Ectoin®

ULTIMATE PROTECTION & REPAIR

ANTI-POLLUTION

OUTSTANDING!

ECO CERT COSMOS CERTIFIED

MADE IN GERMANY
PROTECTED QUALITY

CHINA APPLICABLE

PATENTED INGREDIENT

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Extremolytes for Life

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Latest scientific evidence confirms, that air pollution contributes to premature skin aging like pigmentation and wrinkle formation and may even be the primary cause of ageing in some cases.
Furthermore air pollutants are also linked to causing or worsening acne, atopic dermatitis, eczema, allergic reactions and even skin cancer.

The natural active Ectoin® (INCI: Ectoin) is the only anti-pollution ingredient with clinically proven in vivo efficacy for cosmetics and healthcare products.

Ectoin® shows a dual anti-pollution activity: global protection from various air pollutants and powerful skin regeneration of environmentally damaged skin.

The anti-pollution efficacy of Ectoin® has been proven in vivo in multiple clinical studies with more than 190 test persons!

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Why is Ectoin® an outstanding anti-pollution ingredient?

- clinically proven anti-pollution efficacy
- unique dual anti-pollution action
- multiple benefits beyond anti-pollution
- natural molecule with well-understood mode of action
- convincing characteristics:
  - INCI: Ectoin
  - safe
  - certified and patented
  - very easy to formulate

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Why is Ectoin® an outstanding anti-pollution ingredient?

- anti-pollution efficacy was clinically proven on more than 190 test persons
- claim substantiation by standardized in vivo (clinical) study design for cosmetic AND healthcare applications
- anti-pollution efficacy was shown for various air pollution components – multiple PM sizes were tested, especially ultrafine/nano particles (UFP) as well as soot (carries PAHs), engine exhaust (contains heavy metals & PAHs) and cigarette smoke
- anti-pollution efficacy was confirmed by renowned independent institutes like the *IUF Leibniz Research Institute for Environmental Medicine* and the *Max Planck Institute*

clinically proven anti-pollution efficacy

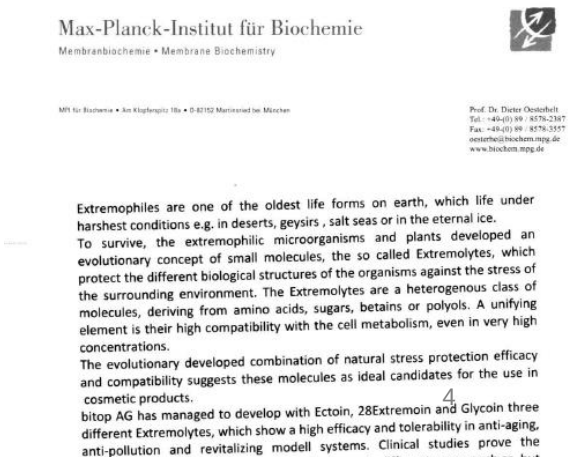
http://www.iuf-duesseldorf.com/pr20161027.html

Pollution and skin: From epidemiological and mechanistic studies to clinical implications

Jean Krutmann [a,b,*], Wei Liu [c], Li Li [d], Xiaochuan Pan [e], Martha Crawford [f], Gabrielle Sore [f], Sophie Seite [g]

relevant for wrinkle formation as well as skin carcinogenesis [47]. Another example is the osmolyte ectoine, which has been used as an active ingredient in cosmetic as well as cosmeceutical products for years and which was recently found to be capable of preventing particle-induced keratinocyte gene expression including MMP-1 expression (Krutmann et al. unpublished data). These studies indicate that it is indeed possible to develop specific cosmetic/cosmeceutical strategies to counteract pollution-induced skin

https://www.ncbi.nlm.nih.gov/pubmed/?term=airborne+particle+exposure+vierk%C3%B6tter+et+al


