**Heterogeneity in lobar and near-acini deposition of inhaled aerosol in the mouse lung**

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**Abstract:**

Laboratory animals are often used to derive health risk from environmental exposure. To do so, it is important to measure not only the total dose of deposited particulates but also their spatial distribution in the lung. A unique database including both high resolution lung anatomy and deposition data in four strains of mice have been recently made available to the research community (Lung anatomy + particle deposition (LAPD) mouse archive: <https://doi.org/10.25820/9arg-9w56>). Using these data, we determined the effect of particle size (0.5, 1 and 2 µm) on the distribution of deposited particles between lobes. Analysis was performed on a total of 34 mice where 3 (16 and 15) animals were exposed to 0.5µm (1µm and 2µm) particles. Lobar deposition (volume) was normalized by the sum of deposition (volume) in each of the five lobes. For each animal, we then calculated the particle deposition to volume ratio for each lobe (). When , particle deposition is proportional to lobar volume; when differs from one, lobar deposition is relatively greater () or smaller () than lobar volume. At the near-acini level, for each animal, frequency distribution is constructed using single-compartment particle depositions. The skewness and standard deviation of the distribution are then calculated and regressed on particle size.

At the lobar level, significant deviation from 1 were found for DV ratio in the cranial lobe (), where deposition was relatively greater than lobar volume. , and were all significantly <1 and lower than (p<0.01). Furthermore, was positively correlated with particle size (p=0.004) and was negatively correlated with particle size (p=0.026). and also show a negative trend with respect to particle size but the regressions were not significant. At the near-acini level, positive correlations are found between particle size and skewness as well as standard deviation of the distributions.

In conclusion, an uneven distribution of deposited particles of the mouse lung at the lobar level and near-acini level is shown. Thus, depending on the lobe, individual lobe analysis to determine overall deposition may either underestimate or overestimate total lung burden, at least for particles in the micron size range. Varying particle sizes can introduce ineligible deviations of the density and spatial homogeneity of aerosol dosimetry measurements at the near-acini level.

1. **INTRODUCTION**

Exposure to airborne particulate matter (PM) plays an important role in initiating or aggravating respiratory and cardiovascular diseases. The understanding of the pathogenic effects resulting from such a PM exposure requires knowledge of the in-situ distribution of deposited pollutants on airway and alveolar surfaces. Such knowledge is also essential in any assessment of the therapeutic effect of a drug delivered by inhaled therapy. Animal models have long been used as surrogates to predict therapeutic effects in humans or possible adverse health effects arising from chemical and/or particulate exposures with mathematical models being often been used to complement experimental studies under different exposure conditions. Additionally, modeling can be used as a tool for interspecies dose extrapolation, an important element in preclinical and toxicological studies.

In recent years, sophisticated subject-speciﬁc computational models of aerosol transport and deposition in the lung have been developed for both humans (De Backer et al., 2008; Hofmann, 2011; Ma & Lutchen, 2009; Vinchurkar et al., 2012; Kuprat et al., submitted 2020) and research animals (refs). These models lack subject-speciﬁc experimental validation and have been mainly validated with averaged *in vivo* deposition data from the literature. As considerable inter-subject variability exists both in airway geometry and in deposition data, there is a need for detailed subject-specific datasets of lung anatomy and site-specific deposition information. Bauer et al. recently provided such data for the mouse lung in a publicly accessible repository, the lapdMouse archive (<https://doi.org/10.25820/9arg-9w56>). This archive provides high-resolution lung models of 34 mice combined with experimental data of local particle deposition and breathing parameters measured during aerosol exposure. These data may not only be used to develop more accurate models of particle deposition in the mouse lung but can also be analyzed to better understand the interplay between lung anatomy and regional aerosol deposition among animals. The mouse is one of the most commonly used animal models in toxicological and preclinical studies. It is thus important to understand heterogeneities in deposition patterns not only within a single mouse lung but also across different strains. This is the focus of this study. In particular, we investigated the effect of particle size on 1) the lobar distribution of aerosol deposition and 2) on deposition patterns at the near-acini level.

