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 ($jNO_2 = 0.28\ min^{-1}$) (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 H_2O_2 uptake by inorganic seed particles was not observed (as shown by the blank results), it is unlikely that more H_2O_2 was taken up by SOA given the hygroscopicity parameter values ($\kappa = 0.53$ for ammonium sulfate vs. $\kappa = 0.006 – 0.2$ 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 be 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:

Boyd, C. M., Sanchez, J., Xu, L., Eugene, A. J., Nah, T., Tuet, W. Y., Guzman, M. I., and Ng, N. L.: Secondary organic aerosol formation from the β-pinene+NO₃ system: effect of humidity and peroxy radical fate, Atmos. Chem. Phys., 15, 7497-7522, 10.5194/acp-15-7497-2015, 2015.

Chan, A. W. H., Kautzman, K. E., Chhabra, P. S., Surratt, J. D., Chan, M. N., Crounse, J. D., Kurten, A., Wennberg, P. O., Flagan, R. C., and Seinfeld, J. H.: Secondary organic aerosol formation from photooxidation of naphthalene and alkylnaphthalenes: implications for oxidation of intermediate volatility organic compounds (IVOCs), Atmos. Chem. Phys., 9, 3049-3060, 2009.

Chan, M. N., Surratt, J. D., Chan, A. W. H., Schilling, K., Offenberg, J. H., Lewandowski, M., Edney, E. O., Kleindienst, T. E., Jaoui, M., Edgerton, E. S., Tanner, R. L., Shaw, S. L., Zheng, M., Knipping, E. M., and Seinfeld, J. H.: Influence of aerosol acidity on the chemical composition of secondary organic aerosol from β-caryophyllene, Atmos. Chem. Phys., 11, 1735-1751, 10.5194/acp-11-1735-2011, 2011.

Chen, C. Y., Peng, W. H., Tsai, K. D., and Hsu, S. L.: Luteolin suppresses inflammation-associated gene expression by blocking NF-kappa B and AP-1 activation pathway in mouse alveolar macrophages, Life Sci., 81, 1602-1614, 10.1016/j.lfs.2007.09.028, 2007.

Eddingsaas, N. C., Loza, C. L., Yee, L. D., Chan, M., Schilling, K. A., Chhabra, P. S., Seinfeld, J. H., and Wennberg, P. O.: α-pinene photooxidation under controlled chemical conditions – Part 2: SOA yield and composition in low- and high-NOx environments, Atmos. Chem. Phys., 12, 7413-7427, 10.5194/acp-12-7413-2012, 2012.

Haddad, J. J.: L-buthionine-(S,R)-sulfoximine, an irreversible inhibitor of gamma-glutamylcysteine synthetase, augments LPS-mediated pro-inflammatory cytokine biosynthesis: evidence for the implication of an I kappa B-alpha/NF-kappa B insensitive pathway, Eur. Cytokine Netw., 12, 614-624, 2001.

Kamimura, D., Ishihara, K., and Hirano, T.: IL-6 signal transduction and its physiological roles: the signal orchestration model, in: Reviews of Physiology, Biochemistry and Pharmacology, Springer Berlin Heidelberg, Berlin, Heidelberg, 1-38, 2004.

Loza, C. L., Craven, J. S., Yee, L. D., Coggon, M. M., Schwantes, R. H., Shiraiwa, M., Zhang, X., Schilling, K. A., Ng, N. L., Canagaratna, M. R., Ziemann, P. J., Flagan, R. C., and Seinfeld, J. H.: Secondary organic aerosol yields of 12-carbon alkanes, Atmos. Chem. Phys., 14, 1423-1439, 10.5194/acp-14-1423-2014, 2014.

Matsunaga, K., Klein, T. W., Friedman, H., and Yamamoto, Y.: Involvement of Nicotinic Acetylcholine Receptors in Suppression of Antimicrobial Activity and Cytokine Responses of Alveolar Macrophages to Legionella pneumophila Infection by Nicotine, The Journal of Immunology, 167, 6518-6524, 10.4049/jimmunol.167.11.6518, 2001.

Mohr, C., Huffman, J. A., Cubison, M. J., Aiken, A. C., Docherty, K. S., Kimmel, J. R., Ulbrich, I. M., Hannigan, M., and Jimenez, J. L.: Characterization of Primary Organic Aerosol Emissions from Meat Cooking, Trash Burning, and Motor Vehicles with High-Resolution Aerosol Mass Spectrometry and Comparison with Ambient and Chamber Observations, Environmental Science & Technology, 43, 2443-2449, 10.1021/es8011518, 2009.

Ng, N. L., Kroll, J. H., Chan, A. W. H., Chhabra, P. S., Flagan, R. C., and Seinfeld, J. H.: Secondary organic aerosol formation from m-xylene, toluene, and benzene, Atmos. Chem. Phys., 7, 3909-3922, 10.5194/acp-7-3909-2007, 2007.

Petters, M. D., and Kreidenweis, S. M.: A single parameter representation of hygroscopic growth and cloud condensation nucleus activity, Atmos. Chem. Phys., 7, 1961-1971, 10.5194/acp-7-1961-2007, 2007.

Rogge, W. F., Hildemann, L. M., Mazurek, M. A., Cass, G. R., and Simoneit, B. R. T.: Sources of fine organic aerosol. 1. Charbroilers and meat cooking operations, Environmental Science & Technology, 25, 1112-1125, 10.1021/es00018a015, 1991.

Tuet, W. Y., Fok, S., Verma, V., Tagle Rodriguez, M. S., Grosberg, A., Champion, J. A., and Ng, N. L.: Dose-dependent intracellular reactive oxygen and nitrogen species (ROS/RNS) production from particulate matter exposure: comparison to oxidative potential and chemical composition, Atmos. Environ., 144, 335-344, http://dx.doi.org/10.1016/j.atmosenv.2016.09.005, 2016.

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, 10.5194/acp-17-839-2017, 2017.

Witkamp, R., and Monshouwer, M.: Signal transduction in inflammatory processes, current and future therapeutic targets: A mini review, Veterinary Quarterly, 22, 11-16, 10.1080/01652176.2000.9695016, 2000.

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-2019™) 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 $\overline{OS}_c$) 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 l 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 seen 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:

Arashiro, M., Lin, Y. H., Sexton, K. G., Zhang, Z., Jaspers, I., Fry, R. C., Vizuete, W. G., Gold, A., and Surratt, J. D.: In Vitro Exposure to Isoprene-Derived Secondary Organic Aerosol by Direct Deposition and its Effects on COX-2 and IL-8 Gene Expression, Atmos. Chem. Phys. Discuss., 2016, 1-29, 10.5194/acp-2016-371, 2016.

Baltensperger, U., Dommen, J., Alfarra, R., Duplissy, J., Gaeggeler, K., Metzger, A., Facchini, M. C., Decesari, S., Finessi, E., Reinnig, C., Schott, M., Warnke, J., Hoffmann, T., Klatzer, B., Puxbaum, H., Geiser, M., Savi, M., Lang, D., Kalberer, M., and Geiser, T.: Combined determination of the chemical composition and of health effects of secondary organic aerosols: The POLYSOA project, J. Aerosol Med. Pulm. Drug Deliv., 21, 145-154, 10.1089/jamp.2007.0655, 2008.

Cerezo, J., Zúñiga, J., Bastida, A., Requena, A., and Cerón-Carrasco, J. P.: Atomistic Molecular Dynamics Simulations of the Interactions of Oleic and 2-Hydroxyoleic Acids with Phosphatidylcholine Bilayers, The Journal of Physical Chemistry B, 115, 11727-11738, 10.1021/jp203498x, 2011.

Chen, C. Y., Peng, W. H., Tsai, K. D., and Hsu, S. L.: Luteolin suppresses inflammation-associated gene expression by blocking NF-kappa B and AP-1 activation pathway in mouse alveolar macrophages, Life Sci., 81, 1602-1614, 10.1016/j.lfs.2007.09.028, 2007.

Gao, D., Fang, T., Verma, V., Zeng, L., and Weber, R.: A method for measuring total aerosol oxidative potential (OP) with the dithiothreitol (DTT) assay and comparisons between an urban and roadside site of water-soluble and total OP, Atmos. Meas. Tech. Discuss., 2017, 1-25, 10.5194/amt-2017-70, 2017.

