ACTISEANE®

A unique combination
of natural algal growth substances

*

Reinforces skin metabolism

Keeps vitality to mature skin

Gelyma

The data presented in this document ACTISEANE® (pp. 1-19)
are offered solely for your consideration and investigation.
No guaranty is expressed or implied.

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

GELYMA – Parc d'Affaires Marseille Sud (C4) – 1 Boulevard de l'Océan – 13009 Marseille – France.
Phone (33) 496 140 982 – Fax (33) 496 140 983 – E-mail : gelyma @ wanadoo.fr.

SUMMARY

GELYMA – Parc d'Affaires Marseille Sud (C4) – 1 Boulevard de l'Océan – 13009 Marseille – France.
Phone (33) 496 140 982 – Fax (33) 496 140 983 – E-mail : gelyma @ wanadoo.fr.

INTRODUCTION

Aging is an inevitable, progressive and irreversible degeneration of biological functions. It is universal among all living organisms.

Like all organs, the skin is affected by the aging process.

Skin changes become visible over the years. They are due to a combination of several factors occurring during

- intrinsic aging which is largely genetically determined
- extrinsic aging caused by environmental exposure.

Intrinsic aging (chronological aging) is defined by the clinical, histological and physiological decrements that occur in sun-protected skins of older individuals and affect specially rate of epidermal turnover, dermal thickness, thermoregulation, mechanical protection, immune responsiveness, sensory perception, sebum production, vascular activity (Gilchrest & Yaar, 1992 – Br. J. Dermatol. 127 (suppl. 41): 25- 30; Gilchrest, 1996 – Br. J. Dermatol. 135: 867-875).

Extrinsic aging (photo-aging) is the superposition of photo-damage on the aging process. It is caused by frequent and prolonged UV radiation exposure, smoking, wind... It results breakdown of collagen and elastic fibres in the skin, fine and coarse wrinkling, irregular pigmentation, dryness, roughness.

Other effects may be occurred over such as hormonal changes, especially during the menopause of women.

Over the years,

- The skin loses its elasticity because the dermis produces fewer and fewer collagen and elastin fibers. It results the weakening of connective tissue and the apparition of wrinkles.
- The skin becomes drier and thinner. As a consequence, it can no longer retain enough moisture and protective coating.
- The skin defends itself with difficulty against the attacks of free radicals for the natural defence system gradually declines.
- The skin develops actinic damage in the epidermis as well as in the dermis caused by chronic exposure to ultraviolet light.

It is thought that the menopause is not associated with any specific changes in the skin. However, the decrease of oestrogen levels would be partly responsible for the dryness, thinning and decreased elasticity of skin seen (Bleiker & Graham-Brown, 1999 – J. Br. Meno. Soc. 5:111-115).

Use of hormone replacement therapy has been shown to improve many of these parameters (Bonté, 2001 – Phytothérapie 25-28) especially phytoestrogens, which mimic the action of normal sex hormones are employed for various skin care treatments.

GELYMA – Parc d'Affaires Marseille Sud (C4) – 1 Boulevard de l'Océan – 13009 Marseille – France.
Phone (33) 496 140 982 – Fax (33) 496 140 983 – E-mail : gelyma @ wanadoo.fr.

Phytoestrogens are plant chemicals naturally found in higher plants, but neither in fungi or algae. In seaweeds, scientists have vainly researched compounds acting as phytoestrogens, such as flavonoids (*cf* Glombitza, 1977 in Marine Natural Products Chemistry: 191-204, Plenum Press, N.Y.; Markham, 1988 – Techniques of flavonoid identification, London, Academic Press).

However, interesting phytohormones (plant growth substances) are present there.

Plant growth substances are chemicals which regulate growth and development of higher plants. They act at concentrations lower than for nutrients (*e.g.* vitamins).

They belong to different chemical groups.

Auxins frequently show an indole structure.
Gibberellins are cyclic diterpenes.
Abscissic acid is a small sesquiterpene.

Moreover, it is well known, since 1956, that the phytohormones of synthetic origin induce stimulating activity without incompatibility for skin (Rovesti & Cocchini, 1956 – Parfumerie moderne, 48:86-91).

Although algae are regarded as a less advanced group of plant than higher plants in spite of the great diversity in morphology and in biochemical systems, it is well established that they contain the same plant growth substances as those present in higher plants (Bradley, 1991- J.Phycol., 27:317-321; Evans & Trewavas, 1991 – J. Phycol. 27:322-326).

Several types of plant growth substances have been identified there, *e.g.* abscisic acid, auxins (indoleacetic acid, phenylacetic acid), cytokinins, gibberellins.

However, it is important to recall that algal phytohormones are not comparable to phytoestrogens. There are no flavonoid in seaweeds such as genistein.

By taking advantage and synergizing the properties of two aqueous extracts prepared from the symplasm of the brown seaweeds *Ascophyllum nodosum* and *Halopteris scoparia*, both rich in plant growth substances (phytohormones), it has been possible to develop

ACTISEANE®

that can help counter several factors that contribute to skin aging including slower cellular turnover in the epidermis and dermis, deterioration of the dermal fibres and prevention of dryness.

ACTISEANE® also modulates the cytokine balance, and consequently strengthens skin wound healing and soothes inflammation.

GELYMA Patent FR 2 837 386.

GELYMA – Parc d'Affaires Marseille Sud (C4) – 1 Boulevard de l'Océan – 13009 Marseille – France.
Phone (33) 496 140 982 – Fax (33) 496 140 983 – E-mail : gelyma @ wanadoo.fr.

ALGAL SOURCE

Ascophyllum nodosum (Linnaeus) Le Jolis

Ascophyllum nodosum (Linnaeus) Le Jolis (from Greek *askos*: wine-skin and *phullon*: leaf) belongs to the phylum *Heterokontophyta*, the class *Phaeophyceae,* the order Fucales and the family *Fucaceae*.

➤ Morphology & Biology

The thallus (Fig.1) appears bushy, irregularly branched with bluntly serrated margins. It is fixed to substratum by means of a disciform basal holdfast. It is yellow-brown to olive-green in colour and can reach 150 cm in length and approximately 1cm in width.

