Generation of biogenic secondary organic aerosols for the assessment of their health impacts

Introduction | Experimental system | Particle size analysis | Oxidative potential | Biological assays | Conclusion

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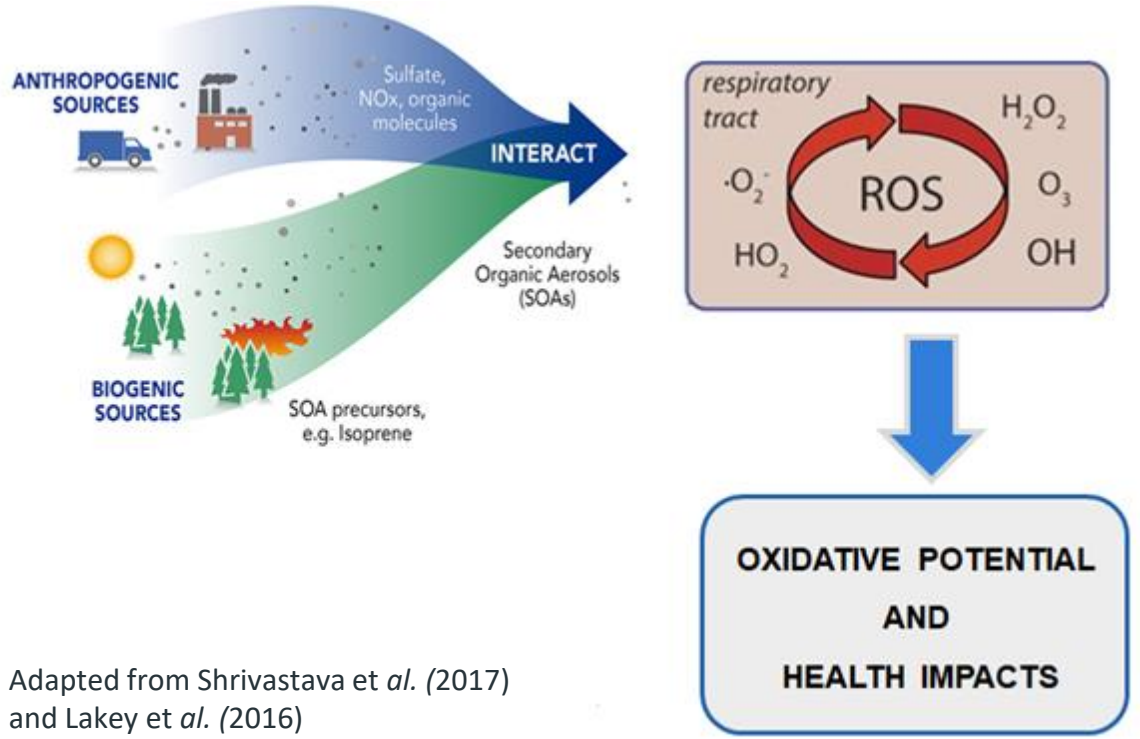
Secondary organic aerosols (SOA) are formed *in situ* in the atmosphere by the oxidation of **VOCs**, particularly biogenic ones[1]. In the Mediterranean region, the family of monoterpenes, to which **limonene** belongs, is believed to strongly contribute to the formation of these ultrafine particles[2]. After inhalation, SOA could lead to an abnormally high production of **reactive oxygen species** (ROS) causing **oxidative stress** [3,4].

The **objectives** of this project are:

- Generation of SOAs under controlled conditions by limonene ozonolysis,
- Their physico-chemical characterisation,
- Evaluation of their toxicity by acellular and cellular methods.