Haddad, J. J.: L-buthionine-(S,R)-sulfoximine, an irreversible inhibitor of gamma-glutamylcysteine synthetase, augments LPS-mediated pro-inflammatory cytokine biosynthesis: evidence for the implication of an I kappa B-alpha/NF-kappa B insensitive pathway, Eur. Cytokine Netw., 12, 614-624, 2001.

Kamimura, D., Ishihara, K., and Hirano, T.: IL-6 signal transduction and its physiological roles: the signal orchestration model, in: Reviews of Physiology, Biochemistry and Pharmacology, Springer Berlin Heidelberg, Berlin, Heidelberg, 1-38, 2004.

Khmelinskaia, A., Ibarguren, M., de Almeida, R. F. M., López, D. J., Paixão, V. A., Ahyayauch, H., Goñi, F. M., and Escribá, P. V.: Changes in Membrane Organization upon Spontaneous Insertion of 2-Hydroxylated Unsaturated Fatty Acids in the Lipid Bilayer, Langmuir, 30, 2117-2128, 10.1021/la403977f, 2014.

Kuznetsov, A. V., Kehrer, I., Kozlov, A. V., Haller, M., Redl, H., Hermann, M., Grimm, M., and Troppmair, J.: Mitochondrial ROS production under cellular stress: comparison of different detection methods, Analytical and Bioanalytical Chemistry, 400, 2383-2390, 10.1007/s00216-011-4764-2, 2011.

Lin, Y.-H., Arashiro, M., Martin, E., Chen, Y., Zhang, Z., Sexton, K. G., Gold, A., Jaspers, I., Fry, R. C., and Surratt, J. D.: Isoprene-Derived Secondary Organic Aerosol Induces the Expression of Oxidative Stress Response Genes in Human Lung Cells, Environmental Science & Technology Letters, 3, 250-254, 10.1021/acs.estlett.6b00151, 2016.

Lin, Y.-H., Arashiro, M., Clapp, P. W., Cui, T., Sexton, K. G., Vizuete, W., Gold, A., Jaspers, I., Fry, R. C., and Surratt, J. D.: Gene Expression Profiling in Human Lung Cells Exposed to Isoprene-Derived Secondary Organic Aerosol, Environmental Science & Technology, 10.1021/acs.est.7b01967, 2017.

Lund, A. K., Doyle-Eisele, M., Lin, Y. H., Arashiro, M., Surratt, J. D., Holmes, T., Schilling, K. A., Seinfeld, J. H., Rohr, A. C., Knipping, E. M., and McDonald, J. D.: The effects of alpha-pinene versus toluene-derived secondary organic aerosol exposure on the expression of markers associated with vascular disease, Inhal. Toxicol., 25, 309-324, 10.3109/08958378.2013.782080, 2013.

Matsunaga, K., Klein, T. W., Friedman, H., and Yamamoto, Y.: Involvement of Nicotinic Acetylcholine Receptors in Suppression of Antimicrobial Activity and Cytokine Responses of Alveolar Macrophages to Legionella pneumophila Infection by Nicotine, The Journal of Immunology, 167, 6518-6524, 10.4049/jimmunol.167.11.6518, 2001.

Mbawuike, I. N., and Herscowitz, H. B.: MH-S, a murine alveolar macrophage cell line: morphological, cytochemical, and functional characteristics, Journal of Leukocyte Biology, 46, 119-127, 1989.

McDonald, J. D., Doyle-Eisele, M., Campen, M. J., Seagrave, J., Holmes, T., Lund, A., Surratt, J. D., Seinfeld, J. H., Rohr, A. C., and Knipping, E. M.: Cardiopulmonary response to inhalation of biogenic secondary organic aerosol, Inhal. Toxicol., 22, 253-265, 10.3109/08958370903148114, 2010.

McDonald, J. D., Doyle-Eisele, M., Kracko, D., Lund, A., Surratt, J. D., Hersey, S. P., Seinfeld, J. H., Rohr, A. C., and Knipping, E. M.: Cardiopulmonary response to inhalation of secondary organic aerosol

derived from gas-phase oxidation of toluene, Inhal. Toxicol., 24, 689-697, 10.3109/08958378.2012.712164, 2012.

Mukherjee, S., Chen, L.-Y., Papadimos, T. J., Huang, S., Zuraw, B. L., and Pan, Z. K.: Lipopolysaccharide-driven Th2 Cytokine Production in Macrophages Is Regulated by Both MyD88 and TRAM, J. Biol. Chem., 284, 29391-29398, 10.1074/jbc.M109.005272, 2009.

Oberdörster, G., Ferin, J., Gelein, R., Soderholm, S. C., and Finkelstein, J.: Role of the alveolar macrophage in lung injury: studies with ultrafine particles, Environmental Health Perspectives, 97, 193-199, 1992.

Oberdörster, G.: Lung Dosimetry: Pulmonary Clearance of Inhaled Particles, Aerosol Sci. Technol., 18, 279-289, 10.1080/02786829308959605, 1993.

Park, H. M.: Comparing group means: t-tests and one-way ANOVA using Stata, SAS, R, and SPSS, 2009.

Rosenthal, N., and Brown, S.: The mouse ascending: perspectives for human-disease models, Nat Cell Biol, 9, 993-999, 2007.

Sankaran, K., and Herscowitz, H. B.: Phenotypic and functional heterogeneity of the murine alveolar macrophage-derived cell line MH-S, Journal of Leukocyte Biology, 57, 562-568, 1995.

Sullivan, K. E., Cutilli, J., Piliero, L. M., Ghavimi-Alagha, D., Starr, S. E., Campbell, D. E., and Douglas, S. D.: Measurement of Cytokine Secretion, Intracellular Protein Expression, and mRNA in Resting and Stimulated Peripheral Blood Mononuclear Cells, Clinical and Diagnostic Laboratory Immunology, 7, 920-924, 2000.

Takao, K., and Miyakawa, T.: Genomic responses in mouse models greatly mimic human inflammatory diseases, Proceedings of the National Academy of Sciences, 112, 1167-1172, 10.1073/pnas.1401965111, 2015.

Tuet, W. Y., Fok, S., Verma, V., Tagle Rodriguez, M. S., Grosberg, A., Champion, J. A., and Ng, N. L.: Dose-dependent intracellular reactive oxygen and nitrogen species (ROS/RNS) production from particulate matter exposure: comparison to oxidative potential and chemical composition, Atmos. Environ., 144, 335-344, http://dx.doi.org/10.1016/j.atmosenv.2016.09.005, 2016.

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, 10.5194/acp-17-839-2017, 2017.

Wen, Y., Gu, J., Chakrabarti, S. K., Aylor, K., Marshall, J., Takahashi, Y., Yoshimoto, T., and Nadler, J. L.: The Role of 12/15-Lipoxygenase in the Expression of Interleukin-6 and Tumor Necrosis Factor-α in Macrophages, Endocrinology, 148, 1313-1322, 10.1210/en.2006-0665, 2007.

Witkamp, R., and Monshouwer, M.: Signal transduction in inflammatory processes, current and future therapeutic targets: A mini review, Veterinary Quarterly, 22, 11-16, 10.1080/01652176.2000.9695016, 2000.

Wright, C. J., Agboke, F., Muthu, M., Michaelis, K. A., Mundy, M. A., La, P., Yang, G., and Dennery, P. A.: Nuclear Factor-κB (NF-κB) Inhibitory Protein IκBβ Determines Apoptotic Cell Death following Exposure to Oxidative Stress, J. Biol. Chem., 287, 6230-6239, 10.1074/jbc.M111.318246, 2012.

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

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Abstract

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 ($RO_2 + HO_2$ vs. $RO_2 + NO$ 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 ~~anti-oxidant~~antioxidant species (e.g. dithiothreitol, DTT; ascorbic acid, AA). The ~~anti-oxidant~~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_2DCFDA) 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 ~~showed~~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 include~~ 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 (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

100 insights. Furthermore, cellular responses may complement these previously measured DTT
101 activities to elucidate a more complete picture of the health effects of PM.