Large and single air bladders are individually placed as occasional swellings at intervals along axes. There is no midrib.

The stalked receptacles are globular or oval, yellowish in colour.
This alga is perennial and dioecious.

Fig. 1 - Morphology of *Ascophyllum nodosum in situ.* Photo GELYMA.

➤ Ecology & Geographical distribution

Ascophyllum nodosum occurs abundantly on rocks and boulders from the high-water mark to the half-tide level in sheltered bays exposed at low tide.

It is present in the European Northern Atlantic and along the Atlantic coasts of Canada. It is absent along the rocky shores of the Bay of Biscaye.

➤ Composition in marine growth substances

The aqueous extracts of *Ascophyllum nodosum* contain

- auxins, especially indoleacetic acid (Kingman & Moore, 1982 – Bot. Mar. 25 : 149-153 ; Sanderson & Jameson, 1986 – Acta Hortic. 179: 113 ; Sanderson *et al.*, 1987 – J. Plant. Physiol. 129: 363),
- gibberellins (Williams *et al*., 1981 – Proc. VIII th Int. Seaweed Symp. : 760-763),
- cytokinins, especially adenine (Kingman & Moore, 1982 – Bot. mar. 25 : 149-153), cis- et trans-zeatine ribosides, trans-zeatine dihydrozeatine (Featonby *et al.*, 1984 – Bot. mar. 27 : 527-531 ; *Tay et al*., 1985 –Phytochemistry 24 : 2611-2614 ; Tay *et al*., 1987 – J.Plant Growth),
- abscisic acid (Kingman & Moore, 1982 – Bot. mar. 25 : 149-153 ; Boyer & Dougherty, 1988 – Phytochemistry 27 : 1521-1522),
- betaïnes, especially glycine betaïne, γ -aminobutyric betaïne acid, δ- aminovaleric betaïne acid, laminine (Blunden *et al*., 1986 – Bot. mar. 29 : 155-160 ; Blunden *et al*., 1992 – Biochem. Systematics and Ecol. 20: 373-388).

GELYMA – Parc d'Affaires Marseille Sud (C4) – 1 Boulevard de l'Océan – 13009 Marseille – France.
Phone (33) 496 140 982 – Fax (33) 496 140 983 – E-mail : gelyma @ wanadoo.fr.

➤ Utilizations

This alga is called "Knotentang" in Germany, "Knobbed wrack, Yellow Tang or Pigweed" in England,"Griseteng, Knuppetag" in Norway and "Goémon noir" in France.

Its use is ancient. As early as the XVIIth century, it was collected to manufacture soda and iodine. Today, it is a raw material in the alginate industry. It serves in human and animal nutrition and as fertilizer.

Halopteris scoparia (Linnaeus) Sauvageau

Halopteris scoparia (Linnaeus) Sauvageau (from Greek *hals*: sea and *pteron*: wing) belongs to the phylum *Heterokontophyta*, the class *Phaeophyceae,* the order Sphacelariales and the family *Stypocaulonaceae*.

It is also known as *Stypocaulon scoparium* (Linnaeus) Kützing (from Greek *stupa* : the coarse part of flax and *kaulon* : stem and from Latin *scoparius* : broom).

It is named "Seabroom" in England, "Balai de Triton" in France and "Escobilla de mar" in Spain.

➤ Morphology & Biology

The thallus forms large and compact branched tufts, resembling small brooms (Fig.2). It is fixed to substratum by means of rhizoids which show a basal spongious structure. It appears green-brown in colour and can reach 8-15cm in height.

Each axis shows a characteristically prominent apical cell filled with tanniferous compounds. Fructification occurs during winter.

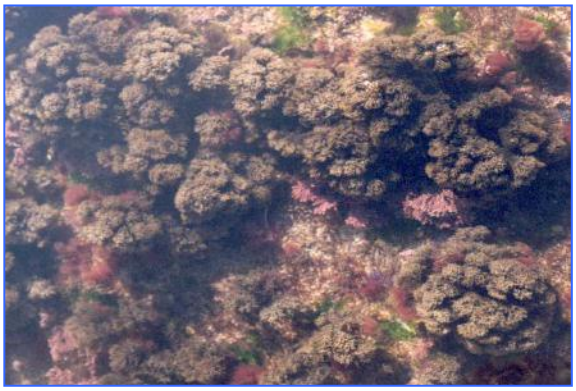


Fig.2 - Population of *Halopteris scoparia*
Photo GELYMA.

➤ Ecology & Geographical distribution

This alga is common and abundant on submerged rocks between the tide-marks and in deeper water. It is present in the English Channel, the Atlantic (from Scandinavia to Marocco) and in the Mediterranean.

➤ Composition in marine growth substances

Halopteris scoparia also contains auxinic substances in significant quantities (Augier, 1970 – C. r. Acad. Sc. Paris, 270 : 3311-3314), especially:

- indolyl-acetonitrile (IAN) (120 µg per kg of fresh algae)
- ethyl indolyl-acetate (IAE) (105 µg) and their precursor tryptophan (45µg).

➤ Utilization

No industrial use is known for this algal species.

GELYMA – Parc d'Affaires Marseille Sud (C4) – 1 Boulevard de l'Océan – 13009 Marseille – France.
Phone (33) 496 140 982 – Fax (33) 496 140 983 – E-mail : gelyma @ wanadoo.fr.

THE ACTIVE INGREDIENT ACTISEANE®

Specifications

on a control batch

- appearance : limpid liquid amber coloured
- odour : typical
- pH : 6.1 ± 1.0
- density : 1.011 ± 0.010
- dry residual (%) : 1.9 ± 0.3
- solubility : soluble in ethanol, propylene glycol, butylene glycol
  : insoluble in oils.
- microbiology : bacteria : < 100 germs / ml.
  : yeasts, moulds : < 100 germs / ml.
  : pathogens : free.

Composition

Ingredients		Amount (%)
Brown algae	*Ascophyllum nodosum + Halopteris scoparia* extract	54
Solvent	water	46
Preservative	*as required*	
Others (antioxidants …)	none	

INCI names
water
Ascophyllum nodosum extract
Halopteris scoparia extract

CAS n° 7732-18-5
CAS n° 84775-78-0

EINECS n° 231-791-2
EINECS n° 283-907-6

Storage

ACTISEANE® 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, ACTISEANE® remains stable for at least 18 months.

