Response to comments from referee #1

We would like to thank the reviewer for the helpful comments and suggestions.
Our response and corresponding modifications are listed below.

General comments:

In this manuscript, the authors assessed the DTT activities of lab-generated SOA using flow tubes from two PAHs (phenanthrene and naphthalene). The mass normalized DTT activities of SOA were compared to those from monoterpenes (limonene and α-pinene). Peroxide content was also determined to assess the contribution from peroxide to OP. Aging effects from oligomerization, heterogeneous oxidation, and mixing with copper were investigated on the DTT activities of SOA. In my opinion, this is an important work. It provides reference to future studies on the DTT activities of SOA and addresses some current open questions regarding OP. It provides a new angle of looking at chemical composition that contributes to OP and health. The manuscript overall is fairly strong and I recommend acceptance of the manuscript after following small corrections are done.

Specific comments:

1. Line 36-37, the authors stated "Oxidative stress has been…, and is often expressed as the oxidative potential (OP)". This sentence is misleading in that "oxidative stress" is "oxidative potential" while these two terms are different. The authors should make sure the definitions of these two terms are clear.

Response: Oxidative potential is the capacity for inhaled air pollutants to cause redox imbalance through consumption of antioxidants and generation of reactive oxygen species (ROS).

Oxidative stress is the redox imbalance induced by oxidative potential (Adler et al., 1999;Finkel and Holbrook, 2000). Hence, the definitions of oxidative potential and oxidative stress in this study were modified as below:

“Oxidative stress has been proposed as one of the main mechanisms for PM toxicity in recent years, and is caused by oxidative potential (OP) (Li et al., 2003b). OP is exhibited as the capacity of inhaled PM to induce oxidative stress, the redox imbalance generated through consumption of antioxidants and production of reactive oxygen species (ROS) (Antiñolo et al., 2015; Shen et al., 2011; Shiraiwa et al., 2012).”

2. Line 37-38, delete “mass normalized”. OP can also be expressed in “volume normalized”.

Response: Modified. Please see line 38 in the manuscript.

3. Section 2.3, the authors quoted McWhinney et al. papers for the DTT protocols, despite that, detailed protocols should be provided either in method section or the supplement. For example, what is the volume of sample added to each well, what is the concentration of DTT solution (i.e. the initial DTT concentration in the reaction), concentration of DTNB, was DTT consumed more than 50%, and etc.

Response: The method section has been expanded to include in more experimental details. Please see line 179-line 189 and Fig. S2c, d in the manuscript.

The volume of SOA sample in each well was 160 μL. The concentration of DTT was 0.2 mM. The concentration of DTNB was 2 mM (10 times in excess) of DTT. Such initial DTT concentration was determined in order to maintain an eventual DTT consumption near/over 50% (Fig. S2c, d) for PAH derived SOA tested in our study.

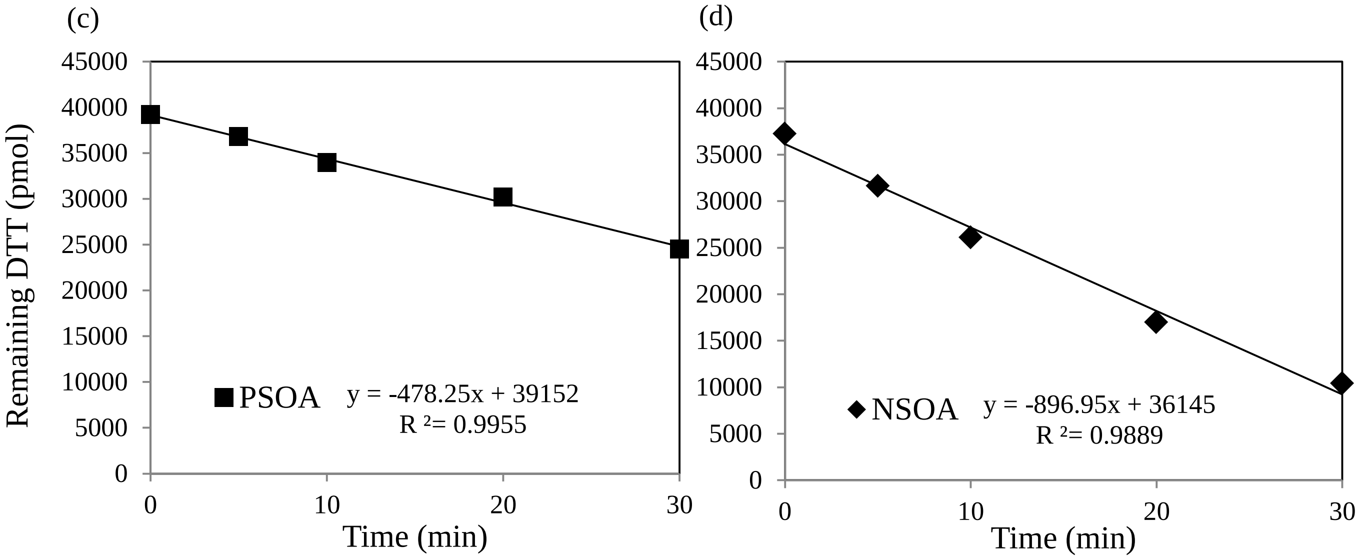
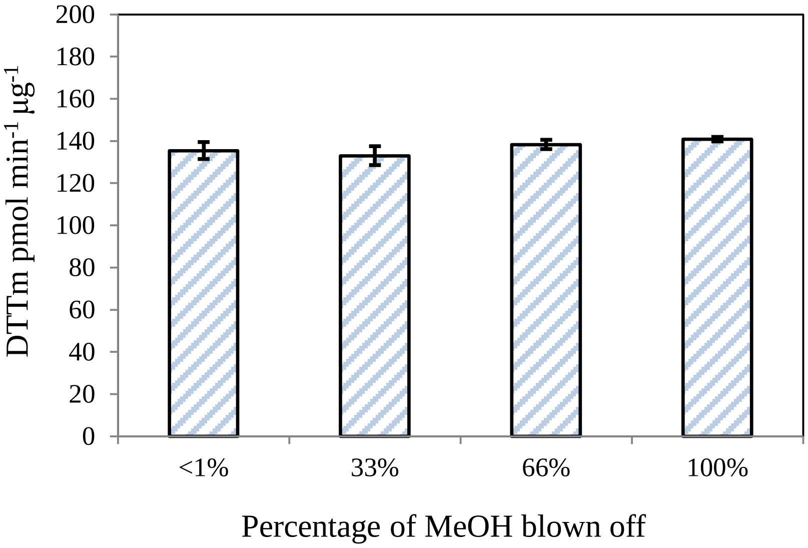