[1] Hallquist et *al.* 2009 [2] Panopoulou et *al.* 2020 [3] Chowdurry et *al.* 2019 [4] Lin et *al.* 2016



Adapted from Shrivastava et *al.* (2017) and Lakey et *al.* (2016)

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Laminar aerosol flow reactor

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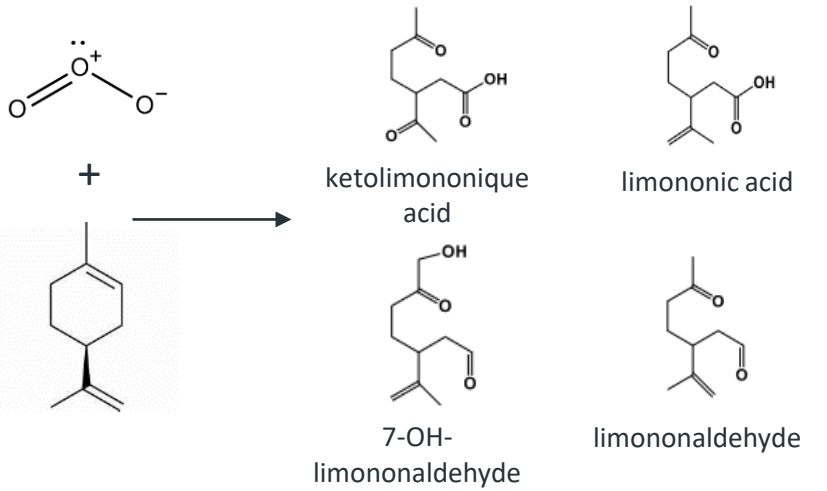
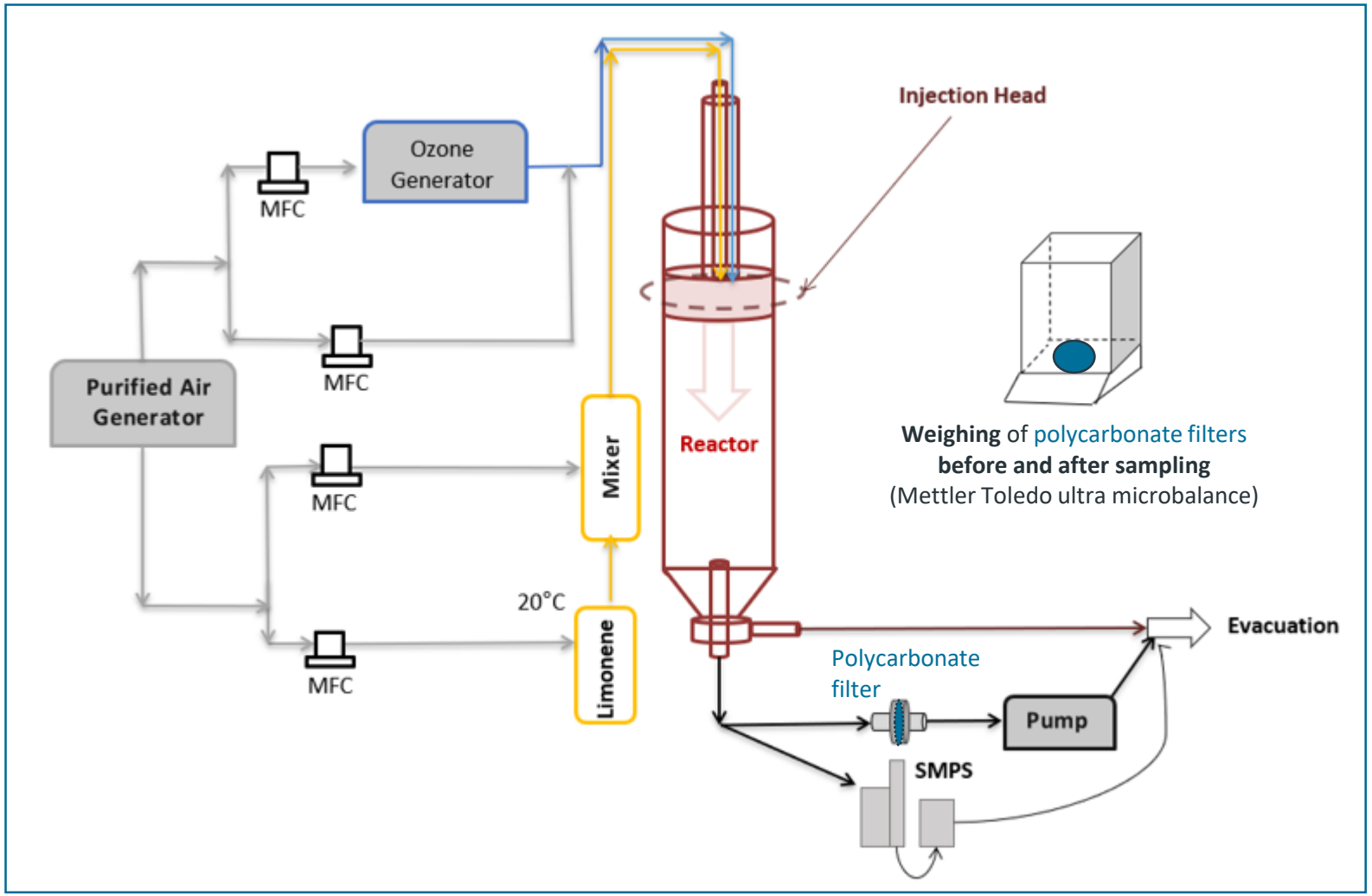
- Length: 100 cm
- Diameter: 10 cm
- Material : Pyrex
- Ozone concentration: 20 ppm
- Limonene concentration: 10 ppm



