ALGOMEGA NP®

Strengthens the skin’s barrier function

*

Improves skin hydration & reduces water loss

Regulates skin desquamation

Guarantees full cell membrane functionality after barrier function damage

Gelyma

The data presented in this document ALGOMEGA NP® (pp. 1-28)
are offered solely for your consideration and investigation.
No guaranty is expressed or implied.

No responsibility or liability for any consequences
arising from the use of these data can be accepted,
including possible infringement of any patent.

SUMMARY

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.

INTRODUCTION

Cutaneous hydration is regarded as the main cosmetic preoccupying of 80% of the consumers. It affects all age groups and all cutaneous regions *e.g*. face, body, hands and feet.

The concept of skin hydration is often associated to a psychosensorial concept:

- a well hydrated skin appears sweet and supple,
- a rough skin feels tight and irritable. It is characterised by a flaky and scaly surface.

Dry skin may be due to many possible causes (Pierard, 1997 – Cosmétologie 14: 48-51) that are

- exposure to different agents *e.g*. excessive baths with harsh soaps, climatic conditions such as wind, extremes of temperature and air-conditioning,

- natural origin *e.g.* aging,

- pathological conditions *e.g*. certain pathologies involve dry skin such as psoriasis, atopic dermatitis, uremic xerosis and HIV,

- physiological origin due to unbalanced diets, tobacco consumption, drug absorption such as laxative or diuretics.

Whatever exogenous or endogenous factors, several clinical studies indicate that dry skin results from:

- deficiency of epidermal lipids and /or sebaceous lipids,

- deficiency of natural moisturizing factors and sometimes

- disturbed lipid composition.

The skin barrier is then no longer intact. Loss of its integrity is a central factor in the development of dry skin conditions.

Consequently, treatment of dry skin is more than just a cosmetic problem.

In order to help resolve this problem, GELYMA has developed ALGOMEGA NP®.

The purpose of ALGOMEGA NP® is to supply lipids and sterols, specially polyunsaturated fatty acids of classes:

- omega-6 (*e.g.* linoleic acid) and

- omega-3 (*e.g.* eicosapentaenoic acid and docosahexaenoic acid),

indispensable to maintain the integrity of epidermal structures and to restore the barrier function.

ALGAL SOURCE & ALGOMEGA NP®

ALGOMEGA NP® is a reparative moisturizer based on the synergistic combination of liposoluble fractions extracted from the association of two algae rich in omega-3 fatty acids:

- a microalga : *Nannochloropsis oculata* (Droop) Hibberd and

- a macroalga : *Porphyra umbilicalis* (Linné) Kützing

in a vegetable oil : *Silybum marianum* seed oil , well-supplied with linoleic acid.

Some biological and biochemical characteristics of these three components are given before to detail efficacy tests.

The microalga *Nannochloropsis oculata*

➤ The genus *Nannochloropsis*

The genus *Nannochloropsis* Hibberd includes eukaryotic microalgae belonging to the phylum *Heterokontophyta* and the class of *Eustigmatophyceae*.

It is characterized by a typical fine structure showing one plastid by cell only. Its chemical composition also presents peculiarities with the presence of one chlorophyll : chlorophyll a and the abundance of violaxanthine and lipids.

It is present in all oceans throughout the world (Antia & Cheng, 1982 – J. Phycol. 15: 57-62 ; Maruyama *et al*., 1986 – Japan. J. Phycol., 34 : 319-325).

➤ The species *Nannochloropsis oculata*

Nannochloropsis oculata (Droop) Hibberd, named at first *Nannochloris oculata* Droop and incorrectly "marine *Chlorella*", is a picoplanctonic unicellular microalga of 0.2-2µm in diameter.

Numerous papers concern its chemical composition : Maruyama *et al*., 1986 – *Ibid* ; Suen *et al*., 1987 – J. Phycol., 23 : 289-296 ; Sukenik & Carmeli, 1989 – J. Phycol., 25 : 686-692 ; Volkman *et al.*, 1993 – J. Phycol., 29 : 69- 79 ; Dunstan *et al*., 1993 – J. Applied Phycol., 5 : 71-83 ; Schneider & Roessler, 1994 – J.Phycol., 30 : 594-598 ; Gelin *et al.,* 1997 – Phytochemistry, 45 : 641-646 ; Veron *et al*., 1998 – J. Phycol., 34 : 273-279 ; Zou *et al*., 2000 – Eur. J. Phycol., 35 : 127-133.

The amount in lipids ranges from 0.34 to 0.76 pg $cell^{-1}$ with 0.32 to 0.66 $pg.cell^{-1}$ of polar lipids (Volkman *et al,* 1993 – *Ibid* ; Veron *et al*., 1998 – *Ibid*). The eicosapentaenoic acid content could be modulated from 20% to 40% of the total extractable fat by varying the culture conditions.

Sterols are also present with cholesterol as major compound (Veron *et al*., 1998 - J. Phycol., 34: 273-279).

This species is used as a feedstuff in the aquaculture industry (Watanabe *et al*., 1983 – Aquaculture, 34: 115-143 ; Enright *et al*., 1986 – J. Exp. Mar. Biol. Ecol., 96 : 1-13). As a consequence of its high fat and eicosapentaenoic acid contents, it provides a highly nutritional feed in the food chain for fish hatchlings.

In ALGOMEGA NP®, the lipidic fraction of *Nannochloropsis oculata* is advantageously associated to the lipidic fraction extracted from the red macroalga *Porphyra umbilicalis.*

The macroalga *Porphyra umbilicalis*

Porphyra umbilicalis (L.) Kützing (from Greek: *porphura*: purple dye) belongs to the phylum *Rhodophyta*, the class *Bangiophycideae*, the order Bangiales and the family *Bangiaceae*.

The genus *Porphyra* C.Agardh 1824 includes approximately 25 species, nearly ubiquitous and often in great abundance. It is very difficult to distinguish the individual species.

Por*phyra umbilicalis* is regarded as the type of *Porphyra* species. It includes different forms such as *linearis, vulgaris, laciniata.*

It is described as “common *Porphyra*”, “nori Breton” in France, “laver” in England; “slack” in Scotland, “sloke” in Ireland, and “Chishima-kuronori” in Japan.

➤ Morphology & Biology

The macroscopic thallus consists of erect and membranaceous fronds, clustered together and divided into several lobes (Fig.1).

It is fixed to substratum by means of a small disc, composed of numerous rhizoidal outgrowths from the lower cells of erect parts.

Blades constituted by a single cell layer can reach 60 cm in height.

Their colour is purple red when they grow fully submerged and yellowish brown when they grow out in intertidal sites. In a dried state, blades are very thin and violet in color.

Fig. 1 - Morphology of *Porphyra umbilicalis* Herbarium sheet GELYMA.

The reproductive cycle consists of two successive generations:

(1) either a well developped gametophyte

(2) or a reduced sporophyte known as *Conchocelis*, consisting of numerous filaments of uninucleate cells.

This alga is monoecious in Europe (Conway & Cole, 1977 – Phycologia, 16:205-216; Kapraun & Freshwater, 1987 – Phycologia, 26:82-87).
Spermatangia form a pale deliquescing marginal band whereas the carpospore groups are scattered in clusters over the general surface of the blade.

An important breakthrough in the understanding of the life history of this alga was the recognition by the English Phycologist Kathleen Drew (1949 – Nature, 164: 748) that the spores of *Porphyra umbilicalis* developed into shell-penetrating filamentous plants called *Conchocelis rosea*.

➤ Chemical composition

A large number of investigations concern the constituents of *Porphyra umbilicalis*.

In fact this macroalga shows an interesting composition in:

- carbohydrates: 40% dry weight
- proteins: 19.2% dry weight
- vitamins especially
  - vitamin B12 : 291 $mg.kg^{-1}$,
  - vitamin B2 : 23.08 $mg.kg^{-1}$ and
  - vitamin A : 13.4 $mg.kg^{-1}$
- choline: 2920 $mg.kg^{-1}$
- inositol: 62 $mg.kg^{-1}$

cf. Verbist & Biard, 1989 – Lettre Phytothérapeutique n°14, supplément).

Generally, macroalgae contain low quantities of lipids.