1. **METHODS**
   1. *Study data*

The data used in this study were obtained from the Lung anatomy + particle deposition mouse (lapdMouse) archive that has been described in detail elsewhere (Bauer et.al). Briefly, this unique database includes high-resolution anatomical data of the lungs of 34 mice that are linked to three-dimensional particle deposition maps. Mice of both sexes and of four different strains (B6C3F1, BALB/C, C57BL/6 and CD-1) were exposed to fluorescent aerosol particles with diameters of 0.5, 1.0 or 2.0 µm while free breathing in nose-only exposure chambers (Table 1). Following exposure, the lungs of these mice were imaged in a serial block-face imaging cryomicrotome at various wavelengths to isolate deposited particles and lung structure. The images were then processed to identify the 3D airway geometry and location of deposited particles. The airways from the trachea to the terminal bronchi were identified, labeled and represented as a mesh. These data were compiled by Bauer et al. (2020) in the lapdMouse archive that can be accessed at <https://doi.org/10.25820/9arg-9w56>.

* 1. *Data analysis*
     1. *Lobar deposition.*

In order to compare aerosol particle deposition densities across lobes, lobar volume () was normalized by total lung volume () and lobar particle deposition () by total particle deposition in the lung (). The volume-normalized deposition fraction in each lobe (*DVlobe*)was then calculated as the ratio between normalized lobar particle deposition and lobar volume, i.e.

(1)

For each mouse sample, DV ratios were calculated for each lung lobe: left lobe, right cranial lobe, right accessory lobe, right middle lobe and right caudal lobe and denoted as , , , and .

Since is the ratio of normalized particle deposition over normalized lobar volume, it is a good indicator of lobar particle deposition density. If the number of deposited particles is proportional to the lobar volume, ratio is one. If the density of deposited particles in a lobe is higher than the averaged whole-lung deposition density, then is greater than one. Inversely, if aerosol particles are sparsely deposited in a lobe, then is less than one.

* + 1. *Near-acini deposition.*

Bauer et al. (2020) partitioned the lung of each mouse into near-acini structures of ~3 mm3 resulting in ~350 compartments/lung. For each mouse, we ranked these compartments based on the density of deposited particles. Deposition densities (expressed in arbitrary units) ranged from 0 to 4.75, with 99.8% of the compartment having a deposition density ≤4. A forty-bin frequency distribution of near-acini particle deposition was then constructed. Any compartment with a deposition greater than four was considered an outlier and grouped together at the tail end of the distribution. The standard deviation (*SD*) and third moment about the mean (skewness, *Sk*) of the distributions were then calculated:

(2)

(3)

where is the total number of near-acini compartments and is the average near-acini single-compartment particle deposition.

* 1. *Statistical Analysis.*

*2.3.1 Lobar Deposition*

ratios were grouped by particle size and strain. For each group, multiple two-tail T tests were performed to determine if ratios are significantly different from one. Paired five-way ANOVA tests were run to compare the differences of ratios across lobes. Unpaired ANOVA tests were run to compare if ratios are distinctive in different mouse samples with different strain, sex, particle size and exposure time. ratios were also regressed on particle size. Tests with P values smaller than 0.05 are reported as significant.

*2.3.2 Near-acini Deposition*

The third moment and standard deviation of deposition density distributions were regressed on particle size. Unpaired T and ANOVA tests were performed to determine if distribution statistics were different across strains and sex.

**3. RESULTS AND DISCUSSION**

All datasets available in the lapd archive were used in this study except for one (mouse m25, 2 µm aerosol) that was labeled as being of poor quality (quality C). Thus, analysis from 33 datasets are presented here.

*3.1 Lobar deposition*

ratios averaged over all mice exposed to a given particle size (mean ± SD) are listed in Table 2 and individual *DV* ratios are shown in Figure 1 where different strains are identified by different symbols. There were variations in *DV* ratios among lobes and these variations increased with increasing particle size. For mice exposed to 2 μm particles, significant deviation from 1 was found for *DV* ratio in the cranial lobe (), where deposition was relatively greater than lobar volume (P<0.001) while , and were all significantly smaller than one (p = 0.020, p < 0.001 and p < 0.001, respectively). Similar trends were found for animals exposed to 1 µm particles, however significance was not reached for . For animals exposed to 0.5 μm particles, the only *DV* ratio that was significantly different than one was (>1, p = 0.033). Finally, irrespective of particle size, showed no difference from one, indicating that particle deposition in the left lobe is proportional to the lobar volume.