Pack size: 1kg – 5 kg- 10kg

Safety

No animal experimentation.

Standard safety testing has proved that ACTISEANE® is safe for cosmetic use.

ACTISEANE ® does not exhibit a potential for ocular irritation and dermal irritation at the recommended use levels.

cf. Annex pp. 15-18.

GELYMA – Parc d'Affaires Marseille Sud (C4) – 1 Boulevard de l'Océan – 13009 Marseille – France.
Phone (33) 496 140 982 – Fax (33) 496 140 983 – E-mail : gelyma @ wanadoo.fr.

EFFECTIVENESS EVALUATION

ACTISEANE®

Biostimulating properties	Stimulation of keratinocytes proliferation MTT test
	Stimulation of fibroblasts proliferation and metabolism MTT test
Protection of the dermal matrix fibres against proteases deterioration	Anti-collagenase activity Spectrophotometric determination
	Anti-elastase activity Spectrophotometric determination
Hydration of the basal epidermis layers	Anti-hyaluronidase activity Spectrophotometric determination
Protection against UV irradiation	Stimulation of cell viability *In vitro* study on reconstituted skins
Regulation of cytokine balance	Regulation of the IL6 production *In vitro* studies on keratinocytes & reconstituted skins- SkinEthic model
	Inhibition of the IL activity *In vitro* studies on keratinocytes & reconstituted skins- SkinEthic model

A new algal approach
for
treating aging & sun damaged skins

GELYMA – Parc d'Affaires Marseille Sud (C4) – 1 Boulevard de l'Océan – 13009 Marseille – France.
Phone (33) 496 140 982 – Fax (33) 496 140 983 – E-mail : gelyma @ wanadoo.fr.

Biostimulating properties

Aging reduces the ability of keratinocyte proliferation. The epidermis becomes irregularly flattened. Its thickness decreases.

Dermal thickness decreases too. Fibroblasts reduce their number and metabolic activities. They appear globular.

Proliferation of human keratinocytes

Method

Cultivation of human keratinocytes in a suboptimal medium impoverished in nutrients and supplemented with ACTISEANE® (3 concentrations: 2%, 4% and 6%).

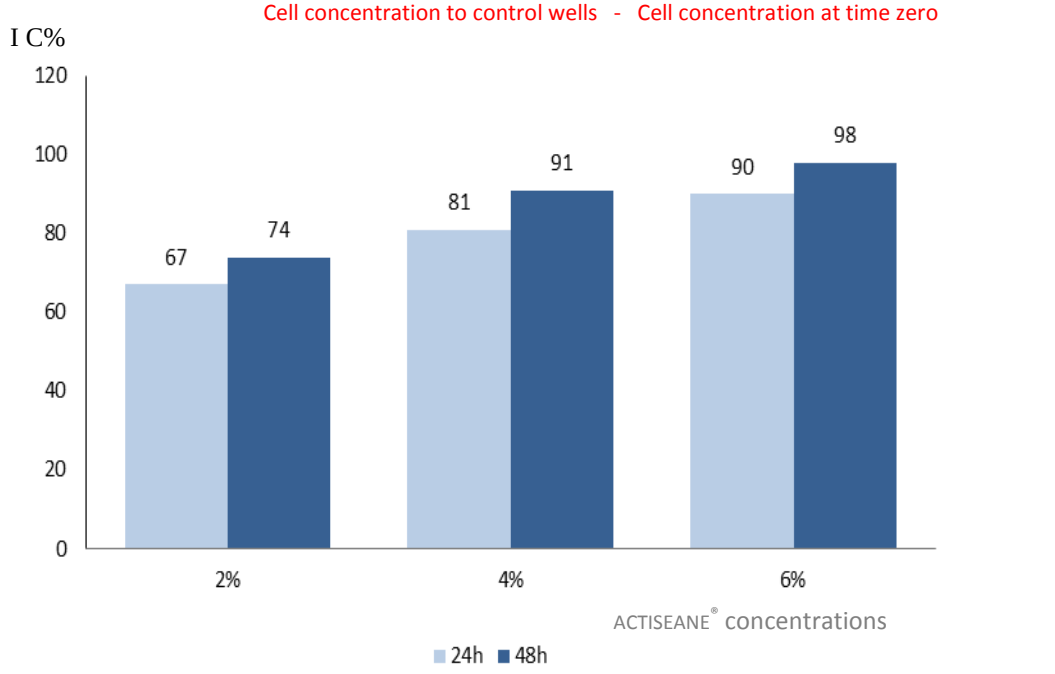
Control is carried out with the suboptimal medium only. After 24h & 48h cultivation, cells quantification by using dye exclusion assay.

Results

Results represent the average of 2 experiments with 6 values for control and each concentration ($\alpha = 0.05$).

The evaluation of the growth index (IC %) is determined from the following equation :

$$IC = \frac{\text{Cell concentration to treated wells} - \text{Cell concentration at time zero}}{\text{Cell concentration to control wells} - \text{Cell concentration at time zero}} \times 100$$



➤ACTISEANE® stimulates the proliferate capacity of keratinocytes.

GELYMA – Parc d'Affaires Marseille Sud (C4) – 1 Boulevard de l'Océan – 13009 Marseille – France.
Phone (33) 496 140 982 – Fax (33) 496 140 983 – E-mail : gelyma @ wanadoo.fr.