Porphyra umbilicalis is an exception. It is characterized by high contents in arachidonic (20:4 n-6) and eicosapentaenoic (20:5 n-3) acids, comparatively to other macroalgal species.

According to Fleurence and collaborators. (1994 – J. Applied Phycol., 6: 527-532), *Porphyra umbilicalis* contains 33.7 $mg.g^{-1}$ of total lipids with 13.5 $mg.g^{-1}$ of fatty acids.

The fat content is distributed as follows:

- neutral lipids : 18.9%
- phospholipids : 25.2%
- glycolipids : 55.9%

with as major fatty acids in all three fractions :

- 18:2 n-6 : 0.4 $mg.g^{-1}$
- 20:4 n-6 : 1.5 $mg.g^{-1}$
- 20: 5 n-3 : 6.7 $mg.g^{-1}$.

Porphyra umbilicalis was chosen due to its interesting lipidic composition.

➤ Ecology & Geographical distribution

The blades of *Porphyra* sp. are frequently observed on rocky shores from polar to tropical seas. Most species are of intertidal occurrence but some inhabit the sublittoral zone.

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 (Fig.2). It occurs in the upper littoral zone on rocks or on other algae. It is also present along the coasts of the Eastern part of the Mediterranean.

➤ Utilizations

Porphyra umbilicalis is harvested and eaten in various recipes.

In France, this red macroalga is authorized for human consumption since 1988.

On the coasts of the United Kingdom, it is also 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.* in Guiry & Blunden, 1991 – Seaweed Resources in Europe : uses and potential, Wiley).

Fig.2 – Population of *Porphyra umbilicalis* in Brittany. Photo GELYMA.

On the Hawaiian Islands, this alga known as "limu" is a popular delicacy.

In Asia, it exists numerous *Porphyra* species, the most important being the species *tenera.*

Porphyra sp. occurs not only on Japanese coasts, but also in many other East Asian areas such as China coasts. They are cultivated in numerous forms and serve for making "sushis" or other delicacies.
Natural *Porphyra* known as "purple laver" was made a part of foodstuff by Japanese people since more than 1,000 years ago.
In Japan, it forms particularly abundant populations in early summer at Cap of Nichimotoro. It is harvested at low tide and use to make "Asakusa-nori".
Historically, this alga was the first seaweed to be cultivated, starting in Tokyo Bay on bundles of wood and bamboo set in shallow water around either 1640 (Miura, 1975 – Advance of Phycology in Japan, pp. 273-304, Junk ed.) or 1736 (Okazaki, 1971 – Seaweeds and their uses in Japan, Tokai Univ. Press).

In China, *Porphyra* cultivation started about 200 years ago (Tseng, 1981 – Proceedings of the Tenth Int. Seaweed Symposium, pp.123-152) on shallow rocks.

The last few decades have witnessed extensive sophistication of the cultivation methods, necessarily based on the previous work of Kathleen Drew.

The solvent *Silybum marianum* seed oil

Silybum marianum (Linné) Gaertner, also known as *Carduus marianus* Linné belongs to the family *Compositaae, Asteraceae*.

It is an annual or biennial herb with different common names : *e.g*. in Great Britain “blessed milk-thistle, lady’s thistle, Mary thistle, St Mary’s milk thistle”, in Germany “Mariendistel”, in Sweden “Mariatistel “and in France “Chardon Marie”.
The species name “*marianum*” honors the symbolic association of the plant with the Virgin Mary. According to a religious legend, the white veins of the leaves represent drops of the Virgin Mary’s milk, fallen there when she nursed baby Jesus.

➢ Morphology & Biology

This plant can reach 1.50 m tall (Fig. 3).

A distinguishing morphological characteristic is the white patches or marbling seen along the veins of the dark green leaves.
The flower head differs from other thistles with the presence of broad leathery bracts tipped with stiff spines.
The seeds are heavy, flat, smooth and shiny.

Fig.3 – Morphology of *Silybum marianum.* Photo GELYMA.

➢ Ecology & Geographical distribution

Silybum marianum is found as dense stands along roadsides and waste areas but is prefers high fertility soils.

This plant is native to the Mediterranean region of Europe. Nowadays, it grows throughout Europe and also in the United States, Canada, New Zealand, Australia, South Africa, Chile and Argentina.

➢ Utilizations

Silybum marianum is among the most ancient of all known herbal medicines. Its use as a liver protecting agent dates at least to the first century.

The active chemical component is silymarin. It is an antioxidant including a combination of three flavonoids.

An injection of silybin (the most active part of the chemical structure of silymarin) is an antidote for *Amanita phalloides* poisoning. “Mariendistel” is kept in German hospitals for emergency treatment of Death Cap mushroom poisonings.

The seeds of *Silybum marianum* contain the highest amounts of silymarin. The whole plant is also used medicinally. It is astringent, bitter, cholagogue, diuretic and stimulant. It is harvested when in flower and dried for later use as homeopathic remedy.

The oil used for the preparation of ALGOMEGA NP® is extracted to seed without heat and any chemical product.
It contains high amounts of unsaturated fatty acids (mono-unsaturated: 22% and poly-unsaturated: 57%). Due to the presence of silymarin (0.25%) and vitamin E (50-60 mg/100g), it shows anti-oxidant properties.

THE ACTIVE INGREDIENT ALGOMEGA NP®

Specifications

on a control batch

- appearance : oily liquid yellow to lightly greenish coloured
- odour : typical
- density : < 1
- solubility : insoluble in water
  : soluble in oils
- microbiology : bacteria : < 100 germs / ml
  : yeasts, moulds : < 100 germs / ml
  : pathogens : free.

Composition

Ingredients		Amount (%)
Solvent	*Silybum marianum* seed oil	93
Microalga + macroalga	*Nannochloropsis oculata + Porphyra umbilicalis* extract	7
Preservative	none	
Others (antioxidants …)	none	

INCI names *Silybum marianum* seed oil CAS n° 84604-20-6 EINECS n° 283-298-7
Porphyra umbilicalis extract CAS n° 223751-76-6
Nannochloropsis oculata extract

Storage

ALGOMEGA NP® should be stored in the original sealed drums, under clean conditions between 15 to 25°C, away from light.

If stored under the recommended conditions, ALGOMEGA NP® remains stable for 12 months.

Pack size: 1kg – 5 kg – 10 kg.

Safety

No animal experimentation.

Standard safety testing proves that ALGOMEGA NP® is safe for cosmetic use.

ALGOMEGA NP® does not exhibit a potential for ocular irritation (IC50 > 50% - Predisafe test) and dermal irritation (no irritant with Human Patch test) at the recommended use levels. It is no phototoxic (*in vitro* test) and no mutagenic (Ames test).

cf. annex pp. 24-27.

EFFECTIVENESS EVALUATION

ALGOMEGA NP®

A synergistic combination of liposoluble fractions of two algae in a vegetable seed oil

Hydration

Fills deficiency in essential fatty acids & reconstitutes the lipid surface film	Anti-oxidant complex rich in unsaturated fatty acids & sterols
Regulates the epidermal water balance	*In vivo* tests Transient thermal transfer
Decreases desquamation. & improves the intercellular cohesion	*In vivo* study D-squames Scanning electron microscopy

Restructuration

Reduces Trans Epidermal Water Loss (TEWL) & reinforces the barrier function	*In vivo* study TEWL measurements Evaporimetry
Improves the structure of cytomembranes after damage of the skin barrier & restores the barrier function	*In vitro* study Cytological study of reconstituted skins submitted to irritation Transmission electron microscopy

Reparative moisturizer
for preventing the dryness & restoring the barrier function
of sensitive and reactive skins

ALGOMEGA NP® fills deficiency in essential fatty acids & reconstitutes the lipid surface film

Fatty acids are the major compounds of oils and alimentary fats. They are also present in Humans and Animals.

