Improvements and Additions to the Multiple Path Particle Dosimetry Model

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1 **Abstract**

2 A little over a decade ago, the first UC Irvine dosimetry conference was held
3 entitled “Frontiers in Aerosol Dosimetry Research”. At that time, mechanistic
4 dosimetry models, such as the multiple path particle dosimetry (MPPD) model,
5 primarily focused on humans, with a model for the Long Evans rat also being
6 available. In the intervening years, advances have occurred on a number of
7 fronts that have led to improvements and additions to the user-friendly MPPD
8 software program that is available free to the public. Here, we review a number of
9 the expanded capabilities of the MPPD model. These include:

- 10 • Calculating deposition and clearance simultaneously for up to 4
11 lognormal distributions having different mass median aerodynamic
12 diameters and geometric standard deviations. This feature is useful
13 whenever the exposure atmosphere is multimodal as is often the case
14 with workplace exposure atmospheres and can occur in inhalation
15 toxicology studies if the particulate aerosol is difficult to generate stably.
- 16 • The ability to adjust the total aerosol deposition for the amount of particle
17 “X” when “X” is a subset of the total aerosol. This is particularly important
18 for workplace exposures where the particles of interest (e.g., copper
19 containing particles) are part of the total dust aerosol that was measured.
- 20 • The ability to specify clearance rates and mucous velocities for both
21 animals and humans so any type of particle can be modeled as well as
22 various lung disease states.

- 23 • The addition of dose metrics for the upper respiratory tract and
24 enhancements to dose metrics for the lower respiratory tract.
- 25 • Addition of a lung geometry/morphometry model and physiologic input
26 variables for mice (Balb/c and B6C3F1), Sprague-Dawley rats, male
27 rhesus monkeys, sheep, and pigs. The deposition and clearance of
28 particles in these species can now be examined.
29

1. Introduction

Regulatory bodies make use of interspecies dosimetric adjustments and high to low dose extrapolation in using animal toxicological data to determine reasonable exposure limits for humans. In addition, therapeutic aerosols are examined using dosimetry models for finding effecting treatment regimens. The multiple path particle dosimetry (MPPD) model is a mechanistic dosimetry model that facilitates the calculation of regional and site-specific deposition and clearance of particles. The MPPD model software is available to the public free of charge (<http://www.ara.com/products/mppd.htm>) and is popular with users because of the user-friendly menu-driven selections that can be made for the various input variables.

Since the first publication by Anjivel and Asgharian (1995) using the multiple path modeling approach, the MPPD model has been applied to the human lung (Asgharian et al., 2001), the rat lung (Asgharian et al., 2003), particle deposition (Jarabek et al., 2005; Asgharian and Price, 2007; Asgharian et al., 2014), as well as fiber deposition in humans (Asgharian and Anjivel, 1998). At the time of the first UC Irvine dosimetry conference held in October of 2005, the MPPD model treated Long Evans rats and humans and poorly soluble particles. Since then a number of improvements and additions have been made to the MPPD model software in the treatment of aerosol distributions, clearance parameters, dose metrics, species geometry models, and report output files. Here, we briefly discuss these improvements and additions and give examples of

52 them to make the reader aware of the currently greatly expanded capabilities of
53 the MPPD model for use in studying the deposition and clearance of particles.

54 **2. Aerosol distributions**

55 Workplace dust exposures typically involve a mixture of particles
56 arising from the various manufacturing or industrial processes that are being
57 used in a given facility. Regulatory bodies for worker health protection establish
58 short- and long-term exposure limits for total dust and for some of its
59 components. The short-term exposure limits typically are intended to protect
60 against irritation or acute injury of the nasal passages or the conducting airways
61 of the lower respiratory tract while the long-term limits are intended to protect
62 against the development of chronic effects.

63 Thus, total particle deposition in the respiratory tract relates to total dust
64 exposure standards. However, to protect workers from health effects that can be
65 caused by particles that are a subset of the total dust, such as copper, nickel, or
66 iron containing particles, the amount of such particles depositing in the lungs also
67 needs to be determined. Given the different processes and workplace locations,
68 workplace air is typically a multimodal log normal distribution.

69 Recently, the MPPD software was modified to be able to handle up to 4
70 overlapping log normal distributions, enabling more complex aerosol distributions
71 to be modeled for deposition and clearance calculations. Each of the individual
72 mode size distributions had to be combined into a single size distribution for use
73 in MPPD. Based on the input parameters provided by the user for each mode, a
74 single multimodal size distribution is constructed computationally to reflect the

overall distribution of particle sizes. The size range of this multimodal particle distribution is then subdivided into logarithmically spaced intervals, and the total number of particles in each size interval is calculated in order to assist MPPD calculations of deposition and clearance.

Table 1 gives cascade impactor data for total dust on various stages as well as data for the analysis of the amount of insoluble and soluble copper on these stages in workplace air at a brass recycling and extrusion facility. The facility specializes in extruded brass rod, bars, and wire and is a leading manufacturer of extruded brass shapes. The sampling at this facility involved the casting area for melting scrap brass and casting it into ingots. For additional details on the count distribution data and the procedure for estimating the overall particle density of the total workplace dust aerosol, see Miller et al. (2013). The analyses in Table 2 show that the cascade impactor sample required 3 overlapping lognormal distributions to fit a continuous function to histograms based on particle mass per size interval. Two fine mode lognormal distributions (i.e., a MMAD mode $< 2.5 \mu\text{m}$) were present that accounted for about 80 % of the total dust. For the casting area, the distribution with a MMAD mode of about $10.2 \mu\text{m}$ represented 19.4 % by mass of the total dust.

