

Response to comments from referee #1 (Manuscript Ref. NO: acp-2017-214)

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Thank you for your valuable comments. We modified the manuscript accordingly.

General comments: This manuscript presents a laboratory chamber study to investigate the contribution of particulate oxidizers in secondary organic aerosols to the oxidative potential measured dithiothreitol (DTT) assays. The significance of quinones, organic hydroperoxides, and peroxyacyl nitrates (PANs) in SOA samples from various precursors and conditions was characterized, and directly compared to the measured DTT activity. The authors report that the presence of particulate organic hydroperoxides can explain most of the DTT consumption of isoprene SOA (almost 1:1 correlation), and partially for toluene SOA, while the amount of PANs and quinones appears to be negligible in the SOA systems investigated here. With completely different approaches, it is intriguing to see the results reported in this study to be closely consistent with Kramer et al. (Atmos Environ, 2016) who directly measured the DTT activity of synthesized isoprene-derived hydroperoxide (ISOPOOH) standard and reported a very strong DTT response (almost comparable to the quinone compound and 3 order of magnitudes higher than other major isoprene SOA constituents).

Overall, this is a well-planned study and the manuscript is well written. I have a few specific questions and suggestions are listed below for the authors' clarification and consideration. I am in support of publication once these questions have been addressed.

Specific comments:

1. Page 5, Line 24: should the storing condition here be 4 °C instead of -4 °C?

Response: This sentence was modified and reads now,
“Before chemical analysis, all SOA samples were stored in a refrigerator at 4 °C.”

2. Page 6 Line 2: how the reaction between DTT and SOA could be quenched by adding 1 mL trichloroacetic acid (1 % w/v)? Was this to quench specific components in SOA samples before measuring the remaining DTT with DTNB? More detailed explanation is needed as it is not clear here.

Response: Trichloroacetic acid is a strong organic acid ($pK_a = 0.5$) and commonly used as a quencher of thiol oxidation (Cho et al., 2005, Fang et al. 2014, Curbo et al. 2013) by decreasing pH. Trichloroacetic acid is crystal. It is easy to prepare the quenching solution by weighing trichloroacetic acid. This sentence was modified as below:

“...the reaction between DTT and SOA was quenched by adding 1 mL 1% w/v trichloroacetic acid (a commonly used quencher of thiol oxidation).”

Reference: Cho, et al., Redox activity of airborne particulate matter at different sites in the Los Angeles Basin, Environ. Res., 99, 40-47, 2005.

Sophie Curbo et al., Is Trichloroacetic Acid an Insufficient Sample Quencher of Redox Reactions? Antioxidants & Redox Signaling, 18, 2013.

Ting Fang et al., A semi-automated system for quantifying the oxidative potential of ambient particles in aqueous extracts using the dithiothreitol (DTT) assay: results from the Southeastern Center for Air Pollution and Epidemiology (SCAPE), Atmospheric Measurement Techniques, 2014

3. Page 6 Line 13-17: how the slopes shown in Figure S1 compare to each other statistically?

Response: With significance level $\alpha = 0.05$, the slope of ΔDTT vs. time with sonicator was not significantly different from the one with shaker, tested using the statistical method which is based on the Student t-test (Andrade and Estévez-Pérez, 2014)). This sentence was modified and reads now,

“Tested using the statistical method based on the Student t-test (Andrade and Estévez-Pérez, 2014), the DTT loss rate in blank control during sonication was not significantly different from that with shaker (significance level $\alpha = 0.05$); therefore, in this study, the influence of free radicals generated by sonication on DTT measurement was insignificant.”

4. Table 1. It looks like no seed aerosols were introduced into the chamber experiments. Did the SOA form solely because of nucleation?

Response: No, SOA was formed without seed aerosol.

5. How the chemical composition and DTT activity might change if there are pre-existing seed aerosols?

Response: It is a valuable question to think. With pre-existing aqueous seed aerosols, SOA yields will increase and SOA compositions will also change. We cannot simply answer for this question because aerosol formation is complex. What we can think is that the water content and aerosol acidity can influence the lifetime of unstable compounds, partitioning of organic species, and the reaction with atmospheric oxidants in particle phase.

6. Figure 4: why a higher concentration of potassium phosphate buffer was needed for the Toluene LNOX-17 Nov 2016 sample? Would changing buffer concentrations affect the measured DTT activity?

Response: Please find the last paragraph of Section 3.1.

“Medina-Ramos et al. (2013) reported that the electron transfer rate between glutathione (GSH) and an electrogenerated mediator ($[\text{IrCl}_6]^{2-}$) exhibited a slight acceleration when the phosphate buffer concentration was increased from 0 to 50 mM at pH = 7.0. To achieve the completion of the reaction between particle oxidizers in SOA and DTT, the DTT_m of toluene SOA ($\text{HC}/\text{NO}_x=24$ ppbC/ppb) was measured with a 0.8 mM potassium phosphate buffer in the first step of DTT assay (2 times higher than the typical buffer concentration (0.4 mM)).”

Thus, the higher concentration of potassium phosphate buffer was applied to toluene (17 Nov 2016) sample and accelerated the rate of the reaction between DTT and toluene SOA.

Response to comments from referee #2 (Manuscript Ref. NO: acp-2017-214)

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Thank you very much for the thoughtful and constructive comments. The paper was greatly improved based on your suggestions.

In this work, Jiang et al. studied the oxidative potential of Secondary Organic Aerosol (SOA) generated in laboratory chambers. The authors investigated the role of various oxygenated compounds within complex SOA systems in contributing to oxidative decay of sulfhydryl groups, which is believed to be the main mechanism by which particulate matter causes adverse cardiopulmonary outcomes. The authors systematically varied experimental conditions and carefully measured various functional groups, and relate the composition to OP measured (using the DTT assay). Based on the experimental results, they conclude that organic hydroperoxides (ROOH) are a major source of oxidative potential, and suggest that electron-deficient alkenes (such as acrolein) may also be important too.

The experiments are done with sufficient controls, and carefully interpreted. The DTT activity of SOA has recently become a topic of great interest within the atmospheric community. The work is within the scope of ACP and should be published after considering the following minor comments.

Major comments:

1. The suggestion about electron-deficient alkenes being important is worth noting. Acrolein seems to be the only example that has significant DTT activity. All others are an order of magnitude lower. Are there example compounds that are more relevant to toluene/benzene system? I would imagine that aromatic compounds would yield conjugated compounds, which according to Figure 7 and Section 3.4, would have negligible DTT activity. Also, acrolein has very high vapor pressure and is unlikely to be in the particle phase.

Response: Acrolein, methyl acrolein, 2,4-hexadienal and mesityl oxide were chosen as model compounds to show how electron-deficient alkenes increase DTT activity. The DTT activity of alkenes increases with an electron withdrawing group such as carbonyls but decreases with an electron donating group such as alkyl groups. The electron-deficiency of acrolein is relatively higher than methyl acrolein, 2,4-hexadienal and mesityl oxide (conjugated carbonyl with 2 methyl groups). As discussed in Section 3.4, the toluene SOA contains a large amount of alkene that are coupled with electron withdrawing groups such as carbonyls, nitrates, and carboxylic acids. These electron-deficient alkenes in toluene SOA might contribute to the DTT response.

2. Related to the previous note, in Section 3.1, the decrease in DTT activity is on the same timescale of acrolein. That is not a fair comparison because acrolein is reacting in the gas phase, where the DTT measurement is for compounds in the particle phase, which likely have longer lifetimes than acrolein in the gas phase.

Response: In order to respond to the reviewer, the lifetime of two semi-volatile conjugated carbonyls (4-oxo-2-butenic acid, and 2-hydroxy-3-penten-1,5-dial) that were reported in the previous study (Jang et al., 2001) was estimated using a structure-reactivity relationship for the reaction with OH radicals (chamber condition). The calculation was included in SI (Section S5). If these two electron-deficient carbonyls are oxidized by OH radicals in the gas phase, the estimated lifetime of 4-oxo-2-butenic acid and 2-hydroxy-3-penten-1,5-dial is estimated to be 134 min and 43 min, respectively. The actual lifetime of these compounds will be shorter than our estimation since they can also be oxidized in particle phase.

Reference: Jang, M. and Kamens, R. M.: Characterization of secondary aerosol from the photooxidation of toluene in the presence of NO_x and 1-propene, *Environ. Sci. Technol.*, 35, 3626-3639, 2001.

3. On that note, why is it called “electron-deficient alkenes”? Why not “unsaturated carbonyls”? Reaction with DTT is likely on the oxygenated group, rather than on the C=C double bond. Also, from a fundamental perspective, does the C=C double bond need to be adjacent to the C=O group? Have the authors investigated, say, 3-penten-2-one?

Response: Not all unsaturated carbonyls are reactive to DTT. The reactivity of an alkene is determined by the contribution of all substituents. If conjugated carbonyls have electron-donating groups such as alkyl, alkenyl, and ester group, they are not anymore electron-deficient alkenes and their reactivity with DTT is low. Thus, we prefer to use electron-deficiency of alkenes (Nair et al.) for their reactivity to DTT.

Reference: Nair, D. P. et al., The thiol-michael addition click reaction: a powerful and widely used tool in materials chemistry, *Chem. Mater.*, 26, 724-744, 2014.

4. Also, if acrolein indeed contributes high DTT activity, I would imagine that SOA from 1,3-butadiene would have very high DTT activity. Have the authors done any experiments to suggest that is the case?

Response: Acrolein was tested to describe the reactivity of functional group in organic compound. Actually, acrolein may not be present in aerosol due to their high volatility. The SOA yield of 1,3-butadiene is as low as 0.4-0.5% for 1.0-2.2 ppm 1,3-butadiene (Angove et al.). In addition, the major aerosol products (75-80%) of 1,3-butadiene are not electron-deficient alkenes, based on the study of Angove et al.

Reference: Angove, D.E. et al., The characterization of secondary organic aerosol formed during the photodecomposition of 1,3-butadiene in air containing nitric oxide, *Atmospheric Environment*, 24, 2006

5. Another suggestion for additional experiments (optional but would really strengthen the argument): if the authors can pass ozone over the SOA (e.g. collected on the filter), the ozone should selectively remove the double bonds. This way the authors can isolate the contribution to DTT from these electron deficient alkenes. While this may create additional organic hydroperoxides, the authors can easily correct for that increase by measuring total OHP with the NPBA assay.

Response: The electron-deficiency of alkenes increases the reactivity to DTT while electron-deficiency can significantly reduce reactivity to ozone. In addition, the products can be off-gassing from the filter by passing ozone through the filter and increase artifacts in chemical assays. Some products that have weak reactivity to DTT may become reactive to DTT due to the ozone reaction.

6. Does the UNIPAR model describe the abundance of electron deficient alkenes under low-NO_x? I would imagine under high NO_x there would be more electron deficient alkenes where C-C bond scission and ring-opening reactions are dominant, but the “unaccounted” DTT activity seems to be higher under low NO_x for toluene (from Fig. 6).

Response: In order to response to reviewer, the quantity of conjugated compounds was estimated using the UNIPAR model. The quantity of conjugated compounds in low-NO_x toluene SOA is similar to or slightly more than that in high-NO_x toluene SOA. Within the error bar, the “unaccounted” DTT activity of low-NO_x toluene SOA was close to that of high-NO_x toluene SOA (Fig. 6 in the old version, Fig. 5 in revised manuscript).

7. I understand that the proposal about measuring DTT_m as a proxy for maximum capacity (described in Section 3.1) is useful for a chemist. But I wonder what the biological relevance is. What is the lifetime of particles deposited in the lungs? I am not a lung expert, but I wonder if mucociliary clearance would make the lifetime shorter than 2 hours, and therefore DTT_m at 2 h may not be that relevant.

Response: The DTT assay enables the evaluation of aerosol reactivity to a sulfhydryl group using a small amount of aerosol mass and simple chemical procedures, and illustrates of the potential toxic mechanisms of aerosols. However, chemical assay is limited to a screening method and the results of chemical assay need to be compared with *in-vitro* and *in-vivo* studies. According to the study of Oberdorster et al. (1988), the lung clearance of particles ranges from several minutes to one day, depending on the solubility, particle size, etc. Binding with and to tissue and cellular components can increase the retention halftimes of particles to days and months. Also, the lifetime of particles in biological systems can also be affected by the lung diseases. The clearance of particles can be retarded if the lung is not healthy.

Reference: Oberdörster G., Lung Clearance of Inhaled Insoluble and Soluble Particles, Journal of Aerosol Medicine, 1, 1988.

8. For DTTm to be meaningful, DTT must be in large excess compared to amount of SOA. What is the estimated ratio of DTT to SOA (100 μ L of DTT, 700 μ L of SOA solution)? Is DTT in large excess? This is not an issue when looking at the catalytic activity, because both DTT and quinones can be regenerated through catalytic cycles. But this issue arises when it is a one-step reaction with these particulate oxidizers.

Response: The excess amount of DTT was used to determine oxidative potential of SOA. A sentence was added and reads now,

“To ensure the pseudo-1st-order reaction between DTT and redox-active species in SOA, the SOA mass applied to DTT assay was constrained to ensure that the DTT consumption remained less than 50 % of the initial DTT concentration.”

9. If hydroperoxides (and PANs) are indeed unstable at room temperature (as suggested in Section 2.3.2), did the decrease in ROOH amount correspond to a decrease in DTTt or DTTm?

