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Atmospheric Chemistry and Physics Discussions

# *Interactive comment on* "*In Vitro* Exposure to Isoprene-Derived Secondary Organic Aerosol by Direct Deposition and its Effects on *COX-2* and *IL-8* Gene Expression" *by* Maiko Arashiro et al.

**Anonymous Referee #2**

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This paper assesses the toxicity of isoprene SOA through exposure of human lung cells to SOA formed in a chamber. The SOA is deposited directly onto cells and inflammatory biomarkers are monitored. Additional tests with resuspended filter-collected SOA confirms the response is due to particles and not gases formed or originally injected into the chamber (NOx, O3, VOCs). Toxicity is inferred from comparison of the biomarker responses to a seed aerosol (approx. 170 ug/m3 of MgSO4 and H2SO4) to the seed aerosol plus SOA (approx. 170 ug/m3 of acid seed + 30 to 40 ug/m3 isoprene SOA). By essentially noting an increase in the ratio of these biomarkers (SOA+seed/seed) the authors conclude isoprene SOA is toxic to humans. Combined with an earlier paper (Kramer et al., 2016), the authors are asserting that isoprene



SOA is toxic. The results of this paper should be of great interest to the air quality community considering the large implications of what is being proposed; biogenic SOA is toxic, and possibly as toxic as diesel emissions (Kramer et al, 2016). Unfortunately, the results are not highly convincing and I fear that these types of publications generally mislead the community since they leave the impression that biogenic SOA is a health hazard, when really, in this case for example, all they show is that cells responded to very high concentrations of a form of SOA produced in these laboratory experiments. For this reason I do not believe this paper should be published without some major discussion up front qualifying the results.

So, how toxic is isoprene SOA formed under these conditions, is it a health concern? As noted above, a reasonable conclusion from this work is simply for these concentrations, which are much higher than ambient, human lung cells responded, period. If these results could be directly compared to other forms of SOA, than some discussion of relative toxicity could be presented and a context provided. Lack of context is a major flaw and makes the paper results nearly impossible to interpret (see more on this below).

Furthermore, these authors recently published a related manuscript (Lin et al., ES&T letter, 2016), except in Lin et al SOA is formed from reactive uptake of MAE and IEPOX and more genes are measured. In a sense, the materials presented here could have been easily folded into Lin et al to provide more context and would have made a much stronger publication (for both papers). How does one put the findings reported in this work in the context of those reported in Lin et al? Why is this paper not cited in this work?

The following are some major issues.

What type of SOA is being formed? It is not clear chemically, exactly what type of isoprene SOA is being produced in these experiments. Put another way, how does this isoprene compare to what one would be exposed to the ambient environment

(maybe specify specific types of locations). It is not clear how just the presence of certain isoprene tracers observed in both the chamber and at YRK confirm the SOA is identical to ambient (at least identical to what was measured at YRK).

More specifically, it seems that isoprene OA presented in this paper is formed with NO injected into the chamber, with no additional HO2 source. Was isoprene decay measured over time? Under what NOx conditions are most isoprene reacted, and what does the RO2 react with? Self-reaction, with NO, or with HO2? From Figure 1, about half of the SOA is formed where there is NOx. Even after NO is zero, given the large amount of isoprene injected (several ppm), the RO2 + RO2 could be prevalent. It's not clear how "low-NOx" products (RO2+HO2) can be formed in these experiments, and that IEPOX-derived SOA can account for 80% of the SOA formed here. Is an HO2 source added to the chamber? Presumably the SOA in Yorkville is formed under low NOx conditions. More discussed regarding the chamber reactions are needed to justify relevancy to ambient data.

Compare the SOA in these experiments to that presented in their previous paper in Atmos Env (Kramer et al., 2016) where these authors assert that isoprene SOA is as toxic as diesel, based on the DTT assay. It seems the experimental conditions are similar to the manuscript here. However, apparently 2-methylglyceric acid is formed in these experiments (Figure 2 of this manuscript), but not in Kremer et al (Figure 2)? Why? Please provide detailed and specific comparisons on the chemical form of the isoprene formed in these two studies.

Are experiments done under dry or humid conditions?

Issues with Cell Details: The passage numbers used in these experiments seem very high. Please comment on the passage numbers and how determined.

From the results, it doesn't look like the time point is maximized for COX-2. Why was the specific time point used in these experiments they chosen? Is it representative of exposure? Is it to maximize gene expression, etc?



For the filter resuspension exposure, the cells are seeded 2 days prior to exposure and there's no mention of media change. If nutrients are not replenished are the cells highly stressed?

Did the cells exhibit inflammatory responds to the acid seed? Ie, what was the fold increase in the biomarkers for the dark seed experiments to the cells exposed to a completely clean chamber? This might give some sense as to the importance of the fold increase in SOA relative to dark (just seed) aerosol.

Issues with context: The authors state that a dose of 0.067 ug/cm2 to their simulated lung surface is sufficient to induce a response. What is the relevance of this number? Ie, can it be compared to ambient concentrations in any manner, or to say a minimum dose for responses of differing aerosol components in which similar health endpoints were measured? The lung surface area is very large. To have this kind of dose spread throughout the lung would require exposure to an enormous mass of isoprene SOA. The number 0.067 ug/cm2 has little meaning without some context (see more on lack of comparison to other work below).

The final line of the paper illustrates the limitations with lack of context, it states: Taken âĂĺtogether, this study demonstrates that atmospherically relevant compositions of isoprene-derived âĂĺSOA can induce adverse effects, suggesting that anthropogenically-derived acidic sulfate aerosol âĂĺmay drive the generation and toxicity of SOA âĂĺ

This seems too strong a statement, all one may infer from this work is that if you expose lung cells to very high doses of the specific type of isoprene SOA formed in these expts (see questions how atm representative it is), they respond. But cells will respond to many things. Context through relative toxicity could have been provided by doing two identical experiments, but with differing SOA types. Say isoprene vs some aromatic species found in incomplete combustion. There is some discussion near the end of the paper attempting such a comparison, ie comparison to aged diesel exhaust

(Lichtveld et al, 2012), but no definitive answer on the relative toxicity of isoprene SOA can be made because the contrast does not involve identical experiments, (ie, different cell lines were used) making it is difficult to conclude that any observed differences are due solely to the exposure of differing SOA chemical composition. I believe same, applies with Hawley et al, who used primary cells and not a cell line.

The authors further support their observations of inflammatory response due to isoprene SOA by noting they also find that the DTT response for SOA is higher than diesel ((Kramer et al., 2016). What they fail to note is that other analysis, based on ambient data, show a DTT response to isoprene SOA, but it is vastly smaller than the DTT responses to other sources, such as those from incomplete combustion (Verma et al., ES&T, 2015). This again demonstrates the limitation of this work due to lack of context; yes there may be a response to isoprene SOA, but how important is it? These authors may note that the Verma work involved only water-soluble extracts, whereas their experiments involved methanol, and so the difference could be due to non-water soluble isoprene SOA components. But the authors note here that the SOA constituents are "water-soluble (lines 329-330)... and remain well mixed in the cell medium".

Typos: Line 307, should it be Fig 4 and following, Fig 4 should be Fig 5?

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