Anonymous Referee #1

Received and published: 25 July 2012: ACPD 12, C4997–C4999, 2012

Reviewer's Comments:

Extensive literature exists regarding how gases and particles interact during the ageing of anthropogenic emissions in the atmosphere. Indeed the challenge is if and how gas and particle interactions affect the actual toxicity of PM of different source. A lot of toxicological studies focus on single species, or single primary pollutants coming from an identified source, in the search for causality for health outcomes observed in epidemiology studies. Thus it is true that these approaches ignore secondary pollutants and the complexity of the ambient air we breathe. Indeed it has been demonstrated that PM collected in summer or winter in the same urban area produces specific season related adverse effects. The PM samples collected in winter and summer seasons differ for dimensions, chemical and microbiological composition. Moreover the amount of photochemical ageing that occurs in a given system is dependent upon environmental variables such as the intensity of the sunlight, atmospheric pressure, temperature, and humidity. Thus, a part form the specificity of the seasons related chemicals linked to PM, air pollution mixtures aging in summer sunlight could accompany the increase in toxicity. This study was designed to extend the demonstration of the existence of "effect modification" to VOC systems that are more like those in large urban areas. The authors here report results which clearly show that when a non-toxic PM is added to these complex oxidized-VOC systems, the PM becomes toxic to cells in the PM-only biological exposure system. This is direct proof that in situ generation of gas-phase VOCs that are toxic to cells exposed in the gas-only biological exposure system can, in the presence of non-toxic PM, modify that PM to be toxic to cells. By extension to real urban atmospheres, the atmospheric oxidation of ambient primary VOCs can make otherwise non-toxic PM become toxic in the lungs of exposed humans.

Comments:

The experimental design is simple enough and reliable. Cultured human epithelial lung cells, type A549, has been used as single biological receptor model. The authors have demonstrated that it was possible to create a highly complex, urban-like system that consists of a mixture of primary, secondary, tertiary and beyond oxidized products, even if they failed to totally identify and quantify all of the components. Nevertheless, these systems remain representative of urban-like environments and are, therefore, useful in demonstrating the existence of PM "effect modification," even if the cause of the observed effect cannot be explicitly identified.

The authors discuss toxicity and inflammatory events sustained by photochemically produced gaseous toxicants which can in turn modify non-toxic PM. Although partially out from the scope of this investigation the authors should take into account to test these toxicants also in

immortalized human bronchial epithelial and pulmonary endothelial cells. In this scenario it might be the case that these toxicants elicit different adverse effects in A549 alveolar epithelial cells and in bronchial epithelial cells. Moreover, these studies should be completed in in vitro air-blood barrier model in order to assess the interplay between pulmonary microvascular endothelial cells and alveolar epithelial cells cultured on the opposite sides of a permeable filter support. Indeed the compartmentalization of the barrier-forming bilayer allows mechanisms of lung injury to be studied in both the epithelial (intra-alveolar) and the endothelial (intravascular) compartments.

This study provides nevertheless important insight to unravel the specific chemical causes of gas-phase toxicity by itself and demonstrates that oxygenated compounds are major components in the evolution and transfer of toxicity. These results should be considered adequate to support the authors' hypothesis.

Author's Reply:

Thank you for including your summary of the paper. We find such feedback very helpful in assessing the degree to which we were successful in conveying our results. We would also like to thank you for your comment about the importance of this work, and that our results were adequate to support our conclusions for this demonstration-of-principle work.

We also appreciate your suggestion regarding the application of alternate and/or multiple cell types from varied regions of the body. We share your desire to unravel the interplay between various parts of the cardio-pulmonary organ system. We feel that such studies will be vital to fully understand the complex interactions between inhaled pollutant exposure and organism-level health outcomes (such as heart disease). We did not perform exposures to multiple tissue types in this study to avoid overwhelming the demonstration of effect for the atmospheric processes of interest with biological data. Therefore, by limiting the number of endpoints and tissue types included in the study, our focus (and hopefully that of the reader) remained on the demonstration of the physio-chemical phenomenon's effect on a measured biological response. For that purpose, we agree with you that the biological response measurements we selected are adequate. We do hope, however, to expand the findings presented here to include precisely the types of inter-cellular signaling you have described in your comments.