMMON NAMES

This alga has several common names :

Agal agal, Agal agal besar, Chilin-t'sai,Cottonii, Eucheuman, Guso, Kirinsai, Adik goma, Adik Kalas, Bohol, Purdoy, Tambalang, Vanguard Giant,

and trade names

Alvarezii, Cottonii, Inerme, Interme, Striatum, Procrusteanum.

MORPHOLOGY

The thallus of *Kapppaphycus alvarezii* shows a simple discoid hold-fast from which arises a main axis with irregular branches. This cylindrical axis can reach 3 cm in diameter (Figs 1-2).

Axes are multi-axial and may contain a central medulla of iso-diametric cells mixed with rhizoids. In cross section, the medulla consists of large rounded cells interspersed with very small, thick-walled cells.

The morphology of *Kappaphycus alvarezii* changes with habit. Thalli range from terete to foliose. They may be tall and loosely branched with few blunt or pointed terminate branchlets or they may form the "spinosum" type where thalli may be densely branched and covered with coarse spinose branchlets. Some are compressed, linear to encrusting and mostly prostrate.

Thalli can reach up 2m tall. Their color is green or yellow.

Figs 1-2 - Morphology of *Kappaphycus alvarezii*

BIOLOGY

Reproductive life cycle includes three successive generations, as in *Chondrus crispus*, the life cycle being triphasic with gametophyte (n), tetrasporophyte (2n) and carposorophyte (2n).

As others *Eucheuma species*, *Kappaphycus alvarezii* shows intense regeneration ability that is useful for industrial cultivation. It is able to double in size every 15 to 30 days.

Natural populations of *Kapppaphycus alvarezii* grow just below the O tide line to the upper subtidal portion of reef areas on sandy-corally to rocky substrates where water movement is slow to moderate.

Kapppaphycus alvarezii is an invasive species in some locations such as in Hawaii and elsewhere it has been introduced for cultivation. Its fast growth rate makes it able to easily overgrow and outcompete native algae and coral species which is incredibly destructive to coral reef ecosystems. However this species is also able to provide habitat for a diverse community of animals (invertebrates and fishes).

ECOLOGY & GEOGRAPHICAL DISTRIBUTION

This alga takes its origin from Malaysia (Sabah). The type *Kappaphycus* species occurs naturally in the Sulu Sea and the Sulu Archipelago.

It has been naturalized in several western and central Pacific localities for farming purposes.

CHEMICAL COMPOSITION

Kappaphycus alvarezzi contains all essential nutrients such as minerals with abundance of sodium (23.4 mg per 1000 mg), potassium (12.44mg), magnesium (23.56 mg) and richness in calcium (3,565 gm/ 100 g).

It produces kappa carrageenan but environmental parameters affect the molecular structure.

The highest content would be in the depths of 20 cm (56.31% DW) and the lowest in 400 cm (17.10%) (Akmal Iskandar *et al*. 2014 – J. Appl. Biotechnol. 2).

Both haploid (n) and diploid (2n) plants show similar carrageenans. This is in contrast to *Chondrus* and other various Gigartinales.

The quality of carrageenan depends on physical and chemical treatments of algae (Chandramishra *et al*. 2006, Seaweed Res. India, 28: 113-117).

The major fatty acids are linolenic acid and linoleic acid. The major amino acid is lysine. (Rajasulochana *et al*. 2010 – ARPN J. Agricultural & Biological Science, 5: 1-12).

BIOACTIVITIES

Kappaphycus alvarezzi offers anti-oxidant activity (Fayaz *et al*. 2005- J. Agric. Food Chem. 53: 792-797; Kumar S.K. *et al.* 2008- Food Chem. 107: 289-295).

According to Kanatt S.R. *et al*. (J. Microbiol. Biotechnol. Food Sc. 2015 doi10.15414/jmbfs 2015.5.1.1-6), this alga also offers in addition to antioxidant properties, antimicrobial activity against *Staphylococcus aureus* and *Bacillus cereus.*

Composite films prepared using aqueous extract of *Kappaphycus* and poly vinyl alcohol (PVA) show excellent barrier properties against UV light. The films present good mechanical characteristics measured in terms of tensile strength, % elongation and puncture strength with low oxygen transfer as well as good antioxidant and antibacterial activity. So such aqueous extracts of this alga could be used to prepare bioactive food packaging films useful for food industry.

Kappaphycus alvarezzii also holds antibacterial activities particularly against *Staphylococcus epidermis* (Mansuya P. *et al*. 2010- J. Exp. Sciences 1 (8): 23-26).

Other properties concern anti-cyclooxygenase and anti- lipoxidase activities (Makkar F. & K. Chakraborty 2017 Natural product Research 31 (10): doi.org/10.1080/14786419.2016.1230113).

The sulfated polygalactans from *Kappaphycus alvarezii* would present antidiabetic and anti-inflammatory potential Interesting for developing functional food ingredient in nutraceutical products (Makkar F. & K. Chakraborty 2017 – Int. J. Food Properties 20 (6): doi 0org/10.1080/10942912.2016. 1209216).

USES

Kappaphycus alvarezii is a major raw material for the manufacture of Kappa-carrageenan.

The farming of *Kappaphycus* had undergone important changes during the last decades.

Different methods were tried in the past from the very simple bottom culture to the more sophisticated types using some form of support such as the raft method, the fixed of-bottom method the tubular net method and the fixed off bottom monoline method.

Today *Kappahycus alvarezii* is intensely cultivated satisfying the demand of the carrageenan industry (Figs 3-4).

The introduction of *Kappaphycus* cultivation to tropical countries continue due to the high production values realized, coastal villages searching for alternative livelihoods, and the increased global industrial demand for carrageenan. (Bindu M.S. & I.A. Levine 2011- J. Appl. Phycol. 23 (4): 789-796).

The productivity can be very important. By flotting in a good location, it can reach 43 tons DW per hectare and per year.

In 2002, the production of *Kappaphycus alvarezii* would reach 114,300 dried tons including 60,000 tons in Philippines and 48,000 tons in Indonesia.

Figs 3-4 Intensive farming of *Kappaphycus alvarezzi* in Asia.

Kappaphycus alvarezzii residual biomass (from colloid industry) would be in an interesting source of bioethanol (Kahambhaty *et al*. 2012 – Bioresour. Technol. 103: 180-185).

Due to its particular polysaccharide composition, different preparations including this alga have been patented for:

- ▶ human consumption for preparing cookies *e.g.* PH 22016000927 (U1) 2017-06-02 or carrot cake *e.g.* Patent PH 22016000924 (U1) 2017-06-02
- ▶ cosmetic applications for

-improving rough skin	PH 11279071 (A) 1999-10-12
-reducing irriration	JP 2000281527 (1à 2000-10-10
-anti-aging effect	CN 106309236 (A) 2017-01-11
-moisturizing	CN 105769643 (A) 2016-07-20

SILAB deposited several patents using this alga for preventing skin ageing, skin tightening and film forming properties : FR 2986430 (A1) 2012-02-06; WO 2013117859 (A2) 2013-08-15; US 2015025036 (A1) 2015-01-22; WO20160050741 (A) 2016-01-29; WO 2017129780 (A1) 2017-08-03.

GELYMA developed

▶ SEA MOIST COMPLEX® for improving hydration of dry skin and ameliorating the epidermal functions.

SEA MOIST COMPLEX®

Fights back against dry skin

by

replenishing depleted moisture

▶ ALGYL®

- ALGYL® in combination *Gigartina stellata/ kappaphycus alvarezii* extract and *Corallina officinalis* extract

ALGYL®

Marine guardian
of
skin barrier functionality
*
Multifaceted ways of action
for a complete skin barrier protection

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: June 16, 2020

SUBJECT: Lithothamnion Calcareum Powder

Biotech Marine. 2015. Manufacturing process Phycocorail™ (Lithothamnion Calcareum Powder).

Biotech Marine. 2017. Statement Phycocorail™ Composition File (Lithothamnion Calcareum Powder).

Palmer Research. 2003. Etude de la tolerance cutanee aigue d'un produit cosmetique chez le volontaire adulte: Patch-test 24 heures occlusif. Pycocorail (contains 57-61% Lithothamnion Calcareum Powder).

Seppic. 2001 Protocol. HET-CAM Test: Pycocorail® (contains 57-61% Lithothamnion Calcareum Powder).

Confidential

MANUFACTURING PROCESS
PHYCOCORAIL™

HARVESTING *(Lithothamnion Calcareum)*

↓

DRYING

↓

GRINDING

↓

MICRONISATION

↓

IONIZATION

↓

MIXTURE ← MANNITOL → ZINC SULFATE → DIATOMACEOUS EARTH

↓

PACKAGING

↓

QUALITY CONTROL

Production Manager
Jean-Marc CATROUX

BIOTECHMARINE (06/08/2015)

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Statement PHYCOCORAIL™ COMPOSITION FILE

(In accordance with EN 45014 General criteria for supplier's declaration of conformity)

We declare, by the present one, that the following product supplied by BIOTECHMARINE:

PHYCOCORAIL™

INCI NAME (USA): Lithothamnion Calcareum Powder*– Mannitol – Diatomaceous Earth - Zinc Sulfate
**update by PCPC to have the most accurate scientific name for the algae compared with the former INCI name in Lithothamnium Calcarum Powder*

Composition

Components	Components usual Name	Function	% (Concentration range)
Lithothamnion Calcareum Powder* **update by PCPC to have the most accurate scientific name for the algae compared with the former INCI name in Lithothamnium Calcarum Powder**		Active	57,0 - 61,0
Mannitol	D-Mannitol	Active	29,0 - 31,0
Diatomaceous Earth	Kieselguhr, soda ash flux-calcined	Active	9,0 - 11,0
Zinc Sulfate	Zinc Sulfate heptahydrate	Active	0,7 – 1,5

This certificate was established to the best of our knowledge and/or according to our suppliers' statements, at the date of this certificate. It is however your responsibility to abide by any clause of any regulations, which may apply to the product that you manufacture, or to the use you make of our product.

Document approved at Pontrieux, on June 26, 2017

By Laëtitia LE GUILLOU
Regulatory & Documentary Affairs from BIOTECHMARINE

Nota
The analytical specifications warranted are only those mentioned on the certificate of analysis supplied with each delivery of the product.
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PALMER RESEARCH

Etude de la tolérance cutanée aiguë d'un produit cosmétique chez le volontaire adulte: Patch-test 24 heures occlusif

Version n° 01/003 du 06 février 2003

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Etude : TCP24H/PA-03/0063 (1030033PA)

Produit : PHYCOCORAIL 209338

Contains 57-61% Lithothamnion Calcareum Powder

Promoteur : **SECMA BIOTECHNOLOGIES MARINES**
ZI
BP65
22260 PONTRIEUX
FRANCE

St Etienne, le 06 février 2003.

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RESUME DU RAPPORT D'ETUDE

Promoteur : SECMA BIOTECHNOLOGIES MARINES **Adresse : ZI** **BP65** **22260 PONTRIEUX** **FRANCE**	**Produit : PHYCOCORAIL 209338** **Code PALMER : PA-03/0063**

ETUDE DE LA TOLERANCE CUTANEE AIGUE D'UN PRODUIT COSMETIQUE CHEZ LE VOLONTAIRE ADULTE : PATCH-TEST 24 HEURES OCCLUSIF

Date de l'étude	L'étude s'est déroulée du 29 au 31 janvier 2003.	
Lieu de l'étude	PALMER Research 13 rue Le Chatelier 42100 ST ETIENNE	
Objectif	Déterminer le potentiel irritant primaire d'un produit cosmétique après application unique sous pansement occlusif pendant 24 heures chez le volontaire.	
Méthodologie	Etude en ouvert.	Nombre de sujets : 11 à peau sensible.
Critères d'inclusion	Peau indemne de toute lésion dermatologique, sujet non allergique.	• Durée de l'application : 24 heures. • Condition d'utilisation : pur
Critères d'évaluation	Détermination du score d'irritation moyen : $I.I.M = \frac{\text{score total des réactions (érythème + œdème)}}{\text{nombre total de volontaires}}$ Les réactions sont cotées de 0 à 3.	
Méthodes d'analyse	Classement du produit en fonction de son I.I.M : Si I.I.M < 0,20 Non Irritant Si 0,20 ≤ I.I.M < 0,50 Légèrement Irritant Si 0,50 ≤ I.I.M < 1 Moyennement Irritant Si I.I.M ≥ 1 Irritant	
Conclusion	L'indice d'irritation moyen du produit **PHYCOCORAIL 209338** est égal à **0** aux lectures 30 minutes et 24 heures. Il est donc **non irritant.**	
Investigateur : **Dr Florence DURAFOUR,** ***Dermatologue***		

Introduction

Certificat d'authenticité des résultats

1 - INTRODUCTION

A la demande de la société **SECMA BIOTECHNOLOGIES MARINES – ZI - BP65 - 22260 PONTRIEUX - FRANCE**, nous avons évalué sur 11 volontaires adultes, la tolérance cutanée aiguë ou potentiel irritant du produit :

PHYCOCORAIL 209338

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 27 janvier 2003 un échantillon du produit que nous avons référencé sous le code PALMER Research **PA-03/0063**.

L'essai a commencé le 28 janvier 2003 pour s'achever le 31 janvier 2003.

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 Florence DURAFOUR, *Dermatologue*.

Date : 13.02.03 Signature :

Ce rapport a été audité par l'Unité Assurance 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 : 14.02.03 Nom : Moncharin Isabelle

Signature :

Protocole expérimental

3 - PROTOCOLE EXPERIMENTAL

L'essai a été réalisé au protocole interne référence TCP24H.

3.1 - Volontaires

3.1.1 - Caractéristiques des sujets inclus

- ✓ 11 sujets à peau sensible ont été inclus dans l'essai,
- ✓ dont 10 de sexe féminin et 1 de sexe masculin,
- ✓ âgés de 18 à 60 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 un produit cosmétique,
- ✓ 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,
  - Néo-codion® et/ou corticoïde < 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 ou personne se sachant déjà sensibilisée au produit (si la formulation du produit est connue du Laboratoire PALMER).
- ✓ 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

Le produit a été appliqué, pur , une seule fois, sur une surface d'environ 50 mm^2 de peau de la zone scapulaire de chaque volontaire, à la dose d'environ 0,02 ml, imprégnant la surface d'une rondelle de papier filtre déposée dans la cupule du patch.

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

Le produit a été maintenu en contact avec la peau pendant 24 heures consécutives.

Cette application a été effectuée 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 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 le produit étudié 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)
E = 1 : érythème léger (bien visible)
E = 2 : érythème modéré
E = 3 : érythème important

Œdème « O » :

O = 0 : absence d'œdème
O = 0.5 : œdème très léger (palpable, à peine visible)
O = 1 : œdème léger (visible)
O = 2 : œdème modéré (net) avec ou sans présence de papules ou vésicules
O = 3 : œdème important (surface débordant la zone d'application) avec présence de vésicules ou présence d'une bulle

Les modifications de structure cutanée (dessèchement, rugosité, épaississement, réflectivité) pouvant être liées à la nature même du produit étudié 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 :

?+ = douteux
\+ = net
++ = modéré
+++ = 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 (IIM) à chaque temps de lecture, selon le rapport :

$$\mathbf{IIM} = \frac{\mathbf{\Sigma \text{ des cotations (érythème + œdème)}}}{\mathbf{\text{Nombre de sujets}}}$$

Cet indice ainsi obtenu (maximum 12), permet de classer arbitrairement le produit cosmétique étudié selon le barème d'interprétation suivant :

IIM	Classe
< 0.20	**Non irritant (NI)**
0.20 ≤ IIM< 0.50	**Légèrement irritant (LI)**
0.50 ≤ IIM< 1	**Moyennement irritant (MI)**
IIM ≥ 1	**Irritant (I)**

Les valeurs individuelles et la catégorie de produits cosmétiques et d'hygiène à laquelle appartient le produit étudié ont également été prises en compte pour une conclusion adaptée dans les conditions de l'essai (24 heures sous pansement occlusif).

