SUN'ALG®

Helps shield your skin
from damaging effects of sun exposure

*

Strengthens the skin's defence mechanisms

Offers high protection against both UVA - UVB radiation

Prevents the formation of sun burn cells

Fights against inflammatory skin reactions

Diminishes redness & soothes sunburns (erythema)

Gelyma

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SUMMARY

Page

Introduction 1

Algal source 3

Dunaliella salina 3
Haematococcus pluvialis 8

The active ingredient SUN'ALG® 13

Specifications 13
INCI nomenclature 13
Distinctive composition 14
Stability studies 17
Free radical scavenger potential 18
Storage 18
Safety..... 18

Effectiveness evaluation 19

SUN'ALG® strengthens the skin's defence mechanisms..... 20

SUN'ALG® offers protection against both UVA-UVB radiation 31

SUN'ALG® prevents the epidermal cells apoptosis
associated to DNA damage (formation of sun burns cells) 35

SUN'ALG® fights against inflammatory skin reactions 37

SUN'ALG® diminishes redness & soothes sunburns 39

Conclusion & Cosmetic benefits 44

Cosmetic applications 44

Annex 45

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INTRODUCTION

UV radiation appears as one of the most harmful environmental factors.

Skin UV exposure generates an excess of oxidative stress due to the formation of reactive oxygen species affecting the integrity and stability of subcellular structures, cells and tissues. This leads to multiple deleterious cutaneous effects from the painful sunburn to photoaging and at least the dangerous skin cancer.

Moreover, UV irradiation induces skin inflammation which in turn leads to the release of inflammatory cytokines that can accelerate photoaging too.

Consequently, the protection against UV-induced damage and photoaging is becoming a fundamental part of topical skin care for the prevention of skin aging. Most skin aging, up to 90%, would be due to photoaging.

One strategy for safeguarding the skin from UV radiation is the use of sunscreen to protect against both UVA and UVB and to counteract the formation of reactive oxygen species.

Many synthetic sunscreens are available in the market but they can cause possible adverse side effects such as allergic reactions or further possible free radical reactions.

So the development of other photoprotective agents with multifunctional strategies for preventing and reversing photoaging appears more significant because of the consumer's preference for natural products.

In order to limit UV-induced damage and fight signs of photoaging, GELYMA proposes SUN'ALG®, an unusual and innovative combination of natural bioactive ingredients.

SUN'ALG® combines

- *Pongamia glabra* seed oil (Karanga seed oil) that provides a primary shield against UV radiation, playing as a natural sunscreen solvant thanks to its important absorption ability especially for UVB but also for UVA
- a plankton extract that brings additional corrective efficacy against the oxidative stress .

This plankton extract results in the association of two microalgal extracts that have not been chosen by random.

- *Dunaliella salina* extract is rich in carotenoids, mainly in β-carotene
- *Haematococcus pluvialis* extract is also rich in carotenoids, chiefly in astaxanthin.

Thereby SUN'ALG® contains several kinds of carotenoids extracted from two different microalgae.

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The cooperative activities *Pongamia glabra* seed oil and microalgal extracts bring a superior and efficient protection against photooxidative damage.

The beneficial effects of SUN'ALG® have successfully been demonstrated by using the cosmetogenomic analysis completed by *in vitro*, *ex vivo* and *in vivo* studies.

Due to its unique natural ingredients combination, SUN'ALG® helps to

- provide a maximal protection against daily attacks against UV rays and
- maintain skin health and appearance by fighting against premature signs of photoaging.

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ALGAL SOURCE

The algal part of SUN'ALG® is based on the properties of the two microalgae *Dunaliella salina* & *Haematococcus pluvialis.*

Dunaliella salina

➤ Classification

Dunaliella salina belongs to

Empire	*Eukaryota*
Kingdom	*Plantae*
Phylum	*Chlorophyta*
Class	*Chlorophyceae*
Order	Chlamydomonadales
Family	*Dunaliellaceae*
Genus	*Dunaliella* Teodoresco 1905
Species	*salina* (Dunal) Teodoresco 1905.

Basionym *Protococcus salinus* Dunal.

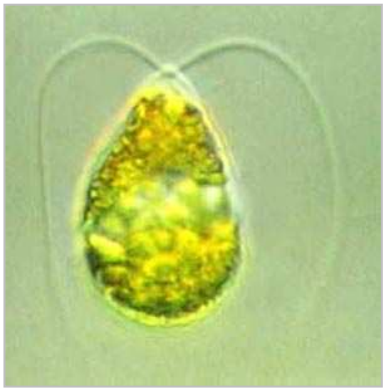
The genus *Dunaliella* includes about 28 species generally widely distributed in marine or brackish waterhabitats. Some species may be found in freshwater or slightly brackhish habitats and also in inland salterns and brine lakes. A number of varieties and forms are recognized (Borowitzka M.J. & C.J. Silva 2007- J. Applied Phycol., 19: 567-590).

➤ Morphology

Dunaliella salina is a unicellular, biflagellate and uninucleate alga without cellulosic cell walls. It is morphologically similar to *Chlamydomonas* with the main difference being the absence of a cell wall in *Dunaliella*.

Cells without rigid cell walls are enclosed solely by a thin elastic plasma membrane. As a result the cell's morphology is strongly influenced by environmental changes.

The cell size is 12-16 x 24-25 µm. Two flagella are apically inserted, equal in length, and usually exhibit a homodynamic pattern of beating. The cell contains a single, large cup-shaped chloroplast with a central pyrenoid, a nucleus, mitochondria and small vacuoles. The chloroplast accumulates large quantities of β-carotene so that the cells appear orange-red rather than green.

A  B 

Morphology of
Dunaliella salina

A - From Roscoff Station
B - From M. Borowitzka
Bio 301

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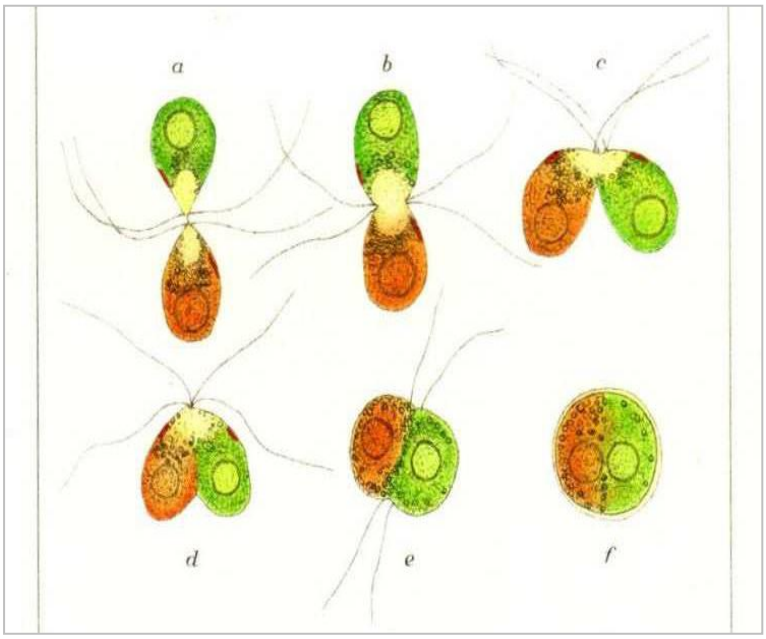
➤ Biology, ecology & geographical distribution

Dunaliella salina can reproduce asexually, sexually and through division of motile vegetative cells.

Sexual reproduction is rarely observed in cultures but more often in the field. It is isogamy.

The zygote is green or red and surrounded by a very thick, smooth wall of sporopollenin. After a resting stage, the zygote nucleus divides meiotically, forming up to 32 cells which are liberated through a rupture in the mother cell wall (Borowitzka M.A. *et al.* 1982 – Annual meeting Australasian Soc. For Phycology & Aquatic Botany: 18).



Zygote formation

From Lerche W. – 1937
Arch. F. Protidtenkd 88 :236-268.

Dunaliella salina occurs in extremely saline habitats and in seaside rock pools in which the salt concentrations significantly exceeds that of normal seawater.

It appears as one of the most environmentally tolerant eukaryotic organisms known; It can develop with a salinity range from seawater (3% NaCl) to NaCl saturation (31% NaCl), and a temperature range from <0 °C to >38 °C (Ginzburg 1987 – Adv. Bot. Res. 14: 93-183). It also shows a very wide pH tolerance ranging from pH 1 to 11.

Sexual reproduction is affected by salt concentration. Low salt concentrations of 2% to 5% induced sexual activity whereas higher salt concentration of 30% decreases sexual reproduction.

In order to survive in salt fields, *Dunaliella salina* contains high concentrations of

- ► β-carotene to protect against the intense light, and
- ► glycerol to provide protection against osmotic pressure.

Dunaliella salina is found in:

- Europe: Balearic Islands, Germany, Romania and Spain.
- Africa: Egypt and Tunisia.
- South-west Asia: Iran.
- Australia and New Zealand: New Zealand, Victoria.

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➤ Chemical composition

The *Dunaliella* cells are composed of minerals, proteins, carbohydrates, lipids, pigments and vitamins.

Carbohydrates include extra-cellular polysaccharides that protect cells from dessication. These polysaccharides studied by J.Dai (Thesis - 2008) reveal an uronic acid content and a sulphated part. It would exist four major constituent monosaccharides: galactose, glucose, xylose and fructose (Mishra A. & B. Jha 2009 –Biores. Technol., 100: 3382-3386; Mishra A. *et al.* 2011- Carbohydrate Polymers 83 (2): 852-857).

Glycerol reaches up to 10% of the dry cell weight. It includes monogalacto-glycerol, digalacto-glycerol and diacyl-glycerol.

Glycerol is produced via two metabolic processes (1) intracellular synthesis through a photosynthetic product and (2) metabolism of starch in the cell (Chen H. *et al.* 2012 – PloS ONE DOI 10.1371/journal.pone.0037578). Glycerol synthesis from starch is regulated through osmotic changes. High extracellular salt concentration drives the synthesis of glucose. Osmotic stress affects enzyme activity of several key enzymes of the glycerol metabolic pathway *e.g.* glycerol-3-phosphate dehydrogenase, glycerol-3- phosphate phosphatase, dihydroxyacetone reductase and dihydroxyacetone kinase. These enzymes regulate glycerol requirements of the cell by responding to osmotic stresses.

Lipids reach 6-18% of the dry cell weight (depending to the habitat).

The fatty acids include palmitic acid, 3 – trance acid hexadecanoic, linoleic acid and arachidic acid. This composition depends on cultivation conditions of *Dunaliella salina* (Al-Hasan *et al.* 1987- J. Gen. Microbiol., 133: 2607-2616).

Dunaliella salina contains about 10% DW of carotenoids but this concentration depends on the effects of salinity. It increases with increasing salinity.

The carotenoids are in the form of droplets (plastoglobuli) located at the chloroplast periphery. They consist of a mixture of the cis- and trans-isomers of β-carotene (Ben-Amotz A. *et al*. 1988 – Plant Physiol. 86:1286-1291).

A typical composition of *Dunaliella salina* (in % total beta carotene) is

15-cis-β-carotene	10
9-cis-β-carotene	41
All-trans-β-carotene	42
Other isomers	6

(Ben-Amotz A. *et al*. 1982 – J Phycol. 18: 529-537).

This differs from the β-carotene produced from carrots and from synthetic β-carotene.

In *Dunaliella salina*, the β-carotene seems to act as photo-protective sunscreen protecting the chlorophyll and the cell DNA from the high irradiance (Ben –Amotz A. *et al*. 1989 - Plant Physiol. 91: 1040-1043).
It would be also possible that the β-carotene acts as a "carbon sink" to store the excess carbon produced during photosynthesis under conditions when growth is limited whereas photosynthetic carbon fixation must continue (*cf*. Boromitzka, M.A. – FAO document).

In addition to high level of β-carotene, *Dunaliella salina* contains

- thiamine, pyridoxine, riboflavin, nicotinic acid, biotin and tocopherol (vitamin E).
- sterols, especially ergosterol. Their content (0.89% DW) decrease with increasing salinities (Francaville M. *et al*. 2010- Biosource Technol., 101:4144-4150).

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➤ Bioactivities & utilizations

Due to its important source of β-carotene and glycerol, *Dunaliella salina* offers opportunity for commercial production because it remains still the richest and best natural source of β-carotene.

Chidambara K.N. & G.A. Ravishanter (2006- in Recent Advances on Applied aspects of Indian Marine Algae) have listed the different sources of carotenoids that prove easily the advantages of *Dunaliella*.

Source	Amount present (mg/100 gms of edible substance)
Apricots	1.6
Asparagus	0.5
Broccoli	1.5
Carrot	6.6
Chicory	2.0
Cress	5.6
Kale	5.3
Mango	2.9
Melons	2.0
Spinach	4.9
Sweet potato	5.9
Dunaliella	up to 5000

Advantages of carotenoids production from *Dunaliella*.

- The algae can be cultivated easily and quickly when compared to plants.
- Produces very high quantity compared to other sources 3.0 -5.0 % w/w on dry weight basis (Jankhe 1999).
- Have both *Cis* and *Trans* isomers of carotenoids for high bioavailability and bioefficacy (Yeum and Russel 2002).

The β-carotene is used in the

- food industry (Dufosse L. *et al*. 205- Trends Food Sci. Technol. 16:389-409)
- cosmetic and pharmaceutical industries (*cf.* table here after from Chidambara K.N. & G.A. Ravishanter - 2006- in Recent Advances on Applied aspects of Indian Marine Algae).

Research work	Referance
β- Carotene regulated gene expression in human endothelial cells	Anna Polus *et al.* 2004.
β- Carotene helps in Asthma	Moreira *et al.*2004.
Acts as prooxidants in human cell line	Paola Palozza *et al.* 2003.
β- Carotene helps in stress conditions in human	Brigitte *et al.* 2003.
Oxidized β - carotene helps in cancer prevention	Shu-Lan Yeh and Miao-Lin Hu . 2003.
β- carotene decreases leukocyte superoxide dismutase activity and serum glutathione peroxidase concentration in humans	Carla *et al.* 2003
β- carotene helps to prevent systemic inflammatory response and increased lipid peroxidation	Quasim *et al.* 2003.
β- carotene acts as hepatoprotective in rats.	Manda and Bhatia 2003.
β- carotene hepl in malignant pleural mesothelioma	Salih Emri *et al.* 2003.
β- carotene along with other vitamins helps in prevention of Cardiovascular disease	Deepak *et al.* 2003.
β- carotene helps in modulating the signals in apoptosis	Paola Palozza *et al.* 2004.
β- carotene protects human skin from UV damage	Hans *et al.* 2001

Consequently, *Dunaliella salina* extract appears very interesting for cosmetic purposes.

A powder of *Dunaliella salina* associated with Dead Sea minerals has shown anti-wrinkle activities (Ma'Or Z. *et al.* 2000 – J. Cosm. Sci., 51: 27-36).

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The interest of this microalga for commercial production also concerns its nutrient profiles and biofuel potentialities (Patent WO2014/003530A1).

The first pilot *Dunaliella* cultivation as a commercial source of β-carotene has been established in the USSR (Massyuk, N.P., 1966 - Ukr. Bot.Zh.23: 12-19) and later as a source of glycerol (Ben-Amotz A. *et al*. 1982 – Experiential 38:49-52).

Nowadays the β-carotene is produced from *Dunaliella* on a commercial scale in Australia, the USA and Israel. Other cultures are also made in other countries such as Egypt.

The two largest producers in the world are Western Biotechnology Ltd (Perth, Western Australia) and Betatene Ltd (Melbourne, Victoria) in Australia.

The extreme environment in which this microalga grows makes the open-air large scale culture much easier compared to other unicellular algae.

The large open ponds used for the culture of *Dunaliella salina* at Hutt Lagoon, Western Australia, by Cognis Nutrition and Health. The largest ponds are about 250 ha in area.

According to FAO document (from M. Borowitzka), the productivities of β-carotene would be of 30–40 g dry weight m^{-2} day^{-1} for large-scale outdoor cultures. However, many of the commercial production systems do not regularly achieve productivities of even 30 g dry weight.m^{-2}.day^{-1}. In fact, productivities are often substantially less.

