Published in final edited form as:

Inhal Toxicol. 2014 December ; 26(14): 829–842. doi:10.3109/08958378.2014.935535.

Computational Modeling of Nanoscale and Microscale Particle Deposition, Retention and Dosimetry in the Mouse Respiratory Tract

B. Asgharian^{1,*}, O.T. Price², M. Oldham^{3,†}, L.C. Chen⁴, E.L. Saunders⁴, T. Gordon⁴, V.B. Mikheev⁵, K.R. Minard⁶, and J. G. Teeguarden⁶

¹Applied Research Associates, Inc., Raleigh, NC

²Applied Research Associates, Inc., Arlington, VA

³Virginia Commonwealth University, Richmond, VA

⁴Department of Environmental Medicine, New York University School of Medicine, NY

⁵Battelle Memorial Institute, 505 King Avenue, Columbus, OH 43201-2696

⁶Pacific Northwest National Laboratory, Richland, WA

Abstract

Comparing effects of inhaled particles across rodent test systems and between rodent test systems and humans is a key obstacle to the interpretation of common toxicological test systems for human risk assessment. These comparisons, correlation with effects and prediction of effects, are best conducted using measures of tissue dose in the respiratory tract. Differences in lung geometry, physiology and the characteristics of ventilation can give rise to differences in the regional deposition of particles in the lung in these species. Differences in regional lung tissue doses cannot currently be measured experimentally. Regional lung tissue dosimetry can however be predicted using models developed for rats, monkeys, and humans. A computational model of particle respiratory tract deposition and clearance was developed for BALB/c and B6C3F1 mice, creating a cross species suite of available models for particle dosimetry in the lung. Airflow and particle transport equations were solved throughout the respiratory tract of these mice strains to obtain temporal and spatial concentration of inhaled particles from which deposition fractions were determined. Particle inhalability (Inhalable fraction, IF) and upper respiratory tract (URT) deposition were directly related to particle diffusive and inertial properties. Measurements of the retained mass at several post-exposure times following exposure to iron oxide nanoparticles, micro and nanoscale C60 fullerene, and nanoscale silver particles were used to calibrate and verify model predictions of total lung dose. Interstrain (mice) and interspecies (mouse, rat, human)

*Corresponding Author Address: Applied Research Associates, Inc., 8537 Six Forks Road, Suite 600, Raleigh, NC 27615-2963, basgharian@ara.com, Phone: (919) 582-3463.

†Currently at Altria Client Services, Richmond, VA

DECLARATION OF INTERESTS

The funding for this study was provided by NIH grants U19-ES019544 (J.G. Teeguarden), U01 ES020126-03 (E.L. Saunders, L.C. Chen, T. Gordon), and in part by the Defense Threat Reduction Agency via contract DTRA01-03-D-0014-0030 (B. Asgharian, O.T. Price). The authors report no declarations of interest.

differences in particle inhalability, fractional deposition and tissue dosimetry are described for ultrafine, fine and coarse particles.

INTRODUCTION

Inhalation studies are the gold standard for assessing the potential risks of exposure to air-borne particulates. Typically, biological responses observed in the respiratory tract or other tissues after inhalation are correlated with measures of external exposure (e.g. mg/m³) or inhaled dose (mg/kg). Biological response in these tissues may be correlated to these measures of external exposure, especially for a single particle type within a single species. However, responses are expected to be directly related to more biologically-based measures of retained dose in the lung such as cellular or tissue dose (Brown et al., 2005, Teeguarden et al., 2007, Oberdorster et al., 2005). Cellular or tissue dose of particles, the preferred measures for correlation with response for purposes of risk and hazard assessment (Andersen and Krewski, 2009), have the advantage of being a more credible basis for comparison of doses across particle types, species and experimental systems (Hinderliter et al., 2010, Teeguarden et al., 2007). The amount of inhaled particles reaching the cells of the respiratory tract is highly dependent on the particle characteristics (size, density, shape), breathing parameters, lung volumes, and the anatomical characteristics of the respiratory tract (Anjilvel and Asgharian, 1995, Asgharian et al., 2001a, Asgharian et al., 2006a, Asgharian et al., 2006b, Asgharian and Yu, 1989, Jarabek et al., 2005). In addition, particles that land on airway surfaces are translocated or removed from the airways by mucociliary clearance, macrophage engulfment, direct penetration through the lung tissues or other processes like dissolution or particle degradation, thus, modifying the tissue and cell dose (Oberdorster et al., 2005).

For practical reasons (e.g. cost) and the ability to manipulate genetics in supporting mechanistic studies, the mouse is a common experimental model for toxicological studies of macroscale and nanoscale particulates (Benson et al., 1987, Benson et al., 1995, Dunnick et al., 1989, Gillespie et al., 2010, Korsak et al., 1998). Differences in respiratory tract geometry (Oldham et al., 1994), breathing parameters (Flandre et al., 2003, Reinhard et al., 2002, Tankersley et al., 1994), and clearance kinetics (Mall, 2008) among mice and between mice, rats and humans, will result in differences in the deposited and retained tissues doses in the respiratory tract of these species. Among mice, differences may also result in inter-strain variability in tissue dose. Since mice are considerably smaller than humans, breathe at significantly lower flow rates, and are obligatory nasal breathers, there is a size-selective bias in particle entry to the respiratory systems of the two species. Not all particles that enter the human nasal passages on inhalation are capable of entering the nasal airways of mice. Thus, special consideration must be given to particle inhalability when extrapolating mouse deposition measurements to human exposure scenarios.

Quantitative studies of the relationships between particle size, respiratory tract geometry, breathing parameters and clearance kinetics have been conducted in several species. Fewer datasets on deposition and clearance of particles in the respiratory tract of mice are available than for other species (Dill et al., 2004, Kuehl et al., 2012, Oberdorster and Finkelstein,

1995, Raabe et al., 1988). Menache et al. (1995) developed particle-size dependent, inhalability curves for humans and a group of small animals based on measurements of particle deposition in the respiratory tract. Model comparison showed a significantly lower inhalability in small animals than in humans. Hence, the first requirement in the assessment of internal cellular or tissue dosimetry of particulates in mice is to develop mouse-specific inhalability models. Extending our understanding of particle dosimetry in the mouse respiratory tract from the effect of inhalability to regional (upper and lower respiratory tract and the pulmonary airways) or cellular dosimetry requires methods for direct measurement or calculation of deposited dose at this level of biological detail. Analytical methods such as magnetic particle detection (MPD)(Minard et al., 2013) and inductively coupled mass spectrometry (ICPMS), can be used to measure deposited dose in the whole lung, but without the regional resolution necessary to determine cellular or tissue doses. Electron or optical microscopy can be applied to tissue sections but resulting estimates of tissue dose are semi-quantitative, and the approach is resource intensive, even for assessing tissue doses in relatively small regions of the lung.

The difficulty of obtaining regional, tissue and cellular level particle dose information by experimental means has been overcome computationally for rats and humans (Koblinger and Hoffmann, 1993, Koblinger and Hofmann, 1995, Anjilvel and Asgharian, 1995, Asgharian et al., 2001a, ICRP, 1994). These efforts produced computational models of particle deposition dose called the Multipath Particle Deposition Model (MPPD) (Anjilvel and Asgharian, 1995, Asgharian and Anjilvel, 1998, Asgharian et al., 2001a) that are now widely used in the research and regulatory science communities (Robinson et al., 2008). Dosimetry models such as MPPD, once verified, can provide an inexpensive, quick, and efficient means to provide the internal estimates of tissue dose, the missing link between external measures of exposure and response. These simplified models of respiratory tract geometry, ventilation, and respiratory aerosol transport are constructed by dividing the respiratory tract into various regions based on anatomical function and adopting a dichotomous airway branching structure. Each airway is approximated as a straight, cylindrical tube. Lung ventilation is the net effect of airway resistance, lung compliance, flow inertia, and other physiologic effects, determined by either calculating lobar expansion rates or from measurements of pressure drop and lung impedance. Regional particle losses in tracheobronchial and pulmonary airways are estimated from first principles (mass and momentum balances). The development of a mechanistic model to predict deposition of inhaled particles comprises four steps we have applied previously in the development of such models for the rat and human: first, semi-empirical predictive equations are found to predict inhalability and deposition in upper respiratory tract (URT; nasal, nasopharyngeal, pharyngeal, and laryngeal); second, the lower respiratory tract (LRT; tracheobronchial and pulmonary airways) structure (e.g. airway diameters, lengths) is reconstructed mathematically (including respiratory bronchioles if appropriate); third, a mechanistic model for LRT ventilation is developed based on the physiology of respiration; and finally, a cross-sectional area averaged, mass-balance transport equation is developed for inhaled particles with a parameter for particle removal to predict airway and regional deposition.

