23 | 09 | 21

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Claim Support for Anti-Pollution Cosmetics

The proderm Webinar on In Vivo Anti-Pollution Testing

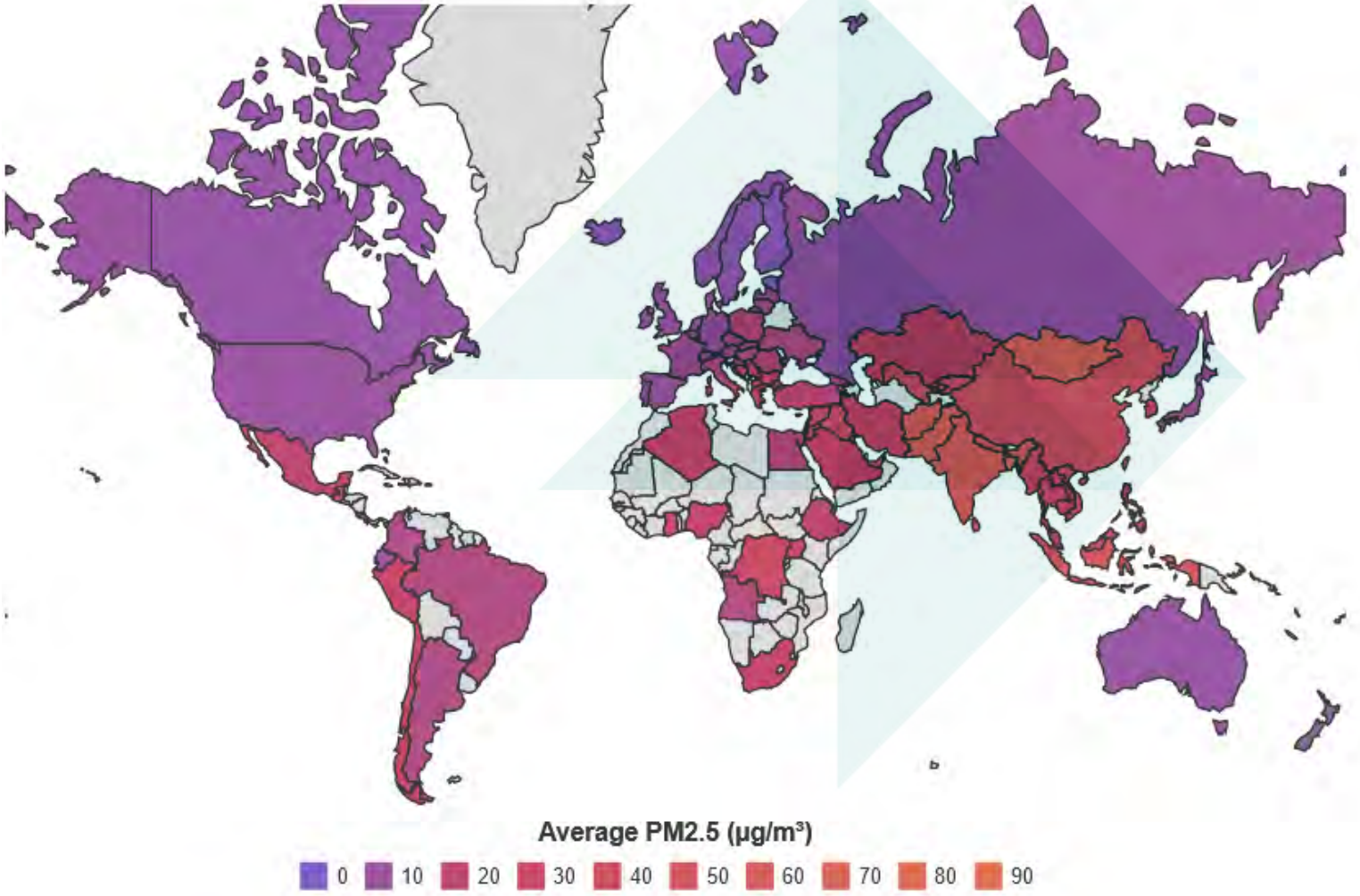
Stephan Bielfeldt

Agenda

- Environmental pollution: an overview
- How can anti-pollution claims be supported?
- Validation: Results of ESR studies ex vivo
- Validation: In vivo findings
- In vivo human study designs
- Conclusion and prospect