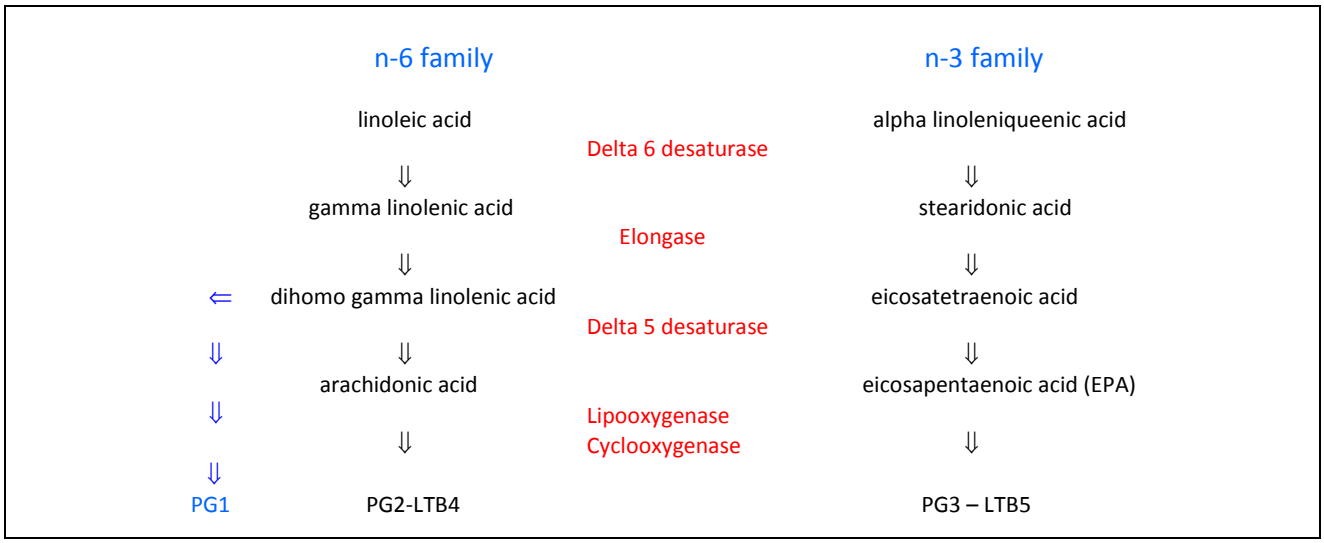
They exist as triglycerides and phospholipids. Some of them are not synthetized by organisms. They must be brought by feeding. They are known as “essential fatty acids” (EFA).

➤ Essential fatty acids

All essential fatty acids are polyunsaturated compounds falled into two families:

- n-6 family with linolenic acid as leader member
- n-3 family under the control of □-linolenic acid.

These two families shows different biological properties with successive desaturation and elongation reactions which are catalysed each one by a specific enzyme (*cf*. the following figure by Monpoint *et al.,*(1992 – Ann. Dermatol.Venereol., 119 : 233-239).



PG : prostaglandins - LTB : leukotrienes

The metabolism of essential fatty acids occurs in all tissues, especially in skin but the liver plays a major function (Métais, 1982 – Cah. Nut . Diet., 17 : 223-234).

In skin, this metabolism is limitated because the epidermis contains neither delta 6 desaturase, nor delta 5 desaturase. Moreover, due to active epidermal turn over, essential fatty acids are not stocked for a long time.

Consequently, the epidermis is directly dependent to the synthesis of essential fatty acids made by liver and to their transport *via* blood towards skin (Chapkin *et al*., 1986 – J. Invest. Dermatol., 86 : 468 ; Ziboh & Chapkin, 1987 – Arch. Dermatol., 123 : 1686a-1690a ; Horrobin, 1989 – J. Am. Acad. Dermatol., 20 : 1045-1053).

Essential fatty acids have both structural and functional roles

They are part of all cell membranes and responsible to their fluidity. Moreover they also occur in other events such as:

- inflammatory processes (Ford-Hutchinson & Chan, 1985 – Br. J. Dermatol., 113, suppl. 28 : 95-97 ; Bouclier & Hensby, 1986 – Ann. Dermatol. Venereol., 113 : 1289-1293),

- immunomodulation (Mertin *et al.*, 1985 – Int. Arch. Allergy Appl. Immunol., 77 : 390-395),

- during the proliferation and differentiation of keratinocytes (Varley, 1983 - Epidermal lipids. Biochemistry and physiology of the skin, ed. Lowell & Goldsmith, vol. 1, Oxford Univ.Press).

When the amount of essential fatty acids becomes deficient, numerous cutaneous problems appear (Martini, 1995 – *Ibid*, Elias *et al.*, J. Invest. Dermatol., 74 : 230-233 ; Wertz & Downing, 1982 – Science, 217 : 1261-1262 ; Schmidt, 1995 – bedc, 3 : 203-206).

When essentiel fatty acids are topically applied, they are metabolized by skin (Prottey *et al.*, 1975 – J. Invest. Dermatol., 64 : 228-234 ; Friedman *et al.*, 1978 - J. Pediatr., 92 : 604-607). The barrier function is rapidly restored (Mao-Qiang *et al.*, 1996 – J. Invest. Dermatol., 106 : 1096-1101).

The diffusion of fatty acids throughout epidermal cell layers would be rapid (Mao-Qiang *et al.*, 1995 – Arch. Dermatol., 131 : 809-876).

So, a topical application of essential fatty acids provides excellent preconditions for:

- restoring the barrier function (and consequently hydration),
- regulating desquamation,
- regenerating the barrier function after irritation.

➤ Sterols

In human epidermis, the most important sterol is cholesterol which represents 14% of the total lipids of the *stratum corneum*.

Sterols present in plants, also named as phytosterols, show similar functions than those of cholesterol in Humans, *e.g.* the maintenance of the structure and function of cytomembranes.

ALGOMEGA NP® is rich in unsaturated fatty acids & sterols

Methods

The analysis of fatty acids was performed by the Company SILLIKER SA (95031 Cergy-Pontoise Cedex) and the analysis of sterols by EUROFINS Scientific Tests Center / ATS (13851 Aix-en-Provence)

Results

Results are grouped here after.

Fatty acids

The fatty acid composition (% of the total fatty acids) of ALGOMEGA NP® is undermentionned.

Fatty acids		Amounts (%total fatty acids)	
Saturated acids			
Myristic acid	C 14 :0	< 0.1	16,8 %
Pentadecanoic acid	C15 :0	< 0.1	
Palmitic acid	C 16 :0	8.2	
Margaric acid	C 17 :0	< 0.1	
Stearic acid	C 18 :0	5.4	
Arachidic acid	C 20 :0	3.2	
Behenic acid	C 22 :0	<0.1	
Monounsaturated acids			
Palmitoleic acid	C 16 :1	< 0.1	28,1%
Heptadecanoic acid	C 17 :1	< 0.1	
Oleic acid	C 18 :1	27.0	
Gadoleic acid	C 20 :1	0.9	
Erucic acid	C 22 :1	0.2	
Polyunsaturated acids			
Linoleic acid	C 18 :2	50.8	53,9 %
Linolenic acid	C 18 :3	0.2	
Eicosapentaenoic acid	C 20 :5	2.3	
Docosahexaenoic acid	C 22 :6	0.6	

- ALGOMEGA N P® shows a good natural unsaturated fatty acid balance (82% of the total fatty acids).
- ALGOMEGA NP® restores the lipid barrier in the *stratum corneum*, offering essential fatty acids vital for healthy skin functionning.
- ALGOMEGA NP® reconstitutes the lipid surface film and reequilibrates lipid deficient skins.

In ALGOMEGA NP®, oleic and linoleic acids come in a majority from *Silybum marianum* seed oil.

Linoleic acid (50.8% of total fatty acids) is an omega-6 fatty acid which is particularly crucial for the formation and maintenance of skin.

It contains certain ceramides, especially ceramide 1 which strengthens the links between skin lipids and corneocytes.

In addition, linoleic acid is the starting material for the biosynthesis of certain prostaglandins which have important immunoregulatory functions and influence epidermal keratinization.

In ALGOMEGA NP®, eicosapentanenoic (EPA) and docosahexaenoic (DHA) acids are from marine origin. They are important structural components of the family omega-3 fatty acids.

Stérols

The phytosterol composition (in relative %) is dominated by beta-sitosterol and delta 7 stigmasterol.

