cosmetic active ingredients
bitop – who we are

Key Facts

- Founded in 1993
- Located in Witten (near Cologne), Germany
- 36 employees
- Science driven company
- Ultra-high quality bioprocessing
- Raw material production (medical & cosmetics)
- Medical device development
- Production facility: 2500m²
what we do: innovation in partnership with nature

<table>
<thead>
<tr>
<th>raw material</th>
</tr>
</thead>
<tbody>
<tr>
<td>▶️ raw material = extremolytes</td>
</tr>
<tr>
<td>▶️ raw material for health care, personal care industry and life science</td>
</tr>
<tr>
<td>▶️ raw material production according to ISO 13485 (medical devices)</td>
</tr>
<tr>
<td>▶️ Ectoin®: worldwide only manufacturing site</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>finished products</th>
</tr>
</thead>
<tbody>
<tr>
<td>▶️ medical devices containing Ectoin® (worldwide licensing and distribution):</td>
</tr>
<tr>
<td>OTC products for</td>
</tr>
<tr>
<td>▶️ eye &amp; nose, skin, lung</td>
</tr>
<tr>
<td>▶️ allergy, dryness, skin diseases (e.g. neurodermatitis), lung diseases (COP, asthma)</td>
</tr>
</tbody>
</table>
highly specialized and world market leader…

we ❤️ remolytes and science!
Extremolytes and their natural origin...

Life under enormous stress in extreme and hostile conditions: extremophilic microorganisms and plants - the oldest life-forms on earth.

Extremolytes are molecules which are produced by extremophilic plants or microorganisms (bacteria, algae etc.). The plants and microorganisms use the molecules (extremolytes) to protect themselves in their hostile habitats.

Extremolytes = stress-protection molecules
general benefits of our cosmetic actives

- 100% natural origin
- pure molecules
- Ecocert-certified
- China approved
- low molecular weight / small molecule
- 100% GMO free substances
- in-house manufacturing process (according to ISO 13485)
- multi-functional
- very safe (broad safety data; meeting all regulatory requirements)
- very deep and broad study data (various in-vivo and in-vitro; independent laboratories)
- effective at low dosage and very easy to formulate (broad pH-Range, high temperatures)
INTRODUCTION

MODE OF ACTION
**mode of action:**

- Ectoin® is a strongly water binding (structuring) substance.
- A solution containing Ectoin® increases the number of neighboring water molecules.
- The structure of water is stabilized by Ectoin® (kosmotropic property).

Complex formed by water molecules and Ectoin: **Ectoin® Hydro Complex**

Reference: D.Paschek, G.Sadowski, TU Dortmund Germany
mode of action: proteins stabilization

Stabilization of biomolecules with Ectoin®

A) protein in water: number of water molecules is small at the surface of the protein.
B) protein in aqueous Ectoin® solution: number of water molecules is increased by formation of Ectoin® water complexes, hydrophobic interactions are increased, compression and thus stronger stabilization of protein
mode of action

extracellular tissue surface

intracellular space
mode of action: membrane stabilization

B) Ectoin® Hydro Complex causes increased interactions of head groups (bilayer) with water. Lipid molecules are mobilized and the fluidity is increased.
Membrane stabilization and increase in membrane fluidity due to Ectoin.

A) lipid monolayer in water: The monolayers are stabilized by hydrophilic interactions within head groups.

B) lipid monolayer in aqueous Ectoin® solution: Ectoin® Hydro Complex causes increased interactions of head groups with water. Lipid molecules are mobilized and the fluidity is increased.
Ectoin® inhibits the activation of stress-response: no release of stress mediators into the cell —> no activation of damaging inflammatory-cascade
**mode of action: protection mechanism**

In the native situation, the skin epithelium including extracellular matrix is exposed to damaging stress factors (e.g. UV radiation, particulate matter, heat, cold ect.)

![Diagram](image)

**Prevention of stress mediator (ceramide) release by Ectoin®.**

A) without Ectoin®: External stress factors (like UV-irradiation) cause membrane damage, water loss and release of stress mediators (ceramides), which act as second messenger for inflammatory reactions.

