We thank the reviewers for their time and comments. Below are detailed responses to each comment. The responses are italicized and the modified texts are in red.

Response to anonymous referee #1 comments:

1) **Page 4 Line 73:** The authors state that there are many gaps. What are the gaps? What is the specific gap this work is attempting to address?

*Thank you for your comment. There are currently too many gaps to include a comprehensive list. The current work focuses on addressing the relative toxicities of different SOA systems, which was mentioned in lines 97 – 104. We have added an additional sentence in this section to clearly state the gap the current work is attempting to address.*

Line 75: “Despite these findings, there are still many gaps in knowledge regarding PM-induced health effects. *The current work will focus on the relative toxicities of different SOA systems, as field studies have repeatedly shown that SOA often dominate over primary aerosols (e.g., PM emitted directly from combustion engines) even in urban environments…*”

2) **Page 4 Line 76-81:** the authors state that health studies focus on primary emissions rather than SOA, but then cited more SOA studies than primary studies. Seems contradictory. In fact, there is now a lot of attention on SOA. I suggest rephrasing.

*We have rephrased this section accordingly.*

Line 86: “Furthermore, in recent years, there have been an increasing number of studies on the health effects of SOA formed from the oxidation of emitted hydrocarbons, demonstrating their potential contribution to PM-induced health effects…”

3) **Page 5 line 95:** Why were IL-6 and TNF-alpha chosen as the biomarkers? There are many other markers (such as HO-1, IL-17). Are these biomarkers better indicators of oxidative stress and better linked to health endpoints than others? Given that there is a nuanced response shown in Fig. 4, perhaps the choice of IL-6 and TNF-alpha was deliberate, but as a reader I am not sure why.

*We chose to measure IL-6 and TNF-α due to their central roles in cellular responses to stimuli and high production in MH-S cells. We have included a brief justification for choosing these specific biomarkers.*

Line 106: “…cytokines indicative of the inflammatory response. TNF-α is a hallmark biomarker involved in triggering a number of cellular signaling cascades. More specifically, TNF-α is involved in the activation of NFκB, which regulates the expression of a variety of genes involved in inflammation and cell death, and the activation of protein kinases, which
regulate various signaling cascades (Witkamp and Monshouwer, 2000). IL-6 has both pro- and anti-inflammatory effects, and may directly inhibit TNF-α (Kamimura et al., 2004). Furthermore, both cytokines are produced at relatively high levels in MH-S cells, ensuring a high signal-to-noise ratio and thus reliable measurements (Matsunaga et al., 2001; Chen et al., 2007).”

4) Page 8 Line 149: 45% relative humidity is still quite dry. I would not label it as “humid”.

*We prefer to label these experiments as “humid”, as they can be considered relatively humid compared to our “dry” experiments (45% RH vs. 5% RH).*

5) Page 8 lines 154-158: does an acidic seed affect the background ROS production? Or is there sufficient buffer that cells are exposed to the same pH?

*It is unlikely for the acidic seed to affect background ROS/RNS production because the mass of seed per volume of media is low. Additionally, no changes in media color were observed during the extraction process. Since the cell culture media (RPMI-1640) contains phenol red, which is an indicator of pH, any significant changes in pH would result in an observable change in color. RPMI-1640 also uses a sodium bicarbonate buffer system to maintain physiological pH, so cells should be exposed to the same pH for all samples.*

6) Page 8 line 161: What is zero air? Is this purified air? How is the air purified?

*We have modified all instances of “zero air” to “pure air” and included how the air is purified at the first mention of pure air.*

Line 171: “Chambers were flushed with pure air (generated from AADCO, 747-14) for ~24 hrs…”

Line 183: “…passing pure air over the solution until it fully evaporated.”

Line 186: “Naphthalene was injected by passing pure air over solid naphthalene flakes…”

7) Page 8 line 169: presumably this concentration of OH is yielded only upon irradiation for the specific set of chamber lights.

*Yes, this is the OH concentration yielded upon irradiation with the specific set of chamber lights (jNO₂ = 0.28 min⁻¹) (Boyd et al., 2015). This value is comparable to typical values for OH concentrations obtained in previous chamber studies (e.g., Eddingsaas et al., 2012; Loza et al., 2014; Ng et al., 2007; Chan et al., 2009; Chan et al., 2011).*
8) Page 10 line 212: why is 24 hrs chosen? What happens if cytokine levels were measured earlier or later? Are there recovery effects of exposure?

We chose to measure both cytokines at 24 hrs to enable comparison at the same time point as ROS/RNS measurements (optimized in Tuet et al. (2016)) and because the production levels of both cytokines are relatively high at this time point for MH-S cells. Previous studies have shown that TNF-α and IL-6 production peak around 4 and 24 hrs, respectively (Haddad, 2001). Measuring at an earlier or later time point results in a decreased response, which may indicate recovery effects. We have modified the manuscript to clarify.

Line 236: “following manufacturer’s specifications (ThermoFisher). This time point was chosen to enable comparison with ROS/RNS levels (also measured at 24 hrs, optimized in Tuet et al. (2016)) and to ensure a high signal for both cytokines. Previous literature have shown that TNF-α and IL-6 production peak around 4 and 24 hrs, respectively (Haddad, 2001). However, while TNF-α production peaks earlier, the signal at 24 hrs is well above the detection limit of the assay, and previous studies have utilized this time point to measure both cytokines (Haddad, 2001; Matsunaga et al., 2001). Nonetheless, it should be noted that these measurements represent a single time point in the cellular response….”

9) Page 12 line 247: H2O2 is unlikely to be taken up by inorganic seeds particles on a Teflon filter (as shown by the authors’ results), but may be taken up if there are organics coated on the filter. Is it possible there is further heterogeneous reactions of H2O2 on the organics, given the H2O2 concentrations are 3ppm?

Since H2O2 uptake by inorganic seed particles was not observed (as shown by the blank results), it is unlikely that more H2O2 was taken up by SOA given the hygroscopicity parameter values (κ = 0.53 for ammonium sulfate vs. κ = 0.006 – 0.2 for organic compounds) (Petters and Kreidenweis, 2007).

10) Page 12-13 lines 259-268: This is a central finding of this manuscript: the carbon backbone seems to play a bigger role than formation conditions. While I do not dispute the results, this finding is hard to rationalize. Formation conditions will affect mostly the functional groups that go onto the molecule (there may be small changes in the backbone with fragmentation pathways), while precursor identity will determine the size and shape of the backbone. ROS is likely produced through electron transfer to/from the functional groups interacting (or reacting) with O2, H2O, antioxidants and NAPDH. It is therefore difficult to imagine that the functional group matters less than the backbone structure. Also, by that logic, reactions that change the molecular structure (such as oligomerization, fragmentation) would change the cellular ROS quite significantly. Is there any evidence of that?

Thank you for your comment. We believe there was some confusion in this section. When we discussed the “carbon backbone”, we intended for “carbon backbone” to include both the
carbon chain length and functionalities. Furthermore, we refer to the “carbon backbone” of oxidation products, rather than the precursor compound. We have modified the manuscript to clarify these points. We also note that the referenced section (lines 259 – 268) refers to findings from a previous study, where the chemical oxidative potentials of these SOA systems were measured (Tuet et al., 2017). In that study, precursor identity was found to influence oxidative potential more significantly than formation condition. We bring this up here to highlight potential differences between chemical and cellular assays. In the current study, both precursor identity and formation condition influenced the level of cellular response, and products with similar functionalities and carbon chain length may induce similar responses. Oligomerization and fragmentation reactions influence the O:C ratio (and hence $\overline{OS}_C$), of SOA. We did observe a correlation between $\overline{OS}_C$ and ROS/RNS production, shown in Fig. 3.

Line 27: “…which suggests that the chemical structure (carbon chain length and functionalities) of photooxidation products may be important…”

Line 295: “DTT may only be sensitive to larger differences arising from different precursors, whereas cellular assays…”

Line 305: “…for SOA precursors whose products share similar chemical structures (i.e., similar carbon chain length and functionalities)…”

Line 529: “…SOA systems whose products share similar functionalities and carbon chain length are likely to induce…”

11) Page 16 line 326: this is an interesting explanation. If fatty acids are really changing cell functions that significantly, meat cooking organic aerosols, which are composed almost entirely of fatty acids, would elicit very strong responses.

Thank you for the suggestion. We have added this as a potential implication.

Line 380: “…lesser response compared to pentadecane SOA exposure. These observations, particularly those for pentadecane SOA, suggest that aerosols from meat cooking may have health implications, as fatty acids comprise a majority of these aerosols (Mohr et al., 2009; Rogge et al., 1991).”

12) Page 16 Line 343: Naphthalene is not “completely” different. For example, IL-6 and TNF-alpha are still somewhat positively correlated at low levels. Perhaps it is just a more distinct pattern.

Thank you for the suggestion. We have modified the manuscript accordingly.
Line 384: “Naphthalene exhibits a **different, more distinct** pattern compared to the rest of the SOA systems…”

13) **Page 18** Line 395-396 and Fig. 3b: what does significant correlation mean? There is an asterisk in Fig. 3b. Does that mean the trend is statistically significant? If so, please provide statistical justification (e.g. 95% confidence interval?). Does it have to be a linear model? Does the correlation still stand if naphthalene SOA (which is the outlier) points are removed? It would seem reasonable to me to remove the naphthalene system if there is reason to believe it has a very different toxicological mechanism.