Beta sitosterol	41.9
Delta 7 stigmasterol	25.5
Stigmasterol	6.6
Campesterol	6.3
Delta 7 avenasterol	5.0
Delta 5 avenasterol	2.3
Fucosterol	0.6
Unidentified compounds	11.8

Sterols play important functions in the epidermal integrity. They also protect against dehydration.

Moreover, β sitosterol shows anti-inflammatory and immunomodulatory properties.

➤ The phytosterols present in ALGOMEGA NP® helps advantageously to the synthesis of epidermal lipids and to the reinforcement of the mechanical properties of the *stratum corneum*.

➤ β sitosterol is the major sterol in ALGOMEGA NP® that brings a soothing effect.

ALGOMEGA NP® is protected from oxidative damage

Essential fatty acids are chemically reactive to oxygen because they contain more double bonds in an equal number of fat molecules than to other fatty acids.

Consequently, ALGOMEGA NP® being rich in fatty acids may undergo oxidations.

So it is imperative to check if it is also able to protect from free radicals.

Method

The used method is the DPPH method according to Lamaison *et al.*, (1988 – Plantes Médicinales et phytothérapie 22: 231-234). The reaction is followed spectrophotometrically at 517 nm.

Results

ALGOMEGA NP® shows an anti-free radical activity with CE50 equal to 16µl/ml of medium.

➤ ALGOMEGA NP® shows efficient anti-oxidative protection.

ALGOMEGA NP® regulates the epidermal water balance

Moisturization is evaluated *in vivo* by measuring transient thermal transfer (TTT) (*cf*. Girard *et al*., 2000-Skin Research and Technology 6:205-213). Transient thermal transfer is, virtually, the property of a body exchanging heat with another body with which it is in contact.

The used apparatus named Hydrascan® has been developed by Laboratoire Dermscan. It includes a very small temperature sensor and a heating element.

A constant thermal pulse, generated by a stimulator located close to the thermal sensor, propagates through the epidermis.
At the same time, the skin temperature is precisely measured (0.1°C), any change in temperature being proportional to the tissue water content (Berardesca *et al.,* 1990 – Acta Derm. Venereol. 70:400-404).
The signal is supplied and processed by related electronic and data processing equipment. The measurement unit is mW/°C.

Three successive series of thermal pulses issued from three increasing powers provide hydration measurements from three increasing epidermal depths from the outer epidermis, the medial layers of the epidermis and the whole epidermis that is not possible with the conventional corneometry.

Method

Study performed by Laboratoire DERMSCAN (69603 Villeurbanne Cedex).

ALGOMEGA NP® is formulated at 5% in a cosmetic base (Glyceryl stearate, PEG-6 stearate and ceteareth-20).
These observations have been performed on 10 volunteers with dry skin (ages: 36 ± 4 years) with one standardised application (2µl/cm²) on forearms on a treated area and a untreated area. Measurement of hydration rate on the two areas has been made at time 1h and 3 hours in controlled temperature (24 ± 2°C) and humidity (40-60%).

Results

The changes in Day/°C (Δ) and in % on mean (Δ%) of the amount of cutaneous hydration are calculated by using the following formula.

$$\Delta = (ZT_{ti} - ZT_{t0}) - (ZN_{ti} - ZNT_{t0})$$

$$\Delta\% = \frac{(ZT_{ti} - ZT_{t0}) - (ZNT_{ti} - ZNT_{t0})}{ZT_{t0} + (ZNT_{ti} - ZNT_{t0})} \times 100$$

with :

ZT	value obtained on the treated area with the tested product.
ZNT	value obtained on the untreated area.
to	before the product application.
ti	at different times after the product application.

Statistical results (p) are relative to the Student test.

Cf. Table here after.

The variations in moisturizing rate by using the cream with 5% ALGOMEGA NP® are:

	Cinetic Times	Variations in J/°C (moy. ± SEM)	Variations in % (moy.)	Significativity
Superior Epidermal cell layers	1hour	+ 0.91 ± 0.27	+ 20%	Yes (p= 0.008)
	3 hours	+ 0.82 ± 0.26	+ 19%	Yes (p= 0.012)
Superior & median epidermal cell layers	1 hour	+ 0.85 ± 0.29	+ 16%	Yes (p= 0.017)
	3 hours	+ 0.69 ± 0.19	+ 13%	Yes (p= 0.005)
Total epidermis	1 hour	+ 0.79 ± 0.23	+ 14%	Yes (p= 0.023)
	3 hours	+ 0.55 ± 0.19	+ 10%	Yes (p= 0.017)

➤ ALGOMEGA NP® is an excellent moisture agent for the whole epidermis.

ALGOMEGA NP® decreases desquamation & improves the intercellular cohesion

The desquamation is a fundamental physiologic process intended to protect the barrier function of the skin.

In a normal and healthy skin, this regular elimination of superficial corneocytes is imperceptible. It is incessantly compensated by the divisions of the proliferative layer and the upward cellular maturation. In a dry skin, an abnormal desquamation leads to a disruption of the barrier function.

Desquamation results from a double mechanism (1) the destruction of the membranes of corneocytes and (2) the enzymatic degradation of corneosomes (Harding *et al.,* 2000 – Int. J. Cosm. Sci. 22: 21-52). Layers of corneocytes can be removed. The characteristics of the revealed part of the *stratum corneum* can then be investigated by tape-stripping and after that observed by optical or electron microscopy.

Method

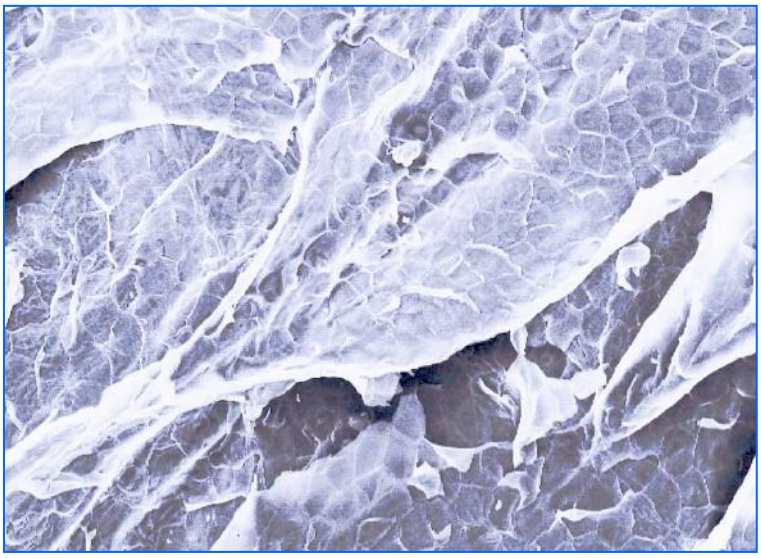
This study was performed on 3 healthy Caucasian volunteers (designated 1-2 and 3) , 3 females ranging from 55 to 60 years old. The tested products are a cream with 3% ALGOMEGA NP® and a placebo cream (without ALGOMEGA NP®) applied in one application (2 µl/ cm²) on leg.

D-squame technique consists of applying sticky membrane to the skin surface using a pressure of 20 Kpa (about 100 g) after 2 hours deposit during 5 seconds. Disks are then submitted to different steps: fixation with 2.5% glutaraldehyde in 0.1M cacodylate buffer during 1 hour, then washing and postfixation by 2% osmium tetroxide in the same buffer during 30 minutes. After dehydration and washing in hexamethyldisilazane (HMDS), cells are gold coated and observed by using a scanning electron microscope (Stereoscan Leica S 440).

Results

Results are illustrated on plates 1 to 3.

