A Step towards Standardisation of Air-Liquid Interface Exposures using a Model Diesel Aerosol

Questions?
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Motivation

- Air pollution → cardiovascular and respiratory diseases
- In vitro toxicological studies with ambient particles are needed
- Air-liquid interface vs submerged exposures
- Reference Aerosol needed for aerosol toxicology community (comparability)
- Are long-term Exposures possible?

➢ **Aim:** To investigate the in vitro effects of model particles and develop an optimized exposure protocol for the cell exposure system.

Lenz, Karg, et al., 2012 BioMed Research International
Methods – Exposure setup

Source

- dieselCAST
  - Benchtop device [1]
  - Two flames
    - Propane flame to heat up diesel [2]
    - Diffusion flame diesel [3]
  - 50-60 µL/min fuel [4]

- Dilution
  - Porous Tube Diluter [5]
  - Ejector Diluter [6]
  - Uses purified compressed air
Methods – Exposure setup

Source
- dieselCAST flame soot generator:
  - 2 x 6h
- Ambient filtered air:
  - 2 x 18h

Exposure System
- Vitrocell™ aerosol exposure station
  - Flow 50 mL/min
  - Humidity 85%
  - Particle deposition through diffusion

Cell monoculture
- A549 monoculture:
  - 48h Exposure
  - Endpoints:
    - Cell viability
    - Gene expression
    - Genotoxicity

Timeline
- 24h Cells on insert
- 24h Cells at ALI
- 6h diesel
- 18h Ambient filtered air
- 6h diesel
- 18h Ambient filtered air
Results – Aerosol characterisation

Particle mass (Aethalometer\(^6\), online Black Carbon)
\(\rightarrow 270 \mu g/m^3\) (avg over 6h, n = 4)

Size Distribution (SMPS\(^6\), online)
\(\rightarrow\) Bimodal distribution with peaks at 140 nm and 550 nm

Particle number (CPC\(^5\), online)
\(\rightarrow 3 \times 10^4/cm^3\) Particles (avg over 6h, n = 4)

\(^5\) CPC: Condensation Particle Counter; Particle growth through condensation to optical detectable sizes

\(^6\) SMPS: Scanning Mobility Particle Sizer; Size distribution through sequential analysis of selective narrow particle sizes (coupled to CPC\(^5\))

\(^6\) Aethalometer: Black Carbon mass; Light absorption on Filter through deposited particles
Results – Aerosol characterisation

- Gaseous compounds (FTIR\textsuperscript{\textregistered} and FID\textsuperscript{\textregistered}, online)
  - Analysis is ongoing
- Chemical characterisation (offline)
  - OC/EC Analyser
    - Particles are **EC-rich**
  - GC-MS\textsuperscript{\textregistered}
    - Quantification of some PAHs, alkanes, and more
    - Analysis ongoing
  - GCxGC-MS
    - Non-targeted approach
    - What other SVOCs are there?
    - Analysis ongoing

\textsuperscript{\textregistered} FTIR: Fourier-transform InfraRed spectroscopy; absorption spectroscopy of hot-filtered aerosol
\textsuperscript{\textregistered} FID: Flame Ionization Detector; Ion detection by combustion of organic carbon in a hydrogen flame ("as propane")
\textsuperscript{\textregistered} GC-MS: Gas chromatography–mass spectrometry; Schnelle-Kreis et al., Anal Bioanal Chem (2011)
Results – Cell viability

- Microscopy
  - Visual inspection of cells
- Alamar Blue Assay
  - Cell metabolism
- LDH Assay (Lactate Dehydrogenase)
  - Membrane disruption

- Good cell viability
- Similar cell viability in different settings

Cell viability

Dose: 270 µg/m² Black Carbon and 3*10⁴/cm² Particles

Controls: i) Incubator control w/ HEPES, w/o CO2; ii) Incubator control w/ CO2
  iii) Positive controls: T-X (LDH), TNFa and HQ (qPCR), H₂O₂ (Comet)
Results – Gene expression

• Gene expression analysis
• Oxidative stress
  – HMOX1 (or HO-1) and SOD2 are first responders to stress
• Inflammation
  – Three cytokines
  – Interleukin 1 beta is upregulated
• Cytochrome P450 (CYP1A1)
  – Induced by PAHs

> Cells respond to the prolonged exposure

Gene Expression Analysis

Dose: 270 µg/m³ Black Carbon and 3*10⁴/cm³ Particles
Controls: i) Incubator control w/ HEPES, w/o CO2; ii) Incubator control w/ CO2
iii) Positive controls: T-X (LDH), TNFa and HQ (qPCR), H₂O₂ (Comet)
Results – Genotoxicity

- Genotoxicity in A549 cells using Comet Assay
  - dieselCAST induces high genotoxicity in A549 cells

**Comet Assay**

- **Dose:** 270 µg/m$^3$ Black Carbon and $3 \times 10^4$/cm$^3$ Particles
- **Controls:**
  1. Incubator control w/ HEPES, w/o CO2
  2. Incubator control w/ CO2
  3. Positive controls: T-X (LDH), TNFa and HQ (qPCR), H$_2$O$_2$ (Comet)
Summary

- dieselCAST
  - Model diesel Aerosol
  - 2 x 6h exposure (overnight filtered ambient lab-air)
- Cell Exposure system
  - 48h total runtime possible
  - Different settings tested in 2 repetitions (modules, buckets)
- A549 monoculture
  - At Air-Liquid Interface
  - Cells in monolayer
Conclusion/ Outlook

- **Stable** aerosol over 4 days (6h/day)
- No cytotoxicity/ **good cell viability**
- Increase in **oxidative stress**, (pro-)**inflammation**, and xenobiotic metabolism
- **Genotoxicity**
- More testing needed if dieselCAST suitable as reference aerosol
- **Aerosol Characterisation**
  - Offline measurement of **SVOCs**
  - Online gaseous characterisation
- **Repetition** of these experiments (for statistical analysis)

Thank you for your attention

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