→ The generated SOAs contain about **66 w% of carbon** as generally found for SOA from terpenes[5].



[5] Lchhabra et *al.*, 2011

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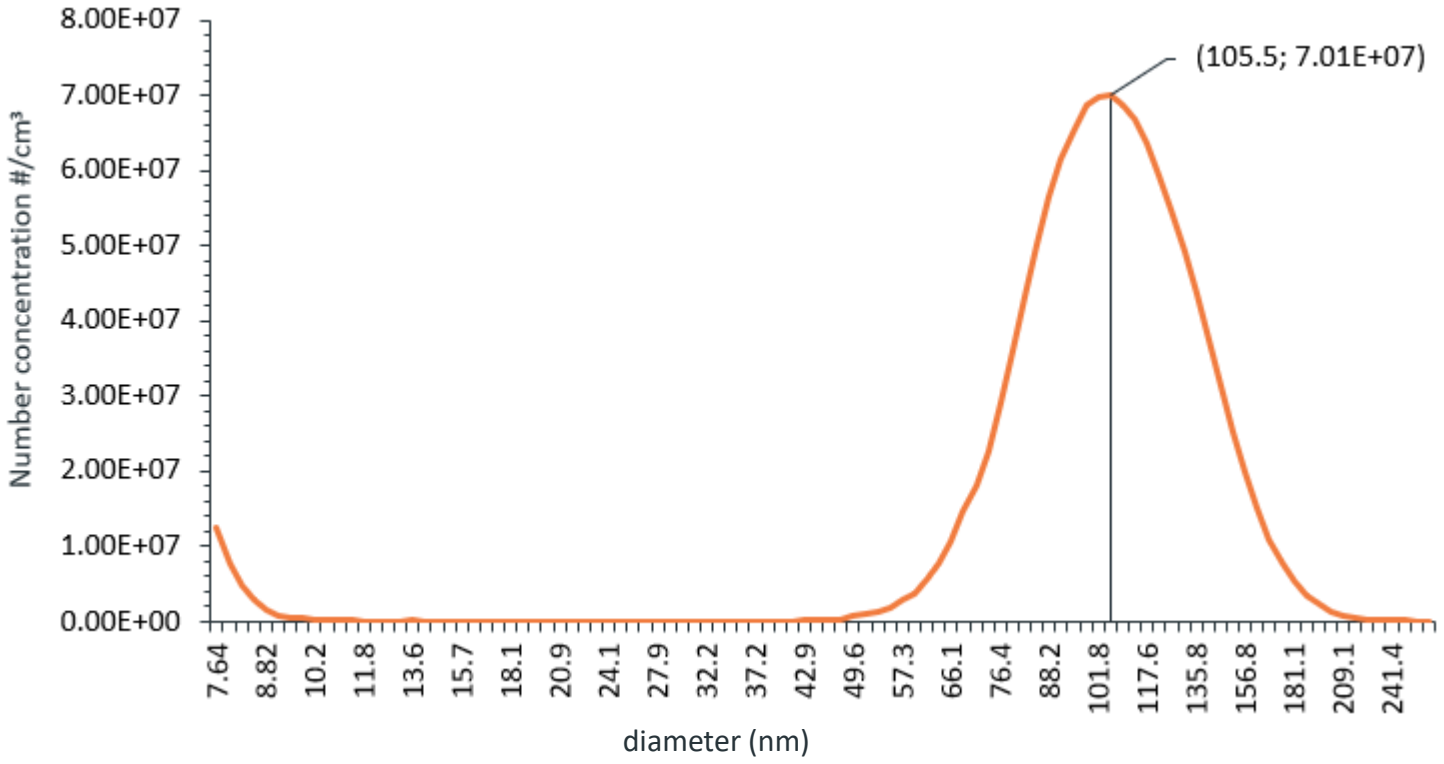