Response: The QA/QC of chemical assays used in this study was reorganized and included in the supporting information (Section S4). The stability of H_2O_2 and peracetic acid with storage time was measured by NPBA assay (Fig. S8) and by DTT activity (Fig. S4). When the solution was kept at 4 °C over a 6-day period, the decrease of DTT response of peracetic acid was 22% and the decrease of NPBA assay was 11%. However, SOA samples of this study were analyzed within 24 h after collection. The decrease of DTT response and NPBA response of peracetic acid stored at 4 °C were insignificant compared to those of the fresh solution. Thus, we think that instability of organic peroxides will not influence of our chemical analysis. The QA/QC information of the stability of compound was shown below:

The stability of peroxides. To investigate the impact of the stability of organic compounds on DTT activities, the DTT consumptions (Δ DTT at reaction time=100 min) by 400 μ L H_2O_2 (100 μ M) and 300 μ L peracetic acid ($\text{CH}_3\text{C}(\text{O})\text{OOH}$; 100 μ M) were measured as a function of storage time. As shown in Fig. S4, Δ DTT of H_2O_2 and $\text{CH}_3\text{C}(\text{O})\text{OOH}$ stored at room temperature (RM) decreased up to 80 % and 36 % after an 8-day storage, respectively. Δ DTT of $\text{CH}_3\text{C}(\text{O})\text{OOH}$ stored at 4 °C also decreased 22% after a 6-day storage.

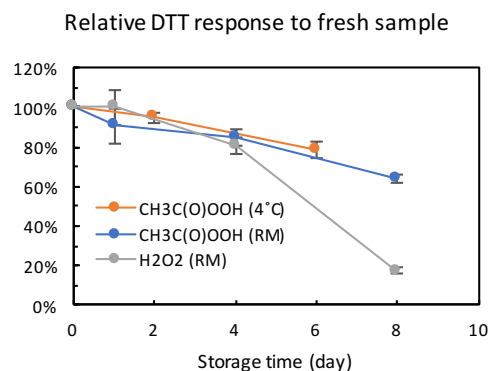


Figure S4. The DTT responses of H_2O_2 and peracetic acid stored at room temperature (RM) or 4 °C. The errors were estimated using the standard deviation of three replicates.

The stability of peroxides. To investigate the impact of the stability of peroxides on NPBA assay, the NPBA responses for 400 μ L H_2O_2 (100 μ M) and 300 μ L peracetic acid (100 μ M) were

measured as a function of storage time. Fig. S8 illustrates that the NPBA response of H_2O_2 and $\text{CH}_3\text{C}(\text{O})\text{OOH}$ stored at room temperature decreased upto 70 % and 27 % after an 8-day storage, respectively. The decrease of the NPBA response of $\text{CH}_3\text{C}(\text{O})\text{OOH}$ stored at 4 °C was similar to that stored at room temperature.

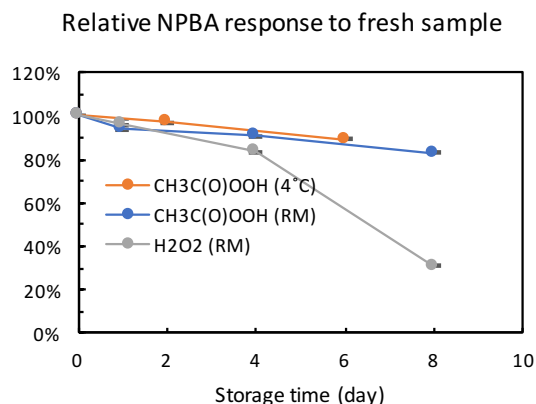


Figure S8. The NPBA responses of H_2O_2 and peracetic acid stored at room temperature or 4 °C. The error bar was estimated using the standard deviation of three replicates.

10. There are large fluctuations in experiment temperatures (more than 20K). Are there systematic differences in chemical composition?

Response: Thanks much for your comment. The UF-APHOR chamber uses the ambient sunlight and meteorological conditions. Thus, the temperature profile for each experiment has a diurnal pattern. Although temperature can influence the chemical composition of SOA due to the impact of temperature on the gas-particle partitioning of organic products, temperature is less influential on DTTt than aging time or NO_x levels. For example, DTTt values of toluene were modulated mainly by aging time (Fig. 3 in old version, Fig. 2 in revised manuscript). For isoprene SOA, DTTt was affected mainly by NO_x conditions. We included the discussion of temperature on DTT activity in the revised manuscript (Section 3.1).

“The DTT_t values of isoprene SOA was, however, higher than those observed by Tuet et al. (Tuet et al., 2017b) and Kramer et al. (Kramer et al., 2016). This difference might be caused by the degree of aerosol aging under different NO_x conditions, initial OH radical sources, humidity and temperature.”

Reference: Tuet, W. Y., Chen, Y., Xu, L., Fok, S., Gao, D., Weber, R. J., and Ng, N. L.: Chemical oxidative potential of secondary organic aerosol (SOA) generated from the photooxidation of biogenic and anthropogenic volatile organic compounds, *Atmos. Chem. Phys.*, 17, 839-853, 2017b. Kramer, A. J., Rattanavaraha, W., Zhang, Z., Gold, A., Surratt, J. D., and Lin, Y.-H.: Assessing the oxidative potential of isoprene-derived epoxides and secondary organic aerosol, *Atmos. Environ.*, 130, 211-218, 2016

Minor comments:

1. Section 2.2: Please provide define PILS and provide details of the PILS method. Was steam used to grow particles for impaction and collection? If so, is there concern about the high temperatures leading to decomposition of thermally labile compounds important for oxidative potential?

Response:

We added the principal of PILS in Section 2.2 and read now,
“The aerosol particle that enters the PILS grows quickly into a droplet under the supersaturated environment and this droplet is collected on the plate by impaction.”

It is possible that high temperature leads to the decomposition of thermally unstable compounds. However, the residence time of a particle inside PILS is approximately 1 second. In addition, the rapid cooling of water steam by the ambient air sample gives the high supersaturation. The resulting temperature downstream PILS is nearly ambient temperature.

2. Section 2.3.1 Line 1: it is unclear what “two steps” mean in parentheses. Perhaps re-word to “The two-step DTT assay: : :”

Response: This paragraph was modified and reads now

“DTT assay was employed to quantify the oxidative potential of SOA (Cho et al., 2005; Jiang et al., 2016). In the first step (DTT oxidation), a mixture of 700 μ L SOA-PILS sample, 200 μ L potassium phosphate buffer (2 mM, pH=7.4) and 100 μ L DTT (1 mM) was incubated at 37 °C in a sonicator (FS30H Ultrasonic Cleaner, Fisher Scientific). For the second step (determination of the remaining DTT), the reaction between DTT and SOA was quenched by adding 1 mL 1% w/v trichloroacetic acid (a commonly used quencher of thiol oxidation).”

3. Section 2.3.1 Line 2: What does SOA sample mean? Is this the aqueous solution?

Response: “SOA sample” was changed to “SOA-PILS sample” that was the aqueous solution collection by PILS.

4. Section 2.3.1 Page 6 Line 1: What is the pH of the PBS?

Response: The information of pH=7.4 was added to the sentence.

5. Section 2.3.1 Page 6 Line 1: “shaken in a sonicator” is confusing. Particularly since in the later section “shaking” was compared to “sonicating”.

Response: This sentence was modified and reads now,

“In the first step (DTT oxidation), a mixture of 700 μ L SOA-PILS sample, 200 μ L potassium phosphate buffer (2 mM, pH=7.4) and 100 μ L DTT (1 mM) was incubated at 37 °C in a sonicator (FS30H Ultrasonic Cleaner, Fisher Scientific).”

6. Section 2.3.2: Can 4-nitrophenol be formed in high NO_x SOA from toluene? If so, are there any negative controls?

Response: 4-Nitrophenol may be found in toluene SOA. The influence of 4-nitrophenol in SOA on NPBA assay was discussed in Section S4.2 (revised supporting information) and reads now,
“4-Nitrophenol, NPBA product, can also be found in toluene SOA and potentially influences on NPBA data. However, the light absorption of the SOA sample collected using PILS was negligible

at 406 nm, suggesting that NPBA data is not influenced by the light absorbing materials in SOA products.”

7. Section 3.1 heading: remove period after title

Response: The period mark after title was removed.

8. Section 3.3 Line 5: Grek et al. does not provide any evidence that PANs can oxidize sulfhydryl groups. They only include peroxy nitrates as an RNS, but PANs are peroxy nitrates.

Response: For the reaction of PAN with a sulfhydryl group, we cited other references. The new citations are shown below:

Products of the Reaction of Peroxyacetyl Nitrate with Sulfhydryl Compounds, Mudd et al., Archives of Biochemistry and Biophysics, 132, 1969.

Reaction of Peroxyacetyl Nitrate with Glutathione, J. B. Mudd, The journal of Biological Chemistry, 241, 1966.f

9. Section 4 Line 25: remove (DTT modulator: quinones)

Response: “(DTT modulator: quinones)” was removed from this sentence.

10. Section 4 Page 14 Line 8: An assay that is not water-based may not be relevant, since cells are water-based

Response: This sentence was deleted.

11. Table 1 and throughout manuscript: Does HC/NO_x ratio refer to the initial or average HC/NO_x? If it is the average, over what time range is the HC/NO_x calculated?

Response: The HC/NO_x ratio mentioned throughout the manuscript refers to the initial HC/NO_x. The title “HC/NO_x (ppbC/ppb)” in Table 1 was changed to “Initial HC/NO_x (ppbC/ppb)”. The detection of the HC/NO_x ratio over the course of experiment is complex due to the complexity in chemical species and the wall process of organics, nitric acid, and organonitrates.

12. Table 1 Mid-collection time: is this local time?

Response: “Mid-collection time” refers to the local time in Gainesville, Florida. This was clarified in the revised manuscript.

13. Figure 4: why is one data series labeled with experiment date, but others are not?

Response: The date information was removed. The initial HC/NO_x ratio was added.

Response to comments from referee #3 (Manuscript Ref. NO: acp-2017-214)

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We really appreciate the referee for providing these constructive comments. The detailed responses are presented as below.

This study investigated the DTT activity of different types of SOA (toluene, TMB, isoprene, α -pinene) formed in the presence of NO_x. Experiments are conducted in an outdoor chamber facility with different levels of NO_x. Results showed that in the presence of relatively higher levels of NO_x, DTT activity of toluene SOA is 2-5 times higher than other SOA. Isoprene SOA has lower DTT with increasing NO_x. Other SOA appears to be insensitive to NO_x. The results are discussed in the context of different DTT modulator compounds.

This is an interesting study and will be of interest to the greater research community. I have a number of questions regarding the experimental design/protocols and how the data are interpreted. Further, the conclusions need to be better justified.

The experiments are conducted with very high levels of hydrocarbon and NO_x. It is not clear what the NO_x levels in this study mean in terms of the reaction pathways. It appears that the authors assume that under the “low-NO_x” conditions in this study, the dominate fate of the peroxy is reaction with HO₂. It is not immediately clear how this is the case, especially with the high levels of hydrocarbon and NO_x, and without an external HO₂ source. To what extent does RO₂+HO₂ proceed in these experiments and whether organic hydroperoxide should be produced in the first place?

Response: Please find the response to question 11 (a) below.

The results in this study should be compared with prior literature and discussed. For instance, Kramer et al. (2016) found that for isoprene SOA, high-NO_x conditions produce SOA that is more oxidizing compared low-NO_x conditions. But, the results in this work suggest the opposite. Results from SOA formed from different hydrocarbons should also be compared to prior literature when available (e.g., Rattanavaraha et al., 2011, Tuet et al., 2017a; Tuet et al., 2017b). Tuet et al. found the DTT activity of different SOA to be fairly insensitive to RO₂ fate.

Response: Please find the response to question 7(b).

Further, the same authors of this manuscript published a recent paper in Atmos Environ (Jiang et al. 2016) on similar experiments, but the results under similar reaction conditions are not the same as those reported in this study. Please clarify and discuss accordingly.

Response: Please find the response to question 11(c).

It was noted that the SOA yields in this study are consistent with prior studies. SOA yields should be compared in the context of organic mass loadings. It does not appear that the yields in this work are in line with literature. Based on the numbers provided in Table 1, the SOA yields calculated are very different from prior studies. Please see detailed comments below. The implications of these differences should be considered and discussed. If the yields are so different, what does it imply regarding their corresponding SOA composition and their health effects? On a related note, the organic mass loadings (one can calculate them based on Table 1) are high in these experiments, which will result in partitioning of more volatile species into the particle phase compared to ambient. The implications of this on the measured DTT activity in this study and how they can be applied to ambient should also be discussed.

Response: Please find the response to question 6(a), 6(b) and 11(d).

The authors attributed the difference in [OHP]_m between low and high NO_x experiments to sample collection times and associated aging. I do not think this is well-justified. Many other parameters are also changing at the same time.

Response: Please find the response to question 11(d).

Finally, the basis for comparison of DTT activity is time of the sample collection in many cases. Is that chosen to represent OH exposure? But if so, it is not clear that [OH] are constant and comparable across different experiments.

Response: Please find the response to question 11(d).

Overall, I recommend publication with major revisions. More detailed comments below.

Major comments:

1. Page 1, line 14. Define clearly what is considered as high vs. low NO_x conditions in this study.

Response: The high NO_x condition represents the initial HC/NO_x ratio <10 ppbC/ppb. The low NO_x condition represents the initial HC/NO_x ratio >10 ppbC/ppb. We clarified the definition of high and low NO_x conditions in the revised manuscript (Section 2.1, Figure 2 and Figure 5).

