Toxicology 279 (2011) 176–188

Contents lists available at ScienceDirect

Toxicology

journal homepage: www.elsevier.com/locate/toxicol

Poorly soluble particulates: Searching for a unifying denominator of nanoparticles and fine particles for DNEL estimation

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ARTICLE INFO

Article history:
Received 2 September 2010
Received in revised form
29 September 2010
Accepted 22 October 2010
Available online 11 November 2010

Keywords:
Volumetric particle overload
Inhalation toxicology
Biopersistent granular particles
Lung clearance
NEL
DNEL
OEL

ABSTRACT

Under the new European chemicals regulation, REACH (Registration, Evaluation, Authorization and Restriction of Chemicals) a Derived No-Effect Level (DNEL), i.e., the level of exposure above which humans should not be exposed, is defined. The focus of this paper is to develop a weight-of-evidence-based DNEL-approach for inhaled poorly soluble particles. Despite the common mode of action of inhaled insoluble, spherical particulate matter (PM), a unifying, most appropriate metric conferring pulmonary biopersistence and toxicity has yet not been demonstrated. Nonetheless, there is compelling evidence from repeated rat inhalation exposure studies suggesting that the particle displacement volume is the most prominent unifying denominator linking the pulmonary retained dose with toxicity. Procedures were developed to analyze and model the pulmonary toxicokinetics from short-term to long-term exposure. Six different types of poorly soluble nano- to submicron PMs were compared: ultrafine and pigmentary TiO_2, synthetic iron oxide (Fe_3O_4, magnetite), two aluminum oxyhydroxides (AlOOH, Boehmite) with primary isometric particles approximately of either 10 or 40 nm, and MWCNT. The specific agglomerate densities of these materials ranged from 0.1 g/cm^3 (MWCNT) to 5 g/cm^3 (Fe_3O_4). Along with all PM, due to their long retention half-times and associated biopersistence in the lung, even short-term inhalation studies may require postexposure periods of at least 3 months to reveal PM-specific dispositional and toxicological characteristics. This analysis provides strong evidence that pulmonary toxicity (sustained inflammation) is dependent on the volume-based cumulative lung exposure dose. Lung toxicity, evidenced by PMN in BAL occurred at lung doses exceeding 10-times the overload threshold. Furthermore, the conclusion is supported that repeated inhalation studies on rats should utilize an experimental window of cumulative volume loads of respirable PM in the range of 1 μl/lung (no-adverse-effect range); however, not exceeding ≈10 μl/lung that would lead to retention half-times increasing 1 year. This can be targeted best by computational toxicology, i.e., the modeling of particle deposition and lung retention biokinetics during the exposure and recovery periods. Inhalation studies exceeding that threshold volume may lead to meaningless findings difficult to extrapolate to any real-life scenario. In summary, this analysis supports a volume-based generic mass concentration of 0.5 μl $PM_{respirable}/m^3$ × agglomerate density, independent on nano- or submicron-sized properties, as a generic no-adverse effect level in both rats and humans.

1. Introduction

Results from numerous short-term inhalation/aspiration/instillation studies with various types of carbon nanotubes (CNT) have been published (for reviews see Donaldson et al., 2006; Madl and Pinkerton, 2009; Maynard, 2007; Oberdörster et al., 2005, 2007). The degree and kind of aggregation of CNT structures is determined by the rigidity and pliancy of nanotubes and whether their diameters are thin enough to allow their buckling and self-aggregation into low-density, particle-like, intertwined, and often coiled assemblages (Pauluhn, 2009a). Given the differences in the physical shape of agglomerate structures, a categorization into rigid and flexible CNT appears to be among the most straightforward discriminative variable. In addition, the type of assemblage structure and whether it is stabilized by mere agglomeration or some kind of inter-tubular aggregation (physical entanglement) needs to be appreciated. Hence, depending on these characteristics, agglomerate structures of nanotubes may differ appreciably from thin-walled to thick-walled, rigid MWCNT. These properties may be decisive for hazard assessment as the critical toxic principle may either emerge from the individual tube structure (e.g., fiber) or the collective behavior of inhalable assemblages of nanotubes.