Particle size distribution in number of particles depending on their diameter

ONLINE analysis of the particle phase using a scanning mobility particle sizer (SMPS) :

- DMA, TSI, model 3082
- CPC, TSI, model 3750

Results :

- SOA diameter mode : **105.5 nm**
- Mean total mass concentration :

  23 mg/m³
- Mean total number concentration :

  1.94 x 10^7 particles/cm³

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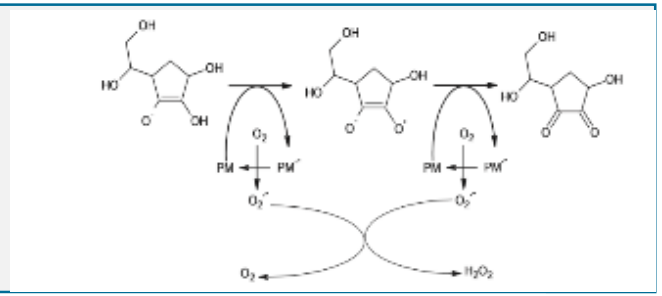
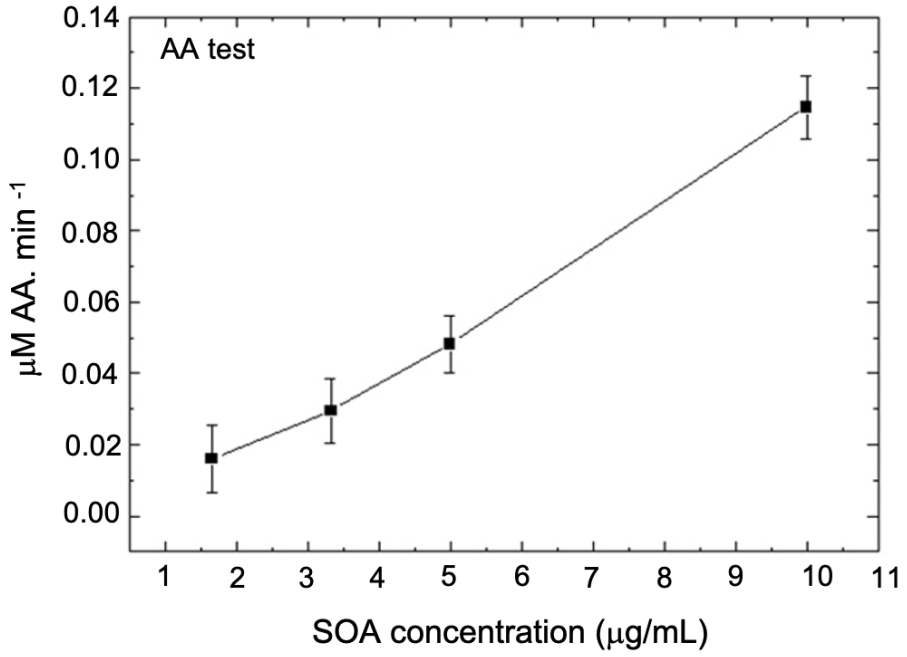
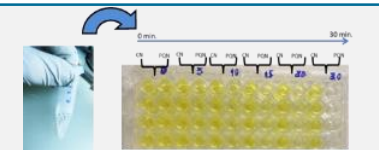
OFFLINE acellular tests of the oxidative potential of AOS :