Max-Planck-Institut für Biochemie
Membranbiochemie • Membrane Biochemistry

Extremophiles are one of the oldest life forms on earth, which life under harshest conditions e.g. in deserts, geysirs , salt seas or in the eternal ice.
To survive, the extremophilic microorganisms and plants developed an evolutionary concept of small molecules, the so called Extremolytes, which protect the different biological structures of the organisms against the stress of the surrounding environment. The Extremolytes are a heterogenous class of molecules, deriving from amino acids, sugars, betains or polyols. A unifying element is their high compatibility with the cell metabolism, even in very high concentrations.
The evolutionary developed combination of natural stress protection efficacy and compatibility suggests these molecules as ideal candidates for the use in cosmetic products.
bitop AG has managed to develop with Ectoin, 28Extremoin and Glycoin three different Extremolytes, which show a high efficacy and tolerability in anti-aging, anti-pollution and revitalizing modell systems. Clinical studies prove the

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Why is Ectoin® an outstanding anti-pollution ingredient?

Ectoin® has a powerful dual anti-pollution action:

1. Ectoin® protects skin form (further) damage by pollution
2. Ectoin® repairs environmentally damaged skin:
   - repairs and enhances broken skin barrier
   - reduces skin inflammation and allergic reactions
   - improves wrinkles
   - reduces skin roughness and scaliness

unique dual anti-pollution action

Protection & repair = effective anti-pollution with visible results

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Why is Ectoin® an outstanding anti-pollution ingredient?

Ectoin® is a powerful und multifunctional active ingredient.
It stops and prevents cell damage and - at the same time - supports regeneration and repair for stressed and aged skin:

- **improvement and protection of skin´s self defense**
- **skin barrier repair**
- **UVA/UVB, visible light, IR-A protection on cellular level**
- **anti-inflammatory, anti-irritant**
- **wrinkle improvement**
- **DNA protection**

multiple benefits beyond anti-pollution

Please see Ectoin® brochure for detailed study data.

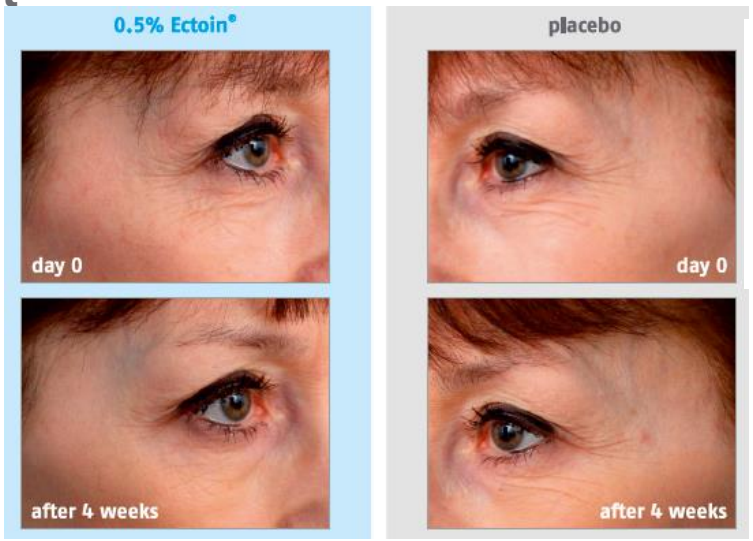

figure: placebo controlled in vivo study - 4 week 2/daily application of 0.5% Ectoin® and placebo on crow´s feet

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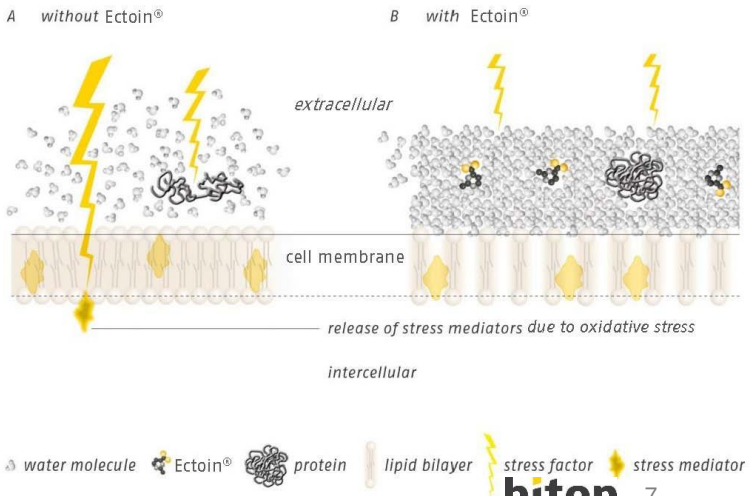
Why is Ectoin® an outstanding anti-pollution ingredient?