**référence bibliographiques :*

- *« Les essais cliniques en dermatologie », Thérapie, 1991, Tome 46, pages 183 à 187*
- *« Dermato-allergologie de contact », G. DUCOMBS, Editions MASSON, 1988 pagez 13 à 16 ; 36-37*
- *« Dermatotoxicology Methods : The laboratory worker's VADEMECUM » ; N. MARZULLI – H. MAIBACH. Ed. Taylor & Francis, 1998.*

Résultats

4 - RESULTATS

Les résultats individuels des lectures à chaque temps expérimental sont regroupés dans le tableau ci-dessous.

PHYCOCORAIL 209338
(patch test 24 heures occlusif – pur)

SUJETS				LECTURES									
				Lecture 30 min après enlèvement du patch occlusif					Lecture 24 heures après enlèvement du patch occlusif				
N°	Identification	Age et sexe (1)	Type de peau	Témoin		Produit à l'essai		Modification de structure	Témoin		Produit à l'essai		Modification de structure
				E	O	E	O		E	O	E	O	
14S05	BRI Ma	44 ans / F	Sensible	0	0	0	0	-	0	0	0	0	-
15S05	SMA Ch	43 ans / F	Sensible	0	0	0	0	-	0	0	0	0	-
16S05	VER Ca	28 ans / F	Sensible	0	0	0	0	-	0	0	0	0	-
17S05	BLA Br	41 ans / F	Sensible	0	0	0	0	-	0	0	0	0	-
18S05	CON Je	19 ans / F	Sensible	0	0	0	0	-	0	0	0	0	-
19S05	LAS Jo	60 ans / F	Sensible	0	0	0	0	-	0	0	0	0	-
20S05	WIL Co	30 ans / F	Sensible	0	0	0	0	-	0	0	0	0	-
21S05	SEB Ha	20 ans / F	Sensible	0	0	0	0	-	0	0	0	0	-
22S05	MON Au	21 ans / F	Sensible	0	0	0	0	-	0	0	0	0	-
23S05	PAT Ni	18 ans / M	Sensible	0	0	0	0	-	0	0	0	0	-
25S05	MIG Co	32 ans / F	Sensible	0	0	0	0	-	0	0	0	0	-

I.I.M	**0**	**0**
Résultats	**non irritant**	**non irritant**

(1) : M = masculin
F = féminin

Conclusion

5 - CONCLUSION

Dans les conditions expérimentales retenues, 30 minutes et 24 heures après l'enlèvement du pansement occlusif, aucun volontaire n'a présenté de réaction d'irritation significative d'une réaction d'intolérance cutanée.

Par ailleurs, aucun effet secondaire n'a été observé.

On peut donc conclure que le produit **PHYCOCORAIL 209338, code PALMER Research PA-03/0063**, appliqué pur et localement sous pansement occlusif pendant 24 heures, sur 11 volontaires adultes ayant la peau sensible, s'est révélé **non irritant.**

Dr Florence DURAFOUR
Dermatologue

STUDY SUMMARY REPORT

Sponsor : SECMA BIOTECHNOLOGIES MARINES **Address :** **ZI-BP65** **22260 PONTRIEUX** **FRANCE**	**Product : PHYCOCORAIL 209338** **PALMER Research code** *: PA-03/0063*

DETERMINATION OF THE IRRITATION POTENTIAL OF A COSMETIC PRODUCT ON HUMAN SUBJECT: 24-HOURS SINGLE OCCLUSIVE PATCH-TEST

Study date:	The study took place from January 29th to January 31st, 2003.	
Study place(s):	PALMER Research 13 rue Le Chatelier 42100 ST ETIENNE	
Objective(s):	Determination of the acute skin tolerance of a cosmetic product by application under occlusive patch over a 24-hours period.	
Methodology:	Open Study.	Number of subjects : 11 with sensitive skin.
Included criteria:	Skin without any dermatological lesion, non allergic volunteer.	• Length of application: 24 hours • Condition of use : pure
Evaluation criteria:	Calculation of an acute irritation index : $M.I.I = \frac{\text{total cutaneous reactions score (erythema + oedema)}}{\text{number of volunteers}}$ Skin responses are scored from 0 to 3.	
Analysis:	Classification of the product according to its M.I.I: if $M.I.I < 0.20$ Non-irritant if $0.20 \leq M.I.I < 0.50$ Slightly irritant if $0.50 \leq M.I.I < 1$ Moderately irritant if $M.I.I \geq 1$ Irritant	
Conclusion:	The irritation index of the product **PHYCOCORAIL 209338** is equal to **0** at the 30-minutes and at the 24-hours readings. It is thus classified as **non-irritant** to human skin.	
Dr Florence DURAFOUR **Dermatologist**		

Phycocorail®

(contains 57-61% Lithothamnion Calcareum Powder)

HET-CAM Test

Tolerance on the chorio allantoïc membrane of a hen's egg

Expert report Tox HETCAM SEPPIC 3493-3495
Phycocorail 2% 10% water a Confidential

1. TEST ITEMS INFORMATION

Test item:	Phycocorail
Batch:	12.04.080
Purity:	Considered as about 100 % dry matter
Tested doses:	2% (TOX13093); 5% (TOX13094) and 10% (TOX13095) w/v dilutions
Vehicle:	Water
pH:	7.4 - 7.7
Physical appearance:	Samples appeared as beige liquids.

2. PROTOCOL

A trial was carried out based on the official method published on 26th December 1996 - Appendix IV - **internal procedure 57CO009 - revised on 26/12/2001.**

• The product was tested on the chorio-allantoïc membrane of fertilised LEGHORN hens' eggs which had been incubated for 10 days at a temperature of 37.8°C (+- 0.5°C) at a humidity of 50 to 60%.

• 0.3 mL of the prepared sample was spread over the chorio-allantoic membrane using a 1 mL pipette. The stop watch was started when the product had been applied. Rinsing with 5 mL of demineralised water was carried out 20 seconds later.

• The objective is to measure the exact moment when the three following phenomena appear (between t0 and 5'):

- °Hyperaemia (Hy): vasodilatation observed from the appearance of new capillaries or the dilation of capillaries which were already visible.
- °Haemorrhage (Ha): Effusion of blood outside of vessels and capillaries
- °Coagulation (Co): Membrane opacity or thrombosis.

• On the basis of the average times (T) obtained on 4 or 6 eggs (depending on the reproducibility of the measurement), a total score (I) is assigned to the tested product, according to the following calculation:

$$I = 5 \times (301 - T_{Hy}) + 7 \times (301 - T_{Ha}) + 9 \times (301 - T_{Co})$$

The classification was carried according to the following chart:

Score	Class
score < 1	*non irritant*
1 ≤ score < 5	*slightly irritant*
5 ≤ score < 9	*moderately irritant*
9 ≤ score < 12	*irritant*
score ≥ 12	*severely irritant*

3. RESULTS AND CONCLUSION

Dose % (w/v)	Reaction time (seconds)			
	HYPERAEMIA	HAEMORRHAGE	COAGULATION	SCORE
2%	301	301	301	0
5%	301	301	301	0
10%	91	91	301	8.4

PHYCOCORAIL (batch 12.04.080) has consequently appeared, under the trial's experimental conditions, to be **NON IRRITANT** in **2%** and **5%** dilutions and **MODERATELY IRRITANT** in **10%** dilution.

Remarks:

- Due to its low solubility and its rapid sedimentation, the 10% dilution was not easy to handle. Nevertheless, the results were reproducible between the eggs and considered as relevant.
- The pure powder form of the product was also tested. Because of its sticky character, it did not allow a good dispersal over the chorio-allantoic membrane. So the results were not considered as relevant.

Catherine PERRIN **Gaelle VINCENT**

Nota

The analytical specifications warranted are only those mentioned on the certificate of analysis supplied with each delivery of the product.

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Subsidiary of the AIR LIQUIDE group

Tox HETCAM SEPPIC 3493-3495 Phycocorail 2% 10% water a Confidential

SPECIFICATION DATA SHEET

Trade name: **SUN'YTOL**

Product: N° W-POLA -00 Version: 1.0 - 2020

Specification: N° S.01 Print date: 01 - 2020

SUN'YTOL is aqueous fraction extracted from the red alga *Polysiphonia lanosa.*

1 – Identification and composition of the substance

Product	N° CAS	N°EINECS	Ingredients %
Water	7732-18-5	231-791-2	67.5
Polysiphonia lanosa extract	-	-	32
Preservative phenoxyethanol	122-99-6	204-589-7	0.5

2 – Characteristics (standard)

Appearance: limpid liquid.

Color: amber.

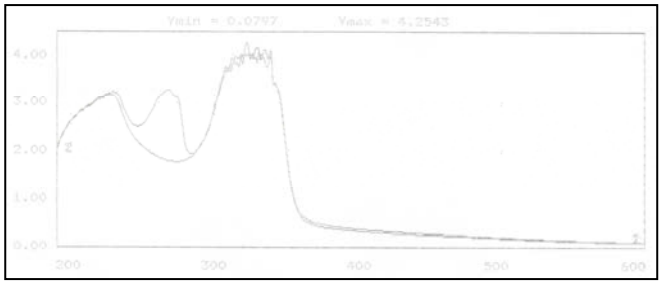
Odour: *sui generis.*

pH: 5.0 ± 1.0.

Relative density: 1.020 ± 0.020.

Dry residuals (%): 4.5 ± 0.1.

UV spectrum (5% in water)



1-without preservative
2- with preservative

Microbiological quality:

Total germs (germs/ml)	< 100.
Pathogens	absence.
Yeasts /moulds	< 100.

Storage: 15°C < store < 25°C.
Validity date: 18 months.

Remark: *No responsibility or liability for any consequences arising from the use of this technical data sheet can be accepted, including possible infringement of any patent.*

GELYMA – Parc d'Affaires Marseille Sud (C4) – 1 Boulevard de l'Océan – 13009 Marseille – France.
Phone: 33 496 140 982 – Fax: 33 496 140 983 – E-mail: gelyma @ wanadoo.fr

RESUME DE RAPPORT D'ETUDE

EVALUATION DE LA TOLERANCE CUTANEE D'UN PRODUIT COSMETIQUE APRES APPLICATION UNIQUE SOUS PANSEMENT OCCLUSIF PENDANT 48 HEURES :
méthode des patchs tests

- **Produit étudié** : SUN' YTOL
- **Promoteur** : GELYMA
- **Objectif de l'étude** : L'objectif de l'étude est d'apprécier la tolérance locale épicutanée d'un produit cosmétique, après application unique sur la peau du dos et sous patch occlusif, pendant 48h, chez des volontaires adultes, sains.
- **Investigateur :** Docteur Mary CREST
- **Lieu de l'étude :** EUROFINS SCIENTIFIC TEST CENTER,
  3 allée des Ingénieurs
  1140 rue André Ampère
  13851 AIX EN PROVENCE cedex 3
- **Dates de l'étude :** du 28/11/06 au 30/11/06 et du 12/12/06 au 14/12/06
- **Méthodologie** :
  - ✓ ***Modalités d'application*** **:**

Zones d'application : dos
Quantité de produit : 0.02 ml
Fréquence et durée : application unique pendant 48 heures.
Conditions d'application : produit déposé dilué à 5% sous patch occlusif.

  - ✓ ***Méthode d'évaluation*** **:**

L'observation clinique des effets provoqués est réalisée, par un dermatologue, après le retrait du patch. La cotation clinique est donnée selon une échelle numérique déterminée, en fonction de l'intensité des phénomènes d'irritation observés (érythème, œdème, sécheresse, vésicule). Le score irritant moyen du produit à l'essai est calculé en faisant la moyenne des cotations obtenues pour l'ensemble des volontaires, permettant ainsi de classer le produit de « non irritant à très irritant ». L'évaluation se fait toujours par comparaison au témoin "négatif" : patch contenant de l'eau déminéralisée.

- **Population :** 11 volontaires adultes, sains.
- **Résultats :** Le score irritant moyen du produit est de 0,0.
- **Conclusion :**

Dans les conditions expérimentales retenues, après application unique de 0.02 ml de produit, sous patch occlusif pendant 48 heures, chez 11 volontaires adultes sains et selon le barème adopté pour l'interprétation des résultats, **le produit SUN' YTOL Lot 06 08 251,** peut être considéré comme **non irritant du point de vue de sa tolérance primaire cutanée.**

Translation 9b – 48 hour patch test SUN'YTOL (Polysiphonia Lanosa Extract)

Summary of Study Report

EVALUATION OF THE SKIN TOLERANCE OF A COSMETIC PRODUCT AFTER A SINGLE APPLICATION WITH OCCLUSIVE DRESSING FOR 48 HOURS: test patch method

Product studied: SUN'YTOL
Sponsor: GELYMA
• Objective of the study: The objective of the study is to assess the local epicutaneous tolerance of a cosmetic product, after single application on the skin of the back and under occluded patch, for 48 hours, with health adult volunteers.

Investigator: Dr. Mary CREST

Place of study: EUROFINS SCIENTIFIC TEST CENTER,
3 allee des lngenieurs 1140 rue Andre Ampere
13851 AIX EN PROVENCE cedex 3

Dates of study: from 11/28/06 to 11/30/06 and from 12/12/06 to 12/14/06

Methodology:
Application modalities:
Application areas: back
Product quantity: 0.02 ml
Frequency and duration: single application for 48 hours.
Conditions of application: product diluted at 5% under occlusive patch.

Evaluation method:
Clinical observation of the effects caused is carried out by a dermatologist after removal of the patch. The clinical rating is given on a determined numerical scale, depending on the intensity of the irritation phenomena observed (erythema, edema, dryness, vesicle). The average irritant score of the test product is calculated by making the average of the ratings obtained for all the volunteers, thus making it possible to classify the product from "non-irritant to very irritant". The evaluation is always done by comparison to the "negative" witness: patch containing demineralized water.

Population: 11 healthy adult volunteers

Results: The average irritant score for the product is 0.0.

Conclusion:
Under the experimental conditions adopted, after single application of 0.02 ml of product, under occlusive patch for 48 hours, in 11 healthy adult volunteers and according to the scale adopted for the interpretation of the results, the product SUN 'YTOL Lot 06 08 251, can be considered non-irritant from the point of view of its primary skin tolerance.

Porphyra umbilicalis

Algae synopsis

Red marine alga

Related actives: HELIONORI®

ALGOMEGA NP® (*P.u*. combined with *Nannochloropsis oculata)*

V.1- 2018

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These data don't pretend to be exhaustive.
They supply scientific pieces of information for conducing to a better understanding of the main characteristics and bioactivities of this algal species.

TAXONOMY

This alga belongs to

Empire	*Eukaryota*
Kingdom	*Plantae*
Phylum	*Rhodophyta*
Class	*Rhodophyceae*
Order	Bangiales
Family	*Bangiaceae*
Genus	*Porphyra* C Agardh 1824
Species	*umbilicalis* Kützing 1843.

