

RAW MATERIAL INFORMATION

NATURA-TEC MARINE CELLSHIELD AP™

Product identification

Manufacturer: Natura-Tec

Country of origin: France

Custom Tariff: 29159070

Manufacturing process and chemical composition

Manufacturing process:

Natura-Tec Marine CellShield AP is obtained by the careful combination of the Caprylic Capric Triglyceride with the Pavlova Lutheri Extract following a maceration process in a closed container at cold temperature under specific agitation during a controlled time. The final product is then packed under nitrogen

The microalgae inoculum of Pavlova Lutheri is cultivated in closed photobioreactor, in our plant on the Riviera, The bioreactors are provided with appropriate sunlight and CO₂

The inoculum multiplies in a specific culture medium and the microalgae biomass is obtained following a certain time of cultivation.

The biomass is then harvested, dried and controlled.

The biomass is then extracted to obtain the Pavlova Lutheri Extract.

Chemical composition:

INCI/USA	INCI/EEC	CAS No.	EINECS / ELINCS No.	Function	%
Caprylic/Capric Triglyceride	Caprylic/Capric Triglyceride	73398-61-5	277-452-2	Active	QSP
Pavlova Lutheri Extract	Pavlova Lutheri Extract	2101635-97-4	--		< 5%

Our **Natura-Tec Marine CellShield AP** does not contain additives

Impurities:

Impurities	Nature	Specification, ppm
Residual solvents		None
Monomers		None
Heavy metals	Pb	<LOQ (LOQ = 0.05ppm)
	As	<LOQ
	Cd	<LOQ
	Hg	<LOQ
	Cr	<LOQ
	Ni	<LOQ
	Others	$\Sigma < 3$ ppm
Pesticides		None

Decontamination by radioactivity

We hereby certify that the product **Natura-Tec Marine CellShield AP** has not been treated with ionizing radiation.

ISO16128 guideline Information

Substance INCI name	Ingredient type	% mass fraction	Natural %*	Natural index	Natural origin index	Organic index	Organic origin index
Caprylic/Capric Triglyceride	Derived Natural	QSP	100	0	1	0	0
Pavlova Lutheri Extract	Natural	< 5%	100	1	1	0	0
Total Natural %	100 %						

Natural %*: percentage of natural origin ingredient

Reach compliance / CLP classification
Reach (CE regulation n°1907/2006)

INCI/USA	CAS No.	EINECS / ELINCS No.	Pre-registration	Registration number
Caprylic/Capric Triglyceride	73398-61-5	277-452-2	Yes	01-2119492306-35-0012
Pavlova Lutheri Extract	2101635-97-4	--	Exempted	

CLP classification

Natura-Tec Marine CellShield AP is not classified under regulation CE 1272/2008

Ingredient of vegetable origin

General description of the vegetable ingredients		
INCI name of the ingredient of vegetable origin	Caprylic/Capric Triglyceride	Pavlova Lutheri Extract
Name of the vegetable (genus – species - family)	Genus: Cocos Species: C. nucifera Family: Arecaceae	Family: Pavlovaceae Genus: Pavlova Species: Pavlova Lutheri
Part used	Fruit	Microalgae inoculum
Geographical origin	Dominican Republic	North sea
Is the plant cultivated or natural?	Cultivated	Cultivated
Is it a regulated vegetal species (CITES, IUCN red list...)?	No	No

Storage conditions

Packaging: Plastic canisters of 1Kg, 10Kg and 25Kg net.
Storage: Store in a cool dry place, below 10°C, away from light in original unopened packaging.
Shelf life: 12 months in original unopened packaging

Toxicological data

From information available, **Natura-Tec Marine CellShield AP** is non-toxic under normal conditions of use.

Cytotoxicity test – MTT test

MTT - In vitro evaluation of the cytotoxicity of a cosmetic product with an assay on Normal Human Epidermal Keratinocyte (NHEK) cell cultures (UNI/EN ISO 10993-5: 2009 (E))

The MTT assay (colorimetric test) evaluates in vitro the viability of cells exposed to different concentrations of the investigated cosmetic product in comparison with untreated cells.

Results: *Natura-Tec Marine CellShield AP is non cytotoxic from 0,1%, to 2,0% of use.*

Regulatory information - Certificates

Cosmetic directive compliance

In conformance to the Regulation n° 1223/2009 in respect to the use in cosmetic products, **Natura-Tec Marine CellShield AP** is exempted of prohibited substances (Annex II) and restricted substances (Annex III). **Natura-Tec Marine CellShield AP** is exempted of phthalates, nonylphenol, alkylphenols, phenol, nitrosamines, glycol ethers.

GMO certificate

We hereby certify that **Natura-Tec Marine CellShield AP** does not contain ingredients from genetically modified organisms in accordance with EC Regulations 1829/2003 and 1830/2003.

Non-animal testing

We hereby confirm that **Natura-Tec Marine CellShield AP** of our manufacture, has not been tested on animals.

Absence CMR

It is certified that the product **Natura-Tec Marine CellShield AP** does not contain carcinogenic, mutagenic or reprotoxic (CMR) substances of categories 1, 2, 3 or 1A, 1B or 2 listed in regulation 1272/2008 and amendments: commission regulation (CE) n° 790/2009 and n°286/2011.

Absence of allergens certificate

It is hereby certified that the product **Natura-Tec Marine CellShield AP** of our manufacture, does not contain any of the 26 allergens listed in the Cosmetic Directive CEE / 76 / 768 and amendments

Absence SVHC

It is certified that the product **Natura-Tec Marine CellShield AP** of our manufacture does not contain substances identified as SVHC featuring in the "REACH candidate list" published.

The "REACH candidate list" is present on ECHA web site at the following link.

Link: http://echa.europa.eu/chem_data/authorisation_process/candidate_list_table_en.asp

BSE statement

We, the undersigned, certify that during the manufacturing process of our **Natura-Tec Marine CellShield AP**:

- No material derived from animal has been used during the manufacture, processing or packing / re-packing.
- There is no risk of cross contamination from products derived from animal during the manufacture, processing or packing / re-packing

Gluten free

We certify that the product **Natura-Tec Marine CellShield AP** that we manufacture is Gluten free.

Microbiological specification

Natura-Tec Marine CellShield AP respects the below specifications :

- Total aerobic mesophilic bacteria < 100 cfu/g/ml
- Yeasts and molds < 100 cfu/g/ml
- Absence of pathogen micro-organisms:
 - Absence of Pseudomonas aeruginosa
 - Absence of Escherichia coli
 - Absence of Staphylococcus aureus
 - Absence of Candida albicans

Nanomaterial free

We hereby certify that our **Natura-Tec Marine CellShield AP** does not contain nanomaterials.

Preservative free

We hereby certify that our **Natura-Tec Marine CellShield AP** does not contain preservatives

Global restriction

Europe	No restriction
USA	No restriction
Canada	No restriction
Japan	No restriction
China	Registered under: Caprylic/Capric Triglyceride (and) Algae Extract - Species: "Pavlova Lutheri"
Australia	No restriction
Korea	No restriction

Efficacy tests

PROPERTIES

Natura-Tec Marine CellShield AP is the result of extensive research by our joint group Biotechnology centre and is produced using our patented closed circuit low carbon footprint photobioreactor technology which ensures consistent quality and purity combined with environmental compliance and minimal carbon footprint (B.E.S.T. - Beautiful Earth Sustainable Technology).

To counteract external aggression to the skin, our Biotechnology center developed a new microalga active, **Natura-Tec Marine CellShield AP**, based on the Pavlova Lutheri species, a tribute to active pioneer women. This species was selected due to its high content in omega 3, long chain EPA and DHA fatty acids, antioxidants, carotenoids and sterols shown by scientists to help the body to fight against the oxidative stress of PM (particulate matter) and offer global skin protection.

