1 A Rabbit Lung Morphology Model for Aerosol Deposition and Clearance

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12
13 ABSTRACT
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15 A rabbit lung morphology model was developed based on data available in the published
16 literature. The airways of the lung were described with a typical path model. The lung
17 airways are grouped by airway generation, which is the number of airway bifurcations
18 leading from the trachea to the terminal airway. The airways in each generation are
19 described by the average number, length, diameter, branching angle, and gravity angle.
20 The geometry of the tracheobronchial (TB) region (generations 1-14) was defined by
21 integrating the data primarily from Kliment (1974), Ramchandani et al. (2001), and
22 Schlesinger and McFadden (1981). Rodriguez et al. (1987) data were used to define the
23 pulmonary (PUL) region. The morphometry data were used in a typical path deposition
24 and clearance model based on the work of Yeh and Schum (1980), Wojciak (1988), and
25 Kimmel et al (2001). Deposition via diffusion, sedimentation, and inertial impaction was
26 modeled for TB and PUL deposition. Deposition in the head and larynx, (i.e.,
27 extrathoracic) was modeled with a function of the diameter, Stokes number, and
28 diffusivity coefficient, and was fit to Raabe (1988) data. A clearance model was
29 constructed, based on mucosal and macrophage movement, and integrated with the
30 deposition model. Predictions were in good agreement with the data. The model was
31 used to explain apparent inconsistencies in a data set collected following inhalation
32 exposure of rabbits to *B. anthracis* spores.
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1. Introduction

For particulate matter and many chemical and biological hazards, inhalation is the major route of exposure. The toxic response to such exposures is often region-specific, rather than the entire respiratory tract being affected in the same manner and to the same degree. Furthermore, at a constant air concentration, regional deposited and retained dose changes depending on several physiological, material, and environmental parameters including particle characteristics, breathing frequency, and tidal volume. In such cases, correlating toxicity with regional deposition and retention rather than external air concentration will result in better dose-response correspondence. In addition, inhalation experiments conducted in animals should be evaluated in terms of deposited and retained dose because lung morphology and deposition and retention patterns differ between experimental animals and humans. In order to account for these differences in deposited and retained dose, mathematical models of the lung morphology, deposition, and clearance can be used.

Statistical models (e.g., a polynomial regression model) can be quite successful at fitting experimental deposition data, but are less useful for extrapolating to different physiological conditions or species because the parameters may have no inherent biological meaning attached to them. Biologically-based models are built using parameters and compartments that have a physiological analogue, and are more appropriate for performing such cross-species and physiological extrapolations. Furthermore, a biologically-based model allows for more straightforward analysis of variability in deposition since model parameter values must fall within physiologically-based ranges that are consistent with life and the dynamics of the animal model's physiology.

The purpose of this work was to develop a mathematical model of region-specific deposition and clearance in the rabbit for interpreting variability in the results of inhalation toxicity studies using rabbits, and extrapolating the results to humans. Several mathematical deposition models have been published, mainly for humans (Hofmann, 1996; Darquenne, 2001; Wojciak, 1988; Yeh & Schum, 1980; Asgharian et al., 2001; Asgharian et al., 2006), and rats (Hofmann, 1989; Anjilvel & Asgharian, 1995), but to the best of our knowledge, no rabbit lung deposition and clearance models have been published.

A biologically-based model was built upon measurements of physiological parameters of the rabbit airways, thus enabling a broad range of exposure scenarios and diverse physiological states to be modeled (e.g., calm versus frightened or agitated experimental animals, or humans). The biologically-based model also allows for more realistic analysis of interindividual variability in deposition since random selection of model parameters must adhere to physiologically-based nominal ranges that are consistent with organism behavior. The model was tested against the rabbit lung deposition data of Gearhart & Schlesinger (1988) and Raabe et al. (1988). Finally, the model was applied to

explain discrepancies in deposition and clearance data from inhalation studies with rabbits.