Homotypic Synonym

Porphyra laciniata f. *umbilicalis* (Kützing) Kleen 1874.

Heterotypic Synonyms

Ulva umbilicalis Linnaeus 1753
Ulva umbilicata S.F.Gray 1821
Ulva latissima var. *umbilicalis* (Linnaeus) C.Agardh 1823
Porphyra laciniata var. *umbilicalis* C.Agardh 1829
Porphyra umbilicata Ruprecht 1850
Porphyra umbilicalis (Linnaeus) J.Agardh 1883
Wildemania laciniata (Lightfoot) DeToni 1892
Wildemania umbilicalis (Linnaeus) De Toni 1897
Porphyra insolita Kornmann & Sahling 1991.

cf. www. algaebase.org

COMMON NAMES

This alga is named in:

-Japan	Chishima-kuronori, Nori, Amanori
-Korea	zakai, gim
-China	zicai
-Britain	Laverbread, Purple laver, Sloak, Slook, Tough Laver
-Portugal	Folhuda
-France	Laitue rouge, Nori.

MORPHOLOGY

The macroscopic thallus consists of membranaceous fronds, clustered together and divided into several lobes, orbiculate to elongate, auriculate and/or cucullate (Figs 1-2).

It is fixed to substratum by means of a small disc, composed of numerous rhizoidal outgrowths from the lower cells of erect parts.

Blades appears reddish brown, brownish, grey brown or olive green in the field. In a dried state, they are very thin and violet in color.

Blades constituted by a single cell layer can reach 60 cm in height.

Fig.1- Morphology of *Porphyra umbilicalis*
Herbarium sheet GELYMA.

Fig 2- Morphology of *Porphyra umbilicalis*
Gametophyte - Photo GELYMA.

BIOLOGY

The reproductive cycle consists of two morphological distinct generations (Fig. 3):

- either a well developed gametophyte
- or a reduced sporophyte known as *Conchocelis*, consisting of numerous filaments of uninucleate cells. This stage was originally thought to be a different species of alga, and so referred to "*conchocelis*" (Fig. 4).

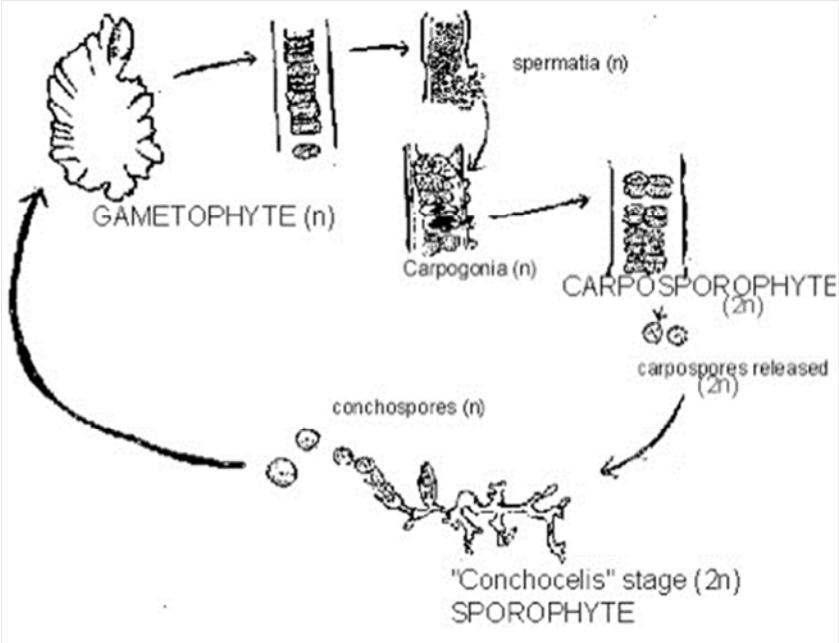


Fig. 3 – Schema of the reproductive cycle of *Porphyra sp.*

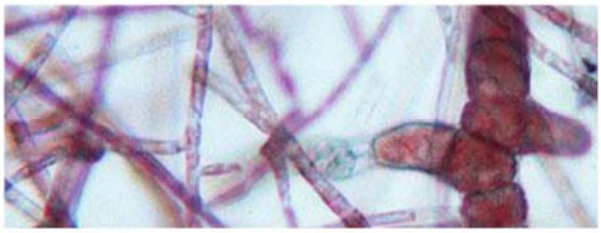


Fig. 4- Morphology of *Conchocelis*
Sporophyte.

This alga is monoecious in Europe (Conway & Cole 1977 – Phycologia, 16: 205-216; Kapraun & Freshwater 1987 – Phycologia, 26: 82-87) that means both male and female gametes are formed on the one thallus. The female gametes while still on the thallus are fertilized by the released male gametes which are non-motile.

Spermatangia form a pale deliquescing marginal band whereas the carpospore groups are scattered into clusters over the general surface of the blade.

As a matter of interest

Discovery about *Conchocelis*

An important break-through in the understanding of the life history of this alga has been the recognition by the English Phycologist Kathleen Drew of the University of Manchester (1949 – Nature, 164: 748).

She studied the life cycle of the red alga *Porphyra umbilicalis*.

She proved that the microscopic phase named *Conchocelis* was not an independent alga, but the diploid stage of the macroscopic haploid stage of *Porphyra umbilicalis.*

Although Kathleen Drew never travelled to Japan, her academic research made a lasting contribution to the development of commercial nori production in the country. Her critical discovery was that at the microscopic *conchocelis* stage, bivalve shells provided essential host environment for the development of penetrating filaments into.

These investigations enabled the Japanese to make increases in the cultivation of Nori to the current level.

Kathleen Drew has been named Mother of the Sea (Figs 5-6)

(*"Titanic musician and palace intruder enter dictionary". BBC News. 2010-05-27. Retrieved 27 May 2010).*

Her work is celebrated each year on April 14. A monument to her was erected in 1963 at the Sumiyoshi shrine in Uto, Kumamoto, Japan. Each year she is honoured by a small ceremony at the site.

Figs 5-6 – Kathleen Drew 1901-1957.

For more informations see:

*-Lund, J. W. G. (1958). "Kathleen M. Drew D.Sc. (Mrs. H. Wright Baker) 1901". British Phycological Bulletin. **1**: iv–12. doi:10.1080/00071615800650021*

-Nori cultivation in www.seaweed.ie/aquaculture/noricultivation.php.

ECOLOGY & GEOGRAPHICAL DISTRIBUTION

Porphyra umbilicalis is common and abundant everywhere on the rocky parts of coasts or on beach pebbles on the Atlantic coasts of Europe (from Scandinavia to Morocco) and North America. It occurs in the upper littoral zone singly or in dense colonies. It is adaptable to conditions on different parts of the rocky shore.

It is also present along the coasts of the Eastern part of the Mediterranean (Figs 6-7).


Figs 6-7 – Populations of *Porphyra umbilicalis in situ*
Photos GELYMA

CHEMICAL COMPOSITION

The chemical composition of *Porphyra umbilicalis* is well known (see in Pereira L. in Seaweed 2011, Nova Science Publishers Inc. as a review).

Major compounds of *Porphyra* are regrouped here after (*cf.* CEVA nutritional data 2015).

- in g/100 g dehydrated

Minerals	6.4 – 39.3
Proteins	10.5 – 44.4
Dietary fibers	24.7 – 55.5
Lipids	0.3 – 5.9
Polyphenols (eq phloropglucinol)	0.03 - 0.53

- in mg/100 g dehydrated

Potassium	168 – 3,271
Sodium	112 – 6,131
Magnesium	5 – 1,907
Phosphorus	139 -803
Calcium	3 - 832
Manganese	1.9 – 9.3
Iron	5.8 – 278.5
Copper	0.3 – 3.5
Zinc	0.9 – 10.0

- in mg/100 g dehydrated

Vitamin A	0.11 – 12.48
Vitamin E	0.9 – 8.7
Vitamin C	2.2 – 192.5
Vitamin B1	0.6
Vitamin B2	0.2 – 3.6
Vitamin B3	2.4 – 10.3
Vitamin B5	0.25
Vitamin B6	0.1 – 1.0
Beta carotene	2,542 – 7, 906

- in µg/100 g dehydrated

Selenium	4.0 – 186.9
Vitamin B8	4.7 – 9.3
Vitamin B9	21.7
Vitamin B12	0.03 – 125.0

The contents in iodine and bromine have been evaluated there (in µg.g-1) :

Iodine	35-102
Bromine	59-116

(Romaris-Hortas V. *et al.* 2009- Talanta 79: 947 - 952).

Among amino acids, essential amino acids are in majority with 39.5 ± 2.7 % of total amount (Sanchez-Machado D.I. *et al*. 2003- Chromatographia 58: 15—163).

Taurine is abundant with about 1.2% (Noda, 1995- J. Appl. Phyco. 5: 255 - 258).

The major polysaccharide is porphyran, a complex sulfated carbohydrate. It is a highly substituted agarose with a linear backbone consisting of 3-linked beta-D-galactosyl units alternating with either 4-linked alpha-L-galactosyl 6-sulfate or 3,6-anhydro-alpha-L-galactosyl units. The composition includes 6-O-sulfated L-galactose, 6-O-methylated D-galactose, L-galactose, 3,6-anhydro-L-galactose, 6-O-methyl D-galactose and ester sulfate. Some of the ester is present as 1-4-linked L-galactose 6-sulfate. Gelling properties depends of the percentage of components.

The amount of fatty acids reaches 13.6 % of the total lipid amount, this alga being a rich source of 20:5 n-3 (Fleurence J. *et al.* 1994- J. Applied Phycol. 6: 527 - 532).

Different pigments exist. Their proportions depend of water depths (in n.mol.cm-² thallus area)

1 m depth	chlorophyll a	1.76	chlorophyll b	0.47
10m depth	chlorophyll a	2.49	chlorophyll b	0.76

(Ramus J. *et al*. 1976 – Mar. Biol. 37: 223 - 229).

Phycobiliproteins include phycoerythrins and phycocyanins. It is known that the red algal species of the eulittoral and the upperlittoral zone have a higher content of phycocyanins, that explains the color more violet of *Porphyra umbilicalis* in certain zones (Luning K. & N.J. Dring 1985- Mar. Biol. 29: 195-200).

Porphyra species contain special compounds named “mycosporine-like amino acids” (MAAs), a group of water-soluble nitrogenous compounds typically absorbing between 310 nm and 360 nm (Singh *et al*. 2008 – Indian J. Exp. Biol. 46: 7 - 17). Each one shows a specific absorbance (Nakamura *et al*. 1982 – J. Chromatogr. 250: 113-118; Bandaranayake, 1998- Natural Product reports: 159-169).

According to Karsten *et al*. (2009- Helgol. Mar. Res. 63: 231 - 238) *Porphyra umbilicalis* contain three main MAAs, palythine, shinorine and porphyra-334, from thalli isolated from the rocky upper littoral zone of the island Helgoland, Germany, in the North Sea.

These results confirm those of (Volkmann M. & A.A. Gorbushina 2006 (FEMS Microbiol Lett. 255: 286 - 295).

BIOACTIVITIES

The species of the genus *Porphyra* have a great nutritional value due to their chemical composition regarded as beneficial to health (*cf.* in Noda – 1993- J. Appl. Phycol. 5: 255-258).

The characteristic taste of hoshi-nori which is favored by most Japanese, would be linked to relative large amounts of free alanine, glutamic acid, aspartic acid and glycine.

The notable levels of taurine (> 1.2%) would help enterohepatic circulation of bile acid, thus preventing gallstone through controlling blood-cholesterol levels.

Porphyra contains high level in C 20 polyunsaturated fatty acids that can act as local hormones in the control of various aspects of metabolism**.**

Porphyran is dietary fiber of good quality. A powder consisting of 20 % nori mixed with a basic diet given orally to rats prevented 1,2-dimethylhydrazine-induced intestinal carcinogenesis. These results were related to porphyrin content of at least 0.4-0.5% in the diet (Yamamoto & Maruyama 1985 - Cancer Lett. 26: 241 - 251).

Porphyran could significantly lower the artificially enhanced level of hypertension and blood-cholesterol in rats (see in Noda 1993- *Ibid*).

Furthermore, a betaine has been isolated from *Porphyra,* and y-butyrobetaine has been shown to significantly lower enhanced levels of plasma cholesterol in rats (Abe & Kaneda 1973 - Bull. Jap. Soc. Sci. Fish. 39: 239).

Porphyra extracts show varying degrees of antioxidative activity according to different extraction media (Ismail A. & T.S. Hong 2002 - Mal. J. Nutr. 8(2): 167 - 177).

Mycosporine-like amino acids (MAAs) act as natural bio-protector against UVA (Shick & Dunlap 2002- 64: 223-262).

According to several investigations, in addition to their involvement in the prevention of UV damage, MAAs might show antioxidant function (Dunlap & Yamamoto 1995 – Comp. Biochem. Physiol., 112B: 105 – 114; Dunlap & Shick 1998 – J. Phycol. 34: 418 - 430; Dunlap *et al.*, 1997 – 4th Int. Marine Biotechnology. Conference, Sorrento).

Other roles might be possible, among others a role linked to the reproductive process like in fungi (Bandaranayake, 1998- Natural Product reports: 159-169).

Recently a Chinese patent has been deposited for improving by cultivation the content of MAAs in *Porphyra* (CN105684880 (A) 2016-06-22).

CULTIVATION OF *PORPHYRA species*

Historically, *Porphyra sp*. was the first seaweed to be cultivated, starting in Tokyo Bay around either 1640 (Miura-1975 – In Advances of Phycology in Japan, eds J. Tokida & Hirose: 273 - 304 – The Hague) or 1736 (Okazaki A. 1971- Seaweeds and their uses in Japan, Tokai Univ.).

The early techniques consisted of setting bundles of bamboo or tree twigs in estuaries on which the spores settled and grew, the mature algae were after harvested (Fig. 8).

The wooden boats used exclusively for Nori working were called “Bekabune”.

These boats were used in Tokyo until 1964.

Fig 8- Nori harvest by Ogata Gekko (1859-1920).

The *Porphyra* cultivation started in China about 200 years ago (Tseng 1981 – Proceedings of the Tenth Int. Seaweed Symposium: 123 - 152) on shallow rocks.

Nori cultivation suffered from unpredictable harvests and had been particularly prone to damage from typhoons and pollution in coastal waters. So bamboo and tree twigs were progressively replaced by nets (Figs 9-10).

Figs 9-10 – Nori cultivation on nets in Japan.

However the discovery by Dr Kathleen Drew in the life history of *Porphyra* allowed to revolutionize Japanese nori aquaculture. The seeding phase (*Conchocelis*) is largely carried out on the oyster shells for Nori farming and thus has induced a firm foundation for the whole industry of Nori that is presently highly mechanised.

About 1kg of ripe *Porphyra* is necessary to seed about 20 000 shells. To seed one net, it is necessary to takes 10 shells. One Seeding Centre may seed up to 20.000 nets in one season. About 50 days after seeding the nets, the fronds are 15-20 cm long. During this period the algae are particularly susceptible to disease and factors such as temperature, salinity.

Experiments of *Porphyra* cultivation in land-based ponds under controlled conditions have been patented in the United States (Patent W0 2005/051073 A1; 2008/013667 A2).