*The method for determining statistical significance was described in the methods section. We have modified the manuscript and figure caption accordingly. The correlation does not hold if naphthalene SOA is removed. However, since other SOA systems (i.e., pentadecane and β-caryophyllene) may also participate in toxicological pathways unique to those SOA systems, we did not exclude naphthalene from the correlation. Furthermore, it is interesting that there exists a correlation between oxidation state and ROS/RNS even though different toxicological mechanisms may be involved.*

Line 436: “Nevertheless, a significant correlation (p < 0.05) was observed…”

Line 592: “…colored by SOA system. * indicates significance, p < 0.05.”

14) **Page 19** lines 404-425: What is the relationship between ROS/RNS and cytokines for these SOA systems? It seems that plotting them against each other would help explain trends in each SOA system, or at least establish whether or not ROS/RNS are linked to upregulation of these cytokines.

*We show the relationship between ROS/RNS and cytokines in Fig. 4, where the ROS/RNS level is influenced by a balance between both cytokines due to pro- and anti-inflammatory effects. We did plot ROS/RNS against cytokine measurements, however, individual correlation plots did not reveal any additional information as the inflammatory markers are involved in pathways with many overlaps and crosstalk. These relationships were only apparent when all three measurements were plotted, as shown in Fig. 4.*

15) **Page 3** Line 52: “anti-oxidant” should be “antioxidant”

*We have modified the manuscript accordingly.*

Line 52: “…redox reactions using an antioxidant species…”

Line 53: “The antioxidant is oxidized…”

Line 401: “…products that promote electron transfer reactions with antioxidants…”
16) Page 7 line 127: “form” should be “from”

We have modified the manuscript accordingly.

Line 149: “SOA formed from the photooxidation…”

17) Page 21 line 464: “RNS/RNS” should be “ROS/RNS”

We have modified the manuscript accordingly.

Line 505: “…produce low levels of ROS/RNS…”

References:


We thank the reviewers for their time and comments. Below are detailed responses to each comment. The responses are italicized and the modified texts are in red. The main comments have been addressed by including a discussion on the limitations of this study and by clarifying our statistical analysis method. The revisions do not affect the conclusions of the manuscript.

Response to anonymous referee #2 comments:

1) **Limitations of this study:** I didn’t see any discussion regarding the limitations of this study, and they mainly cited their own DTT papers throughout the discussion. This would be my most major criticism. You have to be careful to say that your acute exposures here will really translate to the in vivo condition. Specifically, why does one need to be careful in extending the results obtained from in vitro exposures to the in vivo condition? What are the potential issues with extracting filters for resuspension into cell culture? Does the chemistry change, and if so, how might that affect the toxicological response?

*Thank you for your suggestion. We are aware that there are limitations regarding all health studies and have modified the manuscript to include several examples of these limitations. We note that the main objective of this study was to provide perspective on the relative toxicities of different SOA systems. Further studies are required to establish whether results from in vitro assays represent in vivo animal exposures, and from there, whether results from animal exposure studies can be generalized to actual human exposures.*

Line 552: “Additionally, this study confirms…”

Line 562: “…to fully interpret ROS/RNS measurements. Finally, several limitations must be considered before generalizing results from this study to in vivo exposures. For instance, only one cell type was explored in this study, whereas an organism consists of multiple tissues comprised of multiple cell types. Interactions between different cell types and tissue systems were not considered in this study. Furthermore, the doses investigated may not fully represent real world exposures due to differences in exposure routes and potential recovery from doses due to clearance. Nevertheless, this study provides perspective on the relative toxicities of different SOA systems which future studies can build upon.”

2) **Rationale for using murine alveolar macrophages:** I think the authors should provide the rationale for using murine alveolar macrophages for this study. Would certain phenotype of this cell line differ from human alveolar macrophages? How easily relatable is it to human cells? What are limitations of cell lines versus primary cells and would that matter?

*Thank you for your comment. We have included rationale for using this cell type in the manuscript. We chose murine alveolar macrophages as they are the first line of defense against environmental insults, and the particular cell line (MH-S) retains many properties*
of primary alveolar macrophages (e.g., phagocytosis, cytokine production, ROS/RNS production) (Sankaran and Herscowitz, 1995; Mbawuike and Herscowitz, 1989). Furthermore, we have successfully utilized this cell line to investigate the production of ROS/RNS as a result of exposure to ambient PM samples (Tuet et al., 2016). To our knowledge, immortalized human alveolar macrophages do not exist. Mice have also been widely used as a model organism for studying human responses (Rosenthal and Brown, 2007; Takao and Miyakawa, 2015). As for the choice between cell lines and primary cells, primary cells are harvested from multiple animals, which increases the response variability. Results may therefore be less reproducible compared to cell lines.

Line 137: “Exposures were conducted using immortalized murine alveolar macrophages (MH-S, ATCC®CRL-2019TM) as they are the first line of defense against environmental insults (Oberdörster, 1993; Oberdörster et al., 1992). The particular cell line also retains many properties of primary alveolar macrophages, including phagocytosis as well as the production of ROS/RNS and cytokines (Sankaran and Herscowitz, 1995; Mbawuike and Herscowitz, 1989). MH-S cells were cultured…”

3) I noticed that the authors’ cell culture and exposure media contain fetal bovine serum (FBS), which is known to potentially interfere with the ELISA assays. Normally people use serum-free media to avoid such interferences. Do the authors have any control experiments to show that FBS wouldn’t interfere with their ELISA measurement?

We normalized all ELISA responses to a control (cell culture supernatant from cells exposed to stimulant-free media supplemented with FBS) to capture any interferences. For our time point (24 hrs), FBS supplemented media is necessary to prevent serum starvation, which is known to induce oxidative stress (Kuznetsov et al., 2011; Wright et al., 2012). We also disagree that serum-free media is generally used for ELISA measurements, as many previous studies have performed exposures using supplemented media (e.g., Mukherjee et al., 2009; Chen et al., 2007; Sullivan et al., 2000).

4) They use the cell media to extract filters. Since cell media contain a lot of supplementary materials/nutrients, would this affect the fraction of SOA materials extracted? Also, for the reactive products, would they be hydrolyzed before cell exposure?

For oxidative potential measurements, it is known that using different extraction methods (e.g., different solvent, filtration, removing the filter) results in different components extracted and hence yields different oxidative potential measurements (Gao et al., 2017). However, there are limitations for each method. For instance, using an organic solvent requires the subsequent removal of the solvent via evaporation, which may result in loss of unstable components (e.g., semi-volatile organics). In this study, we chose to adapt an extraction method best suited for cellular exposure. While media contains species that would indeed alter the fraction of material extracted, these species are also present in the
alveolar fluid and the extract obtained is biologically relevant. We would also like to note
that plain media (without FBS) was used for extraction and that FBS was supplemented
after filtration of extracts. We did not investigate the hydrolysis of reactive products due to
extraction, however this would be a potential issue for all extraction methods used in offline
analysis. Further studies comparing offline and online analysis are required to investigate
this.

5) Lines 265-266: I think the redox activity is likely more sensitive to the
functionality/electronic configuration of the functional groups, instead of carbon
backbone. If it is carbon backbone, it looks to me that DTT is removed by other
mechanisms such as absorption, but not through redox mechanisms.

The referenced section refers to a previous study, where the chemical oxidative potentials as
determined by DTT consumption were measured for these SOA systems (Tuet et al., 2017).
In this study, we focus on the cellular responses and we find that the precursor identity and
formation condition are both important and affect the cellular responses significantly. We
note that there may have been some confusion in this section, as we intended “carbon
backbone” to include both carbon chain length and functionalities. We have modified the
manuscript to clarify our findings.

Line 27: “…which suggests that the chemical structure (carbon chain length and
functionalities) of photooxidation products may be important…”

Line 295: “DTT may only be sensitive to larger differences arising from different
precursors, whereas cellular assays…”

Line 305: “…for SOA precursors whose products share similar chemical structures (i.e.,
similar carbon chain length and functionalities)…”

Line 529: “…SOA systems whose products share similar functionalities and carbon chain
length are likely to induce…”

6) How these inflammatory responses relate to each other? Are they involved in the same
biological network? They probably need to provide a more detailed biological
background for the biomarkers they measured. For example, TNF-alpha induces IL-8
via NF-κB. This is well known in the toxicological literature. In some of the
toxicological literature, TNF-alpha is used as positive control to stimulate IL-8 in
BEAS-2B cells. I don’t see a clear connection between the endpoints they measured in
this paper and this needs to be more justified. Without a connection to a specific
biological system, it makes it hard (especially for an atmospheric chemist I’m sure) to
understand what your results really mean.
Thank you for your suggestion. We have included justification on our cytokine measurements.

Line 106: “…cytokines indicative of the inflammatory response. TNF-α is a hallmark biomarker involved in triggering a number of cellular signaling cascades. More specifically, TNF-α is involved in the activation of NFκB, which regulates the expression of a variety of genes involved in inflammation and cell death, and the activation of protein kinases, which regulate various signaling cascades (Witkamp and Monshouwer, 2000). IL-6 has both pro- and anti-inflammatory effects, and may directly inhibit TNF-α (Kamimura et al., 2004). Furthermore, both cytokines are produced at relatively high levels in MH-S cells, ensuring a high signal-to-noise ratio and thus reliable measurements (Matsunaga et al., 2001; Chen et al., 2007).”

