VITACHELOX®
SKIN ANTIPOLLUTION
A GLOBAL STRATEGY

in-cosmetics®
global
London • 4-6 April 2017
POLLUTION HAS AN IMPACT ON HEALTH

Air pollution
- Nerve damage
- Lead
- Particulate matter
- Ozone
- Volatile organic compounds

CO

Water pollution
- Bacteria
- Parasites
- Chemicals

SO₂

SO₂(NOₓ)

Respiratory illness
- Gastroenteritis
- Cancer risk
- Nausea
- Skin irritation

Headache
Fatigue

Soil contamination
- Pesticides
AIR POLLUTION HAS AN IMPACT ON HEALTH

• According to the EPA (Environmental Protection Agency) over 142 mil Americans live in areas with poor air quality.

• The WHO attributed 7 millions premature deaths to air pollution exposure.

• It has long been recognized that air pollution affects **skin health** as well. Not only because of UV radiation harmful damage.
Air pollution and skin diseases: Adverse effects of airborne particulate matter on various skin diseases

Kyung Eun Kim, Daeho Cho, Hyun Jeong Park

Abstract
Environmental air pollution exposure causes various health problems, including skin diseases. In this study, we evaluated the effects of particulate matters (PM) on skin cells. The results indicate that PM, especially PM10, causes significant oxidative stress via the production of reactive oxygen species (ROS) and induces DNA damage.

Keywords: Particulate matter, skin injury, oxidant stress, DNA damage.

1. Introduction

Skin protection has become a major concern in today's society, with increased awareness of environmental and lifestyle factors. In recent years, the trend towards natural and organic cosmetics has grown significantly. This increase in demand for skin protection is due to various factors, including the growing awareness of environmental pollution and the health risks associated with it. Environmental pollution is a major source of skin damage, and it is estimated that over 90% of skin damage is caused by environmental factors.

2. Methods

2.1. Particulate matter air pollution and skin damage

Particulate matter (PM) is a major component of air pollution, and it has been linked to various health effects, including skin damage. PM can enter the skin through inhalation or direct contact, and it can cause oxidative stress, inflammation, and DNA damage.

2.2. Customer impact of particulate matters

Customer surveys have shown that a significant number of consumers are concerned about skin damage caused by PM. This concern has led to increased demand for skin protection products, with a particular focus on products that offer natural and organic ingredients.

3. Discussion

The increasing demand for skin protection products highlights the importance of understanding the impact of PM on skin health. Further research is needed to develop effective skin protection strategies that can help minimize the damage caused by environmental pollution.

4. Conclusions

In conclusion, environmental air pollution, especially particulate matter, has a significant impact on skin health. Skin protection products are becoming increasingly popular, and the demand for natural and organic ingredients is on the rise.

References


AIR POLLUTANTS

Outdoor air pollution is comprised of organic and inorganic substances introduced into the atmosphere.

Typically:

• **Heavy metals:** common air pollutants that pose health hazards due to bioaccumulation and reduction of intrinsic defenses

• Gaseous pollutants (as ozone and VOCs)

• Persistent Organic Pollutants (POP) (as pesticides and dioxin)

• Particulate matter (a complex mixture of liquid and/or solid droplets suspended in gas, of which PM10 is the most popular)
WHY ARE AIR POLLUTANTS TOXIC TO THE SKIN?

• Generation of free radicals

• Induction of the inflammatory cascade (dust particles containing heavy metals have been shown to increase the gene expression of pro-inflammatory cytokines)

• Alterations of cutaneous microflora

• Activation of AhR (playing a role in the melanogenesis regulation as well as in the development of inflammatory skin lesions)
WHY ARE AIR POLLUTANTS TOXIC TO THE SKIN?

The reactions induced by air pollutants translate into:

- Accelerated skin ageing
- Atopic dermatitis
- Pigmented spots
A PROPOSED STRATEGY

Heavy metals aggravate the toxic effects of the gaseous pollutants by reducing the natural defense means and accelerate skin ageing.