102 In the present study, alveolar macrophages were exposed to SOA generated under different
103 formation conditions from various SOA precursors. Cellular responses induced by SOA exposure
104 were measured, including intracellular ROS/RNS production and levels of tumor necrosis factor-
105 α (TNF-α) and interleukin-6 (IL-6). Intracellular ROS/RNS production serves as a general
106 indicator of oxidative stress, whereas TNF-α and IL-6 are pro-inflammatory cytokines indicative
107 of the inflammatory response (Henkler et al., 2010; Kishimoto, 2003; Wang et al., 2003). TNF-α
108 is a hallmark biomarker involved in triggering a number of cellular signaling cascades. More
109 specifically, TNF-α is involved in the activation of NFκB, which regulates the expression of a
110 variety of genes involved in inflammation and cell death, and the activation of protein kinases,
111 which regulate various signaling cascades (Witkamp and Monshouwer, 2000). IL-6 has both pro-
112 and anti-inflammatory effects, and may directly inhibit TNF-α (Kamimura et al., 2004).
113 Furthermore, both cytokines are produced at relatively high levels in MH-S cells, ensuring a high
114 signal-to-noise ratio and thus reliable measurements (Matsunaga et al., 2001; Chen et al., 2007).
115 Precursors were chosen to include major classes of biogenic and anthropogenic compounds known
116 to produce SOA upon atmospheric oxidation (Table S1). The selected biogenic precursors include:
117 isoprene, the most abundant non-methane hydrocarbon (Guenther et al., 2006); α-pinene, a well-
118 studied monoterpene with emissions on the order of global anthropogenic emissions (Guenther et
119 al., 1993; Piccot et al., 1992); and β-caryophyllene, a representative sesquiterpene. Both
120 monoterpenes and sesquiterpenes have been shown to contribute significantly to ambient aerosol
121 (Eddingsaas et al., 2012; Hoffmann et al., 1997; Tasoglou and Pandis, 2015; Goldstein and
122 Galbally, 2007). Similarly, the anthropogenic precursors include: pentadecane, a long-chain

alkane; *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_x levels (different predominant peroxy radical (RO_2) fates, $RO_2 + HO_2$ vs. $RO_2 + NO$) 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®CRL-2019™)~~Exposures were conducted using immortalized murine alveolar macrophages (MH-S, ATCC®CRL-2019™) 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_2. For exposure experiments, MH-S cells were seeded at a density of 2 x 10^4 cells $well^{-1}$ 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 ~~form~~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 m^3 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 O_3, NO_2, and NO_x concentrations were monitored using an O_3 analyzer (Teledyne T400), a cavity attenuated phase shift (CAPS) NO_2 monitor (Aerodyne), and a chemiluminescence NO_x 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 ($\overline{OS}_c$) (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 $(NH_4)_2SO_4$ 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_2SO_4) and a dry chamber were used to promote SOA formation via the isoprene epoxydiol (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_2O_2) for RO_2 + HO_2 experiments or nitrous acid (HONO) for RO_2 + NO experiments. For H_2O_2, a 50% aqueous solution (Sigma Aldrich) was injected using the same method described for hydrocarbon injection to achieve an H_2O_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_2SO_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_2O_2 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_2O_2 or HONO uptake, background filters were also collected. These filters were collected when only seed particles and OH precursor (H_2O_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-α, 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 (μg) in the extract for ROS/RNS, TNF-α 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_2O_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 ($OP^{WS\text{-}DTT}$) for these samples, where only precursor identity influenced $OP^{WS\text{-}DTT}$ 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 $OP^{WS\text{-}DTT}$ spanned a wide range, with isoprene and naphthalene SOA generating the lowest and highest $OP^{WS\text{-}DTT}$, 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, $RO_2 + HO_2$ 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 ~~with~~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 RO_2 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 $OP^{WS\text{-}DTT}$ obtained for these SOA samples, where isoprene SOA generated the lowest $OP^{WS\text{-}DTT}$ of all SOA systems studied and the $OP^{WS\text{-}DTT}$ 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

325 are involved in pathways concerning those genes, resulting in a similar response level regardless
326 of SOA formation condition.

327 Similarly, exposure to SOA generated from the photooxidation of α-pinene and *m*-xylene
328 resulted in similar inflammatory responses for all three formation conditions (Fig. 2). These
329 cellular assay results are consistent with results from the DTT assay where the $OP^{WS\text{-}DTT}$ was not
330 significantly different between SOA formed under different formation conditions (Tuet et al.,
331 2017). Response levels induced by these two SOA systems are also similar across all three
332 inflammatory measurements investigated (Fig. 2). This suggests that products from both
333 precursors may induce similar cellular pathways resulting in the production of similar levels of
334 inflammatory markers. Indeed, there are several similarities between products formed from the
335 photooxidation of α-pinene and *m*-xylene. For instance, a large portion of α-pinene and *m*-xylene
336 oxidation products under both $RO_2 + HO_2$ and $RO_2 + NO$ pathways are ring-breaking products
337 with a similar carbon chain length (Eddingsaas et al., 2012; Vivanco and Santiago, 2010; Jenkin
338 et al., 2003). As a result of this similarity, products from both SOA systems may interact with the
339 same cellular targets and induce similar cellular pathways, resulting in a similar response
340 regardless of precursor identity and formation condition. These observations further imply that the
341 chemical structures (e.g., carbon chain lengths and functionalities) of oxidation products may be
342 important regardless of PM source/precursor.

343 A different pattern was observed for β-caryophyllene and pentadecane SOA, where the IL-
344 6 response spanned a much larger range than ROS/RNS and TNF-α (Fig. 2). This is in contrast to
345 the trends observed for the $OP^{WS\text{-}DTT}$ for β-caryophyllene and pentadecane SOA, where $OP^{WS\text{-}DTT}$
346 was similar regardless of formation condition (Tuet et al., 2017). This suggests that there are
347 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-α 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-α 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 β-caryophyllene aerosol, which may be due to the shorter carbon chain observed in known products (Chan et al., 2011). While β-caryophyllene and pentadecane are both C15 precursors, β-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-α 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-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 $RO_2 + HO_2$ and $RO_2 + NO$ 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 $OP^{WS\text{-}DTT}$ measurements. Moreover, a clear increasing trend is apparent for TNF-α and IL-6 produced upon naphthalene SOA exposure, with a higher level of both cytokines observed for aerosol formed under RO_2 + NO dominant and humid conditions. Previously, the effect of different RO_2 fates on SOA $OP^{WS\text{-}DTT}$ 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 ~~anti-oxidants~~antioxidants can result in redox imbalance as measured by $OP^{WS\text{-}DTT}$ 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 $OP^{WS\text{-}DTT}$, 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 $\overline{OS}_c$ (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

439 the effect of the degree of oxidation for SOA formed from the same SOA precursor under the same
440 formation condition to investigate whether atmospheric aging of aerosol (which typically leads to
441 increases in the degree of oxidation) affects inflammatory responses. Finally, the N:C ratio was
442 also determined for SOA systems formed under conditions that favor the RO_2 + NO pathway (Fig.
443 S4) and were found to span a large range. Similarly, there was no obvious trend between N:C ratios
444 and the inflammatory endpoints measured.

445 **Relationship between inflammatory responses.** To visualize whether there exists a
446 relationship between inflammatory markers measured, levels of TNF-α and IL-6 are shown in Fig.
447 4, sized by ROS/RNS. With the exception of naphthalene SOA, the inflammatory cytokine
448 responses for all aerosol systems investigated follow an exponential curve (Fig. 4, shown in black)
449 where there appears to be a plateau for TNF-α levels. Along this curve, ROS/RNS levels also
450 appear to increase with increasing inflammatory cytokine levels to a certain point, after which
451 ROS/RNS levels decrease. These observations are in line with the interconnected effects of both
452 cytokines. While both TNF-α and IL-6 have pro-inflammatory effects that may lead to the increase
453 of ROS/RNS production, the individual pathways are also involved in many complicated
454 stimulation and inhibition loops and there is extensive cross-talk between both pathways. For
455 instance, TNF-α induces the production of glucocorticoids, which in turn inhibits both TNF-α and
456 IL-6 production (Wang et al., 2003). IL-6 also directly inhibits the production of TNF-α and other
457 cytokines induced as a result of TNF-α (e.g. IL-1) and stimulates pathways that lead to the
458 production of glucocorticoids (Kishimoto, 2003). As a result, increases in IL-6 may be
459 accompanied by decreases in TNF-α, resulting in the observed plateau. Furthermore, ROS/RNS
460 levels may represent a fine balance between anti-inflammatory and pro-inflammatory effects. Both
461 cytokines are involved in the acute phase reaction and can affect ROS/RNS levels via pro-

462 inflammatory pathways. IL-6 also exhibits some anti-inflammatory functions and may thus lower
463 ROS/RNS levels as well. These interconnected pathways could account for the observed parabolic
464 pattern for ROS/RNS production. Exposure to naphthalene SOA resulted in responses outside of
465 those observed for other aerosol systems, likely due to the formation of aromatic ring-retaining
466 products as discussed in the previous section.

