Guidance on Suitable Animal Models to Reflect Deposition Efficiency in Humans as a Function of Age

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Abstract
Too frequently small animal models are used to justify and set human therapeutic dosing of new pharmaceuticals. This is done irrespective of the target human population (i.e. age, health state). This work uses a static statistical model to evaluate a range of animal models in comparison to a range of human ages and lung remodeling in order to provide guidance on the most similar respiration mechanics as related to optimal particle delivery.

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Introduction

Animal models are frequently used to model humans in an array of scientific studies, including inhalation studies (Phalen, Oldham and Wolff 2008). These animal models are often used in these health-related studies and the data is then extrapolated to humans because of the limited ability to test on humans (Martonen, Zhang and Yang 1992). However, physiological factors and lung structure in other species can vary substantially from humans. For example, respiratory bronchioles are not present in rats or mice, which will possibly affect particle deposition (Phalen, Yeh and Schum, et al. 1978, Bal and Ghoshal 1988). Additionally, the number of lung generations present in different mammalian species that are used for lung studies is not always the same as in humans (Oldham and Robinson 2007, Maina and Gils 2001, Weinberg, et al. 2005). Moreover, many of the species that are used in lung studies have substantial differences in lung shape from humans. For instance, some studies have shown that many mammalian species (including rats, rabbits, and dogs) have lungs structures that are more monopodial whereas human lungs are typically described as being nearly dichotomous (Phalen, Oldham and Wolff 2008, Phalen, Yeh and Schum, et al. 1978, Weinberg, et al. 2005, Phalen and Oldham 1983). This is because these species have elongated chest cavities while the chest cavity of the human is relatively spherical (Phalen, Oldham and Wolff 2008).

Another difference is that the branching in humans is typically much more symmetrical than that of many laboratory animals (Dahl, et al. 1991). Since these animal models are frequently used to model humans, it is important to also determine particle deposition in the various species that are used in inhalation and dosing studies to see how accurately they represent the deposition in the human. Since there are differences in lung morphometry from infancy to adulthood and as a result depositions are not the same, it is also useful to determine if different animal models more accurately represent humans at different growth stages.

The purpose of this study is to inclusively investigate humans and multiple species, utilizing the same deposition model, to allow for a more direct comparison. This study will also highlight the differences in optimal particle size based on deposition efficiency compared to a volume-averaged efficiency.

This work hypothesizes that the optimal particle size for various animal species will be smaller than for humans thereby calling into question the use of these animal models to determine medication dosing in humans.

Methods

As mentioned earlier, various animal species are frequently used in inhalation and respiratory studies; thus, it is relevant to determine in each case whether or not these species adequately represent humans. Particle deposition calculations will be performed for a subset of animal models and compared with the human results. To perform these calculations, lung morphometry and respiratory conditions are also required for each species that will be compared to humans in this study.

Obtaining lung morphometry as a function of lung generation for various non-human species is difficult because the quantitative data available are limited. Since respiratory conditions in many species are dependent upon body mass, it is necessary to find respiratory conditions and lung morphometry for the same size animal. Therefore, even if lung morphometry is available, finding appropriate respiratory conditions for each species can also be challenging because this information is equally as scarce. As a result of this, the animal models that are utilized in this study include the B6C3F1 mouse, Long-Evans hooded rat, and the Beagle dog because both lung morphometry and respiratory conditions are available. The cast information for the animal species in this study is summarized in Table 1.

