Air pollution: a public health problem then and now

Biological effects of emissions from ship diesel- and gasoline car-engines as well as from wood combustion compliances: Multi-omics characterization of aerosol-exposed lung cells and chemical profiles of the emissions

R. Zimmermann\textsuperscript{1,11}, T.G. Dittmar\textsuperscript{2,11}, T. Kanashova\textsuperscript{2,11}, J. Buters\textsuperscript{3,11}, S. Öder\textsuperscript{3,11}, H. Paur\textsuperscript{4,11}, M. Dilger\textsuperscript{4,11}, C. Weiß\textsuperscript{4,11}, H. Harndorf\textsuperscript{5,11}, B. Stengel\textsuperscript{5,11}, K. Höller\textsuperscript{7,11}, S.C. Sapcariu\textsuperscript{7,11}, K.A. Berube\textsuperscript{8,11}, A.J. Wlodarcyzk\textsuperscript{8,11}, B. Michalke\textsuperscript{9}, T. Krebs\textsuperscript{10,11}, M. Kelbg\textsuperscript{5,11}, T. Streibel\textsuperscript{1}, E. Karg\textsuperscript{1}, J. Schnelle-Kreis\textsuperscript{1}, M. Sklorz\textsuperscript{1}, J. Orasche\textsuperscript{1,11}, P. Richthammer\textsuperscript{1,11}, L. Müller\textsuperscript{1,11}, J. Passig\textsuperscript{1,11}, C. Radischät\textsuperscript{1,11}, S. Smita\textsuperscript{5,11}, J. Orasche\textsuperscript{1,11}, H. Lamberg\textsuperscript{6}, M.-R. Hirvonen\textsuperscript{6,11}, O. Sippula\textsuperscript{6,11}, J. Jokiniemi\textsuperscript{6,11}

\textsuperscript{1}Joint MS Centre, Rostock Univ./Analyt. Chemistry & Helmholtz Zentrum München (HMGU)/CMA, Germany; \textsuperscript{2}Max Delbrück Ctr., Germany; \textsuperscript{3}Technical Univ. Munich (ZAUM), Germany; \textsuperscript{4}Karlsruhe Inst. Technol. (ITC/ITG), Germany; \textsuperscript{5}Univ. of Rostock (Inst. of Piston Machines & Inst. of Physics & Systems Biology), Germany; \textsuperscript{6}Univ. Eastern Finland, Finland; \textsuperscript{7}Univ. Luxemburg, Luxemburg; \textsuperscript{8}Cardiff Univ., UK; \textsuperscript{9}HMGU, Germany; \textsuperscript{10}Vitrocell GmbH, Germany; \textsuperscript{11}HICE-Helmholtz Virtual Institute HICE (www.hice-vi.eu)
Health effects of ambient aerosols

„A Proper Pea-Souper - The Terrible London Smog of 1952“
London smog 1952; Arsenal soccer match (canceled due to low visibility)
Health effects of ambient aerosols

Reasons for London „Killer-Smog“ Episode 1952:

- High emissions of combustion effluents (coal/industry/traffic)
- Weather (typ. winter inversion)

Diagram showing the temperature profile, inversion layer, and mixing depth of the atmosphere during the smog event. The graph illustrates the number of deaths and pollution levels (sulphur dioxide, smoke) during the fog period in December 1952.
Harvard-Six-Cities-Study
Dockery et al. (1993)

- cohort study (8111 Pers.)
- Six US cities with rather different PM10
- Study time: 16 years starting about 75.
- mortality in city with highest PM (~30 µg/m³) was 26% higher compared to cleanest city (~10 µg/m³)
- Re-analysis von Krewski et al. (2000) confirmend the conclusions
Health effects of ambient aerosols

Deposition of particles in the airways of healthy humans

Epidemiological studies suggest that fine (PM2.5) and ultra-fine (< 100 nm) particles are most relevant for health effects (e.g., Wichmann and Peters).
Health effects of ambient aerosols

