IMPACT OF REAL-LIFE OZONE EXPOSURE ON SKIN *IN VITRO* AND *IN VIVO*

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1 INTRODUCTION

Skin is the largest organ directly exposed to environmental insults, like **pollutants** and especially **tropospheric ozone**. At the ground level, ozone concentration can reach **0.1 ppm** during peaks [1]. This pollutant directly interacts with epidermal surface layers. Unsaturated lipids, by ozonolysis reaction, generate very **reactive oxidized molecules**, such as **aldehydes**. Once formed, these species are known to participate to many biochemical reactions impacting cell metabolism in deeper layers [2].

Ozone exposure has been correlated with **disruption of skin integrity and dermatological disorders like atopic dermatitis** [3], but no causal link has been shown yet under real-life ozone exposure. To address this issue, the present study focused on **evaluating the impact of real-life concentrations of ozone on skin** *in vitro*.

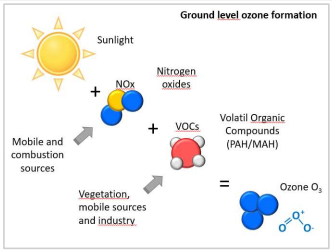


Figure 1. Formation of tropospheric ozone

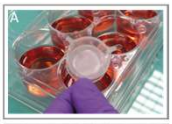
2 MATERIALS & METHODS

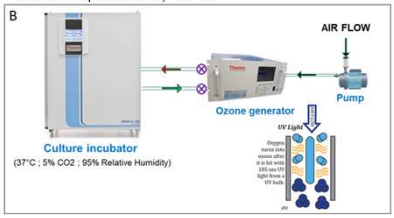
✓ Ozone exposure set up and protocol

Reconstructed human full-thickness skin model T-skin™ was exposed to concentrations of **ozone** from **0.9 ppm to 0.1 ppm**.

Critical points to control :

- ***regular ozone generation and dispersion*** in the cell culture incubator (topical, concentration, duration)
- **culture conditions** of the skin model (compatibility between cell culture and ozone exposure system)





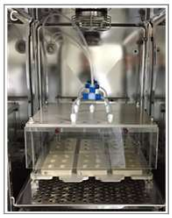


Figure 2. Ozone exposure system on the reconstructed human full-thickness skin model T-skin™.
(A) T-skin™ model, Episkin, Lyon, France. (B) Schematic view of the system with cell culture incubator, ozone generator, analyzer, and pump. Ozone generator consisted in an ultraviolet lamp generating ozone by photolysis of oxygen from air. (C) Ozone exposure chamber in the cell culture incubator.

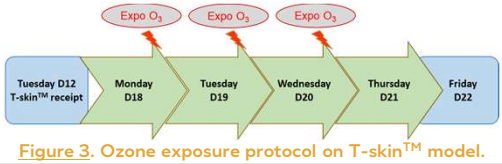
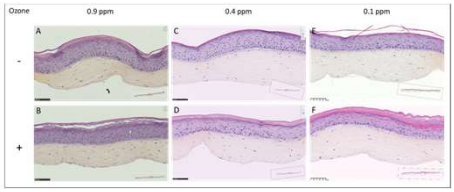


Figure 3. Ozone exposure protocol on T-skin™ model.

3 RESULTS & DISCUSSION

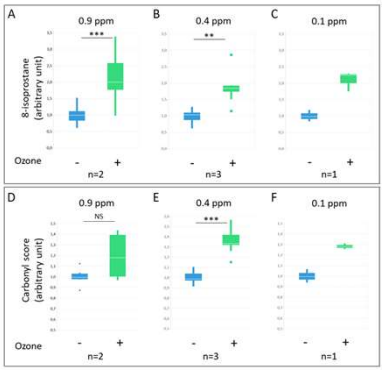
✓ Ozone exposure on skin has low impact on histology



(A, C, E) HES staining of **T-skin™ model** not exposed or (B, D, F) exposed to ozone.

Figure 4. Histological analysis of reconstructed skin exposed or not to ozone at 0.9 ppm, 0.4 ppm and 0.1 ppm.

✓ Ozone exposure induces lipid oxidation and protein carbonylation



(A, B, C) Lipid oxidation was quantified with 8-isoprostane marker. Results are expressed in arbitrary unit.
(D, E, F) Carbonyl score is the quantification of carbonylated proteins normalized by total proteins. Results are expressed in arbitrary unit.

Figure 5. Analysis of lipid oxidation and protein carbonylation on reconstructed skin exposed or not to ozone at 0.9 ppm, 0.4 ppm and 0.1 ppm.

✓ Ozone exposure induces an increase of IL6, IL8 and modulates Filaggrin expression in a dose-dependent-manner

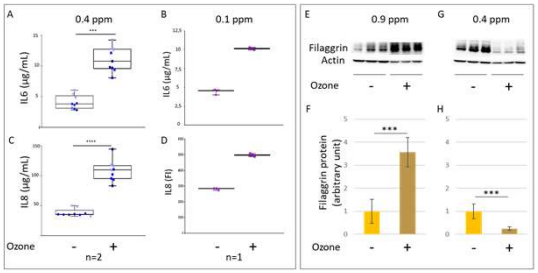


Figure 6. Analysis of inflammation markers (IL6, IL8) and epidermal differentiation marker (Filaggrin) linked to atopic dermatitis on reconstructed skin exposed or not to ozone at 0.9 ppm, 0.4 ppm and 0.1 ppm.
(A, B) Interleukin 6 (IL6) and (C, D) Interleukin 8 (IL8) were quantified after exposure or not to 0.4 ppm or 0.1 ppm of ozone. FI = Fluorescence Intensity. (E, G) Western blot analysis and (F, H) quantification of Filaggrin protein expression levels on reconstructed skins after exposure or not to (E, F) 0.9 ppm or (G, H) 0.4 ppm of ozone.

NS = Not Significant, * P<0.05, ** P <0.01, *** P <0,001, **** P<0.0001

4 CONCLUSIONS

Ozone is a **worldwide urban concern** and its concentration will increase with **global climate warming**. The current study brings for the first time new insights on the **impact of real-life ozone exposure conditions on reconstructed full-thickness skin model.** The increase of **IL6 and IL8 cytokines** expression and the modulation of **Filaggrin** expression after ozone exposure could explain the increase of **atopic dermatitis** prevalence observed after ozone peaks. It is then key for consumers (i) to be aware of skin vulnerability to ozone exposure and (ii) to use daily topical application of specific cosmetic formulations that can protect cutaneous tissues from adverse effects of ozone exposure.

REFERENCES

1. Li K *et al*: **Ozone pollution in the North China Plain spreading into the late-winter haze season**. *Proc Natl Acad Sci U S A* 2021, **118**(10).
2. Ferrara F *et al*: **Redox regulation of cutaneous inflammasome by ozone exposure**. *Free Radic Biol Med* 2020, **152**:561-570.
3. Wang HL *et al*: **Association between air pollution and atopic dermatitis in Guangzhou, China.** *Br J Dermatol* 2021, **184**(6):1068-1076.

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