Atmos. Chem. Phys. Discuss., 10, C11365–C11371, 2010
www.atmos-chem-phys-discuss.net/10/C11365/2010/

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.

H. Shen et al.

canastasio@ucdavis.edu

Received and published: 21 December 2010

Generation of hydrogen peroxide from San Joaquin Valley particles in a cell-free solution

H. Shen 1, A. I. Barakat 2, and C. Anastasio 1 1 Department of Land, Air and Water Resources, University of California, Davis, One Shields Avenue, Davis, CA 95616, USA 2 Department of Mechanical and Aeronautical Engineering, University of California, Davis, One Shields Avenue, Davis, CA 95616, USA

MS No.: acp-2010-544

Answers to Anonymous Referee #2

SPECIFIC COMMENTS

Q: 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 surrogate 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.

A: We thank the reviewer for his or her comments. We agree that the chemistry that occurs in the real ELF of the lung might be quite different from our modeled aqueous solution, although the ability of particles to generate HOOH in our cell-free assay likely corresponds to their ability to generate ROS in vivo. We also agree that our cell-free study has its limitations and that it is hard to extrapolate our cell-free results to potential biological effects; we made both of these points in our ACPD manuscript. The reviewer also makes a good point that the HOOH concentrations we measured in our solutions are unlikely to occur in vivo following PM inhalation, because of HOOH-degrading enzymes in the ELF as well as diffusion of HOOH across cell membranes. However, the concentrations that we measure are a proxy for the total amount (i.e., flux) of HOOH that could be formed from deposited particles in the lungs, and it is likely that this flux is related to toxicity. Thus even if steady-state concentrations of HOOH in vivo are lower than we measure (e.g., because of diffusion across membranes), the flux of HOOH produced in vivo can be estimated based on our results. According to our results HOOH can be continuously generated at relatively high rates so that large amounts of HOOH can be produced within a relatively short time, potentially causing toxic effects. In addition, transition metals from PM can turn HOOH into the more toxic hydroxyl radical, which can cause a variety of cellular damage in the lung. We therefore think that the HOOH formed in the ELF is likely to mediate toxic events in addition to functioning as a signaling molecule.

In light of the reviewers' comments, we have added several sentences to the last section of our Implications section to include these additional uncertainties and the issue of concentration/flux.

TECHNICAL CORRECTIONS

Q: 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.'

A: Yes, 1.0 mM was the final concentration of DSF in the SLF. The sentence was changed to "DSF was added to the SLF to get a final concentration of 1.0 mM".

Q: 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'.

A: We've corrected the HOOH concentration unit to "uM" and mentioned in the text that this level of HOOH was calculated using 24 h of inhalation of particles (hence our previous units). The estimated HOOH concentration is indeed in the "lung lining fluid" (rather than in the bulk "lung") and we have corrected this in the text.

Q: 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.

A: Here we use the maximum HOOH level (e.g., 38 nmol m-3) that was obtained in our extraction (typically after 4 h) to calculate the HOOH concentration in lung lining fluid. We've clarified this point in the text. We have not changed the formatting of "m-3" since

this is the specific format required by the Journal.

Q: Supplementary material appears very similar to the included figures and tables.

A: Yes, the supplementary material is very similar to that in the main text, but they are showing different quantities: we show rates of HOOH formation in the text and maximum levels of HOOH production in the supplementary material.

Q: Typo:'Studay' in Figure S9.

A: We cannot find this typo in Fig. S9 (or in the supplementary material or main text).

Q: 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 _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.

A: HOOH is highly water soluble, with a Henry's law constant at 37 degrees C of 4 $\times$ 10000 M atm–1. Assuming a lung lining fluid volume of 25 mL and a total lung capacity of 6 L, 99.97 % of all of the HOOH in the lung (i.e., in the ELF and lung air) should be present in the ELF. Thus a low concentration of HOOH in exhaled breath condensate does not indicate that ELF concentrations of HOOH are low.

Q: 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?

A: As suggested, we have added a few more details in Section 2.4 about the chemistry of the HPLC method used for HOOH detection. KHP does not participate in the reaction but is used to buffer the pH of the fluorescence reagent to optimize the conditions for

the reaction.

Q: 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.

A: We modified the sentence in Section 2.3 according to the reviewer's suggestion to "Aluminum foil was baked at 400 degrees C for 24 h and then put into the sampler to collect PMcf".

Q: 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'?

A: 1) There was an abundance of O2 in our solutions and accompanying headspace, so O2 availability should not limit the formation of HOOH or other ROS in our conditions. First, our solutions were air saturated, which is the equivalent of 270 μM O2 at 25 degrees C. Second, the 3 mL of air in the head space could supply an additional 6400 μM of O2 to the SLF solution. Clearly there was an enormous excess of O2 present in our sample vials. In addition, our control experiments show that whether the volume of extraction solution in the 7 mL vial was 6 mL or 4 mL, the amount of HOOH produced from the same sample was not affected.

2) We believe that HOOH (and other ROS) formation in our solutions occurs via reaction sequences such as,

M(red) + O2 $\rightarrow$ M(ox) + superoxide; M(red) + superoxide + 2H+ $\rightarrow$ M(ox) + HOOH; M(red) + HOOH $\rightarrow$ M(ox) + hydroxyl radical + OH-; M(ox) + Asc(red) $\rightarrow$ M(red) + Asc(ox)

where M and Asc represent the transition metal and ascorbate, while (red) and (ox)

represent the reduced and oxidized forms, respectively, of these species.

This information was added to the Introduction.

3) A PFA vial is a type of Teflon vial. The vial was normal washed first using soap and ethanol, and then soaked in an acid bath (2M HNO3) for 1h to remove metals followed by rinse with Milli-Q H2O. The text was modified accordingly.

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

A: We removed the word 'approximately' from this sentence.

Q: 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.

A: We agree with the reviewer that citrate should not be labeled as an antioxidant, We've corrected this part of sentence to "other lung fluid antioxidants (e.g. glutathione) and components (e.g. citrate)". We include citrate as a component of lung fluid since the earliest surrogate lung fluids were a mixture of interstitial fluid and blood serum with citrate included. People commonly use citrate as a surrogate for protein, and as an iron mobilizer in asbestos studies. However, we can find no references that have measured citrate in lung fluid, although it has been found in a number of other biological fluids. Regardless, since we did not use citrate in this current work, this is a topic that should be explored in future work.

Please also note the supplement to this comment:
http://www.atmos-chem-phys-discuss.net/10/C11365/2010/acpd-10-C11365-2010-supplement.pdf

---

Interactive comment on Atmos. Chem. Phys. Discuss., 10, 21323, 2010.