<table>
<thead>
<tr>
<th>Model</th>
<th>Age (mo)</th>
<th>Gender</th>
<th>Weight (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>B6C3F1 Mouse</td>
<td>Not Stated</td>
<td>Not Stated</td>
<td>25.6</td>
</tr>
<tr>
<td>Long-Evans Rat</td>
<td>12</td>
<td>F</td>
<td>330.0</td>
</tr>
<tr>
<td>Beagle Dog</td>
<td>17</td>
<td>M</td>
<td>11,600.0</td>
</tr>
</tbody>
</table>

Table 1: Information regarding animal lung casts

For the B6C3F1 mouse, lung morphometry is provided by Phalen (1991). The respiratory conditions, FRC, and TLC for the mouse are determined using correlations provided by Hsieh, Yu and Oberdörster (1999). For the Long-Evans rat, lung morphometry and TLC are provided by Yeh (1979). Respiratory conditions and FRC for the rat are determined using correlations provided in Hsieh and Yu (1999). Even though these correlations are for Fischer rats, body weight between the two rat strains are similar and therefore it is assumed that respiratory conditions and FRC for the Fischer rats are applicable to Long-Evans rats. This assumption is made because lung morphometry for the Fischer rat and respiratory conditions for an appropriately-sized Long-Evans rat are not available. For the Beagle dog, lung morphometry is provided by Yeh (1980). Respiratory conditions and lung volumes are estimated using data provided by Mauderly (1974). The lung geometries for these three animal species are provided in Appendix A. The respiratory conditions, and lung volumes for the three animal species used in this study are summarized in Table 2.
All of the animal lung morphometry models are also based upon a lung at TLC. Therefore, the animal models are also scaled a lung volume of FRC + TV/2. Once again, the diameters are scaled appropriately and the lengths are assumed to remain constant.

The fluid Reynolds number $Re_f$ is necessary to evaluate the fluid flow regime for a particular generation and is

$$Re_f = \frac{U_0 D}{\nu}$$

where $D$ is the diameter of the airway in that generation in m, and $\nu$ is the kinematic viscosity of air in m$^2$/s, which is defined as

$$\nu = \frac{\mu}{\rho_f}$$

In this study, all calculations will be performed with air. This study will evaluate deposition of particles with a geometric particle diameter $d$ from 1.0 µm to 10.0 µm in 1 µm increments. A number of assumptions relating to both the fluid (air or heliox) and the particles are made before beginning particle deposition calculations. First, it is assumed that the particles are spherical and monodisperse, meaning all of the particles are the same size so that no particle distributions need to be taken into consideration. In addition, it is assumed that the particles are homogeneously distributed throughout the inhaled volume. All of the particles are not forced to deposit or see each airway generation. Instead, this study models the inhalation more realistically, where a particular volume is inhaled and the initial section of the volume passes through more generations than the final segment of that volume. It is assumed that the aerosol cloud is charge neutral and electrostatic effects are negligible. This is a reasonable assumption because the high humidity in the lung neutralizes the charge of the particles (Finlay 2001). This study also assumes that the particle growth by hygroscopic effect is negligible. It is assumed the flow is incompressible, which is considered a reasonable approximation in most cases of aerosol inhalation (Finlay 2001). Finally, buccal and nasal depositions are not taken into consideration as this study only evaluates deposition that occurs from the trachea to the deep lung.

Various fluid and particles properties are required for deposition calculations. The air temperature is assumed to be 37ºC (310.15K), which is the temperature of the human body. The particle density $\rho_p$ used in this study is 1.0x103 kg/m$^3$. For air, the following properties are assumed: the density $\rho_f$ is 1.2 kg/m$^3$, the dynamic viscosity $\mu$ is 1.90x10-5 kg/m-s, and the mean free path $\lambda$ is 0.072 µm at 37ºC and 1 atm (Finlay 2001). For heliox, the density and dynamic viscosity are provided by Praxair and are 0.4 kg/m$^3$ and 1.98x10-5 kg/m-s, respectively. The mean free path for heliox is assumed to be the same as air. In addition, the branching angle (of the airways in each generation is assumed to be 38.24º (Finlay 2001).

Particle deposition, particle motion, and fluid dynamics calculations utilized in this study are described by Finlay, 2001. Particle deposition is determined using a statistical mathematical model. Particle motion and fluids dynamics calculations are required to evaluate particle deposition. For particle motion, it is assumed that there is a single particle with a density much larger than the fluid density (Finlay 2001); this particle is assumed to be isolated, so all interactions between particles are neglected. This is generally true except for some dry powder inhalers.

