

HelmholtzZentrum münchen

German Research Center for Environmental Health

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

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Zürich, ETH Combustion Generated Nanoparticles

HELMHOLTZ RESEARCH FOR
GRAND CHALLENGES

Questions?

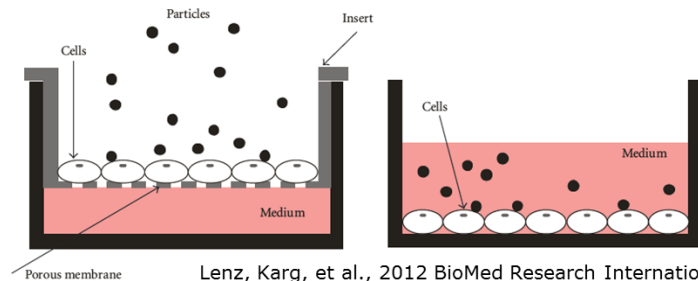
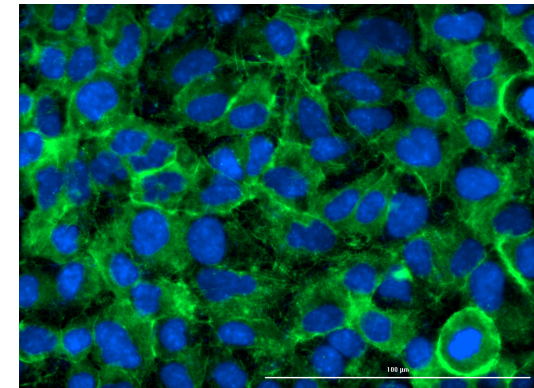
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Motivation

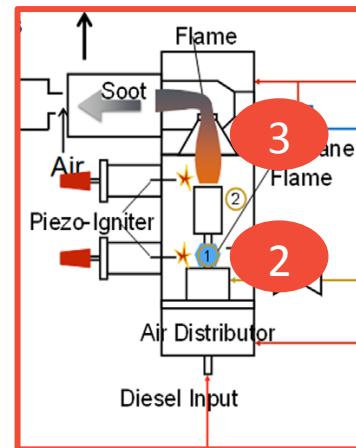
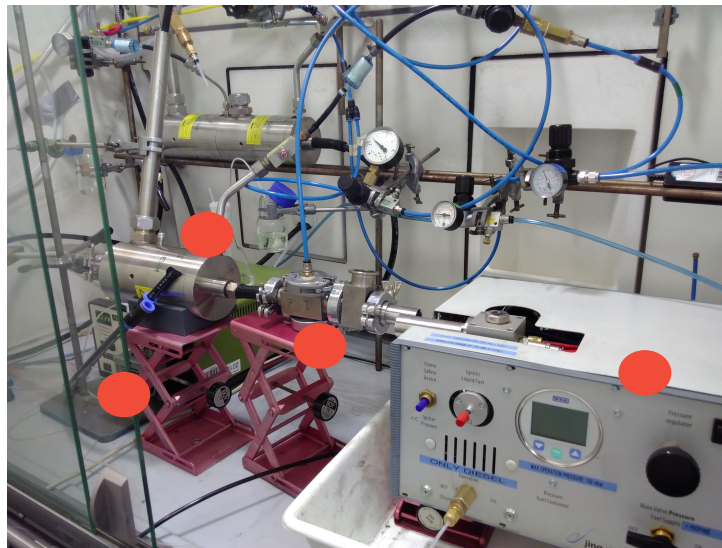
- Air pollution → cardiovascular and respiratory diseases
- In vitro toxicological studies with ambient particles are needed
- Air-liquid interface vs submerged exposures
- Reference Aerosol needed for aerosol toxicology community (comparability)
- Are long-term Exposures possible?
- **Aim: To investigate 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 $\mu\text{L}/\text{min}$ 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