Figure S2. (c) DTT activity of PSOA. (d) DTT activity of NSOA.

4. Line 173, the SOA was extracted in Methanol then blown down to complete dryness before re-dissolving in phosphate buffer. Have the authors assessed the vaporization loss of SOA upon complete dryness? Some Methanol-soluble compounds might not be solubilized in phosphate buffer which is mainly DI. The authors could in a way underestimate the OP of SOA due to artifacts from complete dryness.

Response: This is an important question for oxidative potential assays. In this work, we determined "complete dryness" based on visual inspection. When using the N_2 blow-off system to evaporate methanol, other compounds with relative high volatilities may also evaporate during this process. Based on the reviewer's suggestion, we conducted a series of tests to investigate whether the loss of volatile compounds along with the N_2 blow-off procedure affects the DTT activity of SOA. Four sets of NSOA methanol solutions (with the same SOA amount extracted) were evaporated under the N_2 blow-off system to various extents, with which 100%, 66%, 33%, <1% of the original methanol remained. All the solutions were then replenished with methanol to the same total volume and reconstituted to the experimental concentration with phosphate buffer for measurement of DTT activity. The results are shown below:



Despite the difference in degree of evaporation, there was no significant change in the DTT_m observed in this study. Thus, we deduced the evaporation of volatile SOA compounds during N_2 blow-off procedure did not lead to a significant underestimation of the OP of SOA in this study. It is likely that SOA compounds from naphthalene/ phenanthrene with high volatility may be less oxygenated and less likely to be dominant OP contributor, which is also consistent with our findings in section 3.4. However, it should be noted that this conclusion may not hold for all atmospheric samples, and the contribution of semivolatile compounds to oxidative potential may be significant and could be underestimated with the extraction protocols described in this work.

5. Line 188, should be “Fig. S2(b)”?

Response: Corrected. Please find line 191 in the manuscript.

6. Line 363, peroxide content from α-pinene are 40-100% in this study which is larger than other previous studies (Docherty et al., 2005; Epstein et al., 2014; Mertes et al., 2012) where roughly 20-60% of peroxide are found in α-pinene SOA. Please explain why such large variation in this work and larger values compared to other studies.

Response: Compared to many of the previous studies which used relatively small molecules (i.e. H_2O_2 or -O-O-) for estimating SOA peroxide content (Nguyen et al., 2010;Epstein et al., 2014), the mass of SOA peroxides was calibrated by using benzoyl peroxide as standard in our study. The molecular weight of benzoyl peroxide (242.23 g mol^{-1}) might be larger than the actual averaged molecular mass of peroxides in SOA, leading to a relative higher result. In addition, Mertes et al. (2012) assumed spherical particle geometry density of α-pinene SOA 1.3 g cm^{-3} while our study used a density of 1.25 g cm^{-3}. Using a smaller SOA density might also lead to a higher SOA peroxide fraction. Moreover, according to the study of Mutzel et al. (2013), the process of ultra-sonication might also lead to the decomposition of organic peroxides into hydroxy radical. Those formed hydroxyl radicals are able to further form H_2O_2 and inflate the final SOA peroxide content. At the same time, other studies have shown that organic peroxides decompose into carbonyls and alcohols, which might also result in a lower detected peroxide content. Compare to the study of Docherty et al. (2005), the ultra-sonication time of our study was under 5 minutes while they used 10 minutes, which may explain the differences in results if ultrasonication leads to overall loss of peroxide content. Other factors like the O_3 exposure, temperature and relative humidity during SOA formation may also lead to differences in the calculated peroxide content. Overall, the conclusion from our study is that despite the relatively high peroxide content of α-pinene SOA in our study and in other studies, there is no apparent contribution to DTT activity, suggesting that organic peroxides are not dominant SOA OP contributors in the systems we studied.

