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Fate of inhaled Nano-CeO2 revisited: Predicting the unpredictable

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ABSTRACT

This paper compares the pulmonary kinetics of inhaled nano-CeO_2 from two published repeated inhalation studies of 13-week duration in rats. This database was used to predict the outcome of a 2-year chronic inhalation study with a focus on the no observed adverse effect level (NOAEL) and range of conditions causing kinetic lung overload up to and beyond the maximum tolerated dose (MTD). Modeling identified nano-CeO_2 to be typical poorly soluble, low-toxicity particles (PSLTs), although even partial dissolution may lead to interactions with pulmonary surfactant, eventually resulting in pulmonary phospholipidosis and fibrosis. An earlier model published in 2011 to surpass and replace the traditional Morrow approach focused on kinetic lung overload to simulate the pulmonary fate of inhaled micron-sized PSLT in rats. By misunderstanding or inaction, this earlier model was overlooked as a better hypothesis-based model for dosimetry selection of long-term inhalation studies with the aim of reducing study repetition and animal numbers. While it appears that the primary adverse pathway of the earlier model also applies to nano-CeO_2, the updated model proposed here also accounts for phospholipid-like additional volume loads. Data from a heralded 2-year inhalation study in rats are not yet available, but the study was traditionally modeled to predict the toxicological NOAEL and MTD hallmarks. When completed, this study's data will clarify whether the advanced 21st century modeling proposed here may be more advantageous for design and execution of inhalation studies, compared to simplistic and outdated gross overload models.

1. Introduction

High-quality, short-to long-term repeated-exposure nano-CeO_2 inhalation toxicity studies in rats have been published recently (Keller et al., 2014, Keller, 2015; Schwotzer et al., 2017). These studies provided evidence relating inhaled nano-CeO_2 and associated adverse outcomes. This database was used to predict the no observed adverse effect level (NOAEL) and frank overloading conditions far beyond the maximum tolerated dose (MTD). Details of this model and its applicability for dose selection and prediction of the kinetic hallmarks concerning poorly soluble, low-toxicity particles (PSLTs) were published elsewhere (Pauluhn, 2009, 2010a,b; 2011; 2014a,b,c, 2017).

It seems as though the difference between nano- and bulk-structured CeO_2 is related to their difference in solubility (g per 100 g H_2O): CeO_2-bulk: 0.0007, CeO_2-nano: 0.13, and $CePO_4$: 1×10^{-23} (Clever and Johnson, 1980; 1992; Dahle, 2013). Within the pulmonary microenvironment where phospholipid metabolism is higher than that in any other organ of the body, the phosphatase-mimetic properties of CeO_2 (Xu and Qu, 2014) may scavenge ions of dissolved metal as $CePO_4$ or surfactant-related phospholipid complexes. Any combination of precipitated PSLT-like structures and imbalanced surfactant homeostasis could over-proportionally increase the PSLT-like volume-load of alveolar macrophages (AM). This process then aggravates lung overload-related outcomes. The new evidence generated by Schwotzer et al. (2017) warrants additional kinetic analyses to verify/refute past hypotheses on kinetic overload and classification of nano-CeO_2 as PSLT.

The aim of meta-analyses of past inhalation studies with nano-CeO_2 was to examine the relationship between pulmonary inflammatory endpoints characterized by neutrophils in bronchoalveolar lavage (BAL) and changes in retention kinetics eventually leading to volumetric lung overload. The most recently published sub-chronic study from Schwotzer et al. (2017) offers exceptional opportunities for model validation compared to any other previously published study with PSLTs.