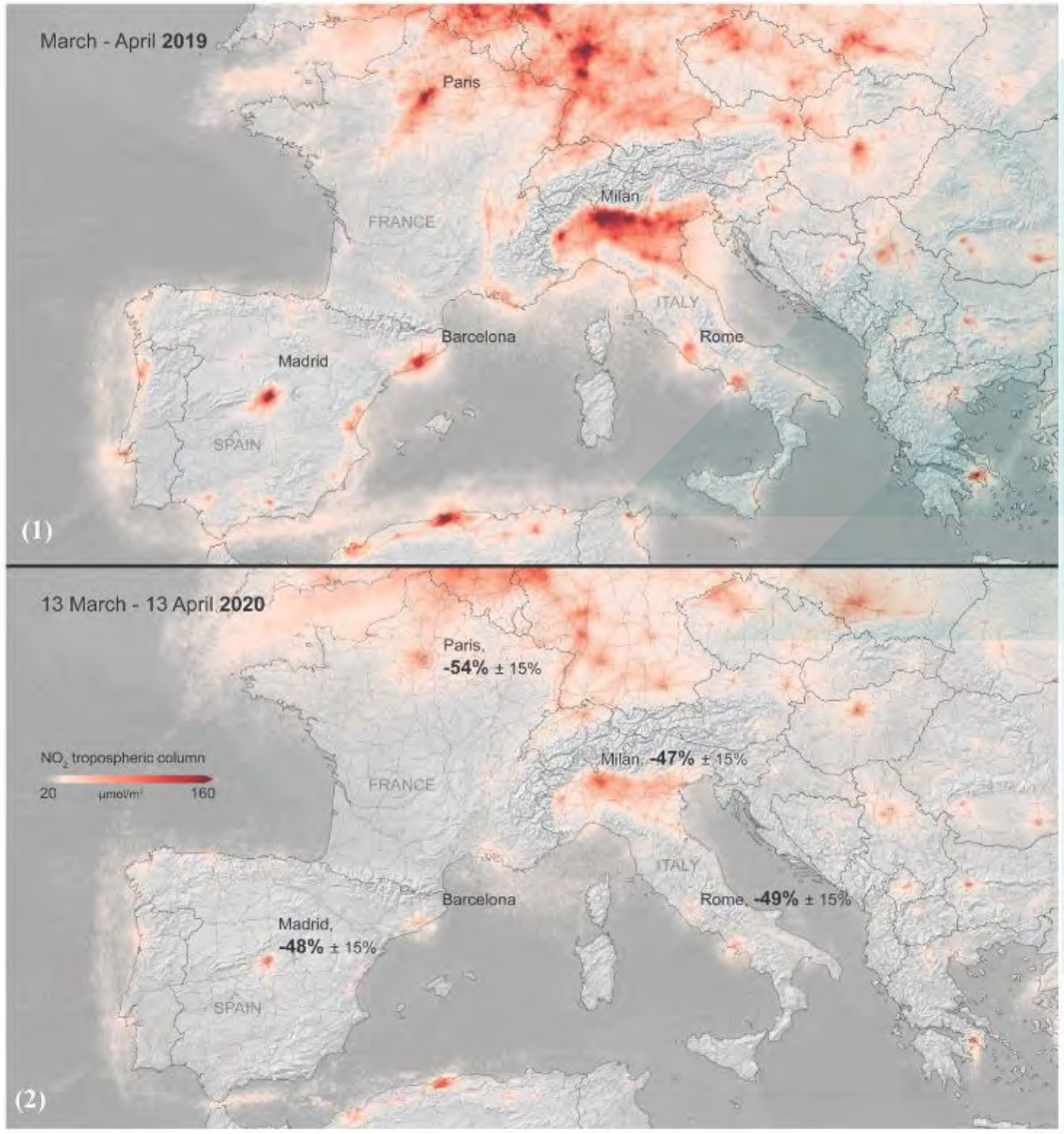
Particulate matter in 2021

Pollution is still a major topic



https://worldpopulationreview.com/country-rankings/most-polluted-countries

The „Corona effect“ was obvious during the lockdown

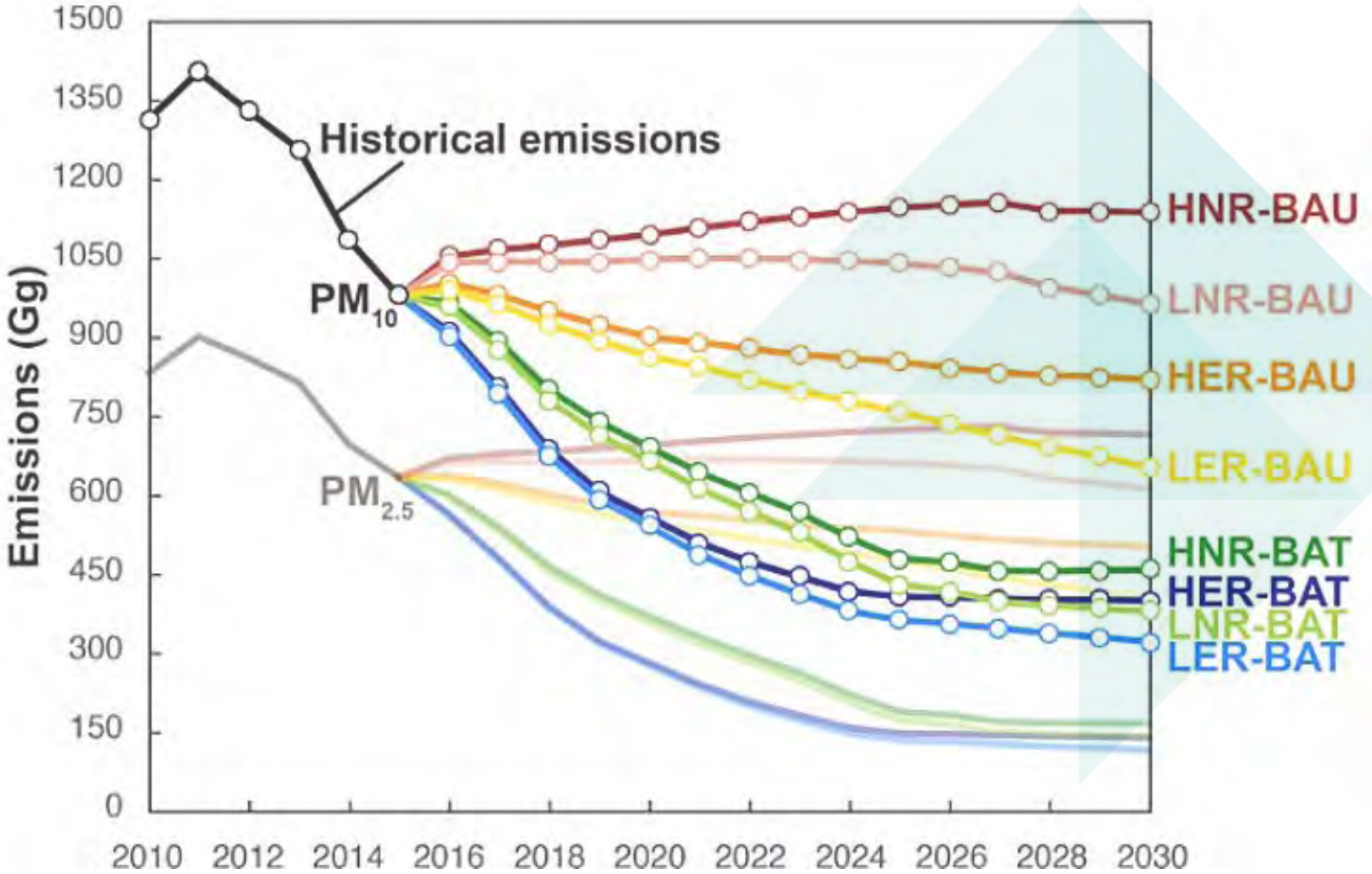


Nitrogen dioxide levels in European countries before (up) and during the lockdown in April 2020.

The mean nitrogen dioxide content in Madrid, Milan and Rome decreased by about 45%, and by 54% in Paris, compared to 2019.

Mousazadeh, M., Paital, B., Naghdali, Z., Mortezania, Z., Hashemi, M., Niaragh, E. K., ... & Emamjomeh, M. M. (2021). Positive environmental effects of the coronavirus 2020 episode: a review. *Environment, Development and Sustainability*, 1-23.

Particulate Matter in China - Forecasts for 2030



Different scenarios for PM 10 emissions from coal power plants.

The future is uncertain

Xiong, T., Jiang, W., & Gao, W. (2016). Current status and prediction of major atmospheric emissions from coal-fired power plants in Shandong Province, China. *Atmospheric Environment*, *124*, 46-52.

The Major Components of Air Pollutions

Toxic gases

- *Sulfur dioxide*
- *Nitrogen oxides*
- *Photochemically derived ozone*
- *Volatile organic compounds*

Particulate matter

- *Respirable particles PM 10 (smaller than 10 µm)*
- *Respirable particles PM 2.5 (smaller than 2.5 µm, reach the alveols)*
- *Particles are carrier of e.g. ROS generating organic compounds*
- *Metals (can catalyse reactions of harmless substances into noxes)*

Indoor pollutants

- *Smoke from indoor combustion (toxic gases and particulate matter)*
- *Cigarette smoke (toxic gases and particulate matter)*

Definition of Environmental Air Pollution

Sources

- Mainly combustion smoke from wood-, coal-, petrol-, gas-burning of Industry, traffic, private firing

Main hazards to skin

- Reactive Oxygen Species (ROS): Superoxide ($O_2\bullet$), hydroxyl (OH •), ozone ($O_{3)}$, singlet oxygen ($^1O_2$), Hydrogen peroxide (H_2O_2)
- Particles loaded with volatile organic compounds (VOCs) and polycyclic aromatic hydrocarbons (PAHs)

https://www.londonair.org.uk/london/asp/PublicEpisodes.asp

WHO Air Quality Guidelines (AQG) of 22 Sept.

New guidelines published yesterday!

Interim target 1 is the old 2005 standard

Table 0.1. Recommended AQG levels and interim targets

Pollutant	Averaging time	Interim target				AQG level
		1	2	3	4	
$PM_{2.5}$, µg/m³	Annual	35	25	15	10	5
	24-hour[a]	75	50	37.5	25	15
PM_{10}, µg/m³	Annual	70	50	30	20	15
	24-hour[a]	150	100	75	50	45
O_3, µg/m³	Peak season[b]	100	70	–	–	60
	8-hour[a]	160	120	–	–	100
NO_2, µg/m³	Annual	40	30	20	–	10
	24-hour[a]	120	50	–	–	25
SO_2, µg/m³	24-hour[a]	125	50	–	–	40
CO, mg/m³	24-hour[a]	7	–	–	–	4

[a] 99th percentile (i.e. 3–4 exceedance days per year).
[b] Average of daily maximum 8-hour mean O_3 concentration in the six consecutive months with the highest six-month running-average O_3 concentration.

https://www.who.int/publications/i

How can anti-pollution claims be supported?

Skin Ageing by Environmental Pollution?

Contribution to acute intoxication

- Our skin barrier strongly limits such effects
- The major acute intoxication is via the lungs

Pollution derived oxidization of skin proteins, skin lipids and further functional skin molecules are the second factor of extrinsic skin aging after photoaging

- Lipid peroxidation (skin barrier, sebum) => impairment of barrier
- Activation of matrix metalloproteinases (MMP's) => collagen cleavage
- Consumption of skin antioxidants (vitamin C, gluthathione, carotenoids ...)

Chronic effect => premature skin aging

- Formation of skin wrinkles,
- Formation of pigmented spots

Efficacy Concepts for Anti-Pollution Cosmetics

Specific skin cleanser to thoroughly remove...

- ...Liquid pollutants
- ...Pollutants dissolved in hydro-lipid film on skin
- ...Particulate matter, even when particle size is small (PM 10, PM 2.5)

Formation of a skin barrier

- Apply products that form a protective film
- Keep toxic and ROS generating molecules away from skin
- Inhibit, or at least reduce penetration of pollutants

Application of antioxidants

- Replace consumed skin own antioxidants
- Inactivate ROS molecules before they can react with the skin

Chelators (e.g. EDTA) to inhibit catalytic heavy metal reactions?

Validation: Results of ESR Studies Ex Vivo

Direct Free Radical Measurements with ESR

- Free radical generation was measured ex vivo directly after application of different doses of UV-light
- Measurement on pig skin (slaughterhouse waste) was performed using Electron Spin Resonance (ESR)
- **Beforehand the skin samples were exposed to two model pollutants**
  - Model 1: Cigarette smoke was applied in a smoke chamber
  - Model 2: Urban Dust was used as the second model pollutant to compare its power to generate free radicals with cigarette smoke

Validation Study Ex vivo: Test Products

Treatment no.	Application on Pig Skin
1	Water (negative control)
2	Pre-treated with water and stressed with cigarette smoke (vehicle, positive control)
3	Pre-treated with a natural hydrophilic phenylethanoid, Antioxidant H1 (1 % in water) and unstressed area (treatment control)
4	Pre-treated with the test substance Antioxidant H1 (1 % in water) and stressed with cigarette smoke
5	Pre-treated with test substance Tocopherol (1 % in EtOH/water 50%/50%)
6	Pre-treated with test substance Tocopherol (1 % in EtOH/water 50%/50%) and stressed with cigarette smoke
7	Pre-treated with test substance Ethylendiamintetraacetat (EDTA, 0.1 % in water)
8	Pre-treated with test substance Ethylendiamintetraacetat (EDTA, 0.1 % in water) and stressed with cigarette smoke
9	Pre-treated with Physiogel (negative control for Urban Dust)
10	Pre-treated with Physiogel and stressed with cigarette smoke (comparator for Urban Dust)
11	Pre-treated with Physiogel and stressed with Urban Dust 5 %
12	Pre-treated with Physiogel and stressed with Urban Dust 2.5 %

Validation Study Ex vivo: Free Radical Formation of Antioxidants and Chelators in Water