B) with Ectoin®: The Ectoin® Hydro Complex protects the membrane against external stress factors, stress mediator release is prevented.

The general protective and hydrating properties against various external stress factors is the basic mode of action of Ectoin®.
mode of action on cellular level- summary

Ectoin® is:

- water binding: it forms specific water-structures (Ectoin® Hydro Complex); it is a kosmotropic substance and thus stabilizes its soundings (“order – maker”)
- membrane stabilizing: it increases the membrane fluidity and stability
- protein stabilizing: proteins are protected from degeneration/damage
- cell protecting: it inhibits the activation of stress-response (release of stress mediators into the cell -> activation of damaging inflammatory-cascade)
Ectoin® is a 100% safe and medically approved ingredient

Ectoin® acts extracellular (outside the cell)
It is not metabolized by the body.

Ectoin® is very effective and safe - it is also an approved substance for medical products:
- for the treatment of inflammatory and serious skin diseases like neurodermatitis, psoriasis, allergy reactions etc.
- for the treatment of lung diseases
- for the treatment of allergic reactions in the eye and nose
- for the moisturization of eyes
- …

Ectoin® is non allergenic and Ecocert certified.
It is approved for the usage in babycare.
market sectors

- skin care:
  - modern anti-aging formulations
  - preventive skin care
  - dermaceuticals / cosmeceuticals / dermocosmetics
  - special purpose skin care & cosmetics (allergy, diabetic, baby…)
  - natural skin care
- sun care: completes UV-, IR- and visible light protection on cellular level
- hair care
- color cosmetics
- oral care
The protection concept

Why protection and prevention are the key parameters to anti aging.
This man was a truck driver for over 40 years of his life. This part of his face was always facing the „inside of the truck“ – it was protected.
The other part of his face was always facing the window.

It was not protected.
Brenda and Barb

Age: 52

A trifecta of smoking, sunning, and lower weight means that Brenda (left) looks significantly older than Barb (right), who lives near her in Ontario, Canada. ("I love being called the younger one," Barb says, laughing.) Brenda has smoked half a pack a day for 14 years, while Barb has never smoked. Brenda also reported seven times more sun exposure (primarily over eight to ten weeks of the year for 30 years, at an estimated 14 hours a week, versus two hours a week for Barb). "The festoons of loose skin" under Brenda's eyes are attributable to her cigarette habit, says Dr. Bahman Guyuron, a plastic surgeon at Cleveland's Case Western Reserve University. "When I see that [on patients], I don't have to ask if they smoke. I know they do."
Jeanne and Susan
Age: 61

Jeanne (left) is the twin who commented that her sister looks “ten years older” — in fact, the researchers upped that to an 11-and-a-quarter-year edge. The reasons for Susan’s condition are that she smoked for 16 years of her life, sunbathes, and weighs 15 pounds less. Since her 20s, Susan (right) has spent as much time as she could in the sun (she has plenty of opportunity on Florida’s east coast, where she moved more than a decade ago). Jeanne, meanwhile, has aimed for “as little exposure as possible.” Besides causing Susan’s dark, patchy discoloration and age spots, UV light also reduces skin’s elasticity, increasing wrinkles and deepening creases, Guyuron says. (Department of Plastic Surgery, University Hospitals Case Medical Center)
Janet and Jean
Age: 54

Jean (right) looks older than Janet (left), Guyuron says, noting her darker undereye area, deeper smile lines, and “more numerous and deeper vertical frown lines.” The probable reason, his study suggests, is that Jean is divorced (after 27 years of marriage), whereas Janet has been married for more than three decades; nothing else measured in the history of these Chicago-area sisters was different. In general, marital trouble, separation, and worries about money and finding a new partner can result in stress levels that affect appearance, Guyuron says. (Department of Plastic Surgery, University Hospitals Case Medical Center)
skin aging: latest scientific knowledge and evidence

extrinsic skin aging is caused by 3 major damaging factors:

- UVA- & UVB-radiation, IR-radiation and visible light
- traffic and industry related particulate matter (environmental pollution)
- tobacco smoke

Ectoin® reliably protects the skin from all of these damaging factors!
global protection and skin improvement: overview

