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^{1,11}, T.G.DITTMAR^{2,11}, T.KANASHOVA^{2,11}, J.BUTERS^{3,11}, S.ÖDER^{3,11}, H.PAUR^{4,11}, M.DILGER^{4,11}, C. WEIß^{4,11}, H.HARNDORF^{5,11}, B STENGEL^{5,11}, K.HILLER^{7,11}, S.C.SAPCARIU^{7,11}, K.A.BERUBE^{8,11}, A.J. WLODARCYZK^{8,11}, B.MICHALKE⁹, T.KREBS^{10,11}, M.KELBG^{5,11}, T.STREIBEL¹, E.KARG¹, J.SCHNELLE-KREIS¹, M.SKLORZ¹, J.ORASCHE^{1,11}, P.RICHTHAMMER^{1,11}, L. MÜLLER^{1,11}, J.PASSIG^{1,11}, C. RADISCHAT^{1,14}, S. SMITA^{5,11}, J. ORASCHE^{1,11}, H. LAMBERG⁶, M.-R. HIRVONEN^{6,11}, O. SIPPULA ^{6,11}, J. JOKINIEMI ^{6,11}

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Emil Nolde, 1910, Tugboat on the Elbe (Kunsthalle Hamburg)



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"A Proper Pea-Souper -The Terrible London Smog of 1952"



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London smog 1952; Arsenal soccer match (canceled due to low visibility)

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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. **o** (2000) confirmend the consclusions



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Deposition of particles in the airways of healthy humans



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Sponge-like structure of the lung

Lung surface: ca. 80 m²

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- Basal membrane thickness: ${\sim}0.1~\mu m$
- Number of alveoli: ca. 300 million

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Size of alveoli: 50 – 250 µm





E. Weibel, 2013

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E. Weibel, 2013

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PAMPs (Damage Associated Molecular Patterns) CAPS, Gout, Tissue Repair, Tumorigenesis, Type 2 Diabetes]

R.

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- 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?



1952, ~850 μg TSP/m³

Health effects of ambient aerosols

Paris 2014

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Enidemiology: Today aerosols (air London "Killer-Smog")

Beijing 2013 ~450 µg PM₁₀/m³

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

 \rightarrow 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?



Helmholtz Virtual Institute - HICE: Research on Aerosol and Health



HICE Konsortium (2012-2017):

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¹¹HICE – Helmholtz Virtual Institute of Complex Molecular Systems in Environmental Health-Aerosols and Health





- 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

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HICE concept: Interdisciplinary research to address health effects of aerosols

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Aerosol Sources







Advanced ALI lung cell culture & animal exposure



Multi-Omics, toxicol.-assays



Comprehensive phys. & chem. aerosol characterization



Biostatistics & Chemometry





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Characterization of biological effects of combustion aerosols: Exposure & Analyses

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Characterization of biological effects of combustion aerosols: Exposure & Analyses



Biological effect analysis:

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

Characterization of biological effects of combustion aerosols: Exposure & Analyses

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HICE-MOBILAB: Mobile biosafety class S2 cell culture & exposure Lab

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Ship emissions & health effects

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shipmanagementinternational.com

Ship emissions and human health: A global problem



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

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HICE ship diesel engine campaign: Operation of engine and aerosol properties

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HICE ship diesel engine campaign: Characterization of the Aerosol





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HICE ship diesel engine campaign: Characterization of the Aerosol







HICE ship diesel engine campaign: Characterization of the Aerosol





ALI PM-deposition dose ship experiments:

(estimation after Comouth et al., 2013, J Aerosol Sci): $D_{HFO} \sim 56 \text{ ng PM/cm}^2$ and $D_{DF} \sim 28 \text{ ng PM/cm}^2$

→ Note: Higher dose for the HFO PM - deposition case!

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HICE ship diesel engine campaign at U Rostock: Characterization of the Aerosol

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Organic compounds: HFO PM much more complex than DF PM





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

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- 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

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selected markers → biological pathways

Effect	HFO	DF
Inflammation	۸	-
Oxidative Stress	^	-
Cell homeostasis	^	-
Response to chemicals	↑	↓ ↓
Cellular stress response	^	^
Motility	↑	↑
Endocytosis	↑	↑
Cellular signaling	MAPK, TGF beta, PDGF, EGF, GPCR	ID, kinase cascade
Energy metabolism	-	^
Protein synthesis	-	¥
Protein degradation	-	۸
RNA metabolism	-	¥
Chromatin modifications	-	^
Cell junction and adhesion	-	₩↑ *

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multi 'omics data analysis 🗦 biological pathways



Effect	HFO	DF
Inflammation		-
Oxidative Stress	↑	-
Cell homeostasis		-
Response to chemicals	↑	₩↑
Cellular stress response	^	↑
Motility	↑	↑
Endocytosis	^	↑
Cellular signaling	MAPK, TGF beta, PDGF, EGF, GPCR	ID, kinase cascade
Energy metabolism	-	^
Protein synthesis	-	¥
Protein degradation	-	^
RNA metabolism	-	¥
Chromatin modifications	-	^
Cell junction and adhesion	-	↓ ↑*

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multi 'omics data analysis 🗲 biological pathways



regulation of anti-apoptosis

Prot.

Effect	HFO	DF
Inflammation		-
Oxidative Stress	^	-
Cell homeostasis	^ \	-
Response to chemicals	↑	₩
Cellular stress response	↑	↑
Motility	↑	↑
Endocytosis	↑	↑
Cellular signaling	MAPK, TGF beta, PDGF, EGF, GPCR	ID, kinase cascade
Energy metabolism	-	↑
Protein synthesis	-	¥
Protein degradation	-	↑
RNA metabolism	-	¥
Chromatin modifications	-	↑
Cell junction and adhesion	-	↓ ↑*

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



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

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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)







Mosser and Edwards, Nature Reviews Immunology 2008 Nature Reviews | Immunology



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



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

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LDH assay for acute cytotoxicity
→ Higher acute cytotoxicity of
DF particles for macrophages!

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



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

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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



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

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http://nl.dreamstime.com, https://pixabay.com





- 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!)

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Outlook

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 <u>cell lines (ALI) and animals</u> 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 Transcrptomics) visisble
- ➔ 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.

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Outlook

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New improved ALI-exposure technology (B) for lung cell exposure with mechanically generated dust (concrete dust, break-/clutch-wear etc.), C3-BMBF 2020 Project

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Ship campaign (diesel, heavy fuel oil), Rostock

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

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Funding:

- Uni Rostock and State of Mecklenburg Vorpommern
- Helmholtz Zentrum München
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- Deutsche Gesetzl. Unfallversich. (DGUV)
- Bundeskriminalamt (BKA)
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 (Virtual Helmholtz Institute HICE)



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ρħofôniôn: The Photo Ionisation Mass Spectromtry Company

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Multi-Component Trace Gas and Aerosol Analysers







Contact: walte@photonion.de







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