2. Methods

2.1. Methods and background

Results from a series of repeated-exposure inhalation studies on rats

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with nano-CeO_2 were recently published (Keller et al., 2014, Keller, 2015; Pauluhn, 2017; Schwotzer et al., 2017). The focus of these studies was on pulmonary dosimetry, kinetics, and associated adverse pulmonary outcomes. These nano-CeO_2 NM-212 studies in rats were sponsored by NANoREG,81|0661/10|170 and included repeated-exposure inhalation studies of 1-, 4-, 13-, and 52-week duration (Keller et al., 2014; Keller, 2015). An additional 13-week study with identical nano-CeO_2 exposure concentrations was reported by Schwotzer et al. (2017). The studies by Keller and Schwotzer occurred at inhalation facilities of BASF and FhG, respectively. Methodological details were published by the authors (for abbreviations and locations, see original papers). Based on the information given, these studies served the purpose of scaffolding a 2-year nano-CeO_2 inhalation study. The pre-studies were aimed at generating data to identify exposure levels low enough to be tolerated without adverse effects up to those causing lung-inflammation, in both the absence and presence of lung overload. At the outset of the studies, the occurrence of lung overload relied upon the approach proposed by Morrow (1988) and Morrow et al. (1996). Published data regarding the outcome of the heralded 2-year inhalation study could not be found at the time of preparation of this paper.

The appropriateness of the Morrow approach was challenged in a previous publication focusing on meta-analysis of the Keller studies (Pauluhn, 2017). The study from Schwotzer et al. (2017) was also subjected to the same type of meta-analysis; however, it held the benefits that lungs were not lavaged prior to lung burden measurements and that in all study groups, measurements of CeO_2 lung burdens and elimination kinetics were pursued. The availability of this set of additional data provides a unique means to resolve yet-contentious issues concerning past studies and interpretations. Difficulties remain to unequivocally define the material density (ρ_M) of airborne agglomerated particles following dispersion into inhalation chambers and their density upon deposition and retention in the alveolar region (ρ_{alv}). Bot densities play exceptionally important roles in inhalation dosimetry modeling: the first for calculating the fractions of particles being deposited in the pulmonary (P) and tracheobronchial (TB) regions, and the second to provide invaluable information on the volume displaced within the phagocyte by the PSLTs themselves and the endogenous material bound to them. Theoretically, for non-adsorptive and totally insoluble structures, ρ_M can be anticipated to be equal to ρ_{alv}. If not, sub-fractions from surfactant may be adsorbed onto the particle-surfaces, facilitating dissolution or precipitation. Especially, the latter case of precipitation increases the particle-associated displacement volume, resulting in $\rho_M > \rho_{alv}$. A pragmatic approach was taken for deriving these parameters.

2.2. Deposition and retention

The accumulated volume- and mass-based lung burdens LB[µl] and LB[mg], respectively, can readily be calculated by the dosimetric (Eq. 1a,b) and kinetic (Eq. (2)) equations given below:

$$LB\ [mg] = C\ [mg/m^3] \times \{f_{(T+TB)}\} \times MV\ [m^3/(rat\text{-}min)] \times t\ [min] \quad (1a)$$

$$LB\ [\mu l] = C\ [mg/m^3] \times \{f_{(T+TB)} \times \rho^{-1}\ [\mu L/mg]\} \times MV\ [m^3/(animal\text{-}min)] \times t\ [min] \quad (1b)$$

$$LB_{\Sigma t} = \Sigma\{LB_0 \times (1\text{-}e^{-kt})\} \text{ with } t_{1/2} = \ln(2)/k \quad (2)$$

t = 0 is the lung burden after the first exposure.

LB: lung burdens; C: actual mass-based breathing zone concentration of CeO_2 solid aerosol; ρ_M: material density of airborne agglomerated particles following dispersion into inhalation chambers (mandatory requirement for MPPD modeling, MMPD: Multiple Path Particle Dosimetry Model); ρ_{alv}: estimated density of agglomerated particles and particle-associated bio-oligomers/-polymers following deposition and uptake by alveolar macrophages, default: $\rho_M = \rho_{alv}$; f: MPPD-modeled fraction of particles deposited in the pulmonary (P) and tracheobronchial (TB) region; MV: respiratory minute volume of nose-only exposed rats (0.29 m^3/kg-rat and 6-h exposure duration, t); k: elimination constant calculated from first-order, one-compartmental elimination half-times ($t_{1/2}$) given by the authors (mass-based metric) or modeled (volume-based metric), and k is re-adjusted after each step of accumulated volume-load as detailed in Pauluhn (2011); $LB_{\Sigma t}$: represents the kinetically modeled volume-based cumulative LB based on the MPPD-modeled P + TB fraction per exposure day (LB_0). The modeling procedure accounts for exposure-free weekends.