Ectoin® protects skin cells against all damaging factors like e.g.:

- oxidative stress (reduction of ROS)
- environmental stress like UV, particulate matter/pollution, heat, cold
- intrinsic and extrinsic aging factors…

Ectoin® improves the skin health and condition — long lasting and self-sustaining (!) by e.g.:

- protection of Langerhans cells (skin immune system)
- boosting the formation of heat shock proteins (hsp)
- stabilizing the skin barrier
- moisture lock and improvement of hydration status
- …
the pathway to real and visible skin improvement

Protection & prevention

Less or no damage and degeneration in cells, membranes, of DNA, tissue, proteins, hair follicles

Improvement & stabilizing

Reduction or prevention of premature aging wrinkles age spot formation hyperpigmentation roughness lose of elasticity cell degeneration hair follicle aging ...

Anti-aging means protection. Ectoin® is an extraordinary protection active. For skin and hair.
**study overview - in vivo**

<table>
<thead>
<tr>
<th>claim</th>
<th>results</th>
<th>usage level</th>
</tr>
</thead>
<tbody>
<tr>
<td>long-lasting hydration/moisturizing (7 days)</td>
<td>Ectoin® showed a dramatic long-term moisturizing effect – skin preserved its hydration status over 7 days!</td>
<td>0,5% and 1%</td>
</tr>
<tr>
<td>protection of Langerhans cells / boost of skin’s</td>
<td>Ectoin® protects 100% of the Langerhans cells in UV-irradiated skin. No damage of Langerhans-Cells at all at 0,5% usage level. Irradiated cell behaves like un-stressed cell!</td>
<td>0,3% and 0,5%</td>
</tr>
<tr>
<td>own self protection</td>
<td></td>
<td></td>
</tr>
<tr>
<td>wrinkling reducing</td>
<td>23% reduction of wrinkle volume</td>
<td>2%</td>
</tr>
<tr>
<td>smoothing</td>
<td>skin roughness was decreased by 86%</td>
<td>2%</td>
</tr>
<tr>
<td>anti-scaling</td>
<td>skin scaling was reduced by 76%</td>
<td>2%</td>
</tr>
<tr>
<td>increasing of skin elasticity</td>
<td>increase of skin elasticity by 16,6%</td>
<td>2%</td>
</tr>
</tbody>
</table>
## study overview - in vitro

<table>
<thead>
<tr>
<th>claim</th>
<th>results</th>
<th>usage level</th>
</tr>
</thead>
<tbody>
<tr>
<td>protection against external stress factors / increase of membrane</td>
<td>increase in membrane stability by 60% due to pre-treatment with Ectoin® for 24 hours</td>
<td>1%</td>
</tr>
<tr>
<td>stability (view study)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>boosts of skin’s self defense - increase of heat shock proteins</td>
<td>Skin cells can protect themselves about 2 to 3 times faster. This guarantees the survival and protection of skin cells during external stress.</td>
<td>1%</td>
</tr>
<tr>
<td>(view study)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>inhibition of second messenger release (view study; view UVA-stress</td>
<td>Ectoin® completely (100%)! prevents UVA induced second messenger release; It inhibits UVA induces damage cascade inside cells and thus the cell aging process in keratinocytes and fibroblasts</td>
<td>1mM = 0,014%</td>
</tr>
<tr>
<td>model)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>inhibition of AP-2 activation and ICAM1 expression</td>
<td>Ectoin® reliably inhibits UV induced initiation of the inflammatory process inside cells</td>
<td>1mM = 0,014%</td>
</tr>
<tr>
<td>block of mitochondrial DNA mutation and damage (view study)</td>
<td>Ectoin® completely (100%)! inhibits UVA radiation-induced mitochondrial DNA mutation</td>
<td>1mM = 0,014%</td>
</tr>
<tr>
<td>UVA and visible-light protection (view overview, view study, )</td>
<td>level of photo protection of Ectoin®: 92,7% (against visible) and 68,9% (UVA/visible)</td>
<td>0,025, 0,05 and 0,0004%, 0,0007%, 0,0014%</td>
</tr>
</tbody>
</table>
## pollution study overview – in vitro