Proliferation of fibroblasts

Method

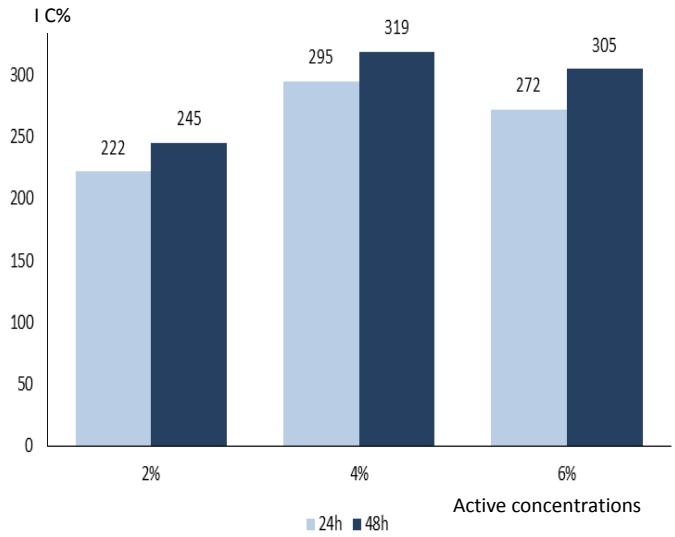
Cultivation of fibroblasts in suboptimal medium with 2.5% foetal calf serum and ACTISEANE® (3 doses: 2%, 4% and 6%).

Control is carried out with the suboptimal medium only. After 24h & 48h cultivation, cell quantification after trypsinization and dye exclusion assay.

Results

Results represent the average of 2 experiments with 6 values for control and each concentration ($\alpha = 0.05$).

Growth index (IC %) is determined by means of the equation previously quoted (*cf.* p. 7).



Stimulation of the mitochondrial activity of fibroblasts

Method

Cultivation of human fibroblasts in an optimal medium followed by suboptimal medium supplemented with ACTISEANE® according to 4 concentrations: 2% - 4% - 6% and 8%.

Two controls without ACTISEANE® are performed, one with the optimal medium, the other with the suboptimal medium. After 24 h treatment, cells are subjected to the MTT assay.

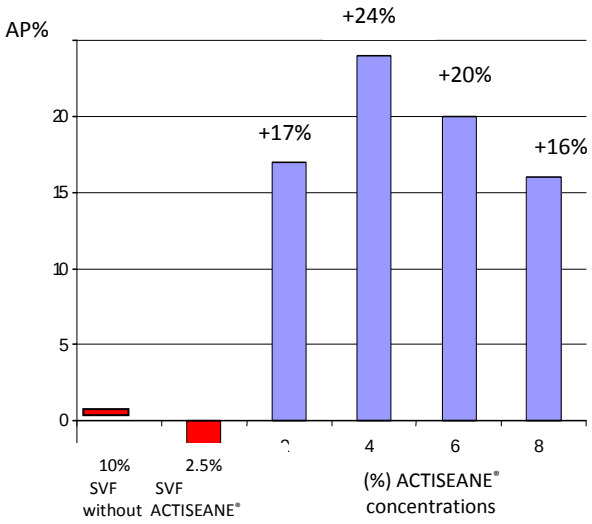
Results

Results represent the average of 2 experiments with 6 values for control and each concentration ($\alpha = 0.05$).

The proliferative activity (AP%) is evaluated

$$AP\% = \frac{\text{OD treated wells} - \text{OD control suboptimal}}{\text{OD control optimal} - \text{OD control suboptimal}} \times 100$$

This equation takes into consideration all parameters of cultivation.



➤ACTISEANE® stimulates the proliferation and the mitochondrial activity of fibroblasts.

GELYMA – Parc d'Affaires Marseille Sud (C4) – 1 Boulevard de l'Océan – 13009 Marseille – France.
Phone (33) 496 140 982 – Fax (33) 496 140 983 – E-mail : gelyma @ wanadoo.fr.

Stimulation of the mitochondrial activity of reconstituted skins

Method

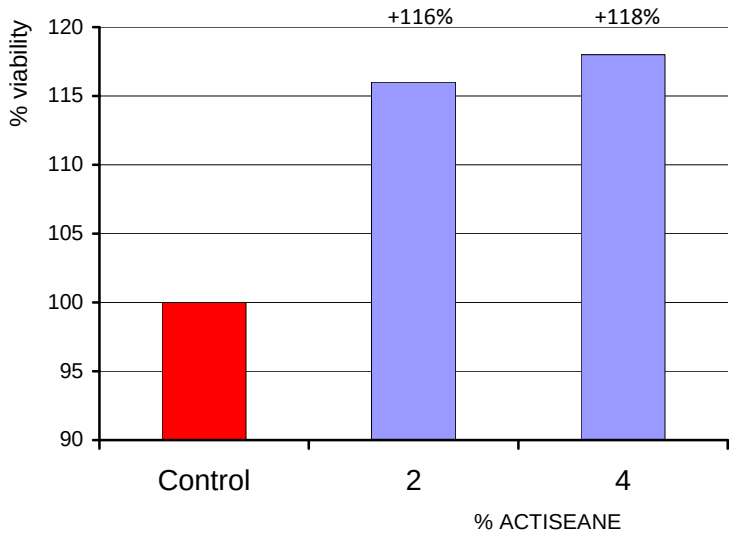
Reconstituted skins (SkinEthic model) are cultured in the presence or the absence of ACTISEANE®.

The MTT test is performed 48 hours after. Optical density is measured in triplicate.

Results

Results are expressed as percentage of viability.

ACTISEANE® enhances the mitochondrial activity of reconstituted skins.



With 2% ACTISEANE®, this stimulation reaches up to +16% compared to control.

Results are validated by two statistical analysis: analysis of variance ANOVA and least significant difference.

- very highly significant difference between ACTISEANE® and control *** $p < 0.001$.
- non significant difference between 2% and 4% ACTISEANE® NS $p > 0.05$.

Accordingly, the recommended use level is 2%.

➤ ACTISEANE® stimulates skin metabolism

By increasing cellular metabolism (keratinocytes & fibroblasts),
ACTISEANE® helps to epidermal restructuration and dermal structure reinforcement.

GELYMA – Parc d'Affaires Marseille Sud (C4) – 1 Boulevard de l'Océan – 13009 Marseille – France.
Phone (33) 496 140 982 – Fax (33) 496 140 983 – E-mail : gelyma @ wanadoo.fr.

Protection of the dermal matrix fibres against protease deterioration

In the skin, elastic fibres with collagen fibres constitute a network developing under the epidermis.