Natura-Tec Marine CellShield AP has a strong protective action against environmental stress which maintains and restores skin integrity and energetic metabolism.

Natura-Tec Marine CellShield AP is an amazingly effective anti-pollution active ingredient with rapid and significant protective effect against outdoor and indoor pollution.

Natura-Tec Marine CellShield AP is a powerful active which strongly improves skin condition by reducing dark spot appearance and increasing skin tone homogeneity.

EVALUATION OF PROTECTIVE EFFECTS AGAINST POLLUTION.

VIABILITY OF KERATINOCYTES INTOXICATED BY URBAN AND INDOOR DUST– IN-VITRO TEST

Aim of study

Evaluation of the protective effects of our **Natura-Tec Marine CellShield AP** from 0,1% to 2% of use against pollution on normal human epidermal keratinocytes (NHEK) during 48 and 72 hours.

More specifically, the effects of the compounds were evaluated on the viability of NHEK intoxicated with urban (Urban dust 1649b) or indoor (Indoor dust 2584) pollutant, following a standard MTT reduction protocol.

Pollutant standards:

1- **INDOOR DUST:** Standard reference Material® 2584 of the National Institute of Standards and Technology (NIST)

Standard Reference Material® 2584

Trace Elements in Indoor Dust

(Nominal Mass Fraction of 1 % Lead)

This Standard Reference Material (SRM) is intended for use in the evaluation of methods and for the calibration of apparatus used to determine lead and other trace elements in dust. SRM 2584 is composed of dust collected from vacuum cleaner bags used in the cleaning of interior dwelling spaces. An SRM unit 2584 consists of 8 g of particulate matter, more than 99% of which passes through a 100 µm sieve (No. 145).

Certified Mass Fractions INDOOR DUST

Element Mass Fraction	(mg/kg)
Arsenic (As)	17.4 ± 4.2
Cadmium (Cd)	10.0 ± 1.1
Chromium (Cr)	135.0 ± 9.1
Lead (Pb)	9761 ± 67
Mercury (Hg)	5.20 ± 0.24

2- **URBAN DUST:** Standard Reference Material® 1649b of the National Institute of Standards and Technology (NIST)

Standard Reference Material® 1649b

This Standard Reference Material (SRM) is intended for use in evaluating analytical methods for the determination of selected **polycyclic aromatic hydrocarbons (PAHs)**, **nitro-substituted PAHs (nitro-PAHs)**, **polychlorinated biphenyl (PCB) congeners**, **chlorinated pesticides**, and **inorganic constituents** in atmospheric particulate material and similar matrices.

Particulate Matter = PM = Complex mixture of small particles, 10 µm of diameter or smaller.

Preliminary cytotoxicity assay

Cell type: NHEK in culture medium
Incubation time: 72 hours
Evaluation parameters: MTT reduction assay and morphological observations with a microscope

Results: **Natura-Tec Marine CellShield AP** is non-cytotoxic from 0,1%, to 2,0% of use.

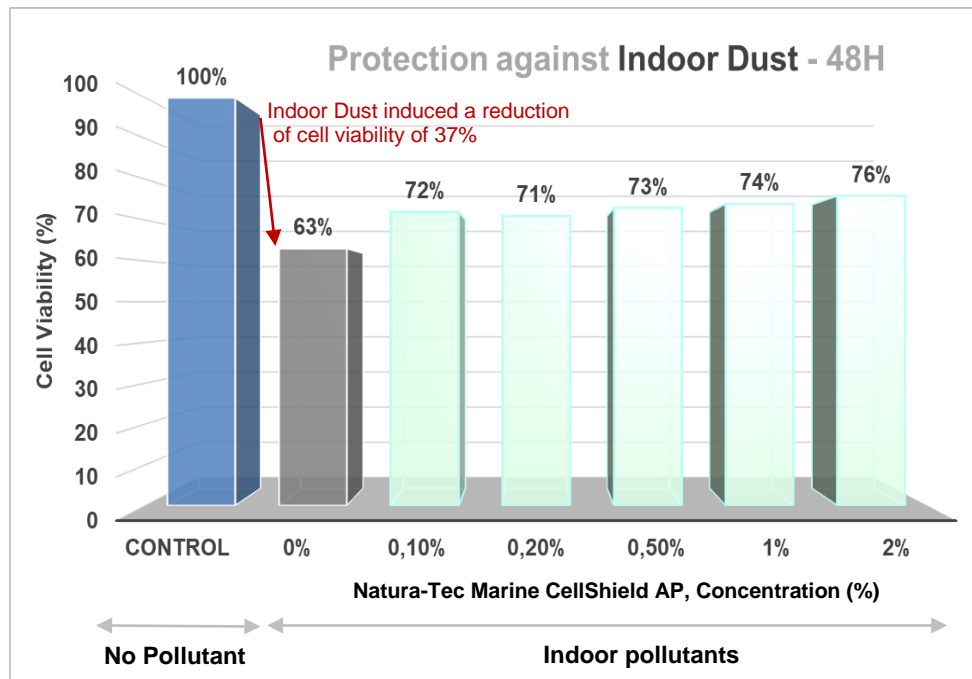
Pollutant effects on cell viability

Under the experimental conditions of this study, the intoxication of the NHEK with urban dust or with Indoor dust induced a strong decrease of cell viability associated with a decrease of cell growth that was clearly observed already after 48 hours of incubation.

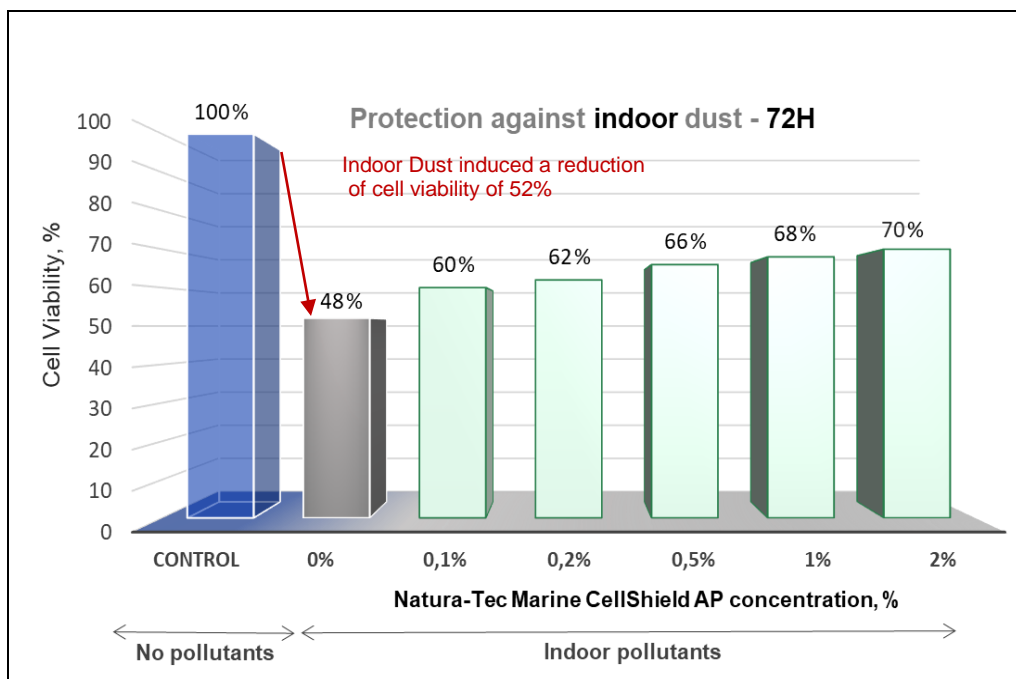
Results after 72 hours add relevance to the test, subjecting keratinocytes to extreme conditions.
The significance of the results obtained at 72 hours confirms the objectivity of this in-vitro test.