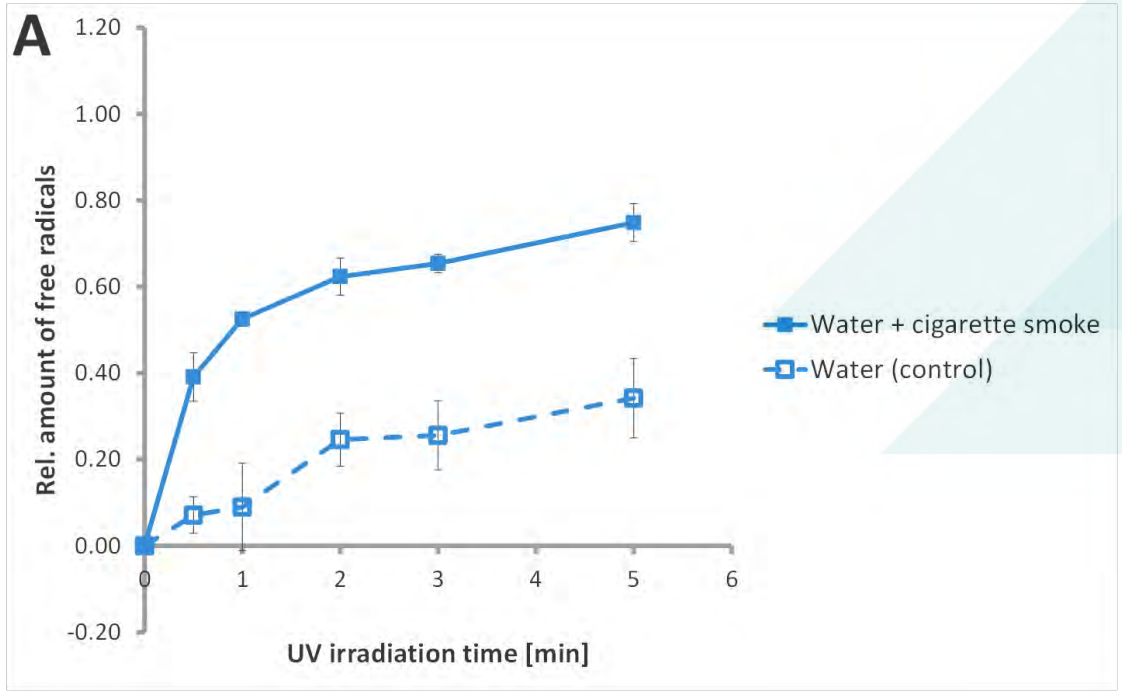
Treatment no.	Application on Pig Skin
1	Water (negative control)
2	Pre-treated with water and stressed with cigarette smoke (vehicle, positive control)
3	Pre-treated with a natural hydrophilic phenylethanoid, Antioxidant H1 (1 % in water) and unstressed area (treatment control)
4	Pre-treated with the test substance Antioxidant H1 (1 % in water) and stressed with cigarette smoke
5	Pre-treated with test substance Tocopherol (1 % in EtOH/water 50%/50%)
6	Pre-treated with test substance Tocopherol (1 % in EtOH/water 50%/50%) and stressed with cigarette smoke
7	Pre-treated with test substance Ethylendiamintetraacetat (EDTA, 0.1 % in water)
8	Pre-treated with test substance Ethylendiamintetraacetat (EDTA, 0.1 % in water) and stressed with cigarette smoke

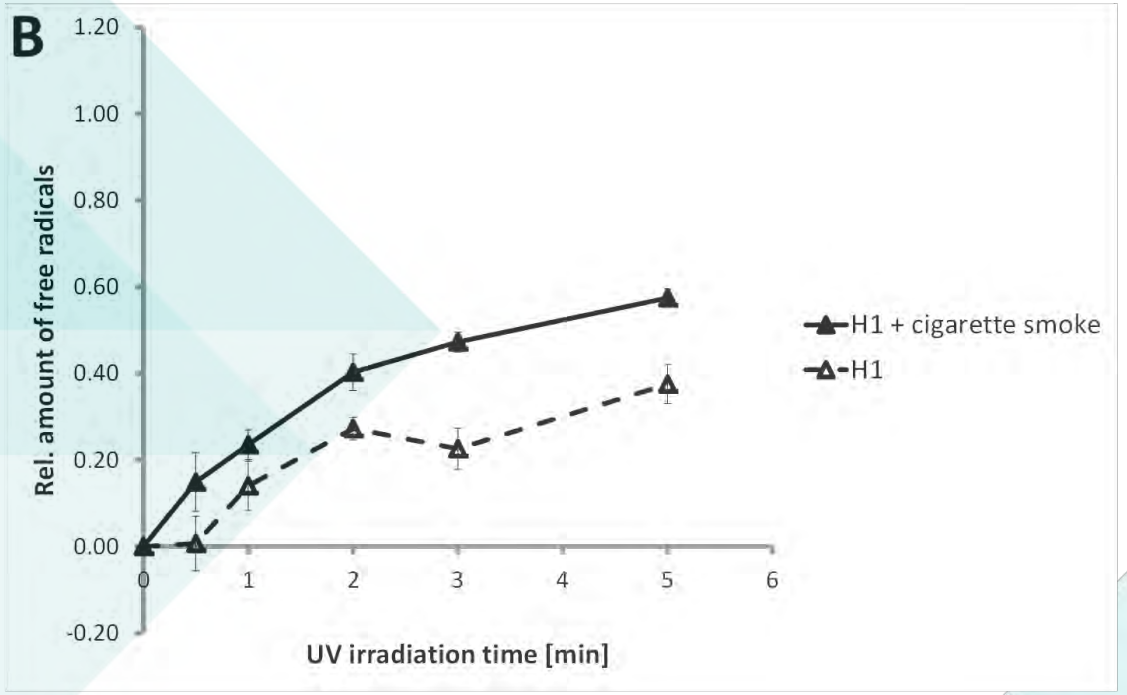
Free Radical Formation of Urban Dust

NIST Standard Reference Material 1649b in a Gel-Formulation

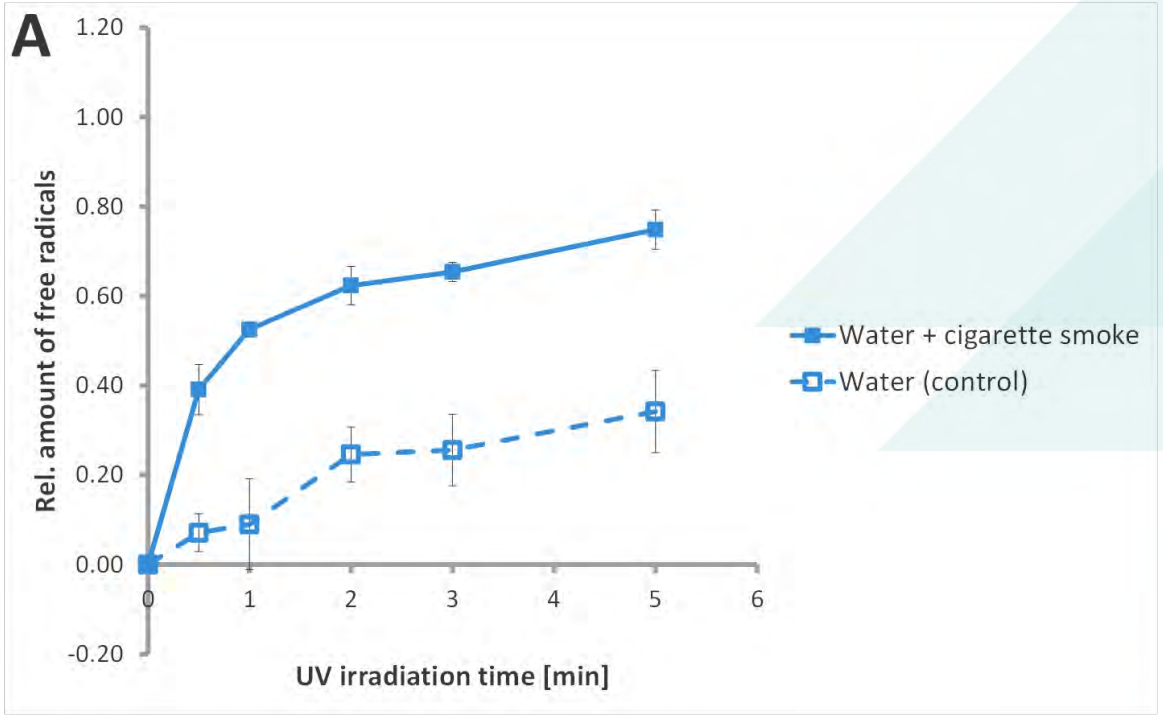
9	Pre-treated with Physiogel (negative control for Urban Dust)
10	Pre-treated with Physiogel and stressed with cigarette smoke (comparator for Urban Dust)
11	Pre-treated with Physiogel and stressed with Urban Dust 5 %
12	Pre-treated with Physiogel and stressed with Urban Dust 2.5 %