During aging, their organisation change. Collagen fibres run predominantly parallel to the dermo-epidermal junction and the skin surface. Elastic fibres decrease in number and diameter. With aging, the elasticity of skin is significantly decreased by elastase activity (which increases) and that results in sagging and wrinkling.

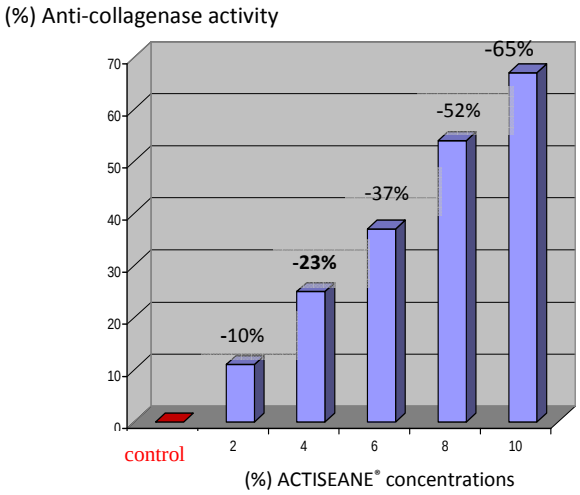
Anti-collagenase activity

Method

The method used (Kit Sircol, Biocolor Ireland) is based on the ability of the dye Sirius Red to quantitatively precipitate collagen.

Results

Results represent the average of 3 values for control and each concentration
(α = 0.05).



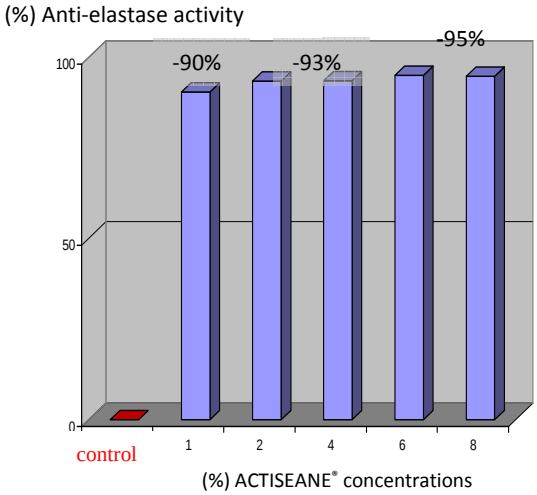
Anti-elastase activity

Method

Porcine pancreatic elastase is assayed spectrophotometrically using [N-succ-(Ala) 3-p-nitroanilide] as the substrate and monitoring the release of p-nitroaniline for 20 min at 25°C.

Results

Results represent the average of 3 values for control and each concentration tested
(α = 0.05).



➤ ACTISEANE® presents dose-dependent anti-collagenase and anti-elastase activities.

ACTISEANE® inhibits the release of proteases that leads to the breakdown of connective tissue.

GELYMA – Parc d'Affaires Marseille Sud (C4) – 1 Boulevard de l'Océan – 13009 Marseille – France.
Phone (33) 496 140 982 – Fax (33) 496 140 983 – E-mail : gelyma @ wanadoo.fr.

Hydration of the basal epidermis layers

The hyaluronic acid constitutes one of the most important structural and functional elements of numerous tissues.

It acts as a “true molecular sponge” because it can absorb a large quantity of water. Its turnover is very high for 24 hours, due to hyaluronidase hydrolysis.

It is important in controlling tissue hydration (Block & Bettelheim, 1970 – Biochem. Biophys. Acta, 201:69).

During aging, its quantity decreases, modifying the viscoelasticity of the skin. In the senescent epidermis, it is even not detectable (Meyer & Stern, 1994 – *Ibid*).

Consequently, it is necessary to prevent hyaluronic acid hydrolysis.

Anti-hyaluronidase activity

Hyaluronidases are endoglucoaminidases which degrade hyaluronan and to a lesser extent other glycosaminoglycans.

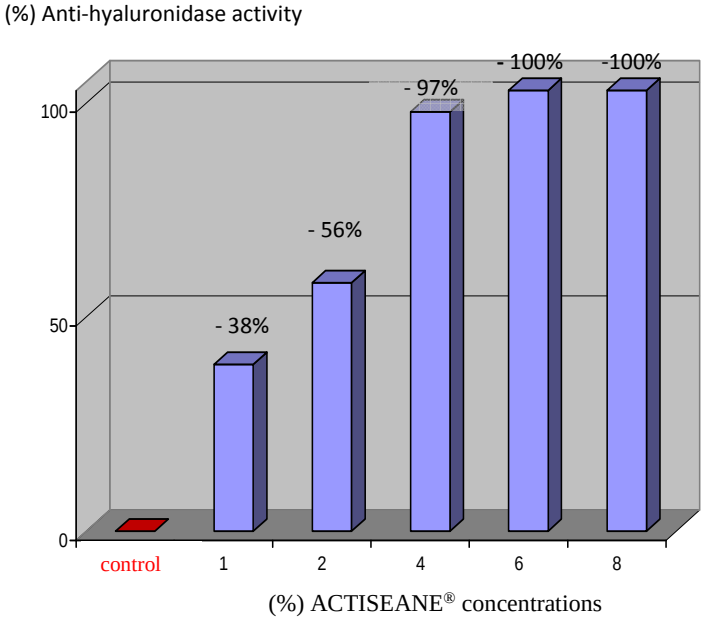
Method

Hyaluronidase activity is determined spectrophotometrically according to the method of Reissig *et al.*, (1955- J. Biol. Chem., 217:959-969).

Results

Results represent the average of three experiments for control and each concentration tested ($\alpha = 0.05$).

They are expressed in % of anti-hyaluronidase activity in presence of 5 concentrations in ACTISEANE® (1% – 2% – 4% – 6% and 8%) compared to control without algal extract.



➤ ACTISEANE® shows a dose-dependent anti-hyaluronidase activity.

Thus, ACTISEANE® preserves moisture balance in the epidermis

GELYMA – Parc d’Affaires Marseille Sud (C4) – 1 Boulevard de l’Océan – 13009 Marseille – France.
Phone (33) 496 140 982 – Fax (33) 496 140 983 – E-mail : gelyma @ wanadoo.fr.

