Generation and characterization of ultrafine soot particles with similar physical but varying chemical properties enabling differential toxicological assessment in human lung cells

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Scientific Context: The contributions of ultrafine particles (UFPs, $D_p \leq 100$ nm) to adverse human health effects are debated [1]. Studies analyzing the composition and biological effects of UFPs from various emission sources remain limited and it is currently not fully understood which physical or chemical characteristics are responsible for their specific response [2]. Whether the physical characteristics contribute most or the particles primarily act as carriers of biologically reactive chemicals, rendering the biological responses is vastly unknown [3]. An additional challenge is that biological response is often influenced by several particle properties at the same time [4]. Thus, the fundamental investigation of such cause-and-effect mechanisms requires sophisticated studies in which the physico-chemical parameters of UFP could be adjusted in a targeted and reproducible manner. Hence, laboratory conditions are a strict requirement to assess these processes and their importance for adverse health effects and thus the production of reliable and reproducible data on UFPs is pertinent [4].

Approach: Generation and characterization of ultrafine soot particles of similar elemental carbon core with changing chemistry i.e., high ($UFP_{high\ OC}$) and low organic content ($UFP_{low\ OC}$) to investigate different biological responses in human alveolar epithelial A549 cells at the Air-Liquid-Interface (ALI). **Methods:** The miniature combustion aerosol standard soot generator (MiniCAST) was used to generate ultrafine soot particles. A catalytic stripper (CS) along with honeycomb activated carbon denuders were used for removing organic compounds depending on their volatility. Two classes of UFP soot, depending on the applied temperature in the CS (residence time 0.35 s) were generated and further characterized. Physical characterization was done concerning particle number concentration, particle mass concentration, mobility diameter and aerodynamic diameter. While the chemical characterization of the derived particles was done in terms of organic (OC)/ elemental carbon (EC) ratio, black carbon (BC) and quantification of polycyclic aromatic hydrocarbons (PAHs). Subsequent exposure of A549 cells at ALI was carried out and the effects of ultrafine soot on metabolic activity, cytotoxicity (LDH release) and xenobiotic metabolism (EROD/ BROD enzyme activity) were assessed using different biological assays. **Results:** The applied approach allowed us to generate two classes of UFPs, which showed similar physical characteristics but distinctly different chemical loading. The detailed chemical and physical analysis indicated significant different OC content for the two classes. For instance, in case of $UFP_{high\ OC}$, EC= 56.2 µgC /m^3; OC= 19.4 µgC /m^3, whereas, for the $UFP_{low\ OC}$, EC= 49.2 µgC /m^3; OC= 5.3 µgC /m^3. However, the differences in particle mass, particle number and size distribution were not significant, for example, mobility diameter for $UFP_{high\ OC}$ was about 44.3 nm and $UFP_{low\ OC}$ was about 35.5 nm). Our results indicate that an increase of the OC content does not change the response on metabolic activity ($UFP_{low\ OC} \approx 56\%$, $UFP_{high\ OC} \approx 59\%$) and cytotoxicity ($UFP_{low\ OC/high\ OC} \approx 27\%$). However, an increase in xenobiotic metabolism seemed to correlate with the PAHs loading of $UFP_{high\ OC}$. The shown approach allowed us to adjust specific physicochemical characteristics of UFP and link them to specific biological responses.

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