Annual production of cultivated Nori reaches around 130,620 tons with 60,000 tons in Japan, 30,165 in China, 20,000 in South Korea and 20,500 in North Korea (*cf*. SURIALINK).

USES

*Porphyra umbilicali*s serves as food and for cosmetic purposes.

Human nutrition

Natural *Porphyra* was made a part of foodstuff by Asian people since more than 1,000 years ago. In China, the consumption of *Porphyra* dates back to 533-544 AD. It was even presented annually to the emperor of China during the Sung dynasty (960-1279 AD) as a gift from the Fujian province.

*Porphyra umbilicali*s is eaten in various recipes. However Nori is not sold in the fresh state but is immediately dried into black sheets for making sushis or other delicacies (Fig. 11).

Fig 11 - Asian delicacies with Nori.

On the Hawaiian Islands, this alga known as “limu” is a popular delicacy too.

On the coasts of the United Kingdom, it is collected for human consumption. “Laverbread” is prepared by warming algae in bacon (or butter in Ireland). It may first made into small cakes coated with oatmeal. It normally takes the place of eggs with bacon for a breakfast dish and it is usually eaten during the week-end. It is particularly popular in the South Wales and in Ireland. (*cf.* Guiry & Blunden 1991 – Seaweed Resources in Europe: uses and potential, Wiley).

In France, this red macroalga was an authorized food since 1988 after its inscription on the positive list established by the French National Council for Health.

Cosmetic uses

Porphyra species are used for cosmetic purposes and several patents have been deposited recently:

-KR 101705801 (A) 2017-02-10
-CN 106176377 (A) 2016-12-07
-CN 105832596(A) 2016-08-10
-US 2007248563 (A) 2007-10-25

some cosmetic compositions being related to sunscreen protection:

-CN 104644511 (A)2015-05-27
-FR 3000894 (A1) 2014-07-18.
-GB 2472021 (A) 2011-01-26

The Patents FR 20010014731 (A1)2001-11-14 ; US 2004228875 (A1) 2004-11-18 concern a product containing an extract of the genus *Porphyra* inducing synthesis of stress protective proteins during physical or physiological stresses or physiopathological aggressions on skin cells

MIBELLE AG COSMETICS offer patented protective compositions against UVA based on mycosporine-like amino acids present in *Porphyra umbilicalis* (CH 20030000761 (B1) 2003-04-30; CH 702571 (B1) 2011-07-29 ; EP 1473028 (A1) 2004-11-03).

GELYMA also uses *Porphyra umbilicalis* in HELIONORI® that contains mycosporine like amino acids (Patents FR 2803201 (A1) 2001-07-06 ; FR 2803201 (B1) 2004-11-26). This active provides a safe alternative to synthetic UVA filters , it prevents the formation of sun burn cells and protects DNA and cell membranes against UVA radiation.

In ALGOMEGA NP® , the extract of *Porphyra* is associated with an extract of a microalga for improving skin hydration and reducing water loss.

HELIONORI®

*

Natural sun protection thanks to

Marine UVA filters (MAAs)

ALGOMEGA NP®

*

Strengthens the skin's barrier function

Personal Care Products Council
Committed to Safety, Quality & Innovation

Memorandum

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

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

DATE: April 14, 2020

SUBJECT: Porphyra Umbilicalis Extract

Mibelle Group. 2020. Technical Data Sheet Helioguard™ 365 (trade name mixture containing 1.25% Porphyra Umbilicalis Extract).

Mibelle Group. 2020. Certificate of Analysis Helioguard™ 365 (trade name mixture containing 1.25% Porphyra Umbilicalis Extract).

Technical Data Sheet
Helioguard™ 365

Mibelle Group Biochemistry
Bolimattstrasse 1
5033 Buchs, Switzerland
Phone +41 62 836 17 31
Fax +41 62 836 14 05

info@mibellebiochemistry.com
www.mibellebiochemistry.com

April 7, 2020

Composition Breakdown / Origin

The product was developed by Mibelle Group Biochemistry, Switzerland in 2003 for cosmetic applications.

Components /INCI (EU/PCPC)	CAS	EC	% w/w
Porphyra Umbilicalis Extract (dry matter)	223751-76-6	/	1.25
Lecithin	8002-43-5	232-307-2	3
Sodium Lactate	72-17-3	200-772-0	0.16
Phenoxyethanol	122-99-6	204-589-7	0.8
Ethanol	64-17-5	200-578-6	15
Aqua/Water	7732-18-5	231-791-2	79.79

Manufacturing

Method of Manufacture

Circular flow extraction of 7.8% dry algae (*Porphyra umbilicalis*) on suction filter.
Extraction solvent: 20% ethanol/ 78.7% water. 0.3% lactic acid 80%, 1% phenoxyethanol

▼

In-process control:
Mycosporine-Like Amino Acids 1.03 -1.49 g/l

▼

Maturation at room temperature, 10 days

▼

Filtration of the supernatant 2.5 µm

▼

Cationic Exchange

▼

Filtration 0.2 µm)

▼

Cross Flow Filtration 10kD Membrane

▼

Encapsulation of the extract into liposomes (water/ethanol/ lecithin) by high pressure homogenization

▼

Packaging

▼

Quality Control

Residues/Impurities

Heavy metals

	mg/kg (ppm)
• Arsenic (As)	<3.0
• Cadmium (Cd)	<0.1
• Lead (Pb)	<1.0
• Mercury (Hg)	<0.1
• Antimony (Sb)	<0.5
• Chromium (Cr)	<1.0
• Nickel (Ni)	<1.0
• Cobalt (Co)	<0.5

Total heavy metals < 20 ppm

Pesticides

The product was screened for the content of pesticides and does not contain pesticides above the concentration limit according to the regulation (EC) No 396/2005.

Other Impurities

The concentration of methanol in ethanol was specified ≤max. 100 ppm by our supplier. Thus the methanol concentration in Helioguard™ ™ 365 is max. 15 ppm .

Due to the manufacturing process of the component phenoxyethanol technical unavoidable traces of the below listed residuals can be present.

- phenol (CAS 108-95-2) max. 0.1 ppm
- ethylene oxid (CAS 75-21-8) max. 0.02 ppm

Use level

Recommended use level	1.0 – 2.0%

Toxicological Review / Physiological Safety

Rat oral LD50 [mg/kg]

No LD50 was conducted. Regarding the composition, a LD50 value of > 2000 mg/kg has to be expected.

Photosensitization

A human photo patch test (tested concentration undiluted) was conducted on 50 volunteers.
On the basis of the test results and under the test conditions, there was no evidence of a primary phototoxic reaction of the product at this concentration.

Ocular Irritant Potential (Het-Cam Test)

The ocular tolerance was tested by the Het-Cam method (tested concentration 10%). According to the JORF classification the product was considered as “moderately irritant” at this concentration.

Cytotoxicity

The cytotoxicity of Helioguard 365 was determined using the MTT assay.
Helioguard 365 does not reduce cell viability at concentrations up to 2%.

Phototoxicity Test

The phototoxicity of Helioguard 365 was determined using UV radiation.
Helioguard 365 does not reduce cell viability at concentration sup to 2% after UV radiation.

Certificate of Analysis
Helioguard™ 365

Helioguard™ 365 is a liposomal preparation of mycosporine-like amino acids (MAA) from the red alga Porphyra umbilicalis which absorbs UV-A light.

Composition

Mycosporine-Like Amino Acids	0.1% (1.0 g/L ± 0.2)
Phospholipids	3%
Ethanol abs.	15%
Sodium Lactate	0.16%
Deionized Water	ad 100%

INCI (EU/PCPC) Declaration

Aqua/ Water (and) Lecithin (and) Alcohol (and) Sodium Lactate (and) Porphyra Umbilicalis Extract (and) Phenoxyethanol

Lot. Nr.	**0830-045** Retested lots are indicated with R1 or R2 in the Lot. Nr. field.	**Expiration date:**	**09/2021**
		Manufacturing date:	**03/2020**
Storage:	**4°C - 8°C in closed containers**	**Retest date:**	**---**

Analytics	Analysis	Specification
Consistency & Appearance	conforms	liquid, slightly opaque
Color	conforms	amber
Odor	conforms	characteristic, ethanolic
pH value	5.2	5.0 - 8.0
particle size	179 nm	60 - 180 nm
MAA content (UV/Vis)	0.95 g/L	0.80 - 1.20 g/L
Density (20°C)	0.9790 g/mL	0.960 - 1.000 g/mL
Bacteriology:		
Total germ count	<10 CFU/g	<100 CFU/g
Yeast / Mould	<10 /g	<100 /g
Pseudomonas aeruginosa	negative	negative in 1g
E. coli / Enterococcus	negative	negative in 1g

Country of Origin: Switzerland

Signature: This certificate of analysis has been generated electronically and is valid without signature.

Mibelle AG Biochemistry, Bolimattstrasse 1, CH-5033 Buchs, Switzerland
Phone +41 62 836 13 46, Fax +41 62 836 14 05

mibelle GROUP biochemistry

SPECIFICATION DATA SHEET

Trade name: HELIONORI®

Product: N° W-POUM-00 Version: 1.0 – 2020

Specification: N° S.00 Print date: 01 - 2020

HELIONORI® is a patented aqueous and concentrated active ingredient prepared selectively from the symplasm of the red algae (*Rhodophyta*) *Porphyra umbilicalis*

1 – Identification and composition of the substance

Product	N° CAS	N°EINECS	Ingredients %
Water	7732-18-5	231-791-2	52
Porphyra umbilicalis extract	223751-76-6	-	48
Preservative	None		

2 – Characteristics (standard)

Appearance: limpid liquid.

Color: reddish brown.

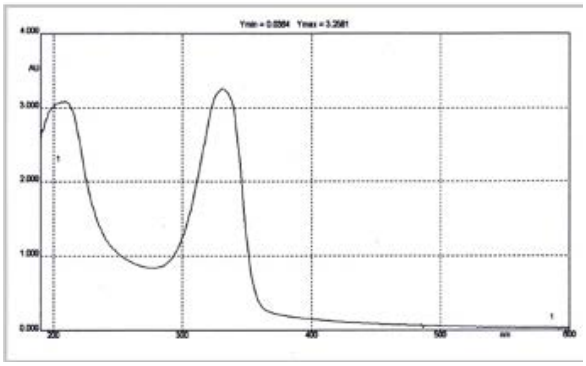
Odour: *sui generis.*

pH: 6.0 ± 1.0.

Relative density: 1.015 ± 0.010.

Dry residuals (%): 3.3 ± 0.6.

UV spectrum (2.5% in water):



Microbiological quality:

Total germs (germs/ml)	< 100.
Pathogens	absence.
Yeasts /moulds	< 100.

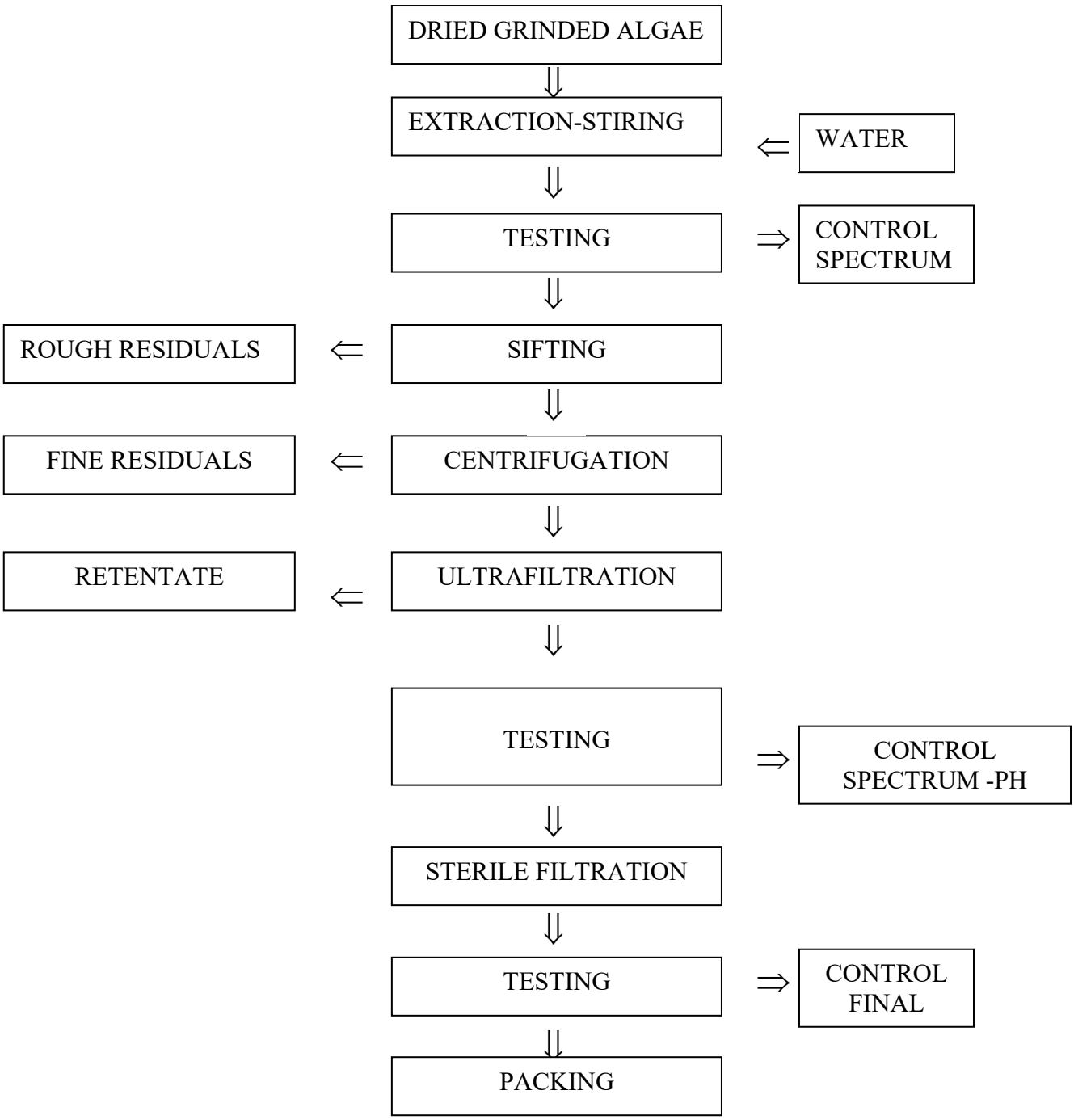
Storage: 15°C < store < 25°C.
Validity date: 6 months
Once opened, the whole drum must be used.

Remark:
No responsibility or liability for any consequences arising from the use of this technical data sheet can be accepted, including possible infringement of any patent.
Any addition of preservatives is at the request and under the responsibility of the applicant, the product being delivered in units of use (1-5 or 10 Kg).