While copper on all cascade impactor stages represented 3.027 % of the total dust sample (Table 1), most of the copper being on the first few stages reflects the presence of large coarse mode particles. The effect of the copper being associated mostly with the larger particles lessens the impact on alveolar copper dose metrics. The method developed by Miller et al. (2013) allows one to

determine the lung burden of a subset of total dust in workplace air, such as those particles containing copper and demonstrates the dosimetric differences between total dust and that of a contaminant of the dust. The added feature of handling up to 4 overlapping lognormal distributions together with computing dose metrics for a subset of the particles in the workplace air allows for more accurate comparisons of animal toxicology data to measured human exposures when assessing potential health effects.

3. Estimating values of physiologic variables

Calculating lung deposition of inhaled particles or the absorption of an inhaled gas requires knowledge of a number of physiologic variables. These include the route, rate, and depth of breathing, the volume of the upper respiratory tract (URT), and the volume of the lungs at FRC. Since the first UC Irvine dosimetry conference, the following species or strains have been added to the airway morphometry options available in MPPD: mice (Balb/c and B6C3F1 strains), Sprague-Dawley rat, male rhesus monkey, pig, and sheep. Subsequent sections provide a brief synopsis of how various physiological variables were determined for these species with the main emphasis on relationships to body weight that have not been previously published. However, the tables are inclusive of the results for all species.

3.1 URT volume

The URT includes from the nares or the mouth down to the beginning of the trachea. The volume of air initially in the URT dilutes particle- or gas-laden inhaled air. The tidal volume air is made up of the mixture of dilution air and

inhaled air that travels to various locations of the lung. In addition to the route and depth of breathing, lower respiratory tract (LRT) deposition is influenced by the volume of the URT. Thus, the URT volume must be known to assess losses of inhaled materials in various LRT locations.

Volume and surface area measurements for the pig and sheep were compiled from computational fluid dynamics (CFD) models of the URT of both species. The pig nasal CFD model was developed from MRI images (2 mm resolution) of an adult male pig weighing approximately 27 kg (Asgharian et al., 2016). The model consisted of both sides of the nose extending from the nostrils through the nasopharynx and also included the larynx and upper trachea. The sinuses were not included in the pig model.

The sheep nasal CFD model was developed from CT images (~0.6 mm resolution) of an adult female sheep weighing approximately 66 kg and consisted of the full nasal passages and sinuses extending from the nostrils through the nasopharyngeal duct. The URT surface geometries for both species were developed using the imaging software 3DSlicer (www.slicer.org last accessed 12/27/15). Computational meshes were generated in each species using the commercial software ICEM-CFD (Ansys, Inc.).

URT surface area and volume measurements were obtained from the surface mesh and volume mesh, respectively, of both species. For the pig, the URT surface area and volume were 882.3 cm² and 137.5 ml, respectively. The sheep URT surface area was 1550.8 cm², and the volume was 202.0 ml. Table 3

gives the body weights and URT volumes of the animal species added to the MPPD software since the last UC Irvine dosimetry conference.

3.2 Breathing parameters

Deposition of inhaled materials in the lung depends on the ventilation rates of the species. Laboratory measurements of ventilation rates include the tidal volume (V_T) and frequency of breathing (f), which when multiplied together determines the minute volume. The subdivision of minute volume into its two components is requisite for accurate deposition modeling since inhaled particles have varying patterns of deposition depending upon the combination of V_T and f .

3.2.1 Breathing frequency with whole body exposures

Toxicological studies in small laboratory animals such as mice and rats are done using both whole body and nose only exposures. Well documented in the literature is that, even with acclimation of the rats to nose only tubes before commencing inhalation exposures to the test compound, the animals breathe at a higher rate than they do when whole body exposure systems are used (Costa and Tepper, 1988; Alexander et al., 2008). Thus, breathing frequency, as well as tidal volume, and minute ventilation, should be established separately for these two types of inhalation exposure systems.

There are limited data in the literature specific for the breathing frequency in larger animals such as pigs and sheep. In a study of respiratory function and pulmonary lesions in pigs infected with porcine reproductive and respiratory syndrome virus, Wagner et al. (2011) used 12 German hybrid pigs as controls. The pigs were 45 to 48 days of age when the study started. Median breathing

frequency ranged from 20 to about 25 bpm over the course of the 21-day post infection period. Over the course of the week preceding exposure to the end of the study, mean body weight increased from 17 kg to 32 kg, with a daily weight gain of 568 g and a standard deviation of 61.

After pigs eat, their respiration rate falls if the data reported in Douglas (1972) are used. He reported f values ranging from 10 to 16 bpm when pigs finished eating. Stahl (1967) did an allometric analysis of various variables, including breathing frequency, across many orders of magnitude in body weight involving a number of different species. His equation for breathing frequency has an R^2 of 0.828 and is given by

$$\text{Breathing frequency (min}^{-1}\text{)} = 53.5 \times (\text{Body Wt (kg)})^{-0.26} \quad (1)$$

Using Eq. 1, a breathing frequency of 24.2 bpm would be predicted for a 21 kg pig, which is within the range reported in the Wagner et al. (2011) study where the animals could be considered to be young adults. Although Hannon et al. (1989) do not report the body weight of 22 pigs they studied, their mean breathing frequency was 20 bpm with a standard deviation of 2.9 and a range from 16 to 25 bpm. In newborn pigs weighing an average of 1.167 kg, Mortola and Noworaj (1985) reported a breathing frequency of 48 bpm. This value is in good agreement with the value of 51.4 bpm one obtains if Eq. 1 is used. Thus, one might reasonably use Eq. 1 to predict the breathing frequency in pigs as a function of body weight. A search on Google Scholar revealed that the range of breathing for adult sheep is predicted to be 12 to 20 breaths per minute. Thus, a reasonable starting point for breathing frequency in adult sheep is 16 bpm

(<http://www.sheep101.info/sheepbasics.html>), which is a range that is in contrast to what one obtains if the equation given by Stahl (1967) is used.