Ectoin® is the epitome of cell and skin protection.
It is a powerful stress-protection molecule – a so called extremolyte.
Ectoin® is found in microorganisms living in the most hostile conditions: salt lakes, the deep sea, deserts, arctic ice and hot springs. These so called extremophiles use Ectoin® as a protection shield from the stress factors of their habitats, like extreme UV radiation, dryness, cold, heat, salinity or chemical stress.

natural molecule with well-understood mode of action

This powerful protection activity is directly transferable to our skin and mucosa.
Ectoin® builds protection shells around skin cells, thus stabilizes cells and their membranes and enhances the cell functions. This leads to a general improvement of cell condition, reduction of ROS and cell degeneration. Ectoin® is able to even completely stop cell damage (in vivo proven)!



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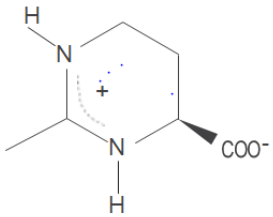
Why is Ectoin® an outstanding anti-pollution ingredient?

- single natural molecule - amino acid derivate
- INCI: Ectoin
- white odorless powder



convincing characteristics

- 100% natural and GMO-free:
  EcoCert, COSMOS and Natrue certified
- safe: approved for medical use (oral, lung inhalation, eye, nose,)
- China applicable
- very easy to formulate:
  - excellently water soluble - dissolves colorless
  - no incompatibilities with other ingredients
  - can be added directly to aqueous phase (hot or cold) prior to emulsification
  - very stable: all temperatures, pH 1-9, light and oxygen stable, shelf life >5 years

- patented anti-pollution efficacy (see page 25)
- biotechnologically manufactured in Germany according to ISO 13485

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air pollution is the world´s biggest environmental health risk

Outdoor air pollution is a major environmental health problem affecting everyone in developed and developing countries alike.

4.2m

deaths linked to long-term exposure to $PM_{2.5}$ – China and India together accounted for 52%

Source: State of Global Air 2017

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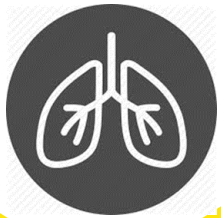

Anti-pollution efficacy and safety of Ectoin® was clinically proven on human lungs (tested persons: 189):

Ectoin® has been tested in multiple clinical studies on patients with lung and airway problems like bronchitis, allergic asthma or pollution induced chronic obstructive pulmonary disease (COPD).
Tested formulation: Ectoin® inhalation solution (INCI: Water, Ectoin, Sea Salt)

1. clinical study with **135 patients**: significant improvement of particle induced bronchitis
2. clinical safety and efficacy study with **18 patients:** significant improvement of hypersensitivity and reduction of lung inflammation.
3. clinical study with **36 patients:** significant and positive effects on lung-inflammation markers.

Scientific Publications:

http://erj.ersjournals.com/content/41/2/433

https://www.dovepress.com/reduction-of-neutrophilic-lung-inflammation-by-inhalation-of-the-compa-peer-reviewed-article-COPD

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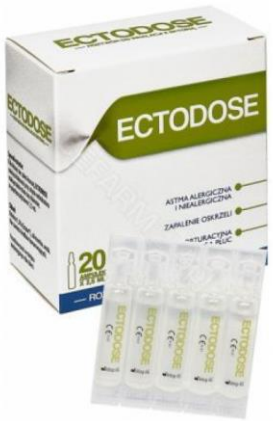
Ectoin® based products for the treatment of pollution induced health problems (medical application)

Ectoin® is the only cosmetic anti-pollution active ingredient, which is also approved for anti-pollution medical applications.