Anti-Pollution Test – against indoor dust

❖ After 48 hours incubation



❖ After 72 hours incubation

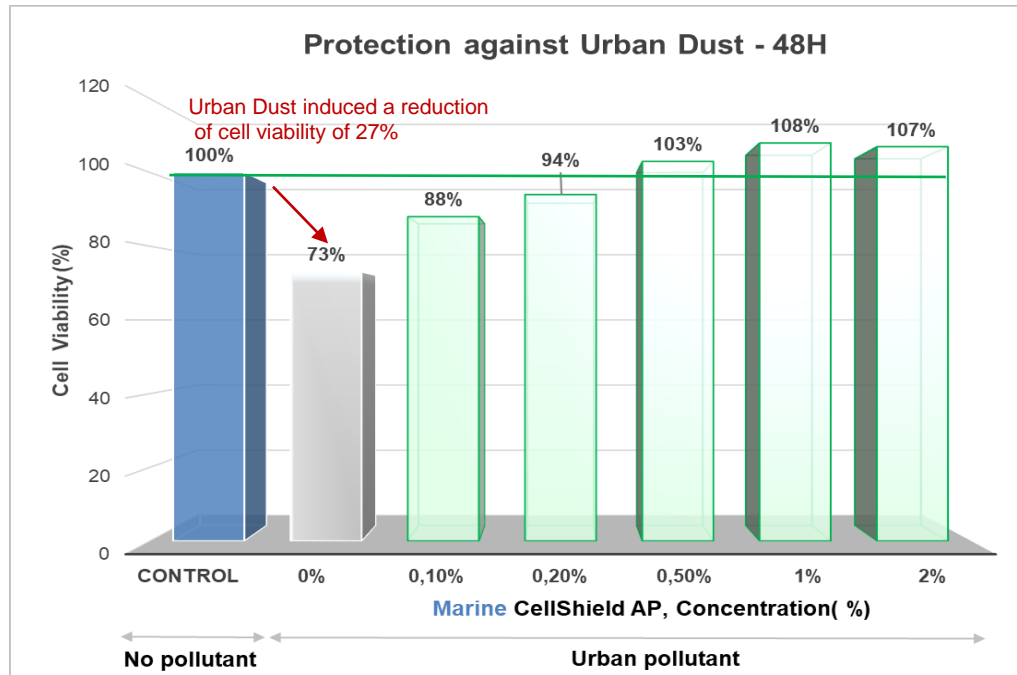


After 48hours and 72 hours incubation, we see that the Indoor dust has a negative impact on the cell viability: pollutants decrease cell viability respectively of 37% and 52%.

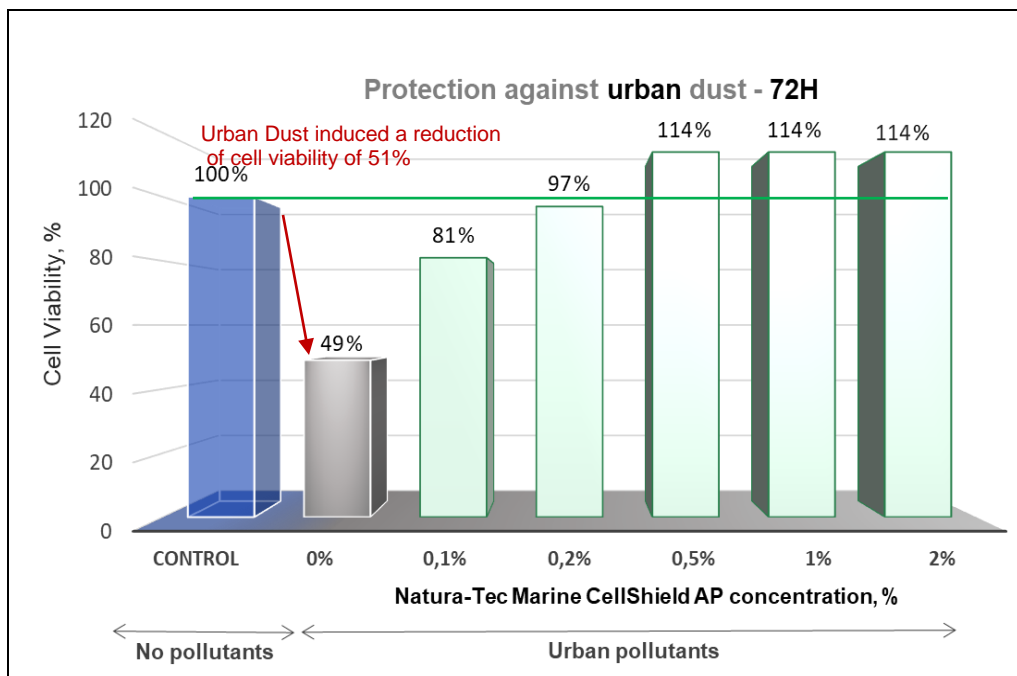
When we use our active, **Natura-Tec Marine CellShield AP**, we see an immediate protective effect when tested at 0,5%, 1%, and 2% already after 48 hours of incubation.

Anti-Pollution Test – against Urban dust

❖ After 48 hours incubation



❖ After 72 hours incubation



After 48 hours and 72 hours incubation, we see that Urban dust has a negative impact on cell viability: pollutants decrease cell viability respectively of 27% and 51%.

When we use our active, **Natura-Tec Marine CellShield AP**, we see an immediate protective effect already with 0,2% after both 48 and 72 hours of incubation.

And then from 0,5% of use, we achieve a maximum cell protection level and observe cell viability greater than in the non-intoxicated condition.

CONCLUSION

After 48 and 72 hours incubation,

Indoor Dust induced reduction of cell viability of 37% and 52% respectively

Urban Dust induced reduction of cell viability of 27% and 51% respectively

These results demonstrate that Indoor pollutants are more aggressive than urban dust.

Against Indoor dust, with 2% of use of **Natura-Tec Marine CellShield AP** we observe an increase of the cell viability of 13% after 48h and 22% after 72h.

This result means a significant protection against indoor pollutants.

Against urban dust, we observe a significant increase of cell viability already with 0,2% of use and from 0,5% of use we can observe a total inhibition of pollution damage.

This active is an excellent shield against urban dust.

Our results indicate that viability and cell stimulation are improved by using our active **Natura-Tec Marine CellShield AP**.

Overall protection from environmental aggression and detoxification effect are essential.

Natura-Tec Marine CellShield AP offers a global protection concept and efficacy to help the formulators develop cosmetics that can help combat the adverse effects of environmental stress and skin exposure to the elements.

Advise for use: 0,2 – 2,0%

In all kinds of formulations for a “Blue” protection claim.

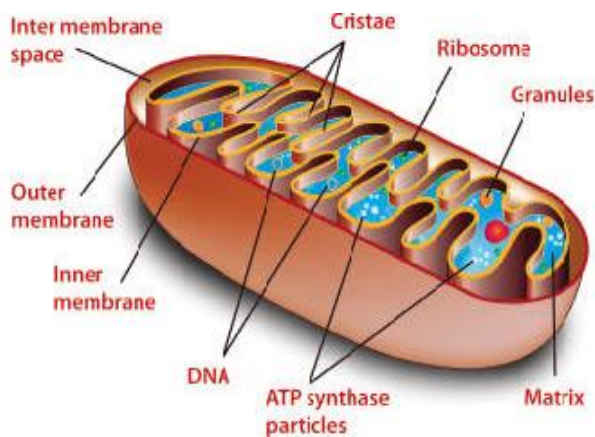
EVALUATION OF THE MODULATING EFFECTS OF NATURA-TEC MARINE CELLSHIELD AP ON NORMAL FIBROBLASTS HUMAN DERMAL UNDER CHRONIC MITOCHONDRIAL STRESS – IN-VITRO TEST



Energetic Metabolism of cells

Mitochondria are small structures inside a cell that generate energy.