2. Page 4, methods.

a. Was seed aerosols used in the experiments? Please state clearly.

Response: We added one sentence to the revised manuscript (Section 2.1): “No seed aerosols were added in this study.”

b. The NO_x levels in this study were very high in all experiments. The authors need to define LNO_x and HNO_x clearly in the methods.

Response: Please find the response to question 1.

c. HONO was used only in toluene experiments, why? The other experiments used NO_x (was that NO or NO₂? Or both? In what ratio?)

Response: The HONO was injected during toluene experiments to accelerate the photooxidation of toluene. NO (2% in N₂, Airgas) was injected as NO_x source in other experiments. We clarified the injection of HONO and NO_x in the revised manuscript (Section 2.1), and read now, “HC and NO (2% in N₂, Airgas) were injected to the chamber before sunrise. For photooxidation experiments of toluene, HONO generated from the reaction of 0.1 M NaNO₂ solution and 10 % w/w H₂SO₄ solution was injected into the chamber as a source of OH radicals.”

d. The authors should provide figures (in the SI) to show the typical time series of hydrocarbon, NO, NO₂, ozone, SOA mass for an experiment using HONO vs an experiment using NO_x. This is critical to give some context regarding the conditions under which the SOA samples were collected for DTT analysis. (for instance, what was the NO_x levels when the SOA samples were collected? This has important implications on the SOA composition and whether the results from different SOA samples can be directly compared.)

Response: The reaction of toluene with OH radicals is much slower than other VOCs. Thus, we added HONO to the chamber at the beginning of the experiment. All toluene experiments were conducted in the presence of HONO except one experiment on Feb/12/2016. For the consistency in toluene SOA data, the toluene experiment without HONO has been removed in the revised manuscript. In order to response to the reviewer, the time profiles of NO, NO_x, HC, ozone, and SOA mass concentrations have been included in supporting information (Figure S1). SOA samples were collected when the SOA mass concentration reached nearly to the maximum point.

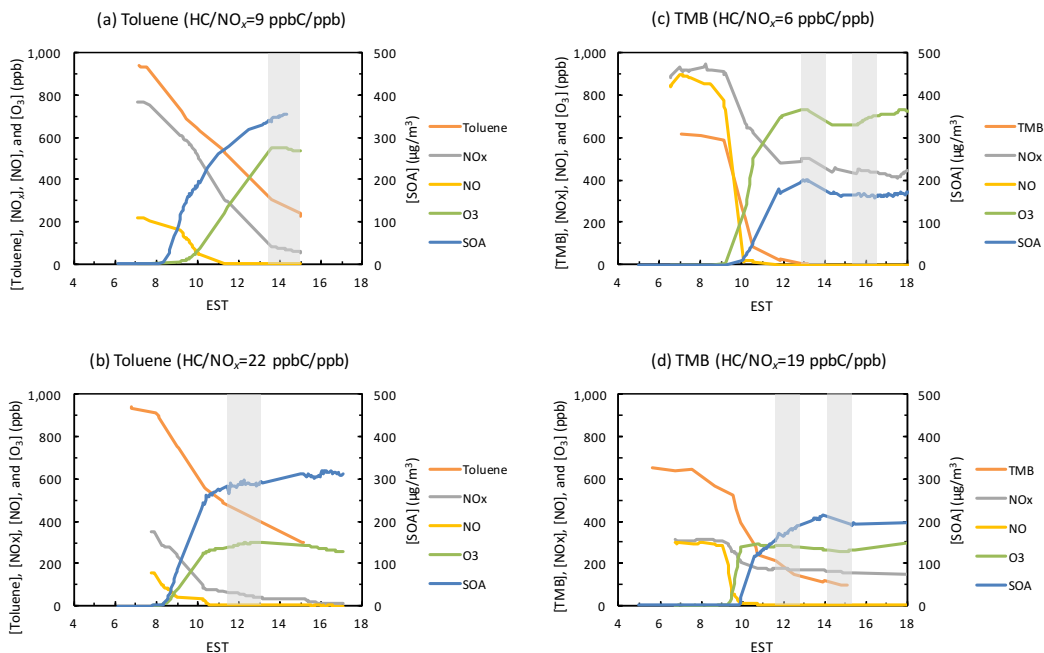


Figure S1. The time profiles of SOA mass concentration, HCs, NO_x, NO and O₃ mixing ratios through the experiments: (a) toluene (HC/NO_x=9 ppbC/ppb); (b) toluene (HC/NO_x=22 ppbC/ppb); (c) TMB (HC/NO_x=6 ppbC/ppb); (d) TMB (HC/NO_x=19 ppbC/ppb). EST, Eastern Standard Time. The shaded areas indicate the time periods of aerosol sampling.

e. What is the collection efficiency of the PILS? How is it calculated? How is it validated? As the DTT values were normalized by mass, the authors need to provide more details to justify the accuracy of the SOA mass values used in the DTT calculations.

Response: The collection efficiency of PILS was published by Orsini et al (2003). We clarified the collection efficiency in the revised manuscript (Section 2.2) as below:

“The sampling efficiency of PILS is greater than 95 % for particle size ranging from 0.03 to 6 μm (Orsini et al., 2003).” A value of 95% was used as the collection efficiency of PILS.

3. Page 6, line 17. It was noted that “The SOA mass applied to DTT assay was constrained to ensure that the DTT consumption remained less than 50 % of DTT₀.” Why?

Response: We modified this sentence as follows:

“To ensure the pseudo-1st-order reaction between DTT and redox-active species in SOA, the SOA mass applied to DTT assay was constrained so that the DTT consumption remained less than 50 % of the initial DTT concentration.”

4. Page 6, line 20 onwards, the NPBA assay. It is not clear how the authors treat (or correct for) potential interference from other SOA components in the NPBA assay measurements.

a. It was noted that absorbance at 406 nm (authors referring to 4-nitrophenol) of SOA before reaction with NPBA was negligible. I do not understand why this is the case. The oxidation of aromatic compounds (such as toluene) by OH in the presence can result in the formation of 4-nitrophenol? If so, why is the absorbance at 406nm negligible?

Response: 4-Nitrophenol can be found in toluene SOA. The influence of 4-nitrophenol in SOA on NPBA assay was discussed in Section S4.2 (revised supporting information) and reads now, “4-Nitrophenol, a NPBA assay product, can also be found in toluene SOA and potentially influences on NPBA data. However, the light absorption of the SOA sample collected using PILS was negligible at 406 nm, suggesting that NPBA data is not influenced by the light absorbing materials in SOA products.”

b. In terms of potential interference from alcohols, it was noted that glycerol aqueous solutions were tested using the NPBA method and no measurable absorption appeared in UV spectrum. The authors then concluded that the UV absorption spectrum was originated from 4-nitrophenol. Multifunctional alcohols can be formed from the oxidation of hydrocarbons. How do the authors justify that the results from glycerol is representative and that the measured absorption is purely from 4-nitrophenol formed by NPBA reactions with organic peroxides, without any interference from other SOA components?

Response: The mechanism of NPBA assay is the production of 4-nitrophenol from the reaction between OOH functional group and NPBA. The SOA contains a lot of OH functional groups, which might also react with NPBA. To make sure that the OH functional groups don't interfere with the detection of hydroperoxides, we did the test of glycerol and found that there was no 4-nitrophenol produced from the mixture of glycerol and NPBA. The influence of multi-alcohols on

NPBA assay was moved to Section S4.2 (revised supporting information) and reads now (next page),

“The influence of multi-alcohols and 4-nitrophenol on NPBA assay. It has been reported that boronic acid can react with multi-alcohols to form colorful products (Kim et al., 2007). SOA products are usually multifunctional and contain multi-alcohols. To examine the possible interference by NPBA-alcohol adducts, the glycerol aqueous solution was tested for the NPBA method. However, no measurable absorption appeared in the UV spectrum.”

c. Why “no more than 10 ug SOA was applied to the NPBA assay”?

Response: We removed this sentence in the revised manuscript.

5. Page 7, line 16 onwards, PAN analysis. Please provide further details to show that PAN are completely hydrolyzed in 15 mins.

Response: The information of hydrolysis time for Griess assay was included in supporting information (S4.3) and reads now,

“Hydrolysis time of PAN products in SOA. In this study, the hydrolysis time for PANs was set to 15 min. To ensure that 15 min is enough to hydrolyse the PANs in SOA products, the Griess response with 15-min hydrolysis was compared to that with 30-min hydrolysis. There was no significant difference in Griess response between a 15-min hydrolysis and a 30-min hydrolysis. “

6. Page 8, line 14-18. SOA yields from different hydrocarbons cannot be compared without the context of organic aerosol mass, as yield is typically a function of organic aerosol mass (Odum equation).

a. In Table 1, while one can calculate the organic mass loading from ΔHC and yield, the authors should also provide the organic mass loadings in the table to facilitate easier comparison of yields from this study to prior studies.

Response: The SOA mass loadings were included in Table 1. The new table is shown in the next page:

Table 1. Outdoor chamber experiment conditions

HC and Date	Initial HC ppb	Initial NO _x (HONO) ^a ppb	Initial HC/NO _x ppbC/ppb	[SOA] _{max} ^b μg/m ³	ΔHC ^c ppb	Y %	Mid- collection time ^d	RH ^e %	Temp ^e K	Chemical assay ^f
<i>Toluene</i>										
13 Feb 2016	641	525 (193)	9	229	403	15.1	13:40	22–63	281–303	DTT
01 May 2016	935	766 (133)	9	348	631	14.6	14:20	18–46	294–316	DTT, PAN
01 May 2016	938	301 (73)	22	292	542	14.3	12:10	21–48	294–315	DTT, PAN
23 May 2016	691	906 (250)	5	148	546	7.1	13:20	18–60	288–315	DTT, Enhance
23 May 2016	735	313 (86)	16	147	421	9.3	15:40	15–22	307–316	DTT, Enhance
18 Aug 2016	640	783 (179)	6	178	517	9.1	12:30	24–61	297–319	DTT, OHP
06 Aug 2016	610	240 (55)	18	75	216	9.2	12:30	43–59	297–305	DTT
18 Aug 2016	342	107 (24)	22	44	227	5.2	14:20	20–38	303–321	OHP
17 Nov 2016	622	179 (43)	24	139	452	8.1	13:20	12–56	282–309	DTT ^g
<i>TMB</i>										
04 Oct 2015	613	920	6	201	613	6.7	14:40	20–43	290–310	DTT
04 Oct 2015	657	310	19	207	542	7.8	13:20	24–46	290–306	DTT
20 Feb 2016	589	1024	5	150	548	5.6	13:00	14–60	282–311	DTT
20 Feb 2016	583	156	34	128	455	5.7	14:40	16–61	282–311	DTT
11 Jan 2016	595	256	21	114	414	5.6	15:50	23–81	274–298	Enhance
<i>Isoprene</i>										
23 Apr 2016	2693	2680	5	352	2693	4.7	12:00	18–48	290–314	DTT
23 Apr 2016	2755	430	32	93	2755	1.2	13:30	23–51	290–312	DTT
14 May 2016	2928	2800	5	406	2928	5.0	14:20	17–47	292–315	DTT, Enhance
14 May 2016	2858	423	34	107	2858	1.3	12:00	25–55	293–312	DTT
22 Jul 2016	2525	2423	5	246	2525	3.5	13:20	20–55	297–320	PAN (gas) ^h
22 Jul 2016	2718	473	29	70	2718	0.9	12:50	23–58	297–320	PAN (gas) ^h
20 Aug 2016	3060	3300	5	279	3060	3.3	12:30	20–58	296–321	DTT, OHP, PAN
20 Aug 2016	3173	583	27	125	3173	1.4	11:50	25–61	297–318	DTT, OHP, PAN
<i>α-Pinene</i>										
25 Feb 2016	319	639	5	257	319	14.5	15:00	21–63	278–299	DTT
25 Feb 2016	323	91	36	650	323	36.1	13:30	25–67	278–298	DTT
18 Jan 2016	257	144	18	223	257	15.6	15:50	25–78	275–297	Enhance

^a For toluene experiments, NO_x was contributed by NO, NO₂ and HONO. The concentration of HONO was estimated using the difference in the NO₂ signal with and without the base denuder (1 % Na₂CO₃+1 % glucose).

^b [SOA]_{max} is the maximum SOA concentration during the aerosol collection.

^c ΔHC is the consumption of HC when the SOA concentration reached to a maximum during the aerosol collection.

^d This column is the mid-collection time (based on the Eastern Standard Time (EST)) of SOA sampling.

^e The RH and temperature conditions shown in the Table 1 were recorded from the beginning of photooxidation (sunrise) until the ending of PILS sampling.

^f The SOA samples were applied to a series of chemical assays, namely DTT assay (DTT), DTT enhancement (Enhance), organic hydroperoxides analysis (OHP), and PAN analysis (PAN).

^g For DTT measurement of toluene SOA sample collected on 17 Nov. 2016, the concentration of potassium phosphate buffer (0.8 mM) in the first step of DTT assay was two times higher than the typical buffer concentration (0.4 mM). The DTT_m of the toluene SOA sample (17 Nov. 2016) is shown in Fig. 3.