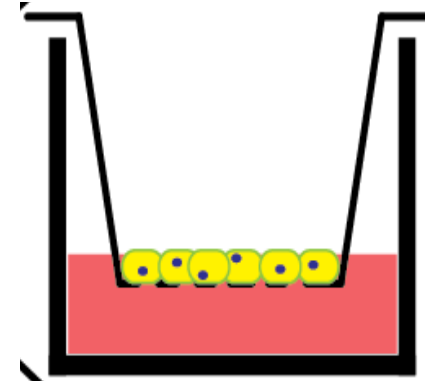
Exposure System



Vitrocell™ aerosol exposure station

- Flow 50 mL/min
- Humidity 85%
- Particle deposition through diffusion

Cell monoclulture

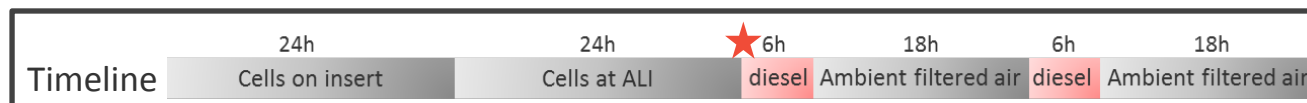


A549 monoclulture:

- 48h Exposure

Endpoints:

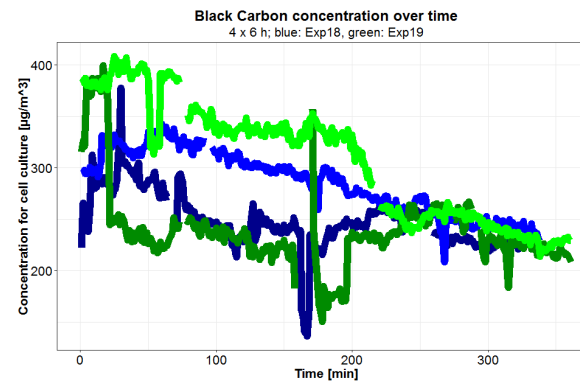
- Cell viability
- Gene expression
- Genotoxicity



Results – Aerosol characterisation



§ **Aethalometer**: Black Carbon mass; Light absorption on Filter through deposited particles



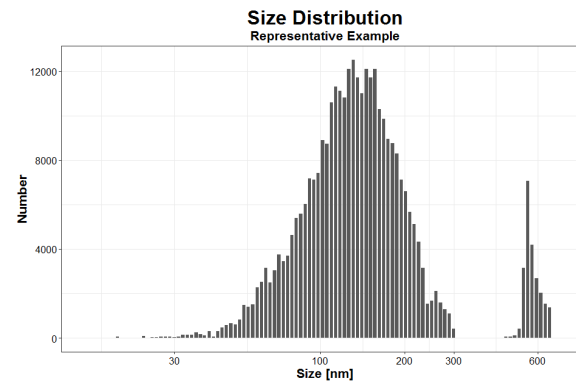
Particle mass (Aethalometer[§], online Black Carbon)

→ **270 µg/m³** (avg over 6h, n = 4)



& **SMPS**: Scanning Mobility Particle Sizer; Size distribution through sequential analysis of selective narrow particle sizes (coupled to CPC[§])

§ **CPC**: Condensation Particle Counter; Particle growth through condensation to optical detectable sizes



Size Distribution (SMPS[&], online)

→ **Bimodal** distribution with peaks at **140 nm** and **550 nm**

Particle number (CPC[§], online)

→ **3*10⁴/cm³** Particles (avg over 6h, n = 4)