<table>
<thead>
<tr>
<th>nm</th>
<th>0,1</th>
<th>1</th>
<th>10</th>
<th>100</th>
<th>1.000</th>
<th>10.000</th>
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<tbody>
<tr>
<td></td>
<td>molecules</td>
<td>droplet aerosols</td>
<td>combustion aerosols</td>
<td>mineral dust</td>
<td>fibre dust</td>
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<tr>
<td></td>
<td>0,2 nm NO₂</td>
<td>10 nm</td>
<td>100 nm</td>
<td>500 nm</td>
<td>2.000 nm</td>
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<tr>
<td></td>
<td>tobacco smoke, SOA</td>
<td>soot</td>
<td>quartz crystals</td>
<td>asbestos</td>
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<tr>
<th>Biological aerosols</th>
<th>biological fragments</th>
<th>virus</th>
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<th>fungal spores</th>
<th>pollen</th>
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<tr>
<td></td>
<td>endotoxin</td>
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<td></td>
<td>80 nm</td>
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<table>
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<tr>
<td></td>
<td>chemical toxicity (e.g. chem reactivity)</td>
<td>allergy</td>
<td>carrier of toxic substances (PAH, metals)</td>
<td>physical toxicity (mechanical irritation)</td>
<td>infection</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Health effects of ambient aerosols

Morphology of fine dust particles: SEM-Photographs

Bacteria & Spores
Wittmaack et al., 2005 und http://www.mpch-mainz.mpg.de

Morphology of fine dust particles:

- SEM-Photographs

Health effects of ambient aerosols
Sponge-like structure of the lung

Lung surface: ca. 80 m²
Basal membrane thickness:
~0.1 µm
Number of alveoli:
ca. 300 million
Size of alveoli: 50 – 250 µm
Health effects of ambient aerosols

Sponge-like structure of the lung

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Number of alveoli:
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Size of alveoli: 50 – 250 µm

Alveolar sacs with blood vessels and macrophages

Interalveolar septum

E. Weibel, 2013
Health effects of ambient aerosols
Health effects of ambient aerosols


TRP

PM

debris from disintegrated macrophages (DAMP)

DAMP/PAMP

Crystalline/ particulate ligands

Lysosomal rupture

NLRP3

ASC

Caspase 1

Activated Caspase-1

"Priming" by proinflammatory stimuli e.g. TLRs, TNF, IL-1β

modified after Schroder & Tschopp, Cell, 2010

DAMPs (Damage Associated Molecular Patterns)
PAMPs (Pathogen-Associated Molecular Patterns)

Inflammatory Response
[Alzheimer's Diseases, Atherosclerosis, Autoinflammatory/ Autoimmune Diseases, CAPS, Gout, Tissue Repair, Tumorigenesis, Type 1 Diabetes, SLE, SIRS, Shock, TNF-Related Diseases, Thrombosis, Vascular Disease]
Health effects of ambient aerosols

- Epidemiology: Today aerosols (air pollution) are the most relevant impact of environment on human health in EU

- Important for health effects (WHO): Combustion aerosols, organics, soot, transition metals, small particles

- Relevant sources: automobiles, trucks, house heating/ bio mass combustion, power stations, industry, ships

Why aerosols are so toxic and what causes the strong acute health effects of ambient particulate matter?

What is the role of organic compounds, soot & carbonaceous fractions?
Health effects of ambient aerosols

- Epidemiology: Today aerosols (air pollution) are the most relevant impact of the environment on human health in EU.

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- Why aerosols are so toxic and what causes the strong acute health effects of ambient particulate matter?

- What is the role of organic compounds, soot & carbonaceous fractions?