<table>
<thead>
<tr>
<th>claim</th>
<th>results</th>
<th>usage level</th>
</tr>
</thead>
<tbody>
<tr>
<td>reduction of pollution induced pigmentation (POMC expression) in Asian skin (view study)</td>
<td>reduction of POMC expression up to 75% in Asian keratinocytes</td>
<td>0,028%</td>
</tr>
<tr>
<td>reduction of pollution induced pigmentation (POMC expression) in Caucasian skin (view study)</td>
<td>reduction of POMC expression up to 53% in Caucasian keratinocytes</td>
<td>0,028%</td>
</tr>
<tr>
<td>reduction of pollution induced wrinkle formation (MMPI expression) in Asian and Caucasian skin (view study)</td>
<td>reduction of air pollution induced MMPI expression up to 42%</td>
<td>0,028%</td>
</tr>
</tbody>
</table>
study overview  - Skinethic® (3D skin model)

<table>
<thead>
<tr>
<th>claim</th>
<th>results</th>
<th>usage level</th>
</tr>
</thead>
<tbody>
<tr>
<td>reduction of sun burn cells (degenerated keratinocyte transforming into cell death); Protection against harmful effects of UVB radiation</td>
<td>Ectoin® significantly reduces the number of sunburn cells. It protects skin cells against harmful effects and damages of the sun.</td>
<td>4%</td>
</tr>
</tbody>
</table>
Ectoin®

PROTECTION & SKIN IMPROVEMENT
protection against external stress factors / increase of membrane stability

- Human erythrocytes are treated for 0, 6, 8 and 24 hours with 1% Ectoin®
- Cells are stressed with 0.04% SDS for 10 minutes
- Cell lysis is determined photometrically via the content of free hemoglobin

Result:

- Increase in membrane stability by 60% due to pre-treatment with Ectoin® for 24 hours
- Ectoin® protects skin from damaging external stress factors

Difference (%) of cell lysis as a function of time pre-treated with 1% Ectoin against untreated control. The experiment is repeated five times.
cell protection: reduction of sun burn cells

24 hours pre-treatment with 4% Ectoin® or placebo

UVB-radiation of organotypic skin (Skinethic®)

histological proof of sun burn cells

Results

Ectoin® significantly reduces the number of sunburn cells.

Ectoin® significantly protects cells against harmful effects and damages of the sun.

Bünger et al, IFSCC 4 (2), 2001
boosts of skin’s self defense - increase of heat shock proteins

Human skin cells produce heat-shock proteins under external stress like UV-radiation or other physical and chemical stress factors. Heat-shock proteins (HSP) recognize, stabilize and metabolize damaged proteins in the skin.

**in vitro study:**
- human keratinocytes pretreated with 1% Ectoin®
- expose to heat-shock for 60 minutes
- control: non treated keratinocytes
- produced stress proteins (HSP 72/72) marked with fluorescent antibodies

**Result:**
- Pretreatment with Ectoin® causes a more rapid supply of protection proteins.
- Skin cells can protect themselves about 2 to 3 times faster than non-treated cells. This guarantees the survival and protection of skin cells against external stress.

Bünger and Beyer, 2001

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Microscopic analysis of stress response (HSP 72/73 expression) in untreated (red) and in Ectoin treated (blue) keratinocytes.
**boost of skin immune system – protection of Langerhans cells**

- **in vivo study**
- treatment twice daily with 0, 0.3% or 0.5% Ectoin® for 14 days
- UV-stress (1.5 MED) of human forearm
- skin preparation and histological proof of Langerhans cells

**Results**

- Ectoin® protects 100% of the Langerhans cells in UV-irradiated human skin
- No damage of Langerhans-Cells at all!

Bünger et al, IFSCC 4 (2), 2001
Protection of Langerhans cells

Results

Ectoin® protects the number of Langerhans cells in UV-irradiated human skin.