Plate 1 Volunteer 1

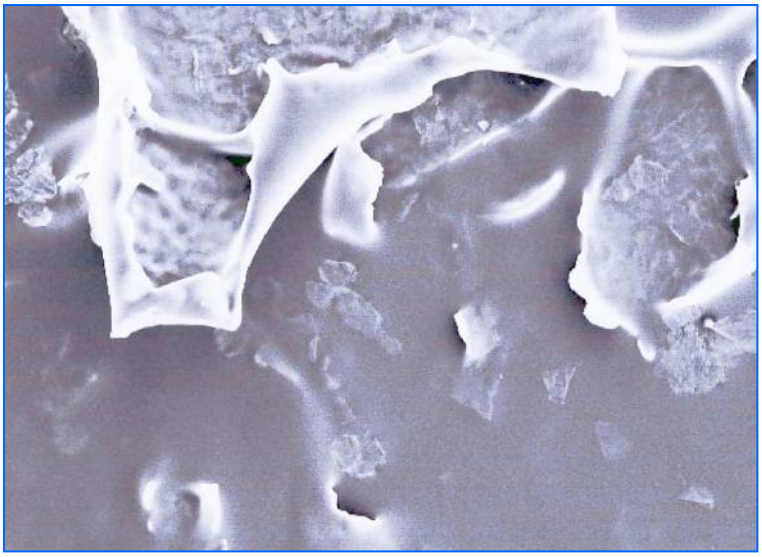


Control

- Larges and firmly agglomerated squames are present

- Isolated corneocytes are never observed.

➤*Stratum corneum* very dehydrated.

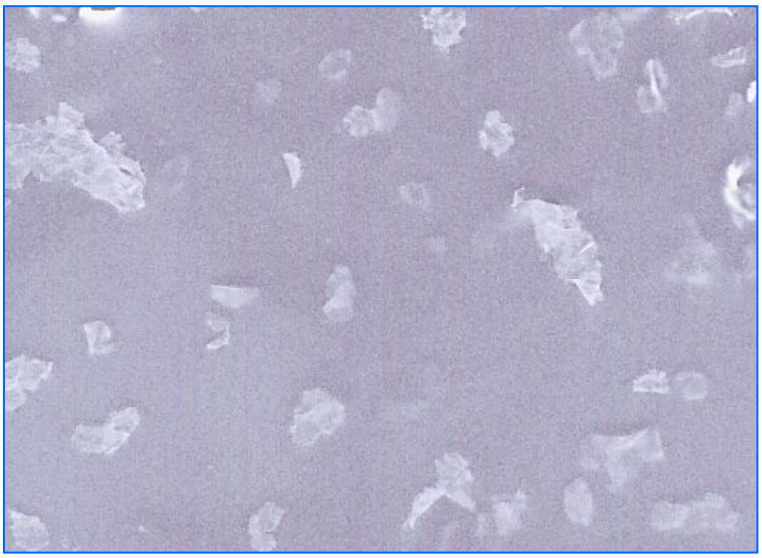


Placebo cream

- Squames appear less closely packed.

- Some isolated corneocytes are visible.

➤*Stratum corneum* with severe desquamation.



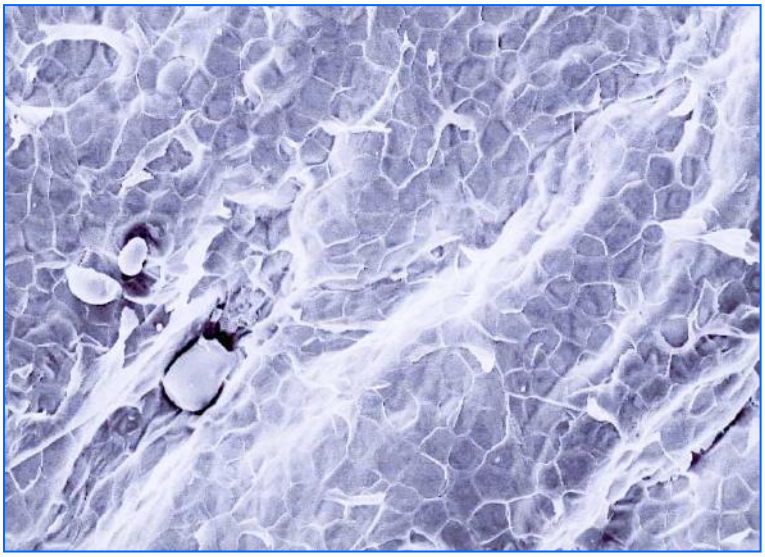
Cream with 3% ALGOMEGA NP®

- Numerous corneocytes are uniformly distributed.

- Some small cellular accumulations are apparent.

➤*Stratum corneum* nearly well hydrated.

Plate 2 Volunteer 2

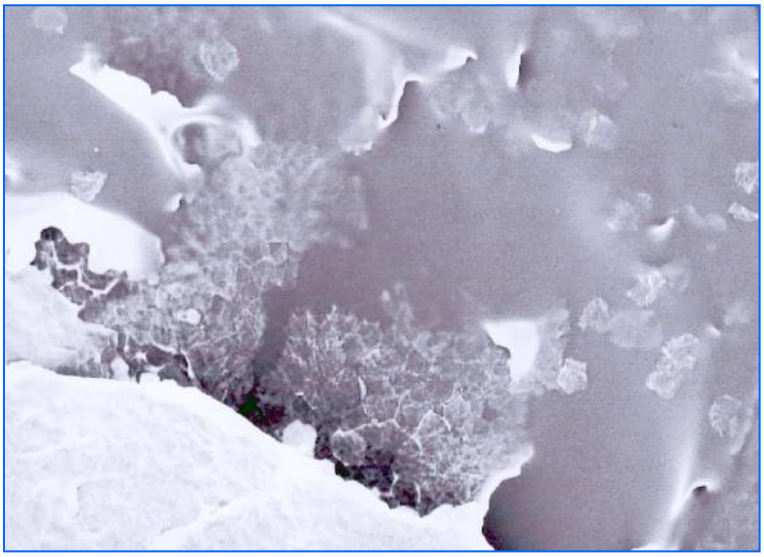


Control

- Squames appear thick , numerous and firmly agglomerated.

- Isolated corneocytes are not observed.

➤ *Stratum corneum* highly dehydrated.

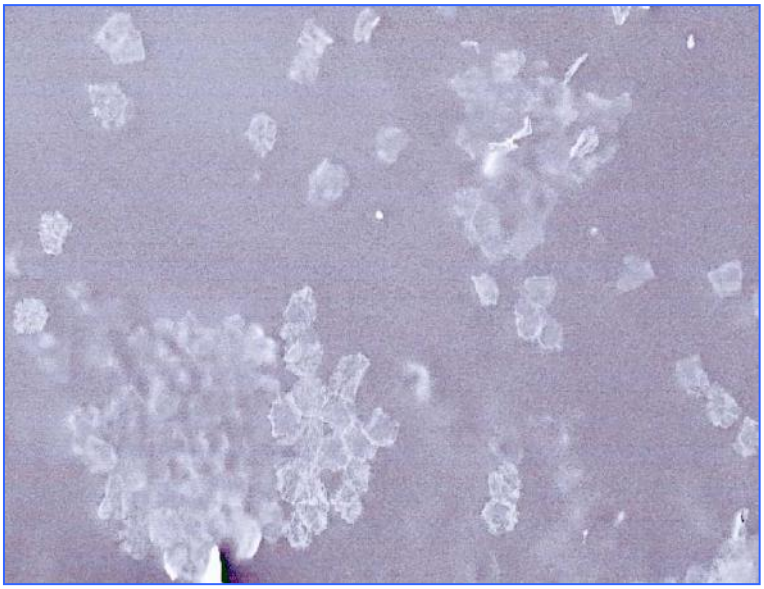


Placebo cream

- Squames seem less closely packed.

- It appears few isolated corneocytes

➤ *Stratum corneum* with still severe desquamation.



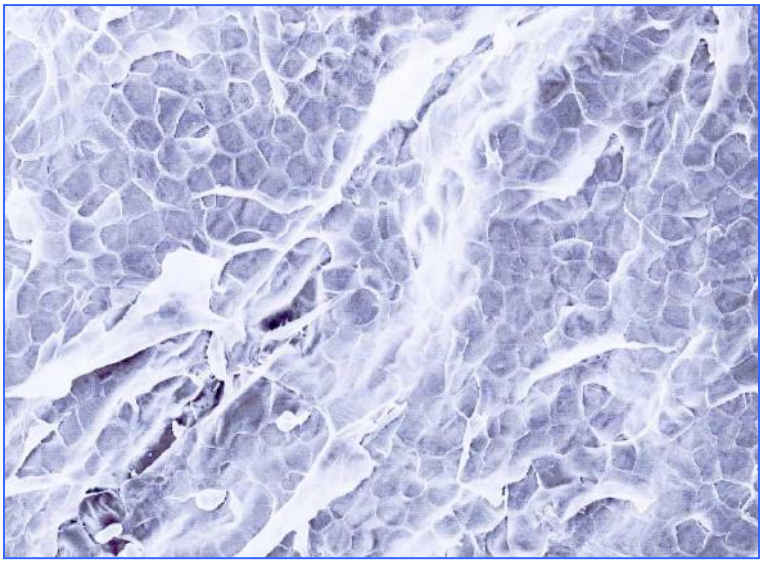
Cream with 3% ALGOMEGA NP®

- Numerous isolated corneocytes seem regularly distributed.

