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

**Introduction**

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.

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\(^1\) Hallquist et al. 2009 \(^2\) Panopoulou et al. 2020 \(^3\) Chowdurry et al. 2019 \(^4\) Lin et al. 2016
**Laminar aerosol flow reactor**

SAGE – IMT Lille Douai

- 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 terpenes⁵.

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⁵ Lchhabra et al., 2011
Introduction

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 10⁷ particles/cm³
OFFLINE acellular tests of the oxidative potential of AOS:

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

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

![Graphs showing AA test and DTT test results](image)
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Introduction

Experimental system

Particle size analysis

Oxidative potential

Biological assays

Conclusion

**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).

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$^2$.

Data represent mean values from two independent experiments in quadruplicate.

LogIC$_{50}$ = 1.2 μg/cm$^2$

IC$_{50}$ = 16.5 μg/cm$^2$

R$^2$ = 0.9

*p < 0.01 to Dunnett’s test
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### Cell measurement of ROS

Seeding of BEAS-2B cells at a density of 2x10⁴ 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
- λ excitation = 485 nm
- λ 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**
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.