Formulation with 0,5% Ectoin® completely inhibits damage of Langerhans cells (compare picture 1 with 4!)

untreated / un-irradiated control
untreated / UV-irradiated
Placebo-treated / UV-irradiated
Ectoin® treated / UV-irradiated

skin preparations; histological proof; Bünger et al, IFSCC 4 (2), 2001
UVA-stress model – the pathway to premature photo aging

UVA irradiation

Cell Membrane

Cytoplasm

Mitochondrion

Cytochrome C

Sphingomyelin

Expression of pro inflammatory genes (ICAM1)

Cascade induced by UVA

modified model of Grether-Beck et al.
human keratinocytes (untreated and pre-treated for 24h with a 1mM solution of Ectoin) exposed to a dose of 30J/cm² UVA-irradiation

- cell harvesting after 1h after irradiation
- determination of second messenger release

Results:

- Pre-treatment with Ectoin® completely prevents UVA radiation induced second messenger release

Grether-Beck et al, 2000
Inhibition of AP-2 activation and ICAM1 Expression (2./3. step in stress model)

- 24 hours pre-incubation of human keratinocytes with 1 mM Ectoin®. Irradiation with 30 J/cm² UVA for 1 hour
- Determination of AP-2 activation and ICAM-1 expression

Result: Ectoin® reliably inhibits the UV induced initiation of the inflammatory process inside cells

Bünger und Driller, Skin Pharmacol Physiol 17: 232, 2004
Block of mitochondrial DNA mutation and damage

UVA-radiation induced mutations of mitochondrial DNA cause aging of cells and tissue and thus wrinkle formation.

Test protocol:
- Fibroblasts were pretreated with 1mM Ectoin® over 24h.
- Human dermal fibroblasts were exposed 3 times per day to 8 J/cm² on 4 consecutive days over 3 weeks.
- The mutations of the mitochondrial DNA were measured with polymerase chain reaction.

Results:
- Pre-treatment with Ectoin® was sufficient to completely inhibit UVA radiation-induced mitochondrial DNA mutation.
sun induced skin aging – more than UVA + UVB...

Visible light and IR irradiation penetrate very deep into the skin and cause damage.

“Visible light” is a major part of sunlight. People are also exposed to “visible light” through computers, televisions and cell phones.
UVA and visible light protection – in vitro study

- photo protective capacity of Ectoin® against UVA/visible and visible irradiations was measured by comet assay
- pretreated with Ectoin® (0.025, 0.05 and 0.1mM)
- keratinocytes were irradiated by combined (UVA/visible) and visible light (dose of 15 J/cm²)

Results:
- all tested concentrations showed a highly significant level of photo protection against UVA/visible and visible light
- level of photo protection: 92.7% (against visible) and 68.9% (UVA/visible)
- Ectoin® completes sunscreen and day care formulations - for a full spectrum protection against UVA- radiation and visible light

IR: untreated, irradiated cells
Controls: untreated, non irradiated

Botta et al, 2008
Ectoin®

IN VIVO STUDIES (ANTI-AGING CLAIMS)
long-term 7 day hydration – in vivo study

0.5% and 1% Ectoin® formulations applied twice a day for 12 days on forearm of 5 volunteers
skin hydration was measured from day 8 to day 12
on day 12, application of Ectoin® was stopped for 7 days; detecting the skin hydration finally at day 19

Results:

- hydration increased up to 200% after 8 days of application
- hydration remained constant until end of testing period although the topical application was stopped on day 12
- actual hydration status was preserved for further 7 days

Ectoin® showed a significant long-term moisturizing effect

Graf et al. 2008
wrinkle reducing properties - in vivo study

Visualization of the smoothing effect by Visioscan

Clinical Trial: test protocol
- double-blind, intra-individual, study with 30 female volunteers (30-60 years)
- normal, dry & sensitive skin
- treatment with 2% Ectoin® cream respectively placebo twice daily for 28 days

Results:
visible and significant reduction of the skin micro relief. Deep structures of were smoothed.
The skin looks healthier and younger.
100% of the subjects demonstrated a significant effect.