- It remains cellular accumulations but not very important comparatively to placebo cream.

➤ *Stratum corneum* less dehydrated.

Plate 3 Volunteer 3

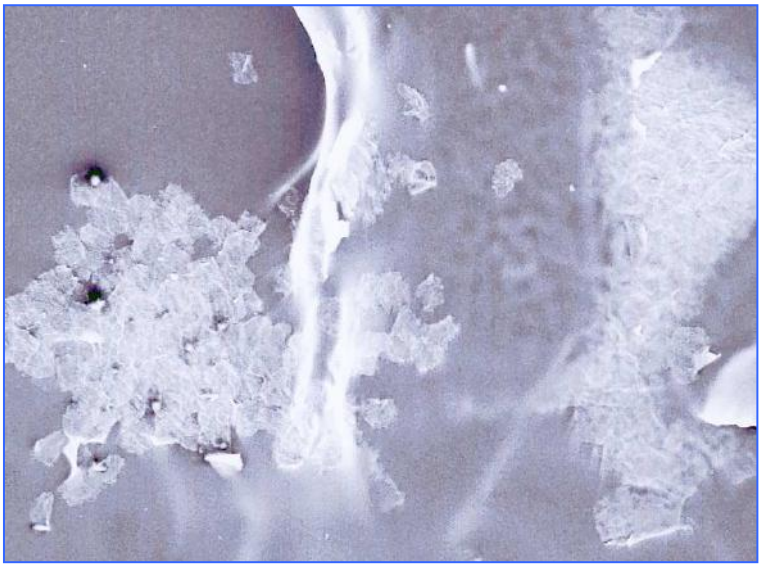


Control

- Thick, larges and compacts squames are apparent.

- Isolated corneocytes are not present.

➤ *Stratum corneum* highly dehydrated.

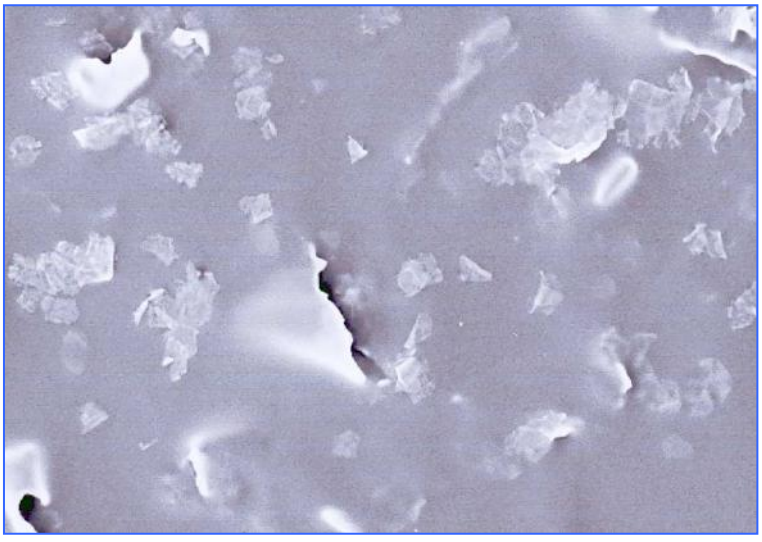


Placebo cream

- Larges squames seem less joined firmy together.

- It exists few cellular isolated agregates.

➤ *Stratum corneum* still rough.



Cream with 3% ALGOMEGA NP®

- Numerous isolated corneocytes are visible near small cellular accumulations.

➤ *Stratum corneum* slightly scaly.

➤ ALGOMEGA NP® regulates desquamation & improves the integrity of *stratum corneum.*

ALGOMEGA NP® reduces Trans Epidermal Water Loss (TEWL) & reinforces the barrier function

Alteration in the *stratum corneum* leads to the loss of the barrier function, with:

- increased transepidermal water loss,

- decreased water content and

- installation of xerosis.

Thereby, the study of the Trans epidermal Water Loss (TEWL) is a good indication of the efficiency and the integrity of the skin barrier function.

Method

Measurements were performed by Laboratory DERMSCAN (69603 Villeurbanne Cedex) using an evaporimeter which measures the amount of water loss by evaporation according to standard guidelines.

The tested cream includes Prodhy 206 base (INCI: Glyceryl stearate, PEG-6 stearate & ceteareth-20) with 5% ALGOMEGA NP®added.

Observations are performed on 12 volunteers with dry skin and about 42 ± 4 years old after one topical application (2µl/cm²) on forearms (treated and untreated areas) and with the following cinetic times : T0, T1h et T3hours. The room temperature is kept at 24 ± 2°C and the ambient air humidity ranges from 40% to 60%.

Results

The variations of water loss (Δ en $g.m^{-2}.h^{-1}$) are calculated by using the following formula :

$$\Delta = (ZT_{\tau\iota} - ZT_{\tau 0}) - (ZNT_{\tau\iota} - ZNT_{\tau 0})$$

with:

ZT	value obtained on the treated area with each tested product
ZNT	value obtained on the untreated area
τo	before the deposit of each product
τι	after the deposit of each product at different times.

Tables (here after) show statistical data: mean, minima, maxima, SEM and 95% confidence intervals (IC95%).

Values of TEWL ($g.m^{-2}.h^{-1}$)

Treated area

Vol	T0	T1H	T3H
1	9,3	11,6	8,0
2	6,2	7,5	5,2
3	3,6	2,8	2,6
4	6,2	7,7	3,7
5	5,6	4,4	4,8
6	3,9	4,8	5,9
7	5,8	5,7	5,4
8	5,7	6,1	5,7
9	5,6	6,3	4,9
10	12,4	3,4	4,6
11	2,8	2,5	2,6
12	5,7	5,5	5,2
Moyenne	**6,1**	**5,7**	**4,9**
Médiane	5,7	5,6	5,1
Minimum	*2,8*	*2,5*	*2,6*
Maximum	*12,4*	*11,6*	*8,0*
SEM	**0,7**	**0,7**	**0,4**
IC 95%	1,6	1,6	0,9

Untreated area

Vol	T0	T1H	T3H
1	10,5	5,0	6,5
2	8,2	6,4	4,9
3	3,0	2,6	2,8
4	4,6	5,0	5,1
5	5,4	4,9	4,8
6	6,6	5,3	5,8
7	4,4	5,9	4,9
8	4,9	4,2	5,6
9	3,5	3,1	4,2
10	16,7	4,1	4,1
11	3,0	3,0	2,7
12	6,4	5,4	6,9
Moyenne	**6,4**	**4,6**	**4,8**
Médiane	5,1	5,0	4,9
Minimum	*3,0*	*2,6*	*2,7*
Maximum	*16,7*	*6,4*	*6,9*
SEM	**1,1**	**0,3**	**0,4**
IC 95%	2,5	0,8	0,8

Variations of TEWL ($g.m^{-2}.h^{-1}$)

Vol	ΔT1H	ΔT3H
1	7,7	2,6
2	3,1	2,4
3	-0,5	-0,7
4	1,1	-2,9
5	-0,8	-0,3
6	2,3	2,8
7	-1,7	-0,9
8	1,1	-0,7
9	1,1	-1,3
10	3,5	4,7
11	-0,2	0,2
12	0,7	-1,0
Moyenne	**1,5**	**0,4**
Médiane	1,1	-0,5
Minimum	*-1,7*	*-2,9*
Maximum	*7,7*	*4,7*
SEM	**0,7**	**0,6**
IC 95%	1,6	1,4
p =	0,068	0,552

% of volunteers with decrease of	**33**	**58**

The reparative effect is positive for 58% volunteers, 3 hours after one application with a variation ranging from -0,3 g. $m^{-2}.h^{-1}$ to -2,9 $g.m^{-2}.h^{-1}$, that means a diminution of -38,4% of the water loss for the last value.