Data showed in Figure 1 and Table 2 compare well with previous studies in rodents. Brain and colleagues (ref) delivered aerosol (MMAD=1.6 µm) to both Syrian golden hamsters and Sprague Dawley rats in animal exposure chambers and determined the distribution of deposited particles through the evenness index (EI) defined as the ratio between normalized lobar deposition and normalized lobe weight. In both species, the EI was larger than one in the cranial lobe (EI = 1.42 in hamsters and EI = 1.51 in rats) while EI in the left lobe was close to one (EI = 0.98 in hamsters and EI=1 in rats). In rats, they also observed an EI < 1 in the right middle, right accessory and right caudal lobes. Morgan et al. (1983 Rad research) exposed SAS/4 mice to 239PuO2 particles with a median aerodynamic diameter of 0.8, 1.5 and 2.2 µm. They observed DV ratios larger than 1 in the cranial lobe and EI < 1 in the caudal and accessory lobe, with deviations from one increasing with increasing particle sizes, in agreement with data from this study (Table 2). Finally, in a more recent study, Yang and colleagues (ACS nano paper) delivered a liquid aerosol with a volume median diameter of 3.5 µm by mechanical ventilation in to C57J/6 mice. Even though the particle size was larger than those used in this study, the DV ratio in each lobe show similar behavior as those observed in the C57J/6 mice included in this study (Figure 2).

The distribution of deposited particles in the lung is closely linked to the distribution of inhaled air among the different regions of the lungs (Bennett et al, 2002, Moller et al., 2009). In humans (milic-emili, JAP 1966) and large animals such as horses (Amis 1984), the dependent lung region gets proportionally a larger fraction of a tidal breath than the nondependent lung regions. In contrast, in small animals such as the rat, the non-dependent lung region has been reported to be better ventilated than the dependent lung region (Rooney et al., Physiol Meas 30, 2009). This may explain the higher relative deposition in the non-dependent lobe (cranial) than in the dependent lobes of the right lung (accessory and caudal) of rodents.

*3.1 Near-acini deposition:*

At the near-acini level, three sample distributions exposed to aerosol particles of different sizes (0.5 μm, 1 μm and 2 μm) are shown in figure 2. Samples exposed to smaller aerosol particles tend to have more near-acini compartments with particle deposition closer to the mean, indicating a more homogeneous distribution. On the contrary, samples exposed to larger aerosol particles tend to have more near-acini compartments with denser depositions, indicating that heterogeneity is introduced with respect to increasing particle size. The skewness and standard deviation of near-acini particle distributions of all mice samples are calculated and plotted against particle size. Statistically, a positive skewness indicates that the distribution is right-skewed, and a numerically large skewness shows that more compartments have denser particle deposition compared to average.

Previous studies have shown that the likelihood of forming localized area of deposition is correlated to the skewness of the particle deposition distribution among all near-acini compartments. (Darquenne et.al) As shown in figure 3A, there is a positive non-linear correlation between skewness and particle size. This indicates that as particle sizes increase, the distributions tend to be skewed more towards the right, with more compartments with particle depositions above average. On the same note, as particle size increases, a more scattered distribution is observed, evident in Figure 3B. Therefore, it is shown that the association between increasing particle size and heterogeneity at the lobar level persists at the near-acini level. Even within the micron range, varying particle sizes can introduce ineligible deviations of the density and spatial homogeneity of aerosol dosimetry measurements.

Fractal analysis?

**4. CONCLUSION**

Analysis was performed on the newly available lapdMouse database to determine the special heterogeneity of aerosol deposition at lobar scale and near-acini scale. There was an uneven distribution of deposited particles among the lobes of the mouse lung. Particularly, cranial lobe receives higher deposition comparing to its volume, and caudal and accessory lobes receive lower deposition. The unevenness increased with increasing particle size (0.5 – 1 µm). Depending on the lobe, individual lobe analysis to determine overall deposition may either underestimate or overestimate total lung burden, at least for particles in the micron size range. At near acini level, larger particle size was associated with higher likeness of formation of hot spots and a less uniform spatial distribution of particle deposition.

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