Indena proposes a strategy about:

- ANTIOXIDANT ACTIVITY
- FREE RADICAL SCAVENGING
- CHELATION
- PROTECTION OF VARIOUS CELLS STRUCTURES
NATURAL ANTIOXIDANTS: A CENTURY-OLD EXPERIENCE ON POLYPHENOLS

<table>
<thead>
<tr>
<th>Grape seed</th>
<th>Milk thistle</th>
<th>Green tea</th>
<th>Curcuma</th>
<th>Bilberry</th>
<th>Cranberry</th>
<th>Arthichoke</th>
<th>Oak</th>
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</table>

![Grape seed](image1)
![Milk thistle](image2)
![Green tea](image3)
![Curcuma](image4)
![Bilberry](image5)
![Cranberry](image6)
![Arthichoke](image7)
![Oak](image8)
THE SCREENING SPOTTED:

- Green tea extract (T)
- Grape seed extract (E)
- Oak wood extract (Q)
DPPH SCAVENGING ACTIVITY
THE IN VITRO EFFICACY

A bunch of botanical actives have been screened for their capacity to inhibit the formation of the free radical DPPH. Here the performance of our best candidates.
CHELATION POTENTIAL
THE IN VITRO EFFICACY

The same actives have been screened for their chelating activity.

Ferrus ions chelation IC50 expressed in ppm
Antioxidant actives Q T E vs EDTA

ppm

Room T 95°C - 15 min 95°C - 30 min

EDTA E T Q
# ANTIPOLLUTION RESULTS

## THE IN VITRO EFFICACY

The best botanical candidates have been screened for their capacity to protect skin cells from the toxic effects of heavy metals.

- Cells viability
- Cells metabolism (protein synthesis) have been evaluated

<table>
<thead>
<tr>
<th>Study name:</th>
<th>Evaluation of the protective effect of cosmetic raw materials against polluting agents</th>
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<tbody>
<tr>
<td>Experimental model</td>
<td>In vitro evaluation of the capability of the tested products to protect skin fibroblasts cultures against toxic effects induced by heavy metals (Pb-Fe-Cr).</td>
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<tr>
<td>Mesured parameters</td>
<td>Cells viability; cells metabolism (protein synthesis)</td>
</tr>
<tr>
<td>Results</td>
<td>Cells treatment with heavy metals showed a marked reduction in cells viability. Ingredients E, Q and T have shown to protect the cells from the toxic effects of heavy metals (Pb-Fe-Cr) (up to +73% in cells viability and +81% in cells metabolism)</td>
</tr>
<tr>
<td>Indications</td>
<td>Multifunctional treatment in anti-pollution topical products, urban protection</td>
</tr>
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</table>

ANTIPOLLUTION RESULTS
THE IN VITRO EFFICACY
ANTIPOLLUTION RESULTS
THE IN VITRO EFFICACY
GRAPE SEED EXTRACT

Cells viability

Cells metabolism

Viability %

+63%

Protein content (µg)

+28%
ANTIPOLLUTION RESULTS
THE IN VITRO EFFICACY
OAK EXTRACT

Cells viability

Viability %

Negative control  Positive control  ACTIVE Q 0.01%  ACTIVE Q 0.005%  ACTIVE Q 0.001%

+38%

Cells metabolism

Protein content (µg)

Negative control  Positive control  ACTIVE Q 0.01%  ACTIVE Q 0.005%  ACTIVE Q 0.001%

+81%
ANTIPOLLUTION RESULTS
THE IN VITRO EFFICACY
GREEN TEA EXTRACT

Cells viability

Viability %

0 20 40 60 80 100 120

Negative control Positive control ACTIVE T 0.01% ACTIVE T 0.005% ACTIVE T 0.001%

+45%

Cells metabolism

Protein content (µg)

0 10 20 30 40 50 60

Negative control Positive control ACTIVE T 0.01% ACTIVE T 0.005% ACTIVE T 0.001%

+72%
VALIDATING THE MODEL
AN INCREMENTAL APPROACH: VITACHELOX®

The winner is... active E for cells VIABILITY
The winners are...active T and Q for cells METABOLISM
How about \((Q+E)+T\)?

\[1+1>2 \ldots +1>3\]
**VITACHELOX®**
**SINERGY FOR ENHANCED PROTECTION**

How about Q+T+E?

