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Development of a Zealand White Rabbit Deposition Model to Study Inhalation Anthrax

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Abstract

Despite using rabbits in several inhalation exposure experiments to study diseases such as anthrax, there is a lack of understanding regarding deposition characteristics and fate of inhaled particles (bio-aerosols and viruses) in the respiratory tracts of rabbits. Such information allows dosimetric extrapolation to humans to inform human outcomes. The lung geometry of the New Zealand white rabbit (referred to simply as rabbits throughout the article) was constructed using recently acquired scanned images of the conducting airways of rabbits and available information on its acinar region. In addition, functional relationships were developed for the lung and breathing parameters of rabbits as a function of body weight. The lung geometry and breathing parameters were used to extend the existing deposition model for humans and several other species to rabbits. Evaluation of the deposition model for rabbits was made by comparing predictions with available measurements in the literature. Deposition predictions in the lungs of rabbits indicated smaller deposition fractions compared to those found in humans across various particle diameter ranges. The application of the deposition model for rabbits was demonstrated by extrapolating deposition predictions in rabbits to find equivalent human exposure concentrations assuming the same dose-response relationship between the two species. Human equivalent exposure concentration levels were found to be much smaller than those for rabbits.

Keywords

Zealand white Rabbit; lung geometry; morphometric variables; particles; deposition modeling

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INTRODUCTION

Bacillus anthracis is gram-positive, non-motile, spore forming bacterium causing anthrax disease on exposure by contact, ingestion, and inhalation (Frazier et al., 2006). The spore is readily reproducible and can be aerosolized to generate micrometer-size particles. Due to its easy generation and long period of viability in air, *B. anthracis* is potentially a biological weapon of great concern in the biodefense community. Inhalation of *B. anthracis* spores is the most effective route of infection. Once deposited on lung airway surfaces after inhalation, the earliest theories for disease onset and progression involved phagocytization of spores by immune cells (primarily macrophages) which are then translocated to lymph nodes where they germinate, proliferate and release toxins although more recent theories suggest that germination, proliferation and release of toxins can also occur within the lung (Hanna and Ireland, 1999; Weiner and Glomski, 2012).

Risk estimates from inhalation of *B. anthracis* are needed in order to prepare for and protect the public during exposure episodes. Collected exposure-response data support the notion that there is a threshold exposure level for adverse effects (Coleman et al., 2008; Coleman and Marks, 2000). Thus, exposure-dose-response data must be collected to establish risk models in people. However, exposure data in humans are scarce because infection rarely occurs naturally. Alternatively, several species have been used as a surrogate for humans in various inhalation studies to collect information on the deposited dose and biological outcome. In particular, rabbits have often been selected as the animal of choice to study anthrax inhalation (Gutting et al., 2012; Twenhafel, 2010; Yee et al., 2010; Zaucha et al., 1998) in part because they are cheaper than nonhuman primates and easier to house and handle. Consequently, inhalation studies in rabbits were conducted to develop risk models (Gutting et al., 2012, 2013) - expressed as statistical relationships between the deposited dose and mortality, defined as lethal dose for 50% of the population (or LD₅₀). Risk models in rabbits can be readily extended to humans if complemented by exposure-dose models both in humans and rabbits. Thus, mechanistic exposure-dose models are required in both humans (Asgharian et al., 2001) and rabbits to predict the deposited dose of anthrax. Risk estimates in people are made based on a metric of dose (deposition fraction, deposited mass, retained mass, etc.) most relevant to the pathology of disease at the initial site of spore deposition. Assuming similar dose-response relationships in humans and rabbits, risk of disease outcome in the population from anthrax exposure is found by calculating the threshold exposure level in people that yields the given dose metric.

