**Heterogeneity in lobar and near-acini deposition of inhaled aerosol in the mouse lung: preliminary analysis of the lapdMouse dataset**

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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 the five distinctive lobes: 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. 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 as outliers and grouped together at the left tail of the distribution as four. 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 are 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 of greater variations () 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 are different across strains and sex.

**3. RESULTS AND DISCUSSION**

*3.1 Lobar deposition:*

Average ratios are calculated and tabulated for mouse samples that are exposed to aerosol particles with particle size of 0.5 μm, 1 μm and 2 μm. In the 2 μm size range, *DV* ratio in the cranial lobe (*)* is found to be significantly greater than one (p < 0.001). The difference is further proved to be significant in all individual strains except in C57BL/6. Meanwhile, , and are all smaller than one (p = 0.020, p < 0.001 and p < 0.001, respectively). Significance in difference remains in all strains. exhibit no difference from one. In the 1 μm size range, most trends revealed in the 2 μm size range persist. However, in the 1 μm size range is not different from one (p > 0.36). In the 0.5 μm size range, the effect sizes of most trends observed with larger particle exposure drop, but remains significantly greater than one (p = 0.033).

Individual ratios for each mouse sample are plotted with respect to different lobes and particle sizes. As figure 1 shows, heterogeneity of particle deposition across lobes exists and exacerbates with respect to increasing particle size. Regardless of particle size and strain, the ratios are always around one, indicating that particle deposition in the left lobe is generally proportional to the lobar volume. Homogeneity of particle deposition in the left lobe can be explained by the shorter path from the trachea to the left lobe. After the airflow enters through the trachea, it only passes one biphication to get to the left lobe, making the aerosol particles subjective to less morphological hinderance and energy lost. Unlike ratios, ratios tend to be greater than one, indicating that particles in the cranial lobe are densely deposited relative to the lobar volume. Over-deposition in the cranial lobe can be explained by the large overall biphication angle. Because for mouse, their cranial lobes locate in the upper half of the right lobes. Such morphological characteristics forces the airflow to make a sharp turn before delivered to and cleared from the cranial lobe, causing more significant energy lost of the aerosol particles. In later correlation studies, a positive correlation is found between and particle size (p = 0.004), indicating that larger particle size tends to worsen aerosol clearance in the cranial lobe. On the contrary, middle, caudal and accessory lobes tend to receive less particle deposition proportional to their volumes. This is likely because middle, caudal and accessory lobes locate at the lower half of the right lobe of the mice, making them less accessible to aerosol particles. Furthermore, a negative correlation is found between and particle size (p = 0.026), suggesting that larger particles have more limited accessibility to the accessory lobe. Therefore, individual lobe analysis to determine total lung particle deposition can either overestimate or underestimate the total lung burden, at least for particles in the micron size range. From figure 1, it is evident that of the four strains of interest, BALB/c comparatively yields the most stable DV ratios across different particle sizes whereas most amount of variations can be observed in C57BL/6. Thus, BALB/c is potentially a better animal model for experiments that require minimized variations in lobar deposition. With that said, despite the difference in amount of variations across strains, no significant correlations are found between DV ratios and strains or sex.

Result from previous studies (Otmar et.al) of aerosol exposure on BALB/c using droplets of diameter 2.5 - 4.0 μm aligns with our findings. Left lobe, proportional to its volume, receives most particles while cranial lobe (superior lobe) disproportionally receives more particles comparing to its volume. The rest three lobes: middle lobe, caudal lobe (inferior lobe) and accessory lobe (postcaval lobe) on average receives less particles comparing to their volumes. However, the results are not significant except in the left lobe. Furthermore, in past studies on the lobar deposition of particles (diameter ranging from 0.5 – 2.5 μm) in mouse, particle size has been found to be associated with deposition (Morgan et.al). Particularly, particle size is positively correlated with deposition in the cranial (right apical) lobe and negatively correlated with deposition in the caudal (diaphragmatic) lobe.

*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.

**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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