1+1+1>3

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<td>In vitro evaluation of the capability of the tested products (VITACHELOX®) to protect skin fibroblasts cultures against toxic effects of polluting agents.</td>
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<td>Mesured parameters</td>
<td>Cells viability; cells metabolism (protein synthesis)</td>
</tr>
<tr>
<td>Results</td>
<td>Up to 78% protection on cells viability: SYNERGISTIC FUNCTION; up to 76% protection of cells metabolism</td>
</tr>
<tr>
<td>Indications</td>
<td>Multifunctional treatment in anti-pollution topical products</td>
</tr>
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VITACHELOX® SINERGY FOR ENHANCED VITALITY

Cells vitality improved by over 78%.
**VITACHELOX®**

**ENHANCED PROTECTION OF CELLS COMPARTMENTS**

Also, the evaluation of the protective properties expands to

- Lipidic damage
- Proteic damage
- DNA damage

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<td><strong>Experimental model</strong></td>
<td>In vitro evaluation of the capability of the tested products (Q+T+E=VITACHELOX®) to protect skin fibroblasts against toxic effects of polluting agents. Heavy metals are used to mimic pollutants (Pc-Fe-Cr)</td>
</tr>
<tr>
<td><strong>Measured parameters</strong></td>
<td>Parameters evaluate the damage on ALL damage markers in most relevant cells components (vitality; lipids, proteins, DNA)</td>
</tr>
<tr>
<td><strong>Results</strong></td>
<td>Enhanced protection on all cells compartments; vitality improved by 78%.</td>
</tr>
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<td><strong>Indications</strong></td>
<td>Multifunctional treatment in anti-pollution topical products</td>
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Lipid protection up to over 66%. MDA is a marker of lipid peroxidation. All tested concentrations were active.
VITACHELOX® COMBINATION FOR ENHANCED PROTECTION on DNA

DNA oxidative protection (8-OHdG)

DNA protection improved by up to 78%. The nucleoside 8-hydroxy-deoxyguanosine (8-OHdG) is a molecular marker of DNA oxidative damage.
PROTEINS protection up to 87%. The carbonylated proteins are regarded as a universal marker of oxidative damage at the protein level.
# VITACHELOX®

**CLINICAL EFFICACY IN PROTECTING THE SKIN FROM ENVIRONMENTAL POLLUTANTS**

<table>
<thead>
<tr>
<th>Study name:</th>
<th>Skin protection from environmental pollutants</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Experimental model</strong></td>
<td>Half face treatment (vs placebo). Randomized application (left/right side) of 2mg/cm² applied by investigator. Skin stripping collecting the different stratum corneum layers. Subjects on their normal routine to remain outdoors in polluted air for 6 hours. Heavy metals analysis.</td>
</tr>
<tr>
<td><strong>Measured parameters</strong></td>
<td>Heavy metals are measured as a marker of pollution since their presence in PM 2.5 and in PM10.</td>
</tr>
<tr>
<td><strong>Subjects</strong></td>
<td>30 subjects (15 tested in Europe and 15 in China)</td>
</tr>
<tr>
<td><strong>Results</strong></td>
<td>The active product has <strong>COMPLETELY PREVENTED</strong> the deposition of heavy metals in the stratum corneum, differently from the placebo formulation.</td>
</tr>
<tr>
<td><strong>Indications</strong></td>
<td>Antipollution, skin protection enhancer, antioxidant, free radical scavenger, skin molecular barrier.</td>
</tr>
</tbody>
</table>

Farcoderm Study 2016/0693
VITACHELOX®

CLINICAL EFFICACY IN PROTECTING THE SKIN FROM ENVIRONMENTAL POLLUTANTS

15 volunteers
Outdoor workers

15 volunteers
Outdoor workers
VITACHELOX®

CLINICAL EFFICACY IN PROTECTING THE SKIN FROM ENVIRONMENTAL POLLUTANTS

Tape 1 is discharged (removes the cream and the dirt).

Tape 2-6: heavy metals are quantified by atomic absorption spectroscopy.

The mechanical barrier provided by the formula itself is made even by the comparison with the placebo formula.

Layers 2-6 are the active skin barrier.