While dosimetry models are available for humans (e.g., Asgharian et al, 2001), there is no inhalation model for rabbits to date despite the use of rabbits in various inhalation studies. The lack of morphometric and physiologic information for the rabbit has hampered such efforts. Emergence of such data will pave the way for the development of a comprehensive inhalation model to interpret experimental studies and predict the inhaled dose to link exposure scenarios with biological outcomes. To extend the available models in humans and other species to rabbits, several key components of the dosimetry model were developed for rabbits. First, particle deposition in the upper respiratory tract (URT) was modeled. Second, the lung geometry for the tracheobronchial (TB) and acinar (A) regions (trees) was constructed based on recently scanned images of the TB geometry and available data on the

acinar region in the literature. In addition, information on lung volumes and breathing parameters were determined as a function of the rabbit weight. Finally, a comprehensive deposition model was developed for inhalation of particles in the respiratory tract of rabbits based on the reconstructed geometry of the lower respiratory tract (LRT) and information on lung and breathing parameters.

MATERIALS AND METHODS

A deposition model was developed to predict the dose of inhaled particles and bio-aerosols such as *B. anthracis* in New Zealand White rabbits. Simplifying assumptions were made to reconstruct the entire geometry of the rabbit and allow rapid and reliable calculations of the deposited dose for the entire respiratory tract. To avoid dealing with structural complexity, lung airways were assumed to be shaped as cylinders. In addition, the alveoli contributed an additional volume in each alveolar duct, where duct dimensions were rescaled by $(FRC / TLC)^{1/3}$ in which FRC is the functional residual capacity and TLC is the total lung capacity. The deposition model was constructed in four steps. The first step consisted of deposition calculations of particles in the URT. The second step involved the construction of the LRT geometry. The third step involved modeling lung ventilation as a means of carrying inhaled particles in and out of the lung. Lung ventilation was based on uniform airway expansion and contraction for slow breathing. A lumped parameter model (network analogy) was used to predict lung ventilation at higher breathing rates (Asgharian et al., 2015). The airflow in each airway was assumed to be fully developed with uniform velocity. The fourth and final step of the deposition model included the formulation of a mathematical model to calculate particle deposition in the LRT. The mathematical model was based on particle population balance or Eulerian description of inhaled particles into and out of the LRT (Asgharian et al., 2001). Particle concentration was uniform across the airway cross-section. Thus, both airflow rate and particle concentration varied only with time and lung depth. A sink term was included in the particle transport equation to account for wall deposition. The sink term was obtained based on deposition efficiencies of particles by inertial impaction, sedimentation, and Brownian diffusion (Asgharian et al., 2001).

Particle deposition modeling in the URT

The URT is defined as the part of the respiratory tract proximal to the trachea. It starts from the nares and includes nasal airway passages, naso-pharynx and larynx extending down to the beginning of the trachea. Inhaled particles deposit in the URT of rabbits by two distinct mechanisms depending on the diameter of the particles. Ultrafine (nano) particles deposit by Brownian diffusion throughout the nasal passages while micrometer (fine) and larger (coarse) diameter particles deposit by inertial impaction. Both deposition mechanisms are present to some extent when sub-micrometer particles are inhaled into the rabbit nasal passages. Particle deposition is expected to reach its minimum in the rabbit nasal passages for sub-micrometer diameter particles (Raabe et al., 1988).

There are no data in the literature on ultrafine deposition of particles either experimentally or computationally. In addition, single *B. anthracis* spore diameter was measured to be sub-micrometer or larger (Carrera et al., 2007; Gutting et al., 2012, 2013). Thus, no attempt was

made in this study to model the deposition of ultrafine particles by Brownian diffusion in the URT of rabbits. Moreover, there are reported measurements on the deposition of fine and coarse particles in the nasal passages of rabbits (Raabe et al., 1988), which could be used to develop a model for deposition of particles in this diameter range. New Zealand white rabbits were exposed briefly to particles between 0.24 and 8.65 μm in resistance diameter and deposition fractions were found for different regions of the respiratory tract. The deposition fractions in the URT were found by adding reported measurements of deposition fractions in the larynx, skull, and G.I. by Raabe et al. (1988). Assuming equal residence times and particle deposition efficiency during inhalation and exhalation in the URT, the following expression was used to calculate deposition efficiencies in the nasal passages (Asgharian et al., 2014).

