Manuscript Number: JAEROSCI-D-20-00128

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

Dear Dr. Darquenne,

Thank you for submitting your manuscript to Journal of Aerosol Science.

I have completed my evaluation of your manuscript. The reviewers recommend reconsideration of your manuscript following minor revision and modification. I invite you to resubmit your manuscript after addressing the comments below. Please resubmit your revised manuscript by August 7, 2020.

When revising your manuscript, please consider the reviewers' comments carefully: please outline every change made in response to their comments and provide a reason for any comments not addressed. Please note that your revised submission may need to be re-reviewed.

To submit your revised manuscript, please log in as an author at <https://www.editorialmanager.com/jaerosci/>, and navigate to the "Submissions Needing Revision" folder under the Author Main Menu.

Journal of Aerosol Science values your contribution and I look forward to receiving your revised manuscript.￼

Kind regards,

Robert Phalen

Special Issue Managing Guest Editor

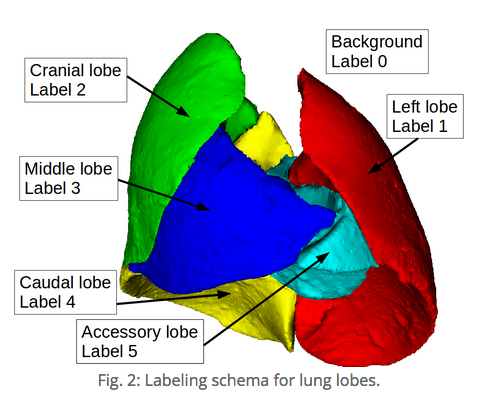
Journal of Aerosol Science

Editor and Reviewer comments:

Reviewer #1: This is a unique study in that using available measurements on particle deposition in the lungs of Balb/c mice, authors examined the hot spots (regions with high deposition). Findings of the study can aid in improved health risk assessment based on localized and not total dose of deposited particles in the lung. It can also inform drug delivery to targeted sites in the lung. The study is well done and this reviewer offers a few comments/suggestions to be addressed to improve the quality of the work.

It would be informative and help reader follow the manuscript if the manuscript includes a diagram of the mouse lung with each lobe indicated in the figure.

A figure has been added to the manuscript in section 2.2.1.



DVlobe depends on animal breathing rates and lung and lobar volumes. Were the data reported or calculated? Please report the information used in your calculations.

DVlobe is the ratio between lobar volume () normalized by total lung volume () and lobar deposition () normalized by total deposition (). All four variables, , , and are preliminarily measured and provided by LAPDmouse dataset.

A new table summarizing animal characteristics is now included in section xxx.

It is noted that DVlobe can also be interpreted as volume-normalized lobar deposition (Dlobe/Vlobe)/(D/V). This definition helps with the interpretation of deposition distribution.

Please define particle deposition density. Is it the same as DVlobe? If deposition density is defined as D/V, the discussion in the paragraph before section 2.2.2 follows easier.

Particle deposition density, in the context of this paper, is equivalent to the DVlobe ratio.

Authors should examine an article that calculates DVlobes to be nearly unity in humans (Asgharian et al., 2004: Journal of Aerosol Science, 17:213-224). The study does not include statistical analysis to determine heterogeneity of deposition.

Particle size distribution often follow lognormal distribution. Why isn't geometric standard deviations used in place standard deviation. Skewness can be calculated in a similar way.

Rather than the distribution of particle size, the statistical distribution addresses in this paper is the distribution of particle deposition. Currently we are not aware that there are literature showing that the particle deposition follows lognormal instead of normal distribution. With that said, the distributions should be bounded by zero and skewed to the right due to the presence of hot spots.

Figure 3: Define units for the x-axis.

The units for the x-axis are arbitrary, normalized based on individual mice.

First line after Figure 4: Change "size" to "sizes".

The paragraph before Figure 5: The discussion appears to apply to humans and not to mice. Better ventilation of apical lobes in humans is the effect of pleural pressure distribution, which is established by the gravity. In rats, lung expansion is expected to be uniform among different lobes because of animal's position. In humans, apical lobes have lower volume (and receive lower flows). I suggest authors compare lobar volumes in rats to confirm the explanation for higher impaction deposition in cranial lobes. Another point to make is that impaction deposition is significant in the first few airway generations of the lung. Flow in the near acini airways is low and impaction does not seem to be a viable deposition mechanism. For particle sizes of this study, it is likely due to sedimentation.

Reviewer #2: This is a very nicely presented study using a recently published database of Mouse lung anatomy and aerosol deposition.  It includes many more animals than in prior studies and explores the effects of sex and strain on observed patterns of aerosol deposition.  The authors find that aerosols with diameters ranging from 0.5 - 2.0 µm consistently deposit to a greater degree in the right cranial lobe.

Major Comments

1) Multiple t-tests performed raise a few issues that should be addressed.  Should the p-value chosen for significance be corrected for multiple comparisons as is commonly done with the Bonferroni correction.  Because the measures of aerosol deposition are relative to each other, they are not independent of each other.  If one lobe has a high deposition, another lobe must have a lower deposition.  I suspect that this non-independence needs to be considered in the statistical analyses.