2. Material and methods

A typical path model was developed based on the rat and human lung deposition models created by Wojciak (1988), which was a refinement of the models of Yeh and Schum (1980) and Schum and Yeh (1980). The morphology model represents the lung as a series of airway generations, starting with the trachea (generation 1), followed by the main bronchi (generation 2), and so on until reaching the alveolar sac, the last generation. Each branching into the next generation leads to a number of identical daughter airways, and all airways in a given generation assume the average properties for the whole generation (i.e., airway length, diameter, etc.). The deposition of inhaled particles due to impaction, sedimentation, and diffusion is calculated along a single, typical path through the series of airways. The deposition probabilities are calculated for inhalation, breath pause, and exhalation separately. Deposition in the tracheobronchial and pulmonary airways is calculated using published equations (Wojciak, 1988; Yeh and Schum, 1980; Schum and Yeh, 1980), while deposition in the head and larynx is calculated using an empirical function. A clearance model was also developed, simulating mucociliary clearance in the head, laryngeal, tracheobronchial regions, and slower, phagocytic clearance by macrophages in the pulmonary airways.

2.1 Tracheobronchial and pulmonary deposition

Equations 1 through 6 are taken from the work of Yeh and Schum (1980). The probability of a particle depositing in the airway via diffusion (P_D) during inhalation and exhalation is computed using equation (1) for laminar flow, and equation (2) for turbulent flow:

$$(1) \quad \begin{aligned} P_D &= 1 - 0.819e^{-7.315x} - 0.0976e^{-44.61x} - 0.0325e^{-114x} - 0.0509e^{-79.31x^{2/3}} \\ P_D &= 2.828\sqrt{x} (1.0 - 0.314\sqrt{x}) \end{aligned} \quad (2)$$

where x is given by:

$$x = (LD)/(2R^2v) \quad (3)$$

L is the length of the airway, D is the diffusion coefficient, R is the radius of the airway, and v is the airflow velocity. The probability of deposition via diffusion during breath pause is computed using equation (4):

$$P_D = 1 - \exp\left(-5.784KTC\tau/6\pi\mu rR^2\right) \quad (4)$$

where K is the Boltzmann constant, T is the temperature (Kelvin), C is the Cunningham slip correction factor, τ is the pause time, μ is the viscosity of air, and r is the particle radius.

The probability of deposition via sedimentation (P_S) is calculated using:

$$P_S = 1 - \exp\left(-4gC\rho r^2\delta\cos(\gamma)/9\pi\mu R\right) \quad (5)$$

where δ , the residence time in the airway, is equal to the pause time for sedimentation during a breath pause, and is equal to L/v for inhalation and exhalation, and γ is the inclination angle relative to gravity.

The probability of deposition by inertial impaction (P_I) on inhalation is calculated by:

$$P_I = 1 - \frac{2}{\pi} \cos^{-1}(\theta St) + \frac{1}{\pi} \sin(2\cos^{-1}(\theta St)), \text{ for } \theta St < 1 \quad (6)$$

where θ is the branching angle into the airway and St is the Stokes' number. For $\theta St \geq 1$, the probability is taken to be 1. There is assumed to be no inertial impaction on breath pause and exhalation.

The probabilities of deposition by diffusion, impaction, and sedimentation are combined to give the total probability of deposition in an airway as follows for inhalation (P_{inh}), exhalation (P_{exh}), and breath pause (P_{paus}), respectively:

$$P_{inh} = P_I + (1 - P_I)(P_D + P_S - P_D P_S) \quad (7)$$

$$P_{exh} = P_D + P_S - P_D P_S \quad (8)$$

$$P_{pause} = P_D + P_S - P_D P_S \quad (9)$$

The impaction probability for inhalation is computed independently of diffusion and sedimentation since the majority of deposition by this mechanism will occur at the bifurcation, ahead of deposition by the other two mechanisms.

2.2 Extrathoracic deposition

Deposition in the head and larynx ($P_{(h,l)}$) is modeled using an empirical function that is fit to the experimental data of Raabe (1988). The model is a function of the particle diffusion coefficient and the Stokes' number:

$$P_{(h,l)} = \frac{c_D D + c_I St}{c_{(h,l)} + c_D D + c_I St} \quad (10)$$

where the constants c_D and c_I are fit to both head and larynx deposition data, c_h is specific to head deposition data, and c_l is fit to specific to larynx deposition data. This is a modification of a previously used head deposition equation that is a function of the particle diameter squared times the air flow rate:

$$P = [(d^2 Q)^\alpha / (c + (d^2 Q)^\alpha)]^\beta \quad (11)$$

where d is particle diameter (μm), Q is air flow rate (mL/s), and α , β , and c are constants (Anjilvel and Asgharian, 1995). This modification was made because equation (11) does not reproduce the observed increase in deposition for particles of smaller diameters (i.e., below $1\ \mu\text{m}$ aerodynamic diameter, see Figure 4).