^h The concentration of gaseous PAN products (collected by an impinger) was measured by the Griess assay.

b. I quickly did the calculations, but found that the yields for the different hydrocarbons are very different from the previous studies cited in the manuscript. For instance, the yield for isoprene SOA photooxidation (with NO_x/isoprene ratio of 5) is all 5% for Kroll et al., Xu et al., and this study. However, the organic mass loading that corresponds to this 5% SOA yield in Kroll et al and Xu et al is an order of magnitude smaller than this study. This means that when plotted in the Y vs. Mo (Odum equation) space, the SOA yields under this specific NO_x/isoprene ratio in this study is substantially lower than all previous studies. Discrepancies also exist for other hydrocarbons. The authors should conduct a detailed comparison with previous studies by showing their data in the yield curve space and comparing with others. The discrepancies should be discussed.

Response: Thanks for point out this comparison.

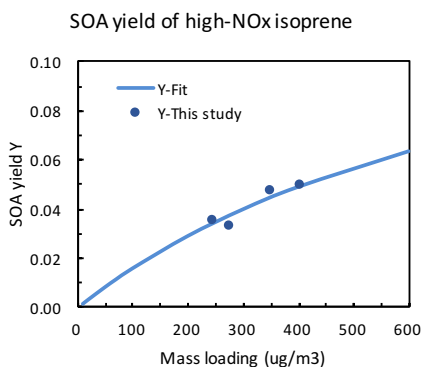
Low NO_x: The SOA yields of low-NO_x isoprene in this study were lower than the values reported by Kroll et al. (2006) or Xu et al. (2014) by several reasons. The HC ppbC/NO_x ratio of our study (low NO_x: 27-34) was much different from the study by Kroll and Xu (NO_x-free). The maximum temperature of the experiments in this study (312-320 K) was higher than the temperature condition in the study of Kroll (around 298 K) or Xu (around 298 K). The temperature effect on SOA yields were discussed in the revised manuscript (Section S3.1) and reads now, “Our SOA yields for isoprene SOA were lower than those reported in previous studies (Carlton et al., 2009; Xu et al., 2014) because the temperatures in our studies were higher than those sourced from indoor chambers.”

In order to response to the reviewer, our isoprene SOA yields at high NO_x conditions (HC/NO_x = 5) were compared to the theoretically predicted SOA yields using the two-product model (Carlton et al. 2009).

$$Y_{\text{high NO}_x} = \frac{0.154}{(K_{\text{OM,high NO}_x}(T)M_0)^{-1} + 1}$$

$$K_{\text{OM,high NO}_x}(T) = K_{\text{ref}} \times \exp\left[\frac{\Delta H_{\text{vap}}}{R} \left(\frac{1}{T} - \frac{1}{T_{\text{ref}}}\right)\right]$$

$K_{\text{ref}} = 0.0020$, $T_{\text{ref}} = 303\text{K}$, M_0 is the total aerosol concentration ($\mu\text{g}/\text{m}^3$). At $T = 313\text{K}$ (a typical temperature during our isoprene experiments), $K_{\text{OM,high NO}_x} = 0.00117$. The observed SOA yields agrees well with the predicted yields as shown below,



Reference: Carlton et al., A review of Secondary Organic Aerosol (SOA) formation from isoprene, Atmos. Chem. Phys., 9, 2009

7. Page 9, line 8-15. Comparison of response of aerosols from different hydrocarbons. This section needs to be expanded to include more discussions (in addition to the description of results).

a. The authors noted that a previous study by Fujitani et al. (2012) with epithelial cells is consistent with this study. Can the cellular results from Fujitani et al. (2012) be directly compared to the DTT in this study? Please discuss.

Response: Because no cellular study was conducted in this paper, we removed the comparison of our results with those of Fujitani et al. In order to response to the reviewer, a new citation was included in the revised manuscript, and reads now,

“The DTT activity of a given SOA can be applied to the assessment of SOA’s ability to oxidize cellular materials. For example, the recent study by Tuet et al. (Tuet et al., 2017a) reported a positive nonlinear correlation between DTT activities and ROS production in murine alveolar macrophages.”

b. How do results in this work compare to previous studies? For instance, recent work from Tuet et al. showed that the response from naphthalene is higher than other hydrocarbons such as m-xylene and α -pinene; McWhinney has also demonstrated previously that showed naphthalene SOA is highly redox-active. It would be useful that the authors provide some context and discuss the DTT activity of different SOA with respect to previous work.

Response: In the previous manuscript, the DTT activity of naphthalene SOA reported by McWhinney was discussed in the Section 1. The DTT activities of SOA in this study were also further compared with other studies (Sect. 3.1, revised manuscript) and reads as:

“The DTT_t values of this study were also compared with those reported in previous studies. The DTT_t values of α -pinene SOA in this study were close to those reported by Tuet et al. (Tuet et al., 2017b). The DTT_t values of isoprene SOA was, however, higher than those observed by Tuet et al. (Tuet et al., 2017b) and Kramer et al. (Kramer et al., 2016). This difference might be caused by the degree of aerosol aging under different NO_x conditions, initial OH radical sources, humidity and temperature.”

8. Page 9, line 25. It was stated that “: : DTT_m of low-NO_x isoprene SOA was much higher than that of high-NO_x isoprene SOA”. This does not seem like it is the case from Figure 4. Some data points overlap and are within uncertainty.

Response: We re-draw this figure to make the difference of DTT_m between low-NO_x isoprene and high-NO_x isoprene clearer. As shown in Figure 3 (revised manuscript), it is obvious that the DTT_m of low-NO_x isoprene SOA was significantly higher than high-NO_x isoprene SOA after a long-time reaction (t=120 min).

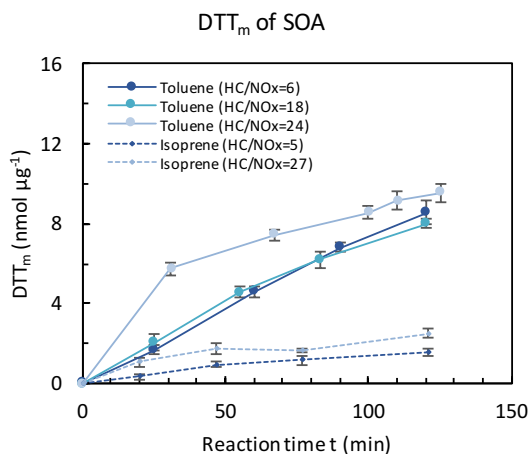


Figure 3. Time profile of DTT_m for toluene and isoprene SOA under different NO_x conditions. To achieve the completion of the reaction between DTT and SOA, the DTT_m of toluene sample (initial HC/NO_x=24 ppbC/ppb collected on 17 Nov. 2016) was measured with a 0.8 mM potassium phosphate buffer in the first step of DTT assay (2 times higher than the typical buffer concentration (0.4 mM)). Each error bar was calculated by $t_{0.95} \times \sigma / \sqrt{n}$ using three replicates, where $t_{0.95}$ is the t-score (4.303 for $n = 3$ replicates) with a two-tail 95 % confidence level.

9. Page 9, line 27. (and page 10) But for the “toluene LNO_x-17 Nov 2016”, the data are not linear? i.e., will the data also start from the origin, if so, considering the origin and the 5 data points in Figure 4, the overall trend is then non-linear? Please discuss.

Response: We re-draw figure 4 by adding lines for each case. The DTT_m of toluene LNO_x (collected on 17 Nov 2016, HC/NO_x=24 ppbC/ppb) is not linear with reaction time and eventually reached to a plateau (Figure 3, revised manuscript). Please find Figure 3 in response to question 8.

10. Page 10, quinones. The authors stated that “the redox cycling of quinones was not the major mechanism underlying DTT consumption by the SOA”. A small contribution of quinones to the total aerosol mass does not necessarily mean they are not important for overall toxicity? Oxidation of the aromatic compounds used in this study can lead to formation of quinones (e.g., Bloss et al., 2005). Many previous studies have pointed to the importance of quinones in ambient PM toxicity. It is not clear how the results in this work should be placed in the context of previous work that pointed to the importance of quinones for PM toxicity. Please discuss.

Response: In the previous manuscript (Section 1), we discussed the difference in compositions between SOA and typical combustion particulates. Combustion particles contain a small amount of metals, PAHs and oxy-PAHs, which can be redox catalyzers. Such compounds in SOA are negligible.

11. Page 11, NO_x conditions.

a. Line 5 onwards. The authors noted that under low NO_x conditions, RO₂ predominately reacts with HO₂, producing hydroperoxides (among other products). This accuracy of this statement will depend on what precisely the “low NO_x conditions” are, as “low NO_x conditions” do not directly (or necessarily) translate to RO₂+HO₂ reactions. It is not clear that RO₂+HO₂ is the dominant reaction under the conditions of this study. The hydrocarbon concentration used in this study is very high (hundreds of ppb to several ppm), there is abundant NO_x (even under “low NO_x” conditions), but no addition HO₂ source (such as H₂O₂). With this, it is not clear how RO₂+HO₂ dominates. Since a large fraction of the discussions in the manuscript hinged on this, it is critical that this is justified clearly in the manuscript. The authors can perform a simple simulation of the relative importance of different RO₂ reactions under their “low” and “high” NO_x conditions.

Response: Please find the definition of low-NO_x and high-NO_x in response to question 1. We clarified the NO_x condition in Figures 2 and 5. As suggested by the reviewer, we simulated the relative significance of RO₂+NO and RO₂+HO₂ at two different HC (ppbC)/NO_x ratios (6 and 25) for toluene. Based on integrated reaction rate (IRR) analysis for several RO₂ species, the reaction of RO₂+NO is very sensitive to NO_x condition: i.e., the IRR at the high NO_x level is 5 times higher than that at the low NO_x. The IRR of RO₂+HO₂ is higher at the low NO_x level but relatively less sensitive than the reaction of RO₂+NO. However, our IRR analysis is for the gas phase oxidation. The composition of aerosol is complex due to the involvement of aerosol phase reactions of various multifunctional organic compounds.

b. The authors shall compare and discuss their results in the context of previous studies. For instance, a recent Kramer et al. (2016) concluded that High-NO_x conditions produce SOA that is more oxidizing compared low-NO_x conditions. This results from this work showed the opposite. Please discuss. Also, Tuet et al. (2017a, 2017b) specifically studied the toxicity of SOA (including isoprene) under RO₂+HO₂ vs RO₂+NO reactions conditions, and found DTT activity of isoprene SOA to be similar under RO₂+HO₂ and RO₂+NO conditions, also for other SOA except naphthalene.

Response: Please find the response to question 7(b).

c. How do the data in this study compare the results from a previous study by the same author (Jiang et al. AE, 2016). For example, the “without denuder” data in Figure 2 Jiang et al AE paper can be compared to those in the current study. But comparing this study and their previous AE publication, the results (in terms of the DTTt values) are different for each hydrocarbon under similar conditions? Please compare and discuss, and confirm self-consistency if that is the case.

Response: The DTTt values reported in this study should be compared to those with denuder in our previous study (Jiang et al. AE, 2016), because the carbon denuder was also applied in this study (Section 2.1). The effect of denuder on DTTt has been explained in our previous study. Overall, there was no difference between the DTTt reported in this study and the values reported by our previous study (Jiang et al. 2016). For example, the DTTt of isoprene SOA (low NO_x) was about 50-60 pmol min⁻¹ μg⁻¹ in this study, which was the same to the values reported before. The DTTt of toluene SOA (with denuder, collected during 13:30-14:50) was reported to be 43 min⁻¹ μg⁻¹ in the previous study, and was lower than the DTTt of toluene SOA (collected in the morning or early afternoon) due to the aging effect. The influence of aging on DTT response was consistent with that reported in this study (Section 3.1).

d. Line 20, and figure 6. What is the x-axis (time) supposed to be a surrogate of? If it is supposed to be a surrogate for OH exposure, then the OH level should be the same in each experiment. Is this the case? The authors simply explained the difference in [OHP]_m between low and high NO_x toluene SOA as the low-NO_x SOA being collected at a later time and resulted in a lower level of [OHP]_m due to further reactions/photooxidation. I do not think the authors can discount other factors, such as the varying RH (maybe SOA composition is different due to different RH?), organic mass loading (when I used the numbers in Table 1 and calculated the mass loadings for the toluene experiments shown in Figure 6, the loadings are very different for the two experiments), etc?

Response: The x-axis is based on local time (EST). In Figure 6, the RH conditions of high-NO_x and low-NO_x experiments for toluene SOA are similar because two experiments were conducted in the same day. As pointed by the reviewer, other factors, like organic mass loading, may also influence the SOA compositions. Overall, we think that the major factors to affect SOA compositions are the NO_x condition and the aging process.

12. Table 1. Was ozone present in these experiments? If so, please include some information here. This should also be specified and discussed clearly in the manuscript, in case some of the SOA is formed from ozonolysis in addition to OH oxidation.

Response: Ozone was not introduced to chamber but was formed during the photooxidation of biogenic HCs (i.e. isoprene and α-pinene). The additional sentences were added to the 1st paragraph in section 3.1 and read now,

“Aromatic hydrocarbons (toluene and 1,3,5-trimethylbenzene) are mainly oxidized by OH radicals while biogenic hydrocarbons (isoprene or α-pinene) by both OH radicals and ozone. Based on the integrated reaction rate (IRR) analysis, the oxidation of isoprene by OH radicals is at least 3 times higher than that by ozone at the low NO_x condition. The oxidation of biogenic hydrocarbons is dominated by OH radicals, particularly, in the morning.

Minor Comments:

1. Page 1, line 26. Clearly state in the abstract under what NO_x conditions the sentence “The amount of organic hydroperoxide was substantial, while PANs were found to be insignificant for both SOA.”

