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Atmospheric Chemistry and Physics Discussions

Interactive comment on "Generation of hydrogen peroxide from San Joaquin Valley particles in a cell-free solution" *by* H. Shen et al.

Anonymous Referee #2

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OVERALL QUALITY

This paper makes valuable contributions to understanding the chemistry that may be involved in the lung following inhalation of ambient PM. It also demonstrates a method for detecting and evaluating the potential toxicity ambient PM.

It showed that 1) urban California PM generate HOOH in a solution that simulates the antioxidant content of human airway lining fluid, 2) that the HOOH generating potential of the PM was greater in the urban than the rural collection site, 3) that the fine PM in the airsheds studied contributed more HOOH (per air volume sampled) than the coarse PM, 4) that there were no seasonal differences in the HOOH generating capacity of the sampled PM, and 5) that physiologically relevant levels of ascorbate (present in lung epithelial lining layer) and copper ions present in the PM samples appeared to be the

most important contributors to the HOOH generation. The expression of HOOH generating capacity in terms of both air volume and milligrams of PM weight is commendable and leads to improved understanding of the role of PM size and mass which are also related to source.

The method used for extracting the PM and detecting HOOH generation in the surrogate lining fluids (SLF) appears to be an improvement over earlier studies.

The work presumes a significant role for HOOH in the mediation of toxic responses and makes an effort to prove that the levels of HOOH that might be generated in vivo are similar to the levels that have been shown to exert toxicity or cellular signaling responses in cell cultures.

SPECIFIC COMMENTS

The ability of dissolved transition metals and oxygen to generate HOOH in an aqueous solution is undoubtedly an important factor to consider and a useful indicator of the potential toxicity of inhaled PM. As noted by the authors, HOOH is not a particularly toxic ROS, and it is believed to be an important signaling molecule in vivo.

An important overall comment is that transition metals catalyze oxidations involving molecular oxygen (often termed 'autoxidations'). The long history of the study of 'Fenton chemistry' that involves HOOH seems to create the belief that HOOH must be generated in order for an oxidation to occur. In fact, HOOH might only be generated in the absence of a substrate (protein or lipid) for more reactive precursors in a reaction cascade starting with molecular oxygen (see below).

The chemistry of the reactions that occur in the epithelial lining fluid (ELF) of the lung may be quite different from the modeled aqueous solution, and the role of HOOH generation may be more for signaling than for mediating the toxic events.

Consideration of the following possibilities is suggested.

The air liquid interface is composed of a lipid-protein-antioxidant layer, while the sur-

rogate fluid used in this study contained no lipid or protein. This difference could lead to a different interpretation of the chemistry than what was reported in the Discussion. Sun et al (2001) showed that when a surrogate lipid-protein-antioxidant solution is exposed to oxygen-18 labeled molecular oxygen (18O2) in the presence of a redox metal-containing fly ash, the 18O-containing reaction products are found in the lipids and proteins. The presence of lipid was necessary for the incorporation of 18O into the protein fraction. The antioxidant enzymes (catalase, glutathione peroxidase and superoxide dismutase) had no effect on the metal-catalyzed incorporation of 18O. Other studies involving metal-catalyzed oxidation were also cited in this paper in which HOOH degrading enzymes were unable to inhibit metal catalyzed oxidations (Khossravi and Borchardt, 1998; Schoneich et al, 1993). It was suggested that the transition metals bind to the protein and lipid and cause oxidative reactions to occur at such close proximity that the antioxidant enzymes are unable to intervene. It was also noted that HOOH degrading enzymes are already present in the ELF (Cantin et al, 1987 and 1990). Also, as noted in the present manuscript, HOOH would diffuse readily across membranes, making the assumption that it would accumulate in the ELF appear invalid.

Thus, although the redox activity of the metals, as demonstrated in the present study, is an important quality of the metal containing ambient PM samples, it is not clear whether HOOH is mediating toxic reactions in vivo or signaling adaptive cellular responses. These ideas would suggest a more cautious approach than presently taken in the manuscript where efforts are seemingly made to prove that the HOOH generated could mediate the toxic responses.

TECHNICAL CORRECTIONS

There are several instances in the paper where the abbreviation for 'molar' and 'moles' appear to be confused. For example, in 2.3.5 it states that '1.0 mM of DSF was added to the SLF' where it should say 'DSF was added to the SLF to a final concentration of 1.0 mM.' In later sections the rate of accumulation of HOOH is often correctly stated as nmoles/hr, but it is sometimes also given as uM/day (page 21339 at the end). A steady

state concentration achieved could be labeled as 'molar' but not a rate of accumulation / time. It should also not state that this estimated concentration is in the 'lung' but in the 'lung lining fluid'. Time needs to be included in some places. For example, in the same page it should state: 'Using the average of the maximum daily HOOH production amounts (38 nmol /m3/ time). Additionally, 'per meter cubed' also should not be expressed as m-3 but as /m3.

Supplementary material appears very similar to the included figures and tables. Typo: 'Studay' in Figure S9.

Not mentioned in the manuscript are several papers that have measured HOOH in expired breath of diseased human subjects (see attached references). Exhaled HOOH concentrations never exceed $\sim$0.8 uM, and these occur only under pathological conditions much more severe than would be encountered by a person breathing ambient air.

Given ideas mentioned above, the discussion of HOOH accumulation in vivo should be greatly modified and shortened.

2.4.15-25 Great detail is given of some aspects of the method, however, the basic chemistry involved is not clear. The chemical basis of the HOOH assay needs to be stated as a 'peroxidase catalyzed oxidation of POPHAA to a fluorescent product in a continuously flow system'. Does the potassium hydrogen phthalate (KHP) participate in the reaction, or is it only an inactive ingredient?

2.3.10 It should be more clear that the baked aluminum foil was added to the impactor of the sampler to collect the coarse PM. The time and temperature used for baking the foil should be given.

2.3.20 The fact that 4 ml solutions of PM in buffer were reacted in a vial with only 3 ml of head space suggests a lack of appreciation that the reaction that is being examined starts with molecular oxygen. Can the authors somehow demonstrate the chemical

reaction that is the source of the HOOH generated? Also, what is a 'PFA vial' and how was it 'acid washed'?

3.3.20 Typo: remove the word 'approximately' from the sentence prior to 'these fractions of HOOH'.

Page 21341 line 8. Citrate should not be labeled an antioxidant. Also, check the discussions of citrate being involved in the chemistry of lung lining fluids. Citrate concentrations are very low absent in normal extracellular fluids.

CITED REFERENCES

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