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### Relationship between *in vivo* and *in vitro* toxicity of six types of carbonaceous nanoparticles

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It is currently believed that the toxicity of inhaled combustion-derived nanoparticles (CDNP) is related to both the surface area of the carbonaceous core of the CDNPs and the amount and type of organic compounds condensed onto the cores. The objective of the present study is to assess whether the inflammatory response of mice (*in vivo* toxicity) to CDNPs can be predicted by the combined information from a cell-free *in vitro* test for oxidative potency and an *in vivo* gene expression analysis targeting inflammation, stress and detoxification related genes.

Six types of carbonaceous particles were either purchased (Diesel SRM-1650a (DEP), PrintexG and Printex90) or generated in our laboratory. The latter comprised ultrafine carbon particles (UfCP) generated by spark-discharge, as well as soot particles with high and low organic content (SootH, SootL) produced by a well-controlled propane diffusion flame (CAST burner). These particle types had widely varying (primary) particle diameter (10-50nm), organic content (OC; 1-20%) and specific BET surface area (43-800m<sup>2</sup>/g). The *in vivo* toxicity was based on the response of BALB/cJ mice (21.1±1g) to particle exposure was based on the influx of polymorphonuclear neutrophils (PMNs) into the lungs 24h after intratracheal instillation of these particles (Stoeger et al., 2006). Using this data we defined the inflammatory efficacy ( $I_{Ef}$  [%PMN/ $\mu$ g]) as the 20% PMN effect level divided by the particle mass causing this effect level. The particles' innate oxidative potency (OxPot) was determined by a cell-free *in vitro* assay the consumption (in nmol) of an antioxidant standard (ascorbate).

We found that OxPot showed a strong linear correlation ( $R^2=0.77$ ) with the *in vivo* inflammatory response ( $I_{Ef}$ ) (Figure 1). The most obvious outlier was high-organics flame soot (SootH, OC=19%), for which the *in vitro* test clearly underestimated the *in vivo* toxicity. Since this was not observed at the same level for DEP, the other high-OC sample (OC=20%), OC alone could not account for this discrepancy. Gene expression analysis of 11 selected detoxification enzymes revealed that the only gene, which was specifically upregulated by SootH (3.9 fold) and DEP (1.6 fold), was the xenobiotic-metabolizing enzyme Cyp1a1. Cyp1a1 is well known to be highly inducible by bioavailable organic compounds, like aromatic hydrocarbons which. Thus the induction of Cyp1a1 by SootH, and to a lesser extent by DEP, indicated that the bioavailability of OC plays an important role for their toxicity. If we include the Cyp1a1 gene expression as independent parameter into a linear model, 94% of the observed variability in  $I_{Ef}$  can be explained by OxPot and Cyp1a1, while OxPot alone only accounts for 77% of the variability.

Thus our data suggests that while organic coating might mitigate *in vivo* inflammatory response by possibly shielding the oxidative potency of the carbon core of CDNPs, CYP1A1 enzyme mediated biotransformation of organics may generate oxidative stress and thus enhance the *in vivo* inflammatory response.

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