Response: This sentence was modified as follows

“Under the NO_x conditions (HC/NO_x ratio: 5-36 ppbC/ppb) applied in this study, the amount of organic hydroperoxide was substantial, while PANs were found to be insignificant for both SOA.”

2. Page 1, line 29. Clearly state what “model compound study” refers to.

Response: This sentence was modified as follows,

“The DTT assay results of the model compounds study suggest that electron-deficient alkenes, which are abundant in toluene SOA, could also modulate DTTm”

3. Page 2, line 8. The author should also cite McDonald et al. (2012, Inhal. Toxicol), McWhinney et al. (2013, ACP), Kramer et al., (AE, 2016), Tuet et al. (2017, ACP), and Tuet et al. (2017, ACPD).

Response: These references were included in the revised manuscript (Section 1).

4. Page 4, line 7-9. The authors should state clearly that only selected (but not all) toluene and isoprene SOA samples are analyzed with the Griess and NPBA assays.

Response: This sentence was modified as follows

“Selected toluene and isoprene SOA samples were immediately applied to the DTT assay and the quantification of particulate oxidizers.”

5. Page 5, line 16. Was 23 May 2016 a typo? Should it be July 22? (based on Table 1)

Response: The date of experiment was corrected as below: “To measure the concentration of PANs in the gas phase, gaseous photooxidation products (22 Jul 2016) were collected using an impinger (filled with 5 mL DI water) at a flow rate of 0.8 L min⁻¹.”

Dithiothreitol Activity by Particulate Oxidizers of SOA Produced from Photooxidation of Hydrocarbons under Varied NO_x Levels

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Abstract. When hydrocarbons are atmospherically oxidized, they form particulate oxidizers, including quinones, organic hydroperoxides, and peroxyacyl nitrates (PANs). These particulate oxidizers can modify cellular materials (e.g., proteins and enzymes), and adversely modulate cell functions. In this study, the contribution of particulate oxidizers in secondary organic aerosols (SOA) to the oxidative potential was investigated. SOA were generated from the photooxidation of toluene, 1,3,5-trimethylbenzene, isoprene, and α -pinene under varied NO_x levels. Oxidative potential was determined from the typical mass-normalized consumption rate (reaction time $t = 30$ min) of dithiothreitol (DTT_t), a surrogate for biological reducing agents. At high NO_x conditions, the DTT_t of toluene SOA was 2–5 times higher than that of other types of SOA. Isoprene DTT_t significantly decreased with increasing NO_x (up to 69 % reduction by changing the hydrocarbon/NO_x ratio from 30 to 5). The DTT_t of 1,3,5-trimethylbenzene and α -pinene SOA was insensitive to NO_x under the experimental conditions of this study. The significance of quinones to the oxidative potential of SOA was tested through the enhancement of DTT consumption in the presence of 2,4-dimethylimidazole, a co-catalyst for the redox cycling of quinones; however, no significant effect of 2,4-dimethylimidazole on modulation of DTT consumption was observed for all SOA, suggesting that a negligible amount of quinones was present in SOA of this study. For toluene and isoprene, mass-normalized DTT consumption (DTT_m) was determined over an extended period of reaction time ($t = 2$ h) to quantify their maximum capacity to consume DTT. The total quantities of PANs and organic hydroperoxides in toluene SOA and isoprene SOA were also measured using the Griess assay and the 4-nitrophenylboronic acid assay, respectively. Under the NO_x conditions (HC/NO_x ratio: 5-36 ppbC/ppb) applied in this study, the amount of organic hydroperoxides was substantial, while PANs were found to be insignificant for both SOA. Isoprene DTT_m was almost exclusively attributable to organic hydroperoxides, while toluene DTT_m was partially attributable to organic hydroperoxides. The DTT assay results of the model

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compound study suggest that electron-deficient alkenes, which are abundant in toluene SOA, could also modulate DTT_m.

1 Introduction

Epidemiological studies have linked human exposure to fine particulate matter (PM_{2.5}, aerodynamic diameter < 2.5 μm) to increased morbidity and mortality from respiratory and cardiovascular diseases (e.g., asthma, myocardial infarction, stroke) (Brook et al., 2010; Chen et al., 2013; Davidson et al., 2005; Jansen et al., 2005; Katsouyanni et al., 1997; van Eeden et al., 2005). Primary combustion particulates (e.g., wood smoke particles, vehicle emissions) are known to be causative agents of these diseases (Danielsen et al., 2011; Nel, 2005); however, increasing attention is also being paid to secondary organic aerosols (SOA) (Fujitani et al., 2012; Jang et al., 2006; Kramer et al., 2016; Lin et al., 2016; McDonald et al., 2010; McWhinney et al., 2013; Tuet et al., 2017a; Tuet et al., 2017b), which are produced from the atmospheric transformation of hydrocarbons (HCs) in the presence of atmospheric oxidants (e.g., NO_x, OH radicals, O₃) (Hallquist et al., 2009). Although SOA comprises a large fraction of PM_{2.5} (20–90 %) (Gelencsér et al., 2007; Kanakidou et al., 2005), its mechanistic role in causing adverse health effects remains unclear.

The toxicity of organic aerosols has been ascribed to the generation of reactive oxygen species (ROS) and the modification of biomolecules (e.g., DNA and cellular enzymes) (Danielsen et al., 2011; Nel, 2005). ROS can induce oxidative stress in pulmonary systems, followed by a cascade of inflammation responses, and ultimately the apoptosis of lung cells (Danielsen et al., 2011; Li et al., 2003; Li et al., 2008). Particulate organic compounds such as quinones and polyaromatic hydrocarbons can react with cellular reducing agents (e.g., NADPH) and form ROS (i.e., H₂O₂ and O₂^{•−}) (Kumagai et al., 2012). To efficiently determine the oxidative potential (the ability to generate ROS) of different types of particulate matter at a laboratory benchtop scale, a low-cost acellular technique, dithiothreitol (DTT) assay, has been widely used (Antiñolo et al., 2015; Cho et al., 2005; Hedayat et al., 2014; Janssen et al., 2014; Kramer et al., 2016; Verma et al., 2015). DTT acts as a surrogate for biological reducing agents owing to its two sulfhydryl groups. Some quinones (e.g., 1,4-naphthoquinone (NQ) and 9,10-phenanthraquinone (PQN)) can efficiently consume DTT via a catalytic redox cycle, during which quinones are reduced to semiquinones or hydroquinones (Chung et al., 2006; Li et al., 2003). Hence, quinone compounds, commonly found

in primary combustion particulates (Danielsen et al., 2011; Jakober et al., 2007), are known to be important contributors to DTT response of combustion particles.

Unlike combustion PM, biogenic SOA and most aromatic SOA (except naphthalene SOA) contain little or no quinones (Forstner et al., 1997; Hamilton et al., 2005; McWhinney et al., 2013; Pindado Jiméñez et al., 2013); however, recent work has shown that the DTT activity of SOA (toluene, 1,3,5-trimethylbenzene (TMB), isoprene, and α -pinene) was high and even comparable to that originating from combustion particulates (e.g., wood smoke particles) (Jiang et al., 2016), suggesting that there must be unidentified mechanisms underlying DTT consumption other than the catalytic act of quinones.

In this study, three groups of SOA products were introduced to explain the mechanistic role of SOA products in DTT consumption (Fig. 1a and 1b). First, non-catalytic particulate oxidizers in SOA, such as organic hydroperoxides (alkyl hydroperoxides and acyl hydroperoxides) and peroxy acyl nitrates (RC(O)OONO₂; PANs), can oxidize sulfhydryl groups in DTT to form disulfides, sulfenic acids (RSOH), sulfinic acids (RSO₂H), or sulfonic acids (RSO₃H) (Grek et al., 2013; Mudd, 1966). These non-catalytic particulate oxidizers are abundant in SOA sourced from various hydrocarbons (Docherty et al., 2005; Sato et al., 2012). Second, catalytic particulate oxidizers, such as quinoid substances, can oxidize sulfhydryl groups through a redox cycle (Cho et al., 2005; Kumagai et al., 2002). A trace amount of quinones can be found in aromatic SOA products (Forstner et al., 1997). Third, electron-deficient alkenes in SOA can react with the sulfhydryl groups of DTT via a Michael addition (Nair et al., 2014). Alkenes substituted with an electron withdrawing group (e.g., conjugated carbonyls) are commonly found in ring-opening products from the photooxidation of aromatic HCs (e.g., toluene) (Jang and Kamens, 2001; Saunders et al., 2003, 1997; Wyche et al., 2009). The contributions of all three groups of SOA products to DTT activity can be influenced by the type of precursor HC (aromatics vs. biogenics) and by NO_x (NO+NO₂) levels (HC/NO_x ratios) (Eddingsaas et al., 2012b; Jang and Kamens, 2001; Wyche et al., 2009; Xu et al., 2014).

Advanced analytical instruments (e.g., aerosol mass spectrometers and liquid chromatograph mass spectrometers integrated with soft ionization) have innovated the characterization of SOA compositions; however, their data are limited to elemental analysis (Xu et al., 2014) or the identification of some chemical species (e.g., carboxylic acids and carbonyls) by a unique fragmentation (Sato et al., 2012; Shiraiwa et al., 2013). Particulate oxidizers (e.g., PANs and

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organic hydroperoxides) are thermally unstable and can decompose during chemical injection at high temperature, making it difficult to characterize SOA compositions using mass spectrometers (Zheng et al., 2011). This difficulty is also compounded by a lack of authentic standards suitable for the analysis of diverse and complex particulate oxidizers.

5 The purpose of this study is to characterize the effect of SOA products on DTT consumption. SOA were generated from the photooxidation of different HCs under varied environmental conditions (NO_x levels) using a large outdoor photochemical smog chamber. The two most abundant anthropogenic HCs (i.e., toluene and TMB) in the ambient atmosphere and the two ubiquitous biogenic HCs (i.e., isoprene and α -pinene) were chosen as SOA precursors. Aerosols were
10 collected using an online technique with a particle-into-liquid sampler (PILS). [Selected toluene and isoprene](#) SOA samples were immediately applied to the DTT assay and the quantification of particulate oxidizers. The amount of PAN was measured using the Griess assay and that of organic hydroperoxides was measured using the 4-nitrophenylboronic acid (NPBA) assay. The contribution of quinones to the oxidative potential of SOA was estimated by the enhancement of
15 DTT consumption in the presence of 2,4-dimethylimidazole, a co-catalyst for the redox cycling of quinones (Dou et al., 2015). In addition to particulate oxidizers, the contribution of electron-deficient alkenes to DTT activity was investigated for aromatic SOA (toluene SOA). Although the chemical assays (e.g., NPBA assay and Griess assay) used in this study have limitations (e.g., providing structural details of organic compounds), they are user-friendly and can accurately
20 quantify the total amount of organic hydroperoxides and PANs, both of which are important for understanding the role of SOA in cellular oxidative stress at the molecular level. The quality control (QC) of the chemical assays used in this study will be discussed.

2 Materials and methods

2.1 Outdoor chamber experiments

25 SOA were generated under natural conditions (ambient sunlight, temperature, and relative humidity) using the University of Florida Atmospheric PHotochemical Outdoor Reactor (UF-APHOR) dual chambers (52 m³ each). Before each experiment, the chambers were flushed with the clean air for 2 days using an air purifier system (GC Series, IQAir Inc.) until the background particle mass concentration was below 1 $\mu\text{g m}^{-3}$. HC and [NO \(2% in N₂, Airgas\)](#) were injected to

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the chamber before sunrise. For photooxidation experiments of toluene, HONO generated from the reaction of 0.1 M NaNO₂ solution and 10 % w/w H₂SO₄ solution was injected into the chamber as a source of OH radicals. HONO produced OH radicals and NO via photolysis. The particle size distribution of chamber SOA was monitored using a scanning mobility particle sizer (SMPS), and was converted to the mass concentration using the SOA density (1.3 g cm⁻³ for α-pinene SOA and 1.4 g cm⁻³ for the other types of SOA) (Ng et al., 2007a; Ng et al., 2007b; Wyche et al., 2009; Xu et al., 2014). SOA were generated under varied NO_x conditions (high NO_x (HC/NO_x<10 ppbC/ppb), low NO_x (HC/NO_x>10 ppbC/ppb); Table 1). No seed aerosols were added to in this study. Other details about chamber experiments can be found in Sect. S1 of the Supporting Information. The typical time profiles of SOA mass concentration, HCs (i.e. toluene or TMB), NO_x, NO and O₃ mixing ratios through the experiments were shown in Fig. S1.

2.2 Sampling method

SOA and background (before chemical injection) samples were collected within a small amount of deionized (DI) water using a PILS technique. The aerosol particle that enters the PILS grows quickly into a droplet under the supersaturated environment and this droplet is collected on the plate by impaction (Orsini et al., 2003). The sampling efficiency of PILS is greater than 95 % for particle size ranging from 0.03 to 6 μm (Orsini et al., 2003). A parallel carbon filter denuder (Sunset Laboratory Inc.) was placed upstream of PILS to remove gaseous compounds. The efficiency of the carbon denuder was measured by comparing the concentrations of toluene and CCl₄ with the carbon denuder to those without the denuder and was found to be almost 100 %. The mass concentration of SOA in the PILS sample was estimated using the chamber SOA mass concentration, the air flow rate of PILS, the total liquid volume collected by PILS, and the collection efficiency of PILS. SOA samples collected by PILS were applied to the chemicals assays described in Sect. 2.3.