Source: EU - Clean Air For Europe (CAFE, 2005)
HICE Konsortium (2012-2017):
T.G. Dittmar²,¹¹, T. Kanashova²,¹¹, J. Buters³,¹¹, S. Öder³,¹¹, H. Paur⁴,¹¹, C. Schlager⁴, S. Mülhopt⁴, M. Dilger⁴,¹¹, C. Weiß⁴,¹¹, S. Diabate⁴, H. Harndorf⁵,¹¹, B. Stengel⁵,¹¹, R. Rabe⁵, K. Hiller⁷,¹¹, S. C. Sapcariu⁷,¹¹, K. A. BeruBe⁸,¹¹, A. J. Wlodarczyk⁸,¹¹, B. Michalke⁹, T. Krebs¹⁰,¹¹, M. Kelbg⁵,¹¹, J. Tiggesbäumker⁵,¹¹, T. Streibel¹, E. Karg¹, S. Scholtes¹,¹¹, J. Schnelle-Kreis¹, J. Lintelmann¹, M. Sklorz¹, M. Arteaga Salas¹,¹¹, S. Klingbeil¹,¹¹, J. Orasche¹, L. Müller¹,¹¹, A. Rheda¹, J. Passig¹,¹¹, T. Gröger¹, G. Abbaszade¹, C. Radischat¹,¹¹, S. Smita⁵,¹¹, J. Orasche¹,¹¹, T. Torvela⁶, P. Tiitta⁶, H. Lamberg⁶, M. R. Hirvonen⁶,¹¹, S. Kasurinen⁶, P. Jalava⁶, O. Sippula⁶,¹¹, J. Jokiniemi⁶,¹¹, R. Zimmermann

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² University of Rostock (Engineering/Physics), D
³ MDC, Berlin, D
⁴ Technical University Munich, D
⁵ ZAUM, Technical University Munich, D
⁶ University of Eastern Finland-Kuopio; FI
⁷ University of Rostock (Engineering/Physics), D
⁸ University of Cardiff, UK
⁹ Helmholz Zentrum München), D
¹⁰ Vitrocell GmbH, Waldkirch, D
¹¹ HICE – Helmholtz Virtual Institute of Complex Molecular Systems in Environmental Health-Aerosols and Health
Health relevance of combustion aerosols: Some scientific questions of HICE

- How is the chemical composition and formation dynamics of relevant combustion aerosols?
- What are the biological effects of gas and particulate phase of the aerosols?
- Why are combustion particles so dangerous and what are the underlying biological mechanisms?
- How does atmospheric aging impact health and environment?

Technological innovations:
- Realistic on-line ALI cell exposure & comprehensive biological effect monitoring (incl. multi-omics)
- Comprehensive aerosol characterization & on-line monitoring of the exposure by e.g. mass spectrometry
HICE concept: Interdisciplinary research to address health effects of aerosols

Aerosol Sources

Advanced ALI lung cell culture & animal exposure

Comprehensive phys. & chem. aerosol characterization

Multi-Omics, toxicol.-assays

Biostatistics & Chemometry

HICE concept: Interdisciplinary research to address health effects of aerosols
Characterization of biological effects of combustion aerosols: Exposure & Analyses

HICE-ALI-Exposure System
(Vitrocell and KIT)
Characterization of biological effects of combustion aerosols: Exposure & Analyses

HICE-ALI-Exposure System (Vitrocell and KIT)
Characterization of biological effects of combustion aerosols: Exposure & Analyses

**Multi’omics-approach**

- Cytotoxicity & Viability (ATP/LDH assays)
- 'Omics – regulation strength (Proteomics/Transcriptomics)
- 'Omics pathway analysis (GO-Terms)
- Analysis of targets Proteins/RNA-Transcrips (Cyp1A1, IL's, JUN, TNFα etc.)
- Verification analysis via specific assays (genotoxicty etc.)
- Verification analysis via primary cell/animal exposure

Modified after: Wu RQ, J Dent Res. 2011
Characterization of biological effects of combustion aerosols: Exposure & Analyses

Biological effect analysis:

- Cytotoxicity & Viability (ATP/LDH assays)
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Characterization of biological effects of combustion aerosols: Exposure & Analyses

Stable isotope labeled cell culture

ALI-Exposure setup

“Transwells” w. A549 or human BEAS2B cells on membrane (18 positions in HICE ALI system)

Native cell culture

Native cell culture

Transcriptomics

Cytotoxicology

Metabolomics

Proteomics (SILAC)