Oldham et al. (1994) developed a mathematical model to predict particle losses in the nasal passages and tracheobronchial airways of B6C3F1 mice. The tracheobronchial airway

dimensions were obtained from measurements of three in-situ prepared lung casts. The model did not include a pulmonary compartment. Hsieh et al. (1999) included the proposed tracheobronchial geometry of Phalen et al. (1991) for B6C3F1 mice in their trumpet deposition model to predict particle deposition throughout the respiratory tract. While predictions were extended to BALB/c mice, no inter-strain differences were addressed due to lack of information on BALB/c mouse specific geometry and physiology parameters. More recently, Oldham and Robinson (2007) constructed the tracheobronchial airway geometry for BALB/c mice based on measurements of three in-situ prepared lung casts. Cast measurements were further analyzed by Madl et al. (Madl et al., 2010) to develop functional relationships for various airway parameters. The tracheobronchial geometry of the BALB/C mouse was completed by attaching to it an eight-generation, typical-path acinar structure that was developed based on the method of Yeh et al. (Yeh et al., 1979). The respiratory tract geometry was used in compartmental for predictions of regional losses of particles (National Council on Radiological Protection and Measurements (NCRP), 1997).

To address particle respiratory tract tissue dose differences in strains of mice used in particulate toxicity studies, and to provide a convenient tool for the prediction of regional tissue doses in the mouse lung, we developed a mechanistic model for transport, deposition, and retention of inhaled particles in the respiratory tract of B6C3F1 and BALB/c mice. Providing the ability to calculate particle dose to each of the 22 regions of the mouse respiratory tract and to alveolar macrophages, the model overcomes a major obstacle to the fields of particulate- and nano-toxicology. Based on the multiple-path that was previously developed for humans and rats (Price et al., 2002), the mouse MPPD model includes strain-specific tracheobronchial airway dimensions and physiological parameters and implements newly-developed expressions for particle inhalability and URT airway deposition. The deposition model was supplemented with measurement-based, semi-empirical models for particle clearance in the pulmonary airways of the lung to determine the fate of inhaled particles. Model predictions were made for various particle sizes at typical breathing conditions and were compared with available in-vivo measurements.

MATERIALS AND METHODS

Development of the mouse MPPD model followed the approach previously reported for humans, non-human primates, and rodents (Anjilvel and Asgharian, 1995, Asgharian et al., 2001a, Asgharian et al., 2012). The model treated particle deposition in the entire respiratory tract of mice, including the upper respiratory tract (URT) and the LRT (lower respiratory tract). The URT started from the nasal openings and extended through the nasal passages, pharynx, and larynx until the beginning of the trachea. The LRT included the conducting airways (tracheobronchial or TB region: trachea, bronchi, and bronchioles) and respiratory airways (pulmonary region or PUL: alveolar ducts and sacs), which together comprised 22 airway generations. Development of the dosimetry model proceeded in several steps, including the assessment of particle inhalability, derivation of semi-empirical relationships for particle deposition in the URT, construction of strain-specific mouse thoracic geometry, and formulation of a mathematical model to predict particle deposition in the full respiratory tracts of mice. In addition, a clearance model for particle removal from the pulmonary region of B6C3F1 mice was constructed based on recent measurements of particle kinetics

in mice airways following short-term nose-only inhalation (National Toxicology Program, 2009a). Predicted deposition and retained dose were verified by comparing to available in-vivo information in the literature (Hsieh et al., 1999; Kuehl et al., 2012; Oldham et al., 2009; Raabe et al., 1988; Teeguarden et al., Submitted), and reported here (silver nanoparticles). Dose-rate was indirectly verified by comparisons to total deposition over a known time interval (dose-rate x time). The model can be used to simulate deposition and retention of individual particle size or distributions of particle sizes during short-term (acute) or extended (chronic) exposures by calculating deposition fraction during a single breathing cycle and extending it to additional breaths by including particle removal and clearance at each time interval. A computer code (Multipath Particle Deposition Model, MPPD) with graphical-user interface was developed based on the dosimetry model and is available for download (<http://www.ara.com/products/mppd.htm>). Table 1 presents the sources for model parameters, equations and data used to calibrate or test the model.

Particle Deposition and Clearance Data

Silver Nanoparticles—FVBN/J mice (The Jackson Laboratory, Bar Harbor, ME) were maintained under IACUC guidelines at the NYU School of Medicine's AALAC-approved animal housing facility in Tuxedo Park, NY. All animals were kept in filter top cages on a 12 hour light cycle with *ad libitum* access to food and water, except during exposure. Animals were exposed to 0.62 and 0.68 mg/m³ of Ag₂O for four hours with two different particle sizes (30 nm and 90 nm respectively) via nose-only inhalation. Particles were generated using a Palas spark generator (Model GFG-1000, Karlsruhe, Germany) from 0.25 in diameter Ag rods of 99.99% purity (ESPI Metals, Ashland, OR) and diluted with HEPA filtered air. Freshly generated nanoparticles (CMD 30 nm, GSD 1.5) were aged (CMD 90 nm, GSD 1.7) in order to allow for agglomeration of particles as previously described in Beckett et al. (Beckett et al., 2005). Particle size was measured using a TSI (St. Paul MN) model 3080 Electrostatic Classifier and a Differential Mobility Analyzer (DMA) with a TSI model 308500 DMA voltage tube for small particles (2–150nm), and a TSI model 308101 DMA voltage tube for larger particles (10–1000nm), and verified via transmission electron microscopy (NYU Microscopy Core). Chamber concentration was measured gravimetrically and verified via energy dispersive X-ray fluorescence, ED-XRF (Thermo Scientific, Quant X, Madison WI). Particle chemistry was examined using x-ray photoelectron spectroscopy (Thermo Scientific K-Alpha, Madison WI). Whole non-lavaged lungs were then digested in nitric acid (Fischer Scientific, Pittsburgh, PA) and analyzed via inductively-coupled plasma mass spectrometry (ICP-MS) for Ag content per gram wet weight.

Iron Oxide Nanoparticles—Teeguarden et al. (Teeguarden et al., Submitted) exposed 30 female BALB/c mice to iron oxide nanoparticles (19.9 mg/m³) with a density of 5.2 g/cm³, count mean diameter of 69 nm, and standard deviation of 1.65 in a nose-only exposure unit for 4 hours. The retained mass in the lungs of these animals were measured immediately, 6 hours, 24 hours, 1 day, and 7 days following the cessation of the exposure. The reported tidal volume was 0.24 cm³.

C60-fullerenes nanoparticles and micro-scale particles—A inhalation exposure study was conducted at the National Institute for Environmental Health Sciences (NIEHS) in

which B6C3F1 mice were exposed to C₆₀ fullerene nano- and micro-sized particles (density of 1.65 g/cm³) for 3 hours per day, 5 days a week, for 13 weeks (National Toxicology Program, 2009a). Two separate sets of exposures were conducted for nano- and micro-sized particles: exposure concentrations of 0.5 mg/m³ and 2 mg/m³ for nano-sized particles (36 and 44 nm count mean diameter, respectively) and 2 mg/m³, 15 mg/m³, and 30 mg/m³ for micro-sized particles (1.1 – 1.3 µm mass median aerodynamic diameter or MMAD). Exposure concentrations were monitored for the nano (RAM: Model RAM-1, MIE Inc, Bedford MA and SMPS was Model 3034, TSI Inc, Shoreview, MN) and micron sized particles (RAM, Casella MicroDust pro (Casella USA, Amherst MA). Gravimetric analysis of materials captured on filters was used to verify exposure concentrations. Animals were removed from the exposure system and sacrificed after 1 day, 2 weeks, 4 weeks, and 8 weeks of post exposure times. The retained masses in the lung were measured and recorded. We acquired the mass burden measurements from NIEHS (National Toxicology Program, 2009a).

Inhalability assessment

During inhalation, particles move toward the opening of the URT in an airflow streamline. The inertia of particles may cause the trajectory of particles to separate from the airflow streamline. If the trajectory of a particle approaching a URT opening deviates significantly from the surrounding airflow streamline moving toward the opening of the URT, the particle may impact on the adjacent facial area. As a result, only a fraction of particles in the streamline, referred to as the inhalable fraction (IF), may reach and penetrate the URT. IF depends on particle size and inhalation flow rate. Asgharian et al. (2003) formulated a semi-empirical relationship for particle inhalability in rats as a function of product of particle aerodynamic diameter (d_{ae}) squared and inhalation flow rate (Q).

$$IF = 1 - \frac{1}{1 + \alpha (d_{ae}^2 Q)^\beta} \quad (1)$$

where $d_{ae}^2 Q$ is the Stokes parameter and is given in g/µm²/s. Given that both rats and mice are obligatory nasal breathers and similar in their facial and nasal structure, equation (1) may also be used for mice by using mouse URT dimensions and breathing information to derive mouse-specific stokes parameters α and β . Parameters α and β were found by equating the

Stokes numbers for rats and mice to find $d_{ae}^2 Q|_{Rat} = \frac{D_{h-Rat}}{D_{h-Mouse}} \times d_{ae}^2 Q|_{Mouse}$, where D_{h-Rat} and $D_{h-Mouse}$ are hydraulic diameters for rat and mouse URT, respectively (Asgharian et al., 2012). Defining hydraulic diameter as the ratio of the nostril surface area to perimeter, a new value of $\alpha = 9.92$ was found in equation (1) for mice while $\beta = -0.7466$ remained the same as for rats. Inhalability in mice is lower than that in rats for the same Stokes parameter. Inhalability drops in mice due to a lower Stokes parameter when compared with rats.