7) Lines 288-290: The authors cite Lin et al. (2016, ES&T Letters), but I think this discussion is really unclear. What genes are similar? What pathways do the authors mean? They should make them clear. Note that Lin et al. (2016, ES&T Letters) only measured oxidative stress-associated genes, but not inflammatory genes in that paper. I noted that Lin et al. (2017, ES&T) just had a newly accepted paper where they found most genes are associated with the Nrf2 pathway, but not much inflammatory response from isoprene SOA exposure under non-cytotoxic conditions. Also, in Lin et al. (2017, ES&T) time course experiments, they found that IL-8 expression is time sensitive. The expression maximized at 9 hr and much lowered at 24 hr, which was also shown in Arashiro et al. (2016, ACP). Their cellular materials were collected 24 hr post-exposure, so they might have missed the peak. How do the authors justify the 24 hr post exposure time? Did they conduct a series of time course experiments to see where things might peak in terms of cellular response? The authors and readers need to realize you may only captured 1 slice in time in how the cells responded.

We have modified the manuscript to clarify this discussion. Specifically, we include an example of a gene whose fold change was similar between the two types of SOA studies in Lin et al. (2016) and discuss how that gene is related to the inflammatory cytokines measured in this study. Oxidative stress plays a crucial role in the inflammatory process, and as such, the oxidative stress related genes measured in Lin et al. (2016) may influence cytokine production. We thank the reviewer for pointing out Lin et al. (2017) and have cited the paper accordingly. We are aware that cytokine production peaks at different time points for different cytokines. In our case, TNF-α peaks around 4 hrs, while IL-6 peaks much later at 24 hrs (Haddad, 2001). We chose to measure both cytokines at the latter time point to allow comparison. Previous studies have shown that the level of TNF-α is sufficiently high at the latter time point for accurate determination (Haddad, 2001; Matsunaga et al., 2001). The manuscript has been modified to include this justification as well.

Line 93: “However, the cellular exposure studies involving SOA focused on SOA formed from a single precursor and included different measures of response (e.g. ROS/RNS,
inflammatory biomarkers, gene expression, etc.) (Arashiro et al., 2016; Lund et al., 2013; McDonald et al., 2010; McDonald et al., 2012; Baltensperger et al., 2008; Lin et al., 2017).”

Line 318: “…the fold change of several genes reported in Lin et al. (2016) are actually similar (e.g., ALOX12, NQO1). Several of these genes directly affect the production of inflammatory cytokines measured in this study. For instance, studies have observed that arachidonate 12-lipoxygenase (ALOX12) products induce the production of both TNF-α and IL-6 in macrophages (Wen et al., 2007). As such, a similar response level regardless of SOA formation condition may be observed depending on the biological endpoints measured. Thus, it is possible that the inflammatory cytokines measured in this study are involved in pathways concerning those genes, resulting in a similar response level regardless of SOA formation condition.”

Line 231: “following manufacturer’s specifications (ThermoFisher). This time point was chosen to enable comparison with ROS/RNS levels (also measured at 24 hrs, optimized in Tuet et al. (2016)) and to ensure a high signal for both cytokines. Previous literature have shown that TNF-α and IL-6 production peak around 4 and 24 hrs, respectively (Haddad, 2001). However, while TNF-α production peaks earlier, the signal at 24 hrs is well above the detection limit of the assay, and previous studies have utilized this time point to measure both cytokines (Haddad, 2001; Matsunaga et al., 2001). Nonetheless, it should be noted that these measurements represent a single time point in the cellular response.…”

8) **Line 305:** what kind of chemical structure do they mean here?

*Thank you for the comment. We have modified the manuscript to clarify.*

Line 340: “These observations further imply that the chemical structures (e.g., carbon chain lengths and functionalities) of oxidation products…”

9) **Line 322-329:** I am not sure about the insertion of pentadecane oxidation products to the membrane. They should at least provide some references to support such a statement. I would expect some cellular response, specifically cytotoxicity, from these products since they are detergent like, which could potentially rupture the cell membrane. Did they see cell death from MTT data for pentadecane oxidation products?

*Thank you for the suggestion. We have included references to support this hypothesis. We did not observe decreases in cellular metabolic activity as measured by the MTT assay (mentioned in lines 282 – 286 in the revised manuscript).*

Line 363: “…could potentially insert into the cell membrane (Loza et al., 2014), as previous studies have shown that fatty acids can feasibly insert into the cell membrane bilayer (Khmelinskaia et al., 2014; Cerezo et al., 2011).”
10) The mechanism of PAH-DNA adduct formation is well known through metabolic activation to diol epoxides. This is not mentioned at all in current discussion.

We mentioned the formation of DNA adducts briefly in the section on naphthalene SOA (lines 420 – 424). The specific mechanism by which these adducts are formed is beyond the scope of this study, but would be interesting to investigate in future studies.

11) Statistical Analysis: One more critical comment relates to the authors statistical analysis. Where are their linear regression results and the associated p values? Also, with multiple groups, one-way ANOVA should be used instead of student’s t test to get p-values (same idea as the increasing type I error with multiple testing). Lastly, when they talked about the trend, I didn’t see any statistical support to differentiate between groups. Are the results really statistically significant?

Based on the reviewer’s comment, we believe the trend referenced refers to Fig. 3. The Pearson’s correlation coefficient is given in the original figure. For clarity, we have modified the manuscript and figure caption to reflect that correlations were evaluated using a 95% confidence interval. Since only two variables (cellular response and bulk aerosol composition, e.g., ROS/RNS and $\text{O}_2^{-}$) were tested, the student’s t-test and one-way ANOVA are actually equivalent (Park, 2009).

Line 436: “Nevertheless, a significant correlation ($p < 0.05$) was observed…”

Line 592: “…colored by SOA system. * indicates significance, $p < 0.05$.”

12) I’m curious why the authors didn’t gravimetrically weigh the filters before and after sampling to insure actual mass on filter for dose-response purposes? If you use the SMPS, you must make assumptions about density to calculate the mass. How was density accurately determined if you did use that approach? Was the SMPS sheath flow conditioned to the appropriate RH used in the chamber?

Mass loadings were low for isoprene and pentadecane SOA. To be consistent, we choose to determine mass by integrating the SMPS volume concentrations for all SOA systems. An aerosol density of 1 g cm$^{-3}$ was assumed to facilitate comparison between studies, since SOA density varies with precursor identity and formation condition. We have added this clarification to the manuscript. For all experiments, the SMPS was connected to the chamber for 2-3 hrs before the start of the experiment to condition the recirculating sheath flow.

Line 201: “…multiplying by the total volume of air collected. SMPS volume concentrations were converted to mass concentrations by assuming a density of 1 g cm$^{-3}$ to facilitate comparison between studies…”
13) Related to #12 above, were extraction efficiencies of aerosol mass from the filters determined by spiking them with representative internal standards? Extracting filters with cell media I may not actually remove a lot of materials (such as oligomers of SOA) from the filters. Why wasn’t organic solvents used, then dried, and then the dried extracts reconstituted with cell media for the exposures? Toxicologists might find your dosing completely uncertain as its hard to gauge how well you removed the SOA from the filters without this information. This is a very important point for Figures like Figure 3. The AMS sees most of the SOA mass but filter extractions may not actually remove all of it for the exposure assessment done here.

Extraction efficiencies were not measured in this study. While different extraction methods are known to result in different constituents being extracted from the PM sample, there are limitations for each method. These are discussed in a recent publication by Gao et al. (2017). For example, using an organic solvent and drying the extract for reconstitution may result in loss of unstable constituents. For this study, we chose an extraction method best suited for cellular exposure.

14) I have a curiosity question. Did the authors observe brown color on some of their filters (like from naphthalene SOA or isoprene SOA)? If so, did you see any trends with brown carbon and your toxicological endpoints?

We only observed brown color on our naphthalene SOA filters. We did not measure brown carbon in this study.

15) Line 81-84: This seems to be an incomplete sentence or poorly worded sentence. Please revise.

Thank you for your comment. We have modified the sentence.

Line 91: “However, the cellular exposure studies involving SOA focused on SOA formed from a single precursor and included different measures of response…”

References:


Inflammatory responses to secondary organic aerosols (SOA) generated from biogenic and anthropogenic precursors

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Keywords: reactive oxygen/nitrogen species, inflammatory cytokines, particulate matter, secondary organic aerosol
Cardiopulmonary health implications resulting from exposure to secondary organic aerosols (SOA), which comprise a significant fraction of ambient particulate matter (PM), have received increasing interest in recent years. In this study, alveolar macrophages were exposed to SOA generated from the photooxidation of biogenic and anthropogenic precursors (isoprene, α-pinene, β-caryophyllene, pentadecane, m-xylene, and naphthalene) under different formation conditions (RO₂ + HO₂ vs. RO₂ + NO dominant, dry vs. humid). Various cellular responses were measured, including reactive oxygen/nitrogen species (ROS/RNS) production and secreted levels of cytokines, tumor necrosis factor-α (TNF-α) and interleukin-6 (IL-6). SOA precursor identity and formation condition affected all measured responses in a hydrocarbon-specific manner. With the exception of naphthalene SOA, cellular responses followed a trend where TNF-α levels reached a plateau with increasing IL-6 levels. ROS/RNS levels were consistent with relative levels of TNF-α and IL-6, due to their respective inflammatory and anti-inflammatory effects. Exposure to naphthalene SOA, whose aromatic ring-containing products may trigger different cellular pathways, induced higher levels of TNF-α and ROS/RNS than suggested by the trend. Distinct cellular response patterns were identified for hydrocarbons whose photooxidation products shared similar chemical functionalities and structures, which suggests that the carbon backbone chemical structure (carbon chain length and functionalities) of photooxidation products may be important for determining cellular effects. A positive nonlinear correlation was also detected between ROS/RNS levels and previously measured DTT activities for SOA samples. In the context of ambient samples collected during summer and winter in the greater Atlanta area, all laboratory-generated SOA produced similar or higher levels of ROS/RNS and DTT activities. These results
suggest that the health effects of SOA are important considerations for understanding the health implications of ambient aerosols.