467 **Comparison with ambient data.** To evaluate how the oxidative potential and ROS/RNS
468 production of the SOA systems investigated compare in the context of ambient samples, the
469 measurements obtained in this study were plotted with those obtained in our previous study
470 involving ambient samples collected around the greater Atlanta area (Fig. 5) (Tuet et al., 2016).
471 These ambient samples were analyzed using the same methods for determining oxidative potential
472 (DTT assay (Cho et al., 2005; Fang et al., 2015b)) and ROS/RNS production (cellular carboxy-
473 H_2DCFDA assay (Tuet et al., 2016)). Furthermore, the same extraction protocol (water-soluble
474 extract) was followed in both studies (Tuet et al., 2016). Results from both studies are therefore
475 directly comparable. Previously, a significant correlation between ROS/RNS production and
476 oxidative potential as measured by DTT was observed for summer ambient samples. In the same
477 study, correlations between ROS/RNS production and organic species were also observed for
478 summer ambient samples, and it was suggested that these correlations may reflect contributions
479 from photochemically produced SOA (Tuet et al., 2016).

480 Fig. 5 shows that laboratory-generated SOA oxidative potential is comparable to that
481 observed in ambient samples, with the exception of naphthalene SOA, which produced higher
482 DTT activities due to its aromatic ring retaining products (Tuet et al., 2017; Kautzman et al., 2010).
483 Laboratory-generated SOA also induced similar or higher levels of ROS/RNS compared to
484 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 measureable 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~~Additionally, 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 necessary 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.

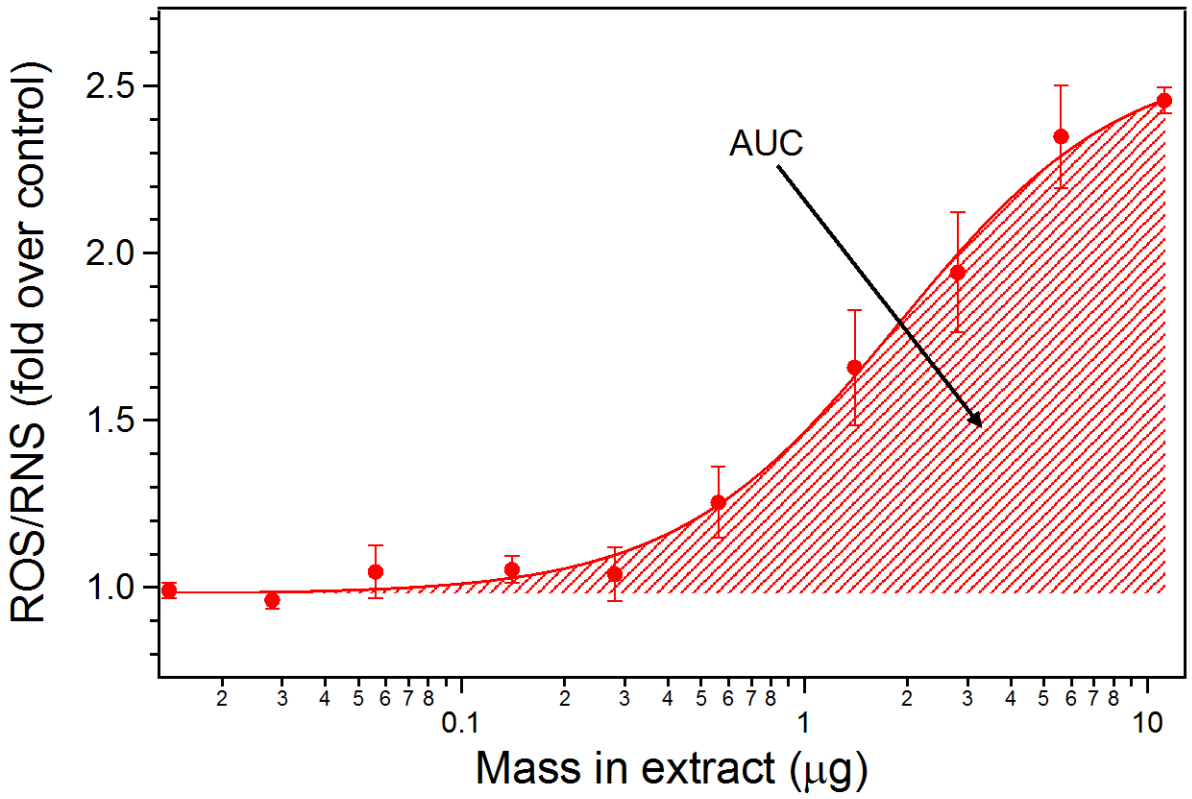
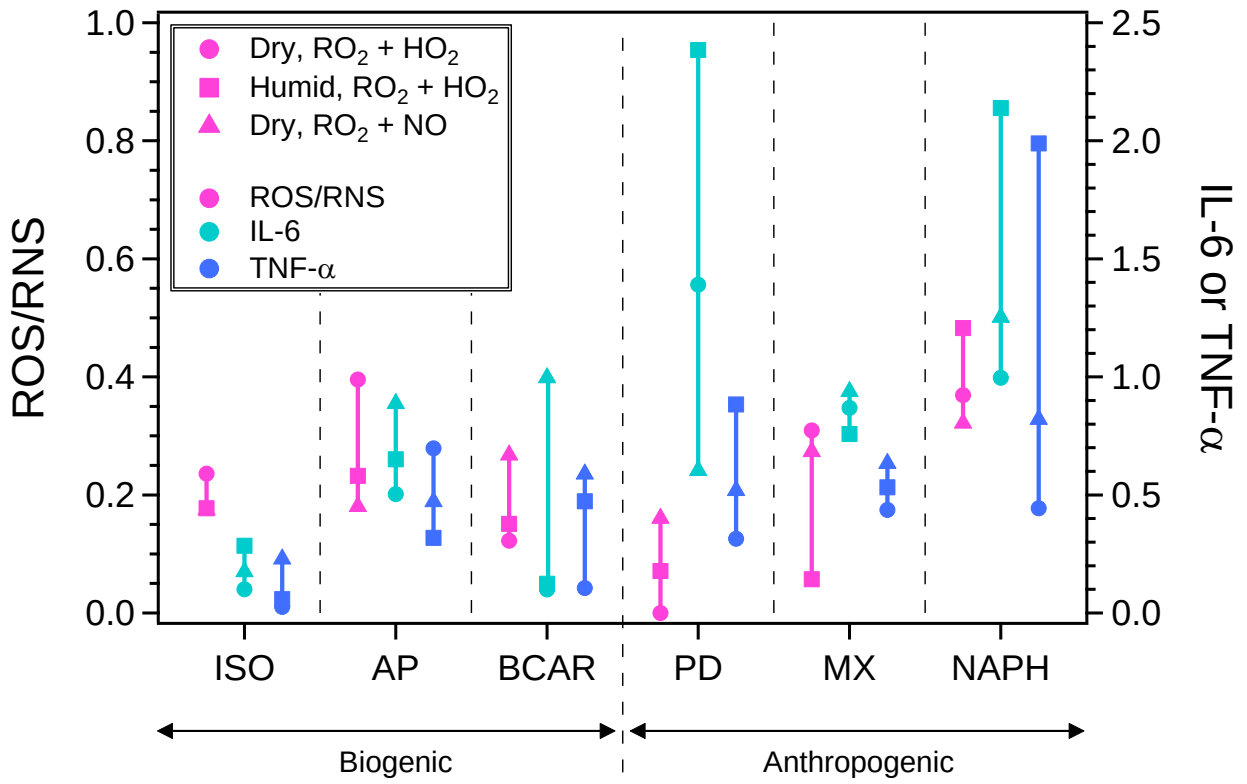
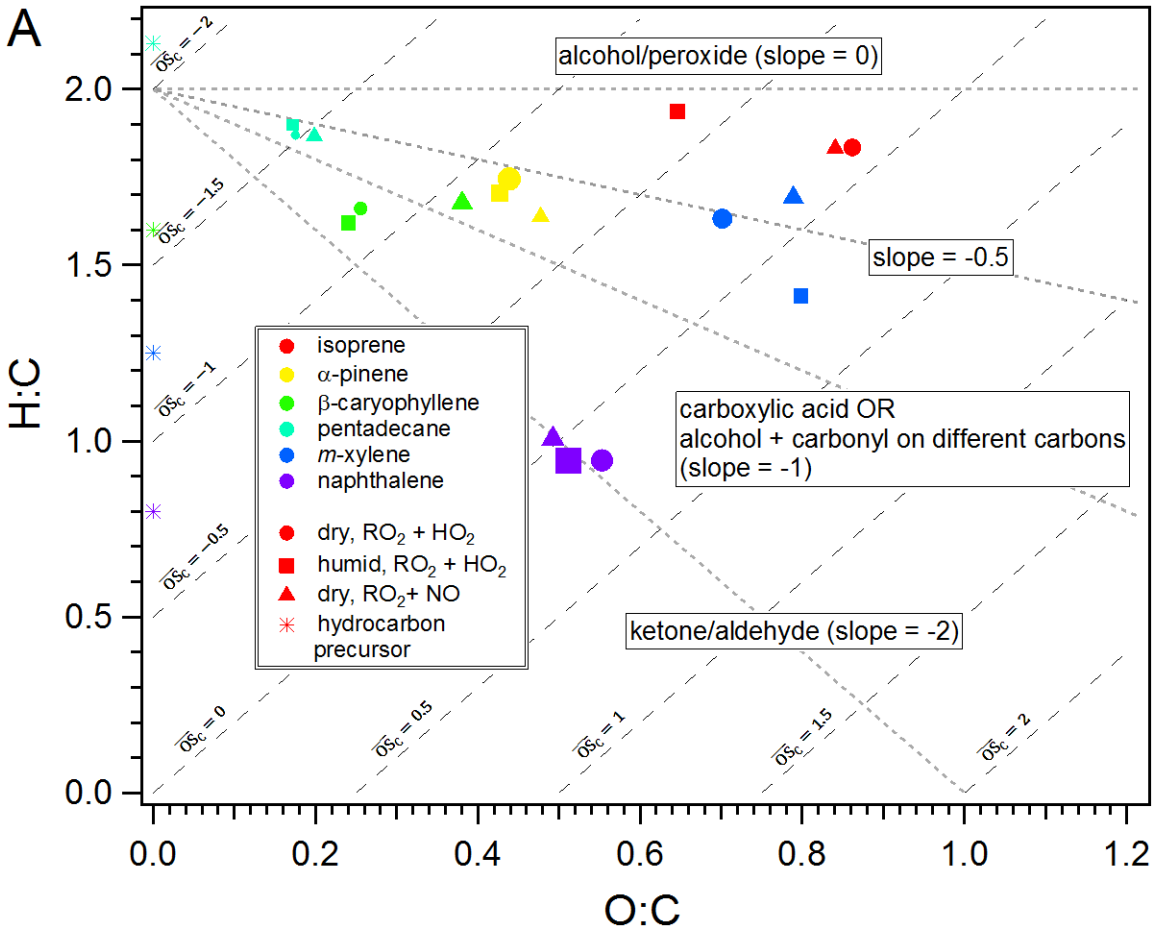