First, it is essential to know the fluid velocity in a particular lung generation. The fluid velocity $U_0$ (in m/s) is

$$U_0 = \frac{MV}{Ac \cdot N}$$

where $MV$ is in m$^3$/s, $Ac$ is the cross-sectional area of an airway in that generation in m$^2$, and $N$ is the number of airways in that generation. Once the fluid velocity is known, the Reynolds numbers for the particle and the fluid can be determined. The Reynolds numbers display the importance of inertial forces to viscous forces and are nondimensional. The particle Reynolds number $Re_p$ is necessary to ascertain the validity of numerous equations and is

$$Re_p = \frac{U_0 d}{\nu}$$

where $d$ is in m and $\nu$ is the kinematic viscosity of air in m$^2$/s, which is defined as

$$\nu = \frac{\mu}{\rho_f}$$

The fluid Reynolds number $Re_f$ is necessary to evaluate the fluid flow regime for a particular generation and is

$$Re_f = \frac{U_0 D}{\nu}$$

where $D$ is the diameter of the airway in that generation in m. The fluid flow regime is laminar when $Re_f < 2300$. Under normal breathing conditions, the flow is laminar in all generations of the lung.

Aerodynamic diameter is often used instead of geometric diameter to describe a particle in an aerosol. Aerodynamic diameter $d_{ae}$ is given as

$$d_{ae} = d \sqrt{\frac{SG}{\rho_f}}$$

where $SG$ is the specific gravity the particle. This equation is only valid when the $Re_p$ is much less than 1 and $d$ is much less than $\lambda$. Both of these conditions are

<table>
<thead>
<tr>
<th>Model</th>
<th>BF (min⁻¹)</th>
<th>TV (ml)</th>
<th>MV (L/min)</th>
<th>FRC (ml)</th>
<th>TLC (ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>B6C3F₁ Mouse</td>
<td>160</td>
<td>1.089</td>
<td>0.00600</td>
<td>0.629</td>
<td>0.976</td>
</tr>
<tr>
<td>Long-Evans Rat</td>
<td>120</td>
<td>2.23</td>
<td>0.535</td>
<td>8.10</td>
<td>14.1</td>
</tr>
<tr>
<td>Beagle Dog</td>
<td>19</td>
<td>244</td>
<td>9.27</td>
<td>545</td>
<td>1363</td>
</tr>
</tbody>
</table>

Table 1: Summary of human respiratory conditions and lung volumes

<table>
<thead>
<tr>
<th>Age (yr)</th>
<th>Gender</th>
<th>BF (min⁻¹)</th>
<th>TV (ml)</th>
<th>MV (L/min)</th>
<th>FRC (ml)</th>
<th>TLC (ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.25</td>
<td>F</td>
<td>30</td>
<td>30</td>
<td>2.38</td>
<td>178</td>
<td>328</td>
</tr>
<tr>
<td>1.75</td>
<td>M</td>
<td>26</td>
<td>81</td>
<td>4.64</td>
<td>232</td>
<td>466</td>
</tr>
<tr>
<td>1.92</td>
<td>M</td>
<td>27</td>
<td>87</td>
<td>4.73</td>
<td>329</td>
<td>599</td>
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<tr>
<td>2.33</td>
<td>F</td>
<td>26</td>
<td>100</td>
<td>5.16</td>
<td>371</td>
<td>699</td>
</tr>
<tr>
<td>3.00</td>
<td>F</td>
<td>24</td>
<td>121</td>
<td>5.80</td>
<td>458</td>
<td>922</td>
</tr>
<tr>
<td>8.67</td>
<td>M</td>
<td>17</td>
<td>278</td>
<td>9.70</td>
<td>908</td>
<td>1990</td>
</tr>
<tr>
<td>9.42</td>
<td>M</td>
<td>17</td>
<td>266</td>
<td>10.09</td>
<td>1573</td>
<td>3243</td>
</tr>
<tr>
<td>14.08</td>
<td>F</td>
<td>16</td>
<td>388</td>
<td>12.06</td>
<td>2918</td>
<td>5362</td>
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<tr>
<td>14.08</td>
<td>F</td>
<td>16</td>
<td>389</td>
<td>12.09</td>
<td>1660</td>
<td>3533</td>
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<tr>
<td>18.00</td>
<td>M</td>
<td>16</td>
<td>447</td>
<td>13.20</td>
<td>1368</td>
<td>2671</td>
</tr>
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<td>21.00</td>
<td>M</td>
<td>14</td>
<td>477</td>
<td>13.73</td>
<td>3281</td>
<td>6656</td>
</tr>
</tbody>
</table>
satisfied in this study. Since the particle density that is chosen is about the same as the density of water, the aerodynamic diameters are determined and are very nearly the same as the geometric diameters, thus there is no affect on the results.