They produce 98% of the energy for the body. For this reason, they are often called the “Energy Powerhouse of The Cell”. They play a fundamental role in the oxidative catabolism leading to the production of a usable form of energy: ATP. ATP (adenosine triphosphate) is the energy molecule. ATP is essential to life – it supplies energy for all reactions in the body. Without it, life cannot exist. Mitochondria also play a determining role in apoptosis, thermogenesis, calcium homeostasis, and many anabolic pathways.



- ❖ **Mitochondria** is an intra-cellular organelle in charge to generate ATP, the key energetic molecule of cells.
- ❖ **Membrane Potential** is the difference of electrical potential existing between the extracellular and intracellular faces of the plasma membrane of any living cell.
- ❖ **ATP (or Adenosine Triphosphate)** is a complex molecule used as a source of chemical energy, it is involved in the functioning of most of metabolic process. ATP is essential in cellular functioning.

Aim of the study

- ❖ In vitro evaluation of the protective effects of our **Natura-Tec Marine CellShield AP** at 0,1%, 0.15%, 0,2% of use against chronic mitochondrial stress on normal human dermal fibroblasts (NHDF).
- ❖ More specifically, the effects of **Natura-Tec Marine CellShield AP** were evaluated on the production of cytosolic ATP, the mitochondrial Membrane Potential and synthesis of procollagen of NHDF intoxicated with a daily exposure to FCCP (*Carbonylcyanide p-trifluoromethoxy phenylhydrazone*).
- ❖ FCCP is a compound that inhibits the mechanism of oxidative phosphorylation (or its compounds), which has a direct impact on the production mitochondrial ATP. FCCP decreases the effectiveness of the mitochondrial ATP synthesis.

EXPERIMENTAL CONDITIONS:

Measured Parameters

1. Cytosolic ATP: measurement of ATP levels by bioluminescence in control and treated cultures.
2. Mitochondrial Membrane Potential: Measurement of Mitochondrial Membrane Potential ($\Delta\psi$) using a fluorescent probe (JC-1) in control and treated cultures.
3. Neosynthesis of procollagen I: measurement by Elisa technique of the [PIP] levels (Procollagen type I Peptide) in the incubation media of control and treated cultures.

Experimental batches:

1x Negative control: NHDF cells not exposed to a mitochondrial stress (-FCCP).

1x Positive control: NHDF cells exposed to a mitochondrial stress (+FCCP).

3x Active: NHDF cells incubated with 3 different concentrations of **Natura-Tec Marine CellShield AP** (0.10%, 0.15% and 0.20%) [REF. 53004 LOT 1801-53004 / 01] and exposed to a mitochondrial stress (+FCCP). Each concentration of the active has been tested in triplicate.

1. Cytosolic ATP rate measurement

Measurement of Cytosolic ATP rate by Bioluminescence assay. The bioluminescence method is based on measurements of adenosine triphosphate (ATP) which is the principal energy carrier in all living organisms and is involved in the functioning of most of metabolic process.

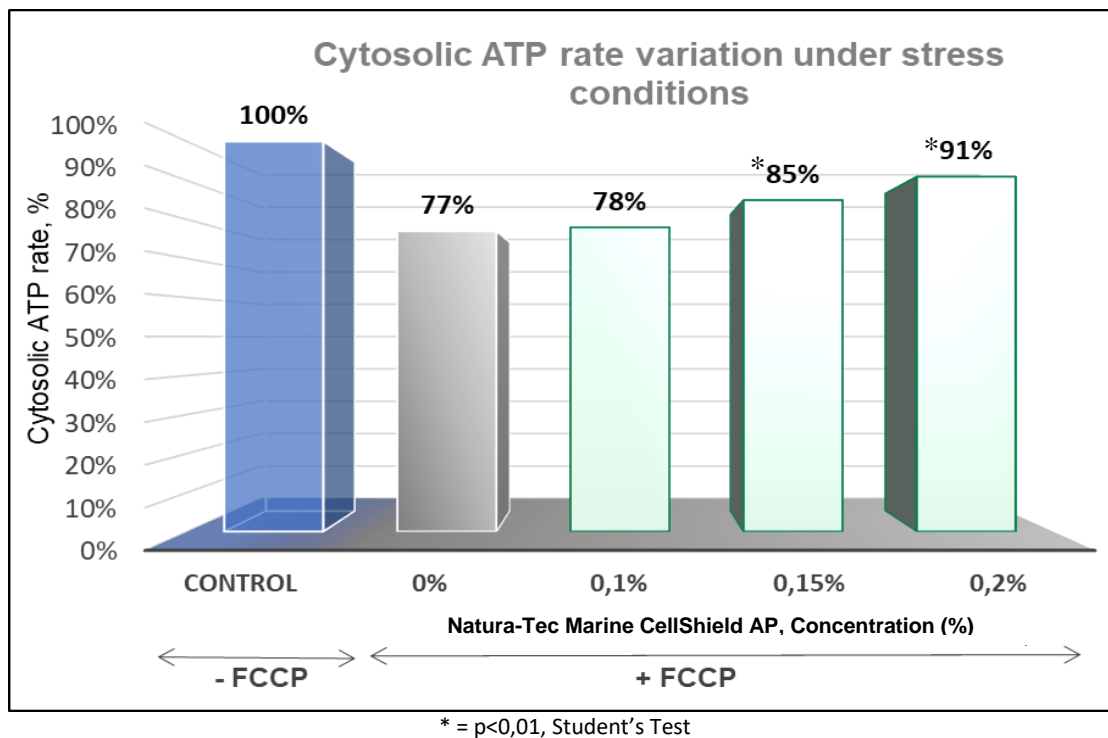
This experiment is performed during 13 days under environmental stress conditions induced by FCCP from 0,1% to 0,2% of use of our **Natura-Tec Marine CellShield AP**.

ATP rate (pmol/well) has been measured in the negative, positive control and treated cultures.

This ATP rate has been corrected from cellular protein levels (μg / well). The results are expressed in pmoles of ATP / μg protein and in percentage (% compared to the negative control (-) FCCP) in the graph below.

RESULTS

Results are expressed in % of cytosolic ATP rate variation after 13 days of incubation.



FCCP (*carbonylcyanide p-trifluoromethoxy phenylhydrazone*) induced a cytosolic ATP rate reduction of 23%, indicating that chronic exposure to FCCP alters cellular ATP synthesis.

Marine CellShield AP induced a significant increase of cytosolic ATP rate of +8% at 0,15% of use and up to +14% at 0,2% of use.

This result shows that the treatment of cells by **Natura-Tec Marine CellShield AP** modulates the decrease of ATP induced by the FCCP. The modulating effect is dose-dependent in the range of tested concentrations.

Natura-Tec Marine CellShield AP restores mitochondrial energetic metabolism.