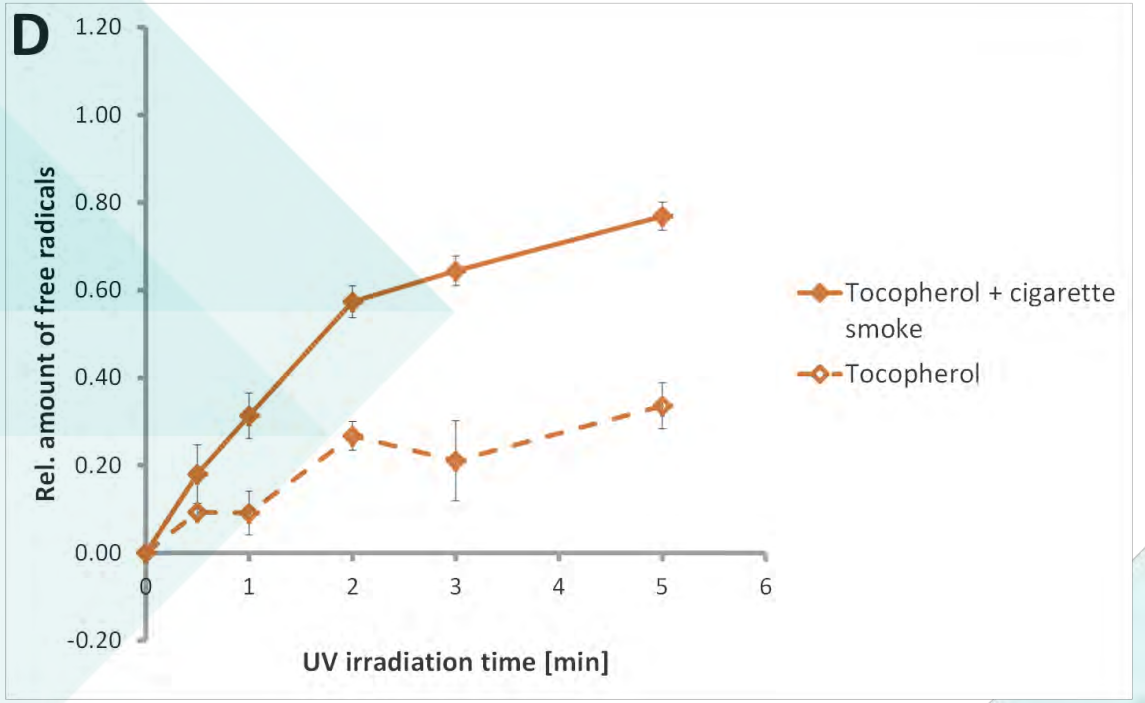
The Hydrophilic Antioxidant H1 Clearly reduces the Formation of Free Radicals



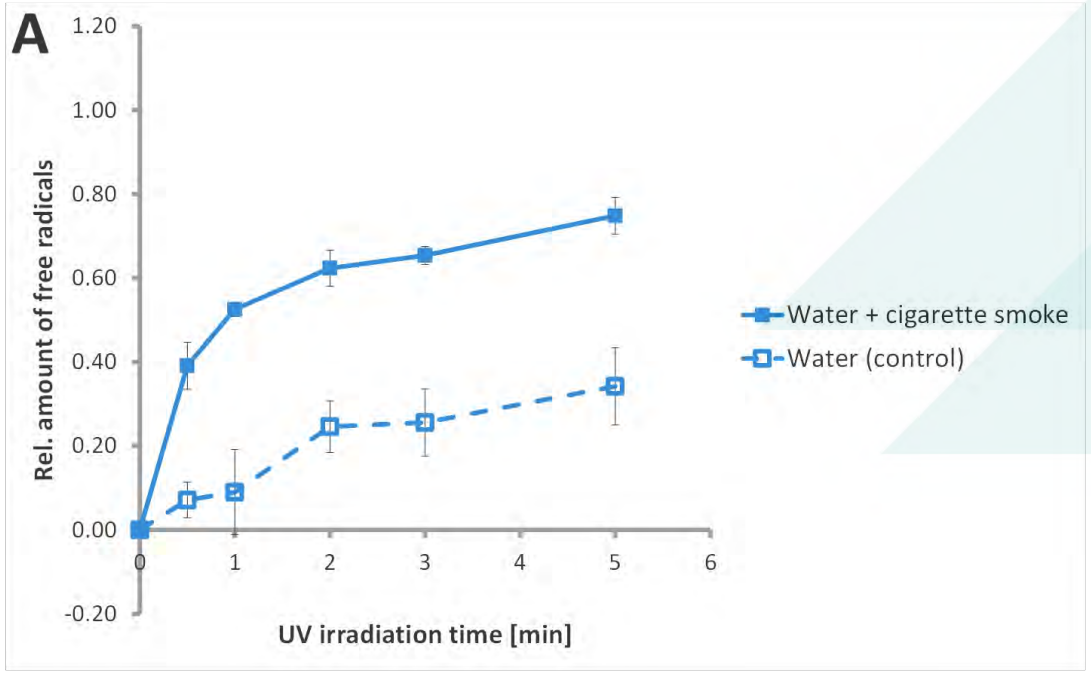


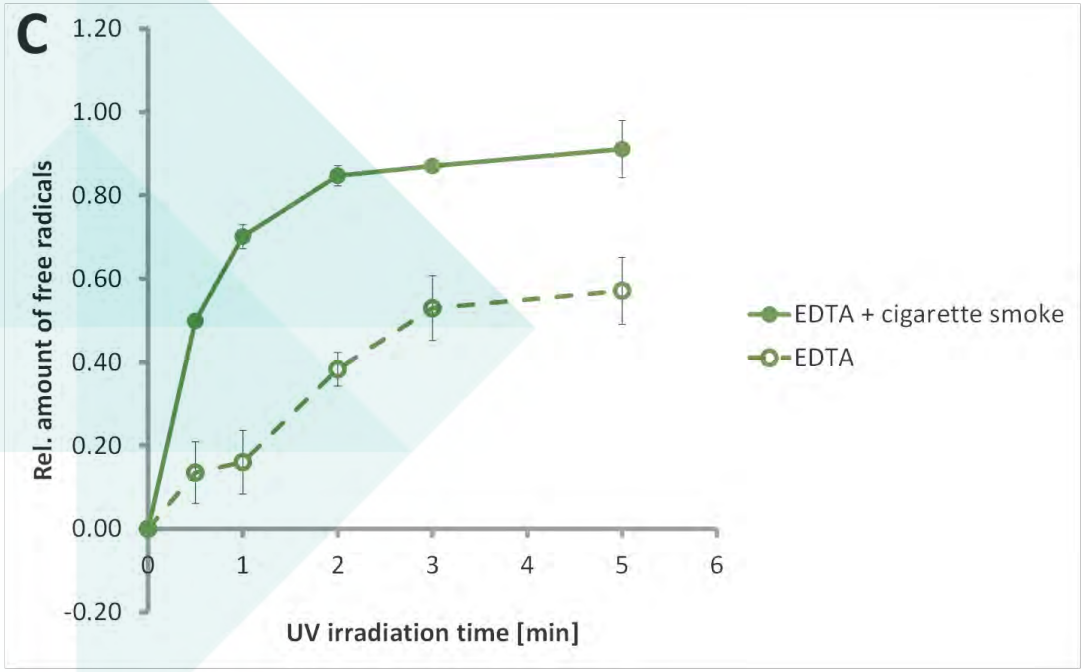
Tocopherol has a Week Effect for Low UV-Doses





EDTA is not Reducing Free Radicals it Behaves Slightly Pro-Oxidative





Induced Free Radicals Compared to No UV-Irradiation

Physiogel as a vehicle generates a comparable amount of free radicals with and without cigarette smoke as water

5 % of urban dust induces approximately the same amount of free radicals as fresh cigarette smoke for 10 minutes

Test sample (no. and short label)	Induced free radicals [%] with ± standard deviation
1. Water (negative control)	22.2 ± 3.2
2. Water + cigarette smoke (vehicle, positive control)	62.1 ± 8.2
3. Antioxidant H1 (1%), (treatment control)	19.1 ± 3.5
4. Antioxidant H1 (1%) + cigarette smoke	34.4 ± 5.4
5. Tocopherol (1%)	20.4 ± 2.8
6. Tocopherol (1%) + cigarette smoke	51.7 ± 3.8
7. EDTA (0.1%)	28.2 ± 7.4
8. EDTA (0.1%) + cigarette smoke	79.4 ± 6.0
9. Physiogel (control for UrbanDust)	23.4 ± 3.7
10. Physiogel + cigarette smoke	62.3 ± 4.7
11. Physiogel + Urban Dust 5 %	54.1 ± 2.2
12. Physiogel + Urban Dust 2.5 %	42.6 ± 8.1