Protection against UVB irradiation

UVB irradiation induces the dangerous formation of sunburn cells. It accelerates premature skin aging and the formation of wrinkles.

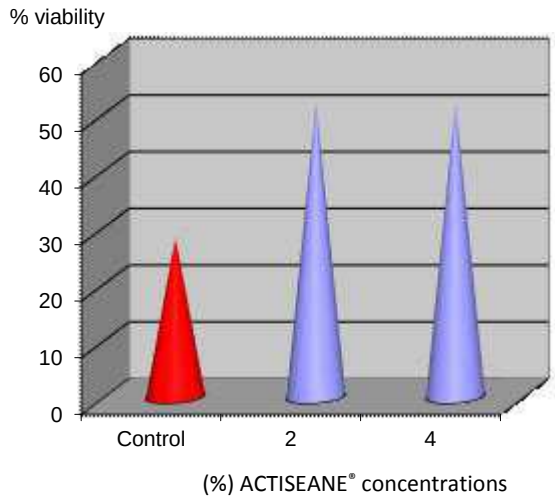
Method

Reconstituted skins (SkinEthic model) are cultured for 5 hours in the presence or the absence of ACTISEANE®.

The UVB irradiation (dose: 300 mJ/cm²) is performed on skins in PBS only. The MTT test performed 48h after irradiation.

Results

This graph shows that 2% ACTISEANE® increases by 24% the viability of reconstituted skins, compared to irradiated control (in red).



Results are validated by two statistical analysis: analysis of variance ANOVA and least significant difference.

- very highly significant difference between ACTISEANE® and control *** $p < 0.001$.
- non significant difference between 2% and 4% ACTISEANE® NS $p > 0.05$.

Accordingly, the recommended use level is 2%.

➤ ACTISEANE® induces a very highly significant protection against UVB,

known to induce the formation of sun burn cells and accelerate premature aging..

GELYMA – Parc d'Affaires Marseille Sud (C4) – 1 Boulevard de l'Océan – 13009 Marseille – France.
Phone (33) 496 140 982 – Fax (33) 496 140 983 – E-mail : gelyma @ wanadoo.fr.

Regulation of cytokine balance

The role of cytokines is essential at the skin level. Some cytokines give positive effect whereas others ones must be inhibited.

The cytokine IL6 is involved in numerous biological processes. In vitro, IL6 stimulates collagen synthesis and the proliferation of keratinocytes and fibroblasts. In vivo, it induces thickness of the *stratum corneum* and helps wound healing.

The cytokine IL1α is released in the early phase of inflammation.

Methods

In the following in vitro tests, IL6 and IL1α are quantified by using ELISA kits (Bender MedSystems, Vienna) on the culture medium of keratinocytes or reconstituted skins (SkinEthic model).

Results

Stimulation of IL6

This table indicates the data (in pg IL6/ml.10000 cells^{-1}) from human keratinocytes submitted or not to UVB irradiation (dose: 20mJ/cm²).

ACTISEANE® (%)	0%	2%	3%	4%
IL6 baseline production	7.42	8.12	7.65	8.60
IL6 induced production	34.69	37.70	41.80	45.79
% increase		+8.7	+20.5	+32

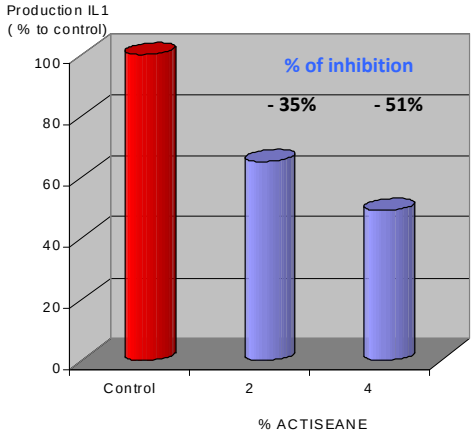
The analysis of variance (n = 4) shows that the values of the baseline production of IL6 are not significantly different.

It also proves the action of ACTISEANE® is very highly significant on the induced production of IL6 in irradiated cells.

Inhibition of IL1 α

This graph demonstrates that ACTISEANE® inhibits the IL1α production in reconstructed skins submitted to UVB irradiation (dose: 300mJ/cm²).

Note 2% ACTISEANE® give inhibition (%) of 35 .



➤ ACTISEANE® regulates the cytokine balance by

- ♦ activating IL6 what improves keratinisation and skin repair
- ♦ inhibiting IL1α synthesis what limits inflammation.

➤ As a result, ACTISEANE® reinforces skin metabolism and repair.

GELYMA – Parc d'Affaires Marseille Sud (C4) – 1 Boulevard de l'Océan – 13009 Marseille – France.
Phone (33) 496 140 982 – Fax (33) 496 140 983 – E-mail : gelyma @ wanadoo.fr.

CONCLUSION & COSMETIC BENEFITS

ACTISEANE® is a patented marine agent based on the synergistic association of two aqueous and calibrated extracts prepared selectively from *Ascophyllum nodosum* and *Halopteris scoparia*, both brown seaweeds being rich in polyphenols and plant growth substances (auxins, gibberellins, cytokinins, abscissic acid).

Thanks to its unique composition in marine plant growth substances, ACTISEANE® targets against both intrinsic aging and extrinsic aging due to UVB induced damage.

It boosts the skin metabolism.

It inhibits elastase, the enzyme which breaks down elastin in the skin.

It also inhibits hyaluronidase activity. This helps reduce skin dryness.

At least ACTISEANE® modulates the cytokine balance, and consequently strengthens skin wound healing and soothes inflammation.

As results, the skin appears fortified and replenished.

GELYMA Patent FR 2 837 386.

COSMETIC APPLICATIONS

This makes ACTISEANE® an effective ingredient for all care products intended for overstretched, tired or mature skins characterized by decreased thickness, dryness and photo-aging signs caused by UV irradiation.

It is also suitable for:

- Face and neck care.
- Repairing and restructuring skin care.

Recommended use levels: 1% - 4%.

