

Title:
Instillations of Different Carbonaceous Nanoparticles Indicate a Surface Area Threshold

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Numerous epidemiological studies have demonstrated an association between elevated levels of ambient particles and morbidity and mortality. Ambient fine-mode PM (<2.5 μm particle diameter, $\text{PM}_{2.5}$) mainly consists of anthropogenic, carbonaceous particles derived from combustion processes. In urban air, fine and ultrafine particles (<100 nm) are most numerous among all particles and represent the highest surface area per mass. This surface can carry large amounts of adsorbed or condensed toxic air pollutants like organic compounds, and transition metals (Oberdorster 2001). Although the mass of ultrafine particles is at ambient background levels below $2 \mu\text{g}/\text{m}^3$, it can increase several fold at locations with high volume of traffic, or during high pollution episodes, where number concentrations higher than 100,000 particles/ cm^3 have been measured (Brand et al. 1992; Oberdorster et al. 2000). An additional source of man made sub-100-nm carbonaceous particles might be seen in the progressive technology of nanosized materials. Despite several differences between engineered nanoparticles and combustion derived ultrafine particles, the same toxicologic principles are likely to apply for both types of nanosized particles, for that reason, we adopt to the proposal of Günther Oberdorster and use for the following the term “nanosized particles” (Oberdorster et al. 2005).

We investigated acute adverse effects of six types of carbonaceous nanosized particles by intratracheal instillation in healthy mice. The following particles were studied: two commercially available pigments, PrintexG and Printex90; two laboratory-made flame soot particles with different organic content, SootL (low) and SootH (high); one spark-generated soot particle, ufCP; and the standardized reference material diesel exhaust (DEP) SRM1650a. These particles cover a size range of 10 to 50 nm. The specific surface area varies between 30 and 800 m^2/g and the organic content between 1 and 20%. For each particle species 3 doses (5, 20 and 50 μg per mouse) have been instilled. To characterize the acute inflammatory events in the lung as caused by the particles, mice were killed 24h after instillation, and bronchoalveolar lavage (BAL) was performed immediately post-mortem. The number of polymorphonuclear leukocytes (PMNs) and the concentration of inflammatory mediators (Interleukin-1beta and the acute phase protein lipocalin-2), in BAL-fluid was used as inflammatory marker. BAL cell differential and cytokine concentration revealed a clear dose-response over the 3 doses. However, even that all six particles were supposed to be similar by their carbonaceous nature, the respective effect levels at a given mass differed quite a lot. Such as for example the diesel and PrintexG particles showed at the highest dose (50 μg) the same inflammatory effect level as ufCP particles at the lowest (5 μg) dose.

To get insight in particle-related attributes, which might be responsible for the different levels of inflammatory responses, primary particle size, organic content, and particle surface area at a given dose were related to the endpoints of lung inflammation. Relating the **primary size** of the particles to the caused inflammatory effect levels, suggested the smaller particles to be more potent than the larger particles. In addition, correlation between **organic content** of the particles and PMN influx ($r = 0.6$), or levels of the inflammatory mediator was found. However, diesel particles, containing the highest fraction of organics, tended to be a less potent effector of inflammation than particles with least organic content, like Printex90. In contrast, significant correlation ($r = 0.9$) became evident when the

inflammation response was related to particle **specific surface area** for all six types of instilled particles.

To all appearances at a closer look at the surface area dose-response curves, within the surface area of 5 to 40 cm² a dose response threshold exists; below of which no significant inflammatory reaction was detected. To confirm this threshold phenomenon, we instilled two additional doses of our most potent particle specimen, the ufCPs at quantities below 5 µg. In concordance to comparatively low surface burdens of the other particles, a BET-surface doses of 4 cm² and 16 cm², representing ufCP particle quantities of 0.5 and 2 µg, did not induce a significant PMN influx or alter basal cytokine concentrations in BALF within 24h. Therefore we suggest for mice, for the acute inflammatory effects caused by instillation of carbonaceous, nanosized particles, a surface area threshold of about 20 cm². Considering a biological size factor of about 1,500 from mouse to man this results in a surface area threshold of about 30,000 cm² for man. It would certainly be most interesting to relate this surface threshold level to particle surface areas encountered at sites of high air pollution, like busy urban areas with particle concentrations of up to 10 µg/m³. Unfortunately, BET surface area of ambient nanoparticles has not been measured so far because of the high quantity required for accurate measurements. A very rough approximation suggests, that lungs burdens of urban residents may exceed 150 cm²/day. Besides the notice, that extrapolation of effects, derived from a single experimental dose administration of model particles, to relevant lower environmental concentrations is very difficult, this calculation suggests a factor of 200 between the surface area threshold and the daily lung burden.

The here observed threshold level corresponds to effects of the acute pulmonary inflammation, others like cardiovascular effects could be more subtle and might thus have much lower thresholds. As a result, particle related cardiovascular effects have been observed in studies where no indication for pulmonary or systemic inflammation could be found (Frampton et al. 2004).

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