URT deposition modeling

The URT filters inhaled particles, limiting penetration of some inhaled particles into the LRT. Due to the lack of data on URT particle deposition in different varieties/strains of

mice, deposition fraction measurements by Raabe et al. (Raabe et al., 1988) in CD1 mice were used to formulate a model for particle loss efficiency in the URT of mice. Raabe et al. (1988) exposed 25- to 35-gram CD1 mice briefly to monodisperse aerosols having aerodynamic sizes in the range of 0.19 μm to 9.57 μm (reported aerodynamic resistance diameters were converted to aerodynamic diameters) in a nose-only exposure chamber for which particle inhalability was assumed negligible. Two study particles were below the coarse range, the rest were in the coarse range. URT particle deposition occurred predominantly by inertial impaction for which inhalability was likely under 100%. Furthermore, deposition by Brownian diffusion could not be assessed from this dataset due to lack of ultrafine size particles in this study. Despite these shortcomings, this dataset presents the only opportunity to obtain an initial estimate of experimental URT deposition. Assuming steady animal breathing and equal deposition efficiency during inhalation and exhalation, URT deposition efficiency by impaction ($\eta_{\text{H-imp}}$) and in LRT airways (η_{L}) by inertial impaction and sedimentation can be calculated from the following equation:

$$\eta_{\text{H-imp}} = \left[\left(1 - \frac{\Delta_{\text{L}}}{2} \right) - \sqrt{\left(1 - \frac{\Delta_{\text{L}}}{2} \right)^2 - \Delta_{\text{H}}} \right] \quad (2)$$

where

$$\eta_{\text{L}} = \frac{\Delta_{\text{L}}}{1 - \eta_{\text{H-imp}}} \quad (3)$$

in which Δ_{L} and Δ_{H} are the calculated deposition fractions by Raabe et al. (1988) in the LRT and URT airways, respectively. Deposition fractions were calculated by dividing measured deposition in URT and LRT by the inhaled mass of aerosols (Raabe, et al., 1988). Since animals' minute ventilation rates were not measured, the empirically-derived equation of Guyton (1947) was used by Raabe et al. (1988) to calculate inhalation flow rate and mass of inhaled particles. A logistic function was selected for URT deposition efficiency as a function of $d_{\text{ae}}^2 Q$ ($\text{g}\mu\text{m}^2/\text{s}$) (Zhang and Yu, 1993),

$$\eta_{\text{H-imp}} = \left[\frac{(d_{\text{ae}}^2 Q)^\alpha}{10^5 + (d_{\text{ae}}^2 Q)^\alpha} \right]^\beta \quad (4)$$

where coefficients $\alpha = 3.96$ and $\beta = 0.117$ were found by fitting equation (4) to the data of Raabe et al. (1988) with $R^2=0.99$. Equation (4) estimates inertial impaction alone. This model is similar to that developed by Oldham et al. (Oldham et al., 1994) and Zhang and Yu (1993), which were also based on the Raabe et al. (1988) dataset. However, these two studies were not used. The deposition efficiency in Oldham et al (1994) was calculated as a function of URT pressure drop, which is not often available. An incorrect form of Guyton's formula was used in Zhang and Yu (1993), requiring reevaluation of their fit coefficients for use in this model.

No experimental data are available on deposition of particles by diffusion in the URT of mice. Cheng et al. (Cheng et al., 1990) derived a semi-empirical relationship for deposition efficiency by diffusion in a rat nasal mold as a function of diffusion coefficient (D in cm^2/s) and inhalation or exhalation flow rate (L/min).

$$\eta_{H\text{-diff}} = 1 - e^{-\alpha D^\beta Q^\gamma} \quad (5)$$

Coefficients α , β , and γ were found by fitting equation (5) to the deposition efficiency data by diffusion in nasal molds (Cheng et al., 1990). Assuming URT structural similarity between rats and mice, the semi-empirical equation (5) for rats was extended to mice by applying an adjustment factor. Examination of the non-dimensionalized form of the mass balance equation (convective-diffusion) revealed that transport and deposition efficiency of particles in the URT depended on the dimensionless parameters, Schmidt (Sc) and Pelect (Pe) numbers. Thus, a dimensionless analog of equation (5) using Sc and Pe in place of D and Q represented the most general form of $\eta_{H\text{-diff}}$. Subsequently, the dimensionless equation was reduced to equation (5) when Sc and Pe were replaced by their definitions. By assuming the same dimensionless model of deposition by diffusion was equally applicable to rats and mice, an adjustment factor was found by equating dimensionless deposition efficiency equations for mice and rats. After simplifications, coefficients β and γ remained the same for rats and mice. However, coefficient α had to be corrected by the adjustment factor $(D_{h\text{-Rat}}/D_{h\text{-Mouse}})^\gamma$. Consequently, equation (5) may be used for mice when $\beta = 0.517$, $\gamma = -0.234$, and $\alpha = 13.688$ and 11.407 for inhalation and exhalation, respectively.

LRT Geometry

To facilitate particle deposition computations in LRT, the LRT structure was assumed to be a collection of cylindrically-shaped tubes arranged as a dichotomous branching structure. The LRT geometry was symmetric and represented by a typical path model. Since rodents exhibited monopodial LRT structure with considerable variation in number of generations per pathway, a dichotomous, symmetric branching structure by which airway number increases by a factor of 2 between consecutive generations led to an overestimation of airway number and hence lung volume. Following Oldham et al. (1994), LRT tracheobronchial airway numbers were calculated by allowing a linear increase in airway number with generation number from generation 5 (trachea = generation 0) to terminal bronchioles for which the number of tracheobronchial airways were known (1505 and 1584 for B6C3F1 and BALB/c mice, respectively). The number of airways between generations 4 and 14 were found to increase by approximately 1.5^n , where n is the generation number. By using available LRT tracheobronchial morphometry in the literature, typical path, symmetric lung geometries for B6C3F1 and BALB/c varieties of mice were constructed in this study. LRT tracheobronchial morphometry for conducting airways was derived from three in-situ prepared LRT casts using identical target LRT volumes for each mouse strain. Airway dimensions included length, diameter, bifurcation and gravity angles. Measurements by Oldham et al. (1994) and Phalen (Phalen, 1991) were used for the B6C3F1 LRT tracheobronchial geometry. The same dataset was also used for lung geometry by Hsieh et al. (Hsieh et al., 1999) to calculate deposition on Ni compounds in the mouse lung. The LRT

tracheobronchial geometry of BALB/c mouse was from measurements of Oldham et al. (1994) and Oldham and Robinson (Oldham and Robinson, 2007).

LRT geometry was completed in both varieties of mice by attaching an 8-generation single-path dichotomous branching pulmonary tree to each terminal bronchiole at generation 14. The structure and morphometry of the symmetric pulmonary tree was reported by Oldham and Robinson (2007) for the BALB/c mouse and Oldham et al. (1994) and Phalen (1991) for the B6C3F1 mouse.

Reconstructed LRT geometries of the two varieties of mice were rescaled twice: first by $(FRC/TLC)^{1/3}$, where FRC is functional residual capacity or lung volume at rest and TLC is the total lung capacity, to adjust airway dimensions to rest conditions, and second by $(1 + V_T/2)^{1/3}$, where V_T is tidal volume, to account for airway size change during breathing. The rescaled LRT geometries were used in calculations of LRT particle deposition.

Physiology parameters

Physiological parameters, included breathing frequency (f), inhalation and exhalation breathing fractions, tidal volume (V_T), total lung capacity (TLC), functional residual capacity (FRC), and URT volume for adult BALB/c and B6C3F1 mice. Physiological measurements from the double chamber plethysmograph study of DeLorme and Moss (DeLorme and Moss, 2002) were used to determine default inhalation and exhalation breathing fractions (f), and V_T for both varieties of mice. TLC was calculated by summing up LRT individual airway and alveolar volumes for BALB/c and B6C3F1 mice. FRC and URT volume were determined by extrapolating from proportional ratios in rats ($FRC/TLC \sim 30\%$ and $URT/TLC \sim 3\%$).

Mechanistic model of particle deposition in the lung

The mathematical model of Anjilvel and Asgharian (1995) was used to calculate particle deposition fraction during a breathing cycle in mouse LRT geometries described above. The model was formulated based on the assumption that airways are shaped as cylinders and expanded and contracted uniformly during a breathing cycle. In addition, particle concentration was uniform across the airway cross section. Particle deposition was accounted for by a loss term in the 1-D transport model (Anjilvel and Asgharian, 1995). The loss term was related to net deposition efficiency of particles by various loss mechanisms for which analytical equations had been described (Yu, 1978). While the deposition model does not indicate a bound for uncertainties associated with model assumptions, it provides average predictions for deposition fractions per generation of the LRT due to variation in airway dimensions and breathing mechanics.

Clearance Modeling

Insoluble particles depositing on mouse lung airway surfaces are generally assumed to be removed by the mucociliary escalator in the bronchial airways and macrophage phagocytosis in the pulmonary region. Clearance of insoluble particles from the conducting airways by mucociliary clearance is rapid and complete within a few days of initial deposition (ICRP, 1994). However, pulmonary clearance is slow and the deposited particles

tend to accumulate (ICRP, 1994). A one-compartmental model in which particle removal was described by first-order clearance kinetics was applied to each pulmonary airway. The retained mass in each pulmonary airway of the mouse lung (m_A) was calculated from the following mass balance equation:

$$\frac{dm_A}{dt} = \dot{r}_A - \lambda m_A \quad (6)$$

where λ is the particle clearance rate constant and $\dot{r}_A$ is the deposition rate of particles given by

$$\dot{r}_A = C \cdot V_T \cdot f \cdot D_F \quad (7)$$

in which C is the exposure concentration of particles and D_F is the deposition fraction in the pulmonary region calculated from the deposition model described in the previous sections. Particle clearance rate constants reflect the ability of macrophages to engulf and carry the deposited particles away and thus depends on the retained mass of particles. The particle clearance rate may be approximated from mass burden measurements taken post exposure by solving equation (6) during two consecutive time points for which λ may be assumed to be uniform,

$$\lambda = \frac{1}{t_2 - t_1} \ln \frac{m_{A1}}{m_{A2}} \quad (8)$$

where subscripts 1 and 2 correspond to time points 1 and 2. The deposited mass in the TB region is effectively removed after about a couple of days post exposure. Thus, measured lung burdens correspond to the retained mass in the pulmonary region, which can be substituted in equation (8) to find the rate constant.