To measure the concentration of PANs in the gas phase, gaseous photooxidation products (22 Jul 2016) were collected using an impinger (filled with 5 mL DI water) at a flow rate of 0.8 L min⁻¹. A filter (13 mm diameter, Pall Life Scientific Pallflex, TX40HI20-WW) was applied upstream of the impinger to remove particles. The impinger samples were then applied to PAN analysis.

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2.3 Chemical assays

Detailed information about chemicals and solution preparation can be found in Sect. S2. To avoid the decay of some unstable SOA products in the aqueous solution, the analytical procedures of DTT, PAN, and organic hydroperoxides assays were completed within 24 h after sampling. Before

5 chemical analysis, all SOA samples were stored in a refrigerator at 4 °C. The limit of detection of each chemical assay is provided in Sect S4. The reaction schemes and quality assurance/quality control (QA/QC) details of chemicals assays are included in Sect. S3 and S4.

2.3.1 DTT assay

DTT assay was employed to quantify the oxidative potential of SOA (Cho et al., 2005; Jiang et al., 2016). In the first step, (DTT oxidation), a mixture of 700 µL SOA-PILS sample, 200 µL potassium phosphate buffer (2 mM, pH=7.4) and 100 µL DTT (1 mM) was incubated at 37 °C in a sonicator (FS30H Ultrasonic Cleaner, Fisher Scientific). For the second step, (determination of the remaining DTT), the reaction between DTT and SOA was quenched by adding 1 mL 1% w/v trichloroacetic acid (a commonly used quencher of thiol oxidation). Then, 0.5 mL 5,5'-dithiobis-15 (2-nitrobenzoic acid) solution (1 mM in methanol) was added to react with the remaining DTT forming 2-nitro-5-thiobenzoic acid (Scheme S1), which produced a yellow color after the addition of 1 mL Tris base buffer-HNO₃ (pH = 8.9, 0.4 M). The absorbance of 2-nitro-5-thiobenzoic acid at 412 nm was measured using a UV/VIS Spectrometer (Lambda 35, PerkinElmer). To ensure the pseudo-1st-order reaction between DTT and redox-active species in SOA, the SOA mass applied to DTT assay was constrained to ensure that the DTT consumption remained less than 50 % of the initial DTT concentration. Background chamber air samples, blank controls (DI water) and positive controls (0.1 µM PQN) were run in duplicates for each set of measurements. The DTT loss in blank and positive controls were shown in Fig. S2 and Fig. S3. To estimate the effect of radicals produced by sonication on DTT assay, the DTT loss rate in blank control during sonication was compared to that during shaking (Edison Environmental Incubator Shaker G24, low speed, 37 °C). Tested using the statistical method based on the Student t-test (Andrade and Estévez-Pérez, 2014), the slope of DTT loss vs. time with sonicator was not significantly different from the one with shaker (significance level $\alpha = 0.05$); therefore, in this study, the influence of free radicals generated by sonication on DTT measurement was insignificant. The blank-corrected DTT consumption (Δ DTT, nmol) was estimated by Eq. (1):

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$$\Delta\text{DTT} = \frac{A_{\text{blk}} - A_{\text{SOA}}}{A_0} \text{DTT}_0 \quad (1)$$

where A_{blk} is the absorbance of the blank control after incubation, A_{SOA} is the absorbance of the SOA sample after incubation, A_0 is the absorbance of the blank control without incubation, and DTT_0 (100 nmol) is the initial moles of DTT.

Deleted: As shown in Fig. S1, the DTT loss rate in blank control during sonication ($0.162 \text{ nmol min}^{-1}$) was close to that during shaking ($0.153 \text{ nmol min}^{-1}$), shaken by an Edison Environmental Incubator Shaker G24, low speed, 37°C ; therefore, in this study, the influence of free radicals generated by sonication on DTT measurement was insignificant. The SOA mass applied to DTT assay was constrained to ensure that the DTT consumption remained less than 50 % of DTT_0 .

5 SOA samples should be applied to chemical assays soon after collection (within 24 h).

To investigate the additivity of DTT response from different types of chemical species, ΔDTT of the blend of several compounds was compared with the sum of ΔDTT originating from individual compounds. Figure S5 proves that the DTT consumption is additive by showing that ΔDTT of the mixture of four model compounds (i.e., acrolein, PQN, H_2O_2 , and tert-butyl hydroperoxides) was

10 consistent with the sum of individuals.

2.3.2 Organic hydroperoxides analysis

The NPBA method, which had been used by Su et al. (Su et al., 2011) for the determination of H_2O_2 , was extended for the quantification of alkyl and acyl hydroperoxides. NPBA reacts with organic hydroperoxides to form a 4-nitrophenol (Scheme S2), which has a large absorption

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15 coefficient at 406 nm (Kuivila, 1954; Kuivila and Armour, 1957; Su et al., 2011). A mixture of 1 mL SOA sample, 100 μL NPBA solution (10 mM in methanol), and 900 μL KOH solution (50 mM) was incubated at 85°C .

Positive control (10 μM H_2O_2) was run in duplicate for each set of measurements. The NPBA method was calibrated using aqueous 4-nitrophenol solutions ranging from 1–40 μM (Fig. S6).

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20 $(\text{CH}_3\text{C}(\text{O})\text{OOH})$, tert-butyl peroxide $((\text{CH}_3)_3\text{COOC}(\text{CH}_3)_3)$, tert-butyl hydroperoxide $((\text{CH}_3)_3\text{COOH})$, and hydrogen peroxide (H_2O_2). Within a 90 % confidence level, the absorbance sourced from the reaction of NPBA with the known amount of organic hydroperoxides or H_2O_2 was covered by the calibration curve (Fig. S6). However, no absorbance at 406 nm appeared in the NPBA+tert-butyl peroxide mixture (data not shown).

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25 alcohols products and 4-nitrophenol that might be formed in SOA has no influence on the NPBA assay. For toluene SOA, the reaction of organic hydroperoxides with NPBA completed within 7 h, and for isoprene SOA within 2 h (Fig. S7). Organic hydroperoxides in aqueous solution are unstable. For example, after a 6-day storage at 4°C , $(\text{CH}_3)_3\text{COOH}$ degraded 10 % (Fig. S8);

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30 therefore, we ensured that SOA samples were applied to chemical assays soon after collection (within 24 h).

2.3.3 PAN analysis

The concentration of PANs was quantified by Griess assay. The Griess reagent, a mixture of sulfanilic acid and n-(1-naphthyl) ethylenediamine dihydrochloride (NEDD), has been widely applied to quantify the concentration of nitrogen oxides in environmental, industrial, and biological systems (Giustarini et al., 2008; Ridnour et al., 2000; Saltzman, 1954). Nitrogen oxides were transformed to nitrites that can form azo dyes when mixed with Griess reagent (Scheme S3) (Giustarini et al., 2008). For PAN analysis, a 300 μ L SOA (collected by PILS) or gas sample (collected by an impinger) was mixed with 300 μ L KOH aqueous solution (50 mM) for 15 min to hydrolyze PANs completely and form nitrites. [As discussed in Sect. S4.3, a 15-min hydrolysis was shown to be enough to hydrolyze the PANs in SOA products.](#) Then, 1 mL Griess reagent (20 mM sulfanilic acid and 5 mM NEDD aqueous solution) was added to the mixture, and allowed to react with nitrites for 15 min. A purple-magenta color formed immediately. [No difference in the absorbance was found between a 15-min reaction and a 30-min reaction.](#) The concentration of PANs was estimated from the absorbance at 541 nm (Ridnour et al., 2000). Positive controls (10 μ M NaNO₂) were run in duplicate for each set of measurements. Griess assay was calibrated using NaNO₂ aqueous solutions with varied concentrations (0.4–50 μ M; Fig. S9).

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2.3.4 DTT activity enhancement

Dou et al. (Dou et al., 2015) showed that by forming H-bonds with hydroquinones, imidazole derivatives are capable of facilitating electrons transfer from hydroquinones to molecular oxygen, accelerating the redox cycling of quinones and enhancing the oxidation of DTT. (Scheme S4). In the DTT enhancement test, a 250 μ L 2,4-dimethylimidazole aqueous solution (5 mM) was mixed with a 450 μ L SOA-PILS sample to get a 700 μ L mixture. Then, 100 μ L DTT solution (1 mM) and 200 μ L potassium phosphate buffer (2 mM) were added to the mixture. The subsequent steps were the same as those used for the DTT assay. The enhanced DTT consumption rate ($t = 30$ min) in the presence of 2,4-dimethylimidazole was measured. Positive controls (2 μ M NQN) were run in duplicates for each set of measurements.

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3 Results and discussion

3.1 DTT activity of SOA

The SOA yield (Y) represents a ratio of organic mass formed to HC consumed (Odum et al., 1996). As shown in Table 1, the Y values of toluene, TMB, isoprene, and α -pinene SOA ranged from 5 % to 25 %, 6 % to 8 %, 1 % to 5 %, and 14 % to 36 %, respectively. Except isoprene SOA, the SOA yields in this study were consistent with those reported in previous studies (Eddingsaas et al., 2012a; Healy et al., 2008; Odum et al., 1996; Sato et al., 2007). Our SOA yields for isoprene SOA were lower than those reported in other studies (Carlton et al., 2009; Xu et al., 2014) because the temperatures in our outdoor experiments were higher than those sourced from indoor chambers. Within the NO_x conditions ($\text{HC}/\text{NO}_x=5\text{-}36\text{ ppbC/ppb}$) in this study, SOA yields of high- NO_x isoprene were much higher than those of low- NO_x isoprene, and SOA yields of other three types of SOA at high NO_x conditions were generally lower than those at low NO_x conditions. Aromatic hydrocarbons (toluene and 1,3,5-trimethylbenzene) are mainly oxidized by OH radicals while biogenic hydrocarbons (isoprene or α -pinene) by both OH radicals and ozone. Based on the integrated reaction rate (IRR) analysis, the oxidation of isoprene by OH radicals is at least 3 times higher than that by ozone at the low NO_x condition ($\text{HC}/\text{NO}_x=17\text{ ppbC/ppb}$). The oxidation of biogenic hydrocarbons is dominated by OH radicals, particularly, in the morning.

The DTT consumption rate, DTT_t ($\text{pmol min}^{-1} \mu\text{g}^{-1}$), was defined as DTT consumption (ΔDTT , pmol) per minute of reaction time (t , min) per microgram of SOA mass (m_{SOA} , μg):

$$\text{DTT}_t = \frac{\Delta\text{DTT}}{m_{\text{SOA}} t} \quad (2)$$

Figure 2 illustrates the DTT_t ($t = 30\text{ min}$) of SOA produced from four different HCs under varied NO_x conditions. Overall, the influence of NO_x on DTT_t varied, depending on the type of HC. The DTT_t of toluene SOA was insensitive to NO_x for samples collected within a similar sampling period, but it decreased with increasing aging time. The DTT_t of toluene SOA reached approximately $70\text{ pmol min}^{-1} \mu\text{g}^{-1}$ by 13:00 under both high NO_x and low NO_x conditions, but decreased by about 40 % in the late afternoon. For aged toluene SOA, the decline of DTT_t might reflect the decay of photooxidation products that could potentially react with DTT (e.g., electron-deficient alkenes can react with OH radicals) (Finlayson-Pitts and Pitts Jr, 2000). The lifetime of two semi-volatile electron-deficient alkenes (4-oxo-2-butenic acid, and 2-hydroxy-3-penten-1,5-dial) that were reported in the previous study (Jang and Kamens, 2001) was estimated using a

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structure-reactivity relationship for the reaction with OH radicals (typically 2×10^{-4} ppb under chamber conditions) (Finlayson-Pitts and Pitts Jr, 2000; Jang and Kamens, 2001; Kwok and Atkinson, 1995). If these two electron-deficient carbonyls are oxidized by OH radicals in the gas phase, the estimated lifetime of 4-oxo-2-butenic acid and 2-hydroxy-3-penten-1,5-dial is estimated to be 134 min and 43 min, respectively (Sect. S5). The actual lifetime of these compounds will be shorter than our estimation since they can also be oxidized in particle phase. Furthermore, some particulate oxidizers might also photochemically decompose with increasing oxidation time (Sect. 3.3). For isoprene SOA, DTT_t was significantly affected by NO_x . There was a 38 % to 69 % decrease in isoprene DTT_t when the HC/ NO_x (ppbC/ppb) ratio was reduced from 30 to 5. Under high NO_x conditions, the DTT_t of less-aged isoprene SOA was about 50 % lower than that of less-aged toluene SOA. However, under low NO_x conditions, the DTT_t of isoprene SOA was comparable to that of toluene SOA. The DTT_t of TMB and α -pinene SOA were much lower than those of toluene and isoprene SOA, and they were not affected significantly by NO_x conditions. The DTT_t values of this study were also compared with those reported in previous studies. The DTT_t values of α -pinene SOA in this study were close to those reported by Tuet et al. (Tuet et al., 2017b). The DTT_t values of isoprene SOA was, however, higher than those observed by Tuet et al. (Tuet et al., 2017b) and Kramer et al. (Kramer et al., 2016). This difference might be caused by the degree of aerosol aging under different NO_x conditions, initial OH radical sources, humidity and temperature. The DTT activity of a given SOA can be applied to the assessment of SOA's ability to oxidize cellular materials. For example, the recent study by Tuet et al. (Tuet et al., 2017a) reported a positive nonlinear correlation between DTT activities and ROS production in murine alveolar macrophages.