Breathing frequency in a number of animal species with body weights much less than those of adult pigs or sheep has been shown to be basically independent of body weight. For example, Asgharian et al. (2012) showed that, for rhesus monkeys weighing up to 4.5 kg, there is no relationship between breathing frequency and body weight for either sex. They found the overall average breathing frequency for the males and females was 39.7 and 39.6 bpm, respectively, with the standard deviation of the males being 6.8 (N=31) and being 10.1 for the females (N=36). For Sprague-Dawley rats, the data in Costa and Tepper (1988) show that the weighted mean breathing frequency determined in a pressure box is 120 breaths per min with a pooled standard deviation of 23.2.

Assuming the overall average breathing frequency is the same for male and female pigs and sheep would be consistent with the absence of gender differences in this variable for a number of animal species. For example, Alexander et al. (2008) reported for cynomolgus monkeys a bpm value of 72 for males and 69 for females. These same investigators found a value of 259 bpm for both male and female CD1 mice, and the weighted mean for Beagle dogs using their Table 2 data gives mean breathing frequencies of 30 and 29 for males and females, respectively. Similarly, Costa and Tepper (1988) show no gender difference in breathing rates for rats.

Given that Stahl (1967) was an allometric analysis across a wide range of body weights while the estimates of breathing frequency in the other studies

discussed above were obtained from data only on the given species and that the predicted breathing frequencies for pigs weighing 17 to 32 kg fall in the range of 20-25 bpm, as reported by Wagner et al. (2011), one way forward at the current time would be to assume that the breathing frequency in pigs and sheep is also independent of body weight over this range of weights.

However, an equally reasonable path would be to use Eq. 1 to estimate breathing frequency for pigs and sheep. Until more definitive data are available for the various species, we have elected to make breathing frequency independent of body weight for the animal species available in MPPD. However, for humans, the changes in ventilatory demand with work or exercise dictate that the user be able to specify the breathing frequency. The recommended breathing frequencies for the various species available in MPPD are summarized in Table 4 together with what the breathing frequency would be if Stahl's (1967) equation was used for the body weights listed in the table. One can see that Stahl (1967) greatly under predicts the breathing frequency measured in smaller species like mice, rats, and monkeys.

3.2.2 Breathing frequency with nose- or head-only exposures

While restraint in nose-or head-only inhalation exposure tubes elevates the breathing frequency of the mice or rats even if they are acclimated in the tubes over several days before exposure to the test compound, there is no apparent reason to suspect gender differences in breathing frequency. Han Wistar rats are the only strain of rats for which breathing frequency has been measured in both males and females where unanesthetized animals were

restrained in head-only tubes; in this instance, Alexander et al. (2008) reported breathing frequencies of 177 for males and 179 for females with standard deviations of 46.2 and 39.4, respectively.

From 3 studies involving 101 male rats (unanesthetized but restrained in head-only tubes) from Sprague-Dawley, Han Wistar, and one other unspecified rat strain (Alexander et al., 2008), the weighted mean breathing frequency was 166 breaths/min with a pooled standard deviation of 39.7 for measurements obtained using head-out plethysmography with a restraint tube. For female rats in Table 2 of Alexander et al. (2008), only 2 studies reported breathing frequency values, with the weighted mean being 158 bpm. However, these authors noted that one of the female rat studies was of questionable reliability because it involved repeated measurements on the same animals over several weeks with body weight increasing but respiratory minute volume decreasing. In addition, the male and female means are not different statistically.

Thus, an average breathing frequency of 166 bpm is recommended for nose-or head-only exposures as the baseline breathing frequency for Sprague-Dawley rats; the pooled standard deviation can be used to examine deposition in animals breathing at one or two standard deviations above or below the mean. Of interest is that Asgharian et al. (2003), using Skornik tubes in nose-only exposures, also reported an average breathing frequency of 166.

The data for breathing frequency in Balb/c and B6C3F1 mice are from restrained whole-body plethysmography studies involving 555 mice (Delorme and Moss, 2002; Flandre et al., 2003; Lofgren et al., 2006). Combining data for

the two strains gives a weighted mean breathing frequency of 276.78 (i.e. 277 bpm). While some restraint is involved, the mice are not nearly as restrained as they would be in a nose- or head-only inhalation exposure system. To our knowledge, there are no experimental data available for breathing frequency under these exposure conditions. Thus, we use the same relative increase for mice as experimentally determined for S-D rats (120 bpm pressure box versus 166 head-only restraint for about a 40% increase) and assume that nose- or head-only breathing frequency for mice is about 388 bpm (i.e., 277×1.4).

3.3 Tidal volume (V_T)

In most instances where dosimetry calculations are of interest, tidal volume is seldom measured. For newborn pigs weighing 1.167 kg, a tidal volume of 14.2 ml reported by Mortola and Noworaj (1985) corresponds to a tidal volume of 12.2 ml/kg. Hannon et al. (1989) report a tidal volume/kg of 10.1 based upon 22 pigs with a standard deviation of 2.08. For the pigs in Table 2 of Bide et al. (2000) and using Eq. 1 to estimate breathing frequency, one derives a tidal volume of 10.5 ml/kg for pigs weighing 197 kg. Protti et al. (2011) determined V_T in anesthetized and paralyzed pigs to be 22 ml/kg, which is much greater than the 11.5 ml/kg value one gets using Bide's equation for minute volume together with Eq. 1 for the 21 kg pigs used by Protti et al. (2011).

Given that Bide et al. (2000) used data from 142 studies on 18 different species spanning a body weight range of more than 4 orders of magnitude to develop a power law allometric equation for minute volume as a function of body weight, one would tend to dismiss the Protti et al. (2011) estimate in view of the

surgical procedure and controlled ventilation that they used in their lung stress and strain study. The equation in Bide et al. (2000) to relate minute ventilation to body weight explains 97.5 % of the variation in the data and is given by

$$\text{Minute Volume (L)} = 0.499 \times \text{Body Wt (kg)}^{0.809} \quad (2)$$

Given the larger number of studies, more species, and greater total number of animals involved in the Bide et al. (2000) analysis, the use of Eq. 2 to estimate minute volume is recommended over using the individual formulas for bpm and tidal volume given by Stahl (1967) and then multiplying the derived values to estimate minute volume.

