Molecular biological effects and toxicity of combustion aerosol emissions on air/liquid-interface exposed human and murine lung cells

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It is known that combustion aerosol emissions are important for public health. In the framework of the Virtual Helmholtz Institute-HICE, the physical and chemical properties of various combustion emissions as well as their biological effects on lung cells were thoroughly investigated and jointly analysed. For addressing the biological activity and the toxicity of the different combustion aerosols, human and murine lung cell cultures were exposed to diluted combustion exhaust fumes by the modern air-liquid interface (ALI)-exposure technology. The ALI-approach allows a very realistic lung cell exposure by simulation the situation in the lung. After 4h exposure the biological response of the exposed lung cells are analysed by a comprehensive multi-omics molecular biological effect-characterisation on the transcriptomic, proteomic and metabolomic level. Up to now emissions of wood combustion, ship engines, small diesel engines and car gasoline engines were investigated. For wood combustion experiments emissions from a masonry heater and a metal stove (beech, birch, spruce and pine log wood) as well a state of the art pellet burner (soft wood pellets) were used. Shipping emissions were generated on a test-bed ship diesel engine, running either on common heavy fuel oil (HFO) or distilled diesel fuel (DF). A small diesel engine for land based mobile application was included as well. Finally a modern car engine operated with gasoline (E10) and ethanol (E85) was investigated. Two special field deployable ALI-exposure-station systems and a mobile S2-biological laboratory were set up and applied for this study. The ALI-exposure station is now commercially available (Vitrocell GmbH). Human alveolar basal epithelial cells (A549, BEAS2B and primary cells) as well as murine macrophages (RAW) were ALI-exposed to freshly diluted combustion aerosols. The cellular effects were then comprehensively characterized (viability, cytotoxicology, multi-omics molecular-biological effects monitoring) and put in context with the chemical and physical aerosol data. The HICE concept is summarized in the literature [1]. The overall cellular response of the combustion aerosols (i.e. the regulation strength on the different 'omics-levels) was compared at a similar aerosol dilution (in most cases 1:40). The dilution/dose of 1:40 (1:100 for HFO) was selected to be below a measurable direct cytotoxicity after the 4h ALI exposure (LDH release- or viability-assay). Figure 1 puts the overall cellular response, measured by the transcriptomic regulation strength (i.e. the concentration changes of m-RNA copies in the cells) of exposed cell with respect to reference experiments in context to the deposited combustion aerosol PM mass.

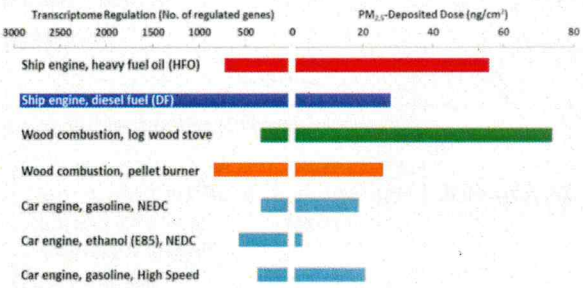

Figure 1: Particle deposition dose (right, measured as deposited PM-mass per surface unit) and overall biological effects strengths (left, measured as the total number of m-RNA transcripts with a fold change larger than $log_2(1.5)$) in human lung cells for different ALI-cell exposure experiments with 1:40 diluted combustion aerosols (1:100 for HFO).

The figure depicts that the overall biological response-strength differed considerably for different aerosol sources and is not well correlated with the deposited PM mass. This is pointing to large differences in the relative toxicity of the aerosol emissions from different combustion sources and fuel types. The latter finding is supported by the detailed analyses of the activated cellular response pathways (GO-term analysis), depicting regulation of pathways such as pro-inflammatory signalling, xenobiotic metabolism, phagocytosis or oxidative stress. The obtained holistic molecular biological results demonstrate the complexity of PM-induced biological effects. The results obtained with the cell cultures have been compared to simultaneously performed animal exposure experiments (BL6 mice) on e.g. the omics-level. Furthermore the direct combustion emission exposure experiments have been accompanied also by simulated atmospheric aging experiments (UV-aging in flow tube)

[1] Oeder et al., PLoS one, 2015, DOI: 10.1371/journal.pone.0126536