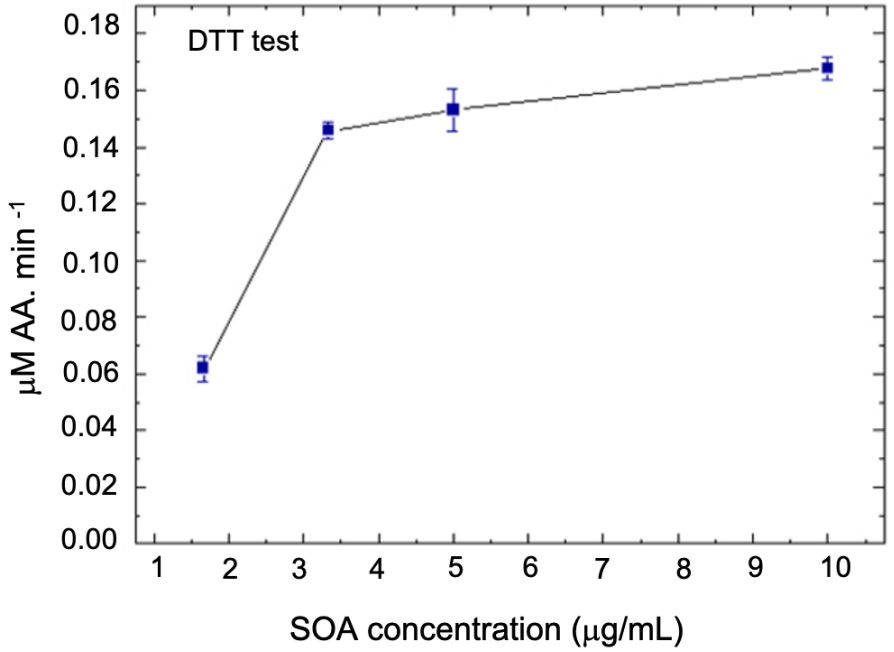
Acid Ascorbic test (AA test)
- Potassium phosphate buffer solution (pH = 7.4)
- Temperature: 37 °C
- C_{final} of AA: 200 µM
- Absorbance: 265 nm





Dithiothreitol test (DTT test)
- Potassium phosphate buffer solution (pH = 7.4)
- Temperature: 37 °C
- C_{final} of DTT: 0.1 mM
- C_{final} of DTNB: 0.14 mM
- Absorbance: 412 nm





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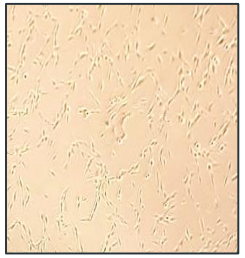
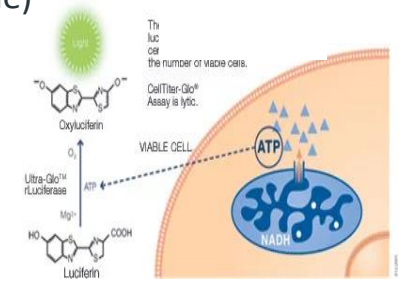
Cell viability test :