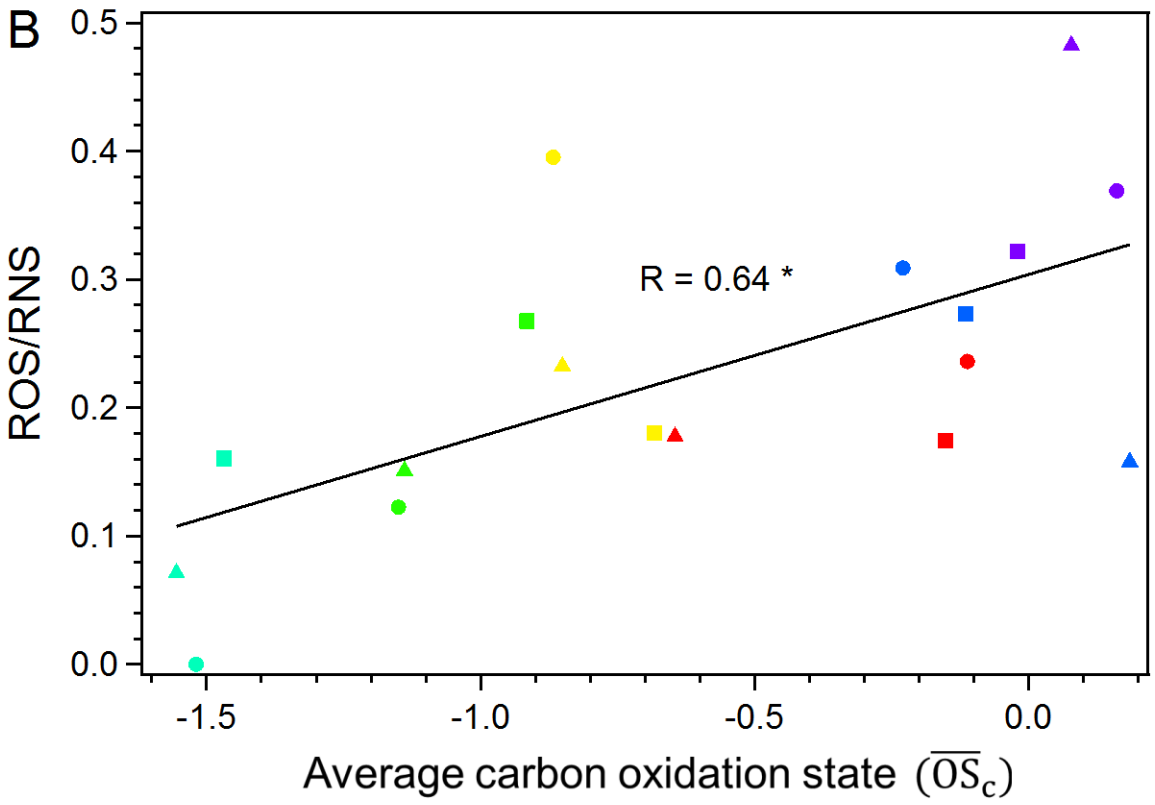
Figure 1. Representative dose-response curve of ROS/RNS produced as a result of filter exposure (naphthalane SOA formed under dry, RO_2 + NO dominant conditions). ROS/RNS is expressed as a fold increase over control cells, defined as probe-treated cells incubated with stimulant-free media. Dose is expressed 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.