An inhalability correction (Ix) was added to the model to account for reduced intake of larger particles into the nose. The model of Menache et al. (1995) was used:

$$Ix = 1 - 1/(1 + e^{c_{Ia} + 0.4343c_{Ib} \log(d)}) \quad (12)$$

where $c_{Ia} = 2.57$ and $c_{Ib} = -2.81$ for five species of experimental animals, including rabbits.

The head and larynx deposition models were fit to the rabbit deposition data of Raabe et al. (1988). Breathing frequencies (F) and tidal volumes (TV) were not measured in these experiments, but a minute volume of approximately $1000\ \text{mL}$ was estimated based on Guyton's formula and the body weights of the rabbits (Guyton, 1947). So, during the head and larynx deposition model fitting process, TV and F were set such that the minute volume was approximately $1000\ \text{mL}$. The best fits were obtained with $TV=20\ \text{mL}$ and $F=50$ breaths per minute. Raabe et al. (1988) also corrected the deposition data for inhalability in the published numbers, so the inhalability correction was not used for our fitting procedure.

2.3 Tracheobronchial and extrathoracic clearance

Since the model calibration and verification was conducted by comparing simulations to data obtained in animals with functioning airway clearance processes, it was necessary to develop clearance models to utilize these data. The mucocilliary clearance model was developed based on the work of Kimmel et al. (2001). The rate of mucocilliary clearance from an airway is given by:

$$dN/dt = v_{muc}N/L \quad (13)$$

where v_{muc} is the mucus velocity, N/L is the number of particles per unit length of the airway. Head, larynx, and trachea mucus velocities are supplied to the model as inputs, and the mucus velocities for the remaining bronchial airways are calculated by surface area scaling from the tracheal mucus velocity:

$$v_{muc,i} = v_{muc,1} A_1/A_i \quad (14)$$

where A is the surface area of the airway, the subscript 1 denotes the trachea, and i denotes the subsequent generation numbers.

2.4 Pulmonary clearance

The pulmonary clearance simulates the phagocytosis of particles in the pulmonary airways by patrolling phagocytic cells, such as alveolar macrophages. This approach was inspired by previously published work on pulmonary clearance (Charnick & Lauffenburger, 1990; Fisher & Lauffenburger, 1987), and represents a simplified version of the chemotaxis model:

$$dN/dt = \rho\chi P v_P D_P \quad (15)$$

where ρ is the particle surface density, χ is the chemotactic index, P is the number of phagocytes present, v_P is the speed at which the phagocyte patrols the space, and D_P is the diameter of the phagocyte. The product of the speed and the diameter gives the surface area swept out by the phagocyte per unit time. The chemotactic index is a measure of how strongly the phagocyte is attracted to the particle, and tells how directly it travels to the particle as it follows the chemotactic gradients. A maximum χ of one means that the phagocyte moves directly to the particle and engulfs it, while smaller values indicate that it makes random deviations from the chemotactic gradient. The chemotactic index was fit to reproduce long term lung clearance data in rabbits (Gearhart and Schlesinger, 1988). The particle surface density is determined from the amount of aerosol deposited in the alveolar region divided by the alveolar surface area for a rabbit, where the surface area is calculated from the airway dimensions and numbers in Figure 1. P is set to be 135 million (Valberg, 1990). A macrophage speed of 3 $\mu\text{m}/\text{min}$ is assumed, based on the recommendation of Fisher and Lauffenburger (1988). Rabbit-specific alveolar macrophage data were not found in our literature search, so the diameter of the macrophage is set to a value of 15 μm , which is similar values reported for rats, guinea pigs, and dogs.

2.5 Morphology

The mechanistic deposition and clearance calculations require a morphology model that has the average number, length, diameter, branching angle, and gravity angle of the airways in each generation. The lung morphology was defined by integrating the data primarily from in situ casting studies by Kliment (1974), Ramchandani et al. (2001), Schlesinger and McFadden (1981), and Rodriguez et al. (1987).

