Laboratory-generated soot with well-defined organic coating for

*in-vitro* cytotoxicity assessment

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Air pollution is the 6th highest-ranking risk factor for death globally.

92%
More than 9 out of 10 of the world’s population lives in areas exceeding WHO guidelines for safe limits.

<1%
In China and India, less than 1% of the population lives in areas meeting the WHO guidelines.

Global map of modelling annual median concentration of PM$_{2.5}$ in µg/m$^3$ http://maps.who.int/airpollution/

Source: WHO 2016

Adapted from “Recent advances in understanding SOA: implications for global climate forcing” M. Shrivastava et al., 2017.
Project goals and experimental setup (I)

Generation of well characterized aged soot particles coated with oxidized organic matter

- Anthropogenic origin (1,3,5-trimethylbenzene, urban site)
- Biogenic origin (α-pinene, suburban)

**Diagram:**

- Combustion Generator (CAST)
- Fresh soot particles
- 1,3,5-trimethylbenzene, α-pinene
- Dilution: 1:10
- Photo-oxidation reactor
- Coated-soot particles
- DMA 1, CPC 1
- DMA
- CPC 2
- Aethalometer, PAX (870nm)
- TEOM
- Diffusion Size Classifier (minidisc)
- EC/OC ANALYSIS, Chromatography

**Estimation of coating thickness**
- Optical properties
- Mass concentration
- Lung-deposited surface area
- Chemistry

References:
M. Vasilatou, Aerosol Science and Technology (2019)
Project goals and experimental setup (II)

(Nano-)Aerosol Chamber for In-Vitro Toxicology, NACIVT (www.nacivt.ch)

54 × 49 × 48 cm (WDH), 30.5 kg

Aerosol (external production/source)

- “All-in-one”, mobile system for direct use at any particle source
- Mimics particle deposition in lungs (T, RH, gas composition, air flow, \( N_p, N_{dep} \))
- Simultaneous exposure of 24 cell cultures
- Custom electronics (aerosol charger, integrated pump, flow control)

Controlled & stable conditions allow long-term exposures

Jeannet N. et al., Nanotoxicology, 2015; Geiser M. et al., Nanomaterials, 2017
Objective and experimental setup (III)

three experimental campaigns took place at Federal Institute of Metrology (METAS)

1st: 13-16/08/2018 (TMB)
2nd: 03-17/12/2018 (TMB)
3rd: 13-24/05/2019 (α-pinene)

equipment of University of Bern transferred to METAS:

- NACIVT chamber
- Cell culture lab (e.g. sterile bench, cell incubator)
- Light microscope

Exposure of realistic cell models to well-controlled, chemically defined synthetic reference aerosols, to identify the aerosol components/properties responsible for adverse respiratory effects.
**Particle delivery strategy**

Exposure time (1h)
- Normal: undifferentiated cells, ALI
- 4h: ~5 days
- 24h: 21-28 days

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**1st campaign**
- 1,3,5-trimethylbenzene (TMB)
- Cytotoxicity depends on organic coating (experiments repeated with additional coating thickness)
- EC (soot core) → not cytotoxic
- dₚ=30 nm, dₚ=90 nm

**2nd campaign**
- 1,3,5-trimethylbenzene (TMB)
- dₚ=30 nm, dₚ=90 nm

**3rd campaign**
- α-pinene
- dₚ=30 nm, dₚ=90 nm

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Particle number concentration remains the same between 30 nm and 90 nm in all three campaigns:

- 30 nm ~ 4 \times 10^5 NP/cm³
- 90 nm ~ 1.3 \times 10^5 NP/cm³
1\textsuperscript{st} and 2\textsuperscript{nd} campaign: TMB as SOA-precursor (I)

**No significant difference in terms of cytotoxicity between incubator control (I.C), p-free air and synthetic air exposed cells**

**Soot particles consisting only of EC (90 nm UnCoated soot) cause no cytotoxic effects**

**Cytotoxicity increases with increasing coating thickness. Max-Coat 90 nm induces the highest cytotoxicity, i.e. release of LDH (mean ± S.E.)**
3\textsuperscript{rd} campaign: \(\alpha\)-pinene as SOA-precursor

- Cytotoxicity increases with coating thickness as in the case with TMB as SOA-precursor.
- General, slightly lower cytotoxic effect with \(\alpha\)-pinene as precursor compared to TMB.

\[R^2 = 0.5116, \quad p-value = 0.0303\]
Out of 102 cytokines and chemokines analyzed, 35 are down- or up-regulated in comparison to incubator control.
Conclusion and outlook

Pilot study funded by FOEN

Particles consisting only of EC (90 nm UnCoated soot) cause no cytotoxic effects. Cytotoxicity increases with coating thickness with TMB and α-pinene as SOA-precursors. α-Coat 90 nm induces the highest cytotoxicity. α-pinene as SOA-precursor cause slightly lower cytotoxic effect compared to TMB.

Ongoing and future activities:

- Transmission electron microscopy (TEM) of cell culture sections and of particles sampled on TEM grids.
- Secretome analysis for the 3rd campaign

The pilot study has led to the EU project: EMPIR AeroTox (http://empir.npl.co.uk/aerotox)

- Confocal microscopy of samples from 2nd and 3rd campaign at the National Physical Laboratory (NPL, UK).
- Analysis of the degree of oxidation of the organic matter by Aerosol Mass Spectrometry (NPL, UK).
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Ongoing analysis: TEM inserts

2nd EXPERIMENTAL CAMPAIGN: TMB as SOA-precursor

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<th>I.C</th>
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<th>Synth. air</th>
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3rd campaign: α-pinene as SOA-precursor

LDH release (%) vs. various treatments:
- Ctrl: incubator control
- Synt. Air: particle-free air
- Exp: experimental conditions
- p-free: particle-free conditions
- UnCoated: uncoated soot
- Min-Coat: minimum coating
- Med-Coat: medium coating
- Coated: fully coated soot

- Soot 90 nm
- Soot 30 nm

Cytotoxicity upon exposure to coated soot much higher than that of incubator control and particle-free air tests -> increase in cytotoxicity is indeed caused by coated soot particles
1\textsuperscript{st} and 2\textsuperscript{nd} campaign: TMB as SOA-precursor (II)

**Good reproducibility between the 1\textsuperscript{st} and 2\textsuperscript{nd} campaign**
Model aerosols:
internal mixture of soot core particles with coating from secondary organic matter

Properties tested:
uncoated vs. coated particles, role of soot core size, coating thickness and coating chemistry

Current project

Spring 2018 - Spring 2019

EMPIR project

Further tests to clarify the role of:
- primary vs. secondary organic coating
- secondary inorganic coating
- internal metal admixtures
- externally mixed dust particles (e.g. from brake abrasion)

Biomedical analyses will be complemented with toxicogenomics and particle uptake studies

June 2019 - May 2022
Generation of aged soot aerosol

SSA = light scattering/light extinction

Prototype miniCAST soot generator
Model 5201 BC
(Jing Ltd., Bern, Switzerland)

Oxidation flow reactor
- UVC lamp
- ozone, OH
- UVC lamp

Particle composition [%]
- Elemental carbon (EC)
- Organic carbon (OC)

Par*cle	composi*on	[%]
- EC/TC [%]
- OC/TC [%]

Organic vapor concentration (ppm)

Number concentration [#/cm³]

Mobility diameter [nm]

SSA = light scattering/light extinction

Prototype miniCAST soot generator
Model 5201 BC
(Jing Ltd., Bern, Switzerland)