23% reduction of wrinkle volume after 4 weeks
skin roughness/ smoothing – in vivo study

- double-blind, intra-individual, study with 30 female volunteers (30-60 years)
- normal, dry & sensitive skin
- treatment with 2% Ectoin® cream respectively leading cosmetic formulation twice daily for 4 weeks
- skin surface analysis with SELS method (Visioscan), described by 5 parameters: roughness, scaling, smoothness, wrinkling and volume

Results

- skin roughness was decreased by 86% with 2% Ectoin® cream
reduction of skin scaling – in vivo study

- double-blind, intra-individual, study with 30 female volunteers (30-60 years)
- normal, dry & sensitive skin
- treatment with 2% Ectoin® cream respectively leading cosmetic formulation twice daily for 4 weeks
- skin surface analysis with SELS method (Visioscan), described by 5 parameters: roughness, scaling, smoothness, wrinkling and volume

**Results**

- skin scaling was decreased by 76% with Ectoin®

Heinrich et al, Skin Pharmacol Physiol 20: 211, 2007
increasing of skin elasticity – in vivo study

- monocentric, randomized, double-blind, vehicle-controlled application test
- 30 female volunteers (30-60 years old) with normal, dry and sensitive skin using cream containing 2% Ectoin® and vehicle 2x/day for 28 days
- elasticity measurement with Cutometer® (in AU) at D0 and D28
- measured parameters: visco-elasticity & biologic elasticity

<table>
<thead>
<tr>
<th>Elasticity</th>
<th>week 0</th>
<th>week 4</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Visco-elasticity</td>
<td>▼</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2% Ectoin®</td>
<td>0,12</td>
<td>0,11</td>
<td>-7,5</td>
</tr>
<tr>
<td>vehicle</td>
<td>0,11</td>
<td>0,12</td>
<td>+3,5</td>
</tr>
<tr>
<td>untreated control</td>
<td>0,14</td>
<td>0,15</td>
<td>+9,1</td>
</tr>
<tr>
<td>Biologic elasticity</td>
<td>▲</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2% Ectoin®</td>
<td>0,84</td>
<td>0,86</td>
<td>+2,4</td>
</tr>
<tr>
<td>vehicle</td>
<td>0,85</td>
<td>0,83</td>
<td>-1,6</td>
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<tr>
<td>untreated control</td>
<td>0,78</td>
<td>0,75</td>
<td>-3,5</td>
</tr>
</tbody>
</table>
Ectoin®

…the truly super active

- Ectoin® enhances the skin condition – even after application is stopped (in contrast: hyaluronic acid – Ectoin® is not metabolized)
- Ectoin® has a global and strong protection approach – it protects the “whole system” (in contrast: e.g. antioxidants)
- Ectoin® is a single pure molecule (in contrast: e.g. algae extract)
- one of the broadest documentations in the industry
- golden standard studies (in vivo, ex vivo, in vitro)
- Ectoin® is used in medical devices against dermal diseases, allergy etc.
- Ectoin® is very easy to formulate:
  - at all temperatures (up to 80°C)
  - at a broad pH-range (1-9)
  - excellent water solubility
- Ectoin® works already at low dosage
### physical & chemical data in a nutshell

<table>
<thead>
<tr>
<th>Property</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dosage</td>
<td>0.3 - 2%</td>
</tr>
<tr>
<td>Appearance</td>
<td>white crystalline powder, odorless</td>
</tr>
<tr>
<td>INCI</td>
<td>Ectoin</td>
</tr>
<tr>
<td>Content</td>
<td>≥ 96% (≤ 4% Hydroxyectoin)</td>
</tr>
<tr>
<td>Solubility</td>
<td>excellent solubility in water</td>
</tr>
<tr>
<td>Chemical name</td>
<td>(4S)-2-methyl-1,4,5,6-tetra- hydropyrimidine-4-carboxylic acid</td>
</tr>
<tr>
<td>Molecular formula</td>
<td>C₆H₁₀N₂O₂</td>
</tr>
<tr>
<td>CAS</td>
<td>96 702-03-3</td>
</tr>
<tr>
<td>Molar mass</td>
<td>142.16</td>
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<tr>
<td>Shelf life</td>
<td>5 years</td>
</tr>
<tr>
<td>Stability</td>
<td>pH 1-9</td>
</tr>
<tr>
<td>Storage conditions</td>
<td>dry place, temperature from 15°C - 25°C</td>
</tr>
<tr>
<td>Pack sizes</td>
<td>1kg, 5kg, 25kg</td>
</tr>
</tbody>
</table>