2.5.1 Lung cast data

Kliment (1974) injected latex into the lungs of rabbits, using a minimum pressure gradient (Kliment, 1974), to produce latex lung casts, and measured the average length and radius of the trachea, main bronchi, and 24 subsequent airway generations of the rabbit lung airways, with the last generation representing alveoli. The percent of conducting airways, respiratory bronchioles, alveolar ducts, alveolar sacs, and alveoli were also estimated for the main bronchi down. The number of airways was estimated by segmental zones, namely the main bronchi, bronchi of orders 2, 3, and 4, respiratory bronchioles, alveolar ducts, alveolar sacs, and alveoli.

Ramchandani et al. (2001) prepared silicone casts of the lungs of three mature rabbit lungs and analyzed the right upper lobe of the lung casts. After formalin fixation for 7 days at an airway pressure of 20 cm H₂O, 10% of the formalin was withdrawn and an equal volume of silicone and catalyst was slowly infused. The acinar regions were trimmed from the casts, and the number of airways and mean and standard deviation of the diameters were catalogued for up to 37 airway generations starting with the lobar bronchus. These data do not describe a typical, or average geometry in which all paths to the terminal airways are the same average length, but rather average diameters for every generation, with some paths being shorter or longer than others. The maximum number of airways occurred at generation 17, indicating this is a common TB path length. Beyond generation 17, the number of airways begins to dwindle. The number of conducting airways per generation and the total number of conducting airways were computed by averaging the numbers from the three right upper lobes examined by Ramchandani et al. (2001), and multiplying by six to extrapolate to the whole lung assuming six similar lobes. Although the authors recognize that different lobes of the lungs will have different morphology, data in other lobes were not available, and this is assumed to represent the best approximation.

Schlesinger and McFadden (1981) analyzed the upper airway morphology of a 4 kg male New Zealand white rabbit using a silicone cast of the lungs reported to be representative of end inspiration state. The length, diameter, and branching angle was measured for each airway from the trachea through the sixth generation. The length data were reported as the average ratio of the longer to shorter daughter airway in each generation. The diameters were presented as the major to minor daughter airway diameter ratio, the major daughter to parent airway diameter ratio, and the minor daughter to parent airway diameter ratio, averaged for each generation. Average branching angles were reported for the minor and major daughter airways for each generation.

Rodriguez et al. (1987) studied the morphology of the rabbit pulmonary acinus of the right upper lobe in a 2.9 kg rabbit. Silicone rubber was instilled into the airways to create a silicone cast of the lungs at an inflation level of about 55% of total lung capacity (TLC). All acini were trimmed from the right upper lobe and the average number of acini, acinar volume, number of branches per acinus, and branching factor (i.e., the average number of daughter airways at a branch) were determined. An average length of 7 branching generations was found for the acini. The branching factor was reported by acinar generation, and closely approximated regular dichotomy for the first five generations, after which it decreased. The average airway length, inner diameter (i.e., excluding alveoli), and outer diameter (i.e., including alveoli) were reported for 9 branching generations of the alveolar ducts and for the alveolar sacs.

In order to combine the airway lengths and diameters reported in the various studies, it is necessary to consider the lung inflation level used in each casting study. Branching angles and gravity angles were assumed to be constant with respect to inflation level. Klimont (1974) created casts by infusing at a minimum pressure gradient, Ramchandani et al. (2001) prepared casts in a typical experimental airway pressure of 20 cm H₂O, Schlesinger and McFadden (1981) used a cast of the end inspiration state of the lungs,

and Rodriguez et al. (1987) created casts corresponding to 55% of TLC. Of the Schlesinger and McFadden (1981) data, only the branching angles were used, so no adjustment was needed for these data. Assuming that a lung under no pressure corresponds to approximately the functional reserve capacity (FRC, the volume at the end of a normal, resting expiration), we further assumed that “minimum pressure” casts used by Kliment (1974) corresponds to approximately the FRC plus the tidal volume (TV), or the volume after a normal, resting inspiration. The FRC is approximately 40% of the TLC (Stahl, 1967) and the FRC plus TV is approximately 60% (Tortora and Grabowski, 1996), so we also assumed that the acinar region casts of Rodriguez et al. (1987) correspond to the FRC plus TV. Ramchandani et al. prepared lung casts at 20 cm H₂O of pressure, which is a typical experimental pressure that is meant to inflate the lungs to a volume typical of normal breathing conditions, so we assumed the inflation level is approximately the same as that of Kliment (1974) and Rodriguez et al. (1987). Since only branching angles, which were assumed to be constant with respect to inflation level, were taken from the data of Schlesinger and McFadden (1981), and all other casts were assumed to correspond to approximately the same level of inflation, no adjustment was made before combining the data.