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doi:10.1016/j.tox.2010.10.009

Unlike conventional poorly soluble crystalline particle structures, MWCNT are present as submicronsized agglomerated arrangements of closely packed CNT which increase the void-space volume creating a novel type of composite low-density PM-structures. Consequently, when phagocytized by alveolar macrophages, much less particle mass is needed to exceed the volumetric overload limit for the diminution of macrophage-mediated clearance (Morrow, 1988, 1992, 1994). Based on Morrow's hypothesis of the volumetric overload of alveolar macrophages, the particle displacement volume rather than surface area appears to be the most critical metric for these types of materials. Hence, agglomerated nanoparticles present in submicronsized form may cause a volumetric overload of alveolar macrophages at lower exposure doses as compared to their micron-sized crystalline counterparts. Yet, no single particle characteristic as a hallmark indicator directing fate and pulmonary toxicity has been identified (Madl and Pinkerton, 2009). However, emerging views suggest that the assemblage displacement volume of MWCNT, which is critical to the impairment of alveolar macrophage-mediated clearance and elicitation of pulmonary inflammation, may dictate the fate and pulmonary response to this type of structures. Considerable efforts have been expended in measuring and modeling pulmonary deposition of inhaled particles in rodents and other species, and several comprehensive reviews have been published (Miller, 2000; Brown et al., 2005; Oberdörster et al., 1992; Oberörster, 2002). This retrospective comparison focuses on a PM-volume-based metric as the most apt denominator to compare PMs of different size, effective density, and structure. The focus of previous approaches was limited to submicronsized, high-density particles (Pauluhn, 2009b) whereas this analysis utilized additional data from recent repeated exposure inhalation studies on rats with nanostructured, low-density materials.

In the context of pulmonary toxicity of poorly soluble particles surface area is often considered to be the leading metric. However, it is hard to believe that the gauge commonly used to determine surface area (N_2) (Klobes et al., 2006) is at any rate reflective of the competitive adsorption of the numerous peptides and proteins present in the lining fluids of the lung. In other words, the biologically effective surface areas are dependent on the gauge size of the most avidly binding endogenous polypeptide, including its competitive displacement. Likewise, especially for particle structures in the submicron to nanometer range, the aggregation properties of assemblages of PM may be changed by preparation and collection. For such complex and irregular shape three-dimensional aggregated objects it becomes increasingly difficult to assign one single physical qualifier for an unequivocal characterization. The phenomena occurring during the contact between nanoparticles and cellular media or biological fluids (dispersion, agglomeration/aggregation, protein adsorption) in relation to the surface properties of the nanoparticles considered are discussed elsewhere in detail (Fubini et al., 2010). From that perspective, the three-dimensional characteristics 'volume' appears to be a better qualifier than PM number or surface area. In this context and following the hypothesis of AM overload, the most directly accessible and mechanism-based variable is the displacement volume of PM within the available pool of phagocyte or AM (volume of distribution, V_d). Therefore, the following risk analysis is solely focused on this variable. Accordingly, surface area has not been considered in this paper as a previous analysis with higher-density particles of different surface areas has demonstrated that the PM mass concentrations and pulmonary inflammation correlated better than surface area concentrations (Pauluhn, 2009b).

The objective of this paper is to analyze whether the somewhat unique pulmonary inflammatory potency of MWCNT assemblages share some unifying characteristics with their higher-density granular biopersistent counterparts when using a volume metric. Based on this rationale, the composite volume of aggregates appears to be the most critical variable of dose. Hence, it is timely to analyze as to which extent current testing paradigms need to be modified to the advancement in toxicological knowledge to appropriately identify and rank the hazards of PMs. Computational toxicology (modeling) has been utilized to better design and predict the outcome of repeated rat inhalation studies. This latter aspect serves the additional objective to improve the design and dose-rationalization of inhalation bioassays across different laboratories. Especially for lung toxicity, the Guidance given in the new REACH regulation (ECHA, 2008a,b,c,d) is lacking prescriptive instructions to arrive at scientifically reasonable assessment factors and NELs (no-effect levels) for the derivation of Occupational Exposure Levels (OELs) for these types of substances. This paper attempts to rationalize a mode-of-action-based, scientific approach.