RESULTS AND DISCUSSION

In each of the sections below, the results of the model development process are presented as simulations of particle deposition along with comparisons to the available experimental data useful for testing or calibrating the MPPD model. Uncertainties are discussed with a focus on experimental data required to advance the MPPD mouse model.

Inhalability

The inhalability model in mice was obtained by extending the rat inhalability model (equation 1) to mice, assuming geometric similarity between the URT anatomy of the two species. To evaluate the particle penetration capability of the mouse URT, inhalability curves were generated and compared with those of rats and humans for multiple particle sizes (Figure 1). Typical resting ventilation rates were used for computations in each species including minute ventilations of 8.3 cm³/s, 2 cm³/s, and 7.5 liters per minute (LPM) for rats, mice, and humans, respectively. No attempt was made in this study to develop an inhalability model for humans, thus, the model by Menache et al. (Menache et al., 1995) was used for human inhalability calculations. As expected, mice had the lowest predicted

inhalability compared with rats and humans. Predicted inhalability in humans remained 100% for particles below 10 μm while dropping sharply for rats and mice, from near 100% for 1- μm particles to below 20% for 10 μm and larger particles. Humans had a much higher predicted inhalability compared to rodents. The trend of the rat and mouse inhalability curves were similar and inhalability values differed by a few percent with the maximum difference of less than 10% around 3 μm particles.

URT Deposition

Deposition in the URT occurs by two primary processes, flow driven inertial impaction and Brownian motion driven diffusion. Deposition measurements in CF₁ mice (Raabe et al. (1988)) were used to develop a semi-empirical equation to predict impaction losses (Figure 2) in the URT (equation 4) for inclusion in the MPPD model. The fitted line passed through most data points and formed a sigmoidal shape, which is typical for particle losses by inertial impaction losses. The predicted URT deposition fraction by inertial impaction was about 5% for 0.07 μm particles but rose sharply to reach 100% at a 20 μm or greater particle size. The URT impaction efficiency model in this study (equation 4) was in close agreement with the model of Oldham et al. (1994) at high flow rates but predicted 5% higher deposition efficiencies at lower flow rates.

The availability of analytical equations to predict the deposition fraction of inhaled particles in the URT by combined mechanisms of Brownian diffusion and impaction in rats, mice, and humans allowed a comparison of expected particle deposition across these species. Such comparisons are important not just when the URT is the target site, but because URT deposition removes material from the airstream prior to entrance to the lower respiratory tract, effecting tissue doses in the LRT. Predicted total URT deposition fractions (Figure 3) for mice, rats, and humans, at typical breathing rates for each species, showed that humans had a lower deposition fraction at all particle sizes compared to mice and rats. Thus, in humans, a higher fraction of inhaled particles pass through the URT (a higher respirable fraction) and reach the LRT compared with rats and mice. Overall, the URT of mice was most efficient in removing particles, except for particles larger than 1 μm . Particles larger than 3 μm could not penetrate rodent URT to reach the LRT. Thus, when studying biological effects of inhaled particulate matter in rodents, particle size should be limited to below 3 μm .

Respirable Fraction in Mice and Comparison to Humans

Interspecies differences in the respirable fraction of inhaled particles, the fraction that passes the URT and reaches the LRT, may contribute to interspecies differences in deposited dose in the LRT for identical exposure conditions. The respirable fraction is frequently used in the risk assessment process for inhaled particles as an approximation for the delivered dose, or the LRT tissue dose for purposes of dose-response analysis when it can account for these interspecies differences. Respirable fractions (Figure 4) for the inhalable size range were predicted to be relatively similar in mouse, rat and humans for particles with a diameter of 100 nanometers or less. The differences in respirable fraction increased for particles larger than 100 nanometers in diameter, with mice having the lowest and human having the highest respirable fraction of inhaled particles. Except for 2- μm and larger size particles, the respirable fraction was lowest in mice because they have the lowest inhalability and highest

URT deposition fraction. The highest respirable fraction was observed in humans at all particles sizes. Respirable fraction of particles was about 90% in humans but dropped below 30% in rats and mice for particles smaller than 0.2 μm . No particles of 3 μm or larger size reached the LRT of rodents. Thus, when using rodents as human surrogates in inhalation exposure studies, the dose-metric for interspecies extrapolation should be based on relevant particle sizes and targeted sites of deposition within the respiratory tract. We note that consideration was not given to the potential for differences between species of hygroscopic growth of particles, which could influence predictions of the respirable fraction.

Comparison of Deposition Predictions with Measurements

Having established particle inhalability, URT deposition (extraction), and respirable fraction for mice for use in MPPD, total predicted LRT deposition was compared to available measures of LRT deposited dose in mice.

Among the limited number of reported inhalation exposure studies in mice, four sets of LRT measurements were considered for comparison to model predictions. These studies were reported by Raabe et al. (1988) (CF₁ mice), Oldham et al. (Oldham et al., 2009) (BALB/c mice), and Kuehl et al. (Kuehl et al., 2012), (B6C3F1 mice), Hsieh et al. (Hsieh et al., 1999) (C57BL/6 mice) and finally in this manuscript (FVBN/J mice). The short exposure duration of a few minutes in some of these studies minimizes particle clearance from URT and LRT. Thus, measurements of LRT burden reflected particle deposition rather than deposition and clearance. Predicted deposition fraction of particles in LRT airways is strongly influenced by LRT parameters and breathing profile of the animal, which were not measured in the first two studies. A breathing frequency of 160 breaths per minute and calculated minute ventilation of 2 mL/s from Guyton (Guyton, 1947) based on an average mouse weight of 30 g were used to compare predictions with measurements of Raabe et al. (1988). Since the selected particle sizes in the study of Raabe et al. (1988) were mostly in micrometer size-range, for which there were high URT and low LRT deposition fractions, deposition fractions in the LRT were converted to deposition efficiency using equation (3). Reported lung and breathing parameters in each study were used in model predictions. Measured minute ventilation rates reported by Delorme and Moss (2002) were used in calculating deposition fractions for comparison to measured deposition rates reported by Oldham et al. (2009).

The reported particle size characteristics and measured breathing parameters (tidal volume and breathing frequency) were used in MPPD for purposes of comparing model predictions to measured LRT deposition reported by Kuehl et al. (2012), Hsieh et al. (1999), and in this report (silver and iron oxide nanoparticles). An average FRC of 0.6 cm³ was used in all model predictions. Figure 5 compares predicted total LRT deposition fractions against experimentally measured values. Also shown in the figure is a line indicating the expectation if the correlation between the two were perfect (line of identity). Plotted data points for Raabe et al. (1988) scattered around the line of identity with a significant departure for two data points with 100% deposition efficiency. These data points corresponded to aerodynamic particle sizes of 5.98 and 9.65 μm for which there were near 100% URT losses and insignificant LRT deposition. While agreement between model

predictions and experimental data for other particle sizes were reasonable, there were some differences which could be attributed to uncertainty in breathing parameters and differences in LRT geometries between this model (BALB/c) and that for CF₁ mice, which were used by Raabe et al. (1988), or FVBN/J mice used for the silver nanoparticle deposition study. Model predictions agreed well with measurements of Oldham et al. (2009) and Kuehl et al. (2012). MPPD predictions compared favorably with the results of the study by Hsieh et al (1999), and for the silver nanoparticles, however, model predictions were slightly lower.

Regional Deposition in the Lung

Regional particle deposition in the LRT was calculated for B6C3F1 and BALB/c mice. To isolate and characterize the influence of mouse strain differences in LRT geometry on regional particle deposition, breathing parameters were held constant in the computations, which included a URT volume of 0.0315 cm³ (Mendez et al., 2010), lung functional residual capacity of 0.3 cm³ (DeLorme and Moss, 2002), tidal volume of 0.2 cm³ (Delorme and Moss, 2002), average breathing frequency of 300 breaths/min, and 0.4 for inspiratory fraction with no pause between inhalation and exhalation (Delorme and Moss, 2002).