2. Measurement of the mitochondrial membrane potential

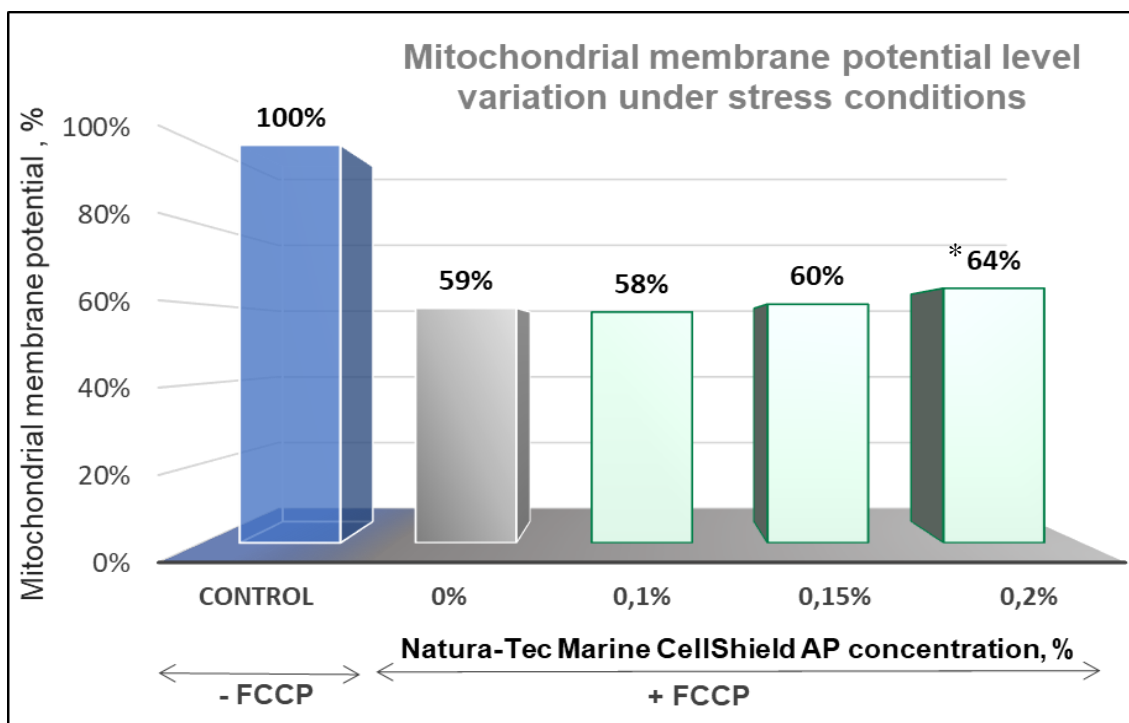
The mitochondrial membrane potential of NHDF cells has been evaluated under environmental stress conditions induced by FCCP (*carbonylcyanide p-trifluoromethoxy phenylhydrazone*) from 0,1% to 0,2% of use of our **Natura-Tec Marine CellShield AP** for 18 days.

Membrane Potential is the difference of electrical potential existing between the extracellular and intracellular faces of the plasma membrane of any living cell.

The mitochondrial membrane potential ($\Delta\psi$) was assessed by measuring the fluorescence intensities IF2 ($\lambda=590\text{nm}$) and IF1 ($\lambda=535\text{nm}$) of the JC-1 probe. The $\Delta\psi$ of the cells is expressed by the ratio [IF2 / IF1].

RESULTS

Results are expressed in % of Mitochondrial membrane potential after 18 days of incubation.



* = $p < 0,01$, Student's Test

FCCP induced a mitochondrial membrane potential reduction of 41%. This result indicates that chronic exposure to [FCCP] is correlated to a fall in mitochondrial membrane potential.

A significant increase (+ 5%) is observed at 0,2% of **Natura-Tec Marine CellShield AP**. This result shows that the treatment of cells by **Natura-Tec Marine CellShield AP** makes it possible to modulate significantly the fall of $\Delta\psi$ during the mitochondrial response to FCCP-induced stress.

Natura-Tec Marine CellShield AP decreases the mitochondrial response to stress.

3. Procollagen type I Peptide (PIP) neosynthesis

The PIP rate of NHDF cells has been evaluated under environmental stress conditions induced by FCCP (*Carbonylcyanide p-trifluoromethoxy phenylhydrazone*) from 0,1% to 0,2% of use of our **Natura-Tec Marine CellShield AP** for 13 days.

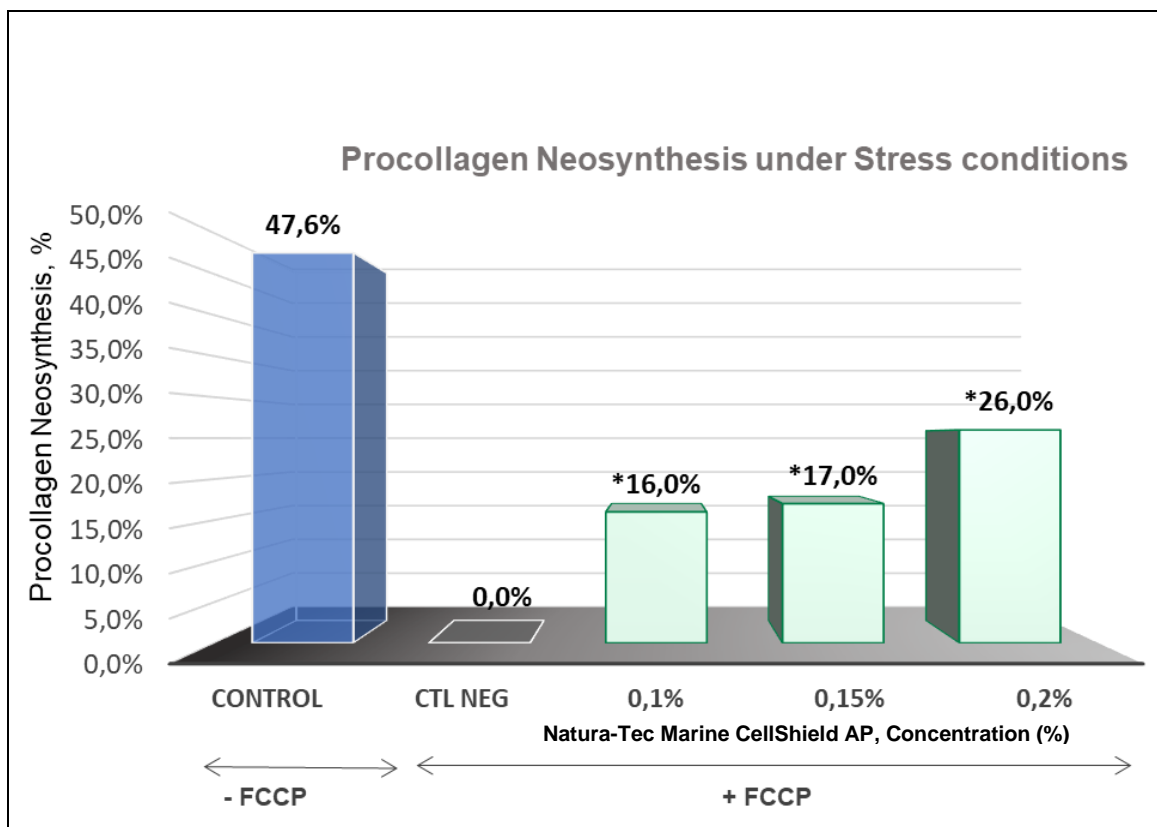
Procollagen I is the precursor of the Collagen type 1 which is the main component of the extracellular matrix of the dermis. Collagen type 1 contributes to provide resistance, elasticity and suppleness to skin.

Due to its action on mitochondrial energetic metabolism, FCCP acts on various metabolic processes as well as Collagen I neosynthesis. FCCP disrupts this pathway by inhibiting the synthesis of one of its precursors, the Procollagen type I Peptide (PIP).

PIP rate (ng/well) has been measured in the negative, positive control and treated cultures. This PIP rate has been corrected from cellular protein levels (μg / well). The results are expressed in ng of PIP/ μg protein and in percentage (% compared to the negative control (-) FCCP) in the graph below.