GELYMA – Parc d'Affaires Marseille Sud (C4) – 1 Boulevard de l'Océan – 13009 Marseille – France.
Phone: (33) 496 140 982 – E-mail: gelyma @ wanadoo.fr – contact @ gelyma.com

Parc d'Affaires Marseille Sud
Bâtiment C4
1, Boulevard de l'Océan
13009 Marseille

FLOW CHART FOR HELIONORI®



Siège social : Parc d'Affaires Marseille Sud, 1 Boulevard de l'Océan, Impasse du Paradou, 13009 Marseille
Tél. 04 96 14 09 82 - Fax 04 96 14 09 83 E mail : gelyma@wanadoo.fr

GELYMA SAS
0001046267

Dossier : CH-10418-00038 / Ech : 18CH010081 / V1

CHIERRY - DEPARTEMENT CHIMIE

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02402 CHATEAU-THIERRY CEDEX
FRANCE

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Mail : contact@fr.upscience-labs.com

GELYMA SAS
Parc d'affaires Marseille Sud C4
1, Boulevardde l'Ocean

13009 Marseille
FRANCE

RAPPORT D'ESSAI FINAL
EXTRAIT LIQUIDE - HELIONORI

Date réception client :
Date fabrication :
N° lot client : 1802120
Fournisseur :
N° lot fournisseur :
Tonnage :
DLUO :

Demandeur : Mme PELLEGRINI Liliane
N° commande :
N° client :
N° optim :
N° étude :
Réf. commerciale : DS18CT001071
Tiers :

Date réception labo : 15/03/2018

Masse brute (g):

Observations :

ANALYSES CHIMIQUES

Determination	Rés/brut	Rés/sec	Incertitude	Cible	Mini	Maxi	Conforme
MINERALISATION MICRO ONDES Méthode interne - ELTRACES-H - CT	Réalisée						
ARSENIC Méthode interne - ELTRACES-H - CT	3679 µg/kg		736 µg/kg				
CADMIUM Méthode interne - ELTRACES-H - CT	<10 µg/kg						
MERCURE Méthode interne - ELTRACES-H - CT	<10 µg/kg						
PLOMB Méthode interne - ELTRACES-H - CT	<10 µg/kg						

Conclusion :

Validé le : 20-03-18

Melle BRU CAMILLE

Superviseur

Le code à 2 lettres indique le site UPSCIENCE sur lequel a été réalisée l'analyse : CT = site de Chierry, SN = site de Saint-Nolff.

En cas de déclaration de conformité à la spécification, celle-ci ne prend pas en compte l'incertitude associée aux résultats.
Si ce rapport fait mention de résultats de mycotoxines, ils sont corrigés du taux de récupération. Ce rapport d'essai ne concerne que l'échantillon soumis à essai.
Si ce rapport fait mention de résultats de pesticides, ils ne sont pas corrigés du taux de récupération si celui-ci est compris entre 70 et 120 %.
« # » : analyse faite plusieurs fois

Translation 7c Porphyra Umbilicalis Extract

Chemical Analysis

Determination	**Results/Units**	**Uncertainty**
Microwave mineralization Internal method ELTRACES-H - CT	Completed	
Arsenic Internal method ELTRACES-H - CT	3679 µg/kg	736 µg/kg
Cadmium Internal method ELTRACES-H – CT	<10 µg/kg	
Mercury Internal method ELTRACES-H – CT	<10 µg/kg	
Lead Internal method ELTRACES-H - CT	<10 µg/kg	

Conclusion: Validated 03/20/2018 Melle BRU CAMILLE

Supervisor

The 2-letter code indicates the In vivo Labs site on which the analysis was carried out: CT = site of Chierry, SN = site of Saint-Nolff.

In case of declaration of conformity to the specification, this does not take into account the uncertainty associated with the results.
If this report mentions mycotoxin results, they are corrected for the recovery rate. This test report only concerns the sample submitted for testing.
If this report mentions pesticide results, they are not corrected for the recovery rate if it is between 70 and 120%.

"#": Analysis made several times

HELIONORI®

For a natural protection against UVA

TOXICOLOGICAL DATA

Product: HELIONORI®

INCI names water CAS n° 7732-18-5 EINECS n° 231-791-2
Porphyra umbilicalis extract
CAS n° 223751-76-6

Product	N° CAS	N°EINECS	Ingredients %
Water	7732-18-5	231-791-2	52
Porphyra umbilicalis extract	223751-76-6	-	48
Preservative	None		

GELYMA – Parc d'Affaires Marseille Sud (C4) – 1 Boulevard de l'Océan – 13009 Marseille – France.
Phone (33) 496 140 982 – E-mail: gelyma @ wanadoo.fr.

Ocular irritation Het Cam test : weakly irritating

Method

The Het Cam test is applied to the vascularized chorioallantoic membrane (CAM) for 6 eggs on day 10 of embryonation. The reaction times are observed for the three endpoints : haemorrhage, lysis and coagulation within a 5-min observation period.

Results

eurofins | ATS

N° d'étude : 48285F01.DOC
Version : 1
Page : 2

RESUME DE RAPPORT D'ETUDE

EVALUATION DU POTENTIEL IRRITANT D'UN PRODUIT PAR APPLICATION SUR LA MEMBRANE CHORIO-ALLANTOÏDIENNE DE L'ŒUF DE POULE : *Méthode du Het Cam*

- **Produits étudiés :** HELIONORI
- **Promoteur :** GELYMA
- **Objectif de l'étude :** Evaluer le potentiel irritant du produit étudié
- **Méthodologie :** Le principe en est basé sur l'observation à l'œil nu, par une personne entraînée, des effets irritants (hyperémie, hémorragie, coagulation / thrombose), pouvant survenir dans les cinq minutes suivant le dépôt du produit sur la membrane chorio-allantoïdienne d'œuf de poule embryonné, au dixième jour d'incubation. L'étude a été réalisée selon le mode opératoire ATS M1918LAC.
- **Dates de l'étude :** 8/07/02
- **Résultats :**

Dénominations	Réf	N°ATS	Concentration testée	RESULTATS	
				Score	Classement
HELIONORI	011205	48825	PUR	2	Faiblement irritant

- **Conclusion :**

Le produit HELIONORI Lot 011205, n° de prestation 48825, testé en mode pur, peut être considéré comme acceptable du point de vue de sa tolérance primaire oculaire.

Stéphanie RIVOIRE
Responsable tests d'innocuité et d'objectivation
StephanieRivoire@Eurofins.com

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➤ HELIONORI® without any dilution appears weakly irritating (score = 2).

Phototoxicity *In vitro* test : no phototoxic

Method

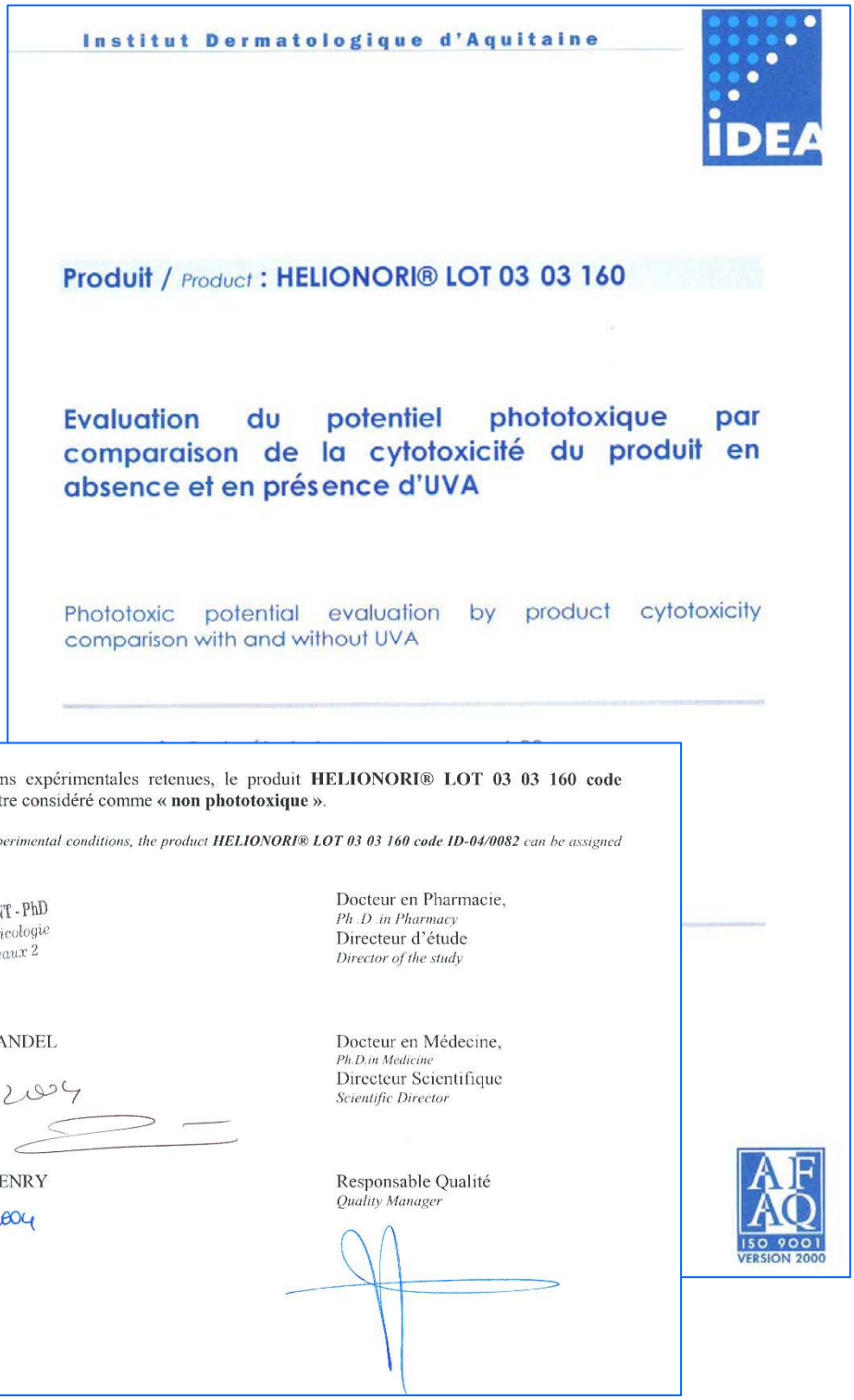
This method is an alternative to animal experimentation.

The principle is based on the product cytotoxicity comparison with and without non cytotoxic UVA dose exposure.

After a cell monolayer (fibroblast Balb/c3T3 clone) incubation with the assessed product (7 concentrations) and irradiation with UVA, cytotoxicity is calculated with the help of Neutral Red Uptake.

This investigation is carried out according to the

Results



Institut Dermatologique d'Aquitaine

IDEA

Produit / *Product* : HELIONORI® LOT 03 03 160

Evaluation du potentiel phototoxique par comparaison de la cytotoxicité du produit en absence et en présence d'UVA

Phototoxic potential evaluation by product cytotoxicity comparison with and without UVA

Dans les conditions expérimentales retenues, le produit **HELIONORI® LOT 03 03 160 code ID-04/0082** peut être considéré comme « **non phototoxique** ».

*Under the retained experimental conditions, the product **HELIONORI® LOT 03 03 160 code ID-04/0082** can be assigned as "non phototoxic".*

Isabelle BAUDRIMONT - PhD
Laboratoire de Toxicologie
Université Bordeaux 2

Docteur en Pharmacie,
Ph .D .in Pharmacy
Directeur d'étude
Director of the study

Pascale DENIS-KANDEL
13.02.2004

Docteur en Médecine,
Ph.D.in Medicine
Directeur Scientifique
Scientific Director

Marie-Christine HENRY
12.02.2004

Responsable Qualité
Quality Manager

AFAQ ISO 9001 VERSION 2000

➤ HELIONORI® is not phototoxic under the experimental conditions.

Genotoxicity *In vitro* test : 3D Assay : no direct genotoxicity

Method

This chemiluminescent 3D Assay is an ELISA-like assay, realized by the well known company S.F.R.I.(St Jean d'Illac, France), by using plasmid DNA adsorbed on sensitized microplates as the substrate.
This method is based on a repair reaction of DNA (Salles *et al.*, 1995 – Analytical Biochemistry 232:37-42; Patent FR n° 95003230).DNA lesions are repaired by the excision repair pathway which implies an incision-excision reaction followed by DNA repair synthesis.

In the present experiment, these lesions were performed by UVB irradiation (three tested doses: 0.1 J/cm², 0.5 J/cm² and 1j/cm²). HELIONORI® is added according to 5 concentrations: undiluted, 50- 25-10 and 2%. The standard was chlorpromazine, a neuroleptic drug known to be photoreactive with production of free radicals.

Results

The ability of a molecule to alter DNA is measured by the reparation ratio R

R = RLU sample at a known dilution / RLU solvent alone
RLU: Relative Light Units

When R is inferior to 2, there is no genotoxicity,
When R is superior to 2, there is a significant genotoxicity.

Results represent the mean of two independent experimentations. They are expressed comparatively to control (irradiated or no-irradiated solvent).

HELIONORI® shows a reparation ratio inferior to 2 without irradiation, that means it is no genotoxic directly with the experimental conditions.

After irradiation, all ratios are nearly to 1 whatever the tested concentration.
When irradiation doses increase from 0 to 1J/cm², ratios do not increase significantly.

Compounds	Concentrations	Reparation ratio R			
		0 J/cm²	0.1 j/cm²	0.5 J/cm²	1J /cm²
HELIONORI®	Undiluated	0.91	0.42	0.41	0.42
	50%	1.08	0.52	0.67	0.73
	25%	1.02,	0.77	0.84	1.12
	10%	0.87	0.69	0.82	1.01
	2%	0.86	0.68	0.81	1.00
Chlorpromazine	0.01 mM	0.89	5.06	4.79	4.04
	0.001 mM	0.87	2.55	2.79	2.98

➤ HELIONORI® shows no genotoxicity directly or after UVB irradiation.

GELYMA – Parc d'Affaires Marseille Sud (C4) – 1 Boulevard de l'Océan – 13009 Marseille – France.
Phone (33) 496 140 982 – E-mail: gelyma @ wanadoo.fr.

Personal Care Products Council
Committed to Safety, Quality & Innovation

Memorandum

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

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

DATE: April 10, 2020

SUBJECT: Studies on an Eye Cream with 0.0375% Rhodymenia Palmata Extract

Institute for In Vitro Sciences, Inc. 2013. Tissue Equivalent Assay with Epiocular™ Cultures (Eye Cream with 0.0375% Rhodymenia Palmata Extract).

TKL Research. 2013. Human Cummulative Irritation Patch Test (Eye Cream with 0.0375% Rhodymenia Palmata Extract).

FINAL REPORT

Study Title

TISSUE EQUIVALENT ASSAY WITH EPIOCULAR™ CULTURES

Test Articles

[redacted]

Eye cream with 0.0375% *Rhodymenia palmata* extract

Authors

Greg Mun, B.A.
Jennifer R. Nash, M.S.

Study Completion Date

6 March 2013

Performing Laboratory

Institute for In Vitro Sciences, Inc.
30 West Watkins Mill Road, Suite 100
Gaithersburg, MD 20878

Study Number

[redacted]

[redacted]

Laboratory Project Number

[redacted]

TISSUE EQUIVALENT ASSAY WITH EPIOCULAR™ CULTURES

SUMMARY

IIVS Test Article Number	Sponsor's Designation	Conc.	t_{50} (hours)		pH
			Preliminary (1 August 2012)	Trial 1 (8 August 2012)	
[redacted]					
Eye cream	[redacted]	Neat	> 16	19.3	5.0
[redacted]					
Positive Control	0.3% Triton®-X-100	NA	21.2 minutes	21.9 minutes	NA

NA – Not Applicable

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STATEMENT OF COMPLIANCE

The Tissue Equivalent Assay With EpiOcular™ Cultures, of the test articles, [redacted] was conducted in compliance with the U.S. FDA Good Laboratory Practice Regulations as published in 21 CFR 58 and the principles presented in the OECD series on Good Laboratory Practice in all material aspects with the following exceptions:

The identity, strength, purity and composition or other characteristics to define the test articles have not been determined by the testing facility. The certificates of analysis were not provided by the Sponsor.