7. Line 379, please explain why the number of DTT_m of benzoyl peroxide is 38 pmol/min/ug while that in Table 2 is 160 pmol/min/ug.

Response: Thanks for pointing this out. The value of "160" was a typo. After various testing, the DTT_m of benzoyl peroxide in our study was eventually measured to be 37 pmol min^{-1} μg^{-1}. Corresponding information was corrected in the manuscript. Please find the updated Fig.3, Table 2 and line 382 in the manuscript.

8. The initial DTT at t=0 (18000 pmol) in various types of peroxides in Figure 3 are different from that in blank controls (~37000 pmol, Figure S2). Please explain why the authors chose different initial DTT amount to begin with between blanks and samples.

Response: The DTT_m for peroxides are generally lower than other tested chemicals (redox-active) in this study. We determined this initial DTT concentration (in excess) for peroxide based on an estimation of eventual DTT consumption percentage. However, in order to measure DTT activity under the same initial conditions, we repeated the OP measurement of peroxides (120 μM) with the same initial DTT concentration (0.2 mM) as other DTT experiments conducted in this study, such that they are comparable. The results are shown in the updated Fig.3.

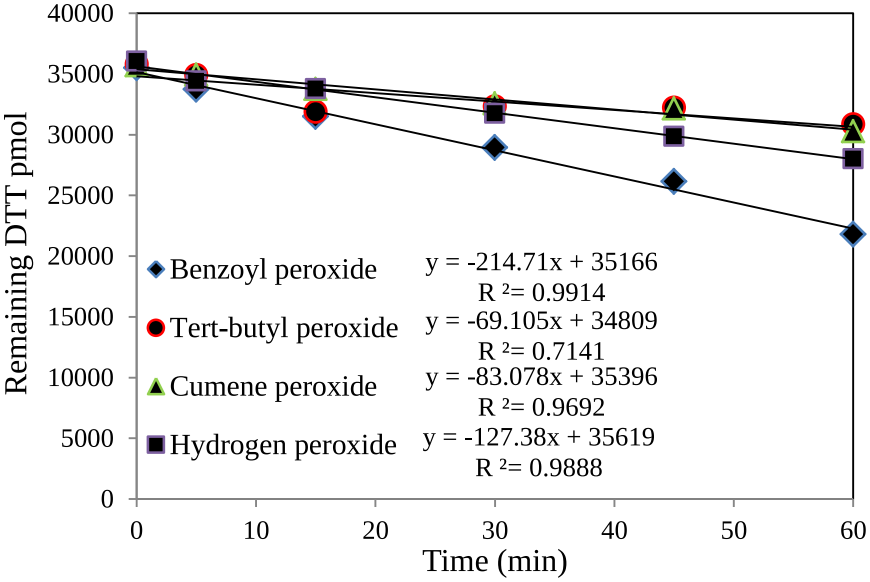


Figure 3. DTT activity of various types of peroxides (hydrogen peroxide, cumene peroxide, tert-Butyl peroxide, benzoyl peroxide). With the same initial concentration of peroxide (120 μM), benzoyl peroxide has the highest DTT activity (converted to DTT_m of 37 pmol min^{-1} ug^{-1}).

9. Figure 6, asterisk missing?

Response: Corrected. Asterisks were added onto Fig.6.

10. Figure 9, can the authors comment on the delta decrease of T2 relaxation larger in the lower δ(^{1}H) range than higher range with Cu added to SOA compared to SOA alone? This is not observed in 1,2-NQN case (Figure 11)

Response: Thank you for the comment. We apologize for not clarifying the regions in ^{1}H NMR of the SOA data. The peaks at lower δ (^{1}H) region (3-5ppm) are mainly associated with protons on aliphatic hydroxyl, while those from 6-9 ppm are mainly from aromatics. As quinones (1,2-NQN) in this study is purely aromatic, there are no groups that resonate lower than 6 ppm. We have now updated it in the manuscript to clarify the NMR regions.

We clarified the description in the revised manuscript (section 3.5) as below:

"……interactions between copper and SOA components. In general, protons adjacent to and within aliphatic hydroxyl groups resonate between 3-5 ppm, while aromatic groups and double bonds arise between 5-9 ppm. Before the addition of copper, NSOA T2 relaxation time show a range of values consistent with a complex material with a diversity of functional groups and dynamics. It was after the addition of copper, both of the aliphatic hydroxyl and aromatic region showed a decrease in T2 and inherit roughly similar relaxation values. This suggested copper is able to influence a wide range of SOA components, with both structural categories (aliphatic hydroxyl and aromatic) involved in copper binding to some extent."

References

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