578

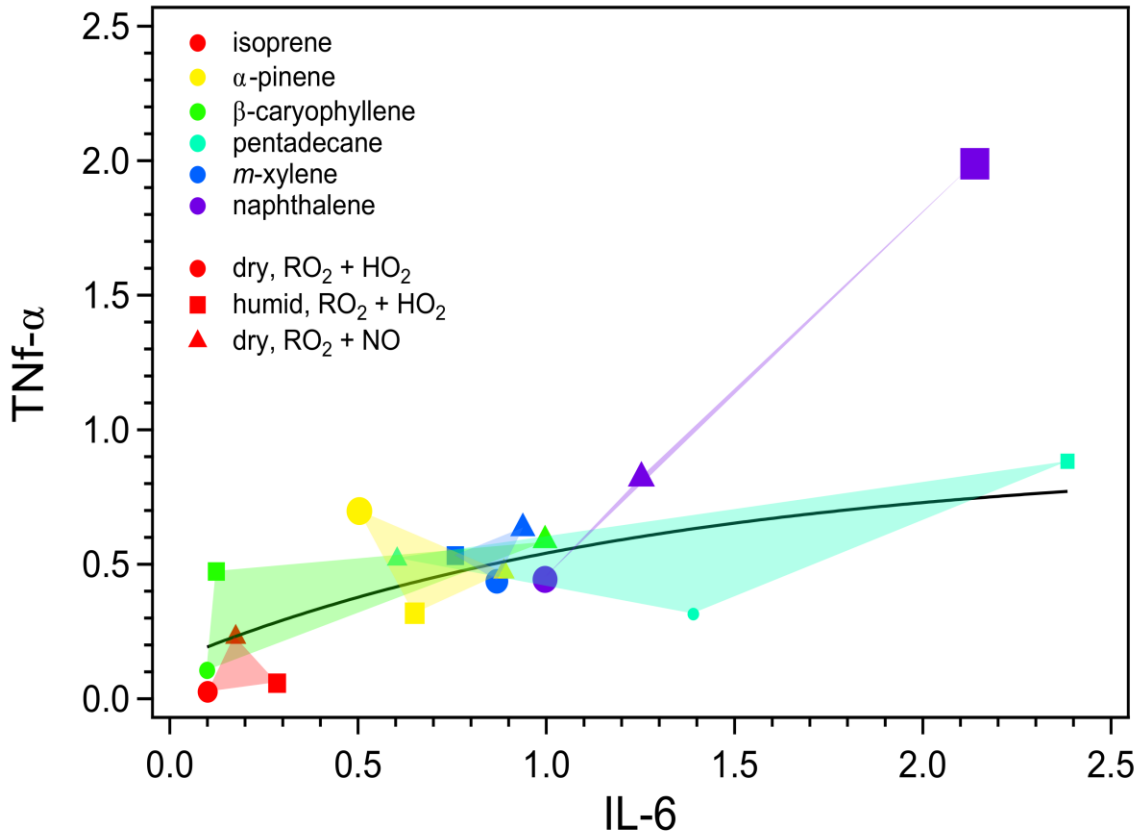
579 **Figure 2.** Area under the dose-response curve for various inflammatory responses induced as a
580 result of SOA exposure: **ROS/RNS**, **IL-6**, and **TNF-α**. SOA were generated from various
581 precursors (ISO: isoprene, AP: α-pinene, BCAR: β-caryophyllene, PD: pentadecane, MX: *m*-
582 xylene, and NAPH: naphthalene) under various conditions (circles: dry, RO_2 + HO_2; squares:
583 humid, RO_2 + HO_2; and triangles: dry, RO_2 + NO). Lines connecting the same inflammatory
584 response for SOA generated from the same precursor under different formation conditions are also
585 shown.


A
alcohol/peroxide (slope = 0)
slope = -0.5
carboxylic acid OR
alcohol + carbonyl on different carbons
(slope = -1)
ketone/aldehyde (slope = -2)
isoprene
α-pinene
β-caryophyllene
pentadecane
m-xylene
naphthalene
dry, RO2 + HO2
humid, RO2 + HO2
dry, RO2+ NO
hydrocarbon precursor
H:C
O:C


B
R = 0.64 *
ROS/RNS
Average carbon oxidation state

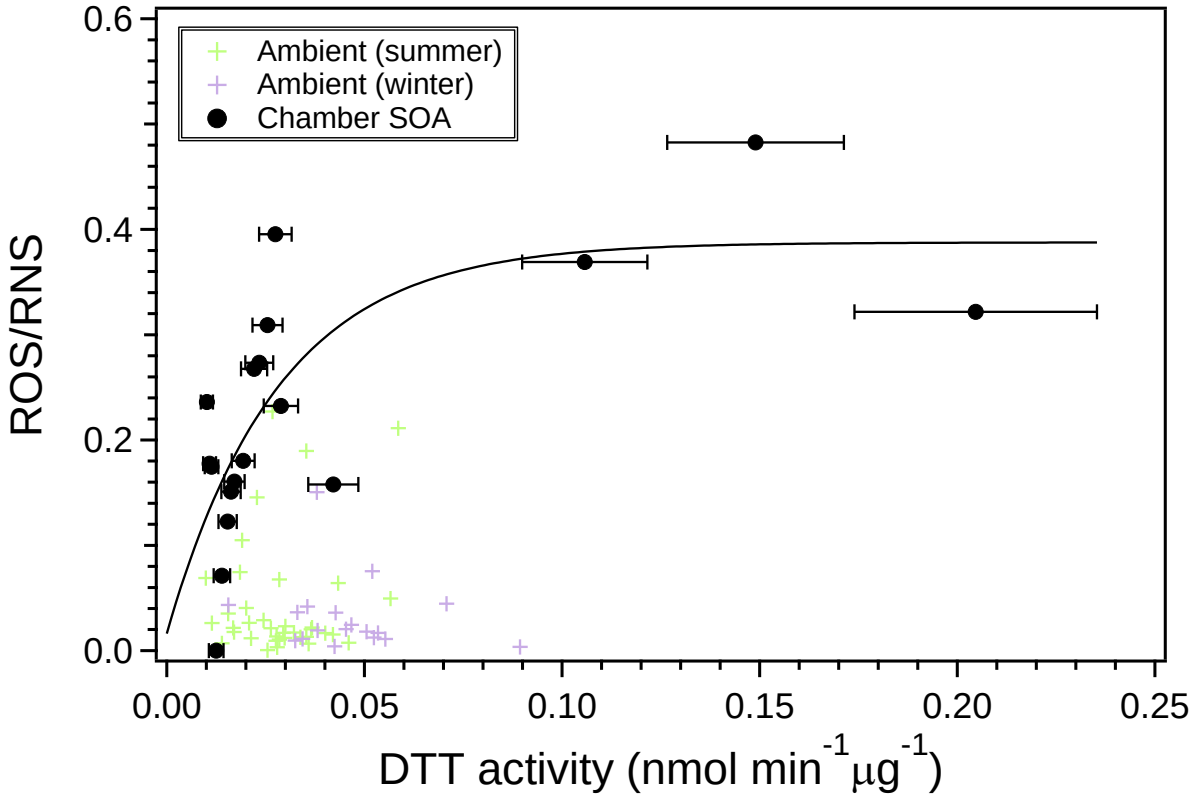
586

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, $RO_2 + HO_2$; square: humid, $RO_2 + HO_2$; and triangle: dry, $RO_2 + NO$). SOA precursors are shown as stars, colored by SOA system. * indicates significance, $p < 0.05$.



593

594 **Figure 4.** Area under the dose-response curve per mass of SOA for various inflammatory
595 responses induced as a result of SOA exposure. Data points are sized according to ROS/RNS level.
596 SOA were generated from various SOA precursors (red: isoprene, yellow: α-pinene, green: β-
597 caryophyllene, light blue: pentadecane, blue: *m*-xylene, and purple: naphthalene) under various
598 conditions (circles: dry, $RO_2 + HO_2$; squares: humid, $RO_2 + HO_2$; and triangles: dry, $RO_2 + NO$).
599 A fitted curve excluding naphthalene data is shown as a guide. Shaded regions for each system,
600 colored by SOA precursor, are also shown to show the extent of clustering and provide a
601 visualization for the different patterns observed.



602

603 **Figure 5.** ROS/RNS production and intrinsic DTT activities for chamber SOA and ambient
604 samples collected around the greater Atlanta area. All samples were analyzed using the method
605 outlined in Cho et al. (2005) and Tuet et al. (2016). Ambient samples are colored by season as
606 determined by solstice and equinox dates between June 2012 and October 2013 (Tuet et al.,
607 2016). A fitted curve for laboratory-generated samples is shown as a guide.

608 **Table 1.** Experimental conditions.