The stability of the test articles under the storage conditions at the testing facility and under the actual test conditions has not been determined by the testing facility and is not included in the final report.

Greg Mun, B.A.
Study Director

6 March 2013
Date

QUALITY ASSURANCE STATEMENT

Study Title: Tissue Equivalent Assay With EpiOcular™ Cultures

Study Number: [redacted]

Study Director: Greg Mun, B.A.

This study was divided into a series of in-process phases. Using a random sampling approach, Quality Assurance monitored each of these phases over a series of studies. Procedures, documentation, equipment records, etc., were examined in order to assure that the study was performed in accordance with the U.S. FDA Good Laboratory Practice Regulations (21 CFR 58), and the OECD Principles of Good Laboratory Practice and to assure that the study was conducted according to the protocol and relevant Standard Operating Procedures.

The following are the inspection dates, phases inspected and report dates of QA inspections of this study:

Phase Inspected	Audit Date(s)	Reported to Study Director	Reported to Management
Protocol and Initial Paperwork	26-July-12	27-July-12	30-July-12
Addition of MTT	09-Aug-12	09-Aug-12	09-Aug-12
Draft Report and Data	21,24-Sep-12	24-Sep-12	25-Sep-12
Final Report	05-Mar-13	05-Mar-13	05-Mar-13

This report describes the methods and procedures used in the study and the reported results accurately reflect the raw data of the study.

Renee Forde, Ph.D.
Quality Assurance

05March 2013
Date

SIGNATURE PAGE

TISSUE EQUIVALENT ASSAY WITH EPIOCULAR™ CULTURES

Initiation Date:	26 July 2012
Completion Date:	6 March 2013
Sponsor:	[redacted]
Sponsor's Representative:	[redacted]
Testing Facility and Study Director Address:	Institute for In Vitro Sciences, Inc. 30 West Watkins Mill Road, Suite 100 Gaithersburg, MD 20878
Archive Location:	[redacted]
Study Director:	6 March 2013 Greg Mun, B.A. Date
Laboratory Manager:	Nathan R. Wilt, B.S.
Laboratory Supervisor:	Allison Hilberer, M.S.

TEST ARTICLE RECEIPT

	IIVS Test Article Number	Sponsor's Designation	Physical Description	Receipt Date	Storage Conditions*
	[redacted]				
Eye cream	[redacted]		off-white cream	23 July 2012	room temperature
	[redacted]				

* - Protected from exposure to light

TISSUE EQUIVALENT ASSAY WITH EPIOCULAR™ CULTURES

INTRODUCTION

The EpiOcular™ Human Cell Construct (MatTek Corporation) was used to assess the potential ocular irritancy of the test articles. The MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) conversion assay, which measures the NAD(P)H-dependent microsomal enzyme reduction of MTT (and to a lesser extent, the succinate dehydrogenase reduction of MTT) to a blue formazan precipitate, was used to assess cellular metabolism after exposure to a test article for various exposure times[1]. The duration of exposure resulting in a 50% decrease in MTT conversion in test article-treated EpiOcular™ human cell constructs, relative to control cultures, was determined (t_{50}).

The purpose of this study was to evaluate the potential toxicity of the test articles, supplied by [redacted] as measured by the conversion of MTT by EpiOcular™ human cell constructs after exposure to a test article for various exposure times. The laboratory phase of the study was conducted from 31 July 2012 to 9 August 2012 at the Institute for In Vitro Sciences, Inc. After a time range finding assay, the test articles were tested in a valid definitive assay to determine the time of exposure to a test article, which resulted in the t_{50} endpoint.

[1] Berridge, M.V., Tan, A.S., McCoy, K.D., Wang, R. (1996) The Biochemical and Cellular Basis of Cell Proliferation Assays That Use Tetrazolium Salts. **Biochemica** 4:14-19.

MATERIALS AND METHODS

Receipt of the EpiOcular™ Human Cell Construct Model

Upon receipt of the EpiOcular™ Human Cell Construct Kit (MatTek Corporation), the solutions were stored as indicated by the manufacturer. The EpiOcular™ human cell constructs were stored at 2-8°C until used. On the day of dosing an appropriate volume of EpiOcular™ human cell construct assay medium was removed and warmed to approximately 37°C. Nine hundred µL of assay medium were aliquoted into the wells of 6-well plates. The six-well plates were labeled to indicate test article and exposure time. The samples were inspected for air bubbles between the agarose gel and cell culture insert prior to opening the sealed package. Cultures with air bubbles covering greater than 50% of the cell culture insert area were not used. The 24-well shipping containers were removed from the plastic bag and their surfaces were disinfected with 70% ethanol. The EpiOcular™ human cell constructs were transferred aseptically into the 6-well plates. The EpiOcular™ human cell constructs were then incubated at 37±1°C in a humidified atmosphere of 5±1% CO_2 in air for at least one hour. The medium was then aspirated and 0.9 mL of fresh medium were added to each assay well below the EpiOcular™ human cell construct. The plates were returned to the incubator until treatment was initiated. Upon opening the shipping bag, any remaining unused tissues were briefly gassed with an atmosphere of 5% CO_2/95% air and placed back at 2-8°C for later use.

Test Article Preparation

As instructed by the Sponsor, each test article was administered to the test system without dilution.

Assessment of Direct Test Article Reduction of MTT

Each test article was added to a 1.0 mg/mL MTT (Sigma) solution in warm Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 2 mM L-glutamine (MTT Addition Medium) to assess its ability to directly reduce MTT. Approximately 100 µL of each test article were added to 1 mL of the MTT solution and the mixtures were incubated in the dark at 37°C for one to three hours. If the MTT solution color turned blue/purple, the test article was presumed to have reduced the MTT.

The test articles were not observed to reduce MTT in the absence of viable cells.

pH Determination

The pH of each neat liquid test article was measured using pH paper (EMD Chemicals Inc.). Initially, the neat test article was added to pH paper with 0-14 pH range in 1.0 pH unit increments to approximate a narrow pH range. Next, each neat test article was added to pH paper with a narrower range of 0-6 and 5-10 pH units with 0.5 pH unit increments to obtain a more accurate pH value. The pH values obtained from the narrower range pH paper are presented in Table 1.

Time Range Finding Assay

A time range finding assay was performed to establish an appropriate exposure time range to be used in the definitive assay for each test article. Four exposure times of 1, 4, 8, and 16 hours were tested in the time range finding assay. One culture was treated per exposure time with 100 µL of the appropriate test article or control. The negative control, 100 µL of sterile, deionized water (Quality Biological), was exposed for 16 hours. The positive control, 100 µL of 0.3% Triton®-X-100 (Fisher), was exposed for 5, 15, and 45 minutes (one culture per exposure time). The exposed cultures were then incubated for the appropriate amount of time at 37±1°C in a humidified atmosphere of 5±1% CO_2 in air.

After the appropriate exposure time, the EpiOcular™ cultures were extensively rinsed with Calcium and Magnesium-Free Dulbecco's Phosphate Buffered Saline ($Ca^{++}Mg^{++}$-Free DPBS) and the wash medium was decanted. After rinsing, the tissue was transferred to 5 mL of Assay Medium for a 10 to 20 minute soak at room temperature to remove any test article absorbed into the tissue. A 1.0 mg/mL solution of MTT in warm MTT Addition Medium was prepared no more than 2 hours before use. Three hundred µL of MTT solution were added to designated wells in a prelabeled 24-well plate. The EpiOcular™ constructs were transferred to the appropriate wells after rinsing with $Ca^{++}Mg^{++}$-Free DPBS. The trays were incubated at 37±1°C for approximately three hours in a humidified atmosphere of 5±1% CO_2 in air.

After the incubation period with MTT solution, the EpiOcular™ cultures were blotted on absorbent paper, cleared of excess liquid, and transferred to a prelabeled 24-well plate containing 2.0 mL of isopropanol in each designated well. The plates were sealed with parafilm and stored in the refrigerator (2-8°C) until the last exposure time was harvested. The plates were then shaken for at least two hours at room temperature.

At the end of the extraction period, the liquid within the cell culture inserts was decanted into the well from which the cell culture insert was taken. The extract solution was mixed and 200 µL were transferred to the appropriate wells of a 96-well plate. Two hundred µL of isopropanol were added to the two wells designated as the blanks. The absorbance at 550 nm (OD_{550}) of each well was measured with a Molecular Devices Vmax plate reader.

Definitive Assay

Based on the results of the time range finding assay, four exposure times were chosen for the definitive assay. The exposure times for the test articles were 8, 16, 20, and 24 hours. The exposure times were chosen such that generally two exposure times were expected to result in survivals lower than 50% and two exposure times were expected to result in survivals greater than 50%. In general, the negative control exposure times were selected to fit the range of the test article or positive control exposure times. The negative control (100 µL of sterile, deionized water) was exposed for 0.25, 4, 8, and 24 hours. The positive control (100 µL of 0.3% Triton®-X-100) was exposed for 5, 15 and 45 minutes. The procedures used to conduct the definitive assay were essentially the same as for the time range finding assay with the exception that at least duplicate cultures were dosed per exposure time.

Presentation of Data

The raw absorbance values were captured. The mean OD_{550} value of the blank wells was

calculated. The corrected mean OD_{550} value of the negative controls was determined by subtracting the mean OD_{550} value of the blank wells from their mean OD_{550} values. The corrected OD_{550} value of the individual test article exposure times and the positive control exposure times was determined by subtracting the mean OD_{550} value of the blank control from their OD_{550} values. The individual % of Control values were averaged to get the mean % of Control value. All calculations were performed using an Excel spreadsheet. The following percent of control calculations were made:

$$\% \text{ of Control} = \frac{\text{corrected } OD_{550} \text{ of Test Article or Positive Control Exposure Time}}{\text{appropriate corrected mean } OD_{550} \text{ of Negative Control}} \text{ X } 100$$

Exposure time response curves were plotted with the % of Control on the ordinate and the test article or positive control exposure time on the abscissa. The t_{50} value was interpolated from each plot. To determine the t_{50}, the two consecutive points were selected, where one exposure time resulted in a relative survival greater than 50%, and one exposure time resulted in less than 50% survival. Two select points were used to determine the slope and the y-intercept for the equation $y=m(x) + b$. Finally, to determine the t_{50}, the equation was solved for $y=50$.

Criteria for a Valid Test

The definitive assay will be accepted if the tissues pass the quality control test at the MatTek Corporation. According to MatTek, the tissues will be considered acceptable if the positive control compound, 0.3% Triton®-X-100, causes a t_{50} within the established acceptable range of 12.2 to 37.5 minutes.

RESULTS AND DISCUSSION

Time Range Finding Assay

A time range finding assay was performed, consisting of four exposure times 1, 4, 8, and 16 hours for the test articles supplied by [redacted] The exposure time response curves are included in Appendix B. Based upon the results of the time range finding assay, four exposure times were selected for each test article for the definitive assay (see Materials and Methods). The t_{50} results for the time range finding assay are reported in Table 1, under "Preliminary".

The test articles were not observed to reduce MTT directly in the absence of viable tissue.

Definitive Assay

Four exposure times were treated in duplicate for each test article. The exposure times for the test articles were 8, 16, 20, and 24 hours. The negative control was also exposed in duplicate for 0.25, 4, 8, and 24 hours. Table 1 summarizes the t_{50} results of the definitive Tissue Equivalent Assay With EpiOcular™ Cultures for the test articles and the positive control, 0.3% Triton®-X-100, under "Trial 1". The exposure time response curves are included in Appendix B. The MatTek's t_{50} for the positive control was 23.48 minutes. Since this positive control fell within the established acceptable range at MatTek Corporation (12.2 – 37.5 minutes), the assay results were accepted.

Table 1

IIVS Test Article Number	Sponsor's Designation	Conc.	t_{50} (hours)		pH
			Preliminary (1 August 2012)	Trial 1 (8 August 2012)	
[redacted]					
Eye cream [redacted]		Neat	> 16	19.3	5.0
[redacted]					
Positive Control	0.3% Triton®-X-100	NA	21.2 minutes	21.9 minutes	NA

NA – Not Applicable

HUMAN CUMULATIVE IRRITATION PATCH TEST

TKL STUDY NO. [redacted]

[redacted]

CONDUCTED FOR:

[redacted]

DATE OF REPORT:

January 24, 2013

201.587.0500 • www.tklresearch.com

TKL Research, Inc. 365 West Passaic Street, Rochelle Park, NJ 07662

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SIGNATURES

This study was conducted in compliance with the requirements of the protocol and TKL's Standard Operating Procedures, and in the spirit of GCP ICH Topic E6.[1] The report accurately reflects the raw data for this study.

Jonathan S. Dosik, MD
Principal Investigator

1/22/13
Date

Kathleen Georgeian
Kathleen Georgeian
Director, Dermatologic Safety Testing

1/23/13
Date

Michelle Medina
Manager, Dermatologic Safety Testing

1/23/13
Date

STATEMENT OF QUALITY ASSURANCE

This report has been reviewed by the TKL Research, Inc (TKL) Corporate Quality Assurance Department and the report accurately reflects the raw data for this study.

Quality Assurance

1/24/13
Date

[1] ICH Topic E6 "note for the guidance on Good Clinical Practices (CPMP/ICH/135/95)" – ICH Harmonised Tripartite Guideline for Good Clinical Practices having reached Step 5 of the ICH Process at the ICH Steering Committee meeting on 1 May 1996.

TITLE OF STUDY

Human Cumulative Irritation Patch Test

SPONSOR

[redacted]

STUDY MATERIALS

[redacted] Eye Cream containing 0.0375% *Rhodymenia palmata* extract.

[redacted]

DATE STUDY INITIATED

October 22, 2012

DATE STUDY COMPLETED

October 29, 2012

DATE OF REPORT

January 24, 2013

INVESTIGATIVE PERSONNEL

Jonathan S. Dosik, MD - Dermatologist
Principal Investigator

Kathleen Georgeian
Director, Dermatologic Safety Testing

Michelle Medina
Manager, Dermatologic Safety Testing

CLINICAL SITE

TKL RESEARCH, INC
1 Palmer Terrace
Carlstadt, NJ 07072

SUMMARY

An eye cream containing 0.0375% *Rhodymenia palmata* extract was [redacted] evaluated neat to determine their ability to cause irritation to the skin of volunteer subjects with normal skin using a 7-day semi-occlusive cumulative irritation patch study. Distilled water served as a negative control and 0.75% SLS served as a positive control. Thirty-eight (38) subjects completed the study.

The dermatologist was present on the final study day for evaluation.

This study determined the following irritation scores and associated classifications:

TSIN #	Irritation Scores: Total	Irritation Scores: Overall Average Skin Grade (N=39 Enrolled)	Cumulative Irritation Index
SLS 0.75%	117.5	0.4361	0.1090
[redacted]			
Eye cream [redacted]	2.5	0.0094	0.0023
[redacted]			
Distilled Water	1.0	0.0038	0.0009
[redacted]			

Source: Table 4, Appendix 1, and Data Listing 3, Appendix II

Under the conditions employed in this study, the subjects showed no evidence of irritation to [redacted] an eye cream containing 0.0375% *Rhodymenia palmata* extract.