It is used e.g. for the therapy of particle induced COPD and asthma.
Products in the market are e.g. “PARI ProtECT inhalation solution” (Pari, Germany) and “Ectodose inhalation ampoules” (Solinea, Poland).



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theguardian

Source: https://www.theguardian.com/environment/2016/sep/16/new-inhaler-protects-lungs-against-effects-of-air-pollution

New inhaler protects lungs against effects of air pollution

Inexpensive over-the-counter product could help millions of people avoid worst health effects of breathing toxic air, say scientists

School children take part in an anti-air pollution campaign in London, UK. Photograph: Chris Radburn/PA

Ectoin® anti-pollution inhaler
watch video on youtube:
https://www.youtube.com/watch?v=O1tvJzCcbYQ

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Air pollution and skin aging

air pollution is causing:

- **pigment spots and hyperpigmentation**
- **coarse wrinkle formation**

Source: Vierkoetter et al., 2010

twin research (smoker vs. non-smoker) gives a hint…

Source: Okada et al. 2013

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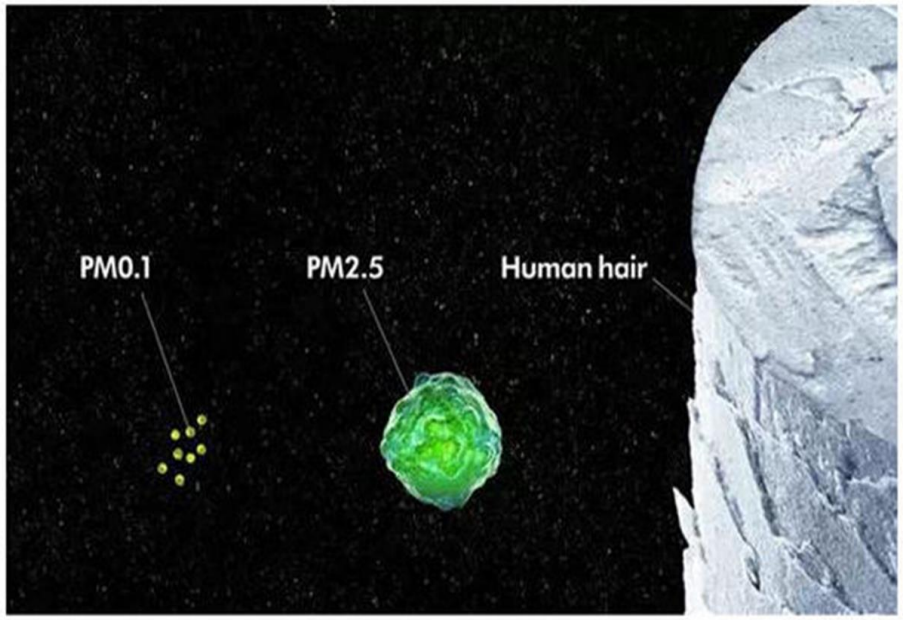
Pollution and skin aging: why you should focus beyond PM2.5:

Particulate matter consists to 80 % of ultrafine particles (UFP) like PM0.1.

The highest level of concentration of toxic compounds is related to ultrafine particles (PM0.1 and smaller).

Particle sizes PM0.1 and smaller can penetrate into our bloodstream and enter cells.



However - size is not everything!

The PM size of an individual particle does not allow for a complete conclusion about its skin toxicity.
The toxicity of a pollution particle is additionally determined by its form and substance, in other words - the chemistry and physics of a particle play also an important role.

Ultra fine pollution particles (UFP) loaded with PAHs (polycyclic aromatic hydrocarbons) have been shown to be the main cause for pollution induced skin pigmentation and pigment spot formation (Vierkoetter et al, 2010). Test persons living in urban areas with high levels of PAHs had a 22 percent increase in pigmented spots. Furthermore also a significant decrease of wrinkle formation was detected. PAHs can be found in soot, industrial pollutants, emissions of engines, or cigarette smoke.