To examine the influence of lung airway geometry on particle deposition in the LRT of B6C3F1 and BALB/c mice, deposition fractions of particles between 0.01 µm and 10 µm were predicted for endotracheal breathing (i.e., bypassing the nasal passages). Predicted deposition fractions are given in Figure 6 for tracheobronchial (TB), pulmonary airways/tissue (PUL), and for the entire LRT. In both mouse strains, the shapes of regional deposition fraction curves were similar and corresponded to those for humans and rats. LRT particle deposition was larger in BALB/c than that in B6C3F1 mice. Despite some differences in magnitude, the predicted deposition pattern in the TB was similar between the two strains of mice. Brownian diffusion operating in the TB airways contributed to a noticeable deposition of nanoparticles. Impaction driven deposition of micron scale particles was quite high in the TB region, and was considerably higher in B6C3F1 mice than in BALB/C mice, which was likely due to differences in TB airway dimensions. Predicted pulmonary deposition fractions were nearly identical for the two strains of mice for particles smaller than 0.7 µm but drifted apart for larger particles. The high filtering of inhaled nano and microscale particles in the TB region reduced the number of particles that reached the PUL region, reducing deposition fractions in the PUL region. There was a peak in the deposition fraction curves for particle sizes around 3 µm to 4 µm in the PUL region, beyond which deposition fractions fell sharply to zero. Despite having similar geometry and volumes, PUL deposition fractions were significantly lower in BALB/C mice than in B6C3F1 mice because of the TB filtering effect. Overall, compared to fine and coarse particles, the predicted deposition fraction of nano particles was less sensitive to the strain of mouse.

Predicted regional deposition fractions of particles with sizes between 0.01 and 10 µm in the URT and LRT of both mouse strains for normal nasal breathing are presented in Figure 7. Breathing parameters were the same as those in the prior calculations (Figure 6). The role of the nose in particle inhalability given by equation (1) was considered in these calculations. Comparison of predicted deposition fractions in the LRT region via nasal breathing with

those for endotracheal breathing (Figure 6) showed similar patterns of deposition with particle size. However, predicted deposition fractions in Figure 7 were significantly lower because fewer particles reached the LRT due to URT deposition by Brownian diffusion (equation 5) for nanoparticles and impaction deposition for larger particles. In addition, reduced inhalability drastically limited the number of micron scale particles that reached the LRT, which could be available to deposit on lung airway surfaces. Consequently, there was little or no deposition of 3 μm and larger particles in the LRT. Overall, predicted deposition fractions of particles in the LRT of mice were substantially different from those for rats (Anjilvel and Asgharian, 1995) and humans (Asgharian et al., 2001a). Thus, inhalation studies conducted in mice should consider, and if necessary adjust, particle size to insure the intended tissue doses are obtained, especially when comparisons to other species or strains are made on a particle number or surface area deposited basis.

URT deposition fractions decreased with increasing particle size in the submicrometer size range (Figure 7) due to reduction in Brownian diffusion. While the same mathematical expressions were used to calculate URT deposition, there was a slight difference in predicted losses for the two mouse varieties, particularly for nanoscale particles. The difference stemmed mainly from URT deposition during exhalation. Since LRT deposition of BALB/c mice was greater than that for B6C3F1 mice, URT deposition for BALB/c mice were slightly lower during exhalation as indicated in Figure 7.

Assessment of the Retained Mass of Nanoparticles in the LRT

Recent measurements of nanoparticle clearance in the mouse lung airways were used to confirm and calibrate MPPD for the mouse. Specifically, two total lung mass burden vs. time data sets were utilized for nano-sized and micrometer-sized particles (National Toxicology Program, 2009b) to calibrate lung slow clearance equations used in MPPD. The rapid clearance of fullerene- C_{60} particles evident in the early time post exposure points is attributed to mucociliary clearance, and is based on kinetic transport of deposited particles in the tracheobronchial airways of the lung (Asgharian et al, 2001b). The slower clearance observed after a few days of post exposure, is attributed to slower processes dominated by alveolar macrophage ingestion and transport and used to calibrate this form of the clearance in MPPD.

Predictions of the retained mass of an aerosol (count mean diameter 69 nm) of iron oxide nanoparticles 6, 24, 1 and 7 days after a four hour nose only exposure in BALB/c mice were made using reported study conditions (Teeguarden et al., Submitted). A lung functional residual capacity (FRC) of 0.6 cm^3 and URT volume of 0.04 cm^3 were used in the MPPD predictions. Mucociliary clearance is the dominant mechanisms of particle removal from the surface of conducting airways of the lung during the first few days. The same mechanistic model of mucociliary clearance used for humans and rats (Asgharian et al., 2001b) was employed to predict the retained mass in the conducting airways. Due to the lack of information on the tracheal mucous velocity in BALB/c mice, a tracheal mucous velocity (0.2 mm/min), about an order of magnitude lower than that in rats, was selected. Model predictions were compared with measurements in Figure 8. Model predictions gave a slightly higher mass in the mouse LRT after 4 hours of exposure. The predicted retained

mass dropped at a slightly faster rate compared to measurements. Model prediction agreed well with the measurements after 7 days of post exposure

Measured retained masses of C₆₀ fullerene nano- and micro-sized particles (density of 1.65 g/cm³) in mice 1 day, 2 weeks, 4 weeks, and 8 weeks after a 13 week exposure e by inhalation (National Toxicology Program, 2009a) were also compared to simulated values. Equation (8) was used to convert mass burdens to clearance rate constants (λ) at different post exposure periods. A semi-empirical relationship was developed for the clearance rate constant as a function of the retained mass of particles in the pulmonary region. Figure 9 shows the fitted line and calculated λ based on the available measurements. While the general trend exhibited a reduction in particle clearance with decreasing mass burden, there was a significant scatter of data particularly at smaller mass burdens. The fitted line passed through the data and represents a first order approximation of clearance kinetics. The fitted line for λ as a function of the retained mass is given by:

$$\lambda = \frac{1}{25.904 + 1844.5 \times m_A^{4.7894}} \quad (9)$$

in which λ is the cleared mass in milligram per day and m_A is the retained mass. Equation (9) was substituted in equation (6) for λ to calculate the retained mass during exposure and post-exposure times in the B6C3F1 mouse pulmonary region using exposure parameters of the NIEHS studies described above. Figure 10 presents the predicted mass burden in the pulmonary region at different exposure times. In addition, burden measurements were plotted in the same figure for comparison with predictions. While the model for the clearance rate constant (equation 9) was based on measurements during post-exposure times, a comparison of predictions with measured mass burdens during the exposure period can provide a means for model verification when no other data are available. Since deposition and clearance models are coupled during the initial exposure phase, comparison of the retained mass between predictions and measurements at the end of exposure and start of post exposure period (13 weeks) is an indirect test for the accuracy of model predictions. Results in Figures 10A and 10B correspond to nano- and micro-sized exposure scenarios. Model predictions in each case showed a gradual rise in the retained mass as a result of competition between deposition and clearance of particles during exposure followed by a smooth decline during the post exposure period. Comparisons between reported measurements and predictions for nanoparticle exposures at the end of the 13 week exposures were excellent for both exposure concentrations of 0.5 mg/m³ and 2 mg/m³ (Figure 10A). Lung burden dropped during post exposure and nearly passed through the measurements. Simulations of retained mass for micro-sized particles were less consistent with the data. The predicted retained mass was about 50% smaller than reported measurement at the end of 13 weeks of exposure for the lowest exposure concentration of 2 mg/m³ (Figure 10B). The difference decreased to 25% at higher exposure concentrations (15 mg/m³ and 30 mg/m³). The discrepancy can be attributed principally to the expected discrepancies in the predicted deposition in the nasal region. The model developed for nasal losses (Figure 4) was based on measurement of Raabe et al. (Raabe et al., 1977) in CD-1 mice, which tended to over predict losses of 1 μ m particles in C57BL/6 mice as indicated by recent measurements (Kuehl et al., 2012).

MPPD Based Regional Tissue Dose Metrics for Extrapolation and Risk Assessment

A software program previously developed to predict regional and local deposition of inhaled particles for several species (Rat, human, monkey) was extended to include the particle dosimetry model for two strains of mice described above. MPPD mouse can be used to predict the deposited and retained doses of inhaled particles on a mass, surface area or particle number basis for the URT, TB and PUL regions, and individually all generations of the respiratory tract. Exposures to a single particle size or a distribution of particle sizes, across the duration of a single breath, or over the course of multiple days or months of constant or episodic exposure can be represented in simulations. Particle dose per gross lung region (e.g., TB, PUL), per generation (e.g., generation 15), on a per lung surface area can be output. MPPD mouse also outputs the particle dose per alveolar macrophage in lung regions containing macrophages. The current version of the model produces an output file and several plots with all these metrics of dose for each simulation. Standard respiratory tract parameters referenced above are used in MPPD mouse as default values. Tables 1 and 2 report airway parameters per generation for B6C3F1 and BALB/c mice. The model is available upon request (ARA Associates, Arlington, VA: <http://www.ara.com/products/mppd.htm>).

Uncertainties and Data Needs

The accuracy of regional and cellular dose metrics predicted by MPPD for the mouse depends on the accuracy of several assumptions made during its development, and the accuracy of other parameters also used to construct or implement the model. Foremost, the fidelity of constructed lung geometries for the two strains of mice to actual geometries is important for realistic predictions of the delivered doses. Currently, the geometry of the entire lung is based on limited measurements of airway parameters along specific pathways of the lung. Symmetric lung structures are developed based on statistical relationships obtained from these measurements, but may not account for variability or the presence of a fraction of the represented geometries that deviate from the typical path represented in the model. In addition, there is limited information regarding intra-strain variations of lung airway dimensions and no information on lung dimensions as a function of age or body mass, which adds to the uncertainty regarding the representativeness of the MPPD mouse geometry. Measurement of URT airway dimensions for both strains of mice or URT deposition measurements would overcome the uncertainties imposed by the absence of these data. Recent enhancement of experimental approaches for lung imaging and new image processing software will enable reconstruction of multiple lung geometries from the trachea down to micrometer-level airways of the lung for additional refinement of MPPD.