Particle deposition occurs in the respiratory tract by three primary mechanisms: sedimentation, inertial impaction, and diffusion. Sedimentation is when the particles deposit in an airway because of gravitational settling. For sedimentation calculations, the fluid velocity profile is assumed to be laminar plug flow. In plug flow, the fluid velocity is the same across any cross-sectional area of the tube. When determining the probability of sedimentation, the terminal settling velocity (velocity at which the particle settles due to gravity) for each particle must be determined. The settling velocity vsettling (in m/s) is

\[ v_{settling} = \frac{C_d \rho_d d^2}{18 \mu} \]

where \( g \) is acceleration due to gravity in m/s², \( d \) is in m, and \( C_d \) is the Cunningham slip correction factor. This equation is also only valid when the Re is much less than 1 and \( d \) is much less than \( \lambda \). The Cunningham slip correction factor is necessary when the particle diameter gets smaller and the mean free path is not much smaller than particle radius (Finlay 2001). The Cunningham slip correction factor is nondimensional and is

\[ C_d = 1 + 2.25 \frac{\lambda}{d} \]

The distance in which the particle will settle in a particular generation \( x_s \) (in m) is given by

\[ x_s = v_{settling} t \]

where \( t \) is the residence time of a particle in that generation in s. It is also necessary to compute \( \kappa \) to determine sedimentation probability. The value of \( \kappa \) is

\[ \kappa = \frac{3v_{settling} L}{4u_d} \cos \theta \]

where \( L \) is the length of an airway in a particular generation in m and \( \theta \) is the branching angle. The probability of sedimentation for laminar plug flow is

\[ P_s = 1 - \frac{2}{\pi} \left[ \cos^{-1} \left( \frac{4}{3} \kappa \right) - \frac{4}{3} \kappa \sqrt{1 - \left( \frac{4}{3} \kappa \right)^2} \right] \]

When using this equation, it is important to note that \( \kappa \) is only a real number when it is less than \( \frac{1}{4} \). Due to this, \( P_s \) is frequently set to 1 when \( \kappa \geq \frac{1}{4} \) because this indicates that it takes a particle longer to travel through the length of the tube than it does for it to travel the diameter of the tube perpendicular to the flow and therefore sedimentation will occur (Finlay 2001).

