Biological effects of ship diesel exposure on human bronchial epithelial cells – effects of gas phase vs. particle phase of different fuels at the air liquid interface -

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Introduction: Combustion aerosols cause a wide range of health effects and influence the pathophysiology of many diseases. The Virtual Helmholtz Institute HICE addresses the health effects of anthropogenic combustion processes. This is performed by means comprehensive characterization of the chemical and physical properties of combustion aerosols as well as the biological effects on cell cultures. To get a comprehensive picture of how these health effects are influenced, the entity of metabolic pathways has to be analyzed. Metabolism strongly depends on metabolic active enzymes and other proteins. The availability of these proteins is determined by the expression of the respective gene. Therefore gene expression represents one first step in regulation of metabolism. As gene expression is highly influancable by environmental agents, we studied within HICE the transcriptome (entity of gene transcripts) of bronchial epithelial cells that were directly exposed to combustion aerosols at the air-liquid interface.

Methods: Within the ship diesel sampling campaign in Rostock, Germany, human immortilized bronchial epithelial cells (BEAS-2B) where exposed to the complete combustion aerosol (including particles) and to the gas phase of the combustion aerosol alone (filtered aerosol). The aerosol was generated in a ship engine being operated with light and heavy fuel oil (LFO and HFO). Cells were exposed in the "HICE exposure system" at the air-liquid interface for 4 hours to clean air diluted exhaust (1:40 for LFO and 1:100 for HFO). Subsequently cells were lysed and frozen. RNA extraction was conducted using Rneasy columns (Qiagen). RNA quantity was measured by UV/VIS spectrophotometry (Nanodrop, Qiagen) and RNA integrity was assessed after electrophoretic separation (Bioanalyzer, Agilent). Whole transcriptome analysis was performed on Sure Print G3 human gene expression microarrays using single color Cy3 labeling (Agilent). Statistics on transcriptome data and pathway analysis were done using GeneSpring software (Agilent).

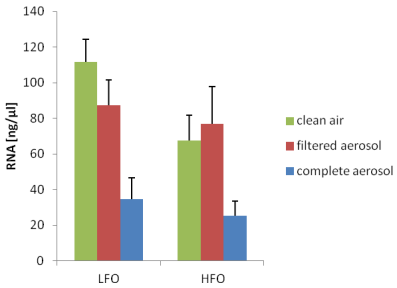

Figure 1. RNA yield after ship diesel exposure

Results: Exposure to the combustion aerosol caused clear decrease in total RNA, indicating cellular toxicity of LFO. Though higher diluted, HFO caused the same toxic effect compared to LFO. Especially changed were genes that code for proteins that are necessary for translation of RNA into proteins (cytoplasmic ribosomal proteins), for proteins of the energy metabolism (electron transport chain and oxidative phosphorylation), for cellular signal transduction (G-protein coupled receptors), for phase I and phase II enzymes of xenobiotic metabolism (cytochrome P450, phase-1-functionalization of compounds, metapathway biotransformation), for proteins of immunomodulatory interactions and for blood clotting. For most of the genes regulation was much stronger after exposure to the complete combustion aerosol than to the gas phase of the aerosol alone.

Conclusion: Ship diesel combustion aerosol increased the general activity status of the bronchial epithelium (translational activity, energy metabolism) and induced specific cellular signaling. The induction of xenobiotic metabolism indicates the presence of organic compounds and increases the risk of an activation of these substances into toxic metabolites. Further health hazard stems from the induction of blood clotting. These effects seem to be caused by the particles or by substances that are attached to the combustion particle cores, because the gas phase alone had much weaker effects.

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