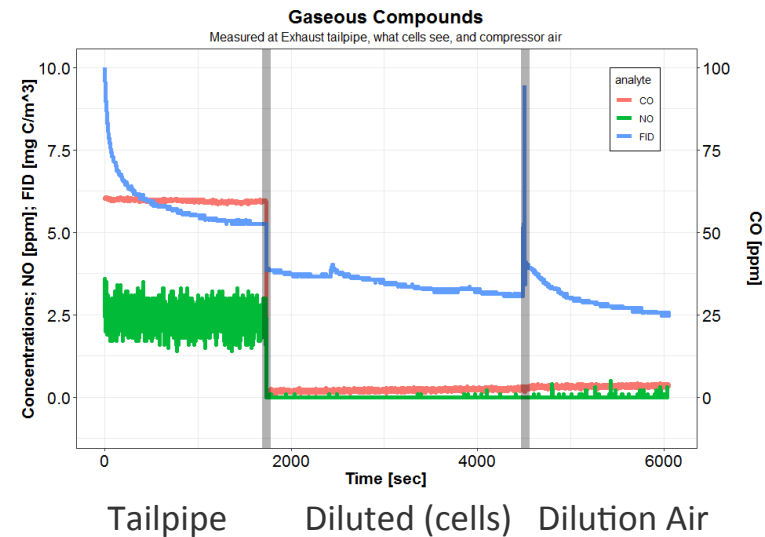
Results – Aerosol characterisation

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

[§] **FTIR**: Fourier-transform InfraRed spectroscopy; absorption spectroscopy of hot-filtered aerosol

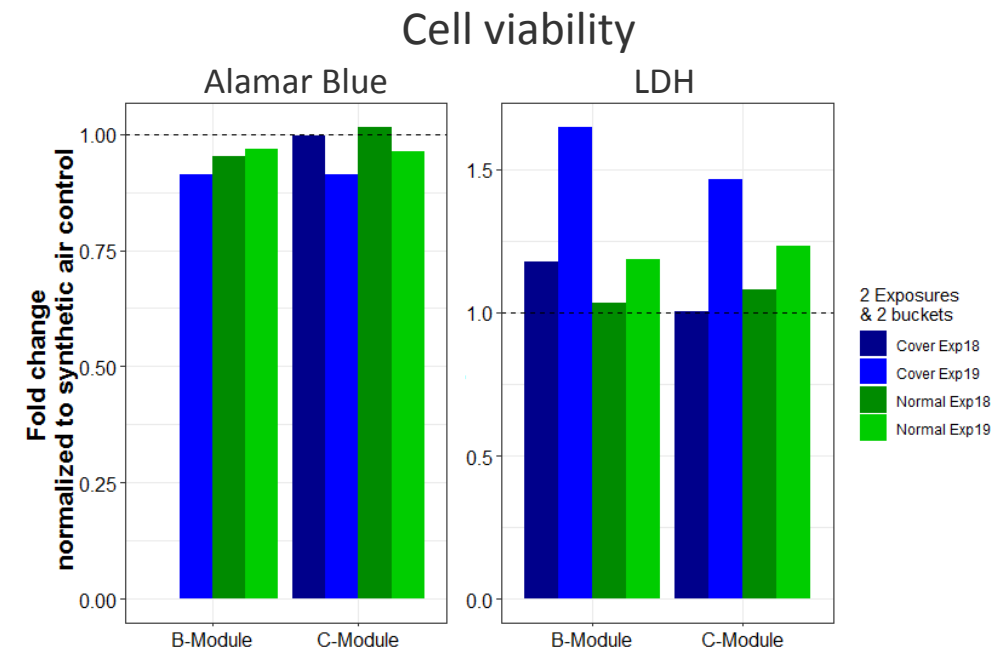
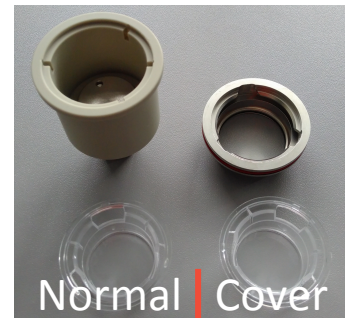
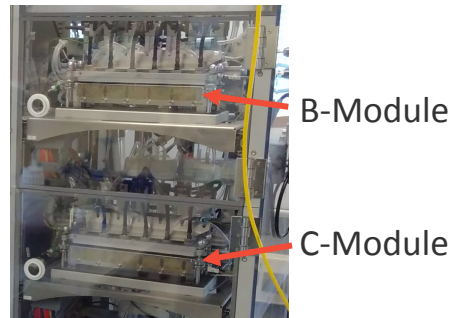
[§] **FID**: Flame Ionization Detector; Ion detection by combustion of organic carbon in a hydrogen flame („as propane“)