Stable isotope labeled cell culture

L4-Lysine and 13C-Glucose

BEAS2B or A549 cells

filtered Aerosol (gas) or clean air

Aerosol (gas + particles)

4 h exposure

BEAS2B, human bronchial epithelium cells

A549, human alveolar epithelium cells

RAW 264.7, murine macrophages
Air pollution by transportation sector

- **Road traffic**
- **Aviation**
- **Shipping**

### Annual Emissions [Tg(C/N/S)/yr]

- **CO₂**: 1122, 175, 222
- **NOₓ**: 8.3, 6.5, 2.2
- **SO₂**: 2.1, 0.075, 0.001
- **PM10**: 2.1, 1.7, 0.001
- **Fuel Consumption**: 1320, 207, 280

**Road traffic**

- **CO₂**: 1122 Tg yr⁻¹
- **NOₓ**: 8.3 Tg yr⁻¹
- **SO₂**: 2.1 Tg yr⁻¹
- **PM10**: 2.1 Tg yr⁻¹

**Aviation**

- **CO₂**: 175 Tg yr⁻¹
- **NOₓ**: 6.5 Tg yr⁻¹
- **SO₂**: 0.71 Tg yr⁻¹
- **PM10**: 0.075 Tg yr⁻¹

**Shipping**

- **CO₂**: 222 Tg yr⁻¹
- **NOₓ**: 2.2 Tg yr⁻¹
- **SO₂**: 2.1 Tg yr⁻¹
- **PM10**: 1.7 Tg yr⁻¹

**Fuel Consumption**

- **CO₂**: 1320 Tg yr⁻¹
- **NOₓ**: 207 Tg yr⁻¹
- **SO₂**: 280 Tg yr⁻¹
Air pollution by transportation sector

A satellite image from 4 March 2009 showing ship tracks — the bright streaks of clouds that form around the particles in ship exhaust — over the northeast Pacific Ocean. The ship tracks are brighter than the natural marine clouds around them because they contain lots of small cloud droplets, which you can see in this zoomed-in image. NASA image by the LANCE/EOSDIS MODIS Rapid Response Team.

Annual Emissions [Tg(C/N/S)/yr]

<table>
<thead>
<tr>
<th></th>
<th>Fuel Consumption</th>
<th>PM10</th>
<th>SO2</th>
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<tbody>
<tr>
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<tr>
<td>Road traffic</td>
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<td>1,7</td>
<td>8,0</td>
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<tr>
<td>Aviation</td>
<td>207</td>
<td>2,1</td>
<td>8,0</td>
</tr>
<tr>
<td>Shipping</td>
<td>1320</td>
<td>0,075</td>
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</tbody>
</table>
Corbett et al., ES&T 2007: Worldwide mortality cases due to pulmonary diseases attributable to ship pollution:~ 60,000 excess mortality cases/a

⇒ ports /costal areas are in particular affected by (marine) shipping pollution
Chemical & Physical Characterization

- On-line gas phase mass spectrometry
- On-line particle sizing and chemical analysis
- Filter & impactor sampling and off-line analysis

Dilution & Cooling

Research Ship-Diesel Engine

HFO

DF

Lung Cell Exposure (Air-Liquid-Interface)

E Comprehensive Molecular-Biological Characterization

- Cytotoxicity
- Transcriptomics
- Proteomics (SILAC)
- Metabolomics
- Metabolic Flux
HICE ship diesel engine campaign: Operation of engine and aerosol properties

![Test cycle and load diagram](image)

![Number and mass concentration of aerosol particles](image)

**Test cycle**
- Load (kW): 80, 60, 40, 20, 0
- Time (min): 0, 50, 100, 150, 200

**Number conc. dN/dlogD (cm³)**
- Number HFO
- Number DF
- Mass HFO
- Mass DF

**Particle diameter (nm)**
- 10¹
- 10²
- 10³
- 10⁴

**Number conc. (mg/m³)**
- 10⁰
- 10¹
- 10²
- 10³

**Mass conc. (mg/m³)**
- 10⁰
- 10¹
- 10²
- 10³
Switching from diesel fuel oil (DF) to heavy fuel oil (HFO) ➞
Increase of Polycyclic Aromatic Hydrocarbons (PAH) in exhaust gas
HICE ship diesel engine campaign: Characterization of the Aerosol