Experiment	SOA precursor	OH precursor	Relative humidity (%)	$[HC]_0$ (ppb)
1	isoprene	H_2O_2	<5%	97
2	α-pinene	H_2O_2	<5%	191
3	β-caryophyllene	H_2O_2	<5%	36
4	pentadecane	H_2O_2	<5%	106
5	*m*-xylene	H_2O_2	<5%	450
6	naphthalene	H_2O_2	<5%	178
7	isoprene	H_2O_2	<5%[a]	97
8	α-pinene	H_2O_2	40%	334
9	β-caryophyllene	H_2O_2	42%	63
10	pentadecane	H_2O_2	45%	106
11	*m*-xylene	H_2O_2	45%	450
12	naphthalene	H_2O_2	44%	431
13	isoprene	HONO	<5%	970
14	α-pinene	HONO	<5%	174
15	β-caryophyllene	HONO	<5%	21
16	pentadecane	HONO	<5%	74
17	*m*-xylene	HONO	<5%	431
18	naphthalene	HONO	<5%	145

609 [a] Acidic seed (8 mM $MgSO_4$ and 16 mM H_2SO_4) was used instead of 8 mM $(NH_4)_2SO_4$

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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

REFERENCES

Akhtar, U. S., McWhinney, R. D., Rastogi, N., Abbatt, J. P., Evans, G. J., and Scott, J. A.: Cytotoxic and proinflammatory effects of ambient and source-related particulate matter (PM) in relation to the production of reactive oxygen species (ROS) and cytokine adsorption by particles, Inhal. Toxicol., 22, 37-47, 2010.

Anderson, J. O., Thundiyil, J. G., and Stolbach, A.: Clearing the Air: A Review of the Effects of Particulate Matter Air Pollution on Human Health, Journal of Medical Toxicology, 8, 166-175, 10.1007/s13181-011-0203-1, 2011.

Arashiro, M., Lin, Y. H., Sexton, K. G., Zhang, Z., Jaspers, I., Fry, R. C., Vizuete, W. G., Gold, A., and Surratt, J. D.: In Vitro Exposure to Isoprene-Derived Secondary Organic Aerosol by Direct Deposition and its Effects on COX-2 and IL-8 Gene Expression, Atmos. Chem. Phys. Discuss., 2016, 1-29, 10.5194/acp-2016-371, 2016.

Bai, Y., Suzuki, A. K., and Sagai, M.: The cytotoxic effects of diesel exhaust particles on human pulmonary artery endothelial cells in vitro: role of active oxygen species, Free Radical Biology and Medicine, 30, 555-562, http://dx.doi.org/10.1016/S0891-5849(00)00499-8, 2001.

Baird, W. M., Hooven, L. A., and Mahadevan, B.: Carcinogenic polycyclic aromatic hydrocarbon-DNA adducts and mechanism of action, Environmental and Molecular Mutagenesis, 45, 106-114, 10.1002/em.20095, 2005.

Baltensperger, U., Dommen, J., Alfarra, R., Duplissy, J., Gaeggeler, K., Metzger, A., Facchini, M. C., Decesari, S., Finessi, E., Reinnig, C., Schott, M., Warnke, J., Hoffmann, T., Klatzer, B., Puxbaum, H., Geiser, M., Savi, M., Lang, D., Kalberer, M., and Geiser, T.: Combined determination of the chemical composition and of health effects of secondary organic aerosols: The POLYSOA project, J. Aerosol Med. Pulm. Drug Deliv., 21, 145-154, 10.1089/jamp.2007.0655, 2008.

Baritaki, S., Apostolakis, S., Kanellou, P., Dimanche-Boitrel, M. T., Spandidos, D. A., and Bonavida, B.: Reversal of Tumor Resistance to Apoptotic Stimuli by Alteration of Membrane

643 Fluidity: Therapeutic Implications, in: Advances in Cancer Research, Academic Press, 149-190,
644 2007.

645 Bates, J. T., Weber, R. J., Abrams, J., Verma, V., Fang, T., Klein, M., Strickland, M. J., Sarnat,
646 S. E., Chang, H. H., Mulholland, J. A., Tolbert, P. E., and Russell, A. G.: Reactive Oxygen
647 Species Generation Linked to Sources of Atmospheric Particulate Matter and Cardiorespiratory
648 Effects, Environmental Science & Technology, 49, 13605-13612, 10.1021/acs.est.5b02967,
649 2015.

650 Boyd, C. M., Sanchez, J., Xu, L., Eugene, A. J., Nah, T., Tuet, W. Y., Guzman, M. I., and Ng, N.
651 L.: Secondary organic aerosol formation from the β-pinene+NO₃ system: effect of
652 humidity and peroxy radical fate, Atmos. Chem. Phys., 15, 7497-7522, 10.5194/acp-15-7497-
653 2015, 2015.

654 Brunekreef, B., and Holgate, S. T.: Air pollution and health, Lancet, 360, 1233-1242, 2002.

655 Bruns, E. A., El Haddad, I., Slowik, J. G., Kilic, D., Klein, F., Baltensperger, U., and Prévôt, A.
656 S. H.: Identification of significant precursor gases of secondary organic aerosols from residential
657 wood combustion, Scientific Reports, 6, 27881, 10.1038/srep27881

658 http://www.nature.com/articles/srep27881#supplementary-information, 2016.

659 Burnett, R., Brook, J., Dann, T., Delocla, C., Philips, O., Cakmak, S., Vincent, R., Goldberg, M.,
660 and Krewski, D.: Association between particulate-and gas-phase components of urban air
661 pollution and daily mortality in eight Canadian cities, 2001.

662 Canagaratna, M. R., Jimenez, J. L., Kroll, J. H., Chen, Q., Kessler, S. H., Massoli, P.,
663 Hildebrandt Ruiz, L., Fortner, E., Williams, L. R., Wilson, K. R., Surratt, J. D., Donahue, N. M.,
664 Jayne, J. T., and Worsnop, D. R.: Elemental ratio measurements of organic compounds using
665 aerosol mass spectrometry: characterization, improved calibration, and implications, Atmos.
666 Chem. Phys., 15, 253-272, 10.5194/acp-15-253-2015, 2015.

667 Castro, L., and Freeman, B. A.: Reactive oxygen species in human health and disease, Nutrition,
668 17, 161-165, 2001.

669 Cerezo, J., Zúñiga, J., Bastida, A., Requena, A., and Cerón-Carrasco, J. P.: Atomistic Molecular
670 Dynamics Simulations of the Interactions of Oleic and 2-Hydroxyoleic Acids with
671 Phosphatidylcholine Bilayers, The Journal of Physical Chemistry B, 115, 11727-11738,
672 10.1021/jp203498x, 2011.

673 Chan, A. W. H., Kautzman, K. E., Chhabra, P. S., Surratt, J. D., Chan, M. N., Crounse, J. D.,
674 Kurten, A., Wennberg, P. O., Flagan, R. C., and Seinfeld, J. H.: Secondary organic aerosol
675 formation from photooxidation of naphthalene and alkylnaphthalenes: implications for oxidation
676 of intermediate volatility organic compounds (IVOCs), Atmos. Chem. Phys., 9, 3049-3060,
677 2009.

678 Chan, A. W. H., Chan, M. N., Surratt, J. D., Chhabra, P. S., Loza, C. L., Crounse, J. D., Yee, L.
679 D., Flagan, R. C., Wennberg, P. O., and Seinfeld, J. H.: Role of aldehyde chemistry and
680 NO_x concentrations in secondary organic aerosol formation, Atmos. Chem. Phys.,
681 10, 7169-7188, 10.5194/acp-10-7169-2010, 2010.

682 Chan, M. N., Surratt, J. D., Chan, A. W. H., Schilling, K., Offenberg, J. H., Lewandowski, M.,
683 Edney, E. O., Kleindienst, T. E., Jaoui, M., Edgerton, E. S., Tanner, R. L., Shaw, S. L., Zheng,

M., Knipping, E. M., and Seinfeld, J. H.: Influence of aerosol acidity on the chemical composition of secondary organic aerosol from β-caryophyllene, Atmos. Chem. Phys., 11, 1735-1751, 10.5194/acp-11-1735-2011, 2011.

Charrier, J. G., and Anastasio, C.: On dithiothreitol (DTT) as a measure of oxidative potential for ambient particles: evidence for the importance of soluble transition metals, Atmos. Chem. Phys., 12, 9321-9333, 10.5194/acp-12-9321-2012, 2012.