[&] **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**



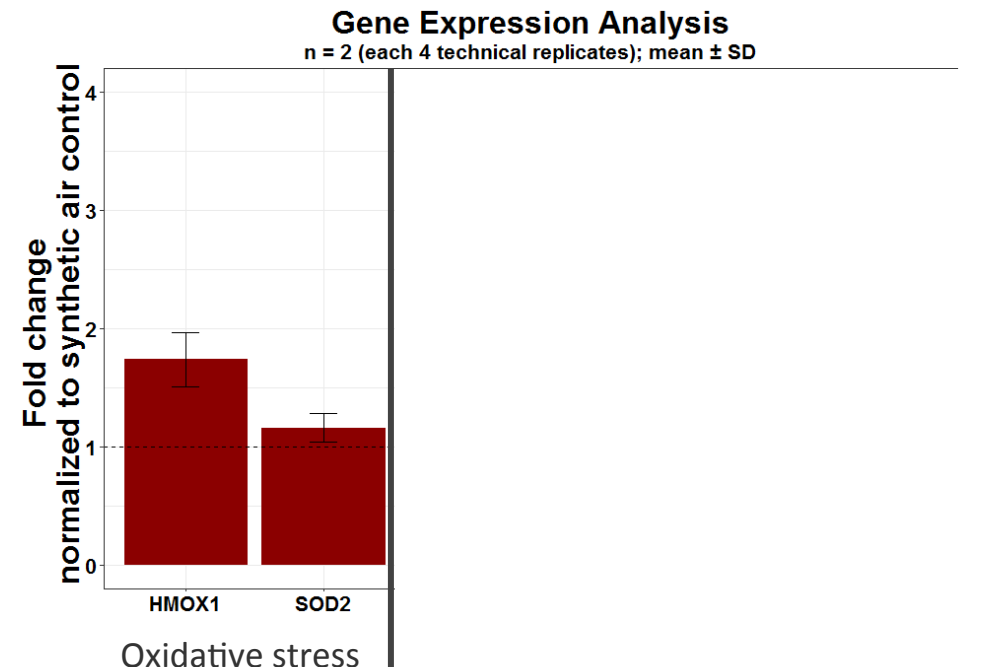
Dose: 270 $\mu\text{g}/\text{m}^3$ Black Carbon and $3 \cdot 10^4/\text{cm}^3$ Particles

Controls: i) Incubator control w/ HEPES, w/o CO_2 ; ii) Incubator control w/ CO_2

iii) Positive controls: T-X (LDH), TNF α and HQ (qPCR), H_2O_2 (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

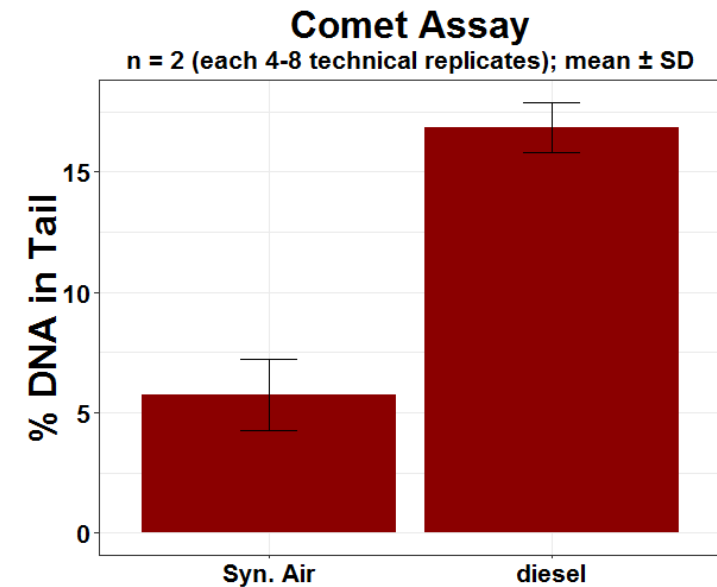
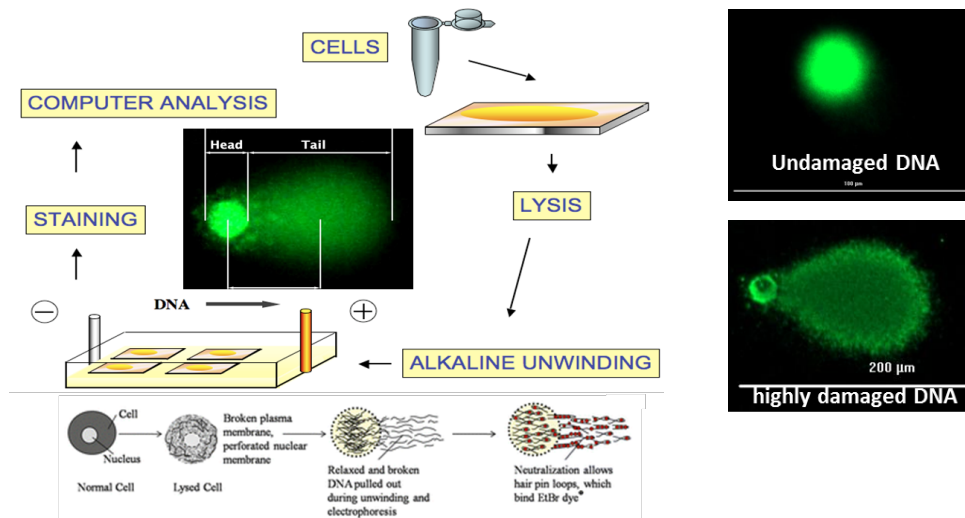