The current model for lung ventilation assumes non-moving airways, and steady airflow rather than inhalation/exhalation cycle among other simplifications. These assumptions may impact airflow distributions and airflow inertia, and consequently the predicted particle deposition. More sophisticated models of lung ventilation that account for these processes would overcome these limitations but construction of such detailed lung ventilation models based on airway resistance, lung compliance, and airflow inertia are only plausible and useful if lung geometry is fully represented.

The total amount of particles in any region or generation in the lung is the time-dependent product of competing mechanisms of deposition and removal from the airways. All particle deposition measurements, like those used here to calibrate the model, inherently include both deposition, and depending on the exposure period and post exposure period, some clearance of deposited particles. There is little information in the literature on mucociliary clearance velocities at different airways of any strain of mouse lung. We have used the available data to estimate these rates for the TB region, but the limited amount of data adds to the uncertainty of dose predictions and impedes direct comparison of model predictions with measurements. Studies that characterize mucus velocities in the TB region and quantify clearance rates of particles, at multiple exposure concentrations, via macrophage ingestion and movement up the respiratory tree are essential elements of a research program designed to reduce uncertainty in particle dosimetry models like MPPD. Because the physical-chemical characteristics of particles can also affect clearance, MPPD is best applied to particles similar to those used to calibrate the model when deposition and clearance are both modeled.

Evolving Particle Deposition Modeling Approaches

Some limitations imposed by the simplification of the respiratory tract necessitated in developing deposition models like MPPD can be overcome using approaches that are both more computationally intensive and require a much greater level of anatomical and physiological detail. Particle and chemical deposition models using computational fluid dynamics (CFD) approaches with detailed numerical calculations of the airflow and particle deposition becomes feasible are being developed for some compartments of the animal respiratory tracts such as the rat (Fischer F344) nose and upper airways (Kimbell et al., 1997, Kimbell and Subramaniam, 2001, Kimbell et al., 2001, Schroeter et al., 2006). If the complete geometry (e.g. Airway dimensions, bifurcation and gravity angles) of the respiratory tract extending from nasal and oral openings to the alveolar sacs were available, one requirement for the CFD approach would be met. However, at present, two obstacles render CFD modeling of particle deposition of the complete respiratory tract impractical if not impossible. First, conducting simulations of air flow through three dimensional representations of airways containing millions of airways currently exceeds available computational resources. Hybrid approaches are being developed to overcome this challenge, which might also be overcome with advances in computing. In addition, the respired airflow, which is initiated by the expansion of the most distal and compliant airways of the respiratory tract cannot be resolved at the resolution of CFD modeling due to the lack of data on deep lung pleural pressure variation. Thus, micro-scale, detailed computational modeling capturing the true complexity of the airways, airflow and particle deposition is not currently a viable option for assessment of overall deposition of inhaled particles in the murine respiratory tract. At present, the approach taken here expressed in MPPD, is the most advanced and comprehensive approach that is feasible.

Concluding Remarks

A particle deposition and clearance model was developed for BALB/c and B6C3F1 mice. Model predictions showed that submicrometer particles were capable of penetration and deposition in the LRT of mice, which was consistent with the limited in-vivo data. Ultrafine

particle deposition fraction was more significant in alveolar airways than in the TB region. The deposition fraction of 1 μm or larger particles was only a few percent. Particles larger than 3 μm were not capable of penetrating beyond the mouse URT. Comparison of model predictions for the two varieties of mice showed that while deposition patterns were similar, there were differences in the predicted deposition fraction. Thus, strain-specific deposition models should be developed for mice to study predicted dose and biological end points. Limited comparison of model predictions with the retained mass measurements gave satisfactory agreement for the burden at the end of 13 week exposure period for which one dataset was available.

Acknowledgments

The authors would like to thank Nigel Walker of the National Institute of Environmental Health Sciences and the National Toxicology Program for providing the deposition and clearance data for C60, and to the Battelle Multiscale Toxicology Initiative for providing the iron oxide particle deposition and clearance data.

References

- ASGHARIAN B, PRICE OT, McClellan G, Corley R, Daniel REinstein DR, Richard E, Jacob RE, Harkema J, Carey SA, Schelegle E, Hyde D, Kimbell JS, Miller FJ. Development of a rhesus monkey lung geometry model and application to particle deposition in comparison to humans. *Inhalation Toxicology*. 2012; 24:869–899.
- ANDERSEN ME, KREWSKI D. Toxicity testing in the 21st century: bringing the vision to life. *Toxicol Sci*. 2009; 107:324–30. [PubMed: 19074763]
- ANJILVEL S, ASGHARIAN B. A multiple-path model of particle deposition in the rat lung. *Fundam Appl Toxicol*. 1995; 28:41–50. [PubMed: 8566482]
- ASGHARIAN B, ANJILVEL S. A multiple-path model of fiber deposition in the rat lung. *Toxicol Sci*. 1998; 44:80–6. [PubMed: 9720144]
- ASGHARIAN B, HOFMAN W, BERGMANN R. Particle deposition in a multiple-path model of the human lung. *Aerosol Science and Technology*. 2001a; 34:332–339.
- ASGHARIAN B, HOFMANN W, MILLER FJ. Mucociliary clearance of insoluble particles from the tracheobronchial airways of the human lung. *Journal of Aerosol Science*. 2001b; 32:817–832.
- ASGHARIAN B, KELLY JT, TEWKSBUURY EW. Respiratory deposition and inhalability of monodisperse aerosols in Long-Evans rats. *Toxicol Sci*. 2003; 71:104–11. [PubMed: 12520080]
- ASGHARIAN B, PRICE O, MCCLELLAN G, CORLEY R, EINSTEIN DR, JACOB RE, HARKEMA J, CAREY SA, SCHELEGLE E, HYDE D, KIMBELL JS, MILLER FJ. Development of a rhesus monkey lung geometry model and application to particle deposition in comparison to humans. *Inhal Toxicol*. 2012; 24:869–99. [PubMed: 23121298]
- ASGHARIAN B, PRICE O, OBERDORSTER G. A modeling study of the effect of gravity on airflow distribution and particle deposition in the lung. *Inhal Toxicol*. 2006a; 18:473–81. [PubMed: 16603478]
- ASGHARIAN B, PRICE OT. Deposition of ultrafine (nano) particles in the human lung. *Inhal Toxicol*. 2007; 19:1045–54. [PubMed: 17957545]
- ASGHARIAN B, PRICE OT, HOFMANN W. Prediction of particle deposition in the human lung using realistic models of lung ventilation. *Journal of Aerosol Science*. 2006b; 37:1209–1221.
- ASGHARIAN B, YU CP. Deposition of Fibers in the Rat Lung. *Journal of Aerosol Science*. 1989; 20:355–366.
- BECKETT WS, CHALUPA DF, PAULY-BROWN A, SPEERS DM, STEWART JC, FRAMPTON MW, UTELL MJ, HUANG LS, COX C, ZAREBA W, OBERDORSTER G. Comparing inhaled ultrafine versus fine zinc oxide particles in healthy adults: a human inhalation study. *Am J Respir Crit Care Med*. 2005; 171:1129–35. [PubMed: 15735058]