- Number conc. dN/dlogD (cm$^{-3}$)
- Mass conc. dM/dlogD (mg/m$^3$)

Particle diameter (nm)

- △ number HFO
- △ number DF
- △ mass HFO
- △ mass DF

Mass integral (mg/m$^3$)
HICE ship diesel engine campaign: Characterization of the Aerosol

deposited mass HFO: ~ nano particles

deposited mass DF: ~ larger aggregates
HICE ship diesel engine campaign: Characterization of the Aerosol

ALI PM-deposition dose ship experiments:
(estimation after Comouth et al., 2013, J Aerosol Sci):
\[ D_{\text{HFO}} \approx 56 \text{ ng PM/cm}^2 \quad \text{and} \quad D_{\text{DF}} \approx 28 \text{ ng PM/cm}^2 \]

⇒ Note: Higher dose for the HFO PM - deposition case!
HICE ship diesel engine campaign at U Rostock: Characterization of the Aerosol

Organic compounds: HFO PM much more complex than DF PM
Analysis of shipping PM: Concentration at the ALI-cell exposure experiment

- HFO particles contain more metals, PAH, organics (OC)
- DF particles contain more “pure” elemental carbon (BC, EC)
HICE ship diesel engine campaign: Biological effects on human lung cells

Transcriptome

Proteome (SILAC)

Metabolome

Significance (replicates)

Intensity

Regulation: down - up

HICE ship diesel engine campaign: Biological effects on human lung cells

Transcriptome

Proteome (SILAC)

Metabolome

Significance (replicates)

Intensity

Regulation: down - up
HICE ship diesel engine campaign: Biological effects on human lung cells

- **Stronger biological activity** (regulation strength) of DF-PM on all hierarchical biological levels (transcriptome, proteome, metabolome)
- **Note**: Dose of HFO-PM & content of toxic constituents is higher
HICE ship diesel engine campaign: Biological effects on human lung cells

regulation ➔ selected markers ➔ biological pathways

<table>
<thead>
<tr>
<th>Effect</th>
<th>HFO</th>
<th>DF</th>
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<tbody>
<tr>
<td>Inflammation</td>
<td>↑</td>
<td>-</td>
</tr>
<tr>
<td>Oxidative Stress</td>
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<td>-</td>
</tr>
<tr>
<td>Cell homeostasis</td>
<td>↑</td>
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</tr>
<tr>
<td>Response to chemicals</td>
<td>↑</td>
<td>↓↑</td>
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<tr>
<td>Cellular stress response</td>
<td>↑</td>
<td>→</td>
</tr>
<tr>
<td>Motility</td>
<td>↑</td>
<td>→</td>
</tr>
<tr>
<td>Endocytosis</td>
<td>↑</td>
<td>→</td>
</tr>
<tr>
<td>Cellular signaling</td>
<td>MAPK, TGF beta, PDGF, EGF, GPCR</td>
<td>ID, kinase cascade</td>
</tr>
<tr>
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<tr>
<td>Protein synthesis</td>
<td>-</td>
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<tr>
<td>Protein degradation</td>
<td>-</td>
<td>→</td>
</tr>
<tr>
<td>RNA metabolism</td>
<td>-</td>
<td>↓</td>
</tr>
<tr>
<td>Chromatin modifications</td>
<td>-</td>
<td>→</td>
</tr>
<tr>
<td>Cell junction and adhesion</td>
<td>-</td>
<td>↓→</td>
</tr>
</tbody>
</table>