Introduction

Particulate matter (PM) exposure is a leading global risk factor for human health (Lim et al., 2012) with numerous studies reporting associations between elevated PM concentrations and increases in cardiopulmonary morbidity and mortality (Li et al., 2008; Pope III and Dockery, 2006; Brunekreef and Holgate, 2002; Dockery et al., 1993; Hoek et al., 2013; Anderson et al., 2011; Pope et al., 2002). A possible mechanism for PM-induced health effects has been suggested by toxicology studies, wherein PM-induced oxidant production, including reactive oxygen and nitrogen species (ROS/RNS), initiates inflammatory cascades thus resulting in oxidative stress and cellular damage (Li et al., 2003a; Tao et al., 2003; Castro and Freeman, 2001; Gurgueira et al., 2002; Wiseman and Halliwell, 1996; Hensley et al., 2000). Furthermore, prolonged stimulation of these inflammatory cascades may lead to chronic inflammation, for which there is a recognized link to cancer (Philip et al., 2004). Together, these findings suggest that a possible relationship exists between PM exposure and observed health effects.

Various assays have been developed to study PM-induced oxidant production, including cell-free chemical assays that measure the oxidative potential of PM samples (Kumagai et al., 2002; Cho et al., 2005; Fang et al., 2015b) as well as cellular assays that measure intracellular ROS/RNS produced as a result of PM exposure (Landreman et al., 2008; Tuet et al., 2016). Cell-free assays simulate biologically relevant redox reactions using an antioxidant species (e.g. dithiothreitol, DTT; ascorbic acid, AA). The antioxidant is oxidized via electron transfer reactions catalyzed by redox-active species in the PM sample and its rate of decay serves
as a measure of the concentration of redox-active species present (Fang et al., 2015b). On the other hand, cellular assays utilize a fluorescent probe (e.g. carboxy-H$_2$DCFDA) that reacts with ROS/RNS and the measured fluorescence is proportional to the concentration of ROS/RNS produced as a result of PM exposure (Landreman et al., 2008; Tuet et al., 2016). Both types of assays have been utilized extensively to characterize a variety of PM samples and identify sources that may be detrimental to health (Verma et al., 2015a; Saffari et al., 2015; Fang et al., 2015a; Bates et al., 2015; Li et al., 2003b; Tuet et al., 2016). In particular, numerous studies suggest that organic carbon constituents, especially humic-like substances (HULIS) and oxygenated polyaromatic hydrocarbons (PAH), may contribute significantly to PM-induced oxidant production (Li et al., 2003b; Kleinman et al., 2005; Hamad et al., 2015; Verma et al., 2015b; Lin and Yu, 2011). Furthermore, recent measurements of ROS/RNS production and DTT activity using ambient samples collected in summer and winter around the greater Atlanta area showed that there is a significant correlation between summertime organic species and intracellular ROS/RNS production, suggesting a possible role for secondary organic aerosols (SOA) (Tuet et al., 2016). The same study also reported a significant correlation between ROS/RNS production and DTT activity for summer samples, while a relatively flat ROS/RNS response was observed for winter samples spanning a similar DTT activity range (Tuet et al., 2016). These results highlight a need to consider multiple endpoints as a simple correlation may not exist between different endpoints, especially cellular responses that may result from complicated response networks.

Despite these findings, there are still many gaps in knowledge regarding PM-induced health effects. While The current work will focus on the relative toxicities of different SOA systems, as field studies have repeatedly shown that SOA often dominate over primary
aerosols (e.g., PM emitted directly from combustion engines) even in urban environments (Zhang et al., 2007; Jimenez et al., 2009; Ng et al., 2010). Many prior health studies have focused on the effects of primary emissions (e.g., PM emitted directly from combustion engines) (Kumagai et al., 2002; Bai et al., 2001; McWhinney et al., 2013a; Turner et al., 2015) rather than those of SOA formed from the oxidation of emitted hydrocarbons (McWhinney et al., 2013b; Rattanavaraha et al., 2011; Kramer et al., 2016; Lund et al., 2013; McDonald et al., 2010; McDonald et al., 2012; Baltensperger et al., 2008; Arashiro et al., 2016; Platt et al., 2014). The cellular exposure studies that do explore SOA focused on SOA formed from a single SOA precursor and included further, in recent years, there have been an increasing number of studies on the health effects of SOA formed from the oxidation of emitted hydrocarbons, demonstrating their potential contribution to PM-induced health effects (McWhinney et al., 2013b; Rattanavaraha et al., 2011; Kramer et al., 2016; Lund et al., 2013; McDonald et al., 2010; McDonald et al., 2012; Baltensperger et al., 2008; Arashiro et al., 2016; Platt et al., 2014; Gallimore et al., 2017). However, the cellular exposure studies involving SOA focused on SOA formed from a single precursor and included different measures of response (e.g., ROS/RNS, inflammatory biomarkers, gene expression, etc.) (Arashiro et al., 2016; Lund et al., 2013; McDonald et al., 2010; McDonald et al., 2012; Baltensperger et al., 2008; Lin et al., 2017). As a result, there is a lack of understanding in terms of the relative toxicity of individual SOA systems. Recently, Tuet et al. (2017) systematically investigated the DTT activities of SOA formed from different biogenic and anthropogenic precursors and demonstrated that intrinsic DTT activities were highly dependent on SOA precursor identity, with naphthalene SOA having the highest DTT activity. As a result, a systematic study on the cellular responses induced by these SOA systems may provide similar
insights. Furthermore, cellular responses may complement these previously measured DTT activities to elucidate a more complete picture of the health effects of PM.

In the present study, alveolar macrophages were exposed to SOA generated under different formation conditions from various SOA precursors. Cellular responses induced by SOA exposure were measured, including intracellular ROS/RNS production and levels of tumor necrosis factor-α (TNF-α) and interleukin-6 (IL-6). Intracellular ROS/RNS production serves as a general indicator of oxidative stress, whereas TNF-α and IL-6 are pro-inflammatory cytokines indicative of the inflammatory response (Henkler et al., 2010; Kishimoto, 2003; Wang et al., 2003). TNF-α is a hallmark biomarker involved in triggering a number of cellular signaling cascades. More specifically, TNF-α is involved in the activation of NFκB, which regulates the expression of a variety of genes involved in inflammation and cell death, and the activation of protein kinases, which regulate various signaling cascades (Witkamp and Monshouwer, 2000). IL-6 has both pro- and anti-inflammatory effects, and may directly inhibit TNF-α (Kamimura et al., 2004). Furthermore, both cytokines are produced at relatively high levels in MH-S cells, ensuring a high signal-to-noise ratio and thus reliable measurements (Matsunaga et al., 2001; Chen et al., 2007).

Precursors were chosen to include major classes of biogenic and anthropogenic compounds known to produce SOA upon atmospheric oxidation (Table S1). The selected biogenic precursors include: isoprene, the most abundant non-methane hydrocarbon (Guenther et al., 2006); α-pinene, a well-studied monoterpene with emissions on the order of global anthropogenic emissions (Guenther et al., 1993; Piccot et al., 1992); and β-caryophyllene, a representative sesquiterpene. Both monoterpenes and sesquiterpenes have been shown to contribute significantly to ambient aerosol (Eddingsaas et al., 2012; Hoffmann et al., 1997; Tasoglou and Pandis, 2015; Goldstein and Galbally, 2007). Similarly, the anthropogenic precursors include: pentadecane, a long-chain
Alkanes; m-xylene, a single-ring aromatic; and naphthalene, a poly-aromatic. These compounds are emitted as products of incomplete combustion (Robinson et al., 2007; Jia and Batterman, 2010; Bruns et al., 2016) and have considerable SOA yields (Chan et al., 2009; Ng et al., 2007b; Lambe et al., 2011). In addition to precursor identity, the effects of humidity (dry vs. humid) and NO\textsubscript{x} levels (different predominant peroxy radical (RO\textsubscript{2}) fates, RO\textsubscript{2} + HO\textsubscript{2} vs. RO\textsubscript{2} + NO) on SOA cellular inflammatory responses were investigated, as different formation conditions have been shown to affect aerosol chemical composition and mass loading, which could in turn result in a different cellular response (Chhabra et al., 2010; Chhabra et al., 2011; Eddingsaas et al., 2012; Ng et al., 2007b; Loza et al., 2014; Ng et al., 2007a; Chan et al., 2009; Boyd et al., 2015). Finally, correlations between bulk aerosol composition, specifically elemental ratios, and cellular inflammatory responses were investigated to determine whether there is a link between different inflammatory responses and aerosol composition.