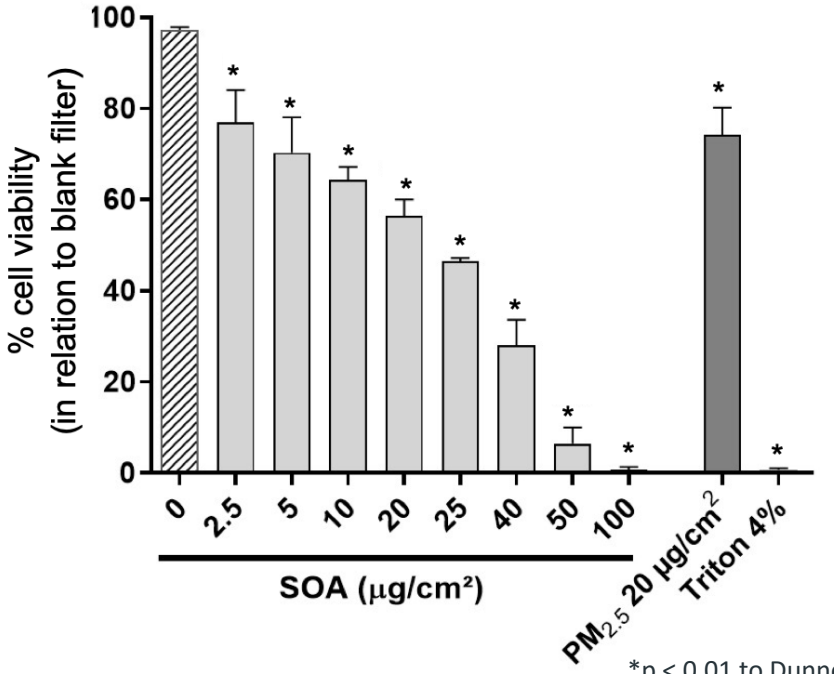
Human bronchial epithelial cells (BEAS-2B cell line)

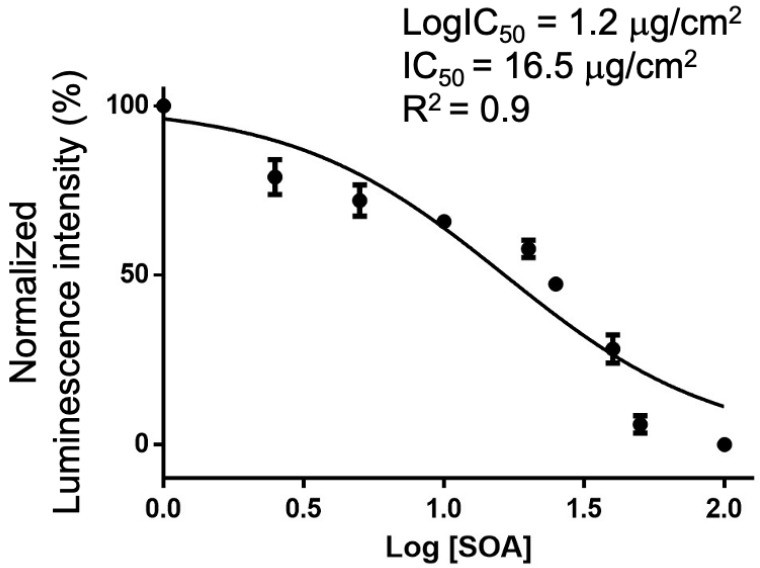




Intracellular ATP concentrations of BEAS-2B cells were determined using the CellTiter-Glo luminescent cell viability kit (Promega).

Data represent mean values from two independent experiments in quadruplicate.





*p < 0.01 to Dunnetts'test

Result :

Significant decreases of BEAS-2B cell viability are reported after exposure to increasing SOA concentrations and the calculated IC_{50} value was 16.5 µg/cm^2.

