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Surface area is the biologically most effective dose metric for acute nanoparticle toxicity in the lung

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ABSTRACT

In this study we provide guidance on the biologically most relevant dose metric for pulmonary toxicity of biopersistent, spherical nanoparticles (NPs). A retrospective analysis of nine *in vivo* studies on particle-induced, acute pulmonary toxicity in animal models (mouse, rat) was performed encompassing five different types of nanomaterials (polystyrene, titanium dioxide, carbonaceous materials, transition metal oxides (Co, Ni, Zn) and hydrothermally synthesized α-quartz) with a wide range of primary particle diameters (9–535 nm) and mass-specific BET surface areas (6–800 m^2/g). The acute influx of polymorphonuclear neutrophils (PMNs) into the lungs after intratracheal instillation of NPs was chosen as a toxicological endpoint for acute lung inflammation. The allometrically scaled toxicological data were investigated with respect to various dose metrics, namely (primary) particle number, joint length, BET and geometric surface area, volume and mass.

Surface area is identified as the biologically most relevant dose metric for spherical NPs explaining about 80% of the observed variability in acute pulmonary toxicity ($R^2=0.77$). None of the other dose metrics explains more than 50% of the observed variability in pulmonary inflammation. Moreover, using surface area as the dose metric allows identification of material-based toxicity classes independent of particle size. Typical materials without intrinsic toxicity – here referred to as low-solubility, low-toxicity (LSLT) materials – show low surface-specific toxicity with an EC_{50} dose of 175 m^2/g-lung (geometric mean; $\sigma_g=2.2$), where EC_{50} represents the dose inducing 50% of the maximum effect (here 30% PMN). In contrast, transition metal oxides (here Co, Ni, Zn) – materials known for their intrinsic toxicity – display a 12-fold enhanced surface-specific toxicity compared to LSLT particles ($EC_{50}=15$ m^2/g-lung).

This analysis implies that surface-related modes of action are driving acute pulmonary toxicity for the types of NPs investigated here. The relevance of other dose metrics such as number and volume is acknowledged in the context of different modes of action, namely shape-induced toxicity (fiber paradigm) and extremely high particle lung burden (overload conditions), respectively. So which dose metric should be monitored by aerosol scientists involved in health related aerosol exposure measurements? The short answer is – all of them (except length), but there is a strong preference towards surface area.

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1. Introduction

Epidemiological studies have described correlations between the mass concentration of ambient particulate matter (PM) and increased morbidity and mortality in adults and children (Salvi, 2007; Stone, Johnston, & Clift, 2007). The tremendous economic success of consumer products containing nanomaterials (e.g. sun screen, tooth paste, tires) has raised concerns regarding consumer exposure to inhaled nanostructured materials. However, very little epidemiological data are available for engineered nanomaterials. The few studies performed at industrial sites of nanoparticle (NP) production (mainly carbon black) did not show conclusive evidence of particle-induced health effects (Boffetta et al., 2004). However, most of these studies suffer from a lack of accurate exposure data or even personalized lung-deposited dose information (Peters, Ruckerl, & Cyrys, 2011).

Improved exposure studies are under way, but it is still unclear which of the possible dose metrics is best suited for predicting the adverse health outcome of NP exposure. While most (ambient) epidemiological studies were based on mass as the dose metric, there are also a few short-term epidemiological studies indicating that early, transient exacerbations are associated with the number concentration of ultrafine ambient particles rather than the mass of ambient PM (Peters et al., 2011). In toxicology, mass has been used as the dose metric, since mass is the biologically effective dose metric for soluble toxins. However, for non-soluble (or poorly-soluble) soot or engineered NPs only the molecules detached from the surface or located at the surface of the NPs are interacting with the biological fluids and tissue. Hence, surface area is likely to be a biologically more relevant dose metric for biopersistent nano-/microparticles than mass. This was confirmed by numerous cell-based in vitro and animal-based in vivo toxicity studies (Oberdörster, Oberdörster, & Oberdörster, 2005; Stoeger, Schmid, Takenaka, & Schulz, 2007; Stoeger et al., 2009; Waters et al., 2009). However, some toxicological studies have also suggested particle number or volume as the most relevant dose metric for NPs (Donaldson et al., 2013; Pauluhn, 2011).

State-of-the art aerosol technologies for real-time measurement of all moments of the size distribution (number, length, surface area, volume/mass) of compact aerosols are available (Kulkarni, Baron, & Willeke, 2011). Moreover, the number, joint length (Wittmaack, 2002), surface area and volume/mass of loosely agglomerated NPs ("soot-like" agglomerates/aggregates) can be determined in real-time by multi-instrument approaches (Wang et al., 2010). For non-hygroscopic, compact NPs (<300 nm) the lung-deposited surface area concentration can be monitored directly in real-time by electrometer based measurement techniques (Fissan, Neumann, Trampe, Pui, & Shin, 2007). However, for optimized design of future aerosol exposure measurements related to workplace safety or other health-related issues, it is important to identify the biologically most effective dose metric.

In this retrospective analysis of selected animal studies on acute pulmonary toxicity of intratracheally instilled NPs we provide guidance on the biologically most relevant dose metric for acute lung inflammation. We investigated the correlation between acute pulmonary inflammation as evidenced by the influx of inflammatory cells into the lung and pulmonary (primary particle) number, joint length, (BET and geometric) surface area, volume and mass as dose metrics. Published data were compiled from nine different studies conducted by various laboratories using different animal models (mouse, rat). The data cover some of the most commonly used types of NPs (polystyrene, titanium dioxide, various carbonaceous materials including aged diesel soot, quartz, and transition metal oxides) covering a wide range of primary particle diameters (9–535 nm) and mass-specific BET surface areas (6–800 m^2/g). Retrospective analysis of this unified data set provides insight into the relevance of the various dose metrics for predicting NP-induced pulmonary toxicity.

2. Materials and methods

The study presented here is based on animal studies on acute pulmonary toxicity due to intratracheal instillation of NPs into the lung. More than 60 animal studies on acute pulmonary toxicity published between 2001 and 2009 were screened and nine of them were selected for matching all of the following criteria:

(1) Use of mouse or rat as animal models
(2) Particles were applied to the lungs via intratracheal instillation
(3) Data on the influx of polymorphonuclear cells (PMNs) into the lung as hallmark of inflammation are available for at least one (acute) time point (between 16 h and 24 h after particle application). The number of PMNs and the total number of cells on the lung epithelium was determined via differential cell counting of the bronchoalveolar lavage (BAL) fluid.
(4) Non- or poorly-soluble, smooth, nano-sized (<535 nm) primary particles of spherical shape were used. The state of agglomeration in the applied NP suspension is typically not reported and therefore not considered as criterion here.
(5) Lung-deposited (instilled) mass dose, mass-specific BET surface area and material density are reported (or available).
(6) Accurate information on the primary particle diameter is provided for conversion of the pulmonary mass dose into other dose metrics, namely number, (joint) length, geometric surface area.

As mouse and rat are the most frequently used animal models for particle toxicity studies, we included both rat and mouse data here. NPs were delivered to the lungs via intratracheal instillation, i.e. the anesthetized animals were intratracheally intubated and a defined volume of a NP suspension was squirted directly into the lungs with a syringe via the trachea. While this route of application is physiologically not realistic (not an aerosol, but a liquid bolus of a NP suspension is

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