- BENSON JM, CARPENTER RL, HAHN FF, HALEY PJ, HANSON RL, HOBBS CH, PICKRELL JA, DUNNICK JK. Comparative inhalation toxicity of nickel subsulfide to F344/N rats and B6C3F1 mice exposed for 12 days. *Fundam Appl Toxicol.* 1987; 9:251–65. [PubMed: 3653567]
- BENSON JM.; CHANG, I-Y.; CHENG, YS.; HAHN, FF.; BARR, EB.; MAPLES, KR.; SNIPES, MB. Submitted by Inhalation Toxicology Research Institute (ITRI), Lovelace Biomedical and Environmental Research Institute. Albuquerque, NM: 1995. Species differences in the fate of nickel compounds in the respiratory tract. Final report to NiPERA.
- BROWN JS, WILSON WE, GRANT LD. Dosimetric comparisons of particle deposition and retention in rats and humans. *Inhal Toxicol.* 2005; 17:355–85. [PubMed: 16020034]
- CHENG YS, HANSEN GK, SU YF, YEH HC, MORGAN KT. Deposition of ultrafine aerosols in rat nasal molds. *Toxicol Appl Pharmacol.* 1990; 106:222–33. [PubMed: 2256113]
- DELORME MP, MOSS OR. Pulmonary function assessment by whole-body plethysmography in restrained versus unrestrained mice. *J Pharmacol Toxicol Methods.* 2002; 47:1–10. [PubMed: 12387933]
- DILL JA, LEE KM, MELLINGER KH, BATES DJ, BURKA LT, ROYCROFT JH. Lung deposition and clearance of inhaled vanadium pentoxide in chronically exposed F344 rats and B6C3F1 mice. *Toxicol Sci.* 2004; 77:6–18. [PubMed: 14600283]
- DUNNICK JK, ELWELL MR, BENSON JM, HOBBS CH, HAHN FF, HALY PJ, CHENG YS, EIDSON AF. Lung toxicity after 13-week inhalation exposure to nickel oxide, nickel subsulfide, or nickel sulfate hexahydrate in F344/N rats and B6C3F1 mice. *Fundam Appl Toxicol.* 1989; 12:584–94. [PubMed: 2731671]
- FLANDRE TD, LEROY PL, DESMECHT DJ. Effect of somatic growth, strain, and sex on double-chamber plethysmographic respiratory function values in healthy mice. *J Appl Physiol* (1985). 2003; 94:1129–36. [PubMed: 12571140]
- GILLESPIE PA, KANG GS, ELDER A, GELEIN R, CHEN L, MOREIRA AL, KOBERSTEIN J, TCHOU-WONG KM, GORDON T, CHEN LC. Pulmonary response after exposure to inhaled nickel hydroxide nanoparticles: short and long-term studies in mice. *Nanotoxicology.* 2010; 4:106–119. [PubMed: 20730025]
- GUYTON AC. Measurement of the respiratory volumes of laboratory animals. *Am J Physiol.* 1947; 150:70–7. [PubMed: 20252828]
- HINDERLITER PM, MINARD KR, ORR G, CHRISLER WB, THRALL BD, POUNDS JG, TEEGUARDEN JG. ISDD: A computational model of particle sedimentation, diffusion and target cell dosimetry for in vitro toxicity studies. Part *Fibre Toxicol.* 2010; 7:36. [PubMed: 21118529]
- HSIEH TH, YU CP, OBERDORSTER G. Deposition and Clearance Models of Ni Compounds in the Mouse Lung and Comparisons with the Rat Models. *Aerosol Science and Technology.* 1999; 31:358–372.
- ICRP. Human respiratory tract model for radiological protection: A report of a task group of the International Commission on Radiological Protection. Oxford, UK: Elsevier Sciences, Ltd; 1994.
- JARABEK AM, ASGHARIAN B, MILLER FJ. Dosimetric adjustments for interspecies extrapolation of inhaled poorly soluble particles (PSP). *Inhal Toxicol.* 2005; 17:317–34. [PubMed: 16020031]
- KIMBELL JS, GODO MN, GROSS EA, JOYNER DR, RICHARDSON RB, MORGAN KT. Computer simulation of inspiratory airflow in all regions of the F344 rat nasal passages. *Toxicology and Applied Pharmacology.* 1997; 145:388–398. [PubMed: 9266813]
- KIMBELL JS, SUBRAMANIAM RP. Use of computational fluid dynamics models for dosimetry of inhaled gases in the nasal passages. *Inhalation Toxicology.* 2001; 13:325–34. [PubMed: 11295865]
- KIMBELL JS, SUBRAMANIAM RP, GROSS EA, SCHLOSSER PM, MORGAN KT. Dosimetry modeling of inhaled formaldehyde: comparisons of local flux predictions in the rat, monkey, and human nasal passages. *Toxicological Sciences.* 2001; 64:100–10. [PubMed: 11606806]
- KOBLINGER L, HOFFMANN W. Aerosol deposition calculations for a stochastic lung model. (th Aerosol in Medicine Congress. 1993
- KOBLINGER L, HOFMANN W. Aerosol Inhalation in the Rat Lung .2. Theoretical Predictions of Particle Deposition Patterns. *Journal of Aerosol Medicine-Deposition Clearance and Effects in the Lung.* 1995; 8:21–32.

- KORSAK Z, MAJCHEREK W, RYDZYNSKI K. Toxic effects of acute inhalation exposure to 1-methylnaphthalene and 2-methylnaphthalene in experimental animals. *Int J Occup Med Environ Health*. 1998; 11:335–42. [PubMed: 10028200]
- KUEHL PJ, ANDERSON TL, CANDELARIA G, GERSHMAN B, HARLIN K, HESTERMAN JY, HOLMES T, HOPPIN J, LACKAS C, NORENBURG JP, YU H, MCDONALD JD. Regional particle size dependent deposition of inhaled aerosols in rats and mice. *Inhal Toxicol*. 2012; 24:27–35. [PubMed: 22145784]
- MADL P, HOFMANN W, OLDHAM MJ, ASGHARIAN B. Stochastic morphometric model of the BALB/c mouse lung. *Anat Rec (Hoboken)*. 2010; 293:1766–75. [PubMed: 20652937]
- MALL MA. Role of cilia, mucus, and airway surface liquid in mucociliary dysfunction: Lessons from mouse models. *Journal of Aerosol Medicine and Pulmonary Drug Delivery*. 2008; 21:13–24. [PubMed: 18518828]
- MENACHE MG, MILLER FJ, RAABE OG. Particle Inhalability Curves for Humans and Small Laboratory-Animals. *Annals of Occupational Hygiene*. 1995; 39:317–328. [PubMed: 7793751]
- MENDEZ LB, GOOKIN G, PHALEN RF. Inhaled aerosol particle dosimetry in mice: A review. *Inhalation Toxicology*. 2010; 22:15–20. [PubMed: 21142796]
- MINARD KR, LITCKE MH, WANG W, XIONG Y, TEEGUARDEN JG, THRALL BD. Magnetic particle detection (MPD) for in-vitro dosimetry. *Biosens Bioelectron*. 2013; 43:88–93. [PubMed: 23287653]
- NATIONAL COUNCIL ON RADIOLOGICAL PROTECTION AND MEASUREMENTS (NCRP). NCRP Report. Vol. 125. Bethesda, MD: 1997. Deposition, retention and dosimetry of inhaled radioactive substances.
- NATIONAL TOXICOLOGY PROGRAM. Chemical Effects in Biological Systems Database. National Toxicology Program, National Institutes of Environmental Health Sciences; 2009a. Toxicity Evaluation of Nanomaterial (Fullerene-C60 50 nm) in B6C3F1 Mice.
- NATIONAL TOXICOLOGY PROGRAM. Chemical Effects in Biological Systems Database. Research Triangle Park, NC: U.S. Department of Health and Human services, Public Health Service, National Institute of Health; 2009b. Toxicity Evaluation of Nanomaterial (Fullerene-C60 50 nm) in B6C3F1 Mice.
- OBERDORSTER, G.; FINKELSTEIN, J. Final report to NiPERA. Rochester, NY: 1995. Subchronic inhalation studies with insoluble nickel compounds: A species comparison. Submitted by Department of Environmental Medicine
- OBERDORSTER G, OBERDORSTER E, OBERDORSTER J. Nanotoxicology: an emerging discipline evolving from studies of ultrafine particles. *Environ Health Perspect*. 2005; 113:823–39. [PubMed: 16002369]
- OLDHAM MJ, PHALEN RF, BUDIMAN T. Comparison of Predicted and Experimentally Measured Aerosol Deposition Efficiency in BALB/C Mice in a New Nose-Only Exposure System. *Aerosol Science and Technology*. 2009; 43:970–977.
- OLDHAM MJ, PHALEN RF, SCHUM GM, DANIELS DS. Predicted Nasal and Tracheobronchial Particle Deposition Efficiencies for the Mouse. *Inhaled Particles VII*. 1994:135–141.
- OLDHAM MJ, ROBINSON RJ. Predicted tracheobronchial and pulmonary deposition in a murine asthma model. *Anatomical Record-Advances in Integrative Anatomy and Evolutionary Biology*. 2007; 290:1309–1314.
- PHALEN, RF. Final report submitted to NiPERA. Irvine, CA: University of California, Irvine; 1991. An airway model for the laboratory mouse.
- PRICE, OT.; ASGHARIAN, B.; MILLER, FJ.; CASSEE, FRDEW-SR. Multiple Path Particle Dosimetry Model (MPPD v1.0): A model for human and rat airway particle dosimetry. Bilthoven, the Netherlands: National Institute for Public Health and the Environment (RIVM); 2002.
- RAABE, OG.; YE, H-C.; NEWTON, GJ.; PHALEN, RF.; VELASQUEZ, DJ. Deposition of inhaled monodisperse aerosols in small rodents. In: WALTON, WH.; MCGOVERN, B., editors. *Inhaled Particles IV, Part I: Proceedings of an International Symposium*. Edinburgh, Scotland: Pergamon Press, Ltd; 1977. p. 3-21.
- RAABE OGM, AL-BAYATI A, TEAGUE SV, RASOLT A. Regional deposition of inhaled monodisperse aerosols in small rodents. *Ann Occup Hyg*. 1988; 32:53–63.