RESULTS

Results are expressed in % of Procollagen I synthesis after 13 days of incubation.



* = <0,01 (Student's test)

Under environmental stress stimulation, **Natura-Tec Marine CellShield AP** helps to restore Collagen Type 1 neosynthesis. **Natura-Tec Marine CellShield AP** increases significantly Procollagen synthesis of +16% already at 0,1% and up to 26% at 0,2% of use, compared to negative control. **Natura-Tec Marine CellShield AP** helps to restore Collagen type I neosynthesis.

Natura-Tec Marine CellShield AP acts to protect and maintain skin integrity, counteracting the deleterious effects of stress on skin ageing.

CONCLUSION

This study shows that a daily exposure to FCCP induce a mitochondrial dysfunction associated to a decrease of cytosolic ATP, a fall of the mitochondrial membrane potential and a decrease of collagen synthesis capacity.

Mitochondrial involvement in skin diseases has been proven⁽¹⁾. Improving mitochondrial function can be beneficial for aged skin and can be used to reduce skin disorders.

This study proves the ability of **Natura-Tec Marine CellShield AP** to:

- Restore mitochondrial energy metabolism
- Maintain cellular homeostasis under stress conditions
- Protect one of the major anabolic pathways of fibroblasts which is the synthesis of neocollagen

This study concludes that **Natura-Tec Marine CellShield AP** shows a powerful protecting activity against environmental stress.

(1) Mitochondrial dysfunction: a neglected component of skin diseases.

Exp Dermatol. 2014 Sep;23(9):607-14. doi: 10.1111/exd.12484.

Feichtinger RG1, Sperl W, Bauer JW, Kofler B.

SKIN TONE IMPROVEMENT – IN-VIVO EVALUATION

Aim of the study

Evaluation of the In vivo efficacy of **Natura-Tec Marine CellShield AP** incorporated at 1,5% in a basic formulation to reduce dark spots and to increase skin homogeneity.

Panel Characterization

- ❖ 20 volunteers: Sexe F; all of them presenting facial spots.
- ❖ Fitzpatrick phototype category II-III-IV.
- ❖ Panel FORMULE 1- BATCH: EL171203A. Age from 44 to 74 years old. Average age 56 years old.

FORMULE 1 - with 1,5% of **Natura-Tec Marine CellShield AP** BATCH: EL171203A

AQUA (WATER), **CAPRYLIC/CAPRIC TRIGLYCERIDE**, PRUNUS AMYGDALUS DULCIS (SWEET ALMOND) OIL, CETEARYL ALCOHOL, GLYCERYL STEARATE, POTASSIUM PAMITOYL HYDROLYZED WHEAT PROTEIN, **PAVLOVA LUTHERI EXTRACT**, CARBOMER, TOCOPHEROL, BENZYL ALCOHOL, SALICYLIC ACID, GLYCERIN, SORBIC ACID, SODIUM HYDROXIDE.

	Ingredients	INCI	Formule EL 171203A %
A	Water	Aqua (Water)	78,13
	Tegocarbomer 134	Carbomer	0,60
B	Natura-Tec Emulactive W	Cetearyl Alcohol (and) Glyceryl Stearate (and) Potassium Palmitoyl Hydrolyzed Wheat Protein	4,00
	Natura-Tec Sweet Almond Oil - Refined	Prunus Amygdalus Dulcis (Sweet Almond) Oil	5,00
	Natura-Tec Ultrafeel MCT	Caprylic/Capric Triglyceride	8,00
	Cetearyl alcohol	Cetearyl Alcohol	2,00
C	Tocopherol	Tocopherol	0,10
	Geogard ECT	Benzyl Alcohol (and) Salicylic Acid (and) Glycerin (and) Sorbic Acid	0,50
D	Natura-Tec Marine CellShield AP	Caprylic/Capric Triglyceride (and) Pavlova Lutheri Extract	1,50
E	NaOH (25%)	Sodium hydroxide	0,17

pH = 5,5

Volunteers pre-selection according to the following criteria:

- Healthy subjects.
- Absence of skin pathologies.
- Absence of pharmacological treatments.
- Negative anamnesis for atopy.
- Obligation to avoid the use of other topical products in the testing zone during the test and to avoid the application of creams in the zone to be tested for at least 3 days before the beginning of the test.
- Exclusion: pregnant women and women in breastfeeding period, minors.

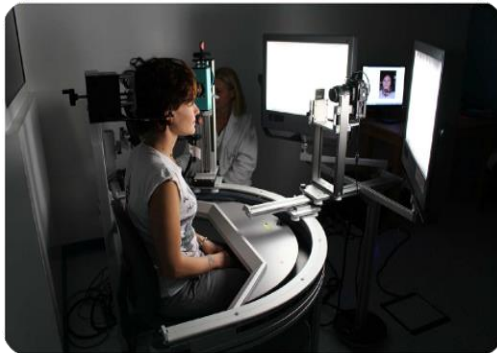
This test has a duration of 30 days, volunteers are going to use daily, morning and evening, the product at home for the whole period of the test.

The instrumental evaluations on the subjects were obtained:

- At T0: measurement of the skin basal values before the beginning of the test.
- At T1: measurement after 30 days of application

Administration of an auto-evaluation questionnaire to volunteers to obtain a judgement from potential customers on product performances.

Experimental procedure



The images, after and before the treatment, are carried out by FRAMESCAN. Frame-Scan is dedicated to the quantitative colorimetric and morphological analysis of calibrated digital photographic images. This software allows the extraction of images characteristics. It analyses samples of images either for usual or advanced analyses as measurements of colorimetry, luminosity, vascularization, pigmentation or homogeneity but also analyses of morphology of elements (pigmentary spots, lashes, wrinkles...). A system identifies with great precision the zone to evaluate. The photo is shot before and after the treatment and finally the differences are photographed. Then it is possible to register the images in the same position before and after the application of the cosmetic product.

The image analysis processing is performed using the following steps:

- SUBJECT POSITIONING for reproducible positioning and repositioning of the face, during a study course and image acquisition using a high-resolution camera with automatic control to insure reproducible parameters of acquisitions.
- SOFTWARE FOR IMAGE ANALYSIS of the skin characteristics using the comparison of the image before and after the treatment of each volunteer:
- MORPHOLOGY ANALYSIS module and selection of ROI (Region of Interest) and extraction of the following parameters:
 1. The morphology of the skin spots is evaluated by measurement of **the Surface average of skin spots**: Average surface of Spot in ROI expressed in %.
 2. **Homogeneity** Haralick: It's the calculation of the homogeneity of a region of interest. It varies in the interval [0, 1]. The surface is homogeneous when the value of the homogeneity approaches 1.