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

where Δ_{LRT} and Δ_{URT} are measured deposition fractions in the LRT and URT. The calculated deposition fractions for different diameter particles were used to construct a deposition model of particles in the URT of rabbits. A logistic expression was adopted for deposition efficiency of sub-micrometer and larger diameter particles in the URT of rabbits as a function of particle inertial parameter, $\rho d^2 Q$, where ρ is the particle mass density, d is the particle diameter and Q is in the breathing flow rate. The following expression was obtained by fitting calculated deposition efficiencies from equation (1) to the logistic function.

$$\eta_{\text{URT}} = 1 - \frac{0.93655}{1 + (\rho d^2 Q / 237.72)^{2.1659}} \quad (2)$$

with a correlation coefficient of $R^2 = 0.965$. A comparison of model predictions with measurements is given in Figure 1. Particle deposition efficiency is near 0.05 for low particle inertia ($\rho d^2 Q < 50$) and rises rapidly with further increase in inertia and reaches a plateau of 1 around $\rho d^2 Q$ of 3000. The deposition pattern is consistent with the expected inertial behavior of particles.

Animals' weight ranged between 2.343 and 3.475 kg in the study of Raabe et al. (1988), and expression (2) is most valid for the given weight range. However, it can be extended to other weights if additional information such as URT surface area and volume are available (Asgharian et al., 2012; Miller et al., 2014).

$$\eta_{\text{URT}} = 1 - \frac{0.93655}{1 + \left(\frac{S/V}{S_0/V_0} \frac{\rho d^2 Q}{237.72} \right)^{2.1659}} \quad (3)$$

where S and V are the URT surface area and volume, respectively, and S_0 and V_0 are the reference values corresponding to those of animals used in the Raabe et al. (1988) study. Surface area and volume of URT vary with animal weight. Equation (3) may be used to predict deposition fraction of inhaled particles in the URT of other animals if the relationship between S/V ratio and body weight is determined.

Construction of LRT geometry

The LRT geometry was developed in two parts. First, scanned images of the rabbit's respiratory tract were assembled to create a three-dimensional TB airways. A 1-D representation of the airways was constructed by assuming a dichotomous airway branching structure with each airway shaped as a cylinder. Second, the acinar tree was constructed from the data provided by Rodriguez et al. (1987). Both the TB airways and acinar tree were rescaled such that the sum of volumes equaled the TLC. The LRT was completed by attaching the acinar tree to the distal end of each terminal bronchiole of the TB tree.

TB airways—The airways parameters for the TB region were obtained from Kabilan et al. (2016). Briefly, the lung cast of TB airways of a male, New Zealand white rabbit weighing 3.7 kg was made and subsequently imaged at 50 micron resolution using μ CT scanner. The lung cast data were segmented using the method of Timchalk et al. (2001) and Carson et al. (2010). The iso-surfaces of the segmented geometry were extracted and used to construct the airway centerlines from which airway dimensions (length and diameter) and orientations (bifurcation and gravity angles) were obtained (Kabilan et al., 2016). The TB geometry comprised of 3875 airways with 1994 outlets (terminal bronchioles). Airway splitting from parent to daughter included all bifurcations, but there were 128 trifurcations and 2 quadfurcations. Zero-length airways were added to convert the tree geometry to a complete bifurcating structure. The entire geometry comprised of 3987 TB airways with a volume of 2.051 cm³.