In a study of lung function impairment in sheep using high peak airway pressures during mechanical ventilation (Kolobow et al., 1987), the control animals were ventilated at a rate of about 10 ml per kg of body weight. These control animals were healthy adult sheep but they had been paralyzed so they could be easily mechanically ventilated. Thus, the tidal volume value per kg of body weight used by Kolobow et al. (1987) is probably on the low side of the normal value for healthy, adult sheep

Using the data in Table 2 of Bide et al. (2000) for non-anesthetized sheep (52.6 kg, 13.9 L minute volume) and assuming an average breathing frequency of 16 bpm, an estimate of tidal volume is 16.5 ml per kg of body weight. Using 52.6 kg as the body weight, Eq. 2 yields a minute volume of 12.3 L. Assuming a breathing frequency of 16 bpm, a tidal volume value of 14.6 ml per kg body weight or 769.6 ml is derived. While Stahl (1967) does not specifically state if the animals in his allometric regression analysis were conscious or anesthetized, the

use of his power law equation in a direct computation of tidal volume gives a value of 474 ml for a sheep weighing 52.6 kg. Using Stahl's power law equation for breathing frequency, this size of sheep would be predicted to breathe at 19.1 bpm giving a minute volume of 9.05 L compared to the 12.3 L minute volume obtained from Eq. 2. Given the larger number of studies, more species, and greater total number of animals involved in the Bide et al. (2000) analysis, the use of Eq. 2 is again recommended to estimate minute volume over using the individual formulas for bpm and tidal volume given by Stahl (1967) and then multiplying the derived values to estimate minute volume.

Since pigs and sheep of different body weights will be used in various studies, the minute volume derived using Eq. 2 can be converted to ml by multiplying by 1000 and then dividing by the value for breathing frequency (e.g., 16 bpm) to provide an estimate of tidal volume. An alternative method is listed in Table 5 that simplifies the result of multiplying by 1000 the ratio of the minute volume equation of Bide et al. (2000) to the equation developed by Stahl (1967) for breathing frequency to obtain a prediction of tidal volume for pigs and sheep. The equations and values for tidal volume for the other species added to the MPPD software are also given in Table 5. Worth noting is that equations for female rhesus monkeys are also given in Table 5; however, the anatomical model was developed from male rhesus computed tomography and morphometric data, so the user is cautioned about using MPPD for female rhesus dosimetry calculations.

3.4 FRC volume

The critical lung volume for deposition calculations is that of functional residual capacity (FRC). The deposition calculations are initiated using the tidal volume to expand the lungs from the lung volume at the end of a breath (i.e., FRC) and to calculate the impact of each mechanism of particle deposition (e.g., impaction, sedimentation, and diffusion) in each lung generation.

Mundie and colleagues (1992) examined 55 normal, Q-fever negative, mixed breed, adult female sheep weighing from 21 to 56 kg and determined the relationship of FRC to various body measurements. The correlation of FRC with lung weight produced an R^2 of 0.766 but only an R^2 of 0.488 with body weight. Various other variables such as body length, circumference, and sternum yielded lower R^2 s with FRC. Using multiple linear regression, Mundie et al. (1992) found the best relationship of FRC was to a combination of body weight and length, but even then the R^2 was only 0.536. They compared their average FRC value to those obtained in 10 other studies where FRC had been determined.

In studies to examine physiologic responses of sheep, FRC and TLC were determined pre- and post-papain exposure in two groups of sheep. One group received papain in a manner designed to produce homogeneous emphysema and the other group was to develop heterogeneous emphysema (Hoffman et al., 2003). However, body weights were not reported.

Since we are most interested in developing an equation that relates FRC to body weight, we combined the data contained in Mundie et al. (1992) and Hoffman et al. (2003) and used two different methods to estimate body weight when a value for this variable was not provided. For the first method, Panel A of

Figure 1 in Mundie et al. (1992) was used to estimate the slope of the linear regression and then inverse prediction was used to develop Equation 3a.

$$\text{Body Wt}_{\text{Mundie}} (\text{kg}) = 15.774 + 13.099 \text{ FRC (L)} \quad (3a)$$

Similarly, from the allometric study of Stahl (1967), the logarithm of the power law equation relating FRC to body weight was determined and the equation rearranged to provide body weight as a function of FRC as given below:

$$\text{Body wt (kg)} = 0.05984 * \text{FRC}^{0.88496} \quad (3b)$$

Using Eqs. 3a and 3b when necessary, Table 6 provides a summary of FRC values determined in various sheep studies. A number of different methods were used to measure FRC. The value reported in Hutchinson et al. (1982) would meet the criterion for an outlier as the same FRC value was obtained as was found in 2 other studies where the body weight was only about half of that in the Hutchinson et al. (1982) study.

Equations 3a and 3b were also used to estimate the individual body weights for the papain pre-exposure groups of sheep studied by Hoffman et al. (2003). As can be seen by the estimated body weight values given in Table 7, the values obtained using Eq. 3b (i.e., the allometric relationship found by Stahl (1967)) average about 15% and 12% more than those from the inverse prediction derived using Eq. 3a based on data in Mundie et al. (1992) for the homogeneous and heterogeneous emphysema groups, respectively.