![Chemical structure](image.png)

**Ectoin®**
Ectoin®
ULTRA STRONG ANTI-POLLUTION
Ectoin®

**key facts**

**benefits for the skin:**
- protection against pollution induced wrinkle formation and hyperpigmentation
- protection against damage by extreme temperature changes
- protection against desiccation

→ Ectoin® is the perfect „urban protection“ active for extreme conditions: pollution, heat/cold, dryness, temperature changes
Ectoin®
STUDY DATA – ANTI POLLUTION
...study background

large „pollution study“ by Vierkoetter et al. 2010: „Airborne Particle Exposure and Extrinsic Skin Aging“ (IUF; Prof. Krutmann)
400 females; 1 year; German urban and rural areas
current study in China (Shanghai)

Summary:
Extrinsic skin aging not longer results only from exposure of skin to solar radiation and to tobacco smoke but also from exposure to ambient particulate matter (air pollution).
Clinical symptoms of extrinsic skin aging include coarse wrinkles, irregular pigment spots, and elastosis.
measured parameters – POMC (Pro-opiomelanocortin)

POMC is a marker gene for hyperpigmentation and age spot formation

POMC expression in keratinocytes is induced by environmental air pollution parameter like fine and ultrafine particles (e.g. diesel exhaust) as well as industrial emissions loaded with heavy metals and noxious chemicals.
measured parameters - MMP1 (matrix metallopeptidase I)

» MMP1 is a marker gene for wrinkle formation

» environmental air pollution parameter like fine and ultrafine particles (e.g. diesel exhaust) as well as industrial emissions trigger MMP1 expression in keratinocytes
Effect on air pollution induced POMC expression - ex vivo study

Test protocol:

- un-treated and pre-treated (24h; 2 mM (0.028%) Ectoin® solution) human epidermal keratinocytes of a female Asian
- stressed with fine and ultrafine carbon black particles and different types of diesel exhaust particles (1.5µgcm⁻²)
- determination of POMC expression in untreated and pre-treated keratinocytes after stress

Results:

- reduction of POMC expression up to 75% in Asian keratinocytes
- Ectoin® dramatically reduces air pollution induced POMC Expression in Asian skin
- reduction of POMC Expression reduces and inhibits air pollution induced hyperpigmentation and age spot formation
**effect on air pollution induced POMC expression - ex vivo study**

**Test protocol:**
- un-treated and pre-treated (24h: 2mM (0.028%) Ectoin® solution) human epidermal keratinocytes of a female Caucasian
- stressed with fine and ultrafine carbon black particles and different types of diesel exhaust particles (1.5µgcm⁻²)
- determination of POMC Expression in untreated and pre-treated keratinocytes after stress

**Results:**
- reduction of POMC expression up to 53% in Caucasian keratinocytes
- Ectoin® dramatically reduces air pollution induced POMC Expression in Caucasian skin
- reduction of POMC Expression reduces and inhibits air pollution induced hyperpigmentation and age spot formation

*(back)*
effect on air pollution induced MMP1 expression - ex vivo study

Test protocol:
- untreated and pre-treated (24h: 2mM (0.028%) Ectoin® solution) human epidermal keratinocytes
- stressed with fine and ultrafine carbon black particles and different types of diesel exhaust particles (1,5µgcm⁻²)
- determination of MMP1 Expression in untreated and pre-treated keratinocytes after stress

Results:
- reduction of air pollution induced MMP1 expression up to 42%
- Ectoin® reduces air pollution induced MMP1 Expression in skin cells
- reduction of MMP1 Expression reduces and inhibits air pollution induced wrinkle formation

MMP1 expression in keratinocytes is induced by environmental air pollution parameter like fine and ultrafine particles (e.g. diesel exhaust) as well as industrial emissions loaded with heavy metals and noxious chemicals.
Asian and Caucasian epidermal keratinocytes stressed with different diesel exhaust particles, nano (ultra fine) particles and fine particles.