Acinar tree—The acinar region contributes most of the volume of the respiratory tract and is made up of thousands of respiratory bronchioles, alveolar ducts, and alveolar sacs, which are covered by millions of alveoli. The complexity of the geometry and enormity of the number of airways make geometric reconstruction of the acinar region a major challenge. Rodriguez et al. (1987) examined the geometry and obtained morphometric parameters of intracinar airways in the silicone rubber cast of a 2.9 kg White New Zealand rabbit. Various acinar parameters such as airway length, inside (duct) and outside (duct plus alveolus) diameters, cast volume, acinar path length and volume, total number of acini, lobar volumes, and total lung capacity (TLC) were obtained from cast measurements. Airway structures of several acini were reconstructed to find the range and mean for the number of acinar generations. The mean number of total generations for the acinar region was reported to be 7. Acinar parameters were reported at 55% of TLC. These measurements were used here to construct the acinar (alveolar) geometry of the rabbit. To retain the overall shape and volume, cumulative acinar volumes and airway path lengths, length-diameter ratio were calculated for the initially reported 10-generations of the acinar geometry (Table 3 of Rodriguez et al., 1987). A typical 7-generation, symmetric acinar geometry was adopted and cumulative volumes, lengths, and length-to-diameter ratios were redistributed among the

seven generations from which volume (diameter) and length were determined for a typical airway in each generation. The volume of alveoli per generation and per duct was found from the difference between total acinar and duct volumes. Acinar airways were assumed to be oriented randomly. Airway dimensions were rescaled to match the calculated acinar volume with that measured by Rodriguez et al. (1987).

LRT geometry—Animals of different weights were used in studies to measure deposition fraction of particles in the URT, and to construct the TB airways and acinar tree. Animals' weights were 2.9 kg (average of 2.343 and 3.475 kg), 3.7 kg, and 2.9 kg in the URT particle deposition, TB airways and acinar tree reconstruction studies, respectively. To get the most utility from available information, the LRT geometry was developed for a 2.9 kg New Zealand white rabbit by scaling TB airways and acinar tree of the LRT to the same TLC. Unlike Rodriguez et al. (1987), the TLC was not reported in the morphometric study of the TB airways (Kabilan et al., 2016). Hence, the volume and dimensions of TB airways were rescaled by the ratio of the measured TB volume of Rodriguez et al. (1987) (5 cm^3 at 55% of TLC) to that calculated for the reconstructed TB airways of Kabilan et al. (2016) (i.e., 2.05 cm^3). Figure 2 gives the rescaled airway lengths (Figure 2A) and diameters of the TB airways (Figure 2B) at TLC. Mean length and diameter are shown by the solid line in Figure 2. There was a significant variation in individual airway dimensions between generations 5 and 30 and more so for the length than diameter. On average, both length and diameter decreased rapidly in the first 5 generations, followed by a slow decline with increasing generation number afterward.

More insight into TB airway structure is gained by counting the number of different airways per generation. Figure 3 gives the number of airways and terminal bronchioles at different generations of the TB tree. There were no terminal bronchioles in the first 5 generations after which the number of terminal bronchioles increased sharply to reach a peak around generation 17 followed by a decline to zero around generation 30. Most airways past generation 25 were terminal bronchioles and half the airways around generations 15 to 17 were terminal bronchioles. The shape of the airway number curve appeared to be similar to a normal distribution for both the airway numbers and number of terminal bronchioles.

The reconstructed typical-path acinar tree was rescaled in proportion to the number of terminal bronchioles identified in the Kabilan et al. (2016) TB airways to preserve the initial TLC. Alveolar (acinar) airway parameters for the New Zealand white rabbit are given in Table I. Airway length decreased gradually with generation number but increased for the last generation (alveolar sacs). Airway diameter stayed relatively constant for all airway generations. Figures 4A and 4B give length and diameter variation from terminal bronchioles through the acinar region. Except for a few airways, the majority of terminal bronchioles have dimensions slightly higher than those of the first respiratory bronchioles, which indicated smooth transition of airway dimensions from the TB to A region. Acinar airways were assumed to be randomly oriented. Thus, random bifurcation and gravity angles were selected in each generation. The rescaled acinar tree was attached to each outlet (terminal bronchiole) of the TB airways to complete the LRT geometry.