The sheep data contained in Tables 6 and 7 were used in various linear regression models to examine the relationship between FRC and body weight. A summary of the regression results is given in Table 8. While one would expect

the R^2 to increase when more data are used in the regression as reflected by comparing the R^2 for the study means regression to the one for the study means plus the individual values reported by Hoffman et al. (2003), a significant change in the slope should not occur if the body weight estimates are accurate. However, Table 8 reveals that the slope using the Mundie et al. (1992) data reflected in Eq. 3a to estimate body weights increases from 0.04647 for the study means regression to 0.069843 when the individual sheep data from Hoffman et al. (2003) are added. In contrast, the slope remains relatively the same if body weights are estimated using Stahl (1967) to develop the inverse prediction given by Eq. 3b (i.e., slopes of 0.042037 versus 0.04583). In addition, Figure 1 shows that, for body weights greater than about 40 kg, the regression model using Eq. 3a consistently under predicts FRC whereas the model using Eq. 3b does not.

Equation 3b is based on Stahl's (1967) allometric analysis over many orders of magnitude; one would expect this equation to provide body weight estimates that go through the mean of the population. In view of this and the above results, the following equation that explains 88.1% of the variation in the data is recommended for estimating FRC in sheep as a function of body weight:

$$\text{FRC (ml)} = 1000(-0.287 + 0.045483 \times \text{Body Wt (kg)}) \quad (4)$$

Unlike for sheep, which cover the same body weight range as pigs until a body weight of about 125 kg is reached, there is a paucity of experimental data reported in the literature for pigs. In a study of lung stress and strain, Protti et al. (2011) reported a FRC of $677 \text{ ml} \pm 96$ in pigs weighing $21 \text{ kg} \pm 3$. Other data found in the literature where FRC was measured either report results as a

percent change from baseline but do not give the baseline value, report FRC but do not provide body weights, or else involve an experimental protocol that may have altered the lung sufficiently that the measured FRC is not likely representative of an unanesthetized pig.

One could use Eq. 4 above to predict FRC values for pigs. However, a comparison of values predicted using Stahl's (1967) allometric analysis of animals over several orders of magnitude revealed differences consistently above Eq. 4 that decrease as body weight increases (e.g., Stahl 30% higher for a 15 kg pig, 4% greater for a 30 kg pig, and only 1-2% greater for pigs weighing 40 kg or more). Thus, a reasonable approach given the absence of more data in pigs is to use Stahl's power law equation to estimate FRC for this species. This equation is

$$\text{FRC, ml} = 24.1 * \text{Body wt (kg)}^{1.13} \quad (5)$$

Table 9 summarizes the recommended values or equations for FRC in the various species that have been added to the MPPD software since the last UC Irvine dosimetry conference held in 2005.

4. Clearance Kinetics

The basic principle upon which clearance models are constructed is that of mass balance (i.e., the mass remaining in a given region is equal to the mass in the region at a prior time + the mass deposited from the airstream during the next time step + the mass coming in from other regions during the time step – the mass leaving the region during the time step). The MPPD model clearance

module for the LRT contains parameters that relate the transfer of material out of the TB and the alveolar regions.

Clearance from the TB region is facilitated by the flow of mucus towards the esophagus where the material is then swallowed. Based upon the peer reviewed literature, the MPPD model provides default values for mucous velocity, but the user can input their own value, particularly if they are studying a diseased animal model or if the human subjects have compromised lung function such as those with chronic obstructive pulmonary disease (COPD).

Clearance from the alveolar region can occur whereby: (1) alveolar macrophages engulf the deposited particles and then move upwards to the mucociliary escalator, (2) the particles dissolve in the surfactant layer lining the alveolar region and move out of the lung by the fluid movement of the portion of surfactant that ends up on the mucociliary escalator, or (3) enter alveolar tissue either via alveolar macrophages and wind up in the lymph nodes or via pinocytosis before entering the blood. Ideally, one has initial mass burdens after exposure ceases that are determined at various time points for one or more exposure concentrations. If one assumes the alveolar clearance rate constants depend on the initial mass burden at the end of exposure (m_{A0}), then $\lambda = \lambda(m_{A0})$.

The MPPD model uses the following functional form for λ :

$$\lambda = \gamma + \alpha \exp(\beta (m_{A0})^\tau) \quad (6)$$

where γ , α , β , and τ are constants estimated from the data. Originally, these constants were determined for poorly soluble particles and could not be changed by the user. However, recently, the MPPD software was modified to allow the

user to specify values for these constants as well as for the mucous velocities for the TB region, thereby extending the clearance modeling of MPPD to any type of particle. Being able to alter the mucous velocities is important when studying the clearance of particles in subjects with compromised clearance such as those with COPD.

Since γ in Eq. 6 represents the background level in the alveolar region absent exposure, this constant needs to be retained if there are endogenous amounts of the particle of interest (e.g., there is endogenous copper in lung tissue, so copper clearance modeling would need to include γ). The number of time points for which there are lung burden data dictates how many parameters can be estimated and how many need to have assigned a fixed value. Ideally, lung burden data will be available at a sufficient number of time points and concentrations such that all parameters can be estimated from the experimental data. Typically, a numerical solver can be used to estimate the optimum value of the parameters for the given lung burden data set.

5. Dose Metrics and Report Files

Normalizing mass deposited or retained at a given point in time to unit surface area allows one to compare species that may have significantly different upper respiratory tract and/or lung sizes and body weights. Originally, MPPD calculated the alveolar mass deposited per unit surface area using only the alveolar ducts. Recently, the software was modified to incorporate the surface area of all of the individual alveoli attached to these ducts for more accurate assessment of mass per unit area dose metric calculations in the alveolar region.

Given that a number of compounds are being regulated based upon their activity in the URT, the MPPD software has also been modified to present URT dose metrics. Quantities such as mass per unit time and mass per unit time per unit area are contained in report files to enable the user to better interpret the deposition of their aerosol from the perspective of assessing human risk or establishing therapeutic drug levels. Other report modifications have also been added to make documentation clearer.

6. Examples of Particle Deposition Patterns

Except for mice, for the species added to the MPPD software since the last UC Irvine dosimetry conference, the user only has to enter the body weight of the species, and then MPPD calculates the values of the various input variables from the mathematical relationships described in earlier sections of this paper. However, if the user has measured a particular variable, such as FRC, and their value is different from that calculated by MPPD, the user can override the value and deposition calculations will be done with the user's value instead.