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Haematococcus pluvialis

➤ Classification

Haematococcus pluvialis belongs to

Empire	*Eukaryota*
Kingdom	*Plantae*
Phylum	*Chlorophyta*
Class	*Chlorophyceae*
Order	Chlamydomonadales
Family	*Haematococcaceae*
Genus	*Haematococcus* Flotow 1844
Species	*pluvialis* Flotow 1844

Heterotypic synomyms

Sphaerella lacustris (Girod-Chantrans) Wittrock
Sphaerella pluvialis (Flotow) Wittrock
Volvox lacustris Girod- Chantrans 1802
Haematococcus pluvialis (Girod-Chantrans) Roststafinski 1875

The genus *Haematococcus* includes five recognized species all found in freshwater habitats.

➤ Morphology

Haematococcus pluvialis is a unicellular alga, more or less oval in shape within a large spherical sheath (Top figure).

Two equal-length flagella arise from the anterior end.

Its protoplast is connected to the wall by fine radiating strands of protoplasm.

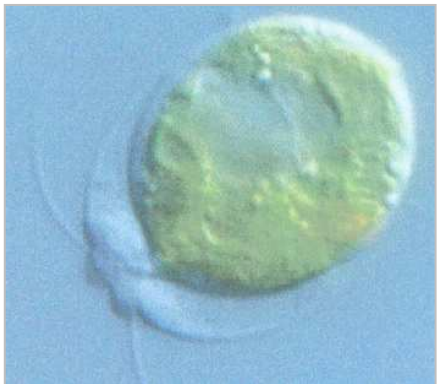
The chloroplast is principally cup-shaped. It contains one or several pyrenoids and a large stigma.

Many contractile vacuoles are present.

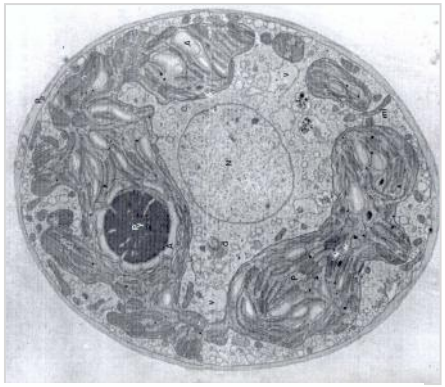
The morphology and reproduction of *Haematococcus pluvialis* are fairly similar to those to *Chlamydomonas* species.

The fine structure of a vegetative cell of *Haematococcus pluvialis* is presented on the bottom figure. A large dark pyrenoid is clearly apparent.

This green stage is obtained under optimal nutritional cultured conditions. Under some environmental conditions, this green micro-alga change progressively in red cells thanks to emergence of secondary carotenoids, astaxanthin. Consequently, the cultures of green cells change progressively in cultures of red cells.



Morphology of green
Haematococcus pluvialis



Fine structure of
a green cell. Photo GELYMA

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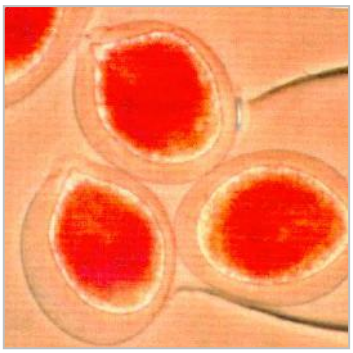
The cell volume increases. Large quantities of secondary carotenoids, mainly astaxanthin appear in the cytosol.

This synthesis is not at the expense of the pigments already present in the green stage.

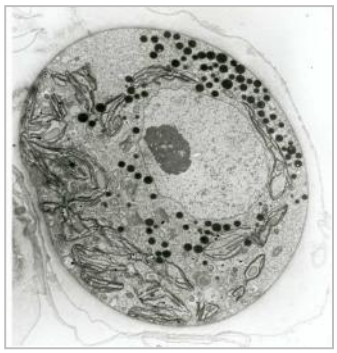
Astaxanthin is accumulated mostly in an esterified form and clearly apparent by electron microscopy in extraplastidic osmiophilic globules, distributed in the hyaloplasm.

Like that of most flagellates, the resting stage of *Haematococcus pluvialis* is characterized by the production of cysts which also contain astaxanthin globules.

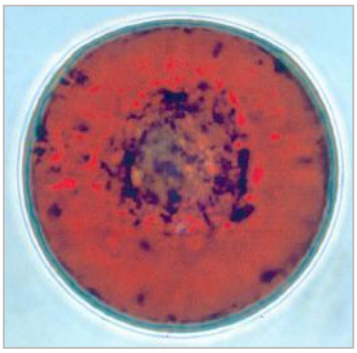
The specific rate of astaxanthin accumulation is a function of the photon flux density cultures are exposed (Lee, Y.R. & C.W. Soh, 1991 – J. Phycol., 2: 575-577).



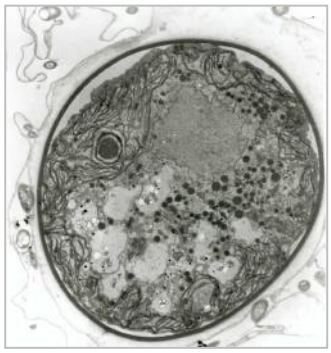
Aspect of red cells in light microscopy



Fine structure of a red cell. Photo GELYMA.



Aspect of a red cyst in light microscopy

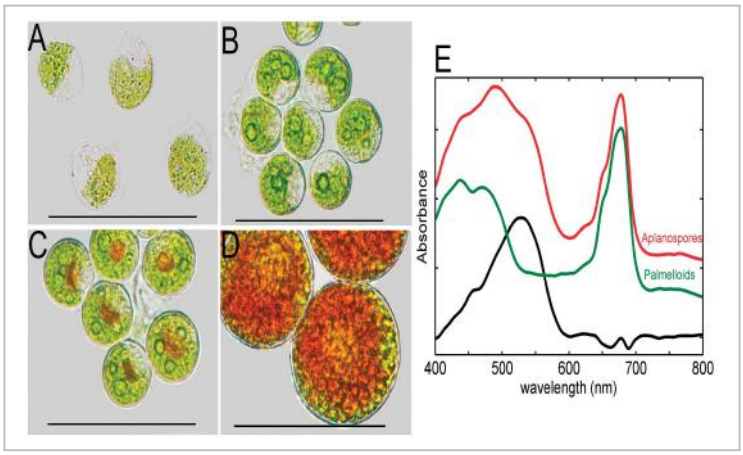


Fine structure of a red cyst. Photo GELYMA

In *Haematococcus pluvialis*, astaxanthin is associated with other pigments, especially β-carotene and chlorophyll.

A recent study describes the distribution of the different pigments in *Haematococcus pluvialis* in various stages of the life cycle (Collins A.M. *et al*. 2011 – PloS ONE 6(9) e24302). Chlorophyll is only found in the chloroplast whereas astaxanthin is identified within globular regions of the cytoplasm. β-carotene is mainly located in the chloroplast whereas astaxanth in the cytosol. These observations imply that β–carotene is a precursor for astaxanthin and the synthesis of astaxanthin occurs outside the chloroplast.

The figure here after shows *Haematococcus* in various growth stages. Transmission light microscopy verified that *Haematococcus pluvialis* cells were cultured as green bi-flagellates (Fig. A) or palmelloids (Fig. B) and induced to accumulate astaxanthin by stressing cells with nitrate-limitation and high-light irradiance.



After 24 hours, red globular regions appeared towards the center of palmelloid cells (Fig. C), and after 5 days of the same treatment, cells had formed aplanospores, appearing mostly red and had significantly enlarged (Fig. D).

The difference between the absorption spectra of green palmelloid cells and red aplanospores (Fig. E) shows a broad band centered at 528 nm that represents the in vivo absorption of astaxanthin and closely resembles previously reported spectra.

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➤ Biology, ecology & geographical distribution

Sexual reproduction of *Haematococcus pluvialis* is isogamous.

Asexual reproduction is made by division of vegetative cells into 4-8 zoospores.

The *Haemaococcus* genus is cosmopolitan. It is reported from all continents except Antarctica.

The species *pluvialis* is found in freshwater habitats and widely distributed in Australia, New Zealand, Artic, Europe, South America, Asia, Pacific Islands and Africa.

➤ Chemical composition

The contents of typical common components of *Haematococcus pluvialis* are listed here after (% DW):

Ash	11.07 - 24.74
Protein	17.30 - 27.16
Carbohydrates	36.9 - 40.0
Lipids	7.14 - 21.22

All amino acids are present with high contents in:

Aspartic acid	1.37 - 2.31
Glutamic acid	1.70 - 2.39
Leucine	1.21 - 1.84

Minerals:

Magnesium	0.85 - 1.4
Iron	0.14 - 1.0
Calcium	0.93 - 3.3.

Numerous vitamins are also present *e.g*. vitamins B1-B2-B6-B12-C-E, biotin and niacin.

The total lipid content of control cells equals 15.61% DW and depends of cultivation conditions.

The fatty acid profile appears similar under all cultivation conditions. The main components consist of palmitic, stearic, oleic, linoleic, linolenic acids but the percentage of fatty acids varies according to culture conditions (% total lipids):

saturated fatty acids	27.81 - 34.8.
mono-unsaturated fatty acid	20.07- 19.91
poly-unsaturated fatty acids	47.23 - 43.15.

These contents prove the potential of this microalga as a biodiesel feedstock (Daminani M.C. *et al*. 2010- Biores. Technol. 101: 3801-3807).

Shengzhao Dong, Yi *et al*. 2014 (Hindawi Publishing Corporation - Scientific World Journal Article ID 694305) have compared different methods of extraction in order to evaluate in *Haematococcus pluvialis* the highest oil yield (33.3 ± 1.1%) and astaxanthin content (19.8 ± 1.1%). The major fatty acid components were oleic acid (13 – 35%), linoleic acid (37 – 43%), linolenic acid (20 – 31%), and total saturated acid (17–28%).

The content in pigments of *Haematococcus pluvialis* varies according to the life cycle. The carotenoid fraction of green vegetative cells consists of mostly lutein (75-80%) and β-carotene (10-20%). In red cysts, the predocimant carotenoid is astaxantin (Renstrom B. *et al*. 1981- Phytochem. 20 (11) 2561-2564).

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The accumulation of astaxanthin also depends on different stress conditions such as

- nutrient deprivation (Kakizono, T. *et al*. 1992 – J. Ferment. Bioengin. 74: 403-405),
- increased salinity (Li, Y. *et al*. 2008- J. Plant Physiol.165:1783-1797; Sarada, R. *et al*. 2002- Process Biochemistry 37: 623-627),
- high irradiance (Kobayashi M. *et al*. 1992- J. Ferment. Bioengin.74:61-63 ; Li, Y. *et al*. 2010 – J. Applied Phycol., 22:253-263) and
- low/high temperature (Tjahjono A.E. *et al*. 1994 – Biotechnology Letters 16:133-138)

as well as combinations of these stresses (Lemoine Y. & B. Schoefs 2010- Photosynthesis Research 106: 155-177).

➤ Bioactivities & utilizations

Haemaococcus pluvialis is exploited for astaxanthin as additives.

Astaxanthin is exempt from certification under the US 21 CFR part 73.35 as a color additive in fish feed. The algal meal is approved by the Food and Drug Administration as a color additive for aquaculture feeds. This meal has also been approved in Japan as a natural food color and as a pigment for fish feeds.

For cosmetic purposes, it exists numerous data and patents proving the efficacy of *Haematococcus* extract and /or astaxanthin

- either in topical application (Arakane K. 2002 - Carotenoid Science 5: 21–24 ; Seki T. *et al*. 2001- Fragrance J. 12: 98–103; Tominaga K. *et al*. 2009 - Food Style 21 13: 84–86. ; Yamashita E. 1995- Fragrance J. 14: 180–185 ; Seki T. *et al*. 2001 Fragrance J. 12:98-103.
- or by oral supplement (Yamashita E. 2002 -. Food Style 21 6: 112–117; Yamashita E. 2006- Carotenoid Science 10: 91–95).

Different functions of astaxanthin in *Haematococcus pluvialis* have been proposed *e.g.*

- acting as a sunshade (Hagen, C. *et al.* 1993- *Plant Cell Environ.* 16: 991-995).
- protecting from photodynamic damage,or minimizing the oxidation of storage lipids (Sun, Z. *et a*l. 1998 -. Proc. Natl. Acad. Sci. USA 95**:** 11482-11488.

An algal extract with 14% astaxanthin would reduce DNA damage and maintain cellular antioxidant status in UVA-irradiated cells (Lyons N.M. & N.M. O'Brien 2002- J. Dermatol. Science 30:73-84).

There is growing commercial interest in the biotechnological production of astaxanthin, due to its antioxidative properties and the increasing amounts needed as a supplement in the aquaculture of salmonoids and other seafood (Lorenz, R. T. & G. R. Cysewski. 2000. *Trends Biotechnol.* 18: 160-167).

However, efficient astaxanthin production by *H. pluvialis* is difficult because of the low cell growth rate, sensitivity of the cells to high hydrodynamic stress and changes in cell morphology under various environmental conditions.

- Under good growth conditions, most cells remain in the vegetative form (zoospore) and accumulate little or no astaxanthin.
- Under stress conditions, the cells change to thick-walled immotile spores (cyst) and, if properly illuminated, accumulate high concentrations of astaxanthin.

Thus, the optimal conditions for vegetative cell growth differ from those under which astaxanthin is synthesized. Separation of cell growth phase from astaxanthin production phase is therefore necessary to

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obtain fairly high cell concentration with high astaxanthin content (Hata N. *et al*. 2001- Journal of Applied Phycology 13**:** 395–402).

Commercial production of astaxanthin from *Haematococcus* has been reported by Cyanotech Corporation and Aquasearch Inc both using a two steps culture:

-the first stage is done photoautotrophically under controlled culture condition in either tubular, bubble column or airlift photobioreactors

- the reddening stage is done in open cultivation ponds.

Aerial shot of Cyanotech facility (from Algae Industries Magazine's 06-2012).

The Japanese company Fuji Chemicals operates as indoor facility in Sweden and in "dome-shaped" bioreactors in Hawaii.

Algatech in Israel uses tubular solar-powered photobioreactors for both the "Green" and "Red" stages in closed, strictly controlled systems from the microalga *Haematococcus pluvialis* according to its patented biocontrolled growing process, exploiting the area's high solar radiation year-round.

General view of Algatechnologie's production plant in the heart of the Negev desert in Israel (from California Academy of Health 05-2014).

Numerous patents describe methods for improving the culture conditions of *Haematococcus pluvialis* (Patents EP1 681 060A1-WO 97/28274; WO 98/00559; WO 03/027267) or for generating mutant strains of this species (EP 1 845 927 A-WO 2008/141757).

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THE ACTIVE INGREDIENT SUN'ALG®

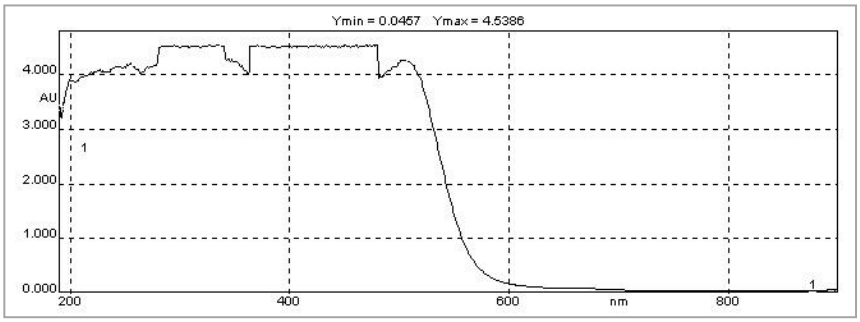
Specifications

on a control batch

- Appearance : limpid liquid, dark red colored

- odour : typical

- UV spectrum



-Density : < 1

- Solubility : soluble in oils

-Microbiology : bacteria : < 100 germs / ml
: yeasts, moulds : < 100 germs / ml
: pathogens : free

- Contents in iodine and heavy metals *(mg/Kg - data from a control batch) (In Vivo Labs – France)*

Iodine	0.09 mg/100ml
Arsenic	71 µg/kg
Cadmium	< 5
Lead	< 30
Mercury	< 2

INCI nomenclature

Assigned INCI name: *Pongamia Glabra* Seed Oil (and) (*Dunaliella Salina/Haematococcus Pluvialis*) Extract.