GELYMA – Parc d'Affaires Marseille Sud (C4) – 1 Boulevard de l'Océan – 13009 Marseille – France.
Phone (33) 496 140 982 – Fax (33) 496 140 983 – E-mail : gelyma @ wanadoo.fr.

ANNEX

Evaluation of ocular irritation

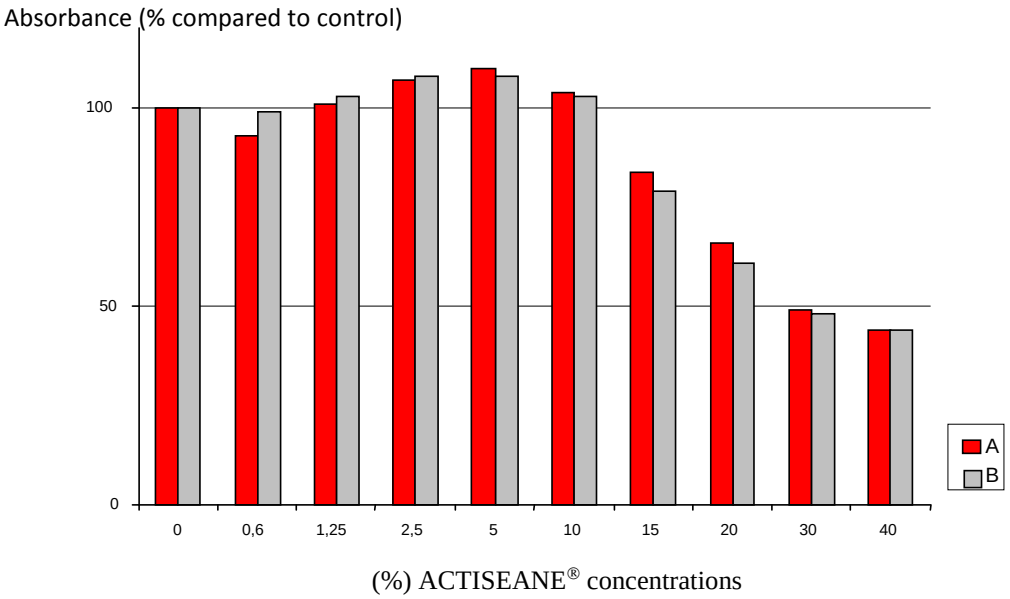
Method

The red neutral uptake test is based on the work of Borenfreud & Puener (J. Tissue Culture Method, 1984 – 9 :7-9).
The fibroblastic line L 929 shows a good correlation with SIRC line cell.
The amount of the vital dye taken up by cells is directly proportional to the number of viable cells in the culture.

Results

After 24h treatment in a medium depleted in nutriments and supplemented with 9 dilutions of ACTISEANE® (two algal collecting batches: A & B), absorbances are measured at 550 nm using a microplate reader.

Results are presented as graphs and expressed as a mean of 12 replicates for each concentration (□= 0.005). Control represents 100% viability.



➤ Good ocular tolerance of ACTISEANE®

➤ The two collecting batches are well-reproducible.

GELYMA – Parc d'Affaires Marseille Sud (C4) – 1 Boulevard de l'Océan – 13009 Marseille – France.
Phone (33) 496 140 982 – Fax (33) 496 140 983 – E-mail : gelyma @ wanadoo.fr.

eurofins | ATS

N° Etude: 191892F01.doc
Version :N° 1
Page:8
P05.0.DOC.00023.01

STUDY SUMMARY

EVALUATION OF THE POTENTIAL IRRITANCY OF A PRODUCT THROUGH ITS APPLICATION ON THE CHORIOALLANTOIC MEMBRANE OF A CHICKEN EGGSHELL: *Het Cam Method*

- **Tested product** : ACTISEANE
- **Promoter** : GELYMA
- **Objective**: To assess the irritant potential of the tested product
- **Methodology**: The principle of this study is based on the visual observation, by a trained person, of the possible irritations (hyperaemia, haemorrhaging, coagulation / thrombosis) that may appear during the five minutes that follow the application of the said product on the chorioallantoic membrane of an embryonic chicken egg after eleven days of incubation.
- **Dates of study :** 12/12/2006
- **Place of study:** EUROFINS ATS, Pôle d'activité d'Aix en Provence
  Actimart, 1140, rue Ampère,
  13851 AIX EN PROVENCE cedex 3
- **Results :**

Denomination	ATS Reference	Initial concentration	Results	
			Score	Classification
ACTISEANE	167112	100%	0	Practically no irritant

- **Conclusion :**

According to the performed experimental conditions, the product ACTISEANE tested by the HET CAM method, at 100 %, can be considered as practically no irritant regarding its ocular primary tolerance.

GELYMA – Parc d'Affaires Marseille Sud (C4) – 1 Boulevard de l'Océan – 13009 Marseille – France.
Phone (33) 496 140 982 – Fax (33) 496 140 983 – E-mail : gelyma @ wanadoo.fr.

Evaluation of cutaneous irritation

Methods

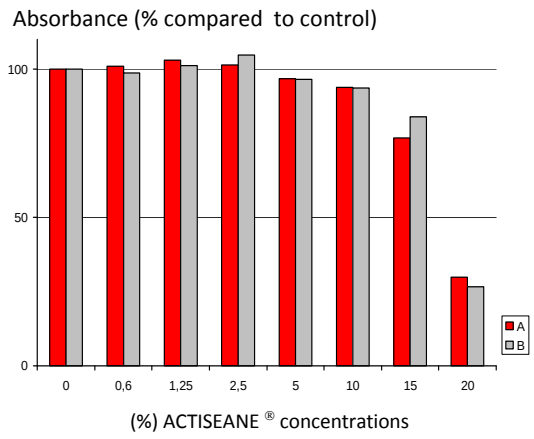
Experiments are performed on two cell types : fibroblasts and human keratinocytes with two batches of algal collections : A and B. The active ACTISEANE® is added to cultivation medium (impoverished in nutriments) during 24hours according to 7 or 9 dilutions : 0.6 – 1.25 – 2.5 – 5 – 10 – 15- 20 - 30 and 40 %. Two colorimetric tests are carried out : total protein determination according to Bradford (1976) and MTT test according to Mosmann (1973) modification Denizot & Lang (1986). Readings are performed using a Dynatech multiwell spectrophotometer.