Furthermore chemicals and metals bind to pollution particles. They are shown to be capable of localizing in mitochondria and generating ROS, leading to skin aging by mitochondrial damage (Vierkoetter et al, 2010).

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Some reflections…

Functional anti-pollution ingredients, which form a film on the surface of the skin (e.g. polymers, emollients) can not prevent penetration of ultrafine particles (e.g. loaded with PAHs) and thus the consequent cell damage.
Ectoin® protects skin also on cellular level. It has been shown (ex vivo), that Ectoin® prevents cell damage caused by ultrafine particle, soot and other pollution components.

The mode of action of anti-oxidants is to neutralize free-radicals. But they can not prevent the formation of free radicals.
Ectoin® can. It prevents and lowers the formation of ROS.

Furthermore, it has been shown, that an intact skin barrier is an effective way to reduce pollution induced skin damage.
Several clinical studies demonstrated excellent skin barrier repair and improvement by Ectoin® .

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ANTI-POLLUTION CLAIM SUBSTANTIATION FOR COSMETIC APPLICATIONS

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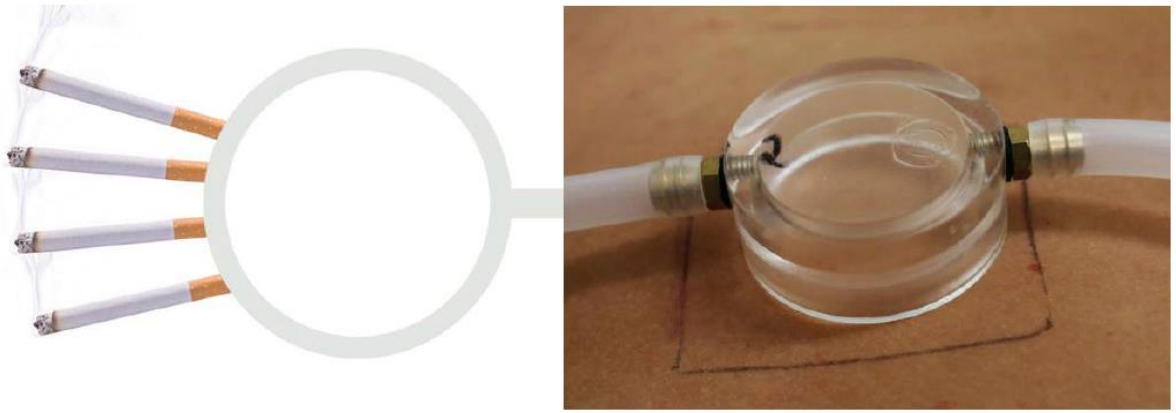
in vivo anti-pollution study

Study protocol

- in vivo study (placebo controlled, randomized, double blind) on volar forearm of six volunteers
- twice-daily application of placebo or **1% Ectoin®** (verum) for 5 days
- on day 5: skin was stressed with cigarette smoke as pollutant for 15 min using a standardized pollution chamber system
- measured parameter: concentration of Malondialdehyde (MDA)

After 5 days of pre-treatment with **1% Ectoin®** and placebo, the skin was stressed with pollution using a standardized cigarette smoke chamber system.



Measured parameter: MDA

MDA results from lipid peroxidation of polyunsaturated fatty acids in the skin and is one of the reactive electrophile species that cause toxic stress. Therefore it can be used as a marker for air- pollution induced skin damage.

Picture 1: in vivo anti-pollution study design.