2.5.2 Branching generations

The number of branching generations in the morphology model was determined by integrating data on the number of conducting airways (Ramchandani et al., 2001; Kliment, 1974), the number of pulmonary airways (Rodriguez et al., 1987; Kliment, 1974), the number of alveoli (Kovar et al., 2002; Kliment, 1974), the branching factor (Rodriguez et al., 1987), the fraction of alveoli in each pulmonary generation (Wojciak, 1988), and the volumes of the acini and total lung (Rodriguez et al., 1987; Kliment, 1974). The procedure is illustrated in Figure 1.

The number of airways was set to 1 for generation one (i.e., the trachea), and 2 for generation two (i.e., the main bronchi). Airway branching in the upper branches of the different lung lobes tends to be dichotomous, but the Ramchandani et al. (2001) data reveal a slight decrease in the branching factor beginning at generation six due to termination of shorter TB pathways. Therefore, the Ramchandani et al. (2001) data were used to define the number and diameter of airways in generations three through six, and branching factors less than or equal to two were used to compute the number of airways in subsequent generations. The values of the branching factors were chosen primarily to approximate the cumulative number of conducting airways, 16,000 (Ramchandani et al., 2001), and the number of acini, 20,000 (Rodriguez et al., 1987). A branching factor of 1.93 was used to compute the number of airways in generations seven through 12, and a factor of two (i.e., dichotomous branching) was used for the last three generations of the tracheobronchial region (13 through 15) and the first three pulmonary generations (16 through 18). These factors were supported by the data of Rodriguez et al. (1987) who measured a branching factor of two for the first four pulmonary generations, after which the branching factor decreased due to termination of shorter acinar pathways, resulting in an overall factor of 1.93.

The final generation (21) represents the alveoli in alveolar sacs. Kovar et al. (2002) estimated the total number of alveoli in rabbit lungs at 50 million. This value was chosen over Kliment's (1974) estimate of 17 million because the methods used by Kovar et al. were more clearly described, and 50 million falls between the numbers for the rat (30 million) and the human (300 million) reported by Yeh and Schum (1980). The alveoli were distributed throughout the pulmonary region using the data of Wojciak (1988) on the fraction of alveoli per pulmonary generation, which was derived based on the original work of Weibel (1963). Wojciak (1988) used eight pulmonary generations for rat and human models, though we use seven for the rabbit based on measurements made by Rodriguez et al. (1987). The number of alveoli in each generation is computed by multiplying the fraction of alveoli per generation times the total number of alveoli for the first six pulmonary generations, and the remaining alveoli are lumped into the final generation. Consistent with the finding of Rodriguez et al. (1987), the branching factor used to compute the number of airways in generations 19 and 20 was reduced to 1.9, which led to a lung volume estimate that falls between the values reported by Rodriguez et al. (1987) and Kliment (1974).

2.5.3 Airway diameters and lengths

Airway diameters were estimated from the data of Ramchandani et al. (2001) and Rodriguez et al. (1987). The diameters of the first 9 generations of airways are those of Ramchandani et al. (2001). The diameters of the last five generations of conducting airways were averages of the Ramchandani et al. (2001) diameters, weighted by the number of airways to match the number of airways in our model. In the alveolated airways, Ramchandani et al. (2001) reported inner diameters and outer diameters. The inner diameter is the diameter of the airway excluding the alveoli, and the outer diameter is the diameter including the additional size of the alveoli. Inner diameters were used for the first 3, partially alveolated airways of the pulmonary region, and outer diameters were used for the more fully alveolated airways (Rodriguez et al., 1987). The diameter of the last generation is the diameter of an alveolus (Kovar et al., 2002).

Airway lengths were derived from the data of Kliment (1974), Rodriguez et al. (1987), and Kovar et al. (2002). The lengths for the conducting airways were derived from Kliment (1974). The lengths of the pulmonary airways were derived from the data of Rodriguez et al. (1987). The length of the last generation is the diameter of an alveolus (Kovar et al., 2002).

Airway lengths and diameters were scaled using the method described by Wojciak (1988). The dimensions were scaled down to the FRC plus TV/2 (i.e., the average volume during respiration) from the cast volume that was estimated to correspond to the FRC plus TV.

