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Atmospheric Chemistry and Physics Discussions

Interactive comment on "Inflammatory responses to secondary organic aerosols (SOA) generated from biogenic and anthropogenic precursors" *by* Wing Y. Tuet et al.

Anonymous Referee #2

Received and published: 30 June 2017

Overall Recommendation:

This study examines an important aspect of atmospheric chemistry that hasn't been well examined in the past. Specifically, it remains unclear what the exact adverse effects (if any) of SOA are on human health. To begin understanding the potential adverse effects of SOA are on human health, this study exposed alveolar macrophages to SOA generated from the photooxidation of biogenic (isoprene, a-pinene, and b-caryophyllene) and anthropogenic (pentadecane, m-xylene, and napthalene precursors under varying condition of NOx level and humidity. Specific cellular responses were measured, including reactive oxygen/nitrogen species (ROS/RNS) production

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and secreted levels of cytokines (TNF-a and IL-8), with the latter known to be related to inflammatory response. I think this paper will eventually be publishable in ACP, but there are a number of revisions that I outline below that need to be considered very seriously by the authors. Some of the revisions relate to justifications missing for the specific cell line used here, comparisons to other literature, and also making more stronger connections to what this all means in terms of biological mechanisms. Many of the potential biological pathways altered by the exposures don't seem to be well justified using citations to the prior toxicological literature. Some of the results (as I expand below) may need to be reanalyzed and conclusions changed based on this re-analysis. Lastly, the conclusions appear muddled and sometimes hard to understand. I think it is really important to clarify the relationship between DTT activity, oxidative stress, inflammation, and downstream health effects. Specifically, particle-bound and particle-induced ROS are not necessarily the same. At this time, I must recommend accept with major revisions noted.

Specific Comments:

1.) Limitations of this study: I didn't see any discussion regarding the limitations of this study, and they mainly cited their own DTT papers throughout the discussion. This would be my most major criticism. You have to be careful to say that your acute exposures here will really translate to the in vivo condition. Specifically, why does one need to be careful in extending the results obtained from in vitro exposures to the in vivo condition? What are the potential issues with extracting filters for resuspension into cell culture? Does the chemistry change, and if so, how might that affect the toxicological response?

2.) Rationale for using murine alveolar macrophages: I think the authors should provide the rationale for using murine alveolar macrophages for this study. Would certain phenotype of this cell line differ from human alveolar macrophages? How easily relatable is it to human cells? What are limitations of cell lines versus primary cells and would that matter?

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3.) I noticed that the authors' cell culture and exposure media contain fetal bovine serum (FBS), which is known to potentially interfere with the ELISA assays. Normally people use serum-free media to avoid such interferences. Do the authors have any control experiments to show that FBS wouldn't interfere with their ELISA measurement?

4.) They use the cell media to extract filters. Since cell media contain a lot of supplementary materials/nutrients, would this affect the fraction of SOA materials extracted? Also, for the reactive products, would they be hydrolyzed before cell exposure?

5.) Lines 265-266: I think the red-ox activity is likely more sensitive to the functionality/electronic configuration of the functional groups, instead of carbon backbone. If it is carbon backbone, it looks to me that DTT is removed by other mechanisms such as absorption, but not through red-ox mechanisms.

6.) How these inflammatory responses relate to each other? Are they involved in the same biological network? They probably need to provide a more detailed biological background for the biomarkers they measured. For example, TNF-alpha induces IL-8 via NF-κB. This is well known in the toxicological literature. In some of the toxicological literature, TNF-alpha is used as positive control to stimulate IL-8 in BEAS-2B cells. I don't see a clear connection between the endpoints they measured in this paper and this needs to be more justified. Without a connection to a specific biological system, it makes it hard (especially for an atmospheric chemist I'm sure) to understand what your results really mean.

7.) Lines 288-290: The authors cite Lin et al. (2016, ES&T Letters), but I think this discussion is really unclear. What genes are similar? What pathways do the authors mean? They should make them clear. Note that Lin et al. (2016, ES&T Letters) only measured oxidative stress-associated genes, but not inflammatory genes in that paper. I noted that Lin et al. (2017, ES&T) just had a newly accepted paper where they found most genes are associated with the Nrf2 pathway, but not much inflammatory

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response from isoprene SOA exposure under non-cytotoxic conditions. Also, in Lin et al. (2017, ES&T) time course experiments, they found that IL-8 expression is time-sensitive. The expression maximized at 9 hr and much lowered at 24 hr, which was also shown in Arashiro et al. (2016, ACP). Their cellular materials were collected 24 hr post-exposure, so they might have missed the peak. How do the authors justify the 24 hr post exposure time? Did they conduct a series of time course experiments to see where things might peak in terms of cellular response. The authors and readers need to realize you may only captured 1 slice in time in how the cells responded.

8.) Line 305: what kind of chemical structure do they mean here?

9.) Line 322-329: I am not sure about the insertion of pentadecane oxidation products to the membrane. They should at least provide some references to support such a statement. I would expect some cellular response, specifically cytotoxicity, from these products since they are detergent like, which could potentially rupture the cell membrane. Did they see cell death from MTT data for pentadecane oxidation products?

10.) The mechanism of PAH-DNA adduct formation is well known through metabolic activation to diol epoxides. This is not mentioned at all in current discussion.

11.) Statistical Analysis: One more critical comment relates to the authors statistical analysis. Where are their linear regression resultss and the associated p values? Also, with multiple groups, one-way ANOVA should be used instead of student's t test to get p-values (same idea as the increasing type I error with multiple testing). Lastly, when they talked about the trend, I didn't see any statistical support to differentiate between groups. Are the results really statistically significant?

12.) I'm curious why the authors didn't gravimetrically weigh the filters before and after sampling to insure actual mass on filter for dose-response purposes? If you use the SMPS, you must make assumptions about density to calculate the mass. How was density accurately determined if you did use that approach? Was the SMPS sheath flow conditioned to the appropriate RH used in the chamber?

13.) Related to #12 above, were extraction efficiencies of aerosol mass from the filters determined by spiking them with representative internal standards? Extracting filters with cell media l may not actually remove a lot of materials (such as oligomers of SOA) from the filters. Why wasn't organic solvents used, then dried, and then the dried extracts reconstituted with cell media for the exposures? Toxicologists might find your dosing completely uncertain as its hard to gauge how well you removed the SOA from the filters without this information. This is a very important point for Figures like Figure 3. The AMS sees most of the SOA mass but filter extractions may not actually remove all of it for the exposure assessment done here.

14.) I have a curiosity question. Did the authors observe brown color on some of their filters (like from naphthalene SOA or isoprene SOA)? If so, did you seen any trends with brown carbon and your toxicological endpoints?

Minor Comments:

1.) Line 81-84: This seems to be an incomplete sentence or poorly worded sentence. Please revise.

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