Placebo formula 0.5% Vitachelox®
VITACHELOX®: A MOLECULAR BARRIER

Chromium

Iron

Nickel

Zinc

Vitachelox® prevents metals deposition on the stratum corneum layers
**VITACHELOX®: A MOLECULAR BARRIER**

Variation of heavy metals content in the stratum corneum

![Graph showing variation of heavy metals content](image)

- Increase of all metals in placebo group.
- Vitachelox® prevented metals deposition on the stratum corneum layers, preventing:
  - Promotion of inflammation
  - Iron mediated photocatalysis
  - Extrinsic ageing
VITACHELOX®: A MOLECULAR BARRIER
# VITACHELOX®

**IN VITRO EFFICACY AS FREE RADICAL SCAVENGER vs BHT IN A COSMETIC FORMULA**

<table>
<thead>
<tr>
<th>Study name:</th>
<th>Determination of Antioxidant Potential (AP)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Experimental model</td>
<td>Antioxidative power (AP): overall antioxidative power by <strong>antioxidant capacity</strong> and the <strong>antioxidant activity</strong>. Vitachelox® and BHT incorporated into a basic cosmetic emulsion both at 0.5%+.</td>
</tr>
<tr>
<td>Measured parameters</td>
<td>Actives incorporated at the same dosage in a cosmetic O/W formulation. The AP (Antioxidant Potential) is expressed in antioxidative units (AU: 1 AU corresponds to the activity of a 1 ppm solution of pure vitamin C as a benchmark). AP=no. Free radicals/mg*min. Retesting at 24 and 48 hours.</td>
</tr>
<tr>
<td>Results</td>
<td>Vitachelox® <strong>18 times</strong> more powerful than BHT</td>
</tr>
<tr>
<td>Indications</td>
<td>Antioxidant, free radical scavenger, skin protection enhancer</td>
</tr>
</tbody>
</table>

Gematria Study
In-AP-09-2016
IN VITRO EFFICACY VITACHELOX®

AS FREE RADICAL SCAVenger vs BHT
DPPH with ESR SPECTROSCOPY

The Antioxidant Power of the product overcomes BHT by 18 times

Vitachelox® reactivity is 17 times faster than BHT

1 AU = arbitrary unit of 1 ppm ascorbic acid antioxidant activity
IN VITRO EFFICACY **VITACHELOX®**

AS FREE RADICAL SCAVENERG vs BHT
DPPH with ESR SPECTROSCOPY

The observed Antioxidant Power of the product **overcomes BHT by 18 times** and this difference stays almost stable after 15 days.

Vitachelox reactivity: 17 times faster than BHT at T0, remaining much faster (10 times faster) after 15 days.

1 AU = arbitrary unit of 1 ppm ascorbic acid antioxidant activity
## VITACHELOX®
### SAFETY PROFILE

<table>
<thead>
<tr>
<th>Test Type</th>
<th>Description</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Skin irritation</strong></td>
<td>In vitro irritation study on a Reconstructed Human Epidermis (RhE) model (EPISKIN™) – (Vitroscreen – report RS 57-16) – according to OECD 439</td>
<td>not irritant</td>
</tr>
<tr>
<td><strong>Eye irritation</strong></td>
<td>Evaluation in the BCOP test following OECD Guideline 437 (resp EU method B47) - (Chemsafe)</td>
<td></td>
</tr>
<tr>
<td><strong>Eye irritation potential</strong></td>
<td>Eye irritation potential on Epiocular (Vitroscreeen) - (Vitroscreen report RS 56-16) - according to OECD 492</td>
<td>not irritant</td>
</tr>
<tr>
<td><strong>Phototoxicity</strong></td>
<td>In vitro 3T3 NRU - (Chemsafe) : non phototoxic</td>
<td></td>
</tr>
<tr>
<td><strong>Skin sensitization</strong></td>
<td>Assessment of sensitizing potential on Keratinosens®- (Vitroscreen report RS 55-16) - according to OECD 422D</td>
<td>not sensitizer</td>
</tr>
<tr>
<td><strong>Mutagenesis</strong></td>
<td>AMES: Bacterial revers mutation test (Chemsafe)/Micronucleus</td>
<td>not mutagenic</td>
</tr>
</tbody>
</table>

In line with current EU regulation 1223/2009
VITACHELOX®

Take Home Message

✓ Natural antioxidants synergy for enhanced protection
✓ Significant chelating activity against heavy metals
✓ Free radical scavenging properties
✓ Improvement of cells vitality
✓ Improvement of cells metabolism
✓ Enhancement of cells protection in different cell-targets
✓ Clinical test for molecular barrier against polluted air
✓ Antioxidant compared to BHT

Patent pending: “Composizioni cosmetiche per la protezione degli agenti inquinanti atmosferici” no. 102016000036493
VITACHELOX®
Take Home Message

0.5% Vitachelox® in formula helps counteract:

- Extrinsic induced ageing
- Iron med. photocatalysis
- Promotion of inflammation