Traditional DTT_t has been used to measure the oxidative potential originating from the catalytic redox cycling of particulate constituents (e.g., quinones and metals) (Charrier and Anastasio, 2012; Cho et al., 2005; Kumagai et al., 2002). When governed by such catalytic reactions, DTT consumption increased linearly with reaction time (Fig. S3). To demonstrate the time-dependency of DTT consumption, the reaction time of DTT assay was extended to 2 h for isoprene SOA and toluene SOA, which both had high DTT_t . The mass-normalized DTT consumption (DTT_m , nmol μg^{-1}) was defined as the ratio of ΔDTT (nmol) to m_{SOA} (μg):

$$DTT_m = \frac{\Delta DTT}{m_{SOA}} \quad (3)$$

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In Fig. 3, the NO_x effect on DTT_m was consistent with that on DTT_i (Fig. 2): no NO_x effect was observed on the DTT_m of toluene SOA, and the DTT_m of low-NO_x isoprene SOA was much higher than that of high-NO_x isoprene SOA.

Figure 3 shows that the increase in DTT_m with time for both isoprene and toluene SOA was non-linear, suggesting that DTT consumption by SOA products was governed by non-catalytic processes. For example, DTT consumption by isoprene SOA was nearly completed within 2 h. For toluene SOA (Toluene HNOX and Toluene LNOX), the increase of DTT_m also appeared to slow down over a 2h reaction, although the DTT_m did not reached a plateau under the same DTT assay conditions (i.e., the same buffer concentration). Medina-Ramos et al. (Medina-Ramos et al., 2013) reported that the electron transfer rate between glutathione (GSH) and an electrogenerated mediator ([IrCl₆]²⁻) exhibited a slight acceleration when the phosphate buffer concentration was increased from 0 to 50 mM at pH = 7.0. To achieve the completion of the reaction between particle oxidizers in SOA and DTT, the DTT_m of toluene SOA (HC/NO_x=24 ppbC/ppb) was measured with a 0.8 mM potassium phosphate buffer in the first step of DTT assay (2 times higher than the typical buffer concentration (0.4 mM)). As shown in Fig. 3, the DTT_m of the toluene SOA (HC/NO_x=24 ppbC/ppb) reached a plateau within 2 h, proving that DTT consumption by toluene SOA was controlled by non-catalytic mechanisms. At high NO_x conditions, the DTT_m (t = 2 h) of toluene SOA was 4–5 times higher than that of isoprene SOA. This difference was about 2 times greater than that for DTT_i (Fig. 2); therefore, we concluded that DTT_m is more suitable than DTT_i for estimating the oxidative potential of SOA, given that DTT_m can determine the maximum capacity of non-catalytic modulators in SOA to consume DTT.

3.2 DTT modulator: quinones

To illustrate the role of quinones in modulating the DTT responses of SOA, the enhanced DTT consumption rate (t = 30 min) in the presence of 2,4-dimethylimidazole was measured. The enhancement factor (pmol min⁻¹ μg-SOA⁻¹ μmol-imidazole⁻¹) was estimated by Eq. (4):

$$\text{Enhancement Factor} = \frac{\Delta\text{DTT}_{\text{mix}} - \Delta\text{DTT}_{\text{SOA}} - \Delta\text{DTT}_{\text{imidazole}}}{m_{\text{SOA}} n_{\text{imidazole}} t} \quad (4)$$

where $n_{\text{imidazole}}$ (μmol) is the moles of 2,4-dimethylimidazole added to the DTT reaction mixture, $\Delta\text{DTT}_{\text{mix}}$ (pmol) is the DTT consumption by the mixture of SOA and 2,4-dimethylimidazole, $\Delta\text{DTT}_{\text{SOA}}$ (pmol) is the DTT consumption by SOA only, and $\Delta\text{DTT}_{\text{imidazole}}$ (pmol) is the DTT consumption by 2,4-dimethylimidazole only. As shown in Fig. 4, the enhancement factors of the

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four SOA were 2–3 orders of magnitude lower than those of the reference quinone compounds (i.e., NQN and PQN), suggesting that the redox cycling of quinones was not the major mechanism underlying the DTT consumption by the SOA. Hamilton et al. (Hamilton et al., 2005) reported that the total amount of identified quinones (i.e., 5-methyl-o-benzoquinone, 2-methyl-p-benzoquinone, 2-hydroxy-5-methyl-p-benzoquinone) from the photooxidation of toluene was less than 0.07 % of the total aerosol mass. In a model compound study, Kumagai et al. (Kumagai et al., 2002) also reported that the oxidation of DTT by most benzoquinones (e.g., 1,4-benzoquinone, 2-methyl-1,4-benzoquinone) was negligible under simulated physiological conditions (pH = 7.5, 37 °C).

3.3 DTT modulator: non-catalytic particulate oxidizers

- 10 In-depth investigations on the roles of non-catalytic particulate oxidizers in DTT consumption were performed for isoprene SOA and toluene SOA, which led to high DTT_t. Organic hydroperoxides and PANs can oxidize sulfhydryl groups (oxidation state of S[−2]) to disulfides (S[−1]) or to even higher oxidation states (S[0], S[+2], S[+4]; Fig. 1b) (Grek et al., 2013; Mudd, 1966; Mudd and McManus, 1969). Under low NO_x conditions, alkyl peroxy radicals (RO₂)
- 15 dominantly react with HO₂ radicals, producing alcohols, alkyl hydroperoxides, and carbonyls (Finlayson-Pitts and Pitts Jr, 2000; Kroll et al., 2006; Ng et al., 2007b). Under high NO_x conditions, RO₂ radicals mainly react with NO generating aldehydes (Finlayson-Pitts and Pitts Jr, 2000). The reaction of aldehydes with OH radicals followed by the reaction with molecular oxygen yields peroxy acyl radicals (RC(O)OO) (Finlayson-Pitts and Pitts Jr, 2000). RC(O)OO can react with
- 20 NO₂ to form PANs and react with HO₂ radicals to form RC(O)OOH (Finlayson-Pitts and Pitts Jr, 2000; Nguyen et al., 2012; Xu et al., 2014). In this study, the concentration of organic hydroperoxides per microgram of SOA were quantified using the NPBA assay, represented by [OHP]_m (nmol μg-SOA^{−1}). The concentration of PANs per microgram of SOA was measured by the Griess assay, represented by [PAN]_m (nmol μg-SOA^{−1}).
- 25 As shown in Fig. 5a, by increasing the HC/NO_x (ppbC/ppb) from 5 to 27, [OHP]_m in isoprene SOA increased 2 times owing to the organic hydroperoxides formed from the RO₂+HO₂ reaction pathway under low NO_x conditions. Under the experimental conditions of this study, the influence of NO_x on [OHP]_m in toluene SOA was insignificant. Presumably, the aging process reduced the significance of the NO_x effect on [OHP]_m. Low-NO_x toluene SOA was collected about 2 h later
- 30 (i.e., a greater degree of aging) than high-NO_x toluene SOA. The organic hydroperoxides in the

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low-NO_x toluene experiment degraded more through photolysis or photooxidation (Lee et al., 2000) than those in the high-NO_x toluene experiment. The effect of the aging process on toluene [OHP]_m was consistent with that on toluene DTT_t (Fig. 2).

As shown in Fig. 5b, [PAN]_m was found to be one order of magnitude lower than [OHP]_m. With the decrease in HC/NO_x (ppbC/ppb) from about 22 to 9, [PAN]_m in the toluene SOA increased 3 times as a result of PANs production from the RO₂+NO reaction pathway under high NO_x conditions (Fig. 1a) (Xu et al., 2014). For isoprene, the moles of both aerosol phase PANs and gas phase PANs per cubic of air volume were significantly greater at higher NO_x levels (Fig. S10). Most PAN products from the photooxidation of isoprene existed in the gas phase and the amount of PAN in particle phase was trivial (Fig. S10); for example, aerosol phase PAN products was only 0.5 % of gas phase PAN products.

To underline the contribution of organic hydroperoxides and PANs to the DTT_m of SOA, the DTT_m values of toluene and isoprene SOA were also compared with the sum of [OHP]_m and [PAN]_m. Figure 5c shows that organic hydroperoxides were the major products that induced the oxidative potential of isoprene SOA. For toluene SOA, only 45–65 % of DTT_m could be ascribed to organic hydroperoxides, and the remaining fraction was attributed to other organic compounds in SOA. We propose that electron-deficient alkenes, abundant in toluene SOA (Jang and Kamens, 2001), can substantially modify sulfhydryl groups in DTT via a Michael addition (Fig. 1b) (Nair et al., 2014). In the next section, the reactivity of electron-deficient alkenes with DTT will be demonstrated using selected model compounds.

3.4 DTT modulator: electron-deficient alkenes

Figure 6 illustrates the DTT_t (*t* = 30 min) of four electron-deficient alkenes (i.e., acrolein, methacrolein, 2,4-hexadienal, and mesityl oxide). Acrolein showed much higher DTT_t than the other compounds. The susceptibility of an alkene to a Michael addition reaction depends on the nature of the electron withdrawing group coupled to the C=C bond (Nair et al., 2014). The methyl group of methacrolein and mesityl oxide is an electron donating group that increases the electron density on the C=C bond; thus, decreasing the reactivity of the C=C bond with DTT. The extended conjugation (C=C-C=C-C(O)H) in 2,4-hexadienal stabilizes the C=C bond leading to an extremely low DTT_t.

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Deleted: To investigate the effect of the interaction among different compounds on DTT response, the additivity of DTT_m by various modulators was tested. As shown in Fig. S5, the DTT_m of the mixture of four model compounds (i.e., acrolein, PQN, H₂O₂, and tert-butyl hydroperoxides) was consistent with the sum of the DTT_m originating from individual compounds, proving that DTT_m is additive.

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The alkenes from the photooxidation of toluene were usually coupled with electron withdrawing groups such as carbonyls, nitrates, and carboxylic acids (Jang and Kamens, 2001). These electron-withdrawing groups enable the alkenes to be reactive with DTT. Compared with toluene SOA, TMB SOA will have more alkyl substituted alkenes owing to the three methyl groups on the aromatic ring, and therefore be less reactive with DTT. This tendency partially explains why the DTT_t of TMB SOA was significantly lower than that of toluene SOA (Fig. 2). Based on aerosol composition predictions using predictive SOA models such as the Unified Partitioning Aerosol Phase Reaction (UNIPAR) model, the mass fraction of electron-deficient alkenes in high-NO_x toluene SOA should be more than 50 % (Im et al., 2014); therefore, the gap between toluene DTT_m and concentrations of non-catalytic particulate oxidizers (Fig. 5c) may be filled by abundant electron-deficient alkenes.

4 Atmospheric implications and conclusions

The influence of NO_x on the oxidative potential of SOA was investigated using DTT_t (Fig. 2). Among four HCs, only isoprene SOA was significantly sensitive to NO_x levels, showing much higher DTT_t at lower NO_x conditions. The DTT_t of toluene SOA was found to be lower with a longer aging time, regardless of NO_x conditions.

For SOA consisting of non-catalytic redox compounds, DTT_m is more appropriate than DTT_t for assessing oxidative potential, because of the non-linear relationship between DTT consumption and reaction time (Fig. 3). A decrease in isoprene DTT_m was observed with increasing NO_x levels,

but no significant NO_x effect on DTT_m was observed for toluene SOA within a 2h reaction. To apply the DTT_m results of this study to ambient atmosphere, DTT_m should be coupled with SOA mass concentrations. Under high NO_x conditions, the DTT_m of toluene SOA was almost 5 times higher than that of isoprene SOA, underlining the importance of toluene in urban areas, despite of its lower SOA yield (Table 1) in the urban environment (i.e., higher NO_x conditions). In spite of relatively low DTT_m for high-NO_x isoprene SOA, isoprene could still play a substantial role in the oxidative potential of ambient urban aerosols, because of its abundance (Guenther et al., 2006) and high SOA yields (Table 1) under high NO_x conditions. The NO_x effect on the DTT_m of isoprene SOA is limited to the NO_x conditions applied in this study, and should be extended to a variety of HC/NO_x ratios in further studies.

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As discussed in Sect. 3.1 and 3.2, the DTT consumption by SOA was not sourced from quinones, which can catalytically yield ROS. Hence, the contribution of non-catalytic particulate oxidizers, especially organic hydroperoxides, to the oxidative potential of SOA was highlighted in this study. Non-catalytic particulate oxidizers account for almost 100 % of isoprene DTT_m, and 45–65 % of toluene DTT_m (Fig. 5c). In addition to non-catalytic particulate oxidizers, electron-deficient alkenes in toluene SOA can potentially react with DTT via a Michael addition (Nair et al., 2014). The results of this study also show that some of the oxidizers (e.g., PANs) formed from the photooxidation of hydrocarbons predominantly exist in the gas phase (Fig. S10). Future studies should further consider how, through absorption into the bio-system, gas phase oxidizers may be effectual for inducing oxidative stress.

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Deleted: Some products may be chemically unstable in aqueous solutions and decay during PILS sampling, inducing a negative artifact on the measurement of DTT consumption; therefore, the development of a cell-free assay without water-extraction of aerosols is needed to improve the assessment of the modulation capacity of SOA on cellular materials.

Supplement. The Supplement related to this article is available.

Competing interests. The authors declare that they have no conflict of interest.

Acknowledgement. This work was supported by a grant (2014M3C8A5032316) from the Ministry of Science, ICT, and Future Planning at South Korea.