Here, we will briefly provide some examples that illustrate the deposition patterns in the lower respiratory tract of various species in comparison to humans. Values of the physiologic variables used in the deposition calculations are summarized in Table 10 except it should be noted that the results for pigs and sheep in comparison to humans are for endotracheal breathing so the URT volume is ignored. This is because the CFD modeling of particle filtration in the URT is still primarily work in progress for these two species. In addition, while values of various physiologic variables are included in Table 10 for rhesus

monkeys, results for this species are not presented as deposition patterns in rhesus monkeys are presented in depth in Asgharian et al. (2012).

Comparisons of tracheobronchial (TB) and alveolar/pulmonary (P) deposition fractions relative to humans for mice (Balb/c and B6C3F1/c) and Sprague-Dawley (S-D) rats spontaneously breathing through the nose are given in Panels A and B of Figure 2, respectively. The TB region deposition fraction patterns are quite different between S-D rats and both strains of mice. The peak TB deposition fraction for particles $> 0.1 \mu\text{m}$ in diameter is about 0.27 in S-D rats and occurs for particles between about 2.5 and $3.2 \mu\text{m}$. In Balb/c mice, however, for particles $> 0.5 \mu\text{m}$ in diameter, deposition peaks at about 0.05 just under $2 \mu\text{m}$ (Fig. 2, Panel A) while for B6C3F1 mice the TB deposition fraction remains fairly constant at around 0.02 for particles up to $2 \mu\text{m}$ in diameter before starting to decrease. Due to a greater filtering efficiency of the URT in S-D rats compared to mice (data not shown), there is significantly more deposition of ultrafine particles that are $< 0.03 \mu\text{m}$ in diameter in both strains of mice compared to S-D rats (Fig. 2, Panel A). Extensive analyses of ultrafine particle (i.e., $< 0.1 \mu\text{m}$) deposition in mice are presented in Asgharian et al. (2014). For humans, the TB deposition fraction has a minimum value of about 0.05 at a particle size of $0.5 \mu\text{m}$, after which there is a slight increase over an extended particle range before beginning to decline after around $4 \mu\text{m}$ (Fig. 2, Panel A).

Pulmonary (P) deposition patterns are quite similar between S-D rats and both strains of mice for fine mode particles (Fig. 2, Panel B), which is considered to be particles $> 0.5 \mu\text{m}$ and $< 2.5 \mu\text{m}$ in diameter. In both mouse strains, there is

an overall steady decline in the P deposition fraction. As with the TB region, P region deposition fractions are significantly greater in the ultrafine mode for Blab/c and B6C3F1 mice in comparison to S-D rats, with peak deposition fractions being about 3 times greater than in S-D rats. Human P deposition fractions are substantially larger than they are in either of the rodent species for particles $> 1\ \mu\text{m}$ in diameter. The P deposition fraction in humans is about 0.2 for particles between 2 and 3 μm . And of course there is coarse mode (i.e., particles $> 2.5\ \mu\text{m}$) deposition in humans, with essentially no deposition of coarse mode particles $> 4\ \mu\text{m}$ in the rodent species (Fig. 2, Panel B).

Comparisons of TB and P deposition fractions relative to humans for sheep and pigs breathing through an end tracheal tube are given in Panels A and B of Figure 3, respectively. As noted earlier, the CFD modeling of the URT for the filtration efficiency of particles in pigs and sheep is a work in progress with some preliminary modeling results presented for pigs in a companion article (Asgharian et al., 2016) in this special issue. Here, we elected to compare the collection efficiency of the lower respiratory tract airways absent any influence of removal of particles by the URT, which gives insights as to the influence primarily of the TB structure on deposition. In sheep, the TB deposition fraction asymptotes at about 0.8, reaching this level at about 4.5 μm (Fig. 3, Panel A). For particles $> 1\ \mu\text{m}$ in diameter, the TB deposition fraction pattern for pigs closely tracks that of humans for end tracheal breathing, with a steadily increasing deposition fraction that has not reached an asymptote even at 10 μm .

Endotracheal breathing also has a significant impact on the pattern of deposition in the pulmonary region (Fig. 3, Panel B). The peak for human deposition shifts to about 4 μm and has a value a little over 0.4, which represents a shift in both location and magnitude compared to spontaneous breathing (see human curve in Fig. 2, Panel B). Although lower in magnitude, the fine mode P deposition fraction curve for pigs is similar to that of humans except for being shifted to the right and reaching a peak at about 6 μm . In the monopodial sheep lung, the peak pulmonary deposition fraction for particles > 1 μm in diameter occurs at around 1.5 μm before steadily decreasing as particle diameter increases.

7. Concluding Remarks

We have briefly reviewed a number of areas where improvements and additions have been made to the MPPD model software since the first UC Irvine dosimetry conference held in the fall of 2005. As imaging capabilities achieve greater resolution, there will be opportunities to expand our knowledge of the lung structure of various species and add refined models to MPPD. Basic physiologic data are needed for a number of species that would supplant the allometric relationships that have had to be used for some of the variables discussed in this paper. Periodic conferences such as the recent UC Irvine dosimetry conference will continue to stimulate development of new information that can be incorporated into mechanistic dosimetry models for the assessment of public health safety and the therapeutic use of drug aerosols.