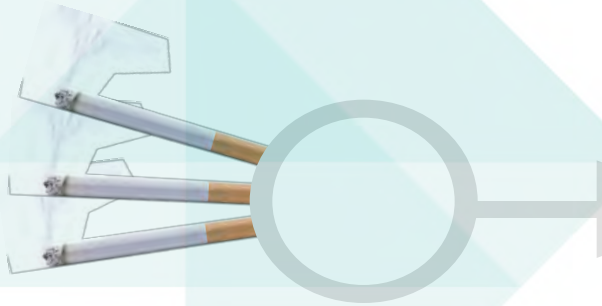
Validation: In vivo findings

The Cigarette Smoke Model

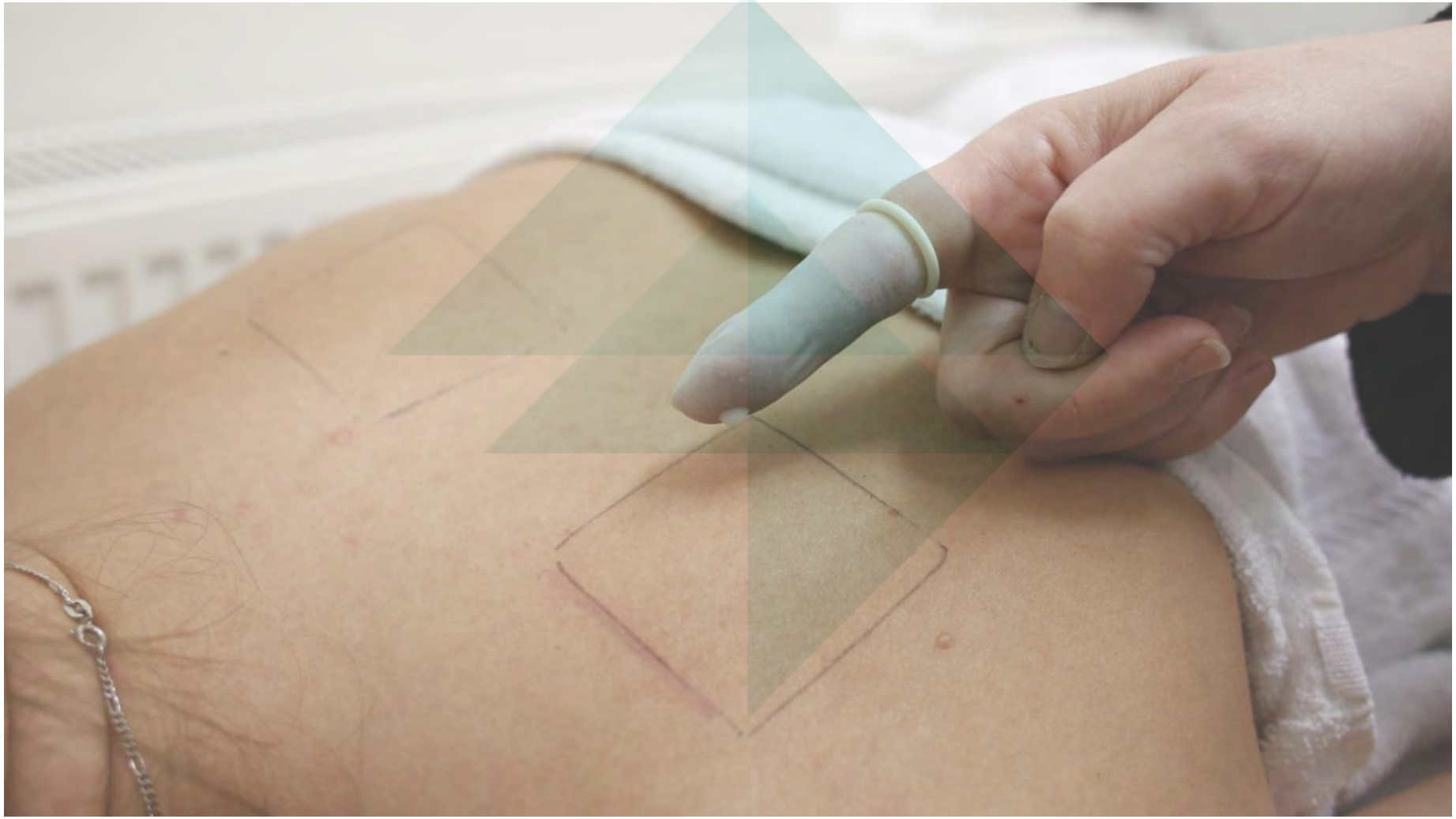
Assessments are performed in vivo on the back or volar forearm

Fresh cigarette smoke is used as the model pollutant

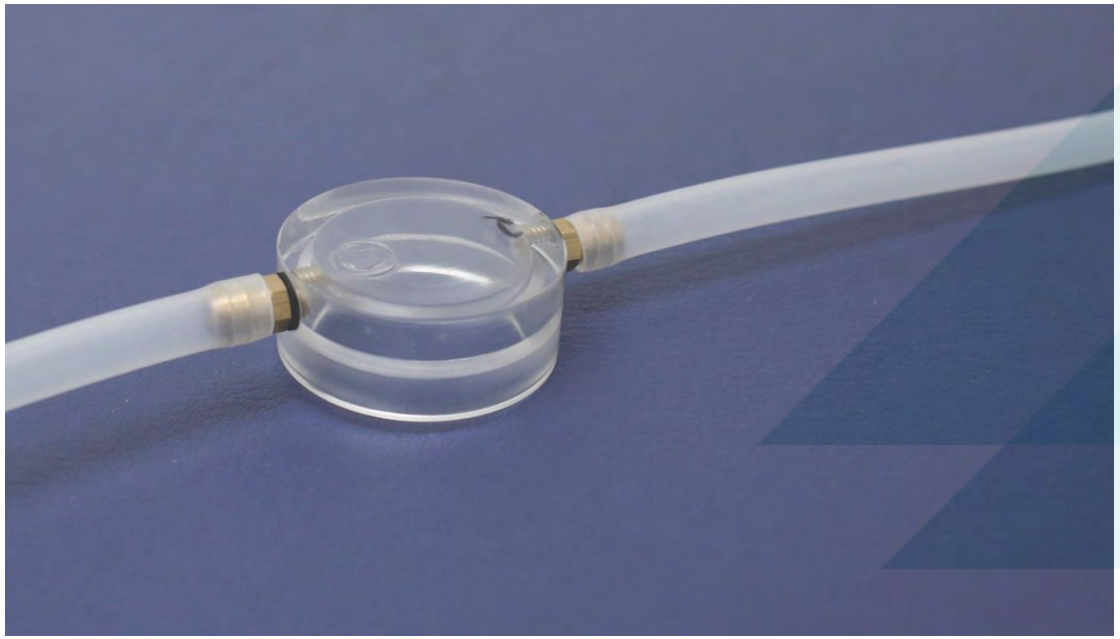
Assessment of oxidized lipids from sebum and / or skin barrier