Instrumental evaluation

The average values were obtained from the instrumental evaluations and the relative decreases/increases in comparison to T0, at the 2 fixed times:

- T0 (before the beginning of the test)
- T1 (after 30 days of treatment)

It was calculated the % variation of the medium instrumental detections with reference to the basal values at the fixed times according to the following formula:

$$\text{Variation \%} = [(v_1 - v_2) / v_2] \times 100$$

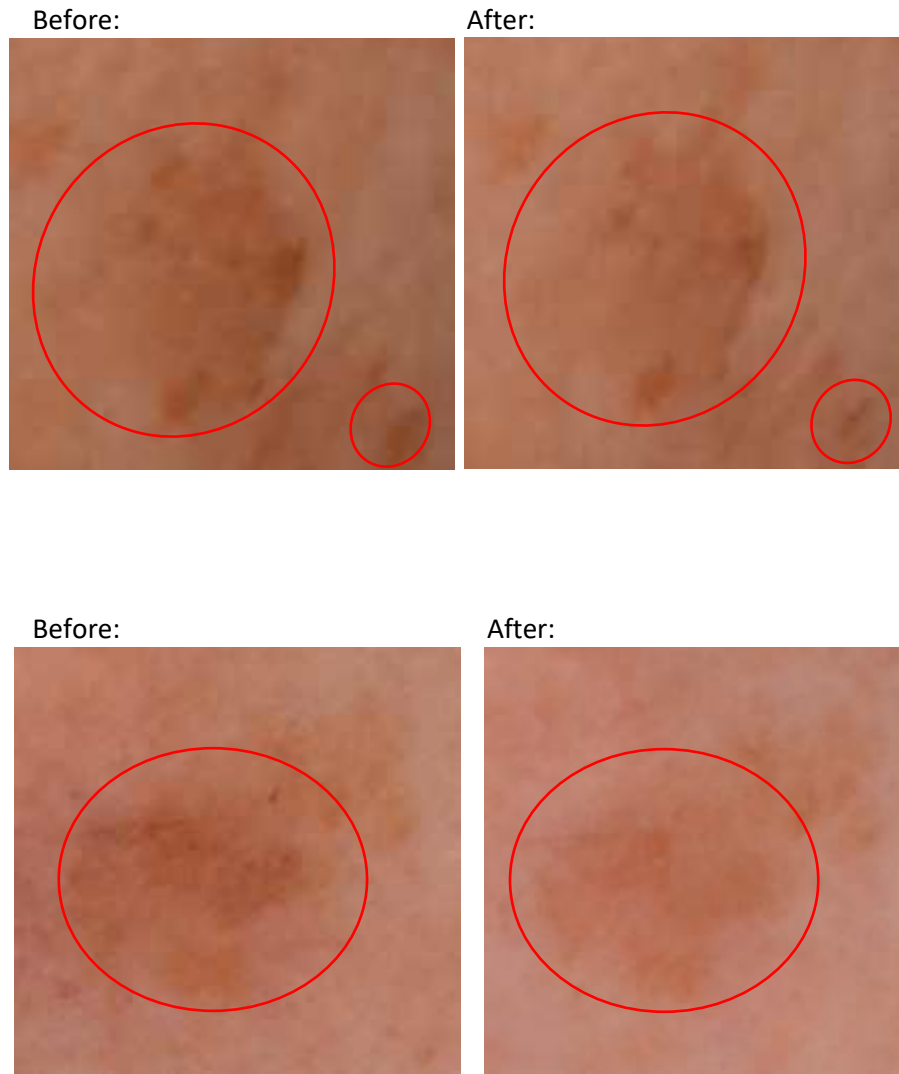
where v_1 is the detection at the considered time and v_2 is the corresponding basal value (T0).

To perform the analysis of the significance, the t- Student test was applied to the significance level $\alpha=5\%$.

1. Results on Skin Spot

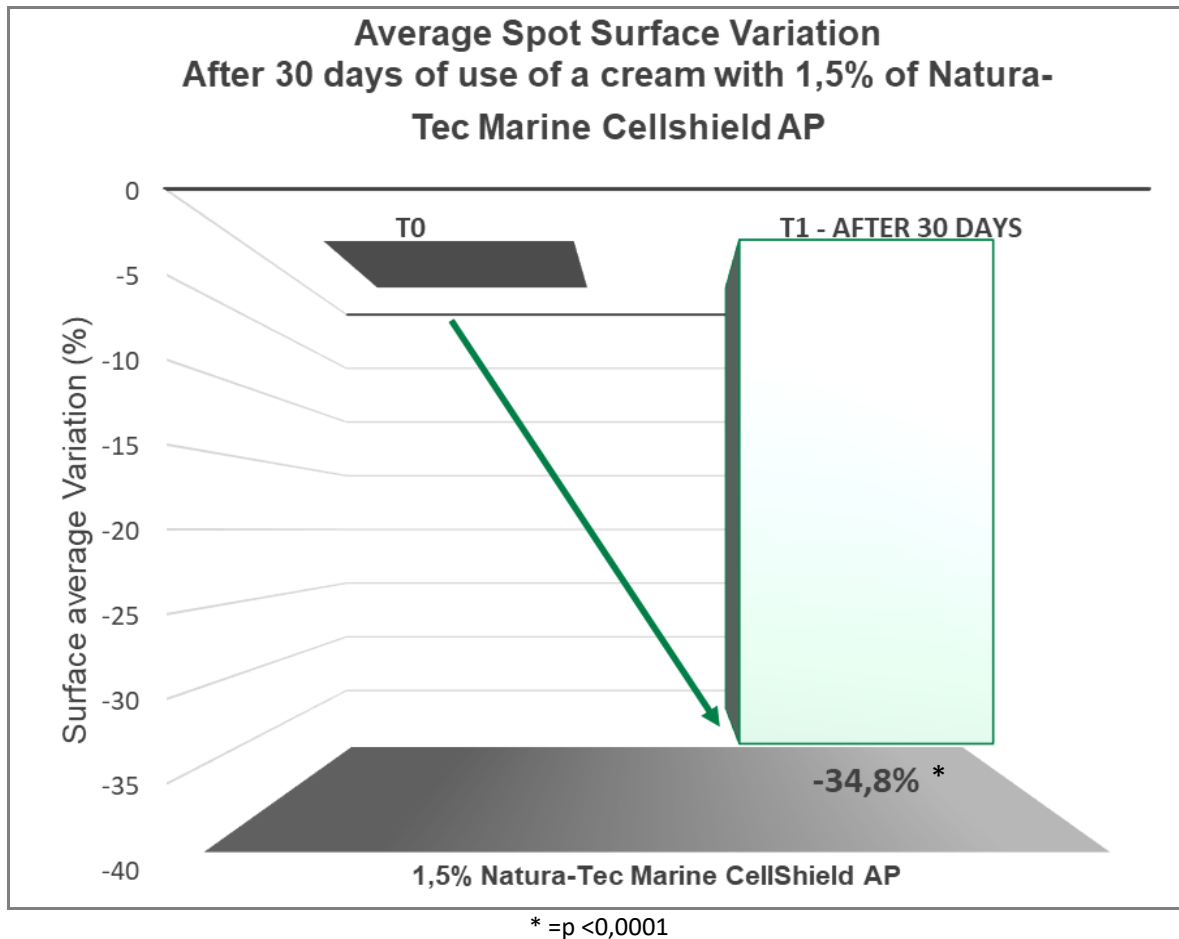
IMAGES CREATED BY FRAMESCAN SOFTWARE

Before/After daily application of a cream at 1,5% for 1 month:



The daily application of a cream containing 1,5% of **Natura-Tec Marine CellShield AP** reduces colour intensity and surface of dark spots intensively after 1 month of use.

The variation of the surface average of skin spot is expressed in %:



In the registered images, the parameter **AVERAGE SPOT SURFACE** is measured (correlated to the average surface of Spot in ROI), the variation is calculated, after 30 days of treatment (T1), in comparison to not treated skin (T0):

With 1,5% of Natura-Tec Marine CellShield AP, the surface of the skin's spots decreases of 34,8%.

The maximum reduction of skin spots surface is up to **60,0%**.

