

Response to the comment to the Anonymous Referee #1

The paper from Arangio et al. measures the concentration of environmentally persistent free radicals (EPFR) and reactive oxygen species (ROS) in size segregated ambient aerosols. EPFR were measured directly by EPR spectrometer, while the ROS were measured by extracting the particles in water and then EPR analysis. As per the reviewer's knowledge, this is first comprehensive measurement of EPFR and ROS in size-segregated aerosols. ROS are an important species in ambient aerosols and could be biologically relevant. In addition to these novel measurements, authors also throw lights on the possible mechanisms of ROS generation through redox cycling between organic compounds and transition metals. An improved understanding of these mechanisms is important to comprehend the aging process of atmospheric aerosols. The paper is well written and easily comprehensible. Therefore, I recommend the publication of this manuscript. However, I have few comments which the authors should consider to make their work better:

Response:

We thank the referee's review and very positive evaluation of this manuscript. The point-by point responses are given below.

Page 2, Line 42: Are there literature evidences that organic radicals also mediate in the oxidative stress? If yes, then authors should include them.

Response:

Some types of organic radicals such as semiquinone and phenoxy radicals are known to play a role in oxidative stress (Pryor et al., 1995; Winterbourn, 2008; Birben et al., 2012). We have added new references.

Page 3, Line 87: Why these two samples were collected for a longer duration? Are the authors not concerned about the loss of semivolatiles during that long sampling duration?

Response:

We agree that semi-volatile compounds may be lost for long sampling duration, which is a common problem of the particle collection using an impactor. Two samples were collected for 48 h in order to obtain sufficiently high mass loadings for all particle size ranges. We have clarified this point in the revised manuscript.

Page 4, Line 128: Authors should somewhere explain these units of spins μg^{-1} , probably in the method section.

Response:

The unit spins μg^{-1} indicates the number of spins (or radicals) per μg of particle mass. We will clarify it in the revised manuscript.

Page 4, Line 129-131: Can authors elaborate on their sentence that EPFR distribution is similar to soot? Do you mean that there is commonality in the sources of two?

Response:

Yes, we think that the sources of soot and EPFR are very similar (e.g., combustion) and EPFR may be often associated with soot particles (Dellinger et al., 2001). We will clarify this point in the revised manuscript.

Page 5, Line 143: Why the samples collected on these two days are significant and discussed separately?

Response:

As explained above, for certain periods we have collected particles for 48 h to collect enough particle mass to perform EPFR and ROS analysis for wide particle diameters (50 nm - 1.8 μm). On the other hand, particles collected for 13 days with a sampling time of 24h were focused on limited particle size range of 50 nm to 500 nm diameter particles.

Page 5, Line 143-150: I am not sure why the authors have discussed the sampling duration separately. The EPFR concentration expressed in units of spin/ μg should not be affected by the sampling duration.

Response:

Indeed the EPFR concentrations are not affected by the sampling duration, but the particle diameters were different. We will clarify it by including the below sentences in the revised manuscript:

“EPFR concentrations contained in particles within the diameter of 50 nm – 3.2 μm collected for 48 h during 26-27 June 2015 was $\sim 2.2 \times 10^{11}$ spins μg^{-1} . EPFR concentrations contained in particles

within the diameter of 56 – 560 nm averaged over the entire measurement period was $2.0(\pm 1.3) \times 10^{11}$ spins μg^{-1} .”

Page 6, Line 176: Is it 41

Response:

Carbon-centred radicals are reduced to 40% in the 1 μm stage. Thanks for point out this typo, we will correct it in the revised manuscript.

Page 6, Line 201: What are the units here for ROS, is it spins/ μg ?

Response:

The unit is μg^{-1} and not spins μg^{-1} , as H_2O_2 is not radical, but it can be still directly compared with concentrations of EPFR and radical forms of ROS (in the unit of spins μg^{-1}) as measured in this study.

Page 7, Line 203-215: I think the authors are completely confused here. DTT assay doesn't measure the ROS in the particle, rather the capability of particles to generate ROS in surrogate biological environment. I am not clear what the authors want to deduce in this discussion and what is the significance of this number of $(2-7) \times 10^{14}$ μg^{-1} of DTT molecules? It is important to note that DTT activity is a completely arbitrary unit and depends on the initial DTT concentration used in the assay.

Response:

The DTT assay measures the consumption rate of DTT molecules due to reactions of redox-active components of particulate matter with antioxidants. The total number of DTT molecules consumed per unit of mass and time are measure of the redox activity or oxidative potential of chemical compounds contained in the particles. The underlying assumption of the DTT assay is that the consumption of one DTT molecule would lead to the generation of one ROS molecule (e.g., H_2O_2). We agree that this assumption has not proved robustly and we are actually planning to investigate this aspect in details in the follow-up study. We think it is still meaningful to make this comparison, but we will refine the sentence to avoid confusion in the revised manuscript.

Page 8, Line 237: Can the authors add references showing HULIS is known to contain substantial amount of quinones?

Response:

We have added the following reference:

Verma, V., Wang, Y., El-Afifi, R., Fang, T., Rowland, J., Russell, A. G., and Weber, R. J.: Fractionating ambient humic-like substances (HULIS) for their reactive oxygen species activity – Assessing the importance of quinones and atmospheric aging, *Atmos. Environ.*, 120, 351-359, 2015.

Page 10, Line 308-310: I don't think that this study shows that ROS can be generated in lung fluid. I think again the authors are confusing between ROS activity (capability of particles to generate ROS) vs. ROS on the particles (measured in this study).

Response:

As pointed out, this study itself did not show that ROS can be generated in the lung lining fluid containing antioxidants, but it did show that the particles can form ROS in water. Several previous studies have shown that redox-active components such as transition metals and quinones can induce formation of ROS species upon interactions with lung antioxidants (Charrier et al., 2014; Charrier and Anastasio, 2011). We will clarify it in the revised manuscript as below:

“Previous studies have shown that redox-active components such as transition metals and quinones can induce ROS formation in surrogate lung lining fluid upon interactions with antioxidants (Charrier et al., 2014; Charrier and Anastasio, 2011). This study also implies that ROS may be released in lung lining fluid upon inhalation and respiratory deposition of atmospheric aerosol particles.”

References:

Charrier, J. G., and Anastasio, C.: Impacts of antioxidants on hydroxyl radical production from individual and mixed transition metals in a surrogate lung fluid, *Atmos. Environ.*, 45, 7555-7562, 2011.

Charrier, J. G., McFall, A. S., Richards-Henderson, N. K., and Anastasio, C.: Hydrogen Peroxide Formation in a Surrogate Lung Fluid by Transition Metals and Quinones Present in Particulate Matter, *Environ. Sci. Technol.*, 48, 7010-7017, 2014.

Birben, E., Sahiner, U. M., Sackesen, C., Erzurum, S., and Kalayci, O.: Oxidative stress and antioxidant defense, *World Allergy Organization Journal*, 5, 1, 2012.

Pryor, W. A., Squadrito, G. L., and Friedman, M.: The cascade mechanism to explain ozone toxicity - The role of lipid ozonation products, *Free Radical Biol. Med.*, 19, 935-941, 1995.

Winterbourn, C. C.: Reconciling the chemistry and biology of reactive oxygen species, *Nature Chem. Biol.*, 4, 278-286, 2008.