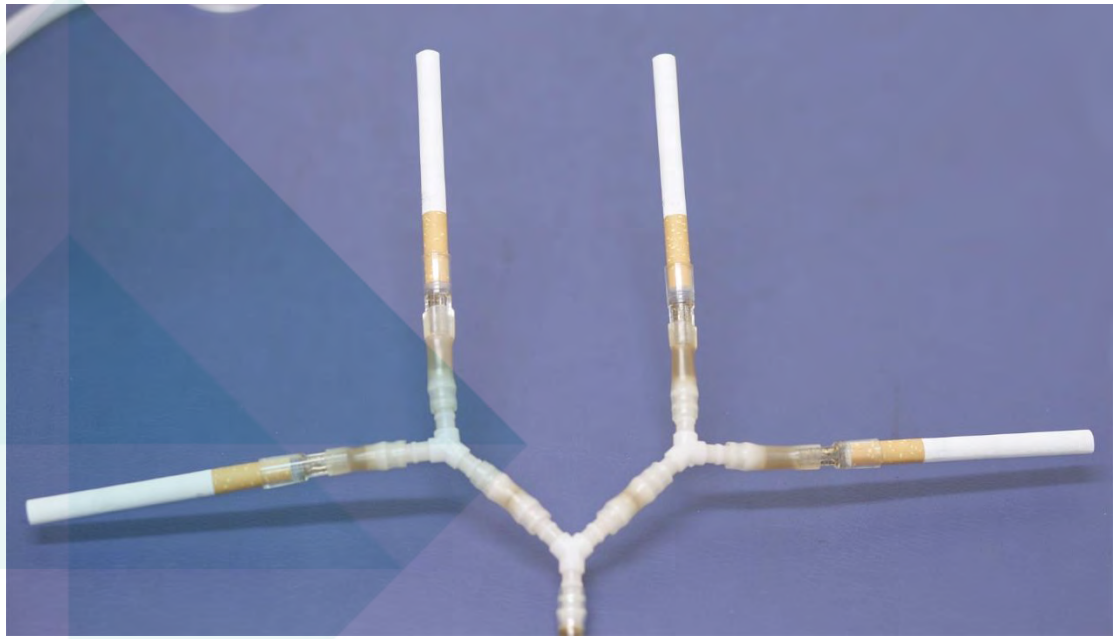


Product application on the back



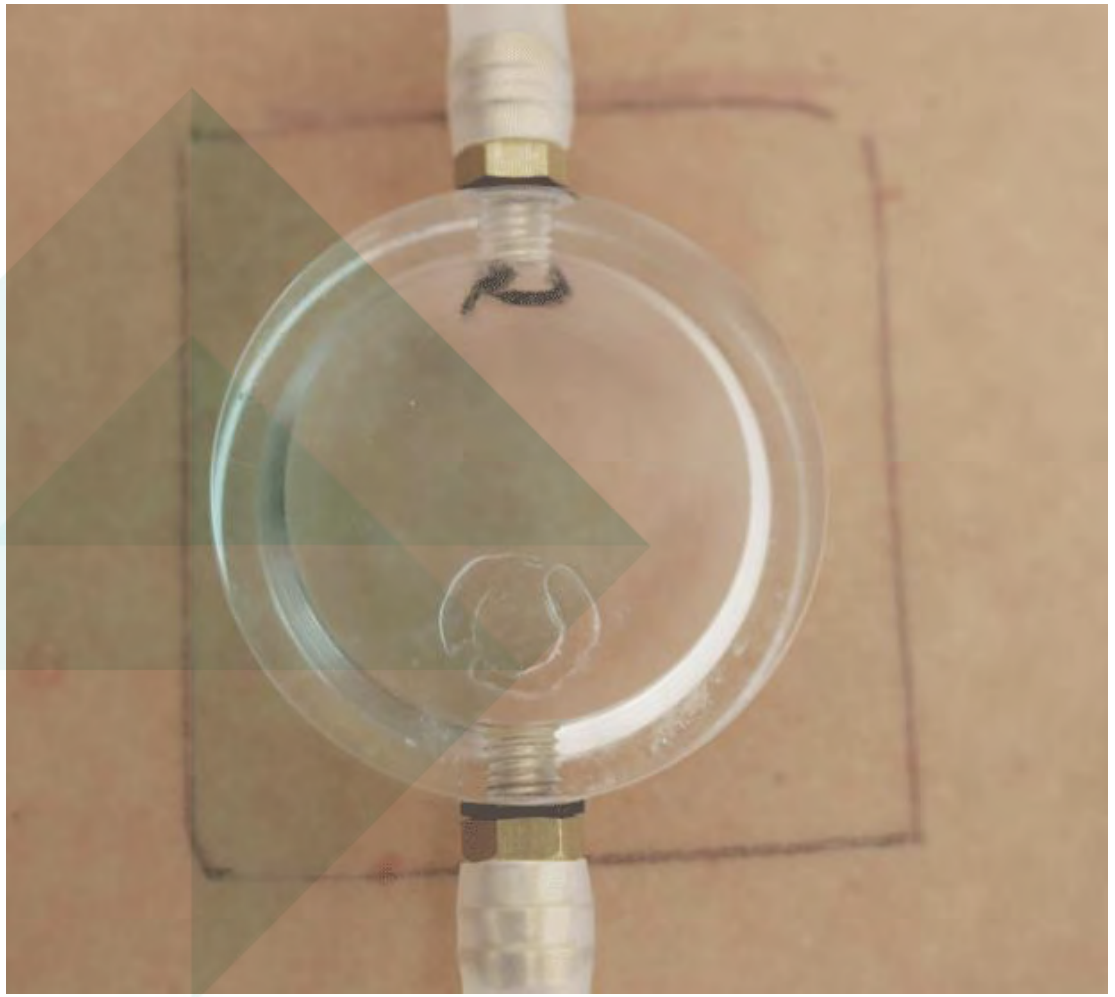
Smoke Application Device





A pump produces negative pressure and transports the smoke to the smoke chamber

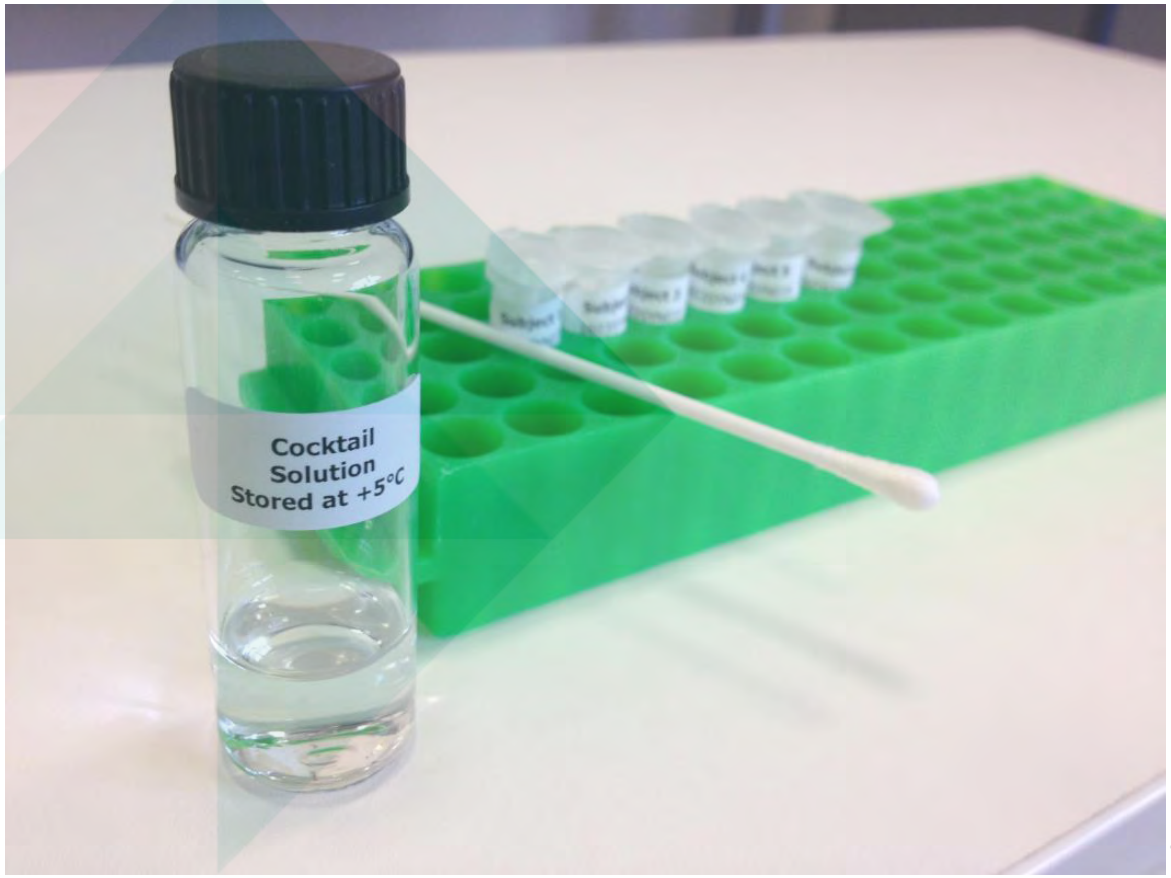
Smoke Chamber



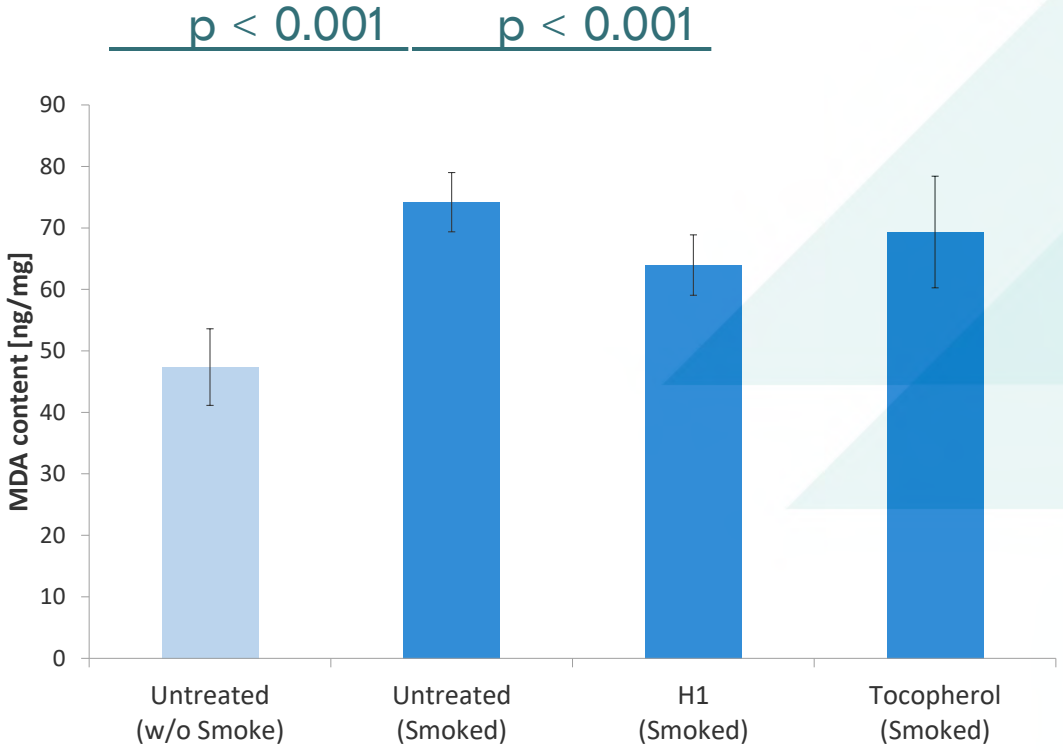
A number of standardised puffs of fresh cigarette smoke is pumped into the chamber on the skin