Dose: 270 µg/m³ Black Carbon and 3*10⁴/cm³ Particles

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

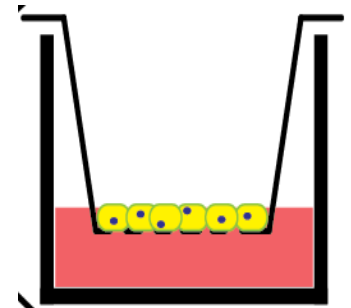
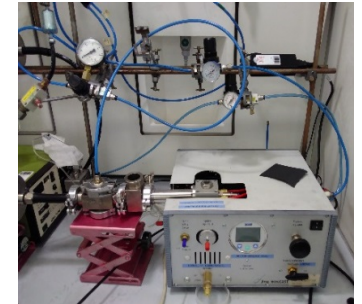
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Summary

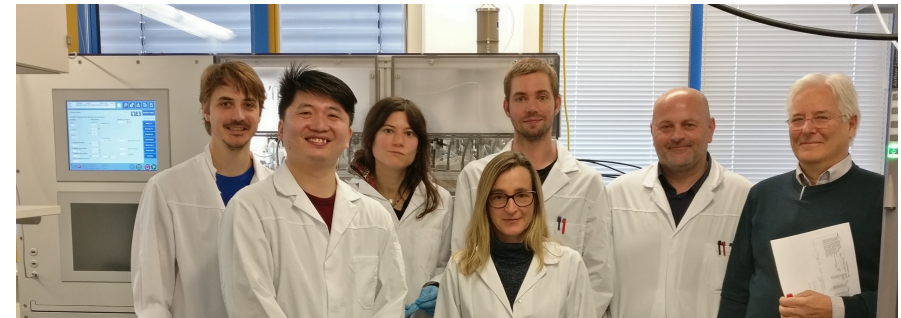
- dieselCAST
 - Model diesel Aerosol
 - 2 x 6h exposure (over night filtered ambient lab-air)
- Cell Exposure system
 - 48h total runtime possible
 - Different settings tested in 2 repetitions (modules, buckets)
- A549 monoculture
 - At Air-Liquid Interface
 - Cells in monolayer



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



Involved people at CMA (alphabetically)

- Anja Huber
- Christoph Bisig, Dr.
- Elias J. Zimmermann
- Erwin Karg, Dr.
- Jürgen Orasche, Dr.
- Gert Jakobi, Dr.
- Nadine Gawlitta
- Ralf Zimmermann, Prof.
- Sebastian Öder, Dr.
- Sebastiano di Bucchianico, Dr.
- Stefanie Bauer, Dr.
- Stephanie Binder

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