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in vivo study - results

Reduction of pollution induced skin damage

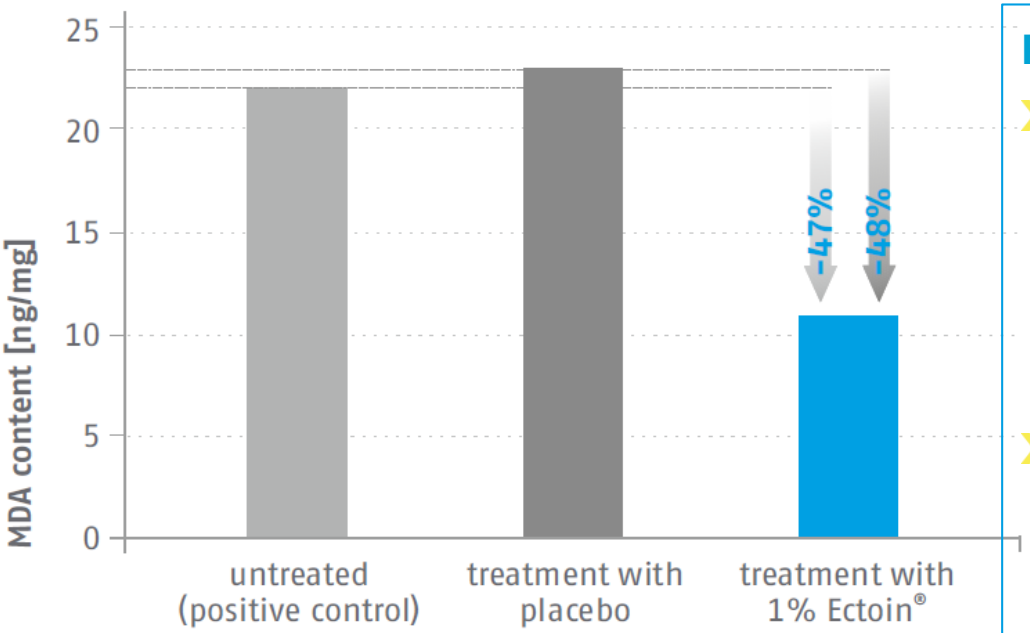

Figure: reduction of pollution induced skin damage

RESULTS:

- after only 5 days of application with **1% Ectoin®** the pollution-induced MDA content in the skin was reduced **by 48% compared to the placebo treated areas**.
- in the same study-set up, a strong anti-oxidant was capable to reduce the pollution-induced MDA content in the skin by only 27% (data provided by cro)

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ex vivo anti pollution study

Study protocol

- fresh human epidermal cells from a female Asian and a female Caucasian donor
- skin cells were untreated or pre-treated with a 2mM Ectoin® solution for 24hours
- pre-treated and untreated skin cells were stressed with different types and sizes of pollution particles
- Measured parameter: POMC up-regulation in skin cells

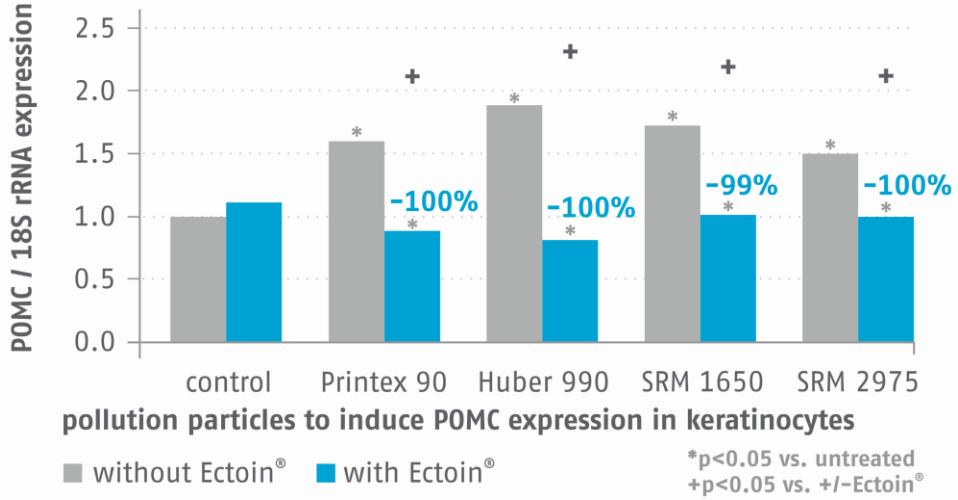

Figure: up-regulation of POMC (marker for pigmentation) in human keratinocytes (Asian and Caucasian) by different ultrafine pollution particles and components

Tested on Asian and Caucasian skin cells.