**Methods**

**Alveolar macrophage cell line.** Immortalized murine alveolar macrophages (MH-S, ATCC\textsuperscript{®}CRL-2019\textsuperscript{TM}) Exposures were conducted using immortalized murine alveolar macrophages (MH-S, ATCC\textsuperscript{®}CRL-2019\textsuperscript{TM}) as they are the first line of defense against environmental insults (Oberdörster, 1993; Oberdörster et al., 1992). The particular cell line also retains many properties of primary alveolar macrophages, including phagocytosis as well as the production of ROS/RNS and cytokines (Sankaran and Herscowitz, 1995; Mbawuike and Herscowitz, 1989). MH-S cells were cultured in RPMI-1640 media supplemented with 10% fetal bovine serum (FBS, Quality Biological, InC.), 1% penicillin-streptomycin, and 50 μM β-mercaptoethanol (BME) at 37°C and humid air containing 5% CO\textsubscript{2}. For exposure experiments, MH-S cells were seeded at a density of 2 x 10\textsuperscript{4} cells well\textsuperscript{-1} onto 96-well plates pre-treated with 10% FBS in phosphate buffered saline.
(PBS, Cellgro). For seeding and all assay procedures thereon, FBS-supplemented cell culture media without BME addition was used as BME is a reducing agent that may interfere with inflammatory measurements.

**Chamber experiments.** SOA formed from the photooxidation of biogenic and anthropogenic precursors were generated in the Georgia Tech Environmental Chamber (GTEC) facility. Details of the facility have been described elsewhere. Briefly, the chamber facility consists of two 12 m³ Teflon chambers suspended within a 21 x 12 ft temperature-controlled enclosure. Black lights and natural sunlight fluorescent lamps surround the chambers, and multiple sampling ports allow for injection of reagents, as well as gas- and aerosol-phase measurements. Gas-phase O₃, NO₂, and NOₓ concentrations were monitored using an O₃ analyzer (Teledyne T400), a cavity attenuated phase shift (CAPS) NO₂ monitor (Aerodyne), and a chemiluminescence NOₓ monitor (Teledyne 200EU) respectively, while hydrocarbon decay was monitored using a gas chromatography-flame ionization detector (GC-FID, Agilent 7890A). Hydrocarbon decay was also used to estimate hydroxyl radical (OH) concentrations. For aerosol-phase measurements, a Scanning Mobility Particle Sizer (SMPS, TSI) was used to measure aerosol volume concentrations and distributions, while a High Resolution Time-of-Flight Aerosol Mass Spectrometer (HR-ToF-AMS, Aerodyne; henceforth referred to as the AMS) was used to determine bulk aerosol composition (DeCarlo et al., 2006). AMS data was analyzed using the data analysis toolkit SQUIRREL (v. 1.57) and PIKA (v. 1.16G). Elemental ratios, including O:C, H:C, and N:C, were obtained using the method outlined by Canagaratna et al. (2015) and used to calculate the average carbon oxidation state ($\bar{OS}_c$) (Kroll et al., 2011). Temperature and relative humidity (RH) were also monitored using a hydro-thermometer (Vaisala HMP110).
Experiments were designed to probe the effects of humidity, RO$_2$ fate, and precursor identity on cellular inflammatory responses induced by different SOA formed under these conditions (Table 1). All chamber experiments were performed at ~25 °C under dry (RH < 5%) or humid (RH ~ 45%) conditions. Chambers were flushed with pure air (generated from AADCO, 747-14) for ~24 hrs prior to each experiment. During this time, chambers were also humidified for humid experiments by means of a bubbler filled with deionized (DI) water. Seed aerosol was injected by atomizing a 15 mM (NH$_4$)$_2$SO$_4$ seed solution (Sigma Aldrich) to obtain a seed concentration of ~20 µg m$^{-3}$. It should be noted that experimental conditions deviate for experiment 7 (isoprene SOA under RO$_2$ + HO$_2$ dominant, “humid” conditions) due to low SOA mass yields. For this experiment, an acidic seed solution (8 mM MgSO$_4$ and 16 mM H$_2$SO$_4$) and a dry chamber were used to promote SOA formation via the isoprene epoxidiol (IEPOX) uptake pathway. This pathway has been shown to contribute significantly to ambient OA and has a higher SOA mass yield compared to the IEPOX + OH pathway (Surratt et al., 2010; Lin et al., 2012; Xu et al., 2015).

SOA precursor was then introduced by injecting a known amount of hydrocarbon solution [isoprene, 99%; α-pinene, ≥ 99%; β-caryophyllene, > 98.5%; pentadecane, ≥ 99%; m-xylene, ≥ 99%; naphthalene, 99% (Sigma Aldrich)] into a glass injection bulb and passing zero pure air over the solution until it fully evaporated. For pentadecane and β-caryophyllene, the glass bulb was also heated gently during hydrocarbon injection to ensure full evaporation (Tasoglou and Pandis, 2015). Naphthalene was injected by passing zero pure air over solid naphthalene flakes as described in previous studies (Chan et al., 2009). OH precursor was then introduced via injection of hydrogen peroxide (H$_2$O$_2$) for RO$_2$ + HO$_2$ experiments or nitrous acid (HONO) for RO$_2$ + NO experiments. For H$_2$O$_2$, a 50% aqueous solution (Sigma Aldrich) was injected using the same method described for hydrocarbon injection to achieve an H$_2$O$_2$ concentration of 3 ppm. This amount yielded OH
concentrations on the order of $10^6$ molec cm$^{-3}$. For HONO injections, HONO was first prepared by adding 10 mL of 1\%wt aqueous NaNO$_2$ (VWR International) dropwise into 20 mL of 10\%wt H$_2$SO$_4$ (VWR International) in a glass bulb. Zero air was then passed over the solution to introduce HONO into the chamber (Chan et al., 2009; Kroll et al., 2005). Photolysis of HONO yielded OH concentrations on the order of $10^7$ molec cm$^{-3}$. NO and NO$_2$ were also formed as byproducts of HONO synthesis. Once all the H$_2$O evaporated (RO$_2$ + HO$_2$ experiments) or NO$_x$ concentrations stabilized (RO$_2$ + NO experiments), the UV lights were turned on to initiate photooxidation.

**Aerosol collection and extraction.** Aerosol samples were collected onto 47 mm Teflon™ filters (0.45 µm pore size, Pall Laboratory). The total mass collected onto each filter was determined by integrating the SMPS time-dependent volume concentration over the filter collection period and multiplying by the total volume of air collected. SMPS volume concentrations were converted to mass concentrations by assuming a density of 1 g cm$^{-3}$ to facilitate comparison between studies. To account for potential H$_2$O$_2$ or HONO uptake, background filters were also collected. These filters were collected when only seed particles and OH precursor (H$_2$O$_2$ or HONO) were injected into the chamber under otherwise identical experimental conditions. All collected samples were placed in sterile petri dishes, sealed with Parafilm M®, and stored at -20 °C until extraction and analysis (Fang et al., 2015b). Collected particles were extracted following the procedure outlined in Fang et al. (2015a) with modifications for cellular exposure. Briefly, filter samples were submerged in cell culture media (RPMI-1640) and sonicated for two 30 min intervals (1 hr total) using an Ultrasonic Cleanser (VWR International). In between sonication intervals, the water was replaced to reduce bath temperature. After the final sonication interval, sample extracts were filtered using 0.45 µm PTFE syringe filters.
(Fisherbrand™) to remove any insoluble material and supplemented with 10% FBS (Fang et al., 2015b).

**Intracellular ROS/RNS measurement.** ROS/RNS were detected using the assay optimized in Tuet et al. (2016). Briefly, the assay consists of five major steps: (1) pre-treatment of 96-well plates to ensure a uniform cell density, (2) seeding of cells onto pre-treated wells at 2 x 10^4 cells well^{-1}, (3) incubation with ROS/RNS probe (carboxy-H_2DCFDA, Molecular Probes C-400) diluted to a final concentration of 10 µM, (4) exposure of probe-treated cells to samples and controls for 24 hrs, and (5) detection of ROS/RNS using a microplate reader (BioTek Synergy H4, ex/em: 485/525 nm). Positive controls included bacterial cell wall component lipopolysaccharide (LPS, 1 µg mL^{-1}), H_2O_2 (100 µM), and reference filter extract (10 filter punches mL^{-1}, 1 per filter sample, from various ambient filters collected at the Georgia Tech site, while negative controls included blank filter extract (2 punches mL^{-1}) and control cells (probe-treated cells exposed to media only, no stimulants).

A previous study on the ROS/RNS produced induced by exposure to ambient PM samples found that ROS/RNS production was highly dose-dependent and could therefore not be represented by measurements taken at a single dose (Tuet et al., 2016). Here, we utilize the dose-response curve approach described in Tuet et al. (2016). For each aerosol sample, ROS/RNS production was measured over ten dilutions and expressed as a fold increase in fluorescence over control cells. A representative dose-response curve is shown in Fig. 1. For comparisons to other inflammatory endpoints and chemical composition, ROS/RNS production was represented using the area under the dose-response curve (AUC), as AUC has been shown to be the most robust metric for comparing PM samples (Tuet et al., 2016).
**Cytokine measurement.** Secreted levels of TNF-α and IL-6 were measured post-exposure (24 hrs) using enzyme-linked immunosorbent assay (ELISA) kits following manufacturer’s specifications (ThermoFisher). *This time point was chosen to enable comparison with ROS/RNS levels (also measured at 24 hrs, optimized in Tuet et al. (2016)) and to ensure a high signal for both cytokines. Previous literature have shown that TNF-α and IL-6 production peak around 4 and 24 hrs, respectively (Haddad, 2001). However, while TNF-α production peaks earlier, the signal at 24 hrs is well above the detection limit of the assay, and previous studies have utilized this time point to measure both cytokines (Haddad, 2001; Matsunaga et al., 2001). Nonetheless, it should be noted that these measurements represent a single time point in the cellular response. All measurements were carried out using undiluted cell culture supernatant. For each aerosol sample, TNF-α and IL-6 were measured over seven dilutions and represented as a fold increase over control. Similarly, the AUC was used to represent each endpoint for comparison purposes.