2.5.4 Branching and gravity angles

Schlesinger and McFadden (1981) provided the only data on branching angles in rabbit lungs. The angles were reported for both the major (larger) and minor (smaller) daughter

395 airways for the first 6 branches from the trachea, which corresponds to generations 2
396 through 7 in our model. This format is appropriate for an asymmetrical or multi-path
397 lung model, but it had to be condensed to a single, average branching angle for each
398 generation for the typical path model. For example, rather than having a minor airway
399 branch at an angle of 60 degrees from the parent airway, and a major branch at an angle
400 of 0 degrees, the average branching angle of 30 degrees is used.

401
402 The branching angle for the trachea is set to zero, and the angles for generations 2
403 through 7 are the average major and minor angles from Schlesinger and McFadden
404 (1981). No other data on the branching angles were available for further generations.
405 However, the rat and human data of Yeh and Schum (1980) both exhibit a trend of
406 increasing angles up to 45 degrees in the pulmonary region. The rabbit was assumed to
407 exhibit a similar trend, and the angles in generations 8 through 14 were linearly
408 interpolated from 35 degrees in generation 7 to 45 degrees in generation 15, and all
409 angles in the remaining pulmonary region were set to 45 degrees.
410 There were no data on the gravity angles in the rabbit, so the rat data of Schum and Yeh
411 (1980) were used.
412

3. Results

3.1 Morphometry model

The rabbit lung morphometry model is shown in Table 1. The model has 21 branching generations, with the trachea in generation one and the alveolar sacs in generation 21. The number and dimensions of the final generation are those of individual alveoli at the alveolar sacs. The transitional bronchioles (i.e., the first airways with alveoli) are in generation 15, so there are 14 conducting airway generations and seven pulmonary generations. Fourteen conducting generations is somewhat less than the average number of 18 reported by Rodriguez et al. (1987), though the data are highly variable and the distribution of transitional bronchiole locations range from generations 13 to 26. Seven pulmonary generations agrees with the average number of 7.2 (range 5 to 10) reported by Rodriguez et al. (1987).

3.2 Head deposition

The simulated head deposition is compared to the experimental data of Raabe et al. (1988) in Figure 4. Our model fits the small and large diameter data well, given the large variability among the data, which may be due to unknown inhalation rates since plethysmographic data were not recorded. Assuming a different flow rate would result in different parameter estimates for the model. Note that the model given in equation (10) predicts an increase below about 0.3 μm due to increasing diffusivity, while equation (11) must go through the point (0,0) and would underestimate the first data point at 0.11 μm . The data exhibit a curious reduction in the relative amount of head deposition at the largest particle diameter of approximately 10 μm , which is accompanied by an increase in the relative amount of particulate in the GI tract (figure 5). Hypothesizing that this is the result of increased mucus secretion and clearance in the head in response to the high levels of deposition, the model was exercised to simulate enhanced mucocilliary clearance in the head for particles above 6 μm , as shown by the dashed lines in figures 4 and 5. The simulations indicate that this is one plausible explanation for these data.

3.3 Tracheobronchial and pulmonary deposition

Figures 6, 7, and 8 show the simulations of tracheal, bronchial, and pulmonary deposition compared to the experimental data of Raabe et al. (1988). Though the data are quite variable, the simulations agree fairly well with the tracheal and pulmonary data. The bronchial simulations in Figure 7 are the most discordant with the data, with nearly 15% deposition of inhaled 0.11 μm particles, and inconsistent percentages measured for the larger particle diameters. However, it seems unlikely that any model with a single set of parameter values for all diameters (e.g., TV, F, mucus velocities) could reproduce all of the bronchial deposition data.

3.4 Clearance

Figure 9 shows the simulation of the clearance of 4.5 μm particles compared to the deposition data of Gearhart and Schlesinger (1988). The model was run with TV=15 mL, F=80 breaths/min, and tracheal mucus velocity = 8 mm/min fitted to the measured data. Figure 9 shows good agreement between the model and the experimental clearance data.

3.5 Application to *B. anthracis* data

The completed model was used to deconvolute a set of experimental data collected in rabbits following inhalation of *B. anthracis* spores (Gutting et al., 2008) (Figure 10). Rabbits were exposed to spores, and then sacrificed at 0, 6, 12, and 24 hours (2 per group). The lungs were removed, and the number of spores counted. The individual animals' exposures were simulated, incorporating individual plethysmographic parameters (tidal volume and breaths per minute). The simulations showed that initial deposition differences among groups based on different tidal volumes (TV) and breathing rates (bpm) resulted in the apparent increase in CFU from 0 to 12 hours. Without simulation, it was not possible to definitively explain the increasing trend, which is contrary to the notion of clearance and vegetation of the spores.