Measured cumulative lung burdens (mass-based) can be modeled when the elimination constant 'k' or the related elimination half-time '$t_{1/2}$' and the fraction of exposure concentration 'C' deposited in the P and/or P + TB region are known. The mass-based particle size distributions of inhaled CeO_2 rely upon cascade impactor analyses. From those, the mass fraction deposited in the lung is calculated by MPPD modeling (Anjilvel and Asgharian, 1995; RIVM, 2002). Convergence of the modeled and empirically determined lung burdens must be sought and is a mandatory prerequisite prior to any modeling. The mass-based data were converted to volume-based concentrations and lung burdens using equation (1b). The apparent material density ρ_M of airborne PSLT could then be estimated from the best fit of the volume-adjusted lung burdens and modeled retention kinetics over the entire study duration (exposure and recovery phases) by equations (1b) and (2). At the outset of modeling, ρ_{alv} was assumed equal ρ_M. However, a lower density for ρ_{alv} was applied in cases where phospholipidosis-like events occurred (see section 2.5). The *ab initio* calculation of the volume-based kinetics of retained particles relative to lung overload are dealt with in previous publications (Pauluhn, 2010a,b; 2011; 2014a,b; 2017). Modeling procedures utilized the same software and approaches as published previously.

As can be deduced from equation (1a),(1b) and (2), a mandatory prerequisite of any scientifically defensible modeling procedure includes an exact determination of the distribution of the mass-based aerodynamic diameters of nano-CeO_2 after high-velocity pressurized air dispersion into whole-body (BASF) or nose-only inhalation chambers (FhG). Notably, the effectiveness of the cascade impactor Series 290 used in these studies is higher than that of any other commercial impactor (as stated in the manufacturer's manual of the device). For particles smaller than 10 µm, the collection efficiency was E = 1 and no corrections for collection efficiencies were necessary. However, for larger particles, the sampling efficiency decreases and required mathematical corrections to adjust for the possible under-sampling of larger particles. Each laboratory produced a different mass-distribution and some fraction of mass beyond the detection range of the 8-stage cascade impactor (lowest/highest cut-off for sampling at 2 LPM: 0.52 µm/21.3 µm, Fig. 1). Despite differing particle-size distributions (Fig. 1), the lung burdens measured at the end of the 13-week exposure period were essentially identical (see result and discussion section, Fig. 6). Collection efficiencies and the use of impaction grease to avoid possible particle bounce and re-entrainment were not mentioned in any publication. Reported concentrations are those from filter analyses. Those from cascade impactor analyses were not reported, though they are called for by contemporary testing guidelines (OECD-TG#412, 2008; OECD#GD39, 2009).

As illustrated in Fig. 1, the particle-size distributions measured were quite polydisperse, making anisokinetic sampling and MPPD calculation-errors more likely. The reported values reflect bulk and true densities and range from 0.25 to 7.13 g/cm^3 (Pauluhn, 2017). Experimental validation of which of the values resembled the ρ_M after dispersion of particles into air was lacking. This data gap was bridged by modeling, as detailed above. The P + TB percentages of nano-CeO_2 given in the studies from Keller and Schwotzer were reported to be approximately 6% and 10%, respectively; however, they haphazardly applied a $\rho_M = 1$. Using the parametrization detailed above, P + TB yielded 13% with $\rho_M = 0.7$ g/cm^3 (Fig. 2). These empirical data-based re-calculated values were applied throughout all modeling procedures presented in