Declaration of Interest

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Table 1. Brass Recycling Facility: Total dust and copper content in cascade impactor sample.^a

Dekati Stage	D_A (μm)	Total Dust (mg/m^3)	Copper ($\mu\text{g}/\text{m}^3$)			Copper as % of Dust	
			Soluble	Insoluble	Total	on Stages	in Total Sample
---	100	ND	ND	ND	ND	-	-
Inlet	30	ND	ND	ND	ND	-	-
1	10	0.063	-	4.90	4.90	7.778	0.825
2	7	0.055	0.26	3.80	4.06	7.382	0.684
3	4.3	-	-	2.60	2.60	7.382	0.438
4	2.7	0.077	-	2.10	2.10	2.727	0.354
5	1.8	0.098	-	1.50	1.50	1.531	0.253
6	1.1	0.093	-	0.54	0.54	0.581	0.091
7	0.7	0.088	-	0.59	0.59	0.670	0.099
8	0.4	0.120	0.46	0.63	1.09	0.908	0.184
9	0.3	-	-	0.25	0.25	0.908	0.042
10	0.2	-	-	-	-	-	-
11	0.1	-	-	-	-	-	-
12	0.06	-	-	0.34	0.34	0.908	0.057
13	0.03	-	-	-	-	-	-
TOTALS ==>		0.594	0.72	17.25	17.97		3.027

^a Data taken from Miller et al. (2013). Bolded numbers are worst-case estimates where no mass was detected on a stage but chemical analyses revealed some copper. D_A is aerodynamic diameter and ND stands for not detected.

Table 2. Airborne particle morphometry in the casting area at a brass recycling facility.^a

A. Mass Distribution

Mode (i)	Fraction _i	MMAD _i (μm)	GSD _i	<i>D_{av(i)}</i> (μm)
1	0.311	0.657	1.201	0.624
2	0.495	2.064	1.550	1.547
3	0.194	10.179	1.200	9.684

B. Count Distribution

Mode (i)	Fraction _i	CMD _i (μm)	GSD _i	<i>D_{av(i)}</i> (μm)
1	0.421	0.157	1.200	0.165
2	0.549	0.283	1.381	0.331
3	0.030	0.696	1.853	1.232

C. Particle Aspect Ratio and Density

	Chicago		
	Mean	Std. D.	C.V. ^b
Aspect Ratio	2.27	0.93	41%
Density (g/cm ³)	4.48	0.64	

^a Data taken from Miller et al. (2013)

^b Coefficient of variation

1 Table 3. Upper respiratory tract volume and body weights of various species and strains available in the MPPD software.

	Body Wt ^a , kg	URT Volume	R ²	Comment
BALB/c & B6C3F1 mice	0.03	0.0322 ml	-	Based on Table 2 of Méndez et al. (2010)
Sprague Dawley rat	0.3	$Y \text{ (mm}^3\text{)} = 9.6302 \times (\text{BWt(g)})^{0.6581}$	0.88	Exponentiation of eq. in Ménache et al. (1995)
Male rhesus monkey	6.5	$Y \text{ (ml)} = 0.56973 + 1.5336 * \text{BWt (kg)}$	0.982	Use for BWt ≤ 12 kg
Sheep	66	202 ml	-	Reported here
Pig	27	137.5 ml	-	Asgharian et al. (2015)
Human (adult)	70	50 ml	-	

^a Except for mice and humans, the user enters body weight for the species and MPPD scales data to the specified weight.

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- 4 Table 4. Breathing frequency values used in MPPD for various species and strains and a comparison with values
 5 obtained using Stahl's equation.

	Body Wt. (kg)	Breathing Frequency, bpm		Source
		Using Stahl (1967)	Assuming Independence of Body Weight	
BALB/c & B6C3F1 mice				Weighted mean of data in Méndez et al. (2010) plus data from Delorme & Moss (2002)
Whole body	0.02	148	277	
Nose- or head-only	0.022	144	388	
Sprague-Dawley rats				
Whole body	0.3	73	120	Miller et al. (2014)
Nose- or head-only	0.3	73	166	Miller et al. (2014)
Male Rhesus monkey	6.5	33	39.7	Asgharian et al. (2012)
Sheep	27	23	16	Derived here
Pigs	27	23	16	Derived here
Humans (adult)	70	18	12 ^a	

^a Applies to adult humans at normal resting ventilation levels.

7 Table 5. Tidal Volume values to use in MPPD for various species and strains.

	Body Wt (kg)	Values and Models for Tidal Volume ^a , ml	R ²	Comment
Balb/c & B6C3F1 mice				
Whole body	0.02	0.218	-	Strain data weighted mean, Méndez et al. (2010) & Delorme and Moss (2002)
Nose- or head-only	0.02	0.218	-	
Sprague-Dawley rats				
Whole body	0.3	Y = 0.7 x BWt (g/100)	-	S-D weighted mean, Costa & Tepper (1988) From Miller et al. (2014)
Nose- or head-only	0.3	Y = 1000(-0.060911+0.0013795* BWt)/166	0.48	
Male Rhesus monkeys	6.5			All rhesus data from Asgharian et al. (2012)
		Y = -23.818 +26.093*BWt -2.1946*BWt ²	0.796	Males, Use for BWt < 5.5 kg
		Y = -61.578 +45.391*BWt -4.3842*BWt ²	0.553	Females, Use for BWt < 4.5 kg
		Y = 1000*Minute Vol/39.7	-	Both sexes, BWt ≥ 5.5 kg for males & ≥ 4.5 kg for females
Minute Vol. Equations as a function of sex and monkey wt.		Y = 1.9108 -24.7378*exp(-1.2479*BWt)	0.731	Both sexes, Use for BWt > 4 kg
		Y = -0.44051 + 3.8434 * Log(BWt)	0.737	Males, Use for BWt ≤ 4 kg
		Y = -2.5302 +1.774 BWt -0.15866*BWt ²	0.59	Females. Use for BWt ≤ 4 kg
Sheep and Pigs	27			
Recommended method		Y = 1000*(0.499 * BWt ^{0.806})/16	0.975	Derived here by multiplying Bide et al. (2000) equation for minute volume by 1000 and dividing by an assumed bpm of 16.
Alternative method		Y = 1000*(0.009327 * BWt ^{1.069})	> 0.99	Derived here by simplifying ratio of Min. Vol equation of Bide et al. (2000) to breathing frequency equation of Stahl (1967) and multiplying the result by 1000.
Human (adult)	70	625	-	Assumed for normal resting ventilation

^a Body weight in model equations for rats is in g; otherwise BWt is in kg.

