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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 #3

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In this paper, the toxicity of isopren -derived secondary organic aerosol (SOA) was examined using the electrostatic aerosol in vitro exposure system (EAVES). The toxicity was evaluated by the lactate dehydrogenase (LDH) assay and also by probing the increase in the inflammatory genes il-8 and cox. Exposures were performed in the light and the dark, for induction of isoprene SOA. The SOA obtained from the EAVES was also compared to PM2.5 collected in Yorkville. Cells maintained in the EAVES system were also compared to cells maintained in regular incubator.

The study is very interesting and provides a new comprehensive approach in understanding the activity of different aerosol components. Overall, the experiments are well described and documented. However, some evidence and logic to explain several is-

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Discussion paper

sues are still lacking. Major issues: 1. The authors have used 1 hour exposure time. How the setting of 1 hour exposure was chosen? Have different time been measured e.g. longer or shorter than 1 hour? 2. “Photochemical aging was allowed for approximately one hour to reach the desired exposure conditions of 30-40 μg m-3 growth of isoprene-derived SOA on the pre-existing 170 μg m-3 of acidified sulfate aerosol” How was this calculation performed? Is this number relevant to real exposure to isoprene SOA? Please also relate to 0.067 ug cm-2.

3. Cytotoxicity measured by LDH is not sufficient for concluding that the isoprene secondary organic aerosol is not toxic. Another assay with a different principal should be performed, such as Hoechst (that interferes with DNA replication and not based on the activity of lactate dehydrogenase enzyme). In addition it would be useful to have an image of the cells before and after exposure?

4. Triton-X 1% raptures the cell’s membrane, causing leakage of the inner content of the cells. Therefore, its use as positive control is not be appropriate. It is better to use other cytotoxic agents that are known to cause cell death. 5. What is the biological significance of the increase expression of il-8 and cox genes? Please describe its relevance to a signaling mechanisms that is relevant to isoprene exposure. Minor issues: 6. Materials and methods: 2.3 section should contain the concentration of all the components in the medium, including antibiotics. 7. Section 2.7: add the formation of cDNA using RT (kit, company etc.) 8. Section 2.7: add the primers sequence for both gene tested. 9. There is no reference to Figure 5 in the text. 10. When relating to genes, please use small italics letters (il-8, cox) 11. In figure 2 the a3 graph (on the right panel) the line is in red. This is probably a mistake. If not please add the purpose for the red line in the legend 12. In the graphs indicating fold change, it would be better to write compared to what in the Y axis and not just the legend. Also add information about the normalizing gene in the legend.

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