**Cellular metabolic activity.** The MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay (Biotium) was used to assess cellular metabolic activity. Briefly, supernatants containing sample extracts were removed after the exposure period and replaced with media containing MTT. Cells were then returned to the incubator for 4 hrs, during which the tetrazolium dye was reduced by cellular NAD(P)H-dependent oxidoreductases to produce an insoluble purple salt (formazan). Dimethyl sulfoxide was then used to solubilize the salt and the absorbance at 570 nm was determined using a microplate reader (BioTek Synergy H4).

**Statistical analysis.** Linear regressions between bulk aerosol composition and cellular inflammatory responses were evaluated using Pearson’s correlation coefficient, and the significance of each correlation coefficient was determined using multiple imputation, which calculated the total variance associated with the slope of each regression. Details of this method
are described in Pan and Shimizu (2009). Briefly, response parameters (i.e. AUCs for each endpoint) were assumed to follow a normal distribution. Ten “estimates” were obtained for each response using the average and standard deviation determined from the dose-response curve fit. These estimates were then plotted against bulk aerosol composition (e.g. O:C, H:C, and N:C) to obtain ten fits, and the slopes and variances generated from these fits were used to calculate the between and within variance. Finally, a Student’s $t$-test was used to calculate and evaluate the associated $p$-values using a 95% confidence interval.

**Results and Discussion**

**Effect of SOA precursor and formation condition on SOA inflammatory response.** To investigate whether SOA formed from different precursors elicited different inflammatory responses, levels of ROS/RNS, TNF-$\alpha$, and IL-6 were measured after exposing alveolar macrophages to SOA generated from six VOCs generated under three formation conditions (Table 1). The AUC per mass of SOA ($\mu$g) in the extract for ROS/RNS, TNF-$\alpha$ and IL-6 are shown in Fig. 2, shaped by SOA formation condition. It should be noted that all responses were normalized to probe-treated control cells to account for differences between endogenous levels of ROS/RNS produced in cells (Henkler et al., 2010). Uncertainties associated with AUC were determined by averaging the AUCs obtained by fitting dose-response data with each point removed systematically, following the methodology described in Tuet et al. (2016). ROS/RNS production was also measured for background filters and found to be within the uncertainty of control cells, indicating that there was no evidence for significant H$_2$O$_2$ or HONO uptake onto seed particles (Fig. S1). Furthermore, exposure to filter extract did not result in decreases in metabolic activity as measured by the MTT assay for all SOA systems investigated (Fig. S2). Since results from MTT may represent the number of viable cells present, changes in inflammatory endpoints did not likely
result from changes in the number of cells exposed (i.e. decreases in response cannot be attributed to cell death).

For all inflammatory responses measured (levels of ROS/RNS, TNF-α, and IL-6), SOA precursor identity and formation condition influenced the level of response, as demonstrated by the range of values obtained from different SOA precursors and different formation conditions (Fig. 2). Despite having a clear effect, no obvious trends were observed for each variable (precursor or formation condition) on individual responses. This is in contrast to that observed for the oxidative potential as measured by DTT (OP\textsubscript{WS-DTT}) for these samples, where only precursor identity influenced OP\textsubscript{WS-DTT} substantially (Tuet et al., 2017). However, this may not be surprising as DTT is a chemical assay, which only accounts for the potential of species to participate in redox reactions (Cho et al., 2005), whereas cellular assays account for many complicated cellular events involved in intricate positive and negative feedback loops. Due to the considerably different classes of compounds chosen as SOA precursors, aerosol compositional changes between different precursors were generally larger than those between different formation conditions of the same precursor (see Fig. 3a) (Tuet et al., 2017). DTT may only be sensitive to larger differences arising from different precursors (i.e. a different carbon backbone), whereas cellular assays could also be sensitive to differences between different formation conditions and chemical composition of the same precursor. Moreover, while Tuet et al. (2017) showed that the intrinsic OP\textsubscript{WS-DTT} spanned a wide range, with isoprene and naphthalene SOA generating the lowest and highest OP\textsubscript{WS-DTT}, these bounds were less clear for cellular responses. While isoprene and naphthalene SOA still generated the lowest and highest inflammatory responses in general, a few exceptions exist (e.g. ROS/RNS levels induced by pentadecane SOA formed under dry, RO\textsubscript{2} + HO\textsubscript{2} dominant conditions, Fig. 2).
Though no apparent trends in individual inflammatory responses were observed as a function of SOA precursor identity or formation condition, several patterns among all three inflammatory responses were observed for SOA precursors whose products share similar chemical structures (i.e., similar carbon backbones—chain length and functionalities). Exposure to isoprene SOA induced the lowest levels of TNF-α and IL-6 among the aerosol systems studied (Fig. 2). Furthermore, isoprene SOA generated from different pathways (i.e. photooxidation under different RO₂ fates and reactive uptake of IEPOX) (Surratt et al., 2010; Xu et al., 2014; Chan et al., 2010) produced similar responses for each inflammatory endpoint. These results suggest that different isoprene SOA products (Surratt et al., 2010; Xu et al., 2014; Chan et al., 2010) may induce similarly low inflammatory responses and are consistent with the intrinsic OP₇WS-DTT obtained for these SOA samples, where isoprene SOA generated the lowest OP₇WS-DTT of all SOA systems studied and the OP₇WS-DTT was similar for all SOA formation conditions explored (Tuet et al., 2017).

This finding is in contrast to a previous study by Lin et al. (2016), where methacrylic acid epoxide (MAE)-derived SOA was found to be substantially more potent than IEPOX-derived SOA. However, while exposure to MAE-derived SOA induced the upregulation of a larger number of oxidative stress response genes than IEPOX-derived SOA, the fold change of several genes reported in Lin et al. (2016) are actually similar: (e.g., ALOX12, NQO1). Several of these genes directly affect the production of inflammatory cytokines measured in this study. For instance, studies have observed that arachidonate 12-lipoxygenase (ALOX12) products induce the production of both TNF-α and IL-6 in macrophages (Wen et al., 2007). As such, a similar response level regardless of SOA formation condition may be observed depending on the biological endpoints measured. Thus, it is possible that the inflammatory cytokines measured in this study
are involved in pathways concerning those genes, resulting in a similar response level regardless of SOA formation condition.

Similarly, exposure to SOA generated from the photooxidation of α-pinene and m-xylene resulted in similar inflammatory responses for all three formation conditions (Fig. 2). These cellular assay results are consistent with results from the DTT assay where the OP_{WS-DTT} was not significantly different between SOA formed under different formation conditions (Tuet et al., 2017). Response levels induced by these two SOA systems are also similar across all three inflammatory measurements investigated (Fig. 2). This suggests that products from both precursors may induce similar cellular pathways resulting in the production of similar levels of inflammatory markers. Indeed, there are several similarities between products formed from the photooxidation of α-pinene and m-xylene. For instance, a large portion of α-pinene and m-xylene oxidation products under both RO_2 + HO_2 and RO_2 + NO pathways are ring-breaking products with a similar carbon chain length (Eddingsaas et al., 2012; Vivanco and Santiago, 2010; Jenkin et al., 2003). As a result of this similarity, products from both SOA systems may interact with the same cellular targets and induce similar cellular pathways, resulting in a similar response regardless of precursor identity and formation condition. These observations further imply that the chemical structures (e.g., carbon chain lengths and functionalities) of oxidation products may be important regardless of PM source/precursor.

A different pattern was observed for β-caryophyllene and pentadecane SOA, where the IL-6 response spanned a much larger range than ROS/RNS and TNF-α (Fig. 2). This is in contrast to the trends observed for the OP_{WS-DTT} for β-caryophyllene and pentadecane SOA, where OP_{WS-DTT} was similar regardless of formation condition (Tuet et al., 2017). This suggests that there are differences between organic peroxides and organic nitrates formed from certain precursors that
influence cellular responses, but are not captured by redox potential measurements. Less is known about the effects of humidity on SOA formation and chemical composition for all SOA systems investigated, as most laboratory chamber studies in literature have been conducted under dry conditions. Specifically here, very high levels of IL-6 were observed post-exposure to pentadecane SOA formed under humid conditions. Prior studies reported opposing findings with some showing a significant effect of water on aerosol formation and chemical composition (Nguyen et al., 2011; Wong et al., 2015; Healy et al., 2009; Stirnweis et al., 2016), while others found little influence (Edney et al., 2000; Boyd et al., 2015; Cocker III et al., 2001). It is clear that humidity effects are highly hydrocarbon-dependent and further studies into the specific products formed under humid conditions are required to understand how these differences in chemical composition may translate to different cellular endpoints. Nonetheless, the known products formed from the photooxidation of these hydrocarbons may provide some insight into the inflammatory responses observed. While there are no prior studies involving pentadecane oxidation products, it is expected that the oxidation products will be similar to those reported in the oxidation of dodecane (i.e. same functionalities with a longer carbon chain) (Loza et al., 2014). It is therefore likely that pentadecane oxidation products resemble long chain fatty acids and could potentially insert into the cell membrane (Loza et al., 2014), as previous studies have shown that fatty acids can feasibly insert into the cell membrane bilayer (Khmelinskaia et al., 2014; Cerezo et al., 2011). This insertion could potentially affect membrane fluidity, which is known to affect cell function substantially although the specific effect depends strongly on the particular modification and cell type of interest (Baritaki et al., 2007; Spector and Yorek, 1985). In some cases, these alterations lead to the induction of apoptosis, which involves pathways leading to the production of TNF-α (Baritaki et al., 2007; Wang et al., 2003). TNF-α can then induce the production of IL-6, which once produced
can also inhibit the production of TNF-\(\alpha\) in a feedback loop (Kishimoto, 2003; Wang et al., 2003). These cellular events are consistent with the observed inflammatory response induced by pentadecane SOA exposure, where there is a high IL-6 response and a lower TNF-\(\alpha\) response. The low ROS/RNS response observed is also in line with these cellular events, as IL-6 exhibits anti-inflammatory functions, which can neutralize ROS/RNS production. These responses are less pronounced for \(\beta\)-caryophyllene aerosol, which may be due to the shorter carbon chain observed in known products (Chan et al., 2011). While \(\beta\)-caryophyllene and pentadecane are both C15 precursors, \(\beta\)-caryophyllene is a bicyclic compound and many SOA products retain the 4-membered ring, resulting in a shorter carbon backbone (Chan et al., 2011). As a result, fewer products may insert into the cell membrane, leading to a lesser response compared to pentadecane SOA exposure. These observations, particularly those for pentadecane SOA, suggest that aerosols from meat cooking may have health implications, as fatty acids comprise a majority of these aerosols (Mohr et al., 2009; Rogge et al., 1991).