Particle deposition also occurs by means of inertial impaction. Deposition occurs via inertial impaction when there is curvature in an airway and the inertia of the particle is too great, resulting in a particle trajectory that no longer follows the fluid flow streamline causing the particle to deposit on the airway wall. The Stokes number determines whether or not inertial impaction will occur and therefore is necessary when evaluating the probability of deposition via impaction. The Stokes number \( St_k \) is nondimensional and is

\[ St_k = \frac{U_d \rho_d d^2 C_c}{18 \mu D} \]

The probability of inertial impaction is given by Chan and Lippmann (1980) and is

\[ P_i = 1.606 St_k + 0.0023 \]

Other than sedimentation and impaction, particle deposition also takes place as a result of Brownian diffusion. Very small particles have Brownian motion occur due to interactions with the molecules of the gas they are carried by. Brownian motion is when a particle collides with the molecules and random walk occurs. This is considered to be diffusion when this takes place with many particles. The root mean square displacement \( x_d \) (in m) describes the distance the particle travels due to Brownian motion and is

\[ x_d = \frac{2}{\sqrt{2 D_d t}} \]

where \( D_d \) is the particle diffusion coefficient in m²/s. The particle diffusion coefficient \( D_d \) is

\[ D_d = \frac{k T C_c}{3 \pi \mu d} \]

where \( k \) is Boltzmann’s constant (1.38x10^-23 J*K⁻¹) and \( T \) is the temperature in K (37 °C=310.15K). To determine the probability of Brownian diffusion, \( \Delta \) is

\[ \Delta = \frac{k T C_c L}{3 \pi \mu u_d R^2} \]

where \( k \) is Boltzmann’s constant and \( R \) is the radius of the airway in a generation in m. The probability of deposition due to Brownian diffusion is given by Ingham (1975) and is

\[ P_d = 1 - 0.819 e^{-14.63 \Delta} - 0.0967 e^{-89.22 \Delta} - 0.0325 e^{-228 \Delta} - 0.0509 e^{-125.92 \Delta^2/3} \]

So far, the equations provided determine the probability of each of these deposition mechanisms occurring alone, which is unrealistic; in reality, these deposition mechanisms occur simultaneously in the lung. Consequently, an empirical relation that calculates the total probability of deposition by taking into consideration all three types of deposition is used. The total probability \( P \) is determined using

\[ P = (P_i^p + P_s^p + P_d^p)^{1/p} \]

where the value of \( p \) (in the exponents) is assumed to be 2 in this study.

The ratio of \( x_d/x_s \) is useful for assessing how important diffusion is when compared with sedimentation. If \( x_d/x_s < 0.1 \), then diffusion becomes negligible and no longer needs to be taken into consideration. When diffusion is not taken into consideration, the total probability becomes

\[ P = P_i + P_s - P_i P_s \]  \hspace{1cm} (21) \]

Once the total probability of deposition is determined in each lung generation for each particle size, it is necessary to quantify the results in a way that is more applicable to dosing. Most studies assume that the par-
articles move through the lung at the same time at an infinite particle density. This is both physically unrealistic and violates several of the assumptions used to develop the statistical models. It was instead chosen in this study to have each model inhale a normal tidal volume where the particles are homogeneously distributed throughout the entire volume. This has the consequence of needing to tag particles to a particular segment of the tidal volume as the last segment of the tidal volume inhaled never reaches the deep lung. This is achieved using the following equation

\[ F_{Vi} = 1 - \frac{\sum Vi-1}{V_{Total}} \]

where \( F_{Vi} \) is the fraction of the adjusted cumulative volume still available, \( V_{Total} \) is the total adjusted cumulative volume, and \( \sum Vi-1 \) is the sum of the adjusted volumes above that generation.

**Results**

Figures 1 (a) and (b) show the traditional alveolar deposition efficiency for the animal species compared to the humans. Despite redefining the alveolar region for the humans, the optimal particle size based on the traditional deposition efficiency remains relatively constant between 2-3 microns all species regardless of body mass. The only ages that do not appear to follow this trend are the infant (F) and 1.75-yr old (M). This trend also applies to the three animal species. It is important to note that deposition efficiencies generally increase as body size increases, although the deposition efficiency in the humans and animal species do not increase at the same rate. For example, even though the mouse is considerably smaller in size than all of the humans, the deposition efficiency for the mouse very closely corresponds to that of the 3-yr old female; the deposition efficiency values for the other animal species increase from here.