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Table 1. Outdoor chamber experiment conditions

HC and Date	Initial HC ppb	Initial NO _x (HONO) ^a ppb	Initial HC/NO _x ppbC/ppb	[SOA] _{max} ^b μg/m ³	ΔHC ^c ppb	Y %	Mid- collection time ^d	RH ^e %	Temp ^e K	Chemical assay ^f
Toluene										
13 Feb 2016	641	525 (193)	9	229	403	15.1	13:40	22–63	281–303	DTT
01 May 2016	935	766 (133)	9	348	631	14.6	14:20	18–46	294–316	DTT, PAN
01 May 2016	938	301 (73)	22	292	542	14.3	12:10	21–48	294–315	DTT, PAN
23 May 2016	691	906 (250)	5	148	546	7.1	13:20	18–60	288–315	DTT, Enhance
23 May 2016	735	313 (86)	16	147	421	9.3	15:40	15–22	307–316	DTT, Enhance
18 Aug 2016	640	783 (179)	6	178	517	9.1	12:30	24–61	297–319	DTT, OHP
06 Aug 2016	610	240 (55)	18	75	216	9.2	12:30	43–59	297–305	DTT
18 Aug 2016	342	107 (24)	22	44	227	5.2	14:20	20–38	303–321	OHP
17 Nov 2016	622	179 (43)	24	139	452	8.1	13:20	12–56	282–309	DTT ^g
TMB										
04 Oct 2015	613	920	6	201	613	6.7	14:40	20–43	290–310	DTT
04 Oct 2015	657	310	19	207	542	7.8	13:20	24–46	290–306	DTT
20 Feb 2016	589	1024	5	150	548	5.6	13:00	14–60	282–311	DTT
20 Feb 2016	583	156	34	128	455	5.7	14:40	16–61	282–311	DTT
11 Jan 2016	595	256	21	114	414	5.6	15:50	23–81	274–298	Enhance
Isoprene										
23 Apr 2016	2693	2680	5	352	2693	4.7	12:00	18–48	290–314	DTT
23 Apr 2016	2755	430	32	93	2755	1.2	13:30	23–51	290–312	DTT
14 May 2016	2928	2800	5	406	2928	5.0	14:20	17–47	292–315	DTT, Enhance
14 May 2016	2858	423	34	107	2858	1.3	12:00	25–55	293–312	DTT
22 Jul 2016	2525	2423	5	246	2525	3.5	13:20	20–55	297–320	PAN (gas) ^h
22 Jul 2016	2718	473	29	70	2718	0.9	12:50	23–58	297–320	PAN (gas) ^h
20 Aug 2016	3060	3300	5	279	3060	3.3	12:30	20–58	296–321	DTT, OHP, PAN
20 Aug 2016	3173	583	27	125	3173	1.4	11:50	25–61	297–318	DTT, OHP, PAN
α-Pinene										
25 Feb 2016	319	639	5	257	319	14.5	15:00	21–63	278–299	DTT
25 Feb 2016	323	91	36	650	323	36.1	13:30	25–67	278–298	DTT
18 Jan 2016	257	144	18	223	257	15.6	15:50	25–78	275–297	Enhance

^a For toluene experiments, NO_x was contributed by NO, NO₂ and HONO. The concentration of HONO was estimated using the difference in the NO₂ signal with and without the base denuder (1 % Na₂CO₃+1 % glucose).

^b [SOA]_{max} is the maximum SOA concentration during the aerosol collection.

^c ΔHC is the consumption of HC when the SOA concentration reached to a maximum during the aerosol collection.

^d This column is the mid-collection time (based on the Eastern Standard Time (EST)) of SOA sampling.

^e The RH and temperature conditions shown in the Table 1 were recorded from the beginning of photooxidation (sunrise) until the ending of PILS sampling.

^f The SOA samples were applied to a series of chemical assays, namely DTT assay (DTT), DTT enhancement (Enhance), organic hydroperoxides analysis (OHP), and PAN analysis (PAN).

^g For DTT measurement of toluene SOA sample collected on 17 Nov. 2016, the concentration of potassium phosphate buffer (0.8 mM) in the first step of DTT assay was two times higher than the typical buffer concentration (0.4 mM).

The DTT_m of the toluene SOA sample (17 Nov. 2016) is shown in Fig. 3.

^h The concentration of gaseous PAN products (collected by an impinger) was measured by the Griess assay.

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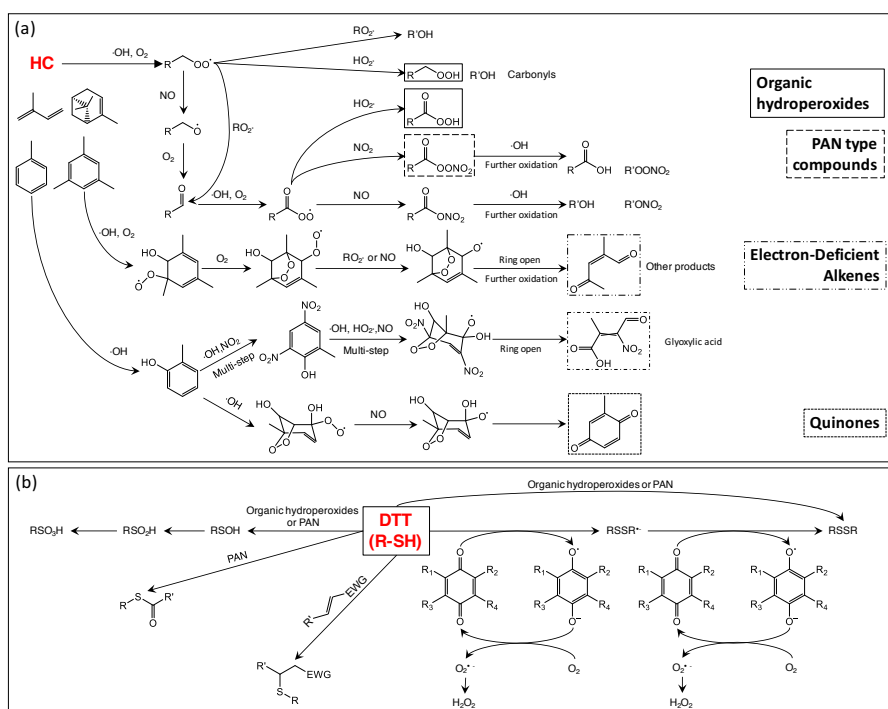


Figure 1. (a) Simplified mechanisms for the formation of alkyl and acyl hydroperoxides, peroxy acyl nitrates (PANs), electron-deficient alkenes, and quinones (Eddingsaas et al., 2012b; Jang and Kamens, 2001; Saunders et al., 2003, 1997; Wyche et al., 2009; Xu et al., 2014). Photooxidation products are not limited to the compounds shown. (b) Possible reaction mechanisms between sulfhydryl groups in dithiothreitol (DTT, represented by R-SH) and SOA products (Grek et al., 2013; Kumagai et al., 2002; Mudd, 1966; [Mudd and McManus, 1969](#); Nair et al., 2014). EWG represents the electron-withdrawing group attached to an alkene.

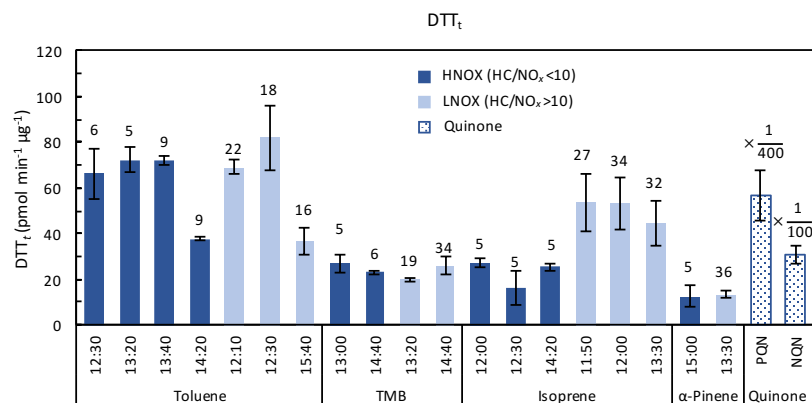


Figure 2. DTT_t of chamber-generated SOA under varied NO_x conditions (HNOX: high NO_x, LNOX: low NO_x) and positive controls (i.e., PQN and NQN). The number above each column represents the initial HC/NO_x ratio. The x-axis represents the mid-collection time (Table 1). The DTT_t of PQN and NQN are divided by 400 and 100, respectively. Each error bar was calculated by $t_{0.95} \times \sigma / \sqrt{n}$, where $t_{0.95}$ is the t-score (4.303 for $n = 3$ replicates) with a two-tail 95 % confidence level.

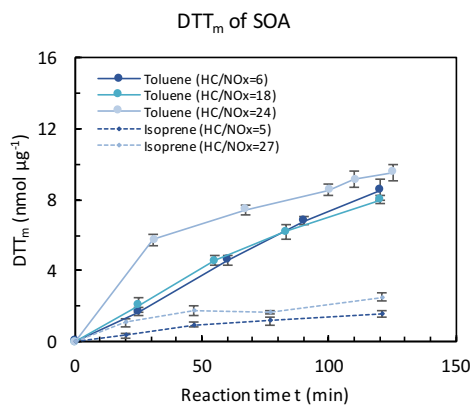


Figure 3. Time profile of DTT_m for toluene and isoprene SOA under different NO_x conditions. To achieve the completion of the reaction between DTT and SOA, the DTT_m of toluene sample (initial $HC/NO_x=24$ ppbC/ppb collected on 17 Nov. 2016) was measured with a 0.8 mM potassium phosphate buffer in the first step of DTT assay (2 times higher than the typical buffer concentration (0.4 mM)). Each error bar was calculated by $t_{0.95} \times \sigma / \sqrt{n}$ using three replicates, where $t_{0.95}$ is the t-score (4.303 for $n = 3$ replicates) with a two-tail 95 % confidence level.

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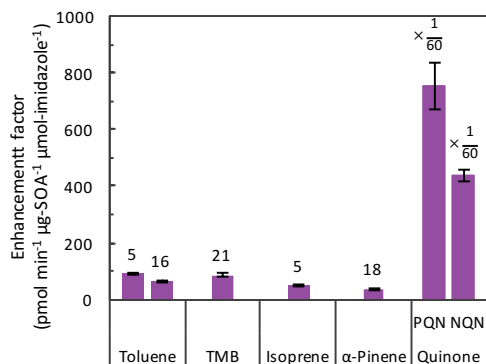


Figure 4. Enhancement factors ($\text{pmol min}^{-1} \mu\text{g-SOA}^{-1} \mu\text{mol-imidazole}^{-1}$) of SOA in the presence of 2,4-dimethylimidazole. The label above each column represents the initial HC/NO_x ratio. The enhancement factor is expressed as the mean ($\pm \sigma$) of three replicates. The enhancement factors of PQN and NQN are divided by 60.

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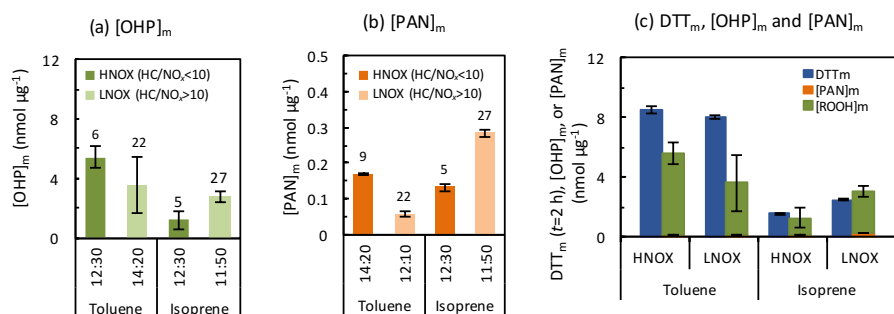


Figure 5. (a) Concentration of organic hydroperoxides in SOA, [OHP]_m (nmol µg⁻¹), measured by 4-nitrophenylboronic acid (NPBA) assay. (b) Concentration of PANs in SOA, [PAN]_m (nmol µg⁻¹), measured by Griess assay. The number above each column represents the initial HC/NO_x ratio. The x-axis represents the mid-collection time (Table 1). (c) Comparison of DTT_m (t = 2 h) with the sum of [OHP]_m and [PAN]_m. The [OHP]_m, [PAN]_m, and DTT_m are expressed as the mean (± σ) of three replicates. HNOX represents high NO_x conditions, and LNOX represents low NO_x conditions.

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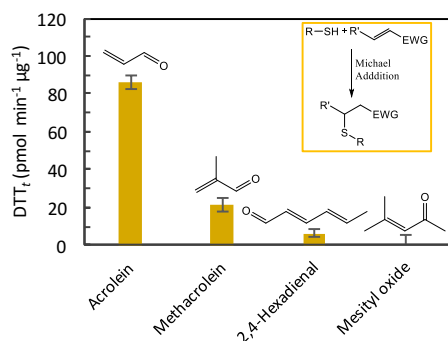


Figure 6. The DTT_t (t = 30 min) of four different electron-deficient alkenes. Each error bar was calculated by $t_{0.95} \times \sigma / \sqrt{n}$ using three replicates, where $t_{0.95}$ is the t-score (4.303 for $n = 3$ replicates) with a two-tail 95 % confidence level. EWG appeared in the mechanism represents an electron withdrawing group (Nair et al., 2014).

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