INCI names	CAS n°	EINECS n°	China IECI listed		Amounts (%)
Pongamia glabra seed oil	-	-	06835	无毛水黄皮（PONGAMIA GLABRA）籽油	96
Dunaliella salina extract	-	-	07377	盐生杜氏藻（DUNALIELLA SALINA）提取物	2
Haematococcus pluvialis extract	-	-	08210	雨生红球藻（HAEMATOCOCCUS PLUVIALIS）提取物	2

Preservative free.

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Distinctive composition

SUN'ALG® combines

- *Pongamia glabra* seed oil (Karanga seed oil)
- a plankton extract made from two different microalgae
  Dunaliella salina
  Haematococcus pluvialis.

Each component complements one another in skin protection.

Benefits provided by *Pongamia glabra* seed oil

Pongamia glabra (synonym *Pongamia pinnata*) is popularly known as "Karanja oil " in Hindi and "Pungai oil" in Tamil. It is a glabrous tree belonging to Leguminosae and found throughout India. It is also distributed in South Eastern Asia and Australia.

In the ayurvedic literature of India, its medicinal properties are well known for the treatment of clinical skin lesions and various skin diseases (*cf*. Balakrishnan & Narayanaswamy 2011 – Int. J. Research of Cosmetic Science 1 (1) 1-12).
Recently its efficient anticancer potential has been proven (Arulvasu *et al*. 2012 – Int. Research J. of Pharmacy 3(8) 131-134).

The cosmetic and biocidal activities would be linked to its special composition in bioflavonoids and fatty acids.

The sunscreen activities of this oil are found to be highly effective in UVB region and moderately effective in UVA region but vary with the nature of solvents used. Thereby *Pongamia glabra* seed oil can be used to formulate sunscreen preparations.

The seed oil used for SUN'ALG® (deodorised grade) shows a SPF Vivo = 20 (*cf.* document COSMECOIL KD–bio 100% - COSMACT-France).

Benefits provided by combined microalgae extracts

The association of two microalgal extracts, rich in carotenoides, has been carefully chosen:

- *Dunaliella salina* extract mainly contains β-carotene,
- *Haematococcus pluvialis* extract chiefly contains astaxanthin.

Thereby SUN'ALG® contains several kinds of carotenoids extracted from two different microalgae.

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➤ Back-ground of the interest of carotenoids

Carotenoids are lipophilic molecules belonging to the category of tetraterpenoids. Structurally they take the form of a polyene hydrocarbon chain which is sometimes terminated by rings and may / may not have additional oxygen atoms attached.

- β-carotene is an unoxygenated (oxygen free) carotenoid
- astaxanthin is an oxygenated carotenoid.

Carotenoids are known to be efficient antioxidants, scavenging singlet oxygen and peroxyl radicals by acting as deactivators of electronically excited sensitizer molecules which are involved in the generation of radicals and singlet oxygen (Truscott T.G., 1990- J. Phytochem. Photobiol. B Biol 6:359-371; Young A.J. & G.M. Lowe, 2001- Arch. Biochrm. Biophys. 385: 20-27H; Beutner S. *et al.* 2001- J. Sci. Food Agric.81 :559-568 ; Stahl W. & H. Sies, 2002- Ski Pharmacol. Appli Skin Physiol. 15 (5):291-296).

The quenching activity of carotenoids would be closely related to the number of conjugated double bonds present in the molecules (Conn P.F. *et al.* 1991- J. Photochem. Photobiol. B Biol. 11:41-47; Schmidt R., 2004- J. Phys. Chem. 5509-5513).

Carotenoids tend to accumulate in lipophilic compartments like membranes or lipoproteins. Their lipophilicity influences their absorption, transport and excretion in the organisms (Stahl W. *et al*. 1993- J. Nutri. 123:847-851; Stahl W. & H. Sies, 2003- Mol. Aspects Med. 24:345-335).

Due to their unique structure, carotenoids posses specific tasks in the antioxidant network such as protecting lipophilic compartments or scavenging reactive species generated in photooxidative processes. These biological benefits would be attributed to specific physico-chemical interactions with membranes (Mc Nultry H.P. *et al*. 2007- Biochim. Biophys. Acta 1768:167-174).

It is well proved that in mixture carotenoids are more effective than single compound for increasing photoprotection as well in topical or in oral application thanks to their synergistic interactions (Böhm F. *et al*. 1998- J. Photochem. Photobiol. B Biol l44:211-215; Pandel R. *et al*. 2013 – ISRN Dermatology ID 930164; Godec A. *et al*., 2014 – Oxidative Medecine and Cellular Longevity ID 860479). This superior protection may be related to the specific positioning of different carotenoids in membranes.

➤ Carotenoids present in SUN'ALG®

Different kinds of carotenoids are present in SUN'ALG®, especially

⇨ β-carotene brought by *Dunaliella salina* extract.

⇨ astaxanthin brought by *Haematococcus pluvialis* extract.

⇨ The β-carotene present in *Dunaliella salina* consists of several isomers with the two main isomers 9-cis and all-trans, showing different functions:

The 9-cis isomer is known as one of the most powerful antioxidant.
The all-trans isomer is a major pro-vitamin A nutrient but with a little antioxidant activity.

Moreover, these isomers are mixed with other carotenoids.

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⇨ *Haematococcus pluvialis* also contains different carotenoids. In the red cysts used for SUN'ALG® preparation, the major pigment is astaxanthin that would be synthetized through the isoprenoid pathway that proceeds through phytoene, lycopene, β-carotene and canthaxanthin before the last oxidative steps to astaxanthin.

Astaxanthin is known as a powerful antioxidant. Astaxanthin has 100-500 times the antioxidant capacity of Vitamin E and 10 times the antioxidant capacity of β-carotene. It would be a stronger antioxidant than lutein, lycopene and tocotrienols.

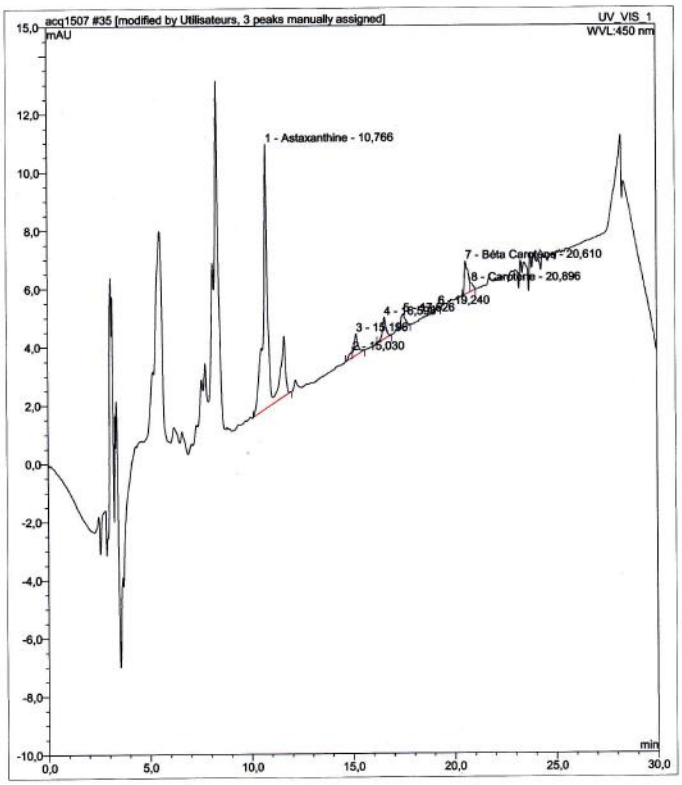
These antioxidant properties would be linked to the unique role of astaxanthin for protecting the cell membrane. This molecule would be able to scavenge radicals both at the surface and in the interior of the phospholipid membrane (Goto S. *et al*. 2001- Biochim Biophys Acta 1512:251-258).

The study of carotenoids present in SUN'ALG® has been performed by HPLC dosage and visible spectrophotometry (*Analysis by Laboratoires AGROBIO – Qualtech group – France).*

SUN'ALG® contains different percentages of carotenoids

Beta carotene	0.34 mg/100 g SUN'ALG®
Free astaxanthin	1.96 mg/100 g SUN'ALG®
Others carotenes	0.27 mg/100 g SUN'ALG®

Profile – Overlay of SUN'ALG® from Integration View is illustrated here after.



This graph confirms the presence of carotenoids in SUN'ALG®.

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Stability studies

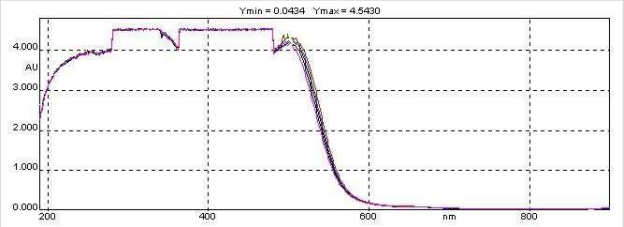
Stability studies have been performed. The graphs here after show results,

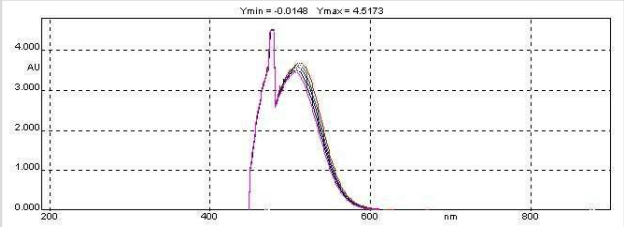
on the left relative to SUN'ALG®

on the right relative to the carotenoid fraction present in SUN'ALG®.

Stability to temperature at 80°C

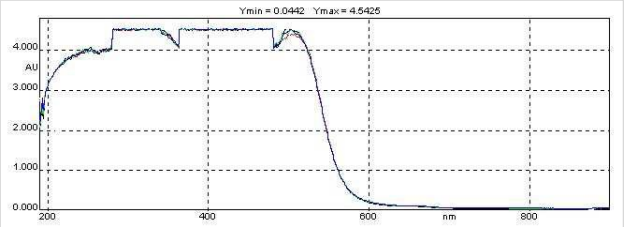
SUN'ALG® at T0 -T 1h - T 2h - T 4h - T 6h

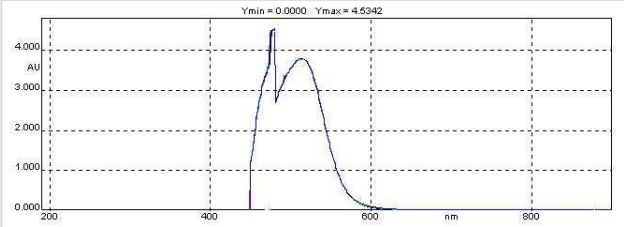




Stability to UVA

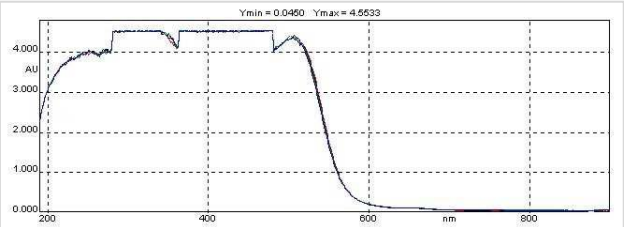
SUN'ALG® under 5 J/cm² - 10 J/cm² - 20 J/cm²

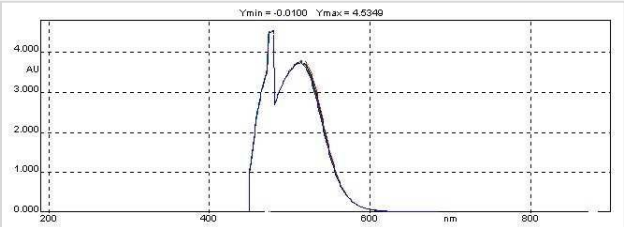




Stability to UVB

SUN'ALG® under 100mJ/cm² - 150mJ/cm² - 200mJ/cm²





The graphs above show that SUN'ALG® does not lose its protective capacities against different kinds of stress *e.g.* temperature, UVA and UVB stresses, under the present experimental conditions.

SUN'ALG® offers a photoprotective composition, stable to temperature and UV irradiation.

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Free radical scavenger potential

The DPPH (2,2-diphenyl-1-picrylhydrazyl) assay has been used for determination of the free radical scavenging properties of SUN'ALG®. It is a stable, free violet-colored radical which is modified in its leuco compound by the substances that trap the free radicals (: scavenger effect).

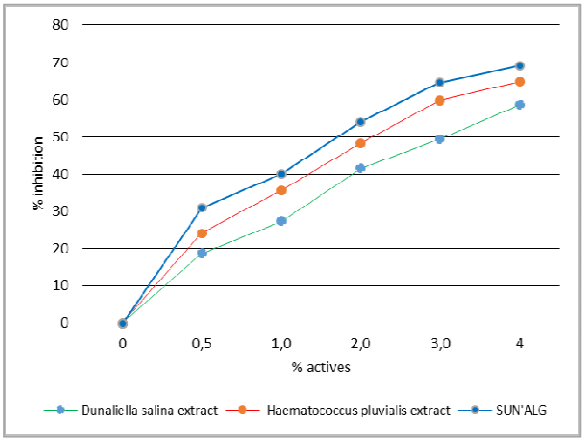
Method

A solution of the purple coloured DPPH-radical was mixed with the test active. The decrease of the absorption was determined photometrically.

Three extracts have been tested: SUN'ALG®, *Haematococcus pluvialis* extract, *Dunaliella salina* extract. These two last extracts contained the same quantity of microalgal biomass than in SUN'ALG® and the same solvent.

Results

Results are given in % of inhibition in relation to control with no active ingredient (average of 3 assays).



IC 50 % inhibition

- *Haematococcus pluvialis* extract 2.08 %
- *Dunaliella salina* extract 2.38 %
- SUN'ALG® 1.85 %

SUN'ALG® shows
the best free radical scavenger potential.

Storage

SUN'ALG® should be stored in the original sealed drums, under clean conditions between 15 to 25°C. In order to avoid microbial secondary contamination, it is recommended to use the whole content of the drum once opened. If stored under the recommended conditions, SUN'ALG® remains stable for at least 18 months.

Pack size: 1kg - 5kg -10 kg.

Safety

No animal experimentation

Standard safety testing proves that SUN'ALG® is safe for cosmetic use.

- Ocular irritation : slightly irritant (Het Cam test)
- Cutaneous irritation : very slightly irritant (Human patch test).
- phototoxicity : no phototoxic (on volunteers).

cf. annex pp 45-47.

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EFFECTIVENESS EVALUATION

SUN'ALG®

A unique combination of natural ingredients for powerful skin protection against UV-induced damage

Cosmetogenomic analysis

SUN'ALG® strengthens the skin's defence mechanisms →
- Gene involved in oxidative stress — AKR1C3 – NQO1
- Gene involved in DNA repair — GADD45A
- Gene involved in skin defense — DEFB4 – LTB4R2
- Gene involved in barrier function — SPRRA – TGM3

In vitro **studies on reconstituted skins**

SUN'ALG® offers high protection against both UVA & UVB radiation →
- Protection against UVA
- Protection against UVB

Ex vivo **studies on human skin explants**

SUN'ALG® prevents epidermal cells apoptosis associated with DNA damage → Reduction of the formation of sun burn cells

SUN'ALG® fights against inflammatory skin reactions → Inhibition of Interleukin IL-8

In vivo **studies**

SUN'ALG® diminishes redness & soothes sunburns → Short-term efficacy
In vivo study on 10 volunteers

SUN'ALG®
- boosts skin self-resistance
- prevents photoaging & sun burn cells formation
- calms inflammatory responses after UV-induced irritation
- diminishes redness & soothes sunburns (erythema)

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SUN'ALG® strengthens the skin's defence mechanisms

A large number of genes participates in the reinforcement of protective mechanisms of the skin.