Lung ventilation and LRT volumes and breathing parameters

Inhaled materials travel to different sites within the respiratory tract by the established airflow within the LRT. The flow field is influenced by airway dimensions, lung volumes, and ventilation pattern. Thus, information on lung dimensions, volumes, and breathing rates are needed for rabbits at different ages (weights) to calculate particle deposition in the lungs. For lung volumes, the measurements of Crossfill and Widdicombe (1961) were used to obtain relationships between FRC and body weight as well as TLC and body weight.

$$\text{FRC} = 0.316228 \times \text{BW}^{0.6}. \quad (4)$$

$$\text{TLC} = 0.288403 \times \text{BW}^{0.74}. \quad (5)$$

where FRC and TLC are in units of cm^3 and body weight is given in grams. Stahl (1967) collected data from the literature on respiratory variables in different species from small rodents to large animals such as cattle. Miller et al. (2014) developed allometric relationships by fitting the data to models of breathing parameters as a function of the weight of the species.

$$\text{BF} = 53.5 \times \text{BW}^{-0.26}. \quad (6)$$

$$\dot{V}_E = 0.499 \times \text{BW}^{0.809}. \quad (7)$$

$$\text{ASA} = 37.325 \times \text{BW}^{0.95948}. \quad (8)$$

where BF is the number of breaths per minute, $\dot{V}_E$ is the ventilation rate (LPM), ASA is the alveolar surface area (cm^2), and BW is the body weight in units of kg in equations (6) and (7) and gram in equation (8). In the absence of information, the above relationships were used to predict breathings rates and alveolar surface area in rabbits.

It has been shown by Yu (1978) that airflow in an airway is obtained from the solution of the continuity (mass balance) equation for the inhaled air and in each airway is proportional to the distal lung volume to that airway. This model provides a means to calculate airflow rate throughout the lung (Asgharian et al., 2001). Airflow inertance becomes significant at higher breathing rates. Assuming various forces on the air to be independent, the lumped parameter method (RLC or electrical network analogy) may be used to solve for the airflow field. Asgharian et al. (2015) found airflow rate in each airway to be proportional to the regional

lung compliance distal to that airway. Subsequently, a mathematical model was developed to calculate lung ventilation throughout the lung.

The above models for low and high breathing rates were also used to find lung ventilation in rabbits. The flow field in each airway was assumed to be steady and fully developed (parabolic) because the Reynolds numbers for the flow were very low for the majority of airways in the lung except for the first few upper airways of the LRT. Hence, average velocity in each airway was found from predicted values for the flow rate in a parabolic flow field. The flow velocity was used in the mass transport equations for particles to find particle deposition in lung airways.

Particle deposition formulation

The mathematical model to predict particle deposition is a one-dimensional (1D) Eulerian description of particle mass balance through each airway of the LRT:

$$\frac{\partial C}{\partial t} + \left(\frac{Q}{A'} - \frac{D}{A'} \frac{\partial A}{\partial x} \right) \frac{\partial C}{\partial x} = D \frac{\partial^2 C}{\partial x^2} - \lambda C \quad (7)$$

where t is the elapsed time, x is the axial coordinate of the airway, C is the airway cross-section averaged particle concentration, D is the diffusion coefficient, A' is the airway cross-sectional area, and λC is the mass of particles deposited on airway walls per unit time per unit volume of the airway (Yu, 1978; Asgharian and Price, 2007). Particle deposition by different mechanisms (i.e., sedimentation, inertial impaction, Brownian diffusion, etc.) is represented by a sink term in the above equation and calculated from expressions derived for a fully developed flow of particles in a cylindrical airway (Asgharian et al., 2001). Equation (7) is solved analytically or numerically to find particle concentration in all airways of the LRT. The deposition fraction of inhaled particles, defined by the fraction of inhaled particles that deposit in an airway, is calculated from the following expression (Asgharian and Price, 2007).

$$DF = \int_0^T \int_0^L \lambda C A dx dt \quad (8)$$

where T is the breathing period and L is the airway length. Regional (TB and A) and total deposition fraction of particles are found by calculating deposition fraction per airway from equation (8) and adding them together in a particular region or for the entire LRT.