Figures 2 (a) and (b) show the volume-weighted alveolar deposition efficiencies for males and females compared to the animal species. The same trends as before redefining the alveolar region exist for humans regarding the deposition efficiency and BMI, age, and gender; however, the optimal particle size for humans has shifted higher for each age as a result of adding the respiratory bronchioles to the alveolar region. These results are anticipated because as previously seen the optimal particle size for respiratory bronchiol deposition efficiency is typically about 2 microns higher for a particular age than the alveolar deposition efficiency. Also, with the addition of the respiratory bronchioles to the alveolar region, there is a larger shift in optimal particle size as related to BMI.
When considering the animal results, the alveolar deposition efficiency and optimal particle sizes for the animals increase as a result of body size increase (from mouse to rat to canine). The volume-weighted deposition efficiency for the mouse, once again, closely corresponds to the results for the 3-yr old female; the optimal particle size for the mouse also very closely matches up to that of the 3-yr old female.

Beyond these results, the correlations between humans and animals are minimal. The rat has volume-weighted deposition efficiency scarcely lower than that of the 8.67-yr old (M) and 9.42-yr old (F), but the optimal particle size for the rat is nearly a micron higher than for these ages. Also, the canine has an optimal particle size similar to the 14.00-yr old (F) and 21-yr old (M), but the deposition efficiency is considerably lower and does not coincide with any of the ages presented in this study. The optimal particle sizes for volume-weighted alveolar deposition efficiency for animals and humans (redefined AL region) are summarized in Tables 3-5.

Table 3: Optimal particle size for redefined AL region for females (volume-weighted)

<table>
<thead>
<tr>
<th>Age (yr)</th>
<th>Gender</th>
<th>Optimal Particle Diameter (µm)</th>
<th>BMI</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.25</td>
<td>F</td>
<td>~3</td>
<td>13.5</td>
</tr>
<tr>
<td>2.33</td>
<td>F</td>
<td>5-6</td>
<td>13.7</td>
</tr>
<tr>
<td>3.00</td>
<td>F</td>
<td>5-6</td>
<td>11.4</td>
</tr>
<tr>
<td>14.00</td>
<td>F</td>
<td>6-7</td>
<td>16.6</td>
</tr>
<tr>
<td>14.08</td>
<td>F</td>
<td>7-8</td>
<td>25.9</td>
</tr>
</tbody>
</table>

Table 5: Optimal particle size for AL region for animal species (volume-weighted)

<table>
<thead>
<tr>
<th>Species</th>
<th>Optimal Particle Diameter (µm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>B6C3F1 Mouse</td>
<td>5-6</td>
</tr>
<tr>
<td>Long-Evans Rat</td>
<td>5-6</td>
</tr>
<tr>
<td>Beagle Dog</td>
<td>6-7</td>
</tr>
</tbody>
</table>

Conclusions

Based on the calculations, obvious differences exist between the three animal species, and between the humans and animals. Additionally, differences exist for the human results when compared to the previous results as a consequence of redefining the alveolar region to include the respiratory bronchioles. When comparing the humans to the animal species, some interesting trends and differences exist.

These results indicate that the mouse represents the volume-weighted deposition efficiency and optimal particle size for the 3-yr old female very well. These results also suggest that the rat and canine do not adequately represent any of the ages presented in this study when considering the volume and optimal particle size for therapeutic deposition. There are a lack of adequate lung geometries and respiratory conditions currently available in literature for other animal species; however, as more animal lung morphometry and respiratory conditions become available, it would be beneficial to compare more animal species to humans to consider a wider range of animal body types and sizes to determine their possible applicability to dosing.

Furthermore, it is obvious from these results that the deposition efficiencies and optimal particle sizes for humans depend upon how the regions of the lung are defined. If the generations that are included in the alveolar region are changed, the deposition efficiency will change. This is also critical when determining and comparing deposition between various models. It is important to define similar lung regions for each model for the results to be accurately compared.