In order to identify the mode of action of SUN'ALG®, we have used cosmetogenomic study that allows look how actives interact with the cell's genes.
The claims are related to the over- or the down expression of specific genes related to skin ageing.

Collaboration Strati CELL (Belgium)

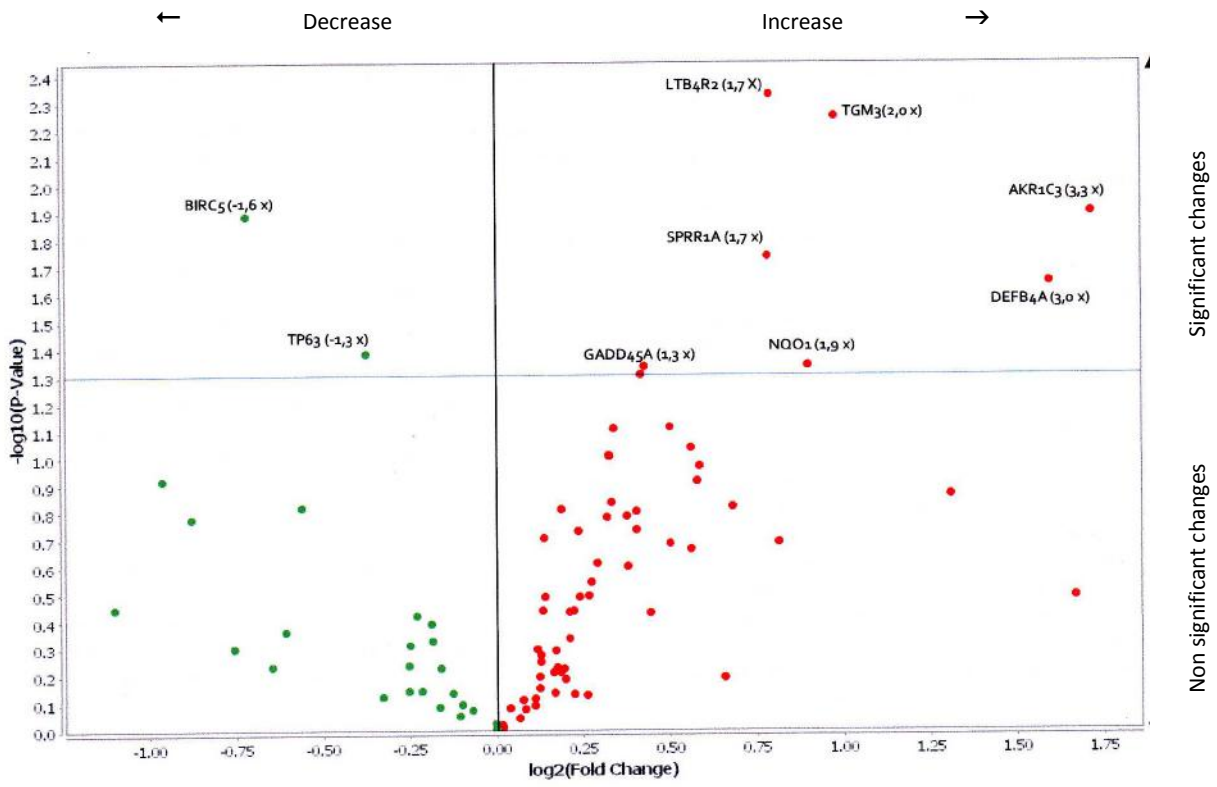
Methods

The experiments were carried out on pigmented reconstituted epidermis. Experimentations were performed in triplicate (n=3). Gene expression (92 genes linked to epidermis biology) was measured by using qRT-PCR and TaqMan card.

SUN'ALG® was tested at 5% in a basic Carbopol gel during 24h and applied at the dose 2mg/cm².The reference molecule for analysis validation was vitamin D3 (100 nM).

Results

Changes of gene expression induced by 5% SUN'ALG® in a basic Carbopol gel are illustrated on the Volcano plot (p-value < 0.5). The red and green points show respectively the increase (RQ >1) and the decrease (RQ <1) of the genic expression.



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Genes showing significant action are listed below.

Assay	Gene names	5% SUN'ALG® in a basic Carbopol gel	
		RQ.	P value
AKR1C3-Hsoo366267_m1	Aldo-keto reductase family 1 member C3	3.2836	0.0128
DEFB4A-Hsoo175474_m1	Beta-defensin 4A (Defensin, beta2)	3.0207	0.0229
TGM3-Hsoo162752_m1	Transglutaminase -3	1.9682	0.0056
NQO1-Hsoo168547_m1	NAD(P)H dehydrogenase [quinone]1	1.2512	0.0988
SPRR1A-Hsoo954595_s1	Cornifin-A (Small proline (rich protein IA)	1.7201	0.0183
LTB4R2-Hso1885851_s1	Leukotriene B4 receptor 2	1.4135	0.0775
GADD45A-Hsoo169255_m1	Growth arrest and DNA damage-inducible protein GADD45 alpha	1.3418	0.0464

SUN'ALG® is able to influence major genes involved in such protective mechanisms, specially mechanisms involved in

-the oxidative response
-the DNA repair
-the anti-microbial defense
-the reinforcement of the epidermis.

These actions are explained in detail following this.

Gene involved in the oxidative response

Free radicals are defined as molecules that contain one or more unpaired electrons, making them unstable and highly reactive (Halliwell B. 1999 - Free Radic. Res. 31: 261-272).

The most important free radicals are Reactive Oxygen Species (ROS) and Reactive Nitrated Oxygen Species (RNOS).

ROS include free radicals such as superoxide, hydroxyl, peroxyl, hydroperoxyl as well as nonradical species such as hydrogen peroxide and hydrochlorous acid.

RNOS include free radicals like nitric oxide and nitrogen dioxide as well as nonradicals such as peroxynitrite, nitrous oxide and alkyl.

In aerobic cells, free radicals are constantly produced mostly as reactive oxygen species. However, cells develop a battery of defense mechanisms to protect them against damage induced by oxidative stress.

Oxidative stress is a consequence of the imbalance between reactive oxygen species (ROS) production and antioxidant capacity. This can occur as a result of either increased ROS generation, impaired antioxidant system, or a combination of both.

This imbalance between prooxidant and antioxidant defenses in favour of prooxidants is associated with the oxidative modification of biomolecules such as lipids, proteins and nucleic acids. (Chance B *et al*. 1979 – Physiol. Rev. 59: 527–605; Djordjević V.B. 2004 - Int. Rev. Cytol. 237: 57-89).

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Once free radicals enter the cytoplasm, biotransformation is required to enhance elimination or inactivation.

The first line of defense against oxidative damage is the induction of stress-response genes, many of which encode antioxidant defense enzymes (Ishii T. *et al*. 2002- in Methods Enzymol., 348:182-190).

For example, one of the best studied transcription factors activated by oxidative stress is the nuclear factor erythroid 2-related factor 2 (Nrf2), which is responsible for the induced expression of a number of antioxidant defense genes (Lee J.M. *et al*. 2004 – J. Biochem. Mol. Biol. 37 (2): 139-143).

In response to oxidative stress, Nrf2 protein translocates to the nucleus and binds to the antioxidant response elements in the promoters of many antioxidant genes. Subsequently, Nrf2 induces a transcriptional program that maintains cellular redox balance and protects cells from oxidative insults (Malhotra D. *et al*. 2010 - Nucleic Acids Res., 38*:* 5718–5734).

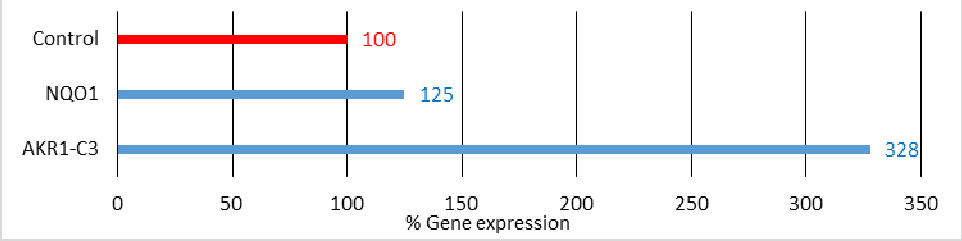
Oxidative modification may be carried out by numerous enzymes such as flavoprotein monooxygenase (FMO) and cytochrome P450 (CYP) enzymes, especially members of the CYP1, CYP2, CYP3, and CYP4 families.

Oxidation often is followed by reductive or conjugative modification by glutathione-S-transferases (GSTs), sulfotransferases (SULTs), UDP-glucuronosyl transferases (UGTs), N-acetyltransferases (NATs), aldo-keto reductases (AKRs), epoxide hydrolases (EPHXs), and NAD(P)H-quinone oxidoreductases (NQOs).

Other cellular enzymes can repair DNA damage induced by ROS (Fruehauf J.P. *et al*. 2007 - Clin. Cancer Res*.* 13: 789-794).

SUN'ALG® is able to overexpress two major genes coding for enzymes involved in the oxidative stress, specially

- NAD(P)H dehydrogenase (NQO1) x 125
- Aldo-keto reductase family1, member C3 (AKR1-C3) x 328.



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➤ NAD(P)H dehydrogenase (quinone) 1 (NQO1)

NAD(P)H: quinone oxidoreductase 1 (NQO1, DT-diaphorase, EC 1.6.99.2) is a homodimeric flavoprotein that utilizes either NADH or NADPH. It catalyzes two-electron reduction and detoxification of quinones and its derivatives. This leads to the cell protection against redox cycling and oxidative stress.

NQO1 is expressed ubiquitously in all the tissues. It is predominately located in the cytoplasm. Under conditions of stress, it migrates to the nucleus associated with mitotic spindles in cells undergoing division (Siegel D. *et al*. 2012 – PloS ONE 7(9): e 4481, Doi: 10.1371). Its gene expression is coordinately induced with other detoxifying enzyme genes in response to antioxidants, heavy metals and radiations (Jaiswal A.K. 2000 – Free Radical Biol. Med. 29: 254-262; Ross D. et al. 2000 – Chem. Biol. Inter. 129:77-97).

This enzyme is generally considered as a detoxification enzyme because of its ability to reduce reactive quinones and quinone-imines to less reactive and less toxic hydroquinones. It is under the control of Nrf2 (Nguyen *et al*. 2009- J. Biol. Chem. 284 :13291-13295).

This enzyme is also antioxidant. Its ability to deactivate many reactive species, including quinones, quinone-imines, and azo compounds, demonstrates its importance as a chemoprotective enzyme.

The functions of NQO1 may not be restricted to metabolism of xenobiotic quinones. NQO1 also plays an antioxidant role *via* the reduction of endogenous quinones such as vitamin E quinone (Siegel D. *et al*. 1997- Mol. Pharmacol., 52:300-305) and coenzyme Q_{10} (Beyer R.E. *et al.* 1996 – Proc. Natl. Acad. Sci. 77: 5216-5220). Reduction of these compounds by NQO1 generates stable hydroquinones with excellent antioxidant properties. Other findings suggest that NQO1 may have an even broader antioxidant role. (Siegel D. *et al.* 2004- Mol. Pharmacol., 65:1238-1247).

➤ Aldo-keto reductase familly1, membre C3 (AKR1-C3)

The carbonyl compound level is reduced *via* the simultaneous action of multiple sets of carbonyl reducing enzymes, such as aldo-keto reductases (AKRs) (Cho M.K. 2013 – Ann. Dermatol. 25 (4): 423–427).

Aldo-keto reductases (AKRs) form a superfamily of proteins characterized by their common three-dimensional structure and reaction mechanisms in catalyzing the reduced nicotinamide adenine dinucleotide (phosphate) (NAD(P)H)-dependent oxido-reduction of carbonyl groups (Jez J.M. *et al*. 1997 - Adv. Exp. Med. Biol. 414 : 579-600; Mindnich R.D. & T.M. Penning 2009 - Human Genomics 3(4) : 362-370).

This superfamily consists of more than 40 known enzymes and proteins. These enzymes catalyze the conversion of aldehydes and ketones to their corresponding alcohols by utilizing NADH and/or NADPH as cofactors. The enzymes display overlapping but distinct substrate specificity. This enzyme may also have a role in controlling cell growth and/or differentiation.

The overexpression of the genes NQO1 and AKR1C3 by SUN'ALG® proves the existence of detoxifying and protective functions towards oxidative and cellular stress.

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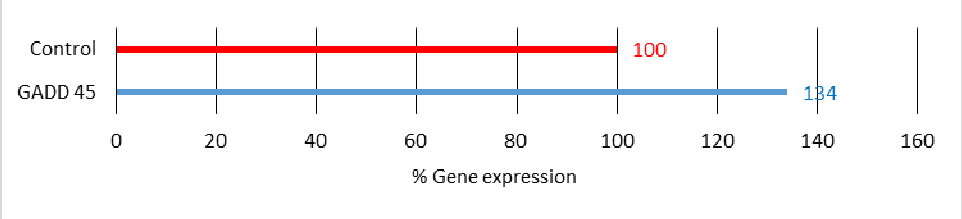
Gene involved in DNA repair

UVB induce damage to a variety of cellular targets including DNA by generating oxygen radicals as well as inducing cyclobutane pyrimidine dimers and purimidine-pyrimidone photoproducts. These forms of DNA damage will, directly or indirectly, indicate the activation of a series of signal transduction cascades comprises of serine/threonine kinases, MAPKs (Hildesheim J. *et al*. 2002- Cancer Research 62: 7205-7315).

One family of genes that is downstream of the MAPKs cascade is the Gadd45 family.

SUN'ALG® is able to overexpress Gadd45 gene involved in DNA repair x 134



➤ Growth arrest and DNA damage-inducible protein (Gadd45 alpha)

This family is composed of three genes: Gadd45a, Gadd45b and Gadd45 g. It is regulated by p53 that plays an important role in regulating DNA repair and in responses to ultraviolet light in keratinocytes (Maeda T. *et al*. 2002- J. Invest. Dermatol. 119 :22-26).

Gadd45 proteins play important roles in modulating diverse molecular pathways of stress signaling in response to a wide variety of extrinsic and physiological stress agents (Cretu, A. *et al*. 2009- Cancer Therapy 7 : 268-276 ; Liu J. *et al*. 2013 - Oxid. Med. Cell. Longevi., 305861).

Their overexpression would result in an increase of stress resistance (Moskalev A. *et al.* 2012 – Cell Cycle 11 (22) 4222-41). Gadd45 would control the balance between cells (DNA repair), eliminate apoptosis and prevent the expansion of potentially dangerous cells (cell cycle arrest, cellular senescence) and maintain the stem cell pool (Moskalev A. *et al.* 2012 – Ageing Res. Rev. 11:51-66).

Gadd45a would be a critical factor protecting the epidermis against UV radiation-induced tumorigenesis by promoting damaged keratinocytes to undergo apoptosis and /or cell cycle arrest, two crucial events that prevent the expansion of deregulated cells (Hildesheim J. *et al*. 2002- Cancer Research 62 : 7205-7315).

By overexpressing Gadd45a gene, SUN'ALG® helps maintaining genomic integrity in UV-exposed skin that would allow an increased resistance of the epidermis against cellular stress.

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Gene involved in skin defence

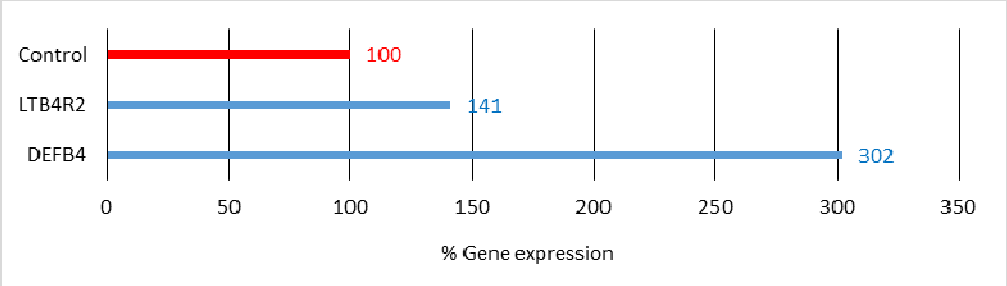
The innate immune system is thought to be one of the first lines of defence against cutaneous pathogens.