1.0 OBJECTIVE

The objective of this study was to assess the potential of the test substances to elicit human skin irritation by repetitive topical application.

2.0 RATIONALE

Cumulative irritancy patch testing is a modified primary irritancy patch test that can detect weak irritants, which require multiple applications to cause a skin reaction. These reactions are due to direct damage to the epidermal cells and no immunologic (allergic) mechanism is involved. This procedure may detect so-called "fatiguing substances" which are mild irritants that cause more strongly positive reactions with successive multiple skin exposure.

3.0 STUDY DESIGN

3.1 STUDY POPULATION

A sufficient number of healthy subjects were enrolled to provide a minimum of 30 completed subjects.

3.1.1 Inclusion Criteria

Individuals eligible for inclusion in the study were those who:

1. Were male or female, between the ages of 18 and 65;
2. Were in general good health as determined by the Medical and Dermatological History Questionnaire (Appendix A of Protocol, see Appendix IV);
3. Read, understood, and signed an informed consent (IC) agreement after being advised of the nature of the study;
4. Were willing to refrain from using lotions, creams, powders, or other skin preparations on the skin in the test area for the duration of the study;
5. Were willing to refrain from exposing skin sites to the sun or going to tanning beds for the duration of the study; and
6. Were willing to refrain from swimming and using hot tubs for the duration of the study.

3.1.2 Exclusion Criteria

Individuals excluded from participation in the study were those who:

1. Had clinically significant active dermatitis or skin disease anywhere on the body (excluding facial acne);
2. Had a history of psoriasis, eczema, or skin cancer;
3. Had a condition or were taking medication(s) which, in the judgment of the Investigator or Designate, made the subject ineligible or placed the subject at undue risk;
4. Had received treatment (chemotherapy, radiation, immune suppressant medications) for any type of cancer within the last 6 months;
5. Had a mastectomy or axillary lymph nodes removed;
6. Had an autoimmune or immune deficiency disease (eg, lupus, myositis, Crohns disease, autoimmune thyroid diseases, autoimmune hepatitis);

7. Were taking any immunosuppressant medication;
8. Had insulin-dependent diabetes;
9. Had asthma or any other chronic respiratory condition requiring daily therapy;
10. Were taking or using any antihistamines or systemic/topical anti-inflammatory medications (eg, ibuprofen, corticosteroid) on a routine or frequent basis. Maximum acceptable dosage should be determined by written laboratory guidelines;
11. Used a topical anti-inflammatory in the patch area within the last 2 weeks;
12. Were receiving allergy injections, expected to start injections before the conclusion of the study, or had the final injection within a week of the study start;
13. Were participating in another dermal study of any kind;
14. Were participating in any clinical study, which, in the judgment of the Investigator, would have potentially affected responses in either study;
15. Had a confirmed skin allergy as a result of participation in a patch study;
16. Had a known sensitivity or allergy relating to the substance(s) being evaluated;
17. Had a known sensitivity or allergy to adhesives, surgical tapes, bandages, etc; and/or
18. Had scars, moles, sunburn, tattoos, etc. in the patch area.

3.1.3 Informed Consent

A properly executed IC document was obtained from each subject prior to entering the study. The signed IC document is maintained in the study file. In addition, the subject was provided with a copy of the IC document (see Appendix III).

3.2 DESCRIPTION OF STUDY

3.2.1 Outline of Study Procedures

The study extended over a 7-consecutive day period with [redacted] product applications and evaluations. On Day 1, the study material was applied to the back under conditions described in Section 3.3.4. Twenty-three hours (±1 hour) later the patches were removed. Twenty to 40 minutes after patch removal the sites were evaluated, the responses recorded, and identical patches applied to the same sites. This was repeated daily for a total of 7 days, including Saturdays and Sundays.

3.2.2 Study Flow Chart

Day	Activities
1	Obtained informed consent, reviewed completed medical screening form, applied patches
2, 3, 4, 5, 6	Staff removed patches, graded, applied patches
7	Staff removed patches, graded

3.2.3 Definitions Used for Grading Responses

Responses were graded using the following numeral equivalents.

Response	Numerical Equivalent
No apparent cutaneous involvement	0

Greater than 0, less than 1 (Faint, barely perceptible erythema OR slight dryness [glazed appearance])	0.5
Faint but definite erythema, no eruptions or broken skin OR no erythema but definite dryness; may have epidermal fissuring	1.0
Greater than 1, less than 2 (Well-defined erythema OR faint erythema with definite dryness, may have epidermal fissuring)	1.5
Moderate erythema, may have a few papules OR deep fissures, moderate-to-severe erythema in the cracks (Cut-off grade, patches are not reapplied)	2.0
Greater than 2 less than 3 (Moderate erythema with barely perceptible edema OR severe erythema not involving a significant portion of the patch [halo effect around the edges], may have a few papules OR moderate-to-severe erythema	2.5
Severe erythema (beet redness), may have generalized papules OR moderate-to-severe erythema with slight edema (edges well defined by raising)	3.0
Greater than 3, less than 4 (Moderate-to-severe erythema with moderate edema(confined to patch area) OR moderate-to-severe erythema with isolated eschar formations or vesicles)	3.5
Generalized vesicles or eschar formations OR moderate-to-severe erythema and/or edema extending beyond the area of the patch.	4.0

The maximum obtainable individual score was a 4.0. Should a reaction of 2.0 have occurred at any point during the study, further patch application on that subject would have been terminated with respect to the product involved. An "NP" symbol and a score of 2.0 would be assigned to all subsequent days.

For each test substance, the average of all inclusive scores was calculated for each completed grade day and reported as the average skin grade for that particular day. An overall average skin grade was calculated (ie, the sum of the daily average skin grades divided by the number of study days) for each test substance.

The Cumulative Irritation Index (CII) was calculated by dividing the total score by the sums of the highest possible score multiplied by the number of subjects multiplied by the number of days.

3.2.4 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.

3.3 STUDY MATERIAL

3.3.1 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 TKL. 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.

3.3.2 Nature of Study Material

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

[redacted]

[redacted]

Special Instructions : Prior to the first application of product, the sites were wiped with 70% isopropyl alcohol. All Patches were prepared upon the subjects' arrival and were used within 15 minutes from patch preparation.

3.3.3 Application of Study Material

Study material was applied to patches as instructed. Patches were applied in a randomized schedule to the infrascapular area of the back, either to the right or left of the midline, or to the upper arm.

3.3.4 Description of Patch Conditions

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

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

4.0 INTERPRETATION

Cutaneous irritation accounts for the majority of cases of contact dermatitis. Reactions consist of local inflammatory responses characterized by erythema and/or edema, or an erosive reaction characterized by local tissue destruction or necrosis. These reactions are due to direct damage to the

epidermal cells and require no prior sensitization. No immunologic (allergic) mechanism is involved.

To qualify as an "irritant", a substance should evoke inflammation on initial exposure (primary irritation) or on repeated exposure to an identical site (cumulative irritation). An irritant substance will cause dermatitis if it is permitted to act in sufficient concentration for a sufficient length of time. Irritant reactions may develop in all subjects, although individual susceptibility varies greatly.

Cumulative irritancy patch testing can detect weak irritants that require multiple applications to produce skin irritation. During and after first contacts with weak irritants, no visible skin alterations are observed. After repeated contact, the skin gradually becomes erythematous; drying and cracking occur; and later, oozing, crusting, and erosion may develop. An eczematous reaction with papules, vesicles, and edema may also develop.

The procedure employed is a modification of that described by Dr. B. M. Lanman[1] at the Joint Conference on Cosmetic Sciences, April 21-23, 1968 in Washington, DC, and further modified by Phillips, et al[2] and Berger, et al.[3]

5.0 PROTOCOL

See Protocol - Appendix IV.

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 (AEs), 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 20 years from completion of the study. Storage is maintained either at a TKL facility in a secured room accessible only to TKL employees, 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 TKL.

7.0 RESULTS & DISCUSSION

Thirty-nine (39) subjects between the ages of 20 and 65 were enrolled and 38 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:		39
Number discontinued:		1
Lost to follow-up:	1	
Number completed:		38

Source: Table 1, Appendix I

The dermatologist was present on the final study day for evaluation.

There were no AEs reported.

[redacted] A protocol amendment was issued on January 8, 2013 with instruction to change the incorrect product descriptions to the correct descriptions provided in the protocol amendment. A copy of the protocol amendment is included in Appendix IV.

A summary of response data are provided in Table 3, Appendix I. A Cumulative Irritation Index by product is provided in Table 4. Individual dermatological response grades are provided in Data Listing 3, Appendix II.

This study determined the following irritation scores and associated classifications:

[redacted]	Irritation Scores: Total	Irritation Scores: Overall Average Skin Grade (N=39 Enrolled)	Cumulative Irritation Index
SLS 0.75%	117.5	0.4361	0.1090
[redacted]			
Eye cream [redacted]	2.5	0.0094	0.0023
[redacted]			
Distilled Water	1.0	0.0038	0.0009
[redacted]			

Source: Table 4, Appendix 1, and Data Listing 3, Appendix II

8.0 CONCLUSION

Under the conditions employed in this study, the subjects showed no evidence of irritation to [redacted] an eye cream containing 0.0375% *Rhodymenia palmata* extract.

9.0 REFERENCES

1 B.M. Lanman, E.B. Elvers and C.J. Howard. "The Role of Human Patch Testing in a Product Development Program" Joint Conference on Cosmetic Sciences, The Toilet Goods Association, Washington, D.C., April 21-23, 1968.

2 L. Philips, M. Steinberg, H.I. Maibach and W.A. Akers. "Comparison of Rabbit and Human Skin Response to Certain Irritants". Toxicol. Appl. Pharmacol. 21.369, 1972.

3 R.S. Berger and J.P. Bowman. "A Reappraisal of the 21-day Cumulative Irritation Test in Man" J. Toxicol. - Cut. & Ocular Toxical. 1 (2). 109-115, 1982.

[redacted]

APPENDIX I

SUMMARY TABLES

Table 1: Summary of Subject Enrollment and Disposition

	N (%)
Subjects enrolled	39
Subjects completed all phases	38 (97.4)
Total subjects discontinued	1 (2.6)
Lost to follow-up	1 (2.6)

Note: All percentages are relative to total subjects enrolled.

See data listing 1 for further detail.

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PRODUCT = R

TKL Study No. [redacted]

Table 2: Summary of Subject Demographics
All Enrolled Subjects

Age	
N (%) 18 to 44	16 (41.0)
N (%) 45 to 64	21 (53.8)
N (%) 65 and up	2 (5.1)
Mean (SD)	47.3 (12.8)
Median	48.4
Range	20.9 to 65.7
Gender	
N (%) Male	14 (35.9)
N (%) Female	25 (64.1)
Race	
Black	3 (7.7)
Caucasian	21 (53.8)
Hispanic	15 (38.5)

See data listing 2 for further detail.

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PRODUCT = R

[redacted]

Table 3: Summary of Dermatologic Response Grades
Number of Subjects by Product

Product = [redacted] Eye cream

	Reading No.						
Response	**1**	**2**	**3**	**4**	**5**	**6**	**7**
0	39	39	39	38	37	37	37
0.5	0	0	0	0	1	1	1
1	0	0	0	1	0	0	0
Average skin grade	0.00	0.00	0.00	0.03	0.01	0.01	0.01
Total evaluable	39	39	39	39	38	38	38
Number absent	0	0	0	0	0	0	0
Number discontinued	0	0	0	0	1	1	1

Overall Average Skin Grade: 0.01

Note: 'Total evaluable' includes subjects with no patch applied.
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PRODUCT = R

[redacted]

Table 3: Summary of Dermatologic Response Grades
Number of Subjects by Product

Product = DISTILLED WATER

	Reading No.						
Response	**1**	**2**	**3**	**4**	**5**	**6**	**7**
0	39	39	39	38	37	38	38
0.5	0	0	0	1	1	0	0
Average skin grade	0.00	0.00	0.00	0.01	0.01	0.00	0.00
Total evaluable	39	39	39	39	38	38	38
Number absent	0	0	0	0	0	0	0
Number discontinued	0	0	0	0	1	1	1

Overall Average Skin Grade: 0.00

Note: 'Total evaluable' includes subjects with no patch applied.
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PRODUCT = R

[redacted]

Table 3: Summary of Dermatologic Response Grades
Number of Subjects by Product

Product = SLS 0.75% LOT# CS71350001

Response	Reading No. 1	2	3	4	5	6	7
0	35	33	21	15	11	11	11
0.5	4	6	10	14	9	2	2
1	0	0	8	8	16	23	23
2	0	0	0	2	0	0	0
Average skin grade	0.05	0.08	0.33	0.49	0.64	0.74	0.74
Total evaluable	39	39	39	39	38	38	38
Number absent	0	0	0	0	0	0	0
Number discontinued	0	0	0	0	1	1	1
Patch not applied	0	0	0	0	2	2	2

Overall Average Skin Grade: 0.45

Note: 'Total evaluable' includes subjects with no patch applied.
Generated on 11/07/12:12:23 by SUMMARY15.SAS / Uses: RESPONSE, PRODLIST, FINAL
PRODUCT = R

Table 3.1: [redacted] Patch Test Grading Scale

Score or Symbol	Description
0	No apparent cutaneous involvement.
0.5	Greater than 0, less than 1.
1	Faint but definite erythema, no eruptions or broken skin or no erythema but definite dryness; may have epidermal fissuring.
1.5	Greater than 1, less than 2.
2	Moderate erythema, may have a few papules or deep fissures, moderate-to-severe erythema in the cracks. **Cut-off Grade - Patches are not re-applied.**
2.5	Greater than 2, less than 3.
3	Severe erythema (beet redness), may have generalized papules or moderate-to-severe erythema with slight edema (edges well defined by raising.)
3.5	Greater than 3, less than 4.
4	Generalized vesicles or eschar formations or moderate-to-severe erythema and/or edema extending beyond the area of the patch.
NA	Not applied
NP	Not patched due to reaction achieved
-	Reading not performed due to missed visit or subject discontinuation

Table 4: Cumulative Irritation Index, by Study Product

Product Index	Product Name or ID	Cumulative Irritation Index	Average Skin Grade
1	[redacted] Eye cream	0.0023	0.0094
[redacted]			
9	DISTILLED WATER	0.0009	0.0038
10	SLS 0.75% LOT# CS71350001	0.1090	0.4361

Note: Cumulative Irritation Index = Total Irritation Score (sum of all days for all completed subjects)/[Highest possible score (=4) x Number of completed subjects x Number of study days (=7)].

Overall average skin grade is calculated as the sum of the daily average skin grades divided by the number of study days.

