

Atmos. Chem. Phys. Discuss., referee comment RC3
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Comment on acp-2021-666

Anonymous Referee #3

Referee comment on "Are reactive oxygen species (ROS) a suitable metric to predict toxicity of carbonaceous aerosol particles?" by Zhi-Hui Zhang et al., Atmos. Chem. Phys. Discuss., <https://doi.org/10.5194/acp-2021-666-RC3>, 2021

The work by Zhang et al. used an online instrument based on the DCFH/HRP assay to measure particle-bound ROS in SOA generated from representative biogenic and anthropogenic precursors. They then collected the same types of SOA on filters and quantified the ROS formation using the same DCFH assay as well as OP using the DTT assay on the filter extracts. They also investigated cytotoxicity via cell viability and cellular ROS production using a DCFH-DA assay from A549 cells exposed to buffer-extracts of these SOA. They found that most of the acellular ROS are short-lived and photochemical aging enhances ROS production and DTT activity. Compared to biogenic SOA, the anthropogenic SOA has a higher acellular and cellular ROS production and higher cytotoxicity. They also concluded that acellular particle-bound ROS could be a suitable metric to predict aerosol particle toxicity and health effects of aerosol given its strong correlation with cellular ROS. Overall, the results are interesting and the paper provides good discussion on related work from literatures and limitations of the study. Below are my comments:

Major comments:

- Please specify what ROS species can be detected by the DCFH/HRP assay? OH, O₂-etc?

also organic peroxides can react with the assay but organic peroxides are not ROS.

- The authors find that naphthalene SOA has a higher ROS content than the biogenic SOA, and it is likely due to quinones and semiquinones in naphthalene SOA that forms superoxide radicals. Is DCFH assay known to be sensitive to superoxide? Semiquinone radical can oxidize DCF radical to form DCF which yields superoxide (Rota et al., provided below) and superoxide forms H₂O₂. How do the authors tell whether the ROS signal is from quinones-DCF chemistry or SOA aqueous chemistry?

Rota C, Chignell CF, Mason RP. Evidence for free radical formation during the oxidation of 2'-7'-dichlorofluorescein to the fluorescent dye 2'-7'-dichlorofluorescein by horseradish peroxidase: possible implications for oxidative stress measurements. *Free Radic Biol Med.* 1999 Oct;27(7-8):873-81. doi: 10.1016/s0891-5849(99)00137-9. PMID: 10515592.

- The analytical methods used in the work (DCFH/HRP, DTT, and cellular DCFH assay) are known to be sensitive to H₂O₂ and/or organic peroxides. Is it possible that peroxides are essentially what the authors are measuring which explains the strong correlations between acellular and cellular ROS?
- section 2.2, details are in Fuller et al. (2014) and Wragg et al., (2016) but it would be useful to briefly discuss what the differences are between online and offline ROS measurements? Are the online extracts filtered? How did you quantify losses inside the denuders? Some descriptions about the DCFH/HRP methods are mentioned in section 2.3, if the online system uses the same method, maybe should move the related method description up to section 2.2.
- line 327, "...ROS components react with HRP seconds after the particles enter the instrument." Some ROS have lifetimes in a range of ns. It would be useful to specify what ROS can the authors capture with the method.
- I am also confused by the authors' use of "particle-bound" and "Short-lived ROS" to describe ROS formation from SOA. My understanding of the online system is that SOA are collected into liquid and then mix with the DCFH probe. Some ROS lifetime can be very short that by the time the samples react with DCFH probe, they might be gone. Particle-bound ROS refer to ROS on the SOA particle. However, the method not only captures the particle-bound ROS with a lifetime longer than the time it takes to travel from PAM to mix with the probe, but also the ROS formed through SOA aqueous chemistry.

Minor comments:

- Offer et al. (2021) are cited many times throughout the manuscript, but according to the reference list, it is a paper under review. Please specify in the main text.
- line 125, could you explain why O₃ are removed prior to online measurement and filter collection?
- line 387, "To the best of our knowledge, the OP of SOA β PIN-SP from this study is the first reported in the literature." Tong et al., EST 2018 paper has provided OP of SOA β PIN.
- line 404, "Compared to naphthalene-derived SOA, β -pinene SOA are expected to contain a negligible amount of quinones but peroxides are suggested to contribute significantly to the OP of β -pinene SOA." The authors cited Wang et al., 2018 and Jiang and Jing, 2018, but these two studies did not use β -pinene SOA. Please correct.