Naphthalene exhibits a completely different, more distinct pattern from compared to the rest of the SOA systems investigated, with a large range observed for both TNF-\(\alpha\) and IL-6 under different formation conditions (Fig. 2). Higher levels of ROS/RNS were also observed as a result of exposure to naphthalene aerosol irrespective of SOA formation condition. Similarly, the OP\(^{WS-}\)\(\text{DTT}\) of naphthalene SOA previously measured by Tuet et al. (2017) was an outlier among all SOA systems investigated, as the measured OP\(^{WS-DTT}\) was at least twice that of the next highest SOA system. These observations are consistent with the formation of specific SOA products such as naphthoquinones, which are known to induce redox-cycling in cells and are formed under both \(\text{RO}_2 + \text{HO}_2\) and \(\text{RO}_2 + \text{NO}\) pathways (Henkler et al., 2010; Kautzman et al., 2010). Consequently, aerosol generated from naphthalene may induce higher levels of inflammatory responses than
other SOA due to this process (Henkler et al., 2010; Lorentzen et al., 1979). However, as shown by the high levels of IL-6, exposure to naphthalene SOA may also induce anti-inflammatory pathways not captured by OP\textsuperscript{WS-DTT} measurements. Moreover, a clear increasing trend is apparent for TNF-\(\alpha\) and IL-6 produced upon naphthalene SOA exposure, with a higher level of both cytokines observed for aerosol formed under RO\textsubscript{2} + NO dominant and humid conditions. Previously, the effect of different RO\textsubscript{2} fates on SOA OP\textsuperscript{WS-DTT} was attributed to the different products known to form under both pathways (Tuet et al., 2017). The same explanation applies for cellular measurements as SOA products that promote electron transfer reactions with antioxidants can result in redox imbalance as measured by OP\textsuperscript{WS-DTT} and the induction of related cellular pathways such as ROS/RNS and cytokine production (Tuet et al., 2017). Finally, naphthalene SOA induced cellular responses outside of those observed for other aerosol systems, with higher levels of all inflammatory markers than other SOA systems. As shown previously for OP\textsuperscript{WS-DTT}, naphthalene may be an outlier due to aromatic ring-containing products, which may then induce different cellular pathways compared to other aerosol systems investigated, the products of which do not contain aromatic rings. Additionally, many known aerosol products formed from the photooxidation of naphthalene have functionalities that resemble those of dinitrophenol, which is known to decouple phosphorylation from electron transfer (Terada, 1990). It is therefore possible that the aromatic functionality present in the majority of naphthalene SOA products results in the involvement of very different cellular pathways, leading to outlier inflammatory endpoint responses. Various products of naphthalene oxidation such as nitroaromatics and polyaromatics are known to have mutagenic properties and may induce the formation of DNA adducts (Baird et al., 2005; Helmig et al., 1992). As such, it is possible that
these products may induce health effects via other pathways as well and naphthalene SOA exposure may have effects beyond redox imbalance and oxidative stress.

Bulk aerosol elemental ratios (O:C, H:C, and N:C) were determined for each SOA system investigated. Different types of organic aerosol are known to span a wide range of O:C, which may be utilized as an indication of oxidation, and the van Krevelen diagram was used to visualize whether changes in O:C and H:C ratios corresponded to changes in levels of inflammatory response (Fig. 3a, S3) (Chhabra et al., 2011; Lambe et al., 2011; Ng et al., 2010). Changes in the slope within the van Krevelen space provide information on SOA functionalization (Heald et al., 2010; Van Krevelen, 1950; Ng et al., 2011). Beginning from the precursor hydrocarbon, a slope of 0 indicates alcohol group additions, a slope of -1 indicates carbonyl and alcohol additions on separate carbons or carboxylic acid additions, and a slope of -2 indicates ketone or aldehyde additions.

As seen in Fig. 3a, the laboratory-generated aerosols span a large range of O:C and H:C ratios. Both SOA formation condition and precursor identity influenced elemental ratios, however, precursor identity generally had a larger effect as evident by the clusters observed for different SOA precursors. Despite these differences in chemical composition, there were no obvious trends between O:C or H:C and any inflammatory endpoint measured. This is similar to that observed for chemical oxidative potential as measured by DTT, where a higher O:C did not correspond to a higher oxidative potential for both laboratory-generated and ambient aerosols (Tuet et al., 2017). This is likely due to the different formation conditions used to generate SOA, which may not be directly comparable. Nevertheless, a significant correlation \( (p < 0.05) \) was observed between ROS/RNS and \( \overline{O}_S \) (Fig. 3b). This positive correlation is not surprising, as a higher average oxidation state would likely correspond to a better oxidizing agent. Future studies should evaluate
the effect of the degree of oxidation for SOA formed from the same SOA precursor under the same
formation condition to investigate whether atmospheric aging of aerosol (which typically leads to
increases in the degree of oxidation) affects inflammatory responses. Finally, the N:C ratio was
also determined for SOA systems formed under conditions that favor the RO$_2$ + NO pathway (Fig.
S4) and were found to span a large range. Similarly, there was no obvious trend between N:C ratios
and the inflammatory endpoints measured.

**Relationship between inflammatory responses.** To visualize whether there exists a
relationship between inflammatory markers measured, levels of TNF-$\alpha$ and IL-6 are shown in Fig.
4, sized by ROS/RNS. With the exception of naphthalene SOA, the inflammatory cytokine
responses for all aerosol systems investigated follow an exponential curve (Fig. 4, shown in black)
where there appears to be a plateau for TNF-$\alpha$ levels. Along this curve, ROS/RNS levels also
appear to increase with increasing inflammatory cytokine levels to a certain point, after which
ROS/RNS levels decrease. These observations are in line with the interconnected effects of both
cytokines. While both TNF-$\alpha$ and IL-6 have pro-inflammatory effects that may lead to the increase
of ROS/RNS production, the individual pathways are also involved in many complicated
stimulation and inhibition loops and there is extensive cross-talk between both pathways. For
instance, TNF-$\alpha$ induces the production of glucocorticoids, which in turn inhibits both TNF-$\alpha$ and
IL-6 production (Wang et al., 2003). IL-6 also directly inhibits the production of TNF-$\alpha$ and other
cytokines induced as a result of TNF-$\alpha$ (e.g. IL-1) and stimulates pathways that lead to the
production of glucocorticoids (Kishimoto, 2003). As a result, increases in IL-6 may be
accompanied by decreases in TNF-$\alpha$, resulting in the observed plateau. Furthermore, ROS/RNS
levels may represent a fine balance between anti-inflammatory and pro-inflammatory effects. Both
cytokines are involved in the acute phase reaction and can affect ROS/RNS levels via pro-
inflammatory pathways. IL-6 also exhibits some anti-inflammatory functions and may thus lower ROS/RNS levels as well. These interconnected pathways could account for the observed parabolic pattern for ROS/RNS production. Exposure to naphthalene SOA resulted in responses outside of those observed for other aerosol systems, likely due to the formation of aromatic ring-retaining products as discussed in the previous section.

**Comparison with ambient data.** To evaluate how the oxidative potential and ROS/RNS production of the SOA systems investigated compare in the context of ambient samples, the measurements obtained in this study were plotted with those obtained in our previous study involving ambient samples collected around the greater Atlanta area (Fig. 5) (Tuet et al., 2016). These ambient samples were analyzed using the same methods for determining oxidative potential (DTT assay (Cho et al., 2005; Fang et al., 2015b)) and ROS/RNS production (cellular carboxy-

H$_2$DCFDA assay (Tuet et al., 2016)). Furthermore, the same extraction protocol (water-soluble extract) was followed in both studies (Tuet et al., 2016). Results from both studies are therefore directly comparable. Previously, a significant correlation between ROS/RNS production and oxidative potential as measured by DTT was observed for summer ambient samples. In the same study, correlations between ROS/RNS production and organic species were also observed for summer ambient samples, and it was suggested that these correlations may reflect contributions from photochemically produced SOA (Tuet et al., 2016).