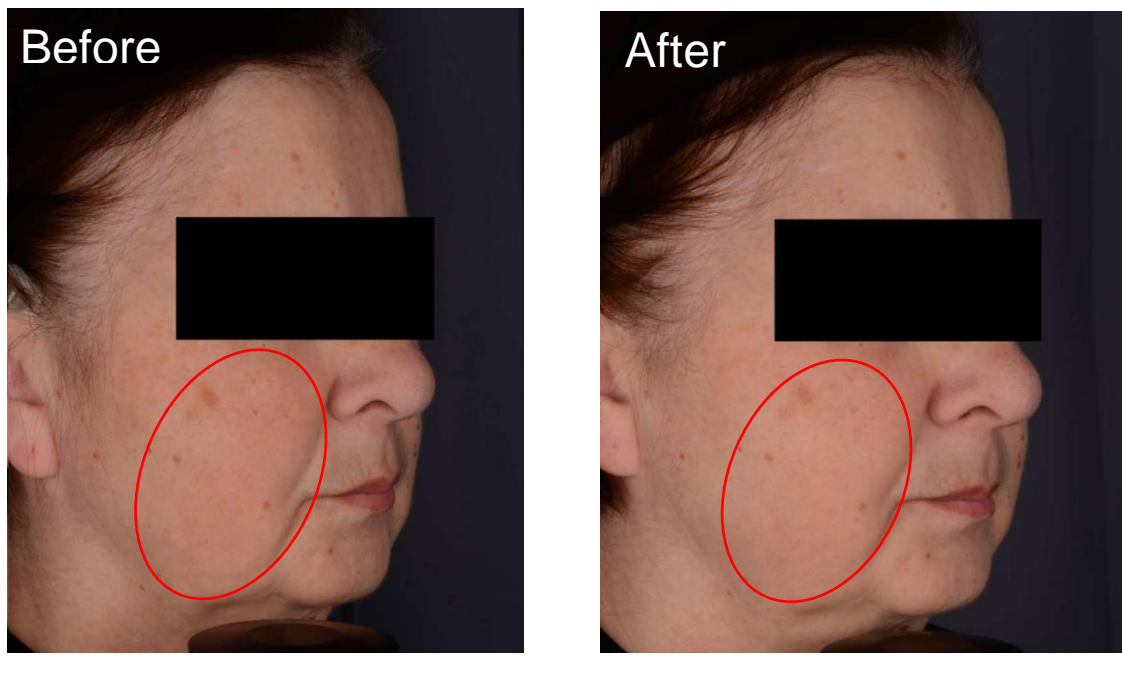
The difference correlated to **SURFACE AVERAGE** in comparison to T0 is statistically significant.

Natura-Tec Marine CellShield AP is able to decrease the surface of skin spots.

2. Results on Skin Homogeneity

IMAGES CREATED BY FRAMESCAN SOFTWARE

Before/After daily application of a cream at 1,5% for 1 month.

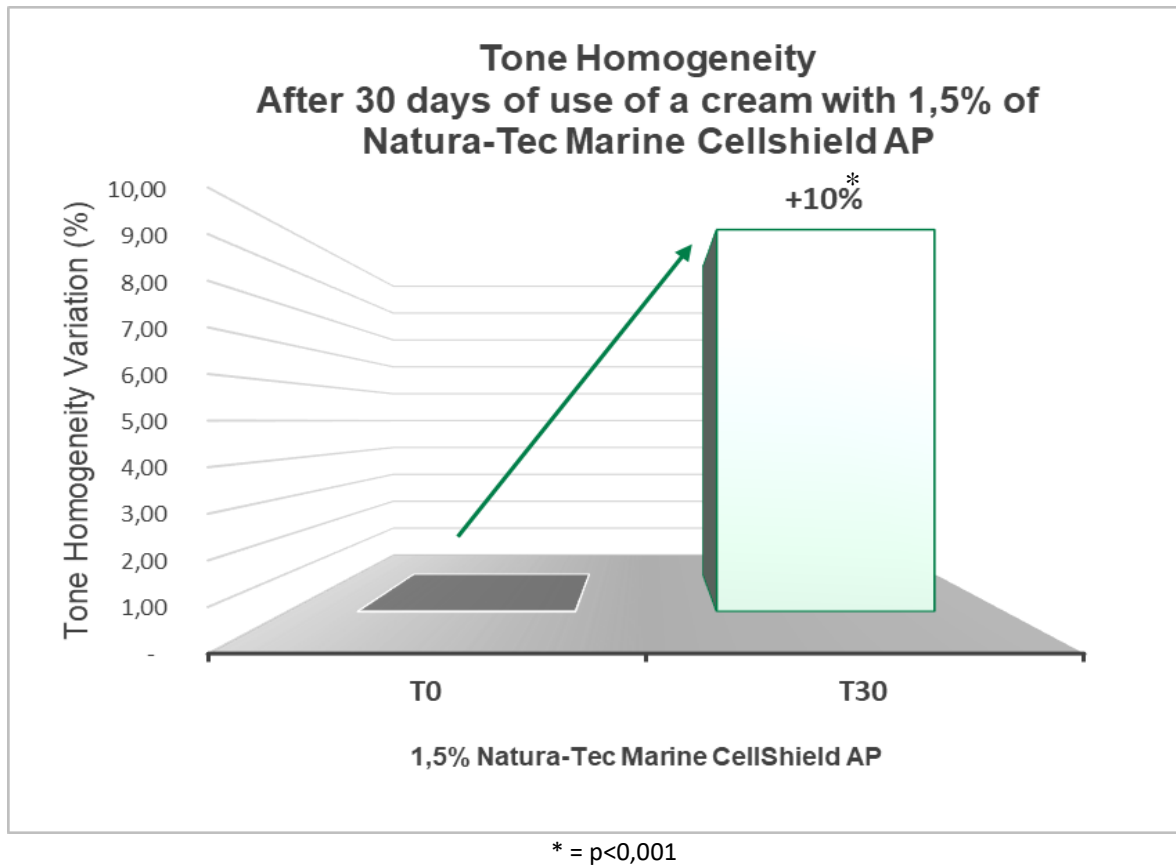


After 30 days of treatment with 1,5% of **Natura-Tec Marine CellShield AP**, skin complexion is unified, and redness are reduced.

Natura-Tec Marine CellShield AP at 1,5% has a global skin lightening activity.

Natura-Tec Marine CellShield AP at 1,5% provides a superior skin tone.

For the skin homogeneity evaluation, the parameter **HOMOGENEITY** is measured, the variation is calculated, after 30 days of treatment (T1), in comparison to not treated skin (T0):



With 1,5% of Natura-Tec Marine CellShield AP, after 30 days of treatment, skin homogeneity increases of **10,0%**. The maximum increase of skin homogeneity is up to 16,0% depending on volunteers. The difference correlated to HOMOGENEITY in comparison to T0 is statistically significant (*p<0,001).

Natura-Tec Marine CellShield AP is able to increase skin homogeneity.

3. Auto Evaluation Study:

After 30 days of treatment with a very neutral cream containing **1,5% of Natura-Tec Marine CellShield AP**, we can conclude as follows:

- **66,7%** of panel note an improvement in the appearance of the skin;
- **83,3%** of panel consider diminished their skin marks;
- **83,3%** of panel consider modified the color of their face skin marks, after the treatment;
- **58,3%** consider that their skin tone was levelled out;
- **75%** of the volunteers are satisfied with the use of this very basic cream with only 1,5% of Marine CellShield AP.

CONCLUSION

Natura-Tec Marine CellShield AP at 1,5% provides an impressive impact on skin appearance. It reduces colour intensity and surface of dark spots by 40% and up to 60% and improves skin homogeneity of 10% and up to 16% depending on volunteers.

Natura-Tec Marine CellShield AP has a strong protective action against pollution and environmental stress which maintains and restores skin integrity and energetic metabolism. Moreover, this is a powerful active which strongly improves skin condition by reducing dark spot appearance and increasing skin tone homogeneity.

Regulatory Affairs Manager: Pascale Goyat
Fréjus, February 2020.



*This document completes the product technical and safety data sheet. Information contained in this notice are based on our current knowledge and relate to the product in the state in which it is delivered
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