4. Discussion

A rabbit lung morphology model was developed, and used with a deposition and clearance model. The morphology model is a typical path model based on data from multiple sources in the literature. The deposition model includes diffusion, inertial impaction, and sedimentation calculations based on the work of Schum and Yeh (1980). An empirical head deposition model was developed which includes terms for deposition via diffusion and inertial impaction. A mucocilliary clearance model was developed for the conducting airways based on the work of Kimmell et al. (2001), and pulmonary clearance modeled after macrophage mediated clearance.

This research effort has focused initially on the development of a typical path model. The typical path model is a simplification of the lungs complex geometry, and its advantage lies in its ease of implementation and relatively low data and computational requirements, while the obvious disadvantage is that it does not capture the full morphological asymmetry and complexity of the actual lungs. Other approaches that account for asymmetric structure include multiple path (Anjilvel and Asgharian, 1995) or lobar (Schum and Yeh, 1980; Yeh and Schum, 1980; Asgharian et al., 2001) models, and stochastic models (Koblinger and Hofman, 1990; Sturm and Hofmann, 2004), though these models are more difficult to implement, are more computationally intensive, and require more detailed morphometric data. Furthermore, the typical path model has been reported to give results that are quite similar to more sophisticated, multiple path models (Asgharian et al., 2006), although this statement was made in reference to the more symmetric human lung. Although the rabbit lung airway is known to be more asymmetric or monopodial than this idealized model, it is expected that, on average, course, regional deposition (e.g., tracheobronchial or pulmonary) will be adequately characterized by this model. An asymmetrical rabbit lung model is currently under

development, which will enable comparisons with the typical path model results for rabbit.

One uncertainty in the morphology model is the use of rat gravity angles for the rabbit lung. These data were not available for the rabbit, so the rat values were used as a surrogate. This assumption of similar angles is reasonable as both the rabbit and rat lung physiology is reported to be strongly monopodial, with similar airway length to diameter ratios (Phalen and Oldham, 1983).

The airways are modeled with fixed lengths and diameters, scaled to correspond to the dimensions at FRC plus one half of the TV. In reality, the dimensions change during the breathing cycle, expanding slightly during inhalation and collapsing during exhalation. The dimensions at one half TV are designed to represent the average airway dimensions throughout the breathing cycle.

A further assumption of the model is that the right apical lobe is representative of all lobes of the rabbit lung. This assumption was necessary due to the availability of only right apical lobe morphometry for some parameters.

Overall, the rabbit lung model simulations were able to reproduce the deposition and clearance profiles observed in Raabe et al. (1988) and Gearhart and Schlesinger (1988). The simulations were in least agreement with the bronchial deposition data. However, the data were highly variable, and plethysmographic data were not available, so it is difficult to determine whether the model is truly incapable of reproducing the bronchial deposition. Despite this, the simulations still capture the general trend of the deposition profiles. The high tracheobronchial deposition of 0.11 μm observed might be due to electrostatic forces, or perhaps hygroscopic growth or agglomeration. The current model does not incorporate these details.

Although not yet published, other researchers have recently developed more detailed rabbit lung morphometry (Corley, 2013). These data will be compared with our model, and eventually used to enhance rabbit lung deposition predictions.

In summary, we have developed a typical path deposition and clearance model of the lungs of rabbits. The model simulations reproduced most of the available experimental data of Raabe et al. (1988), and the lung clearance data of Gearhart and Schlesinger (1988). In combination with human lung models, this lung particle dosimetry model has application to extrapolation of animal inhalation toxicity data to humans. The model was successfully applied to explain irregularities in experimental data with *B. anthracis* spore deposition, confirming the data and enhancing its validity. Future research in this area includes the development of an asymmetrical model of the rabbit lungs for comparison to this symmetric model.

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Figure 1
Illustration of the combination of morphometry data from the various sources to derive the number of airways per branching generation

Figure 2

Illustration of airway diameter estimation is based on data from Ramchandani et al. (2001) and Rodriguez et al. (1987). The diameters for the last five generations of conducting airways were averages of the Ramchandani et al. (2001) diameters, weighted by the number of airways. Inner diameters were used for the first 3, partially alveolated airways of the pulmonary region, and outer diameters were used for the more fully alveolated airways. The diameter of the last generation is the diameter of an alveolus (Kovar et al., 2002).