- Fine, nano and exhaust particles dramatically trigger MMP1, Cyp1A1 and POMC Expression
- Asian skin is more sensitive to POMC-Expression (pigmentation)
- Caucasian skin is more sensitive to MMP1-Expression (wrinkle formation)

Ectoin® is a very effective anti-pollution agent for Asian and Caucasian skin. It dramatically protects skin from air pollution induced pigmentation (age spots, hyperpigmentation) & wrinkle formation.

It has been shown, that Ectoin® also protects the human lung from pollution induced damage (COPD, asthma, allergy/pollens…)

More data available end of 2014.
Ectoin®
HAIR CARE & ORAL CARE
HAIR CARE
effects of Ectoin® on follicular aging process (study by Giesen et al. 2013)

Aim of this study:
- investigate the effect of Ectoin® on hair follicle cells subjected to oxidative stress

Study protocol:
- follicular outer root sheath (ORS) keratinocytes and reconstructed hair follicle models were stressed with sub-lethal doses of hydrogen peroxide

Measured and determined parameters:
- expression of structural proteins and mitochondria relevant genes with and without pre-treatment with Ectoin®
- amount of produced reactive oxygen species (ROS) and the degree of DNA damage (determined by COMET assay)
ROS

Reactive oxygen species (ROS) are chemically reactive molecules containing oxygen. Examples include oxygen ions and peroxides and free radicals. During times of environmental stress (e.g., UV or heat exposure), ROS levels can increase dramatically. This may result in significant damage to cell structures (known as oxidative stress).

Exogenous ROS can be produced from pollutants, tobacco, smoke, drugs, xenobiotics, or radiation.

In general, harmful effects of reactive oxygen species on the cell are most often:

- damage of DNA
- oxidations of polyunsaturated fatty acids in lipids (lipid peroxidation)
- oxidations of amino acids in proteins
- oxidatively inactivate specific enzymes by oxidation of co-factors
Electron structures of common reactive oxygen species (ROS).

Cells have a variety of defense mechanisms to ameliorate the harmful effects of ROS – e.g. superoxide dismutase (SOD) catalyzes the conversion of two superoxide anions into a molecule of hydrogen peroxide (H2O2) and oxygen (O2) \( \rightarrow \) Glycoin\textsuperscript{®} natural!
results

» Ectoin® protects cellular functions against oxidative stress, the resulting DNA damage and impaired keratin synthesis.

» Mitochondrial parameters have been partially restored by Ectoin®.

» Ectoin® exhibits not only a significant restoration of essential cellular parameters under oxidative stress but also a reduction of DNA damage up to 80%.

» Ectoin® antagonizes follicular aging processes.
effects of Ectoin® on follicular aging process

DNA damage in ORS keratinocytes after treatment with Ectoin® followed by hydrogen peroxide

Fig. 2: DNA protection in ORS keratinocytes after 24h treatment with Ectoine followed by challenging the cultures with H₂O₂ (100 μM, 20 min). Values calculated related to %tail DNA. *** p-value ≤ 0.001 (student t-test)

Giesen et al, 2013
effects of Ectoin® on follicular aging process

Gene expression of hair keratins in reconstructed hair follicle models after treatment with Ectoin® followed by hydrogen peroxide stress

Fig. 3: Keratin gene expression after 6h treatment with Ectoine followed by challenging the cultures with H₂O₂ (100 μM, 20 min). Values calculated related to the H₂O₂ control. * p-value ≤ 0.05 (student t-test)

Giesen et al, 2013
effects of Ectoin® on follicular aging process

Gene expression of mitochondrial related genes in reconstructed hair follicle models after treatment with Ectoin® followed by hydrogen peroxide stress

Fig 4: Mitochondria related gene expression after 6h treatment with Ectoine followed by challenging the cultures with H₂O₂ (100 μM, 20 min). Values calculated related to the H₂O₂ control.

Giesen et al, 2013
effects of Ectoin® on follicular aging process

ROS production in ORS keratinocytes after treatment with Ectoin® followed by hydrogen peroxide stress

Giesen et al, 2013