➤ ALGOMEGA NP® decreases TEWL, thus reduces water loss and reinforces the skin barrier.

ALGOMEGA NP® improves the structure of cytomembranes after damage of the skin barrier & restores the barrier function.

The epidermis is a stratified tissue in which the outer layer, the horny layer (*stratum corneum*) is the part which is of significance for the barrier function.

According to Elias (1983 – J. Invest. Dermatol., 80 : 44S – 49S), the 1-20µm thick horny layer is a two-component system, similar to a brick wall (brick and mortar model).

The corneocytes correspond to the bricks and the lipid multilamellar matrix of complex composition present in the intercellular spaces corresponds to the mortar.

Corneocytes are flat and hexagonal-shaped cells.

The lipids of the horny layer essentially consist of ceramides (37%), cholesterol (32%) esters of chloesterol (15%) and free fatty acids (16%) (Norlen *et al*., 1998 – J. Invest. Dermatol., 112 (1) : 72-77).

The composition of free fatty acids changes according the depth of the *stratum corneum* (Bonté *et al*., 1997 – Arch. Dermatol. Res., 289 : 78-82).

These lamellar lipids are of decisive importance for:

- the percutaneous absorption (Elias & Friend, 1975 – J. Cell Biol., 65 : 180-191 ; Smith *et al*., 1982 – J. Invest. Dermatol., 78 : 7-11),

- the intact function of the epidermal barrier and thus for the water impermeability of the skin (Imokawa & Hattori, 1985 – J. Invest. Dermatol., 83 : 282-284 ; Imokava *et al*., 1986 – J. Invest. Dermatol., 87 : 758-761 ; Grubauer *et al.,* 1989 – J. Lipid Res., 30 : 89-96 ; Bonté, 1999 – Recent Res. Devel. Lipids Res. 3 : 43-62).

However, their major function would be to regulate the hydrous flux and to constitute an occlusive barrier without major function in cell cohesion (Haftek, 2000 – In: La Biologie de la peau humaine, ed. D. Schmitt, pp. 23-29).

Their amount decreases with aging and during winter.

In electron microscopy, this lipid molecules are arranged in a highly organized multilamellar fashion with clear and dark alternate bands which correspond to lipophile and hydrophile layers (Elias, 1991 – J. Controlled Release, 15 : 199-208 ; Fartasch *et al*., 1993 – Br. J. Dermatol., 128 : 1-9 ; Lazo *et al*., 1995 – J. Invest. Dermatol., 105 : 296-300).

Various arrangements may be seen (Elias, 1983 – J.Invest. Dermatol., 80 : 44s-49s ;
Landmann, 1986 – J.Invest. Dermatol., 87 : 202-209 ; Fortunier , 1993 – Parfums Cosmétiques Arômes, 110 : 63-66).

The present study by transmission electron microscopy has been performed in order of knowing if a unique topical application of 3% ALGOMEGA NP® on reconstituted human epidermis provides better recovery of cytomembranes after damage to the barrier function by an irritant agent. The chosen irritant is DMSO which is known to induce urticaria and irritation.

Method

Several experiments are performed on reconstituted human epidermis (SkinEthic model-17 days):

- experiment n°1 : cultivation without ALGOMEGA NP® and DMSO treatment for 24 h,

- experiment n°2 : cultivation for 24 hours without ALGOMEGA NP®, DMSO treatment (10% in the culture medium for 12 h), cultivation for 24hours without ALGOMEGA NP® in the culture medium,

- experiment n°3 : cultivation for 24h without ALGOMEGA NP®, DMSO treatment (10% in the growth medium for 12 h), cultivation for 24 h with 3% ALGOMEGA NP®,

- experiment n°4 : cultivation for 24 h with 3% ALGOMEGA NP®, DMSO treatment (10% in the growth medium for 12 h.), cultivation for 24 h without ALGOMEGA NP®.

Samples were prepared for transmission electron microscope observations according to the following steps : fixation with 3% glutaraldehyde in 0.1M cacodylate buffer (pH : 7.4) for 2 h30 at 4°C, washes in buffer, post-fixation in 2% osmium tetroxide solution in the same buffer for 2 h, dehydration in graded ethanol and embedded in Spurr medium.

Ultrathin sections were performed using Reichert UltraCut E ultramicrotome, then contrasted with uranyl acetate and lead citrate solutions and finally viewed in a Zeiss EM 912 electron microscope.

Results

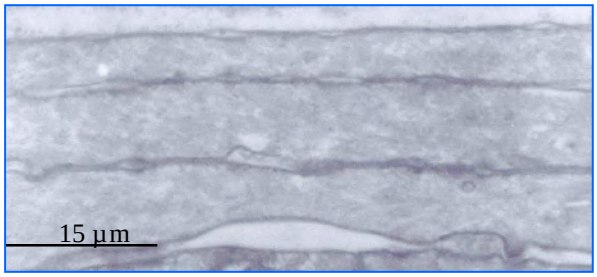
Results are illustrated on the page 22.

Electron micrographs only show superficial cell layers.

- The DMSO treatment (experiment n°2) induces important alterations of the fine structure of cytomembranes. Intercellular spaces become wider comparatively to those of the untreated samples (experiment n°1).

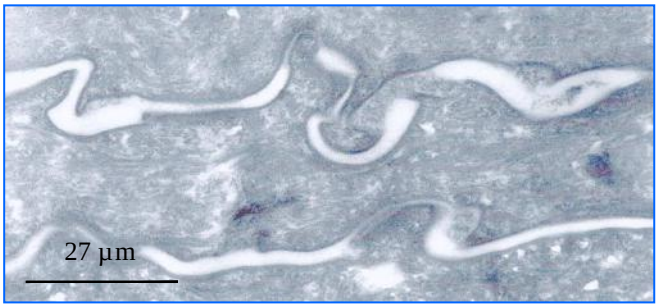
- In presence of ALGOMEGA NP® and whatever its way of introduction, the fine structure of cytomembranes appear better preserved and intercellular spaces more regular (experiments n°3 & 4).

➤ Control without DMSO treatment (*Experiment n°1)*



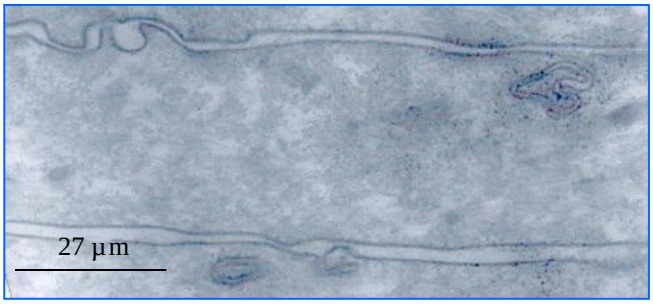
The ultrastructure of cell layers appears well preserved.

➤ Control with MSO treatment for 12 h (*Experiment n°2)*



DMSO treatment induces important damage of cell layers structure. Intercellular spaces are irregularly enlarged between corneocytes layers. The structure of cell membranes is poorly preserved.

➤ ALGOMEGA NP® 3% <u>after</u> DMSO treatment for 12 h *(Experiment n°3)*



➤ ALGOMEGA NP® 3% <u>before</u> DMSO treatment for 12 h *(Experiment n°4)*

ALGOMEGA NP® is able to preserve cell layers structure when the active is applied <u>before or after</u> DMSO treatment. The intercellular spaces between corneocytes layers appear straight and well regular.

➤ ALGOMEGA NP® offers a preventive and a curative protection of the barrier function against damage due to irritant agent.

➤ ALGOMEGA NP® improves the fine structure of epidermal cells that guarantees good functionality of the *stratum corneum.*

CONCLUSION & COSMETIC BENEFITS

ALGOMEGA NP® is a reparative moisturizer based on the synergistic combination of liposoluble fractions extracted from the association of two algae rich in omega-3 fatty acids:

- a microalga : *Nannochloropsis oculata* (Droop)Hibberd

- a macroalga : *Porphyra umbilicalis* (Linné) Kützing

within a vegetable oil : *Silybum marianum* seed oil, well-supplied with linoleic acid (omega-6 fatty acid).