The regulation of immune responses induces the production of antimicrobial peptides, chemokines, and cytokines, including human beta-defensins (hBDs).

Antimicrobial peptides are important components of the innate immunity against microbial infections. They are proposed to participate in the early host defense response against microorganisms. These peptides are usually cationic, and their mechanism of action generally involves membranolytic disruption, permeability, or pore formation against bacteria, fungi, and viruses (Vaara, M. 1992 - Microbiol. Rev. 56: 395-411 ; Boman, H.G. 1995 - Annu. Rev. Immunol., 13:61-92 ; Hancock R.E. *et al.* 1995 - Adv. Microb. Physiol., 37:135-175).

SUN'ALG® is able to overexpress major genes involved in skin defense, specially the genes

- Leukotriene B4 receptor 2 (LTB4R2) x 141
- Beta-defensin 4A (DEFB4) x 302



Leukotriene B4 receptor 2 (LTB4R2)

The LTB4R2 gene encodes the protein Leukotriene B4 receptor 2. It is a low-affinity receptor for leukotrienes, especially leukotriene B4.

Leukotriene B4 acts an endogenous lipid mediator of inflammation derived from arachidonic acid by the sequential activation of cytosolic phospholipase and 5-lipoxygenase.

It shows potent chemoattractant properties. It is involved in the receptor-mediated activation of a number of leukocyte responses including degranulation, superoxide formation and chemotaxis (Burke J.R. *et al.* 1997- Biochem. Biophys. Acta 1359 (1) 80-88).

It is rapidly generated from activated innate immune cells *e.g.* neutrophils, macrophages and mast cells (Ohnishi H. *et al*. 2008 – Allergol. Int. 57 (4) 291-298).

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It plays a central role in neutrophil activation and migration to formyl peptides which are produced at the core of inflammation sites (Afonso P.V. *et al*. 2012 – Dev. Cell. 22 (5) 1079-1091).

Nowadays, it is clearly demonstrated that LTB4 plays a significant role in the control of microbial infections through its ability to activate host innate defenses (Le Bel M. *et al.* 2014 – J. Innate Immun. 6 (2) 158-168).

➤ Beta-defensin 4A (DEFB4)

Defensins include diverse members of a large family of antimicrobial peptides (Ganz T. 2003- Nat. Rev. Immunol., 3(9):710-720).

Defensins play a crucial role in the host defense against microorganisms of many organisms by their capability to permeabilize bacterial membranes.

They are cationic peptides of 28–42 amino acids length containing three intramolecular disulfide bonds (Diamond G. & C.L. Bevins 1998 - Clin. Immunol. Immunopath., 88 :221-225).

Based on the pattern of cysteine connectivity, the mammalian defensins can be divided into two major subgroups termed α- and β-defensins.

> In α-defensins, invariant disulfide bonds are formed in a 1–6, 2–4, and 3–5 order (Selsted M.E. & S.S. Harwig 1989 - J. Biol. Chem., 264:4003-4007), whereas β-defensins exhibit a 1–5, 2–4, and 3–6 cysteine pairing (Tang Y.Q. & M.E. Selsted 1993 - J. Biol. Chem., 268:6649-6653).

The α-defensins were among the first antimicrobial peptides discovered. They are major constituents of the primary granules of mammalian granulocytes (Selsted M.E. *et al*. 1983, - J. Biol. Chem., 258 : 14485-14489; 1985 - J. Clin. Invest., 76:1436-1439). They play a role in inflammation, wound repair, and specific immune responses (van Wetering S. *et al.* 1999 - J. Allergy Clin. Immunol., 104 : 1131-1138).

The β-defensins were originally discovered in mammals as antimicrobial peptides of the airway epithelial cells (Selsted M.E. *et al*. 1993 - J. Biol. Chem., 268 : 6641-6648, Harder J. *et al.*, 1997 - Genomics 46 : 472-475; 2001 - J. Biol. Chem., 276:5707-5713),

Human β-defensin-2 is produced by a number of epithelial cells. It exhibits potent antimicrobial activity against Gram-negative bacteria and *Candida* but not Gram-positive *Staphylococcus aureus.* It represents the first human defense that is produced following stimulation of epithelial cells by contact with microorganisms (*Pseudomonas aeruginosa*) or cytokines (TNF-α , IL-1β) (Schröder J.M. & J. Harder , 1999 - Int. J. Biochem. Cell Biol., 31: 645-651). Moreover, this defensin is known to contribute in vivo to the immunosurveillance of the skin barrier function (Pernet I. *et al*., 2003 - Exp. Dermatol., 12 (6) 755-760).

The overexpression of the genes LTB4R2 and DEFB4 implies that SUN'ALG® is able to contribute to the innate defences of the skin by

- improving the epidermal capacity against microbial attacks
- increasing the ability of cell responses to activate host defenses.

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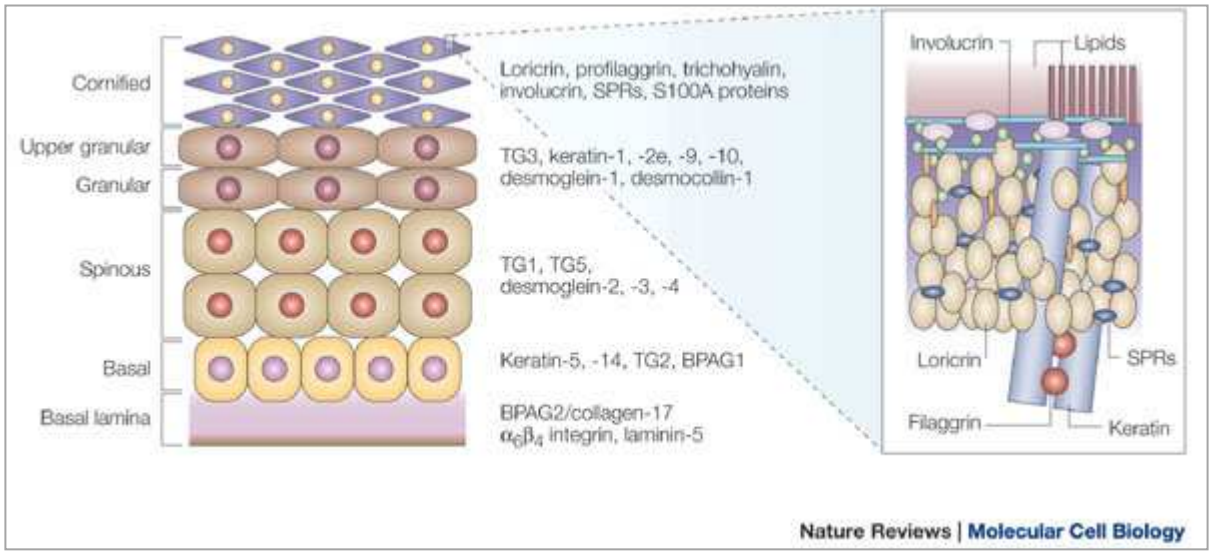
Gene involved in the barrier function

The skin protects our body against various attacks and stresses. The epidermal barrier constitutes the primordial frontline of this defense established during terminal differentiation of the keratinocyte cornified envelope.

Many proteins are essential for epidermal differentiation. They are encoded by genes that constitute the "epidermal differentiation complex" (Darmon, M. *et al.* 1993 - Molecular Biology of the Skin, 1-32, Academic Press) divided on the basis of common genes and protein structures (Kypriotou M. *et al*. 2012 – Exp. Dermatol., 21 (9):643-649) .

The figures here after (from Candi E. *et al.* 2005 – Nature Reviews Molecular Cell Biology 6:328-340) explain clearly the process involved in terminal differentiation and apoptosis in the epidermis.



The proteins that are expressed in particular locations in the epidermis during skin differentiation are shown.

Apoptosis is restricted to the basal layer, whereas cornification occurs in the supra-basal layers, to form a cornified envelope (see inset). BPAG, bullosus pemphigoid antigen; SPR, small proline-rich proteins; TG, transglutaminase.

At the molecular level, proteins are incorporated into the cornified envelope *via* action of epidermal transglutaminases (TGases). These enzymes catalyse the formation of characteristics crosslinks between structural proteins to form the protein part of the cornified envelope.

Additional components such as loricrin, elafin and small proline-rich region proteins (SPRRs) contribute to a mature envelope (Marshall D. *et al*. 2001- PNAS 98: 13031-13063).

These structural proteins including additional components constitute about 7-10% of the mass of the epidermis. Together with lipids (ceramides), they form the complete barrier.

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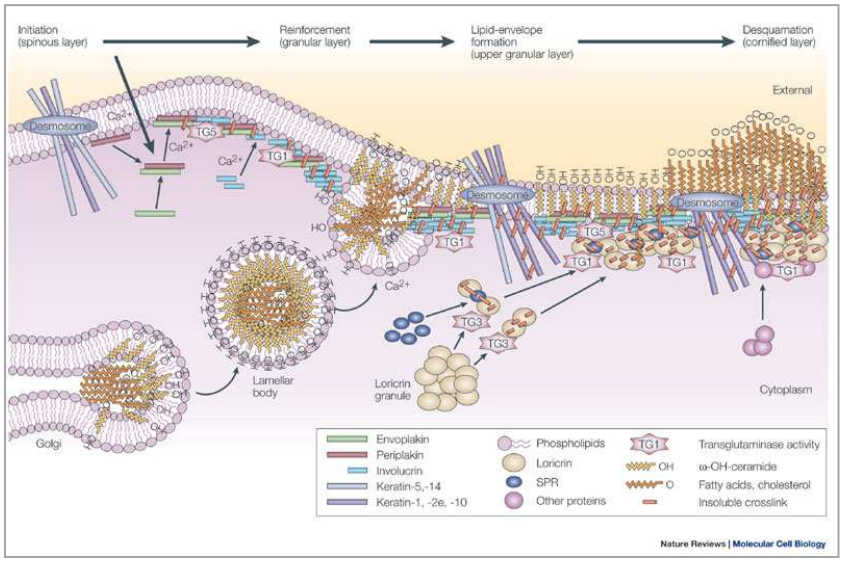


The first step is the initiation stage, which takes place in the spinous layer. It involves the synthesis of the cornified-envelope structural proteins and the synthesis and extrusion into the intercellular space of specific lipids.

Transglutaminases TG1 and TG5 crosslink envoplakin and periplakin under the cell membrane, thereby anchoring them to the desmosome.

The second step is the reinforcement phase, which takes place in the granular layer. It entails the covalent attachment of some lipids to the cornified-envelope proteins and the crosslinking of loricrin to small proline-rich proteins (SPRs) by TG3 and TG1. Heavy crosslinking occurs on the desmosome, where these proteins function as substrates for TGs.

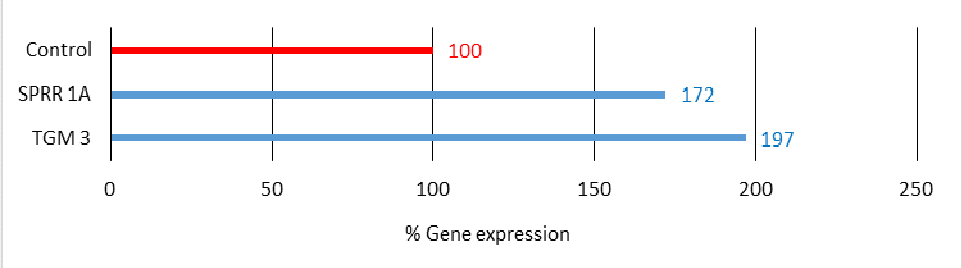
Next, during the formation of the lipid envelope, also in the granular layer, lipids from the lamellar body are attached and crosslinked by TG5 and TG1 on the already crosslinked proteins (envoplakin, periplakin, involucrin) and are exposed on the outside of the membrane. The reinforcement and lipid-envelope-formation steps take place concomitantly.

Finally, the desquamation phase, which takes place in the cornified layer, involves further crosslinking of loricrin and other proteins by TG1 on the protein scaffold, and the extrusion of ω-OH-ceramides, fatty acids and cholesterol.

The physical properties of the cornified envelope depend on the nature of the substrates and on the crosslinks, as well as the lipid deposition. The location of the epidermal layers in which these progressive steps take place is indicated in the previous figure.

SUN'ALG is able to overexpress major genes involved in the epidermal differention :

- Cornifin – A (Small proline-rich protein 1A) (SPPR1A) x 172
- Transglutaminase -3 (TGM 3) x 197



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➤ Cornifin – A (Small proline-rich protein 1A) (SPPR1A)

SPRRs are a family of proteins that are substrates for TG mediated cross-linking of structural proteins into the cornified envelope.

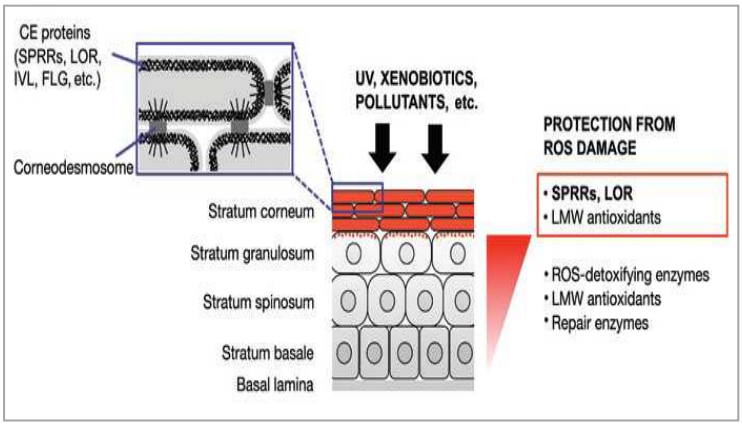
SPRRs (small proline-rich proteins) are involved in the assembly of the cornified cell envelope, a specialized structure beneath the plasma membrane of keratinocytes (Vermejl W.P. *et al*. 2011 – J. Invest. Dermatol. 131 (7):1434-1441).

At least four SPRRs have been described: SPRR1, -2, -3, and -4 (Patel S. *et al*., 2003- Mamm. Genome 14:140-148). Cornifin (SPRR1) first appears in the cell cytosol, but ultimately becomes cross-linked to membrane proteins by transglutaminases.

During late epidermal differentiation, SPPRs are expressed together with other genes of this epidermal differentiation complex that encode structural proteins such as loricrin and filaggrin. In the outer layers of skin, these proteins are cross-linked at the cell periphery by transglutaminases, together with lipids they form the cornified envelope beneath the keratinocyte membrane (Hohl, D. *et al*. 1995 – J. Invest. Dermatol. 104:902-909). So SPPRs are involved in barrier function as an integral part of the cornified envelope.

However a novel function of SPPRs has been reported in ROS defense (Schäfer M. & S. Werner, 2011 – J. Invest. Dermatol., 131: 1409-1411).

SPPRs show ROS-quenching properties due to the high number of cysteine residues in these proteins which directly interact with ROS. They act as antioxidants as it is shown in the figure here after illustrating the reactive oxygen species (ROS) protection mechanisms of the epidermis.
(from Schäfer M. & S. Werner, 2011 – J. Invest. Dermatol., 131: 1409-1411).



The epidermis is exposed to environmental insults such as UV radiation, xenobiotics, and pollutants.

Multiple mechanisms protect keratinocytes from ROS damage induced by these insults. In the *stratum corneum,* several cornified envelope proteins, including loricrin (LOR) and small proline-rich proteins (SPRRs), function as antioxidants together with low-molecular-weight (LMW) antioxi-dants (indicated by red cells).

In addition, a gradient of ROS-detoxifying enzymes, LMW antioxidants, and repair enzymes provides greater protection for suprabasal keratinocytes than for basal keratinocytes (indicated by thered gradient).

Inset: cornified envelope proteins such as SPRRs, LOR, involucrin (IVL), and filaggrin(FLG) form a meshwork at the periphery of keratinocytes in the stratum corneum, which is cross-linked to

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keratin filaments. Corneodesmosomes connect the keratin filaments and the cornified envelope of *stratum corneum* keratinocytes. CE, cornified envelope.