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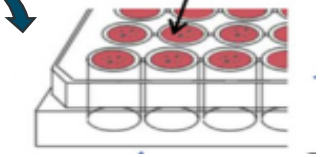
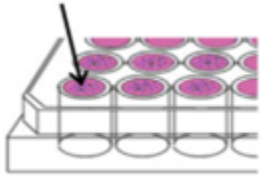
Cell measurement of ROS

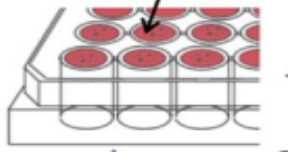
Seeding of BEAS-2B cells at a density of $2x10^4$ cells/well in LHC-9 culture medium (24 h)



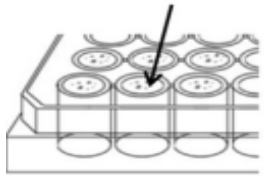
Diluted carboxy-DCFH-DA (10 µM) (40 min. at 37ºC)



Exposure of SOA (24 h)



Replaced with PBS

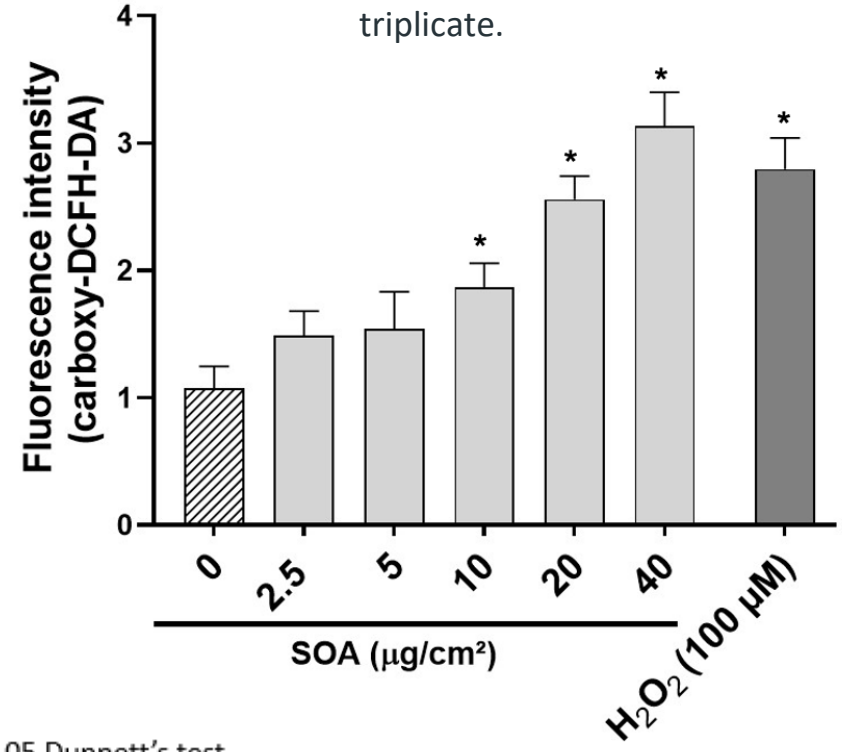


Microplate reader
$\lambda_{excitation}$= 485 nm
$\lambda_{emission}$= 525 nm

Result :

Intracellular ROS generation in BEAS-2B cells shows a tendency to increase starting at 2.5 µg/cm² and was significantly higher in cells exposed to 10 µg/cm² of SOA compared to the control cell.

Data represent mean values from three independent experiments in triplicate.



* $p < 0.05$ Dunnett's test

Quantification of intracellular ROS after SOA exposure during 24 h

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Conclusions :

- Our setup based on a laminar flow reactor gives reproducible and significant amounts of SOA suitable for conducting acellular and cellular toxicological tests.
- The SOA generated in the ultrafine particles size range, can penetrate deeply into the human respiratory system.
- Chemical tests of oxidative potential (AA, DTT) show the ability of SOA to oxidize some target molecules.
- SOA significantly decrease intracellular ATP concentrations and induce ROS production in human bronchial epithelial cells (BEAS-2B).

Perspectives :

- Vary the conditions of SOA synthesis (other VOCs, other oxidants, presence or absence of NOx or inorganic nuclei).
- Study the influence of the chemical composition of SOA on their health impact.
- Extend the study to include SOA freshly collected from the ambient air.
- Investigate the oxidation of some target molecules (proteins, lipids, DNA) in BEAS-2B cells.