Interspecies exposure assessment

Inhalation studies conducted in rabbits are intended to evaluate the outcomes in rabbits followed by extrapolation to people to assess the risk for exposure scenarios. Dosimetry models in rabbits and humans bridge the gap to allow interspecies extrapolation. Risk models in rabbits provide doses that lead to disease/mortality/morbidity outcomes. If the relationships hold in humans, equivalent exposure levels can be found in humans that result

in the same doses and disease outcomes. A relevant dose metric for anthrax exposure is the deposited mass of anthrax spores in a particular region of the respiratory tract. Human equivalent exposure concentration (HEC) can be estimated by (Jarabek et al., 2005):

$$\frac{HEC}{C_R} = \frac{(\dot{V}_E)_R}{(\dot{V}_E)_H} \times \frac{DF_R}{DF_H} \quad (9)$$

where C_R is the rabbit exposure concentration and DF is the deposition fraction in the region of interest in the respiratory tract for humans (H) and rabbits (R).

RESULTS AND DISCUSSION

The four components of the deposition model (URT model for particle deposition, lung geometry, lung physiology, and particle transport model) were combined to construct the deposition model for New Zealand white rabbits. The respiratory tract geometry of rabbits was created from morphometry of multiple rabbits. While the URT deposition model was constructed using measurements in eight rabbits, TB airways and the acinar tree were each based on a single rabbit. Collecting information from different sources to develop a complete geometry of the respiratory tract may impose limitations on rigorous evaluation of the model. However, the proposed model is the first attempt to develop a comprehensive model to predict particle deposition in the entire respiratory tract of rabbits. Model improvements may be made once additional data on the geometry and deposition emerge.

Particle deposition in the URT was limited to fine and coarse particles according to equations (2) or (3) due to lack of data on ultrafine particle deposition, which occurs by Brownian diffusion. This restriction does not impose a limitation on deposition prediction of anthrax spores, which are around one-micrometer in diameter (Gutting et al., 2012, 2013). In this study, deposition fractions and exposure concentrations were calculated for particle diameters 0.1 μm and above in accordance with URT deposition data. Model predictions were based on a 2.9 kg rabbit with lung and breathing parameters calculated from equations (4) through (8) by solving equations (7) and (8) in each airway of the LRT.

Limited measurements are available on particle deposition in the lungs of rabbits. Raabe et al. (1988) reported deposition of 0.19 μm to 8.65 μm resistance diameter particles in eight New Zealand white rabbits with an average weight of 2.9 kg. The deposition results in the URT were used in this study to construct a semi-empirical model for deposition predictions in the URT region. This dataset presents a unique opportunity for comparison of model predictions with measurements in the TB and A regions. The comparison for the TB and A regions are given in Figures 5A and 5B, respectively. There are reasonable agreements between predictions and measurements for a few data points. However, there are significant differences for other cases. Uncertainty regarding study variables (such as minute ventilation, which was not measured and has a significant impact on deposition predictions) may contribute to the differences. In addition, there was a noticeable scatter in the Raabe et al. (1988) deposition data, which may have also contributed to the discrepancies. Generally,

the difference between predictions and measurements did not exceed 0.05 except for one data point.