[redacted]

APPENDIX II

DATA LISTINGS

[redacted]

Data Listing 1: Subject Enrollment and Disposition

	Study Dates					
Subject No.	Screened	1st Applic	Ended	Last Reading #	Completion Status	Days in Study
001	10/22/12	10/22/12	10/29/12	8	C	8
002	10/22/12	10/22/12	10/29/12	8	C	8
003	10/22/12	10/22/12	10/29/12	8	C	8
004	10/22/12	10/22/12	10/29/12	8	C	8
005	10/22/12	10/22/12	10/29/12	8	C	8
006	10/22/12	10/22/12	10/29/12	8	C	8
007	10/22/12	10/22/12	10/29/12	8	C	8
008	10/22/12	10/22/12	10/29/12	8	C	8
009	10/22/12	10/22/12	10/29/12	8	C	8
010	10/22/12	10/22/12	10/29/12	8	C	8
011	10/22/12	10/22/12	10/29/12	8	C	8
012	10/22/12	10/22/12	10/29/12	8	C	8
013	10/22/12	10/22/12	10/29/12	8	C	8
014	10/22/12	10/22/12	10/29/12	8	C	8
015	10/22/12	10/22/12	10/29/12	8	C	8
016	10/22/12	10/22/12	10/29/12	8	C	8
017	10/22/12	10/22/12	10/29/12	8	C	8
018	10/22/12	10/22/12	10/29/12	8	C	8
019	10/22/12	10/22/12	10/29/12	8	C	8
020	10/22/12	10/22/12	10/29/12	8	C	8
021	10/22/12	10/22/12	10/27/12	4	L	6
022	10/22/12	10/22/12	10/29/12	8	C	8
023	10/22/12	10/22/12	10/29/12	8	C	8
024	10/22/12	10/22/12	10/29/12	8	C	8
025	10/22/12	10/22/12	10/29/12	8	C	8
026	10/22/12	10/22/12	10/29/12	8	C	8
027	10/22/12	10/22/12	10/29/12	8	C	8
028	10/22/12	10/22/12	10/29/12	8	C	8
029	10/22/12	10/22/12	10/29/12	8	C	8
030	10/22/12	10/22/12	10/29/12	8	C	8
031	10/22/12	10/22/12	10/29/12	8	C	8
032	10/22/12	10/22/12	10/29/12	8	C	8
033	10/22/12	10/22/12	10/29/12	8	C	8
034	10/22/12	10/22/12	10/29/12	8	C	8
035	10/22/12	10/22/12	10/29/12	8	C	8
036	10/22/12	10/22/12	10/29/12	8	C	8
037	10/22/12	10/22/12	10/29/12	8	C	8
038	10/22/12	10/22/12	10/29/12	8	C	8
039	10/22/12	10/22/12	10/29/12	8	C	8

Key: Completion Status (C=Completed, L=Lost to follow-up, S=Voluntary withdrawal, V=Protocol violation, AE=Adverse event, O=Other)

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[redacted]

Data Listing 2: Subject Demographics

Subject No.	Age	Gender	Race
001	38.5	Female	Caucasian
002	51.9	Female	Hispanic
003	43.0	Female	Hispanic
004	20.9	Male	Black
005	36.3	Male	Hispanic
006	58.4	Female	Caucasian
007	46.6	Female	Hispanic
008	64.2	Male	Caucasian
009	62.8	Male	Caucasian
010	56.6	Female	Caucasian
011	41.1	Female	Caucasian
012	32.1	Male	Caucasian
013	56.7	Male	Caucasian
014	37.6	Female	Caucasian
015	54.0	Female	Caucasian
016	41.1	Female	Black
017	48.2	Female	Hispanic
018	22.4	Female	Caucasian
019	40.9	Male	Hispanic
020	43.2	Female	Hispanic
021	47.1	Female	Caucasian
022	48.4	Female	Caucasian
023	55.8	Female	Caucasian
024	24.5	Male	Hispanic
025	59.7	Female	Hispanic
026	42.9	Female	Hispanic
027	60.4	Female	Hispanic
028	39.7	Female	Caucasian
029	49.1	Female	Caucasian
030	65.7	Male	Caucasian
031	57.5	Male	Hispanic
032	52.0	Female	Caucasian
033	55.8	Male	Caucasian
034	65.6	Female	Hispanic
035	57.2	Male	Hispanic
036	23.1	Female	Hispanic
037	54.0	Male	Caucasian
038	63.7	Female	Caucasian
039	26.4	Male	Black

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[redacted]

Data Listing 3: Dermatologic Response Grades
By Product and Subject

Product = [redacted] Eye cream

Subject Number	Reading No. 1	2	3	4	5	6	7	Total Score
001	0	0	0	0	0	0	0	0
002	0	0	0	0	0	0	0	0
003	0	0	0	0	0	0	0	0
004	0	0	0	0	0	0	0	0
005	0	0	0	1	0.5	0.5	0.5	2.5
006	0	0	0	0	0	0	0	0
007	0	0	0	0	0	0	0	0
008	0	0	0	0	0	0	0	0
009	0	0	0	0	0	0	0	0
010	0	0	0	0	0	0	0	0
011	0	0	0	0	0	0	0	0
012	0	0	0	0	0	0	0	0
013	0	0	0	0	0	0	0	0
014	0	0	0	0	0	0	0	0
015	0	0	0	0	0	0	0	0
016	0	0	0	0	0	0	0	0
017	0	0	0	0	0	0	0	0
018	0	0	0	0	0	0	0	0
019	0	0	0	0	0	0	0	0
020	0	0	0	0	0	0	0	0
021	0	0	0	0	-	-	-	0
022	0	0	0	0	0	0	0	0
023	0	0	0	0	0	0	0	0
024	0	0	0	0	0	0	0	0
025	0	0	0	0	0	0	0	0
026	0	0	0	0	0	0	0	0
027	0	0	0	0	0	0	0	0
028	0	0	0	0	0	0	0	0
029	0	0	0	0	0	0	0	0
030	0	0	0	0	0	0	0	0
031	0	0	0	0	0	0	0	0
032	0	0	0	0	0	0	0	0
034	0	0	0	0	0	0	0	0
035	0	0	0	0	0	0	0	0
036	0	0	0	0	0	0	0	0
037	0	0	0	0	0	0	0	0
038	0	0	0	0	0	0	0	0
039	0	0	0	0	0	0	0	0

Total Score: 2.5
Normalized Total Score: 0.6

Please see table 3.1 for key to scores and symbols.

[redacted]

[redacted]

Data Listing 3: Dermatologic Response Grades
By Product and Subject

Product = DISTILLED WATER

Subject Number	Reading No. 1	2	3	4	5	6	7	Total Score
001	0	0	0	0	0	0	0	0
002	0	0	0	0	0	0	0	0
003	0	0	0	0	0	0	0	0
004	0	0	0	0	0	0	0	0
005	0	0	0	0	0	0	0	0
006	0	0	0	0	0	0	0	0
007	0	0	0	0	0	0	0	0
008	0	0	0	0	0	0	0	0
009	0	0	0	0	0	0	0	0
010	0	0	0	0	0	0	0	0
011	0	0	0	0	0	0	0	0
012	0	0	0	0	0	0	0	0
013	0	0	0	0	0	0	0	0
014	0	0	0	0	0	0	0	0
015	0	0	0	0	0	0	0	0
016	0	0	0	0	0	0	0	0
017	0	0	0	0	0	0	0	0
018	0	0	0	0	0	0	0	0
019	0	0	0	0	0	0	0	0
020	0	0	0	0	0	0	0	0
021	0	0	0	0	-	-	-	0
022	0	0	0	0	0	0	0	0
023	0	0	0	0	0	0	0	0
024	0	0	0	0	0	0	0	0
025	0	0	0	0	0	0	0	0
026	0	0	0	0	0	0	0	0
027	0	0	0	0	0	0	0	0
028	0	0	0	0	0	0	0	0
029	0	0	0	0	0	0	0	0
030	0	0	0	0	0	0	0	0
031	0	0	0	0.5	0.5	0	0	1
032	0	0	0	0	0	0	0	0
033	0	0	0	0	0	0	0	0
034	0	0	0	0	0	0	0	0
035	0	0	0	0	0	0	0	0
036	0	0	0	0	0	0	0	0
037	0	0	0	0	0	0	0	0
038	0	0	0	0	0	0	0	0
039	0	0	0	0	0	0	0	0

Total Score: 1.0
Normalized Total Score: 0.3

Please see table 3.1 for key to scores and symbols.

[redacted]

[redacted]

Data Listing 3: Dermatologic Response Grades
By Product and Subject

Product = SLS 0.75% LOT# CS71350001

Subject Number	Reading No. 1	2	3	4	5	6	7	Total Score
001	0	0	0	0	0	0	0	0
002	0	0	0.5	0.5	1	1	1	4
003	0	0	0	0	0.5	1	1	2.5
004	0.5	0.5	1	2	NP	NP	NP	10
005	0	0	1	2	NP	NP	NP	9
006	0	0	0.5	0.5	1	1	1	4
007	0	0	0	0	0.5	1	1	2.5
008	0	0.5	1	1	1	1	1	5.5
009	0	0	0.5	0.5	1	1	1	4
010	0	0	0	0	0	0	0	0
011	0	0	0	0.5	0.5	0.5	0.5	2
012	0	0	0	0	0.5	1	1	2.5
013	0	0	0.5	0.5	1	1	1	4
014	0	0	0	0	0	0	0	0
015	0.5	0.5	1	1	1	1	1	6
016	0	0	0	0	0	0	0	0
017	0	0	0	0	0.5	0.5	0.5	1.5
018	0	0	0	0	0	0	0	0
019	0	0	0.5	0.5	1	1	1	4
020	0	0.5	1	1	1	1	1	5.5
021	0	0	0.5	1	-	-	-	1.5
022	0	0	0	0.5	0.5	1	1	3
023	0	0	0.5	1	1	1	1	4.5
024	0	0	0.5	0.5	1	1	1	4
025	0.5	0.5	1	1	1	1	1	6
026	0	0	0.5	0.5	1	1	1	4
027	0	0	1	1	1	1	1	5
028	0	0	0	0	0	0	0	0
029	0	0	0	0	0	0	0	0
030	0	0	0	0	0	0	0	0
031	0	0	0	0.5	1	1	1	3.5
032	0	0	0.5	0.5	1	1	1	4
033	0	0	0	0.5	0.5	1	1	3
034	0	0	0	0	0	0	0	0
035	0	0	0	0.5	0.5	1	1	3
036	0.5	0.5	1	1	1	1	1	6
037	0	0	0	0	0	0	0	0
038	0	0	0	0	0	0	0	0
039	0	0	0	0.5	0.5	1	1	3

Total Score: 117.5
Normalized Total Score: 34.6

Please see table 3.1 for key to scores and symbols.

[redacted]

[redacted]

Data Listing 3A: Residual Readings
by Product and Subject
SLS 0.75% LOT# CS71350001

Subject Number	Reading No. 1	2	3	4	5	6	7
004					2	2	1.5
005					2	2	1.5

Please see table 3.1 for key to scores and symbols.
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PRODUCT = R

Personal Care Products Council
Committed to Safety, Quality & Innovation

Memorandum

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

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

DATE: April 23, 2020

SUBJECT: Scientific Literature Review: Safety Assessment of Red Algae-Derived Ingredients as Used in Cosmetics (release date: March 19, 2020)

The Personal Care Products Council has no suppliers listed for the following ingredients included in this report:

Ceramium Rubrum Extract
Chondrus Crispus
Gelidium Pulchrum Protein
Gloiopeltis Tenax Powder
Grateloupia Livida Powder
Hydrolyzed Chondrus Crispus Extract
Hydrolyzed Corallina Officinalis
Pikea Robusta Extract
Porphyra Tenera Extract
Sarcodiotheca Gaudichaudii Extract

The Personal Care Products Council respectfully submits the following comments on the scientific literature review, Safety Assessment of Red Algae-Derived Ingredients as Used in Cosmetics.

Key Issues

The Algae Identification section should state that red algae are in the kingdom Plantae. It is not necessary to describe the other types of algae in this section.

The Algae Identification section states that "Red algae are marine organisms". Although many red algae species are marine organisms, there are other environments where red algae species can be found. For example, the CIR report includes three species (all microalgae) that are not marine species: *Cyanidium caldarium* is a fresh water species found in hot springs; *Porphyridium cruentum* is found in both marine and terrestrial environments; and *Porphyridium purpureum,* for which the algae data base (reference 47) lists the habitat as ubiquitous, is found on moist terrestrial areas such as brick walls. Although *Polysiphon lanosa* is a marine alga, it is also listed as being an obligate

epiphyte of the brown alga, *Ascophyllum nodosum*. Because these species are different from the remaining species in the report (macroalgae found in marine environments), ingredients derived from these four species should be removed from the CIR report. If these ingredients are not removed from the CIR report, information on characteristics and composition of these species should be added to the report.

For example, the following article was found by searching Google for composition of *Cyanidium caldarium*:

Kremer BK. 1982. *Cyanidium caldarium*: A discussion of biochemical features and taxonomic problems. *Br Phycol J* 17: 51-61. (at https://www.tandfonline.com/doi/pdf/10.1080/00071618200650071)

Another example, found by searching Google for composition of *Porphyridium purpureum*:

Li T, Xu J, Wu H, et al., 2019. Growth and Biochemical Composition of *Porphyridium purpureum* SCS-02 under Different Nitrogen Concentrations. *Mar. Drugs*, 17: 124; doi:10.3390/md17020124 (at: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6410139/pdf/marinedrugs-17-00124.pdf).

Google searches for the composition of the other species suggested for removal from the CIR report were not completed.

It is not clear why more composition information about the species with the most uses, *Chondrus crispus* was not included in the report. This review http://www.fao.org/3/x5819e/x5819e05.htm#2.%20distribution,%20ecology%20and%20metabolism found on the internet may provide some additional information and cite additional references that may be useful.

Table 4 - It is not clear why only certain species are included in Table 4 and why the species with the most uses reported to the VCRP, *Chondrus crispus* is not included in this table. The species *Lithothamnion calcareum* and *Lithothamnion corallioides* should also be included in this table as theses species are used to make mineral preparations rather than an organic compound preparation.

Additional Considerations

Introduction - Although these ingredients are frequently sold as more complex mixtures, none of the INCI names included in the CIR report represent more than one species. Therefore, it is not clear why the Introduction states that the ingredients are “derived from one or multiple species of red algae”.

Composition, Gracilaria Verrucosa Extract - It is not clear what the percentage values for MAAs represent. Is this the percent of total composition, or the percentage of amino acids that were specific MMAs?

Composition, Lithothamnium Calcareum Extract - What do the values for the minerals represent? Are they the percent of total composition, or the percent of minerals?

Impurities; Table 6 - As the dose determines whether or not something will be "toxic", please delete the word "toxic" when describing the metal content, and delete "toxic" from the title of Table 6.

Non-Cosmetic Use - Please indicate which species of red algae are used for carrageenan production, and which species are used for agar production.

Acute, Oral, Grateloupia Livida Extract - Reference 16 indicates that Kumming mice with starting weights of 22-26 g were used in the acute study of the *Grateloupia livida* extracts. This information should be added the report and "strain not specified" should be deleted.

Subchronic - What was the "constant volume" used in the 90-day study of Lithothamnion Calcareum Extract?

Anti-Tumorigenicity, Animal - Please do not use the word "autopsied" for any species other than humans. The correct word is "necropsied". This is one explanation of these words from the internet: ""Necro" refers to "dead" and "psy" to study, so necropsy is the "study of the dead." "Auto" refers to "self" so autopsy is "self study." So an autopsy is technically a necropsy, but because a "human is performing it on a human" it is an autopsy."

Cytotoxicity, Gracilariopsis Longissima Extract - What is meant by "significant cytotoxicity"? At what concentration was there a statistically significant increase in cytotoxicity?

Reference 19 - Please look at this reference again. It is from the *Journal of Applied Phycology* not the *Journal of Applied Psychology* as stated in the reference section of the CIR report.