Measured parameter:
POMC = marker for pigmentation

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ex vivo study - results

used pollution particles to induce gene expression in keratinocytes	POMC up-regulation in keratinocytes
Printex90 (ultrafine particles): 0.014µm particle diameter (nanosize)	0%
Huber990 (fine particles): 0.26µm particle diameter	0%
SRM1650 (engine exhaust soot): contains PAHs & heavy metals, particle sizes PM2.5, PM10, PM1	1%
SRM2975 (diesel soot): contains PAHs & heavy metals, particle sizes PM2.5, PM10, PM1	0%

RESULTS:

- Ectoin® completely inhibits pollution induced POMC up-regulation in skin cells.
- Ectoin® is able to down-regulate pollution stimulated pigmentation.

Please see Ectoin® brochure for more ex vivo study data: reduction of pollution induced MMP-1 und Cyp1A1 expression!

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in vitro study – pollen penetration protection

Beside PM, allergens such as pollen are penetrating the human body through the skin, leading to allergic reactions including exacerbation of atopic dermatitis, disturbing the skin barrier and inducing skin aging.

An ideal pollution protection therefore does not only protect the skin from damage of PM, PM and UP, but also from other airborne particles, like allergen loaded pollen.

RESULTS:

- Ectoin® protects epithelial structures from penetration by allergens by up to 50%.
- By the unique action of Ectoin®, the skin barrier´s disturbance is reduced, less allergic reactions occur and the resulting skin aging and damage is ameliorated.
- Ectoin® is the only pollution protection active ingredient, which also reduces allergic reactions induced by airborne allergens such as pollen (shown in vivo).

Please see Ectoin® brochure for more detailed study data!

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Summary

The natural stress protection molecule Ectoin® offers broad and global clinically proven and safe anti-pollution efficacy going further than anti-oxidants and polymers.

Ectoin® shows a dual anti-pollution action: firstly, it protects skin from (further) pollution induced damage and secondly repairs environmentally stressed skin.
This two-step and complementary anti-pollution activity was also clinically proven for healthcare applications (e.g. Ectoin® lung inhalation for the treatment of particle induced asthma).
Ectoin® offers:

- global protection and prevention of PM, UFP, PAH and pollen induced skin damage like skin irritation, pigmentation and wrinkling
- it supports skin barrier repair and reduces skin irritations, inflammations and allergic reactions
- it reduces or prevents UV, visible light and IR-A induced skin damage on cellular level
- it improves wrinkles, skin smoothness and roughness

For powerful anti-pollution and the visible results your customer deserves!

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Anti-pollution claim ideas:

- PM-protection, „pollu-guard“, skin-guard, “PM skin repair”
- fights pollution-aging /PM-aging
- protects the skin from the negative impact of particulate matter
- global urban protection; full spectrum anti-pollution
- protects the skin from the pollution induced cell-answer for pigmentation and wrinkle formation
- shields the skin from pollution-aging
- reduces the visible signs of city-life / urban / PM stress
- global pollution protection (PM10, PM2.5, PM1 and UP)
- pollution and UV-shield for the skin
- acts before damage occurs („pro active“)
- long-lasting protection („skin enhancer“)

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anti-pollution products with Ectoin®

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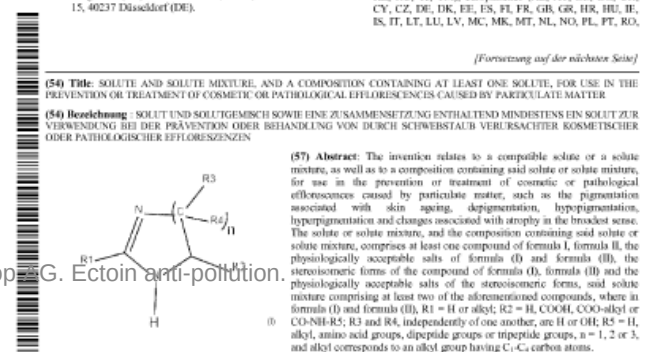
The use of Ectoin® for anti-pollution applications and the treatment of pollution induced skin/mucosa problems is patented / applied for patent:

WO patent application:



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(81) Bestimmungsstaaten *(soweit nicht anders angegeben, für jede verfügbare nationale Schutzrechtsart)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JP, KE, KG, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

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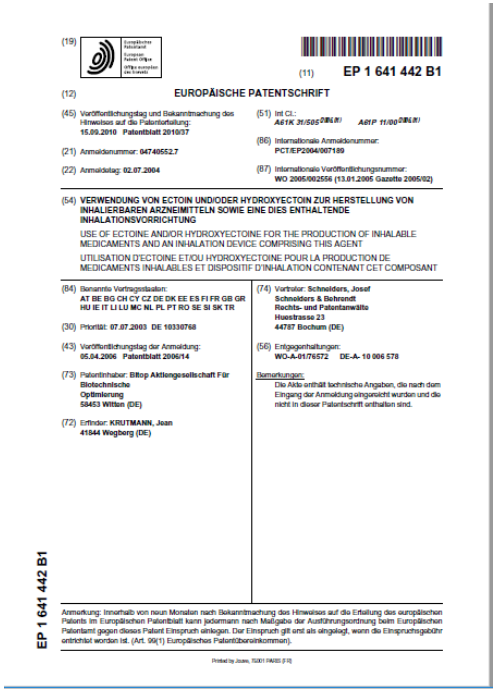
[Fortsetzung auf der nächsten Seite]

(54) Title: SOLUTE AND SOLUTE MIXTURE, AND A COMPOSITION CONTAINING AT LEAST ONE SOLUTE, FOR USE IN THE PREVENTION OR TREATMENT OF COSMETIC OR PATHOLOGICAL EFFLORESCENCES CAUSED BY PARTICULATE MATTER

(54) Bezeichnung : SOLUT UND SOLUTGEMISCH SOWIE EINE ZUSAMMENSETZUNG ENTHALTEND MINDESTENS EIN SOLUT ZUR VERWENDUNG BEI DER PRÄVENTION ODER BEHANDLUNG VON DURCH SCHWEBSTAUB VERURSACHTER KOSMETISCHER ODER PATHOLOGISCHER EFFLORESZENZEN

(57) Abstract: The invention relates to a compatible solute or a solute mixture, as well as to a composition containing said solute or solute mixture, for use in the prevention or treatment of cosmetic or pathological efflorescences caused by particulate matter, such as the pigmentation associated with skin ageing, depigmentation, hypopigmentation, hyperpigmentation and changes associated with atrophy in the broadest sense. The solute or solute mixture, and the composition containing said solute or solute mixture, comprises at least one compound of formula I, formula II, the physiologically acceptable salts of formula (I) and formula (II), the stereoisomeric forms of the compound of formula (I), formula (II) and the physiologically acceptable salts of the stereoisomeric forms, said solute mixture comprising at least two of the aforementioned compounds, where in formula (I) and formula (II), R1 = H or alkyl; R2 = H, COOH, COO-alkyl or CO-NH-R5; R3 and R4, independently of one another, are H or OH; R5 = H, alkyl, amino acid groups, dipeptide groups or tripeptide groups, n = 1, 2 or 3, and alkyl corresponds to an alkyl group having C_1-C_4 carbon atoms.

Granted EU patent:



(19) Europäisches Patentamt
European Patent Office
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(12) EUROPÄISCHE PATENTSCHRIFT

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(54) **VERWENDUNG VON ECTOIN UND/ODER HYDROXYECTOIN ZUR HERSTELLUNG VON INHALIERBAREN ARZNEIMITTELN SOWIE EINE DIES ENTHALTENDE INHALATIONSVORRICHTUNG**

USE OF ECTOINE AND/OR HYDROXYECTOINE FOR THE PRODUCTION OF INHALABLE MEDICAMENTS AND AN INHALATION DEVICE COMPRISING THIS AGENT

UTILISATION D'ECTOINE ET/OU HYDROXYECTOINE POUR LA PRODUCTION DE MEDICAMENTS INHALABLES ET DISPOSITIF D'INHALATION CONTENANT CET COMPOSANT

(84) Benannte Vertragstaaten:
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