Model application to *B. anthracis* spores requires verification of predicted deposition by comparing it against available measurements. Gutting et al. (2012) and (2013) exposed New Zealand white rabbits to *B. anthracis* spores with mass median diameter of $1\ \mu\text{m} \pm 0.3\ \mu\text{m}$ and measured the lung content of anthrax spores. Table II lists measured and predicted deposition fractions. Reported measurements were for two groups of sham vaccinated and vaccinated rabbits. Overall, the agreement between predictions and measurements were reasonable. The reported deposition fractions varied between 1.33% and 4.93%, which were somewhat lower than model predictions of 7.62%. However, as discussed by Kabilan et al. (2016), deposition measurements may have been underestimated due to epithelial cell internalization of the deposited particles in the Gutting (2013) study and the inability of Broncho-alveolar lavage to wash out all deposited particles in the Gutting et al. (2012) study.

To investigate the efficiency of the rabbit lung in removing inhaled airborne particles, deposition fraction of particles in the TB, A, and LRT regions were calculated for particle diameters between $0.1\ \mu\text{m}$ and $10\ \mu\text{m}$. Model predictions for endotracheal breathing (i.e. bypassing the URT region) are given in Figure 6. Deposition fraction was smaller than 0.05 in the TB region for $1\ \mu\text{m}$ and smaller diameter particles. There was a steady increase in TB deposition fraction by inertial impaction for particles larger than $1\ \mu\text{m}$, which exceeded 0.8 for $10\ \mu\text{m}$ particles. Deposition fraction of sub-micrometer particles was generally greater in the A region than that for the TB region but remained below 0.2. As in the TB region, a rapid rise was observed for deposition of micrometer and larger particles in the A region, which was mainly due to gravitational settling. However, the removal of particles in the TB region limited particle deposition in the A region as particle diameter increased. Consequently, the deposition fraction reached a peak in the A region around $4\ \mu\text{m}$ followed by a decrease in deposition fraction to less than 0.01 at $10\ \mu\text{m}$. Overall, deposition fraction of sub-micrometer diameter particles was below 0.2 in the LRT but rose rapidly for fine and coarse particles. Thus, the rabbit lung is inefficient in removal of sub-micrometer particles but highly efficient for that of fine and coarse particles. The analysis assumed no head deposition, the inclusion of which may drastically change LRT deposition characteristics.

Regional deposition fraction of particles in the respiratory tract of rabbits is shown in Figure 7 when nasal filtering of inhaled particles is included in the deposition model. URT deposition fraction of sub-micrometer particles was between 0.1 and 0.15. As a result, particle deposition fractions were further reduced to 0.1 and 0.05 in the TB and A regions, respectively, when compared to the case of bypassing the nasal passages (Figure 6). In addition, particle deposition fraction in these regions was below 0.1 for particles between $1\ \mu\text{m}$ and $4\ \mu\text{m}$, which was due to high deposition of particles in the URT. There was no particle deposition in the TB and A regions for $4\ \mu\text{m}$ and larger diameter particles unlike measurements of Raabe et al. (1988) (Figure 5). Overall, there was limited deposition of particles in the LRT of rabbits (about 0.12 for around $0.1\ \mu\text{m}$ diameter particles and below 0.1 for larger diameter particles).

Deposition fraction per airway generation or with lung depth is given in Figure 8 for particle diameters of 0.2 μm and 2 μm . Deposition fraction was more significant for 2 μm diameter particles than for 0.2 μm in the first 15 airway generations, due to the stronger inertial mechanisms. However, similar deposition fractions were observed for both particle diameters in deep generations of the LRT despite particles being deposited by separate mechanisms. These airways were likely in the A region and deposition occurred by sedimentation for 2 μm diameter particles and Brownian diffusion for 0.2 μm diameter particles. Particle deposition remained fairly uniform in the deep lung for both diameter particles and dropped quickly to zero in the most distal airways of the rabbit lung.