**Note:** The table indicates changes in gene expression with up arrows (↑) for increased expression and down arrows (↓) for decreased expression.
HICE ship diesel engine campaign: Biological effects on human lung cells

multi ‘omics data analysis ➔ biological pathways

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</table>

Z-score

R. Zimmermann et al.
21st ETH Conference on Combustion Generated Nanoparticles, Zürich, Switzerland, June 19th to 22nd 2017
HICE ship diesel engine campaign: Biological effects on human lung cells

multi ‘omics data analysis ➔ biological pathways

> HFO and DF activate different adverse pathways
> Biological response of DF stronger than HFO!
> Oeder et al., PLoS one, 2015

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Validation of ship diesel results: Ship diesel exposure of RAW macrophages

Validation with Macrophage cell model (RAW 264.7, murine M.) Macrophages are phagocytotic innate immune cells heavily involved in inflammatory response

⇒ Same exposure experiment as with the epithelial cells (DF, HFO)

- RAW exp. confirms stronger regulation strength by DF-PM on proteomic level


![RAW 264.7](image.png)
Validation of ship diesel results: Ship diesel exposure of RAW macrophages

LDH assay for acute cytotoxicity

- Higher acute cytotoxicity of DF particles for macrophages!

Proteomics ➔ Inflammatory pathways stronger activated by HFO particles for macrophages!

Graphs showing LDH release and Proteomics results.
LDH assay for acute cytotoxicity

Higher acute cytotoxicity of DF particles for macrophages!

Proteomics ➔ Inflammatory pathways stronger activated by HFO particles for macrophages!

RAW exposure show: DF PM indeed more toxic (acute) than HFO PM

Pathway analysis also confirm results on epithelial cells

Conclusion: Higher overall regulation strengths ➔ higher toxicity!

Sapcariu et al., PLoS one, 2016
Introduction: IMO regulation to reduce Sulfur in shipping fuels – does it help?

Sulphur Emission Control Areas (SECA) by the International Maritime Organization (IMO)

Sulfur limit: 1 % S in fuel (0.1 % in harbor)
Baltic Sea: 0.1 % S in fuel

Suggestion: Don’t play with fuel or engines but filtrate the inhalable particles!
• Successful HICE ship diesel, wood combustion and car exhaust ALI cell and animal exposure field campaigns.

  ➔ comprehensive analysis: Aerosol properties and biological response on human lung cells on the molecular biological level

• Composition/properties of combustion aerosol emissions of the different combustion sources are extremely different

• Biological activity and acute toxicity differs largely as well. Partly unexpected behavior – aerosol components cold be adverse or protective, synergistic effects are likely

• First validation of ALI approach with animal exposure successful

• ➔ Diesel PM highly adverse – Shipping emission problematic!
  ➔ Pellet burners w/o PM precipitation questionable
  ➔ Log wood burner/HFO ships: High PAH emission – genotoxicity
  ➔ Car emissions: Strong contribution of gas phase (in part. ETOH!)
Validation of ALI-cell exposures with animal exposures (mice) and analysis of cells in BALF (Broncho-Alveolar Lavage Fluid), i.e. mainly macrophages

- **HICE-Measurement campaign Nov. 2016:**
  Joint exposure of cell lines (ALI) and animals at the ILMARI facility at University of Eastern Finland (UEF) (with Jokiniemi and Hirvonen research groups)

- 12 h exposure (3x4 h) of healthy male C57BL/6J mice with 1:15 diluted diesel exhaust: Transcriptomic analysis of BALF of macrophages in BALF → Clear effects (Cytotox, Genotox and Transcriptomics) visible

- Validation of animal results via murine macrophage (RAW) and human cell lines (THP1/A549/Beas2B) under progress

*M. Happo (UEF), S.Öder (HMGU) et al.*
New improved ALI-exposure technology (B) for lung cell exposure with mechanically generated dust (concrete dust, break-/clutch-wear etc.), C3-BMBF 2020 Project
Acknowledgements:
HICE-measurement campaigns

Car campaign (gasoline, ethanol), Rostock

Diesel, wood and ageing campaign w. animals, Kuopio

Ship campaign (diesel, heavy fuel oil), Rostock

Wood campaign (pellet, log wood, wood types), Kuopio

2015

2016

2012

2014
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