Fig. 5 shows that laboratory-generated SOA oxidative potential is comparable to that observed in ambient samples, with the exception of naphthalene SOA, which produced higher DTT activities due to its aromatic ring-retaining products (Tuet et al., 2017; Kautzman et al., 2010). Laboratory-generated SOA also induced similar or higher levels of ROS/RNS compared to ambient samples. There are many possible explanations for the observed higher response for some
SOA samples. For instance, individual, single precursor SOA systems were considered in this study, whereas ambient aerosol contains SOA from multiple precursors as well as other species that are not considered in this study (e.g. metals). Interactions between SOA from different precursors is likely to occur and may result in different response levels. Complex interactions between SOA and other species present in the ambient (e.g. metals or other organic species) are also likely involved (Tuet et al., 2016). Previous studies have also suggested the possibility of metal-organic complexes. For instance, Verma et al. (2012) showed that certain metals were retained on a C-18 column, which is utilized to remove hydrophobic components, suggesting that these metals were likely complexed and removed in the process. Further chamber studies involving photochemically generated SOA and metals may elucidate these interactions. Furthermore, there are likely species present in the ambient that do not contribute to ROS/RNS production. That is, while certain species contribute to the mass of PM, there is little to no ROS/RNS production associated with these species. Ambient samples where these species comprise a significant fraction will have a low per mass ROS/RNS production level. Finally, only three SOA formation conditions were investigated in this study. There are multiple other possible oxidation mechanisms that lead to the formation of SOA in the ambient, which were not accounted for in this study. Nonetheless, despite the low ROS/RNS levels observed post SOA exposure, there is an association between ROS/RNS production and DTT activity (Fig. 5). These results suggest that our previous findings based on ambient filter samples may be extended to SOA samples. That is, while the relationship between ROS/RNS production and DTT activity is complex, DTT may serve as a useful screening tool as samples with low DTT activities are likely to produce low levels of RNS/ROS/RNS (Tuet et al., 2016).
**Implications.** Levels of ROS/RNS, TNF-α, and IL-6 were measured after exposing cells to the water-soluble extract of SOA generated from the photooxidation of six SOA precursors under various formation conditions. Although previous epidemiological and ambient studies have found correlations between metals and various measures of health effects (Verma et al., 2010; Pardo et al., 2015; Burnett et al., 2001; Huang et al., 2003; Akhtar et al., 2010; Charrier and Anastasio, 2012), the measured levels of TNF-α, IL-6, and ROS/RNS obtained in this study demonstrate that organic aerosols alone can induce a cellular response. This was previously observed for the oxidative potential as measured by DTT activity as well, where the same laboratory-generated organic aerosol samples catalyzed redox reactions and resulted in measurable DTT decay in the absence of metal species (Tuet et al., 2017).

Results from this study also show that SOA precursor identity and formation condition influenced response levels, with naphthalene SOA producing the highest cellular responses of the SOA systems investigated. As discussed previously, the aromatic functionality present in many naphthalene photooxidation products may be an important consideration for health effects. It may therefore be worthwhile to investigate other anthropogenic aromatic ring-containing precursors as well and to closely study the cellular effects of naphthalene SOA products given its high response. Several patterns were also noted for SOA systems whose products shared similar functionalities and chemical structures. For instance, photooxidation productions from pentadecane and β-caryophyllene share similarities with long chain fatty acids and may participate in membrane insertions, whereas many known products of naphthalene photooxidation are mutagens capable of inducing cellular pathways beyond those that affect cellular redox balance (Baird et al., 2005; Helmig et al., 1992). Given these observations, it may be possible to roughly predict responses based on known SOA products as SOA systems whose products share similar functionalities and
carbon chain length (i.e. similar carbon backbone) are likely to induce similar cellular pathways and produce similar levels of various inflammatory endpoints. Exposure studies involving individual classes of SOA products may elucidate further details as to whether these types of predictions would be plausible. Moreover, such studies could be used to determine whether the hypothesized cellular pathways are indeed involved and whether certain cellular functions are indeed affected by specific products (e.g. membrane insertion by pentadecane photooxidation products and oxidative phosphorylation decoupling by naphthalene photooxidation products).

Mixture effects may be another important consideration as ambient PM contains SOA formed from multiple SOA precursors. As a result, precursor emissions and their corresponding SOA formation potential must be considered to fully assess PM health effects. Furthermore, it may be worthwhile to investigate various prediction models for multi-component mixtures to bridge the gap between laboratory studies and real ambient exposures. For instance, concentration addition may not apply as ambient aerosol is formed in the presence of multiple precursors and the SOA produced may induce response levels completely different from those observed for single precursor SOA systems that comprise the mixture. Interactions between organic components and metal species have also been suggested in previous studies (Verma et al., 2012; Tuet et al., 2016) and may influence responses significantly. While these interactions were not considered in the current study, there may be evidence to support the plausibility of mixture effects as ambient PM samples produced lower levels of ROS/RNS than that of any single SOA system investigated. Laboratory chambers can serve as an ideal platform to investigate mixture effects, as experiments can be conducted under well-controlled conditions where the aerosol chemical composition and health endpoints can be determined.
Finally, this study confirms that while there is not one simple correlation between oxidative potential and cellular responses for different PM samples, the DTT assay may serve as a useful screening tool as a low DTT activity will likely correspond to a low cellular response. Furthermore, while ROS/RNS may serve as a general indicator of oxidative stress, there may be instances where a low level of ROS/RNS does not necessarily indicate a lack of cellular response. In the current study, ROS/RNS levels were associated with levels of inflammatory cytokines for the majority of SOA systems investigated. However, aerosol formed from the photooxidation of pentadecane induced low levels of ROS/RNS production and relatively high levels of both cytokines (i.e. higher than expected given the ROS/RNS level measured). These results suggest that at least one additional measure (e.g. inflammatory cytokines) may be required to fully interpret ROS/RNS measurements. Finally, several limitations must be considered before generalizing results from this study to in vivo exposures. For instance, only one cell type was explored in this study, whereas an organism consists of multiple tissues comprised of multiple cell types. Interactions between different cell types and tissue systems were not considered in this study. Furthermore, the doses investigated may not fully represent real world exposures due to differences in exposure routes and potential recovery from doses due to clearance. Nevertheless, this study provides perspective on the relative toxicities of different SOA systems which future studies can build upon.
Figure 1. Representative dose-response curve of ROS/RNS produced as a result of filter exposure (naphthalene SOA formed under dry, RO_2 + NO dominant conditions). ROS/RNS is expressed as a fold increase over control cells, defined as probe-treated cells incubated with stimulant-free media. Dose is expressed as mass in extract (µg). Data shown are means ± standard error of triplicate exposure experiments. The Hill equation was used to fit the dose-response curve and the area under the dose-response curve (AUC) is shown.
**Figure 2.** Area under the dose-response curve for various inflammatory responses induced as a result of SOA exposure: **ROS/RNS**, **IL-6**, and **TNF-α**. SOA were generated from various precursors (ISO: isoprene, AP: α-pinene, BCAR: β-caryophyllene, PD: pentadecane, MX: m-xylene, and NAPH: naphthalene) under various conditions (circles: dry, RO₂ + HO₂; squares: humid, RO₂ + HO₂; and triangles: dry, RO₂ + NO). Lines connecting the same inflammatory response for SOA generated from the same precursor under different formation conditions are also shown.
Figure 3. van Krevelen plot for various SOA systems sized by ROS/RNS levels (panel A) and correlation between ROS/RNS levels and average carbon oxidation state (panel B). Data points are colored by SOA system (red: isoprene, yellow: α-pinene, green: β-caryophyllene, light blue: pentadecane, blue: m-xylene, and purple: naphthalene), shaped according to formation conditions (circle: dry, RO₂ + HO₂; square: humid, RO₂ + HO₂; and triangle: dry, RO₂ + NO). SOA precursors are shown as stars, colored by SOA system. * indicates significance, \( p < 0.05 \).
Figure 4. Area under the dose-response curve per mass of SOA for various inflammatory responses induced as a result of SOA exposure. Data points are sized according to ROS/RNS level. SOA were generated from various SOA precursors (red: isoprene, yellow: α-pinene, green: β-caryophyllene, light blue: pentadecane, blue: m-xylene, and purple: naphthalene) under various conditions (circles: dry, RO₂ + HO₂; squares: humid, RO₂ + HO₂; and triangles: dry, RO₂ + NO). A fitted curve excluding naphthalene data is shown as a guide. Shaded regions for each system, colored by SOA precursor, are also shown to show the extent of clustering and provide a visualization for the different patterns observed.
Figure 5. ROS/RNS production and intrinsic DTT activities for chamber SOA and ambient samples collected around the greater Atlanta area. All samples were analyzed using the method outlined in Cho et al. (2005) and Tuet et al. (2016). Ambient samples are colored by season as determined by solstice and equinox dates between June 2012 and October 2013 (Tuet et al., 2016). A fitted curve for laboratory-generated samples is shown as a guide.
<table>
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<tr>
<th>Experiment</th>
<th>SOA precursor</th>
<th>OH precursor</th>
<th>Relative humidity (%)</th>
<th>([HC]_0) (ppb)</th>
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<tr>
<td>1</td>
<td>isoprene</td>
<td>(\text{H}_2\text{O}_2)</td>
<td>(&lt;5%)</td>
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\(^a\) Acidic seed (8 mM MgSO\(_4\) and 16 mM H\(_2\)SO\(_4\)) was used instead of 8 mM (NH\(_4\))\(_2\)SO\(_4\)
ACKNOWLEDGMENT

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ABBREVIATIONS

PM: particulate matter; SOA: secondary organic aerosol; ROS/RNS: reactive oxygen/nitrogen species; TNF-α: tumor necrosis factor-α; IL-6: interleukin-6

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