Corrected p values are included in the manuscript based on Bonferroni correction. All results are still significant at the significance level of 0.05.

2) I am confused by the description of the apex-to-base distribution analyses.  The authors state that each near-acini compartment location was determined by its distance to a reference plane that intersects with the carina and is perpendicular to the bisector line between the main bronchi.  I envision this as a transverse plane (traditional CT slice) that is at the level of the carina.  Hence a compartment in the cranial lung region might have the same distance as a compartment in the basal lung region.  The data in figure 5 show compartments with distances of up to 20 mm from the carina.  This seems to be a large distance for a mouse lung if only being measured from the carina.  The data in figure 5 appear to be plotted as a function of location along the cranial to caudal direction rather than the distance from the carina.

As illustrated in figure 5b, the plane intersects with the carina and is perpendicular to the bisector line of the biphication.

3)  I have a tough time deciphering the legends in figure 2.  There appears to be 2 mouse studies (triangles) that are both black (this study).  The triangles indicating mice C57BL/6 suggest that only that strain was used in the figure but I do not get that from reading the text.  The text has C57J/6 mice while the legend has C57BL/6 mice.  Rather than using shades of grey to indicate the study, might the authors use solid markers, open markers, and dotted marker/lines?

4) Why were the 0.5 particles not included in figure 2B?

Only C57BL/6 mice in this figure. It is now clarified in the legend

5) The authors state a significant effect of "height" on the deposition pattern with deposition being greater in the lung apex.  In a prone mouse, the lung apex is at the same level as the lung base so I do not see the effect of height.  The authors later discuss non-dependent and dependent lung regions when they are talking about cranial and caudal regions, respectively.  Again, I do not think of these as non-dependent and dependent regions in a prone mouse.

6) The authors define "hot spots" within near-acini regions.  They then suggest that the hot spots are likely at airway bifurcations to the apical lung regions.  If I understand it correctly, the defined hot spots are in the near-acini compartments exclude the airways and therefore cannot be at airway bifurcations.

7)  Because the hot spots are defined as deposition greater than 2.3 standard deviations above the median, they are going to be much more likely to be present in regions of higher aerosol deposition.  Couldn't these hot spots simply be an accumulation of aerosols in highly ventilated lung regions.  The question is why do the cranial lung regions have a higher specific ventilation than other lung regions.  Traditionally it is thought that specific ventilation is greater in a lung region because either it is more compliant or has a greater transpulmonary pressure than other regions.  Obviously in a prone mouse, this cannot be gravity related.

Minor Comments

1) Abstract, last line - suggest adding "…depending on the lung sample location…"

2) Suggest table 1 present the numbers of animals analyzed in this study rather than the numbers in the database.

3) Last sentence in 2.2.2 should be "… and their spatial locations were recorded."

4) "Data showed in Figure 1 …" should be "Data shown in Figure 1…" 9page 10, line 4).

5) In general, cranial and caudal are better anatomical descriptors than apex and base, respectively.

\*\*\*\*\*

Data in Brief (optional):

We invite you to convert your supplementary data (or a part of it) into an additional journal publication in Data in Brief, a multi-disciplinary open access journal. Data in Brief articles are a fantastic way to describe supplementary data and associated metadata, or full raw datasets deposited in an external repository, which are otherwise unnoticed. A Data in Brief article (which will be reviewed, formatted, indexed, and given a DOI) will make your data easier to find, reproduce, and cite.

You can submit to Data in Brief when you upload your revised manuscript. To do so, complete the template and follow the co-submission instructions found here: www.elsevier.com/dib-template. If your manuscript is accepted, your Data in Brief submission will automatically be transferred to Data in Brief for editorial review and publication.

Please note: an open access Article Publication Charge (APC) is payable by the author or research funder to cover the costs associated with publication in Data in Brief and ensure your data article is immediately and permanently free to access by all. For the current APC see: www.elsevier.com/journals/data-in-brief/2352-3409/open-access-journal

Please contact the Data in Brief editorial office at [dib-me@elsevier.com](mailto:dib-me@elsevier.com) or visit the Data in Brief homepage (www.journals.elsevier.com/data-in-brief/) if you have questions or need further information.

\*\*\*\*\*

MethodsX (optional)

We invite you to submit a method article alongside your research article. This is an opportunity to get full credit for the time and money spent on developing research methods, and to increase the visibility and impact of your work. If your research article is accepted, we will contact you with instructions on the submission process for your method article to MethodsX. On receipt at MethodsX it will be editorially reviewed and, upon acceptance, published as a separate method article. Your articles will be linked on ScienceDirect.

Please prepare your paper using the MethodsX Guide for Authors: <https://www.elsevier.com/journals/methodsx/2215-0161/guide-for-authors> (and template available here: <https://www.elsevier.com/MethodsX-template>) Open access fees apply.

More information and support

FAQ: How do I revise my submission in Editorial Manager?