10 Table 6. Values for FRC in sheep obtained in various studies.

Body Wt., kg ^a	FRC, L	Method ^b	Source
35.9	1.41	N ₂	Mundie et al. (1992)
37.0	1.18	TGV	Needham et al. (1954)
40.0	1.14	TGV	Bossé et al. (1987)
32.0	1.00	TGV	Ahmed et al. (1980)
24.5	1.11	TGV	Abraham et al. (1980)
34.0	1.40	TGV	Wanner et al. (1979)
40.0	1.77	TGV	Fukushima et al. (1990)
35.0	1.31	TGV	Dyer & Snapper (1986)
35.0	1.35	TGV	Wheeler et al. (1990)
29.2	0.75	He	Wanner et al. (1979)
60.0	1.40	He	Hutchinson et al. (1982)
39.2, 45.1 ^c	1.79	Pleth	Hoffman et al. (2003), Group HM
38.9, 44.5 ^c	1.77	Pleth	Hoffman et al. (2003), Group HT

^a Body weight reported or calculated as mean or median value.

^b The methods used to determine FRC: N₂ = nitrogen washout, TGV = thoracic gas volume, He = helium rebreathing, Pleth = plethysmography.

^c The first number for body weight derived using Mundie et al. (1992) and the second number derived using Stahl (1967).

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17 Table 7. Pre-exposure measurements by Hoffman et al. (2003) of FRC and TLC
 18 in sheep before treatment with papain to induce emphysema

A. Homogeneous emphysema group

FRC, L	TLC, L	FRC/TLC	Estimated Body Weight (kg) Using	
			Mundie et al. (1992)	Stahl (1967)
1.78	2.90	0.614	39.1	45.0
1.53	3.04	0.503	35.8	39.4
1.73	3.80	0.455	38.4	43.9
2.05	3.45	0.594	42.6	51.0
1.78	3.45	0.516	39.1	45.0
1.84	2.78	0.662	39.9	46.4

B. Heterogeneous emphysema group

FRC, L	TLC, L	FRC/TLC	Estimated Body Weight (kg) Using	
			Mundie et al. (1992)	Stahl (1967)
1.07	2.64	0.405	29.8	28.7
2.26	3.35	0.675	45.4	55.6
2.06	3.52	0.585	42.8	51.2
2.49	4.10	0.607	48.4	60.6
1.32	3.27	0.404	33.1	34.6
1.39	2.52	0.552	34.0	36.2

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26 Table 8. Relationship of FRC to the body weight of sheep for various data sets
 27 and methods for estimating body weight.

Data Set	Body Wt. from	Linear Model Estimates ^a		R ²
		Intercept	Slope	
Study Means	Mundie	- 0.29833	0.04647	0.452
Study Means	Stahl	- 0.18321	0.042037	0.595
Study Means + Hoffman data	Mundie	-1.042	0.069843	0.771
Study Means + Hoffman data	Stahl	-0.287	0.045483	0.881

^a FRC (L) = Intercept + slope x Body Wt. (kg)

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34 Table 9. FRC values used in MPPD for various species.

	Values and Models for FRC, ml	R ²	Comment
BALB/c & B6C3F1 mice	0.3		Ave. from Crosfill and Widdicombe (1961) (strain not specified) and Yoshida et al. (2009) in C57BL/6J mice
Sprague Dawley rats	$Y = 0.1514 \times (\text{BWt})^{0.55}$	0.958	Exponentiation of equation in Takezawa et al. (1980)
Male rhesus monkeys	$Y = -52.593 + 68.651 \times \text{BWt} - 2.2103 \times \text{BWt}^2$	0.914	Both sexes for BWt ≤ 15 kg; from Miller et al. (2014)
Sheep	$Y = 1000(-0.287 + 0.045483 \times \text{Body Wt})$	0.881	Derived here
Pigs	$Y = 24.1 \times \text{Body Wt}^{1.13}$	0.941	From Stahl (1967)
Humans (adult)	3300		Reference man value; user can specify other value

^a Body weight for Sprague-Dawley rats is in g; body weight for sheep and pigs is in kg.

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37 Table 10. Typical values of physiologic variables for species added to the MPPD
 38 software program for whole body exposures.

Species	Body Wt (kg)	URT Volume (ml)	FRC (ml)	Tidal Volume (ml)	Minute Volume (L/min)	BPM
Balb/c mouse	0.02	0.0332	0.3	0.218	0.0604	277
B6C3F1 mouse	0.02	0.0322	0.3	0.205	0.0568	277
Sprague-Dawley rat	0.3	0.41	3.49	2.1	0.252	120
Male rhesus monkey	6.5	10.54	300	48.8	1.903	39.7
Pig	27	137.5	999	449	7.184	16
Sheep	27	202 ^a	950	449	7.184	16
Human	70	50	3300	625	7.5	12

^a This volume is for a 66 kg sheep.

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Figure Legends

Figure 1. FRC in sheep as a function of body weight. When necessary body weights in Panels A and B were estimated using Eq. 3a and 3b, respectively.

Figure 2. Particle deposition in the lower respiratory tract of mice, Sprague-Dawley rats, and humans as a function of particle diameter with spontaneous nasal breathing. Panels A and B provide results for the tracheobronchial and pulmonary regions, respectively.

Figure 3. Particle deposition in the lower respiratory tract of sheep, pigs, and humans as a function of particle diameter with endotracheal breathing. Panels A and B provide results for the tracheobronchial and pulmonary regions, respectively.

Figure 1A

Figure 1B

Figure 2A

Figure 2B

Figure 3A

Figure 3B