The particle deposition model for rabbits can be used for interspecies extrapolation from laboratory settings to actual human exposure scenarios. Equation (9) gives the relationship between human and rabbit exposure concentrations when dose-response relationship holds for both species. Deposition fractions in rabbits and humans were calculated using the deposition model for rabbits in this study and that for humans (Asgharian et al., 2001). The ratio of HEC to rabbit exposure concentration was obtained based on deposition fraction predictions and minute ventilations for humans and rabbits. The results are given in Figure 9 for the URT, TB, and A regions. The results suggest stringent human exposure levels as compared with that of rabbits. The HEC was about 0.16 times that of the rabbit exposure concentration for the URT region. The human exposure concentration is approximately half that for the URT in the TB and A, and thus LRT regions (about 0.07 of rabbit exposure concentration). This is likely due to the fact that human lungs are more efficient than rabbit lungs for particle deposition. It is noted that the finding is based on using deposited mass as the dose metric. However, different relationships may be obtained if a different dose metric such as retained mass is selected. The relevant dose metric is the one most closely related to disease initiation and progression and in this example is considered to be the deposited mass based on available information in the literature (e.g., Hanna and Ireland, 1999).

CONCLUDING REMARKS

A deposition model for inhalation of particles was developed in rabbits and applied to the scenario of inhalation of *Bacillus anthracis* spores, which are around one-micrometer in diameter. Particle deposition fraction in the lungs of rabbits was found to be relatively low (under 0.2), which was consistent with reported deposition measurements. Together with the deposition model for humans, the rabbit deposition model is a useful tool to interpret collected data in the lab in rabbits or real-life scenarios of human exposure to bio-aerosols and predict disease outcome in people. Model application was given by calculating equivalent human exposure levels based the deposited mass of particles in the lungs of rabbits in laboratory inhalation exposure settings. Further enhancement of the model is envisioned by linking the exposure-deposition model of this study to available risk (dose-response) models in rabbits and ultimately constructing a risk model for humans for use in case of exposure to bio-aerosols and other hazardous agents.

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Figure 1.
Deposition efficiency by inertial impaction of particles in the rabbit URT.

Figure 2.

TB Airway dimensions of Kabilan et al. (2016) at TLC for different airway generation numbers. A. length; B. Diameter. The solid lines represent the mean values for individual length and diameter values (open circle).

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Figure 3.
Number of TB airways of Kabilan et al. (2016) lung geometry for different airway generations.

Figure 4.
Airway dimensions of the rabbit lung extending from the terminal bronchiole to alveolar sac.
A. Length; B. Diameter.

Figure 5.

Comparison of predicted deposition fraction of particles in different regions of a 2.9 kg rabbit with available measurements in the literature, A. TB deposition; B. A deposition.

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Figure 6. Deposition fraction of inhaled particles in different regions of a 2.9 kg rabbit assuming endotracheal breathing.

Figure 7.
Deposition fraction of particles in different regions of a 2.9 kg rabbit via nasal breathing.

Figure 8. Deposition fraction of particles in different generation numbers of a 2.9 kg rabbit via nasal breathing.

Figure 9. Human equivalent exposure concentration calculated based on deposition of 1 μm spore diameter in different regions of the respiratory tract.

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Table I

Acinar geometry of New Zealand white rabbit.

Generation Number	Airway length (cm)	Airway diameter (cm)	Volume of Alveoli per Duct (cm ³)	Bifurcation angle (degrees)	Gravity angle (degrees)
1	0.0447	0.0252	0.0252	45	38.24
2	0.0414	0.0284	0.0284	45	38.24
3	0.0407	0.0259	0.0259	45	38.24
4	0.0350	0.0239	0.0239	45	38.24
5	0.0317	0.0242	0.0242	45	38.24
6	0.0349	0.0287	0.0287	45	38.24
7	0.0744	0.0277	0.0277	45	38.24

Table IIComparison of predicted *B. anthracis* spores deposition with model prediction

Study	Spore diameter	Deposition fraction
Gutting et al. (2013)	1 μm $\pm$ 0.3 μm	0.0433 $\pm$ 0.022 0.0493 $\pm$ 0.008
Gutting et al. (2012)	1 μm $\pm$ 0.3 μm	0.0307 $\pm$ 0.009 0.0133 $\pm$ 0.002
Model prediction	1 μm	0.0762