2. Methods

2.1. Study design and experimental variables

It is important to keep in mind that the model system or testing regimen themselves may influence measured responses irrespective of the particulate material investigated. At present, most of the studies are concerned with pulmonary pathology patterns following single to short-term high-dose bolus dosing. These types of studies have ample room for experimental artifacts which include localized pathology due to particle clumping and irregular particle distribution as a result of dosing of poorly characterized materials and inhomogeneous particle suspensions. Most studies addressing the translocation of particles to extrapulmonary organs do not attempt to critically associate inflammation-related barrier-disruption with increased particle translocation. Compelling experimental evidence supports the view that the study of PM-translocation requires extended postexposure periods and should also include the time-dependent PM-translocation to the draining lung (hilus) lymph-nodes as reference (Pauluhn, 2009c, 2010a,b; Bermudez et al., 2002, 2004). Particle retention and biokinetics, including their dose-dependence, did not receive a great deal of attention yet. Commonly, the procedures used to generate and characterize atmospheres in inhalation studies provide a much higher dosing accuracy and reproducibility than attained by the alternative instillation methods. Therefore, issues related to dosimetry and kinetics can most appropriately be addressed by inhalation protocols.

Published evidence demonstrates that ultrafine TiO_2 particles caused more inflammation in rat lungs than exposure to fine TiO_2 (Ferin et al., 1992; Bermudez et al., 2002, 2004). These differences in toxic potencies seem to be a result of their unique size, surface area/activity and/or crystal properties (Warheit et al., 2005, 2007; Warheit, 2008). In rats, pulmonary inflammatory responses increase precipitously under conditions of apparent decompensated lung overload, a particle-induced depression of clearance as a consequence of an exceedingly high volumetric overload of the alveolar macrophages and associated loss of alveolar macrophage mobility (Morrow, 1988, 1992; Stöber and McClellan, 1997). Overload has been loosely defined as the alveolar burden causing a two- to four-times reduction in alveolar clearance of rats relative to normal clearance rates (ILSI, 2000). Accordingly, it is timely to analyze existing data from repeated exposure inhalation studies in rats to consider as to whether more robust criteria and thresholds for lung overload can be identified and whether the underlying toxic principles apply to humans as well.

This retrospective analysis analyzed and compared different types of PMs examined in 4- and especially 13-week repeated exposure rat inhalation bioassays (for details see Table 1). Studies conducted in compliance with OECD-413 and OECD-GD#39 (OECD, 2009) were given preference. The PM primary particle size ranged from nano- to submicronsized covering an effective density of PM from 0.1 to 5 g/cm^3. The lowest density was represented by multi-walled carbon nanotubes (Baytubes®). Pigment grade magnetite (Fe_3O_4) was the particle with the highest density. Aluminum oxyhydroxide (AlOOH) consisted of agglomerated arrangements of closely packed submicron aggregates consisting of nanoparticles of 10–40 nm. These structures are technically designed to disintegrate into their nanostructures in molten plastic. The principal range of structures addressed in this analysis is illustrated in Fig. 1.

2.2. Exposure regimen and duration of exposure and postexposure periods

The extremes of exposure regimens commonly involved in inhalation toxicity testing are illustrated in Figs. 2 and 3. These representations depict the often experienced experimental challenges involved with repeated exposure inhalation testing: (1) the 'loading phase' may be too short and, due to the surface activity of PMs, surfactant adsorption onto accessible particle surfaces may result in instant, site of initial deposition-dependent surfactant dysfunction and acute alveolitis. In addition to the acute, deposition-related changes, short-term high-dose regimens require thoughtful considerations on dosimetry to arrive at meaningful multiples of