➤ Transglutaminase -3 (TGM 3)

Transglutaminases (TG) are Ca2+ dependent enzymes that catalyse the formation of special bonds between cornified envelope structural proteins to confer the characteristic resistance and insolubility to the skin. They are very important for epithelial barrier formation.

TG1, TG3 and TG5 are known to participate in cornified envelope formation. Both TG1 and TG3 are activated by limited proteolysis during keratinocyte differentiation (Hitomi, K., 2005 – Eur.J. Dermatol. 15(5):313-319).

The overexpression of the genes SPRR1A and TGM3 implies that SUN'ALG® is able to reinforce the epidermal barrier function by respectively

- helping catalyse cross-linking reactions between proteins during the skin cornification
- providing the outer layer with a highly adaptative and protective antioxidant shield.

Summary of this cosmetogenomic analysis

This cosmetogenomic analysis proves that SUN'ALG® is able to influence major genes involved in the protective mechanisms linked to

the oxidative response	therefore	➤ SUN'ALG® detoxifies skin cells
the DNA repair		➤ SUN'ALG® maintains genomic integrity
the antimicrobial defence		➤ SUN'ALG® improves the epidermal ability against microbial attacks
the reinforcement of the barrier function		➤ SUN'ALG® provides an efficient epidermal shield

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SUN'ALG® offers high protection against both UVA & UVB radiation

UVB radiation (280–320 nm) and UVA radiation (320–400 nm) are essential components of sunlight that generate severe oxidative stress in skin cells *via* interaction with intracellular chromophores and photosensitizers, resulting in transient and permanent genetic damage, and in the activation of cytoplasmic signal transduction pathways that are related to growth, differentiation, replicative senescence and connective-tissue degradation.

UVA radiation penetrates deeper into the epidermis and the dermis of the skin and acts indirectly through the generation of reactive oxygen species which subsequently can exert a multitude of effects such as lipid peroxidation and generation of DNA-brand breaks. It is more abundant in sunlight than UVB radiation (95% of UVA and 5% UVB) and therefore exhibits more severe damage than UVB. It appears about 1000 times more effective in producing an immediate tanning effect. Long term UVA exposure can cause premature skin photoaging and produce structural changes in DNA which in turn results in cancerous condition. DNA is not a chromophore for UVA radiation (Rosenstein B.S. & D.L. Mitchell, 1987- Photochem. Photobiol. 45: 775-780), but could be damaged by photosensitization reaction initiated through absorption of UVA by unidentified chromophore.

UVB radiation acts predominantly in the epidermal cell layers of the skin. It is 1000 times more capable of causing sun burn than UVA due to its most energetic wavelengths. It is more genotoxic than UVA inducing direct and indirect adverse biological effects such as free radical production in the skin, cell cycle growth arrest, photoaging and photocarcinogenesis.

Both UVA and UVB radiation are proved to produce DNA damage directly and indirectly through oxidative stress.

Protection against UVA

Methods

Reconstituted human epidermal tissues were studied (in duplicate) topically with 2mg/cm² of gels containing different extracts:

-placebo (basic Carbopol gel)
-SUN'ALG® 2% in the basic Carbopol gel (SA 2%)
-SUN'ALG® 4% in the basic Carbopol gel (SA 4%)
- *Dunaliella salina* extract (same concentration than in SUN'ALG®) in the basic Carbopol gel (DS 2%)
- *Haematococcus pluvialis* extract (same concentration than in SUN'ALG®) in the basic Carbopol gel (HP 2%).

Tissues were irradiated (or not) 24h after cultivation with UVA - dose: 20 J/cm².
After irradiation, they were placed in fresh medium for 24 h. Survival was quantified using the colorimetric MTT assay.

Results

Results are illustrated as percentage against no irradiated control, without any ingredient (Control ni). They represent the average of 2 experiments with 8 values for control and each product.

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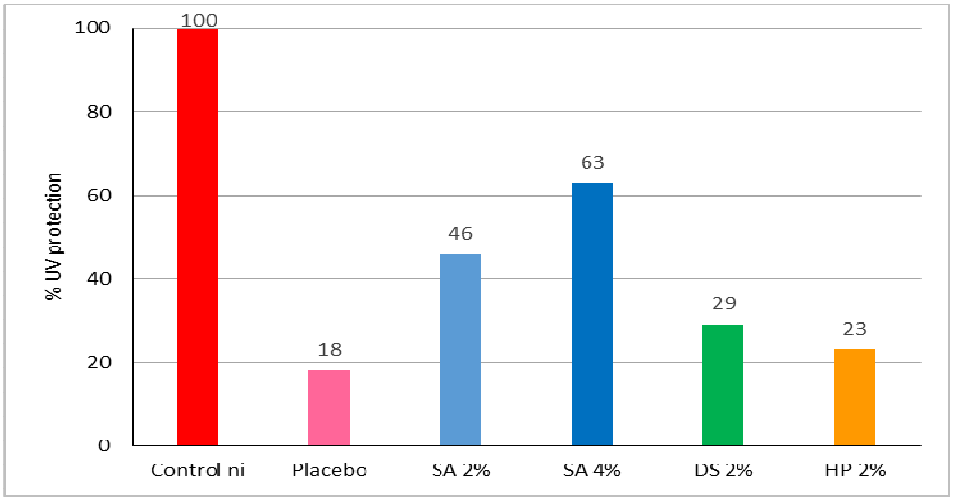

Results are validated by two statistical analysis.
-ANOVA (n=16)

Source Δ	D	υ	V	F
Global	1.9253	5	0.3851	***
Control ni / control i	1.3777	1	1.3777	***
Placebo/algae extract	0.5637	1	0.5637	***
SUN'ALG®/algae extract	0.3276	1	0.3276	***
SUN'ALG® 2% /4%	0.0556	1	0.0556	***
Dunaliella /Haematococcus	0.0072	1	0.0072	***
Residual	0.0043	90	0.00005	

-Least significant difference

$\Delta\alpha = t\alpha \overline{V\ 2\ Vi}\ /n$ n=16 V i= 0.00005

p	tα	Δα	significativity
0.05	2.134	0.0053	*
0.01	2.9467	0.0073	**
0.001	4.0728	0.0102	***

SUN'ALG® provides effective protection against UVA.

Mean decreasing values

Control ni	SA 4%	SA2%	*Dunaliella*	*Haematococcus*	Placebo
0.5000	0.3140	0.2306	0.1443	0.1142	0.0900

Dunaliella salina extract brings a superior efficacy than *Haemacococcus pluvialis* extract in the mix.

Calculated parameters

Im1-m2I	Δc	significativity
Control ni - SA 4%	0.186	***
SA 4% - SA 2%	0.0834	***
SA 2% - *Dunaliella*	0.0863	***
Dunaliella - *Haematococccus*	0.0301	***
Haematococcus - Placebo	0.0242	***

Data validated statistically.

Control ni >>>SA 4% >>> SA 2% >>> *Dunaliella* 2% >>> *Haematococcus* 2% >>> Placebo

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Protection against UVB

Methods

Reconstituted human epidermal tissues were studied (in duplicate) topically with 2mg/cm² of gels containing different extracts:

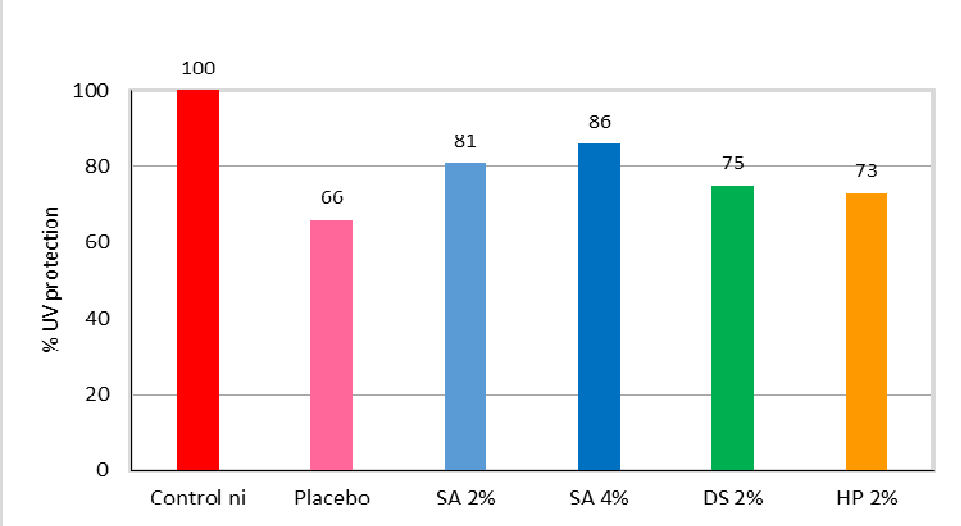
-placebo (basic Carbopol gel)
-SUN'ALG® 2% in the basic Carbopol gel (SA 2%)
-SUN'ALG® 4% in the basic Carbopol gel (SA 4%)
-extract of *Dunaliella salina* (same concentration than in SUN'ALG®) in the basic Carbopol gel (DS 2%)
-extract of *Haematococcus pluvialis* (same concentration than in SUN'ALG®) in the basic Carbopol gel (HP 2%)

Tissues were irradiated (or not) 24h after cultivation with UVB - dose: 200 mJ/cm².

After irradiation, they were placed in fresh medium for 24 h. Survival was quantified using the colorimetric MTT assay.

Results

Results are expressed as percentage against no irradiated control, without any ingredient (Control ni). They represent the average of 2 experiments with 8 values for control and each product.



Results are validated by two statistical analysis.

-ANOVA (n=16)

Source Δ	D	υ	V	F
Global	0.2876	5	0.0575	***
Control ni / control i	0.1925	1	0.1925	***
Placebo/algae extract	0.506	1	0.506	***
SUN'ALG®/algae extract	0.0388	1	0.0388	***
SUN'ALG® 2% /4%	0.0047	1	0.0047	***
Dunaliella extract/Haematococcus extract	0.00095	1	0.00095	**
Residual	0.0098	90	0.00011	

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-Least significant difference

$\Delta\alpha = t\alpha \sqrt{2 Vi}/n$ n = 16 V i = 0.00011

p	tα	Δα	significativity
0.05	2.1314	0.0079	*
0.01	2.9467	0.0109	**
0.001	4.0728	0.01506	***

SUN'ALG® provides effective protection against UVB.

Mean decreasing values

SA 4%	SA2%	*Dunaliella*	*Haematococcus*	Placebo
0.4293	0.4049	0.3733	0.3624	0.3296

Dunaliella salina extract brings a superior efficacy than *Haemacococcus pluvialis* extract in the mix.

Calculated parameters

Im1-m2I	Δc	significativity
Control ni - control i	0.0707	***
SA 4% -SA 2%	0.0244	***
SA 2% - *Dunaliella*	0.0316	***
Dunaliella - *Haematococccus*	0.0109	**
Haematococcus - Placebo	0.0328	***

Data validated statistically.

Control ni >>> SA 4% >>> SA 2% >>> *Dunaliella* 2% >> *Haematococcus* 2% >>> Placebo

SUN'ALG® protects the skin against both UVA and UVB.

The protective abilities of SUN'ALG® and of *Dunaliella salina* extract and *Haematococcus pluvialis* extract are superior to those of the placebo as well for UVA as UVB radiation.

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SUN'ALG® prevents the epidermal cells apoptosis associated to DNA damage (the formation of sun burn cells)

Exposing skin to solar radiation shows the formation of sun burn cells (Daniels F. *et al*. 1961- J. Invest. Dermatol. 37: 351-357).

Sun burn cells are keratinocytes undergoing apoptosis following UVB irradiation (Kulms D. & T. Schwarz, 2000- Photodermatol. Photoimmunol. Photomed 16: 195-201).

Their formation appears as a consequence of UVB-induced DNA damage. However it is also found after irradiation with UVC and high doses of UVA, though to a much lesser extent (Kumakiri M. *et al*. 1977- J. Invest Dermatol. 69: 392-400; Young A.R. 1987 – Photodermatology 4: 127-134).

Sun burn cells show a typical morphologic features such as a shrunken eosinophilic cytoplasm with a condensed pyknotic nucleus which make them easily recognizable in sections (Danno K. & T. Horio, 1987 – Photochem. Photobiol. 45: 683-690).

They are detectable as early as 8h after UV exposure and are maximally expressed after 24-48h (Woodcock A. & I.A. Magnus, 1976 – Br. J. Dermatol. 95: 459-468). Their number would decreased and DNA fragmentation would be reduced in proportion to the UVA/UVB ratio (Green, E.A. *et al*. 2002 – Immunity 16: 183-191)

Protection against the formation of Sun burn cells

The capacity of SUN'ALG® to protect the skin against UV-induced stress was evaluated by determining its influence on the formation of sun burn cells in the skin. Indeed, the presence of sun burn cells appears as the indicator of acute photodamage, specially of UVB-induced DNA damage.

Collaboration SEPhRA (France)

Methods

Basic Carbopol gels with or without addition of SUN'ALG® (2.5% or 5%) were applied on human explants (30 µl/explant) for 24h (2 explants by experimental condition). Explants (from Caucasian femelle 31 years old) were irradited (UVA 8J/cm² + UVB 200 mJ/cm²) then treated again for 24h.

Positive control : Trade cream SPF 50.

Results

Results are illustrated here after. Sun burn cells are shown with *

Explants non irradiated and non treated : none observation of sun burn cells
Explants irradiated and non treated : observation of sun burn cells
Explants irradiated and treated by SPF 50 cream: none observation of sun burn cells
Explants irradiated and treated by 2.5% SUN'ALG®: observation of sun burn cells
Explants irradiated and treated by 5% SUN'ALG®: none observation of sun burn cells.

The basic gel with 5% SUN'ALG® is highly efficient against the formation of sun burn cells.

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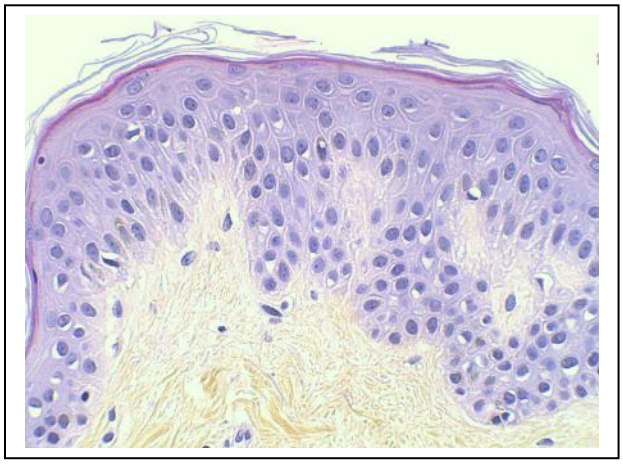
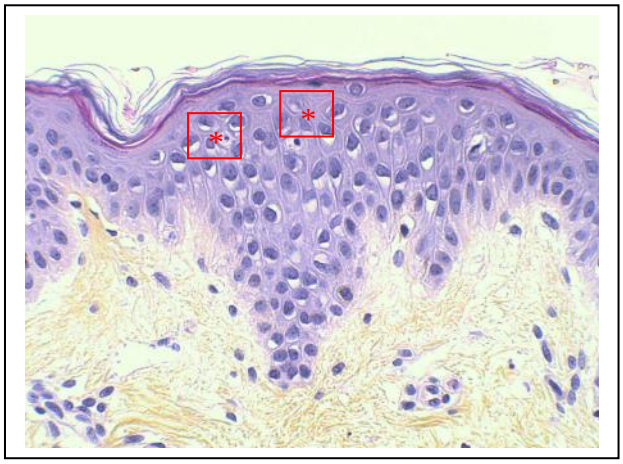
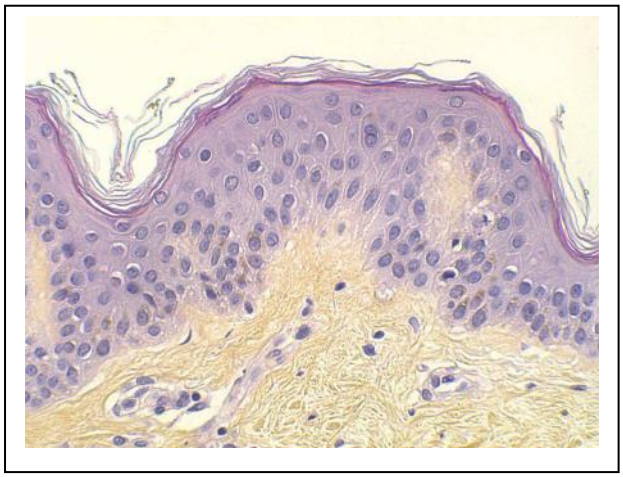