Thanks to its balanced composition in unsaturated fatty acids and phytosterols, ALGOMEGA NP® helps nourish the skin, re-equilibrate lipid deficient skins and replenish skin's moisture barrier, giving it back suppleness and elasticity. So the skin can naturally defend itself against dryness.

ALGOMEGA NP® decreases desquamation and improves the intercellular cohesion.

ALGOMEGA NP® reduces Trans Epidermal Water Loss and reinforces the skin barrier function for more firmness.

ALGOMEGA NP® improves the structure of cytomembranes after damage caused by irritant agent and restores the skin barrier for optimum function.

Thus, ALGOMEGA NP® is able to relieve dryness of sensitive and reactive skins.

COSMETIC APPLICATIONS

The active ingredient ALGOMEGA NP® finds its place in a variety of cosmetic applications for caring properties of dry and sensitive skins *e.g*. creams, lotions, lip care, after sun products.

Skin care :

- Cares for dry, reactive and sensitive skins.
- Lip care.
- After sun care.

Recommended use levels: 3 - 5%.

Vérifié par ECOCERT MATIÈRE PREMIÈRE COSMÉTIQUES

Vérifié par ECOCERT MATIÈRE PREMIÈRE COSMOS APPROVED

CHINA LISTED 2014

Algae extract

ANNEX

Evaluation of ocular irritation

Predisafe test

eurofins | ATS

N° d'étude : 48284F01.DOC
Version : ..... N°1
Page : 2

RESUME DE RAPPORT D'ETUDE

EVALUATION DU POTENTIEL IRRITANT D'UN PRODUIT PAR APPLICATION DIRECTE SUR MONOCOUCHE DE FIBROBLASTES DE CORNEE DE LAPIN : *méthode du rouge neutre*

- **Produits étudiés** : ALGOMEGA NP
- **Promoteur** : GELYMA
- **Objectif de l'étude** : Evaluer le potentiel irritant du produit étudié
- **Méthodologie** : Le principe en est basé sur l'évaluation de la tolérance primaire oculaire des produits cosmétiques, par mesure de cytotoxicité ou viabilité cellulaire, après un temps de contact déterminé, sur des fibroblastes de cornée de lapin. Le rouge neutre, relargué par les cellules, est évalué par mesure de densité optique (DO).
  Cette méthode est inscrite au Journal officiel de la République française selon l'arrêté du 27 décembre 1999.
- **Dates de l'étude :** du 17 au 22 juillet 2002
- **Résultats :**

Dénominations	Réf	Code produit	Concentration testée	RESULTATS	
				IC50	Classement
ALGOMEGA NP	020411	48284	5%	**> 50%**	**Cytotoxicité négligeable**

- **Conclusion :**

➤ The product ALGOMEGA NP®, batch 020411, ref. 48284, tested diluted at 5% in a lipophile solvant shows a very good ocular tolerance.

Stéphanie RIVOIRE
Responsable tests d'innocuité et d'objectivation
StephanieRivoire@Eurofins.com

Eurofins Scientific Tests Center - Pôle Activité Aix-Les Milles - Actimart
1140, Rue Ampère - 13851 AIX-EN-PROVENCE CEDEX3
TEL +33 (0)4.42.39.78.08 - FAX +33 (0)4.42.39.77.81
N° SIRET : 32159112500059 – Code APE : 743 B

Evaluation of cutaneous irritation

Patchs tests on volunteers

eurofins | ATS

N° d'étude : 48286F01.DOC
Version : ..... N°1
Page : 2

RESUME DE RAPPORT D'ETUDE

EVALUATION DE LA TOLERANCE CUTANEE D'UN PRODUIT COSMETIQUE APRES APPLICATION UNIQUE SOUS PANSEMENT OCCLUSIF PENDANT 48H : *méthode des patchs tests*

- **Produit étudié**: ALGOMEGA NP
- **Promoteur** : GELYMA
- **Objectif de l'étude** : Appréciation de la tolérance locale cutanée du produit étudié après un test épicutané réalisé en conditions occlusives, pendant 48h.
- **Lieu de l'étude :** EUROSAFE
  6, rue du Cormier
  35760 SAINT GREGOIRE
- **Date de l'étude :** du 16 au 18 juillet 2002
- **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, type Finn Chambers

✓ ***Méthode d'évaluation*** **:**
L'évaluation clinique par le dermatologue, se fait 30 minutes après le retrait des patchs et 24 heures après la première lecture. La cotation clinique est réalisée en fonction de l'intensité des phénomènes d'irritation observés (érythème, œdème, papules, vésicules, bulles, sécheresse), et selon une échelle numérique donnée.
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 seul.

- **Population :** 14 volontaires sains ont été recrutés et sélectionnés selon des critères d'inclusion et de non inclusion.
- **Résultats :** Le score irritant moyen du produit est de 0.
- **Conclusion :**

➤ The product ALGOMEGA NP®, batch 020411, ref. 48284, tested diluted at 5% in

Eurofins Scientific Tests Center - Pôle Activité Aix-Les Milles - Actimart
1140, Rue Ampère - 13851 AIX-EN-PROVENCE CEDEX3
TEL +33 (0)4.42.39.78.08 - FAX +33 (0)4.42.39.77.81
N° SIRET : 32159112500059 – Code APE : 743 B

Evaluation of phototoxicity

In vitro test

LABORATOIRE

L E M I
Technopole Montesquieu
33650 MARTILLAC - FRANCE

Rapport final n° 2003-DSW646-2-Amendement n°1

Etude du potentiel phototoxique d'une substance sur cellules Balb/c 3T3

(Mode opératoire LEMI n° MB08/102)
(JO des Communautés Européennes L136/90 du 08.06.2000)
(Méthode B.41 de la Directive CE/2000/33

ALGOMEGA NP Lot n° 02 11 20

7. Conclusion

La substance à tester, ALGOMEGA NP Lot n° 02 11 20 (code LEMI : DSW281003), fournie par la société GELYMA, n'est pas phototoxique dans les conditions de l'expérimentation (JO des Communautés Européennes L 136/90 du 08.06.2000 – Méthode B.41 de la Directive CE/2000/33).

Martillac, le 8 décembre 2003

M.-F. HARMAND
Directeur d'étude

➤ ALGOMEGA NP ® is not phototoxic under the experimental conditions.

Reverse mutation assay on Bacteria

Determination of mutagenous activity on
Salmonella Typhimurium his et *Escherichia Coli*

LABORATOIRE

Conformité aux BPL
Selon les directives 88/320/CEE
Et OCDE C/81/30
Décision GIPC du 18 avril 2001

L E M I
Technopole Montesquieu
33650 MARTILLAC - FRANCE

Rapport final n° 2003-DSW646-1

ESSAI DE MUTATION REVERSE SUR DES BACTERIES :

Détermination de l'activité mutagène sur des bactéries « *Salmonella Typhimurium his-* » et « *Escherichia Coli* »
(OCDE n° 471)

ALGOMEGA NP Lot n° 02 11 20

En conclusion :

Le produit à tester, ALGOMEGA NP Lot n° 02 11 20 (Code LEMI : DSW281003), fourni par la société GELYMA ne présente pas d'activité mutagène vis à vis des quatre souches de *Salmonella typhimurium* (TA 1535, TA 1537, TA 98, TA 100) et d'une souche *Escherichia coli* WP2(uvr A⁻) (pKM 101), sans et avec activation métabolique, en accord avec la ligne directrice n° 471 de l'OCDE et la méthode B13/B14 de la directive 2000/32/CE.

Fait à Martillac, le 12 janvier 2004

M.-F. HARMAND
Directeur Scientifique

➤ ALGOMEGA NP ® does not show a mutagenic activity.

Parc d'Affaires Marseille Sud
1 Boulevard de l'Océan
13009 Marseille –France

www. gelyma.com

Contact

☏ (33) 4 96 14 09 82
🖷 (33) 4 96 14 09 83

e-mail gelyma @ wanadoo.fr