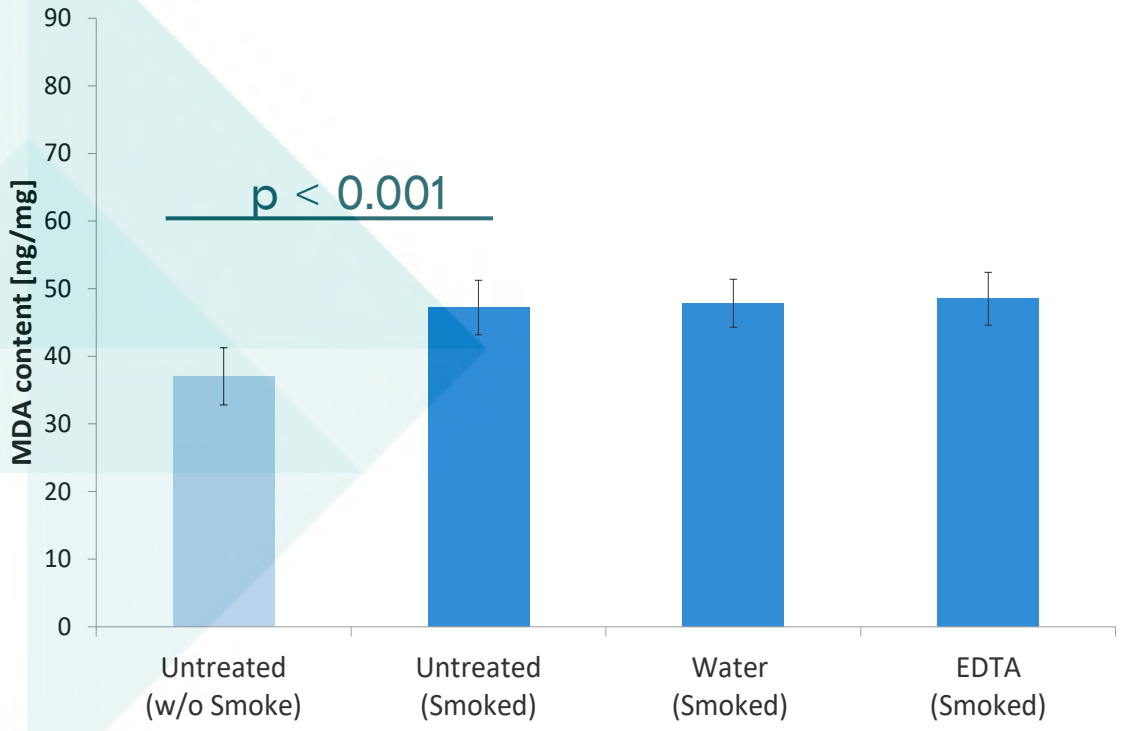
Sampling method to harvest lipids from skin

Swap method with a buffer solution

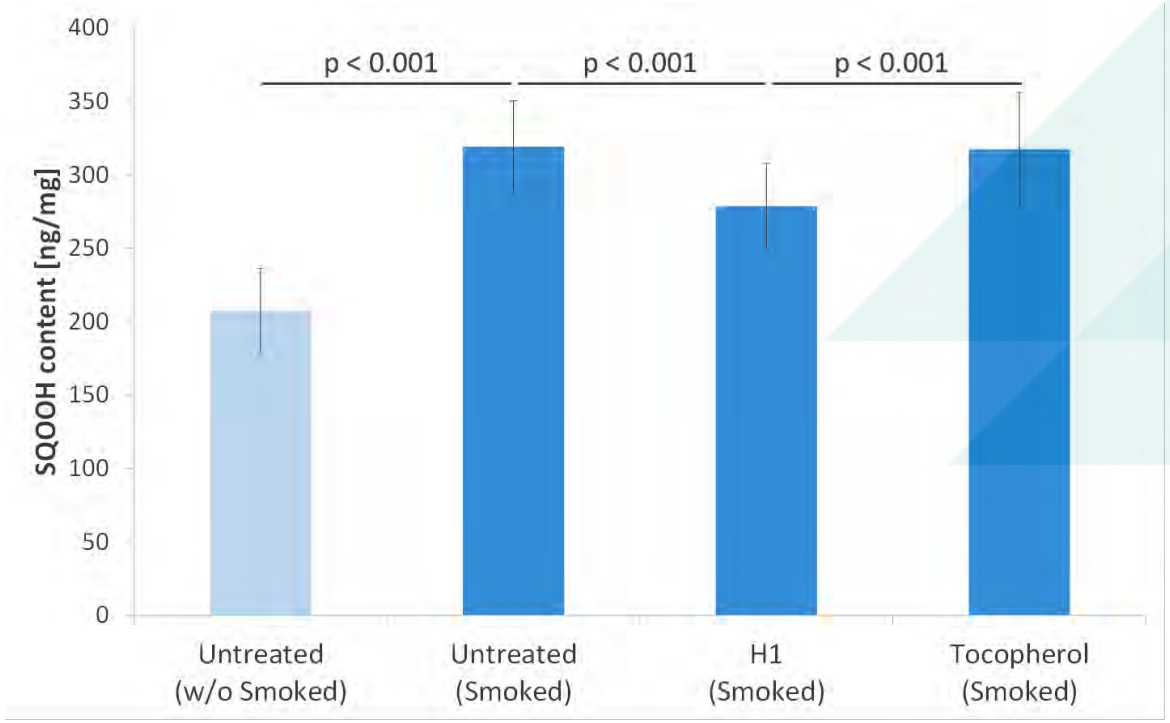


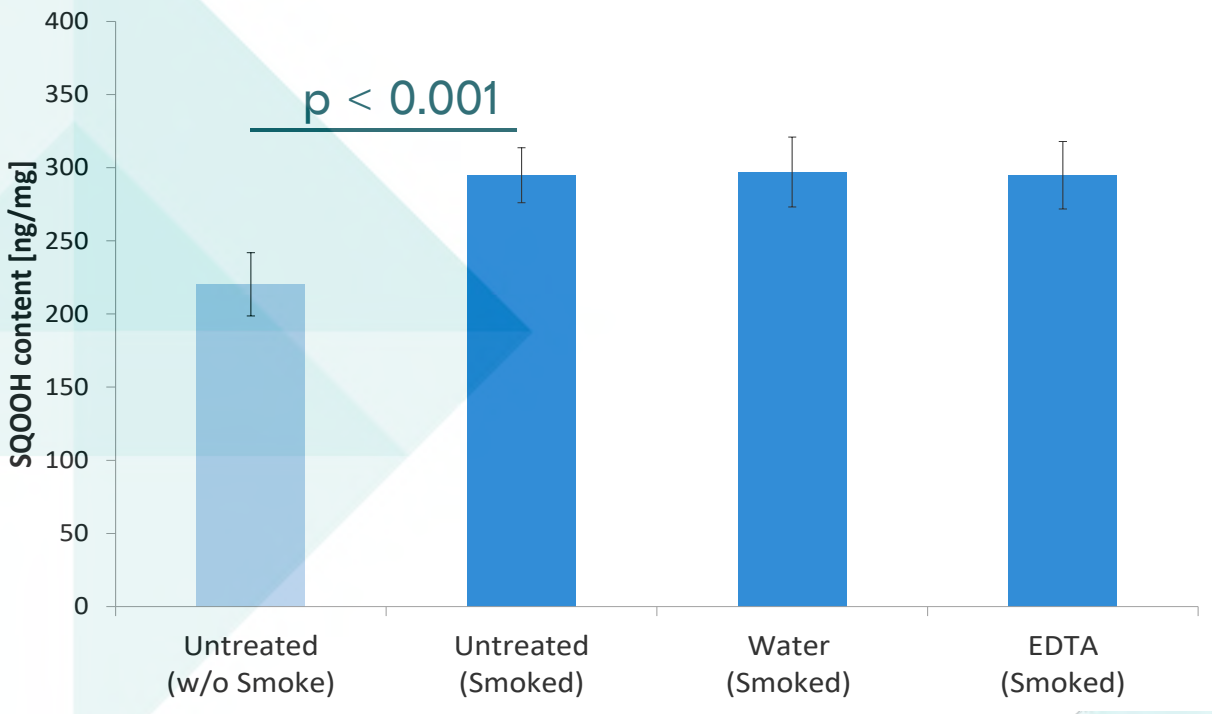
In Vivo Induction of Malondialdehyde by Cigarette Smoke





In Vivo Induction of SQOOH by Cigarette Smoke





More information is found in our publication

Skin Research & Technology

ORIGINAL ARTICLE

Anti-pollution effects of two antioxidants and a chelator—Ex vivo electron spin resonance and in vivo cigarette smoke model assessments in human skin

Stephan Bielfeldt ✉, Katinka Jung, Sabrina Laing, Alain Moga, Klaus-Peter Wilhelm