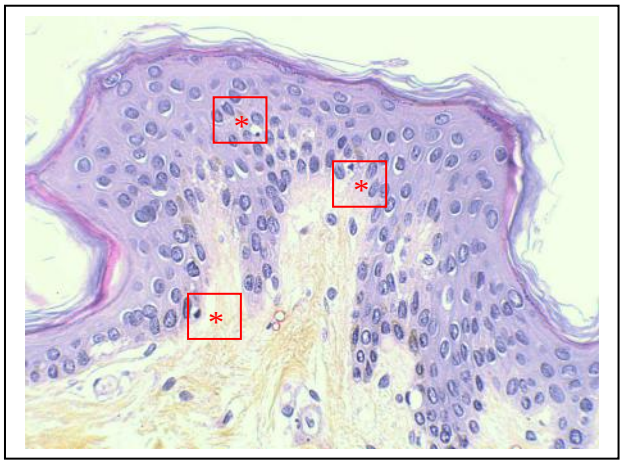
Control - no irradiation - no treatment



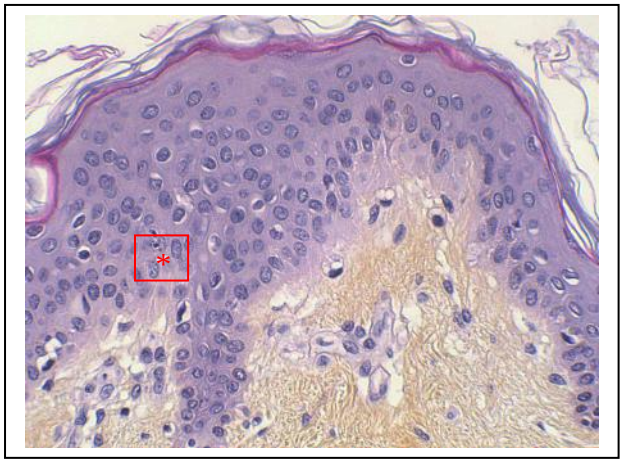
Control - UV irradiation - no treatment



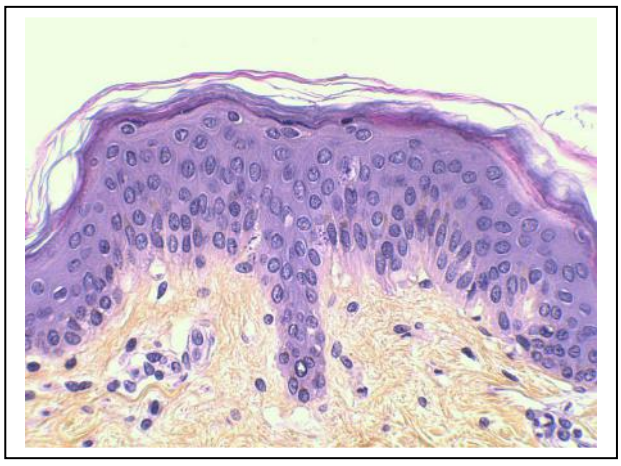
Control cream SPF 50 – UV irradiation



Placebo - UV irradiation



SUN'ALG® 2.5% - UV irradiation



SUN'ALG® 5% - UV irradiation

The basic gel with 5% SUN'ALG® shows a comparable efficacy of the trade cream SPF 50 against the formation of sun burn cells. *

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SUN'ALG® fights against inflammatory skin reactions

UV irradiation induces skin inflammation which in turn leads to the release of inflammatory cytokines and amplifies the irradiation-induced inflammation.

Numerous kinds of cells are capable of producing various cytokines, some of which being mainly induced by UVB whereas both UVA and UVB are able to regulate others.

Among these cytokines, the chemokine IL-8 plays a key role in UVB-induced inflammation acting in a dose and time-dependent manner (Endoh *et al.* -2007- Clinical & Experimental Immunology 148: 161-167).

Inhibition of Interleukin IL- 8

Collaboration SEPHRA (France)

Methods

Basic Carbopol gels without or with addition (5% SA) of SUN'ALG® were applied on human explants (30µl/explant) for 24h (2 explants by experimental condition). The inflammatory stress was performed by PMA (1µg/ml).

Experimental conditions are summarized below.

		24 hours	24 hours	
	Assays	Prior treatment	PMA stimulation	Posttreatment
None stimulation	Control no treatment	/	no	/
	Placebo gel	Topic application 30 µL		Topic application 30 µL
	Gel 5% SUN'ALG®			
	Positive control			
PMA stimulation	Control stimulated	/	Yes PMA 1 µg/mL	/
	Placebo gel	Topic application 30 µL		Topic application 30 µL
	Gel 5% SUN'ALG®			
	Positive control			

Positive control : trade cream SPF 50.

The evaluation of the activity of IL-8 was performed by ELISA testing.

Results

Results are illustrated here after. Data are validated statistically and expressed in IL8 concentrations.

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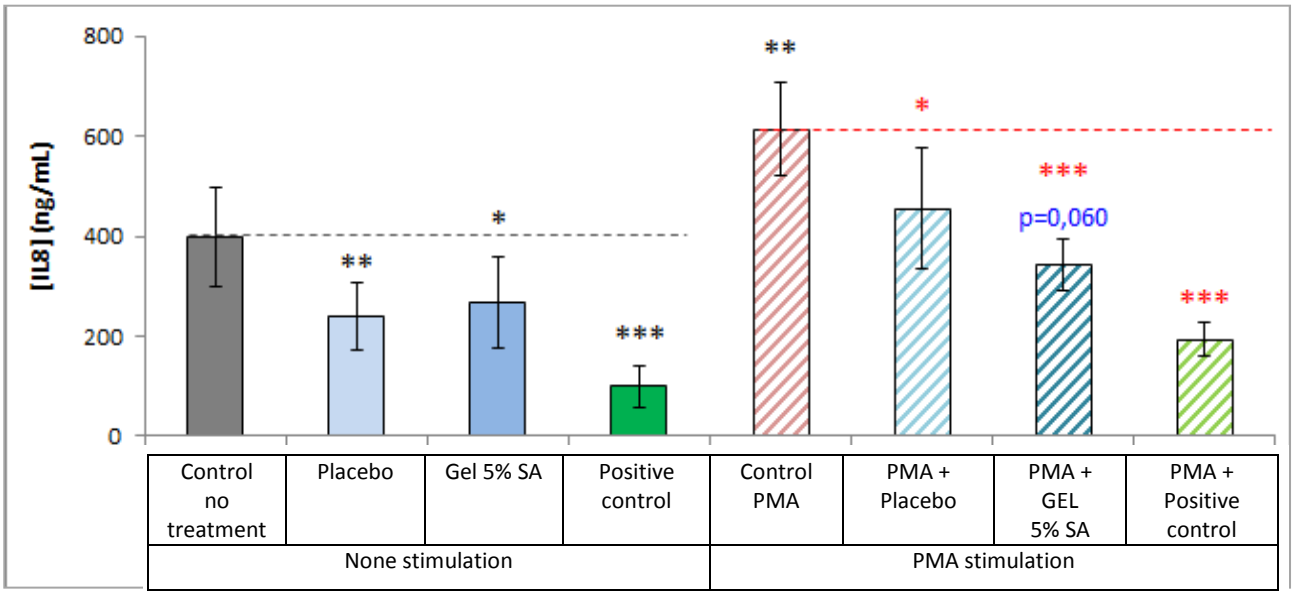

The table below show the percentage of IL-8 inhibition in the different experimental conditions.

Dosage IL-8		Inhibition (%) Mean	SD
None stimulation	Control no treatment		
	Placebo gel	40	16
	Gel 5% SUN'ALG®	33	23
	Positive control cream SPF 50	75	11
PMA stimulation	Control stimulated	54	23
	Placebo gel	26	20
	Gel 5% SUN'ALG®	44	8
	Positive control cream SPF 50	68	6

The validation of the experimentation was demonstrated by the results relative to

- the stimulated control : the release of IL-8 is stimulated by 54% highly significantly
- the positive control cream SPF 50 : the release of IL-8 is inhibited in any case :
  no PMA stimulation 75% inhibition
  PMA stimulation 68 % inhibition.

Note: the tested cream is a complex formulation contrary to the basic Carbopol including 5% SUN'ALG®.

The basic Carbopol gel with 5% SUN'ALG® induces an inhibition too:
no PMA stimulation 33% inhibition
PMA stimulation 44% inhibition.

The basic Carbopol gel with 5% SUN'ALG® inhibits the release of interleukin IL-8 highly significantly.

SUN'ALG® is able to protect the skin against inflammatory stress, indirect consequence of UV radiation and cause of premature skin aging.

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SUN'ALG® diminishes redness & soothes sunburns

Sunburn also called erythema is one of the most obvious signs of UV exposure and skin damage. It is redness of the skin which is due to increased blood flow in the skin caused by dilatation of the superficial blood vessels in the dermis as a result to UV radiation.

UVB radiation is believed to mainly responsible for sunburn but UVA contributes 15-20% to the sunburn reaction in the summer months.

Sunburn can occur in less than 15 minutes. After the exposure, skin may turn red in a little as 30 minutes but most takes 2 to 6 hours, the burn continuing to develop for 24 to 72 hours.

Sunburn increases the risk of premature ageing, dark spots, actinic keratosis and even skin cancer.

So it is important to minimize sunburns.

The present study was aimed to evaluate, on 10 volunteers, the soothing effect of a basic Carbopol gel including 5% SUN'ALG® and the relative placebo on stressed skin irradiated by Solar Simulator (UVA+UVB calculated as MED x 1.5).

The 10 volunteers were male and female, aged from 18 to 65, having medium-white complexion, in particular on phototypes I, II and III classified according to the colorimetric value ITA° (Individual Typology Angle).

In vivo study

Collaborations ABICH (Milano –Italy)

Dr. Samuele Burastero, specialist in Clinical Allergology and Immunology, teacher of molecular allergology at the Faculty of Medicine of the University Vita Salute of the San Raffaele Institute in Milan.

Methods

After a period of about 26 ± 4 hours (T0), on the three irradiated areas the degree of erythema was measured as index of erythema with Mexameter®. Immediately after the measurements, one skin area was treated with the test gel, a second area was treated with the placebo and a third one was not treated (control).

The same parameter (index of erythema) was measured 30 minutes (T0 + 30min), 1 hour (T0 + 60 min) and 2 hours (T0 + 120 min) after the product application, respectively in each area.
Soothing effect was determined by comparing the parameter detected on the treated area with that one detected on the area treated with placebo and on the control site.

Day 0	irradiation
T0	measurement of the basal values of erythema, application of product on one skin site
T0 + 30min	first measurement
T0 + 60 min	second measurement
T0 + 120 min	third measurement.

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The study was carried out under standard environmental conditions for each reading time, monitoring and maintaining constant temperature and humidity.

Test gels were applied on two back skin sites of each volunteer previously irradiated with UVA/UVB radiations and used in no occlusive epicutaneous application. As control area a skin area of the back was identified, where no product was applied after the irradiation.

Three skin sites on the back of each volunteer were irradiated at day 0. Every site was exposed to the same radiations with three beams of light of Solar Simulator having an incremental UV doses second a geometric progression of 1.12.

Gels were conducted in two consecutive days according to the following experimental procedure:

DAY 0
DAY 1 (26 ± 4 hours after irradiation).

The measurement of the skin erythema value E through Mexameter® MX18 is based on the principle of light absorption/reflection by the skin.

The results of measurements are shown on a digital display on a scale from 0 to 999 and expressed with the parameter E (erythema). The probe is very sensitive as compared with visual observation and shows a wide scale of values of erythema (0-999), which allows to accurately monitor extremely little variations in the color of interest.

Erythema value, assessed by hemoglobin content of the skin, was measured on the DAY1 at T0 (26 ± 4 hours after irradiation) before product application, 30 minutes (T0+30min), 1 hour (T0+1hour) and 2 hours (T0+2h) after the product and placebo application. The value was assessed at the same times of observation in an irradiated and untreated area.

The measurements were performed in a room protected from light in order to avoid that light radiation in the environment could affect the measurements. Since the solar simulator has three optical waveguides emitting UV radiation at different power, the erythema was assessed at each measurement time in each one of the three irradiated areas and the average value was extrapolated.

The data obtained using Mexameter ® MX18 probe were expressed as "E" values on a scale from 0 to 999 and the measurements performed at different experimental times were rigorously repeated on the same skin areas.

Photographs of the analyzed skin areas were taken at T0, T0 +30 min and T0 +120 min.

The distributions of the values obtained during the measurements at the different experimental times for the area treated with the product, the area treated with placebo and the untreated area were compared with an intra-group analysis using Student's t test; values of $p < 0.05$ were considered significant.

Results

Under the adopted experimental conditions, the product under study (basic Carbopol gel with 5% SUN'ALG®) has demonstrated short-term efficacy in reducing the erythema value (parameter E) of the UVA+UVB inducted irritation.

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In particular on the area treated with the tested product the erythema value E was reduced by a mean value equal to 3.62%, 7.32% and 15.94% respectively after 30 minutes, 1 hour and 2 hours from the product application in comparison with the levels of Erythema measured at T0.

The reduction of the E parameter on the test product treated area is resulted not statistically significant vs T0 ($p > 0.05$ - Student test) both after 30 minutes (T0 + 30min) and after 1 hour (T0 + 60 min) after the product application, while the reduction after 2 hours (T0 + 120 min) is resulted statistically significant ($p < 0.05$).

In the area treated with the placebo the erythema value E was slightly increased of a mean value equaled to 1.61% (variation not statistically significant, $p > 0.05$); the same parameter resulted decreased of a mean value equal to 0.70% and 1.20% respectively after 60 min and 120 min (variations not statistically significant, $p > 0.05$).

On the untreated area the erythema index E is slightly increased of a value equal to 1.15% (variation not statistically significant, $p > 0.05$) 30 minutes after the product application; the same parameter resulted decreased of a mean value equal to 0.57% and 0.49% respectively after 60 min and 120 min (variations not statistically significant, $p > 0.05$).

The tables below report the means of the Erythema values of the panel of 10 volunteers (table 1), the percentage variations between the Erythema values measured at the different experimental times and the Erythema values measured at T0 and the p values of Student's t test (tables 2 and 3).

Table 1 - Mean values of the Erythema value

Time	Product	Placebo	Untreated
T0	264.5	269.9	263.8
T0 + 30 min	254.9	274.2	266.3
T0 + 1 hour	245.1	268.0	262.3
T0 + 2 hours	222.3	266.6	262.5

Table 2 - Mean percentage variations

Mean % variations	Product	Placebo	Untreated
T0 + 30 min vs T0	-3.62%	1.61%	1.15
T0 + 1 hour vs T0	-7.32M	-0.70%	-0.57%
T0 + 2 hours vs T0	-15.94%	-1.20%	-0.49%

Table 3 - p-value of Student's T test

T test	Product	Placebo	Untreated
T0 + 30 min vs T0	0.2263	0.2990	0.4081
T0 + 1 hour vs T0	0.0768	0.4288	0.4458
T0 + 2 hours vs T0	0.0216*	0.4100	0.4644

* p-value associated to a statistically significant variation ($p < 0.05$).