Figure 3

Illustration of airway length estimation from the data of Kliment (1974), Rodriguez et al. (1987), and Kovar et al. (2002). The lengths for the conducting airways were derived from Kliment (1974). The lengths of the pulmonary airways were derived from the data of Rodriguez et al. (1987). The length of the last generation is the diameter of an alveolus (Kovar et al., 2002).

Table 1. Typical path lung morphology model for the rabbit. Generation 1 is the trachea. Average number, diameter, length, branching angle (β), and gravity angle (γ) for each branching generation derived from multiple sources. γ = degrees from horizontal.

Generation	Number	Diameter (cm)	Length (cm)	β (deg)	γ (deg)
1	1	0.53	5.6	0	4
2	2	0.42	2.2	30	0
3	6	0.26	2.3	30	4
4	12	0.18	0.20	32	19
5	24	0.13	0.094	29	31
6	48	0.098	0.13	38	32
7	93	0.068	0.095	35	29
8	179	0.050	0.074	36	32
9	345	0.039	0.069	38	35
10	666	0.032	0.056	39	32
11	1285	0.029	0.048	40	29
12	2481	0.028	0.048	41	34
13	4962	0.027	0.048	43	32
14	9923	0.028	0.042	44	32
15	19846	0.028	0.040	45	33
16	39692	0.022	0.038	45	32
17	79384	0.021	0.029	45	30
18	158769	0.042	0.027	45	30
19	306424	0.042	0.025	45	30
20	591398	0.041	0.021	45	30
21	37881000	0.010	0.010	45	30

Figure 4. Head deposition simulations. The solid line is the model prediction and the points (mean $\pm$ sd) are the data of Raabe et al. (1988). The dashed line is the model prediction assuming that the mucus clearance rates increased in response to the larger particles and greater head deposition, resulting in enhanced clearance. The model was run for a 45-minute exposure period assuming TV=20 mL and F=50 breaths/min.

Figure 5. Gastrointestinal (GI) tract burden. The GI tract receives the particles cleared in the mucus from head, larynx, and tracheobronchial airways. The solid line is the model prediction and the points (mean $\pm$ sd) are the data of Raabe et al. (1988). The dashed line is the model prediction assuming that the mucus clearance rates increased in response to the larger particles and greater head deposition, resulting in enhanced clearance. The model was run for a 45-minute exposure period assuming TV=20 mL and F=50 breaths/min.

Figure 6. Tracheal deposition in the rabbit. Solid lines are model predictions and the points (mean $\pm$ sd) are the data of Raabe et al. (1988). The model simulates a 45-minute exposure with TV=20 mL and F=50 breaths/min.

Figure 7. Bronchial deposition in the rabbit. Solid lines are model predictions and the points (mean $\pm$ sd) are the data of Raabe et al. (1988). The model simulates a 45-minute exposure with TV=20 mL and F=50 breaths/min.

Figure 8. Pulmonary deposition in the rabbit. Solid lines are model predictions and the points (mean $\pm$ sd) are the data of Raabe et al. (1988). The model simulates a 45-minute exposure with TV=20 mL and F=50 breaths/min.

Figure 9. Clearance of 4.5 μm particles from the rabbit lungs. Solid lines are model predictions and the points are the data of Gearhart and Schlesinger (1988). The model simulates a 10-minute exposure via an oral tube, with $\text{TV}=15\text{ mL}$, $\text{F}=80\text{ breaths/min}$, and a tracheal mucus velocity of 8 mm/min .

Figure 10. Simulation of deposition and clearance of *B. anthracis* spores (colony forming units, CFU) in rabbits. Rabbits were exposed to spores, and then sacrificed at 0, 6, 12, and 24 hours (2 per group). The lungs were removed, and the number of spores counted (CFU, filled circles). The individual animals' exposures were simulated (solid lines, green = 0 hr, black = 6 hr, blue = 12 hr, red = 24 hr), incorporating individual plethysmographic parameters. The simulations explained that initial deposition differences among groups based on different tidal volumes (TV) and breathing rates (bpm) resulted in the apparent increase in CFU from 0 to 12 hours. Data collected by Gutting et al. (2008).