First published: 10 June 2021 | https://doi.org/10.1111/srt.13068

In vivo human study designs

Examples for In Vivo Test Designs: Cigarette Smoke

	Instant Protection	Long term Protection
Subjects	10	
Parameters	Quantification of Squalene Monohydroperoxide (SQOOH), Malondialdehyde (MDA) LC/MS or GC/MS determination by external lab 'Synelvia'	
Test area for barrier lipids	*5* testing areas on the volar forearm	
Test area for sebum lipids	*5* testing areas on the upper back	
Test products	3 cosmetic products, positive control (untreated area exposed to smoke), negative control (untreated area not exposed to smoke)	
Test procedure	Day 1: Product application, 15 min. waiting time, 15 min. exposure to pollution, sampling	Day 1, 2, 3, 4: Product application After 24 h, on day *5*: 15 min. exposure to pollution, sampling

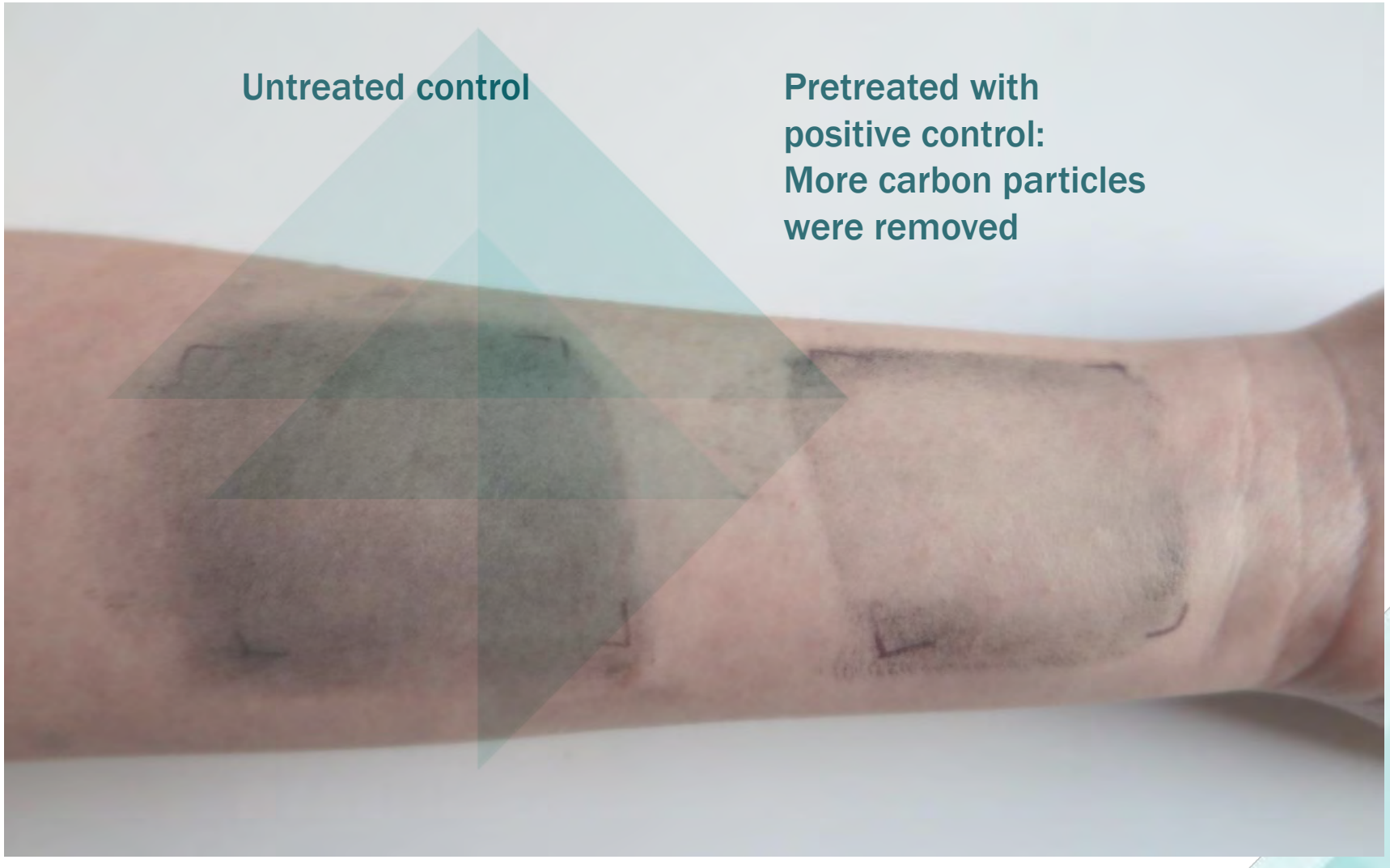
Improved Removal Claim for Leave on Products

Test procedure

Application of a Cosmetic Protection Cream

Application of PM 2.5 carbon particles

Rinsing with hand warm tap water for 15 sec



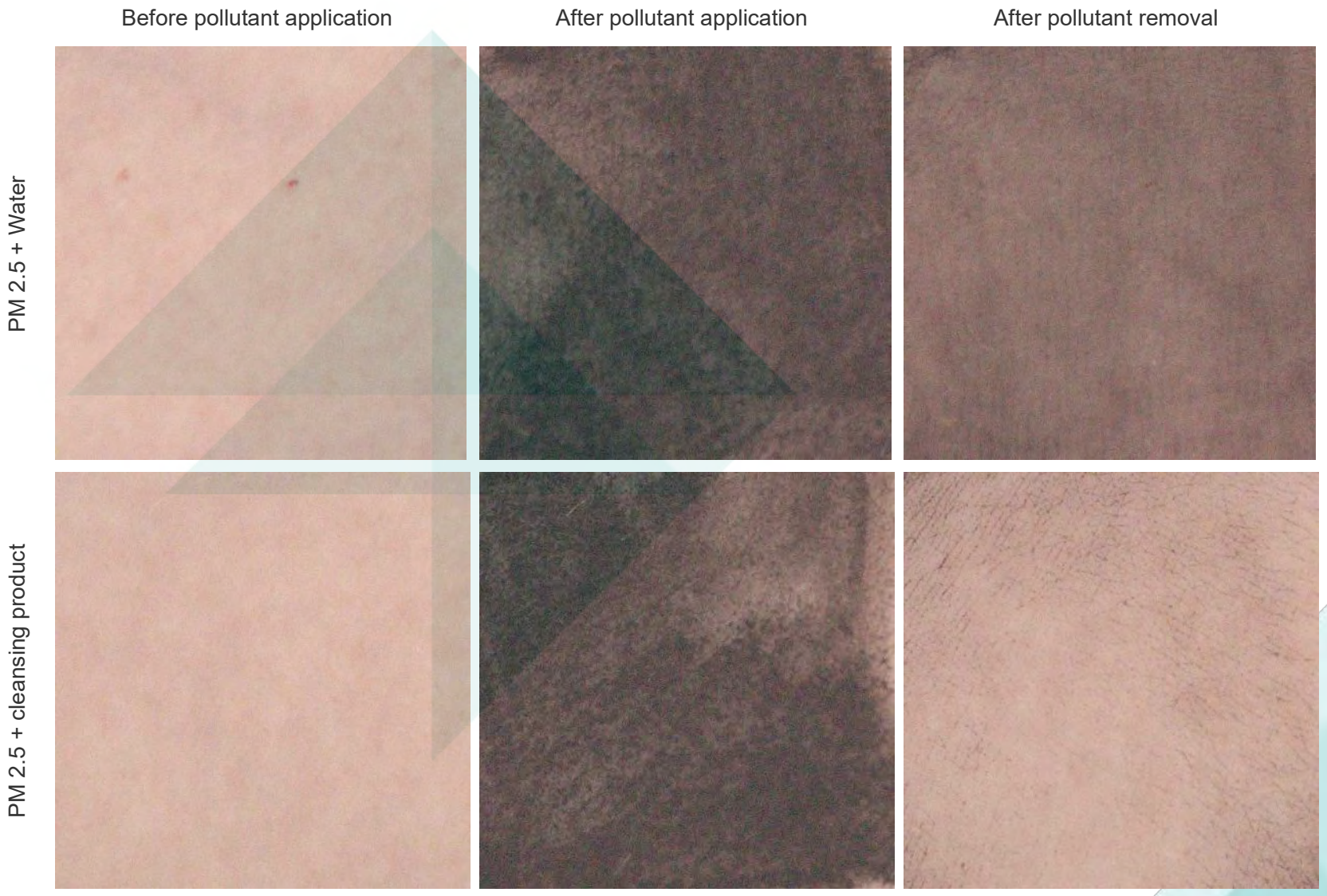
Claim Support for a Cleansing Product

Test procedure

Application of PM 2.5 carbon particles

Standardized washing with an anti-pollution cleanser

Rinsing with hand warm tap water for 15 sec



Conclusions

Ex vivo data on the release of free radicals confirm in vivo findings

...Cigarette smoke and assessment of lipid peroxidation work ex vivo as well as in vivo
...Results on selected antioxidants were found comparable
...The NIST standard and cigarette smoke lead to comparable results

These findings confirm the reliability of our in vivo cigarette smoke model

Cosmetic claims of "anti pollution efficacy" can be assessed successfully by use of in vivo model studies with cigarette smoke

Carbon particulate matter is non invasive and we use it regularly for in vivo claim support of anti-pollution protection-products and cleansers

Prospect

We actually establish an in vivo anti pollution method that uses the ozone radical as the model pollutant

This model will soon expand our portfolio of anti pollution testing

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Questions