- REINHARD C, EDER G, FUCHS H, ZIESENIS A, HEYDER J, SCHULZ H. Inbred strain variation in lung function. *Mammalian Genome*. 2002; 13:429–437. [PubMed: 12226708]
- ROBINSON RJ, SNYDER P, OLDHAM MJ. Comparison of analytical and numerical particle deposition using commercial CFD packages: impaction and sedimentation. *Inhal Toxicol*. 2008; 20:485–97. [PubMed: 18368619]
- SCHROETER JD, KIMBELL JS, ANDERSEN ME, DORMAN DC. Use of a pharmacokinetic-driven computational fluid dynamics model to predict nasal extraction of hydrogen sulfide in rats and humans. *Toxicol Sci*. 2006; 94:359–67. [PubMed: 16984956]
- TANKERSLEY CG, FITZGERALD RS, KLEEGERGER SR. Differential Control of Ventilation among Inbred Strains of Mice. *American Journal of Physiology-Regulatory Integrative and Comparative Physiology*. 1994; 267:R1371–R1377.
- TEEGUARDEN J, MIKEEV VB, MINARD KR, FORSYTHE WC, WANG W, SHARMA G, KARIN NJ, TILTON SC, WATERS KM, ASGHARIAN B, PRICE OR, POUNDS JG, THRALL BD. Comparative Iron Oxide Nanoparticle Cellular Dosimetry and Response in Mice by the Inhalation and Liquid Cell Culture Exposure Routes. *Particle and Fiber Toxicology*. Submitted.
- TEEGUARDEN JG, HINDERLITER PM, ORR G, THRALL BD, POUNDS JG. Particokinetics in vitro: dosimetry considerations for in vitro nanoparticle toxicity assessments. *Toxicological Sciences*. 2007; 95:300–12. [PubMed: 17098817]
- YEH HC, SCHUM GM, DUGGAN MT. Anatomic models of the tracheobronchial and pulmonary regions of the rat. *Anat Rec*. 1979; 195:483–92. [PubMed: 507403]
- YU CP. Exact Analysis of Aerosol Deposition during Steady Breathing. *Powder Technology*. 1978; 21:55–62.
- ZHANG L, YU CP. Empirical Equations for Nasal Deposition of Inhaled Particles in Small Laboratory-Animals and Humans. *Aerosol Science and Technology*. 1993; 19:51–56.

APPENDIX A. MOUSE AIRWAY DIMENSIONS

Table 1

Airway parameters for each generation of B6C3F1 mouse at functional residual capacity.

Generation number	Number of airways	Length (cm)	Diameter (cm)	Branching Angle ¹	Gravity angle ¹	Tracheo-bronchial surface area (cm ²)	Alveolar duct surface area (cm ²)	Alveolar surface area (cm ²)	Total surface area (cm ²)	Number of alveoli
0	1	0.7457	0.1139	0	90	0.2668	0	0	0.2668	0
1	2	0.4223	0.1172	14	78	0.311	0	0	0.311	0
2	4	0.1679	0.0948	18	85	0.2	0	0	0.2	0
3	8	0.069	0.0748	30	77	0.1297	0	0	0.1297	0
4	16	0.0806	0.0648	12	71	0.2628	0	0	0.2628	0
5	32	0.0416	0.0599	10	71	0.2501	0	0	0.2501	0
6	64	0.0357	0.0524	11	60	0.2903	0	0	0.2903	0
7	128	0.0357	0.0449	10	60	0.3838	0	0	0.3838	0
8	256	0.0333	0.0374	16	60	0.459	0	0	0.459	0
9	512	0.0283	0.0333	11	60	0.5349	0	0	0.5349	0
10	1024	0.0274	0.0299	12	60	0.7208	0	0	0.7208	0
11	2048	0.0224	0.0241	18	60	0.7328	0	0	0.7328	0
12	4096	0.02	0.0175	22	60	0.7276	0	0	0.7276	0
13	8192	0.015	0.0116	31	60	0.5611	0	0	0.5611	0
14	16384	0.0108	0.0083	61	60	0.4465	0	0	0.4465	0
15	32768	0.01	0.0082	45	60	0	0.8161	2.075	2.8911	52362
16	65536	0.01	0.0081	45	60	0	1.616	3.882	5.498	104754
17	131072	0.0091	0.0081	45	60	0	2.932	5.949	8.881	209433
18	262144	0.0091	0.008	45	60	0	5.803	17.63	23.433	418867
19	524288	0.0083	0.0079	45	60	0	10.44	29.81	40.25	837585

Generation number	Number of airways	Length (cm)	Diameter (cm)	Branching Angle ^I	Gravity angle ^I	Tracheo-bronchial surface area (cm ²)	Alveolar duct surface area (cm ²)	Alveolar surface area (cm ²)	Total surface area (cm ²)	Number of alveoli
20	1048576	0.0083	0.0078	45	60	0	20.66	59.93	80.59	1675914
21	2097152	0.0075	0.0076	45	60	0	36.4	277.8	314.2	3351084

^I degrees;

Table 2

Airway parameters for each generation of BALB/c mouse at functional residual capacity.

Generation number	Number of airways	Length (cm)	Diameter (cm)	Branching Angle ^I	Gravity angle ^I	Tracheo-bronchial surface area (cm ²)	Alveolar duct surface area (cm ²)	Alveolar surface area (cm ²)	Total surface area (cm ²)	Number of alveoli
0	1	0.852	0.0919	0	90	0.2461	0	0	0.2461	0
1	2	0.384	0.0861	14	105	0.2078	0	0	0.2078	0
2	4	0.1596	0.0794	25	98	0.1592	0	0	0.1592	0
3	8	0.0577	0.0602	26	91	0.08716	0	0	0.08716	0
4	16	0.0802	0.0535	18	78	0.2156	0	0	0.2156	0
5	32	0.0485	0.0434	18	76	0.2117	0	0	0.2117	0
6	64	0.0451	0.0401	12	60	0.2806	0	0	0.2806	0
7	128	0.0393	0.0334	22	60	0.3139	0	0	0.3139	0
8	256	0.0326	0.0301	16	60	0.3616	0	0	0.3616	0
9	512	0.0301	0.0292	9	60	0.5006	0	0	0.5006	0
10	1024	0.0276	0.0267	24	60	0.6472	0	0	0.6472	0
11	2048	0.0234	0.0201	20	60	0.6353	0	0	0.6353	0
12	4096	0.0209	0.0175	16	60	0.7655	0	0	0.7655	0
13	8192	0.0175	0.0134	26	60	0.7557	0	0	0.7557	0
14	16384	0.0134	0.0084	56	60	0.5551	0	0	0.5551	0
15	32768	0.0125	0.0083	45	60	0	1.03	34.07	35.1	523
16	65536	0.0125	0.0082	45	60	0	2.04	67.93	69.97	104
17	131072	0.0117	0.0081	45	60	0	3.769	13.28	17.049	209
18	262144	0.0117	0.008	45	60	0	7.461	26.47	33.931	418
19	524288	0.0109	0.0079	45	60	0	13.71	51.74	65.45	837
20	1048576	0.01	0.0079	45	60	0	25.05	101.1	126.15	1675
21	2097152	0.0092	0.0077	45	60	0	44.94	197	241.94	3351

^I degrees;

Figure 1. Particle inhalability in human, rats, and mice for minute ventilation rates of 7.5 LPM, 8.3 cm^3/s , and 2 cm^3/s respectively.

Figure 2.

Comparison of predicted nasal deposition of particles by the derived semi-empirical relationship for the mouse URT with Raabe et al. (1988) measurements on which the derived semi-empirical relationship is built.

Figure 3. Deposition efficiency of inhaled particles in the URT of human, rats, and mice for minute ventilation rates of 7.5 LPM, 8.3 cm³/s, and 2 cm³/s respectively.

Figure 4.

Comparison of respirable fractions of particles that reach the lungs of humans, rats, and mice at different particles sizes for minute ventilation rates of 7.5 LPM, $8.3 \text{ cm}^3/\text{s}$, and $2 \text{ cm}^3/\text{s}$ respectively.

Figure 5. Comparison of predicted deposition fraction in the mouse lung with available measurements in the literature, or reported here.

Figure 6. Regional deposition fraction of inhaled particles in the LRT of B6C3F1 and BALB/C mice via endotracheal breathing for identical lung and breathing parameters.

Figure 7. Regional deposition fraction of inhaled particles in the LRT of B6C3F1 and BALB/C mice via nasal breathing for identical lung and breathing parameters.

Figure 8.
Comparison of predicted retained mass in the conducting airways mice with reported measurements by Teeguarden et al. (2014) shown by open squares with standard deviation.

Figure 9.

Comparison of predicted clearance rate constants by the curve-fit model (equation (9)) with that obtained based on measured retained mass.

Figure 10.

Retained mass burden in the LRT of B6C3F1 mice for a 13 week exposure followed by 200 days post exposure: A. Nano-sized C60-fullerene particles, B. Microsized C60-fullerene particles.

Table 1

Sources of Model Parameters, Equations and Data for Model Testing

Model Parameter	Mouse Strain	
	BALB/c	B6C3F1
Inhalability Equations	Equations derived for rats (Asgharian et al., 2003) were applied to both strains of mice by adjusting for airway dimensions and physiology.	
	Generalized equations for mice were derived from URT deposition data in CD1 mice for micrometer-sized particles (Raabe et al., 1988). The diffusional transport/loss equations for rats were used for non-sized particles (Cheng et al., 1990) by applying adjustment factor $(D_{h-Rat}/D_{h-Mouse})^{\gamma}$, where	
LRT (TB plus PUL) Airway Dimensions	(Oldham et al., 1994, Oldham and Robinson, 2007)	Oldham et al., 1994, Phalen, 1991)
Transport Equations	(Asgharian et al., 2001a, Asgharian and Price, 2007)	
Clearance Equations	Derived in this manuscript	
Data for Fitting Clearance Parameters	NTP C60, post exposure	
Data for Testing Clearance Model	Iron oxide nanoparticles (Teeguarden et al., Submitted) exposure	
Data for Testing LRT Deposition Model	Raabe et al. (1988); Hsieh et al. (1999); Oldham et al., 2009; Kuehl et al. (2013); Silver nanoparticle data reported here.	