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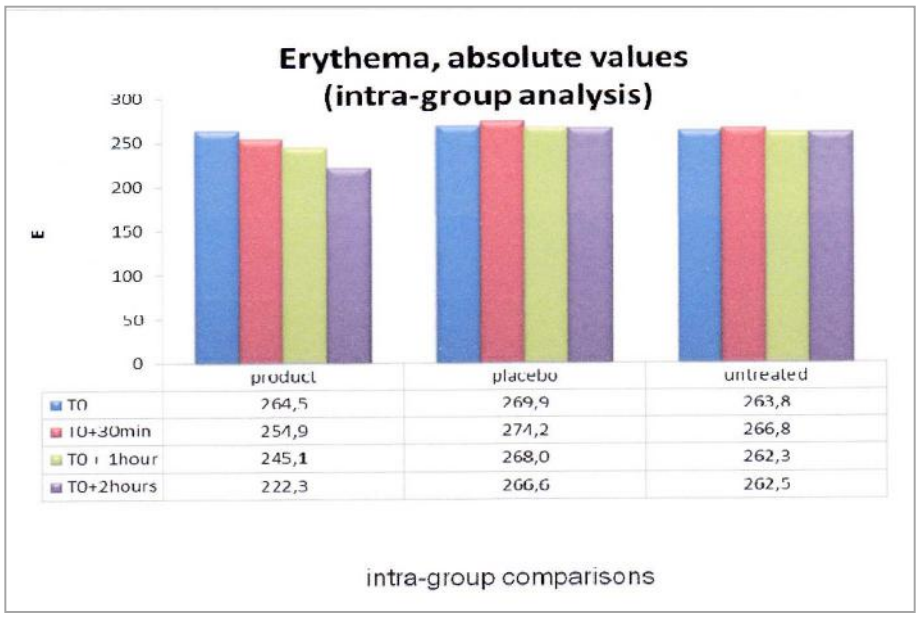
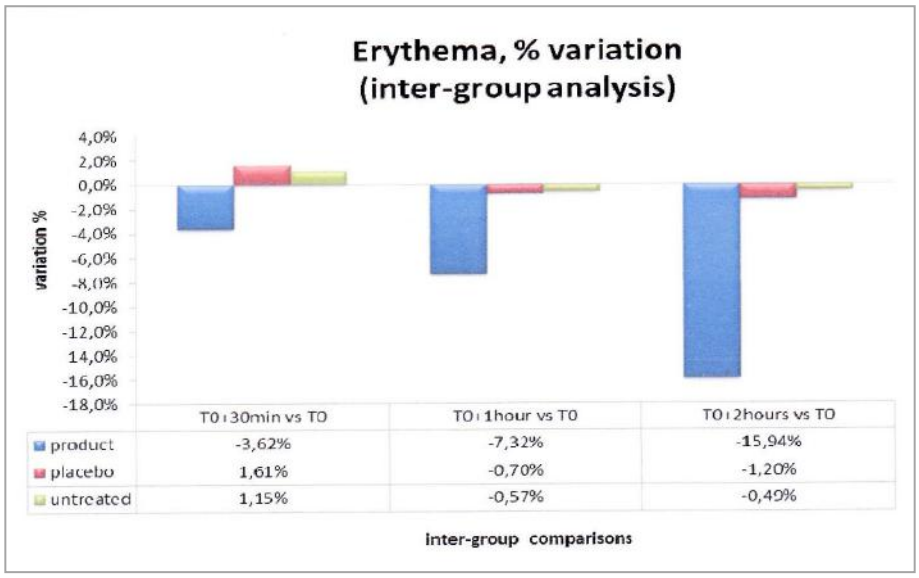
The figure here after illustrates these results .





On the basis of the results obtained under the adopted experimental conditions, the product under study basic Carbopol Gel + 5% SUN'ALG® **,** in the subjects submitted to the test, has demonstrated a short term efficacy (after 30 min, 60 min and 120 min from product application) in reducing the skin erythema at the level of the treated area respect to the erythema value measured at T0, before product application, and respect to the values of the same parameter measured on the area treated with the placebo and on the untreated area.

SUN'ALG® offers a soothing effect which calms local irritations due to UVA+UVB radiation.

By diminishing redness and soothing UV irritation due to sun-exposure (as illustrated here after), SUN'ALG® helps to prevent premature ageing of the skin due to UV radiation.

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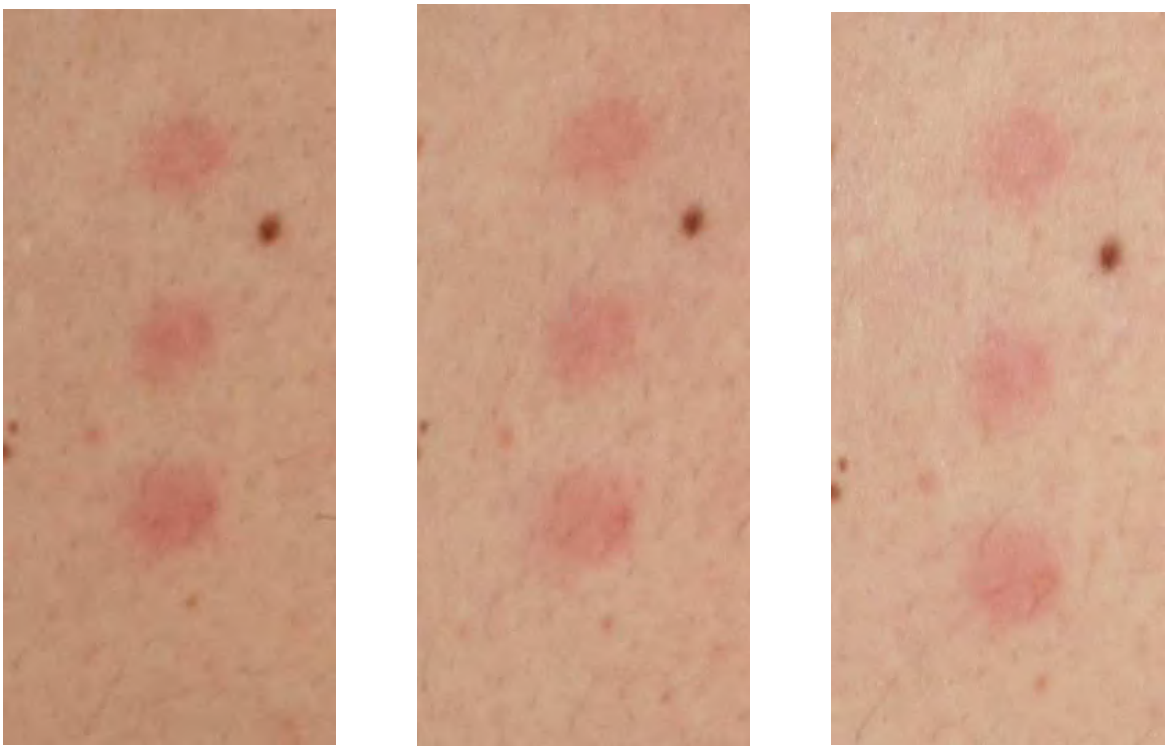
MACROPHOTOGRAPHY

Photographs presented here after illustrate the irradiated areas of the back of two volunteers, treated with the basic Carbopol gel + 5% SUN'ALG® at the different measurement times: 30 min and 120 min.

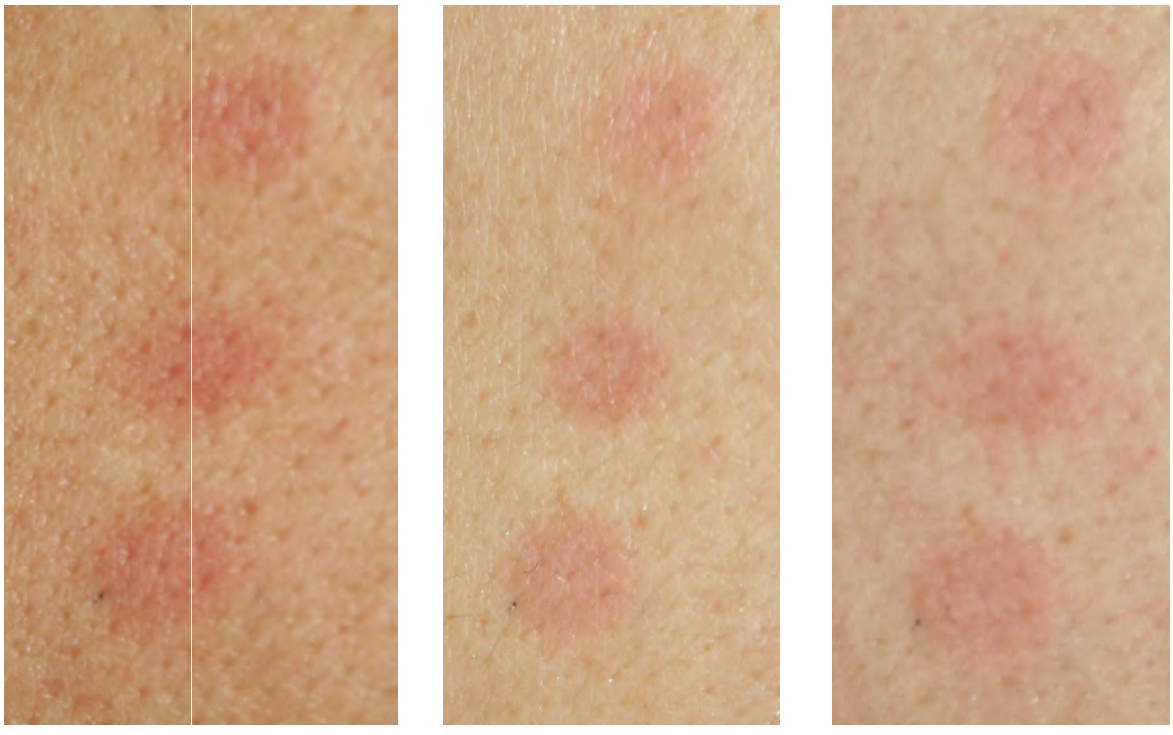
Volunteer ANMA 536

T0 | T0 + 30min | T0 +120 min



Volunteer ROTE 181

T0 | T0 + 30min | T0 +120 min



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CONCLUSION & COSMETIC BENEFITS

SUN'ALG® is based on an innovative natural combination of

- ➤ *Pongamia glabra* seed oil that provides a primary shield against UV radiations, acting as a natural sunscreen due to its important absorption ability especially for UVB but also for UVA, and
- ➤ Two microalgal extracts made from *Dunaliella salina* and *Haematococcus pluvialis*, two microalgae rich in different carotenoids.

This cooperative combination brings a superior and efficient protection against damaging effects of sun exposure. It is stable to temperature and UV radiation and offers free scavenger potential.

Several studies including cosmetogenomic, *in vitro*, *ex vivo* and *in vivo* analysis have successfully proven the beneficial effects of SUN'ALG® that

- ∗ strengthens the skin'defence mechanisms by up regulating various genes linked to the oxidative stress, DNA repair, the antimicrobial defence and the reinforcement of the barrier function,
- ∗ reduces inflammatory skin responses which take place in UV-exposed skin, causing premature skin aging by inhibiting the release of IL-8,
- ∗ provides reinforced protection against both UVA and UVB radiation,
- ∗ prevents the formation of sun burn cells with a high efficacy,
- ∗ minimizes redness and soothes sunburns (erythema) with a short term efficacy (after 30 min, 1 hour and 2 hours from application).

This finally leads to

- ➤ provide an advanced protection against daily attacks against harmful UV rays and
- ➤ maintain skin health and good look by helping to prevent the appearance of lines and wrinkles.

COSMETIC APPLICATIONS

Skin care for daily protection
Sun care
Anti-aging products.

Recommended use level: 2 - 5%.

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ANNEX

Evaluation of ocular irritation

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N° d'étude : 601892F01
Version : 01
Page 1 sur 13
P04.3.DPL.00014.07

RAPPORT D'ETUDE

GELYMA
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Le 28 juillet 2014

EVALUATION DU POTENTIEL IRRITANT D'UN PRODUIT PAR APPLICATION SUR LA MEMBRANE CHORIO-ALLANTOÏDIENNE DE L'ŒUF DE POULE :
Méthode du Het Cam

SUMMARY

The HET-CAM test is an organotypic method to detect the potential irritancy of compounds applied on the surface of the chorioallantoic membrane (CAM) of a fertilized hen's egg. The CAM is a vascular foetal membrane which represents an in vitro model to analyse the effects induced by chemicals that *in vivo* are observed on the conjunctiva.

The principle of this test is based on a visual observation, by a trained person, of the possible end-points (hyperaemia, haemorrhaging, coagulation / thrombosis) that may appear during the five minutes that follow the application of the product on this membrane.

This method is registered in the Official Journal of French Republic (JORF - Decree of 5 April 1971 modified by the decree of 29 November 1996).

In the performed experimental conditions, the **product SUN'ALG, referenced KA-PI**, tested by the HET-CAM method at 100 % and according to the JORF classification, is considered as **slightly irritant**.

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N° SIRET : 33761796300083 – Code APE : 7120 B

Evaluation of cutaneous irritation

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N° Etude : 601891F01
Version : N° 1
Page : 13/16
P05.0.DOC.00017.07

STUDY SUMMARY

ASSESSMENT OF SKIN TOLERANCE OF A COSMETIC PRODUCT AFTER A SINGLE APPLICATION UNDER SEMI-OCCLUDED PATCH DURING 48H ON 10 VOLUNTEERS:
48 hours patch tests

- **Product tested:** SUN'ALG
- **Promotor:** Liliane PELLEGRINI, GELYMA
- **Objective:** Assessment of the skin local tolerance of the studied product after an epicutaneous test performed in semi-occluded conditions, during 48 hours, on healthy adult volunteers.
- **Investigator:** Doctor Chantal SOULIE-REGNIER, dermatologist
- **Place of the study:** EUROFINS ATS
  Pôle d'activité Aix-Les-Milles - ACTIMART
  4 allée des Informaticiens
  1140 rue André Ampère
  13851 AIX EN PROVENCE cedex 3
- **Dates of study:** from 09/07/2014 to 11/07/2014
- **Method:**

✓ ***Application:***
Area: on the back
Quantity of product: 0.02 mL
Frequency and duration: only one application during 48 hours
Conditions of application: product applied pure under semi-occluded patch.

✓ ***Assessment method:***
A dermatologist performs the clinical observation, after the removal of the patches. 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 volunteer, allowing to rank the product from "non irritant to very irritant". The assessment is always made by comparison with the "negative" control.

- **Panel:** 12 healthy adult volunteers.
- **Result:** The average irritant score of the product is 0.083.
- **Conclusion:**

According to the experimental conditions of the study, the SUN'ALG product, referenced KA-PI BATCH 1312200, can be considered as very slightly irritant regarding its primary skin tolerance.

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N° SIRET : 33761796300083 – Code APE :7120B

Evaluation of phototoxic potential

eurofins | ATS

N° d'étude: 659884F01.doc
Version : 1
Page : 1
P05.0.DOC.00050.03

STUDY SUMMARY

TITLE: CLINICAL EVALUATION OF THE PHOTOTOXIC POTENTIAL OF A PRODUCT «SUN'ALG LOT 14 09 100 (491051)», ON 10 SUBJECTS DURING 5 DAYS.

STUDY REFERENCE: ES-1311

PRODUCT: SUN'ALG LOT 14 09 100 (491051)

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

INVESTIGATOR: Dr Marlena NOWAKOWSKA

PROTOCOL: CLINICAL EVALUATION OF THE PHOTOTOXIC POTENTIAL OF A COSMETIC

AIM OF THE STUDY: To evaluate the phototoxic potential of a cosmetic product (tested pure) under dermatological control and under the conditions defined by study's sponsor.

SUBJECTS: 10 healthy volunteers with normal skin corresponding to the inclusion and non-inclusion criteria defined by LISKIN.

STUDY SCHEDULE: November 17th to 21st, 2014

EXPERIMENTAL DESIGN: simple blind and monocentric study.

MAIN TOLERANCE PARAMETERS:

- Irritation potential with and without irradiation
  Erythema, oedema, desquamation, vesicles rated from 0 to 3 by the dermatologist
- Phototoxic potential
  Comparison between irradiated and non irradiated zone

RESULTS:

PRODUCT ZE	PHOTOTOXIC POTENTIAL
SUN'ALG LOT 14 09 100 (491051)	Non-phototoxic (PhTxPo = 0.00)

CONCLUSION:
Under these study conditions, the product «SUN'ALG LOT 14 09 100 (491051)» had a score lower than 0.160 and so it can thus be considered non-phototoxic.

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