Results

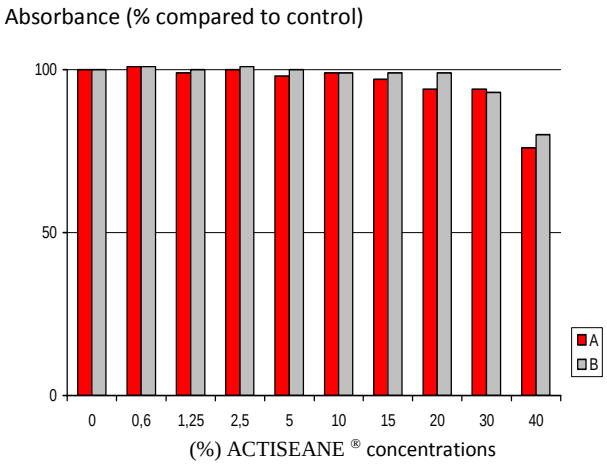
Results are presented as graphs and expressed as a mean of 12 replicates for each concentration (□= 0.005). Control represents 100% viability.

Total protein assay

fibroblasts

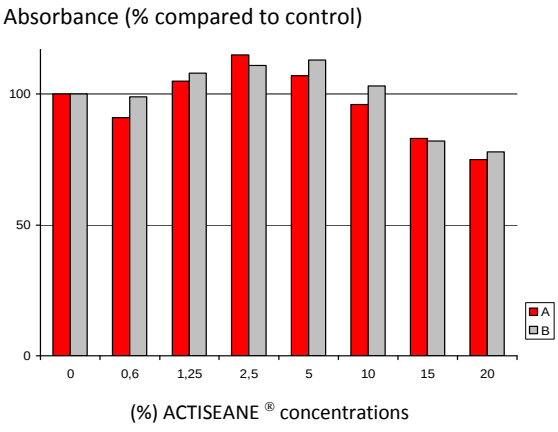


keratinocytes

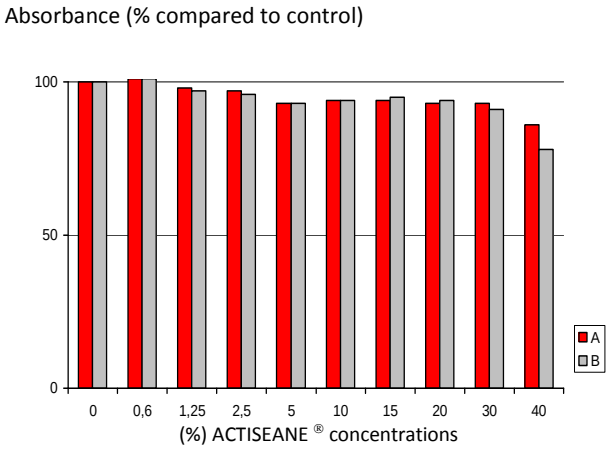


MTT test

fibroblasts



keratinocytes



➤ Good cutaneous tolerance of ACTISEANE®

GELYMA – Parc d'Affaires Marseille Sud (C4) – 1 Boulevard de l'Océan – 13009 Marseille – France.
Phone (33) 496 140 982 – Fax (33) 496 140 983 – E-mail : gelyma @ wanadoo.fr.

eurofins | ATS

N° Etude: 191892F02.doc
Version : N° 1
Page : 15
P05.0.DOC.00017.01

STUDY SUMMARY

EVALUATION OF THE CUTANEOUS TOLERANCE OF A COSMETIC PRODUCT AFTER A SINGLE APPLICATION UNDER OCCLUSIVE PATCH DURING 48 HOURS:
Patch test method

- **Product tested** : ACTISEANE
- **Promoter** : GELYMA
- **Monitor** : Liliane PELLEGRINI, R & D Manager
- **Objective** : Assessment of the cutaneous local tolerance of the studied product after an epicutaneous test performed in occlusive conditions, during 48 hours.
- **Place of the study:** EUROFINS SCIENTIFIC TEST CENTER,
  3 allée des Ingénieurs
  1140 rue André Ampère
  13851 AIX EN PROVENCE cedex 3
- **Investigator** : Doctor Mary CREST
- **Date of study:** from 28/11/06 to 30/11/06 and from 12/12/06 to 14/12/06
- **Methodology:**
  - ✓ ***Application modes:***

Area of application : on the back
Quantity of product : 0.02 ml
Frequency and duration : only one application during 48 hours
Conditions of application : product applied pure under occlusive patch.

- ✓ ***Assessment method:***

A dermatologist makes the clinical observation, after the removal of the patches. The quantification of the cutaneous irritation is given according to a numeric scale (rash, oedema, dryness, blister). The average irritant score of the product to be tested is measured with the average of the quotations obtained for the whole volunteers, allowing ranking the product from "not irritant to very irritant". The assessment is always made by comparison with the "negative" control: patch alone.

- **Population:** 11 healthy adult volunteers.
- **Results:** The average irritant score of the product is 0,0.
- **Conclusion:**

According to the experimental conditions taken into account, after only one application of 0.02 ml of product, under occlusive patch and during 48 hours, on 11 healthy adult volunteers, and according to the scale used for the interpretation of the results, the raw material **"ACTISEANE", Lot 06 08 260, can be considered as not irritant regarding its primary cutaneous tolerance.**

Eurofins Scientific Test Center -Pôle d'activité d'Aix-en-Provence - Actimart - 1140, Rue Ampère - 13851 Aix-en-Provence Cedex 3 - France
TEL +33 (0)4.42.39.78.08 - FAX +33 (0)4.42.39.77.81
N° SIRET : 33761796300067 – Code APE : 743 B

GELYMA – Parc d'Affaires Marseille Sud (C4) – 1 Boulevard de l'Océan – 13009 Marseille – France.
Phone (33) 496 140 982 – Fax (33) 496 140 983 – E-mail : gelyma @ wanadoo.fr.

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

www. gelyma.com

Contact

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

e-mail gelyma @ wanadoo.fr