Chen, C. Y., Peng, W. H., Tsai, K. D., and Hsu, S. L.: Luteolin suppresses inflammation-associated gene expression by blocking NF-kappa B and AP-1 activation pathway in mouse alveolar macrophages, Life Sci., 81, 1602-1614, 10.1016/j.lfs.2007.09.028, 2007.

Chhabra, P. S., Flagan, R. C., and Seinfeld, J. H.: Elemental analysis of chamber organic aerosol using an aerodyne high-resolution aerosol mass spectrometer, Atmos. Chem. Phys., 10, 4111-4131, 10.5194/acp-10-4111-2010, 2010.

Chhabra, P. S., Ng, N. L., Canagaratna, M. R., Corrigan, A. L., Russell, L. M., Worsnop, D. R., Flagan, R. C., and Seinfeld, J. H.: Elemental composition and oxidation of chamber organic aerosol, Atmos. Chem. Phys., 11, 8827-8845, 10.5194/acp-11-8827-2011, 2011.

Cho, A. K., Sioutas, C., Miguel, A. H., Kumagai, Y., Schmitz, D. A., Singh, M., Eiguren-Fernandez, A., and Froines, J. R.: Redox activity of airborne particulate matter at different sites in the Los Angeles Basin, Environmental Research, 99, 40-47, 10.1016/j.envres.2005.01.003, 2005.

Cocker III, D. R., Mader, B. T., Kalberer, M., Flagan, R. C., and Seinfeld, J. H.: The effect of water on gas–particle partitioning of secondary organic aerosol: II. m-xylene and 1,3,5-trimethylbenzene photooxidation systems, Atmos. Environ., 35, 6073-6085, http://dx.doi.org/10.1016/S1352-2310(01)00405-8, 2001.

DeCarlo, P. F., Kimmel, J. R., Trimborn, A., Northway, M. J., Jayne, J. T., Aiken, A. C., Gonin, M., Fuhrer, K., Horvath, T., Docherty, K. S., Worsnop, D. R., and Jimenez, J. L.: Field-Deployable, High-Resolution, Time-of-Flight Aerosol Mass Spectrometer, Analytical Chemistry, 78, 8281-8289, 10.1021/ac061249n, 2006.

Dockery, D. W., Pope, C. A., Xu, X., Spengler, J. D., Ware, J. H., Fay, M. E., Ferris, B. G., and Speizer, F. E.: An Association between Air Pollution and Mortality in Six U.S. Cities, New England Journal of Medicine, 329, 1753-1759, doi:10.1056/NEJM199312093292401, 1993.

Eddingsaas, N. C., Loza, C. L., Yee, L. D., Chan, M., Schilling, K. A., Chhabra, P. S., Seinfeld, J. H., and Wennberg, P. O.: α-pinene photooxidation under controlled chemical conditions – Part 2: SOA yield and composition in low- and high-NOx environments, Atmos. Chem. Phys., 12, 7413-7427, 10.5194/acp-12-7413-2012, 2012.

Edney, E. O., Driscoll, D. J., Speer, R. E., Weathers, W. S., Kleindienst, T. E., Li, W., and Smith, D. F.: Impact of aerosol liquid water on secondary organic aerosol yields of irradiated toluene/propylene/NOx/(NH4)2SO4/air mixtures, Atmos. Environ., 34, 3907-3919, http://dx.doi.org/10.1016/S1352-2310(00)00174-6, 2000.

Fang, T., Guo, H., Verma, V., Peltier, R. E., and Weber, R. J.: PM2.5 water-soluble elements in the southeastern United States: automated analytical method development, spatiotemporal distributions, source apportionment, and implications for heath studies, Atmos. Chem. Phys., 15, 11667-11682, 10.5194/acp-15-11667-2015, 2015a.

Fang, T., Verma, V., Guo, H., King, L. E., Edgerton, E. S., and Weber, R. J.: 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), Atmos. Meas. Tech., 8, 471-482, 10.5194/amt-8-471-2015, 2015b.

Gallimore, P. J., Mahon, B. M., Wragg, F. P. H., Fuller, S. J., Giorio, C., Kourtchev, I., and Kalberer, M.: Multiphase composition changes and reactive oxygen species formation during limonene oxidation in the new Cambridge Atmospheric Simulation Chamber (CASC), Atmos. Chem. Phys. Discuss., 2017, 1-30, 10.5194/acp-2017-186, 2017.

Goldstein, A. H., and Galbally, I. E.: Known and Unexplored Organic Constituents in the Earth's Atmosphere, Environmental Science & Technology, 41, 1514-1521, 10.1021/es072476p, 2007.

Guenther, A., Karl, T., Harley, P., Wiedinmyer, C., Palmer, P. I., and Geron, C.: Estimates of global terrestrial isoprene emissions using MEGAN (Model of Emissions of Gases and Aerosols from Nature), Atmos. Chem. Phys., 6, 3181-3210, 10.5194/acp-6-3181-2006, 2006.

Guenther, A. B., Zimmerman, P. R., Harley, P. C., Monson, R. K., and Fall, R.: Isoprene and monoterpene emission rate variability: Model evaluations and sensitivity analyses, Journal of Geophysical Research: Atmospheres, 98, 12609-12617, 10.1029/93JD00527, 1993.

Gurgueira, S. A., Lawrence, J., Coull, B., Murthy, G. G. K., and Gonzalez-Flecha, B.: Rapid increases in the steady-state concentration of reactive oxygen species in the lungs and heart after particulate air pollution inhalation, Environmental Health Perspectives, 110, 749-755, 2002.

Haddad, J. J.: L-buthionine-(S,R)-sulfoximine, an irreversible inhibitor of gamma-glutamylcysteine synthetase, augments LPS-mediated pro-inflammatory cytokine biosynthesis: evidence for the implication of an I kappa B-alpha/NF-kappa B insensitive pathway, Eur. Cytokine Netw., 12, 614-624, 2001.

Hamad, S. H., Shafer, M. M., Kadhim, A. K. H., Al-Omran, S. M., and Schauer, J. J.: Seasonal trends in the composition and ROS activity of fine particulate matter in Baghdad, Iraq, Atmos. Environ., 100, 102-110, http://dx.doi.org/10.1016/j.atmosenv.2014.10.043, 2015.

Heald, C. L., Kroll, J. H., Jimenez, J. L., Docherty, K. S., DeCarlo, P. F., Aiken, A. C., Chen, Q., Martin, S. T., Farmer, D. K., and Artaxo, P.: A simplified description of the evolution of organic aerosol composition in the atmosphere, Geophysical Research Letters, 37, n/a-n/a, 10.1029/2010GL042737, 2010.

Healy, R. M., Temime, B., Kuprovskyte, K., and Wenger, J. C.: Effect of Relative Humidity on Gas/Particle Partitioning and Aerosol Mass Yield in the Photooxidation of p-Xylene, Environmental Science & Technology, 43, 1884-1889, 10.1021/es802404z, 2009.

Helmig, D., Arey, J., Harger, W. P., Atkinson, R., and Lopez-Cancio, J.: Formation of mutagenic nitrodibenzopyranones and their occurrence in ambient air, Environmental Science & Technology, 26, 622-624, 10.1021/es00027a028, 1992.

Henkler, F., Brinkmann, J., and Luch, A.: The Role of Oxidative Stress in Carcinogenesis Induced by Metals and Xenobiotics, Cancers, 2, 376, 2010.

Hensley, K., Robinson, K. A., Gabbita, S. P., Salsman, S., and Floyd, R. A.: Reactive oxygen species, cell signaling, and cell injury, Free Radical Biology and Medicine, 28, 1456-1462, http://dx.doi.org/10.1016/S0891-5849(00)00252-5, 2000.

767 Hoek, G., Krishnan, R. M., Beelen, R., Peters, A., Ostro, B., Brunekreef, B., and Kaufman, J. D.:
768 Long-term air pollution exposure and cardio-respiratory mortality: a review, Environ Health, 12,
769 43, 2013.

770 Hoffmann, T., Odum, J., Bowman, F., Collins, D., Klockow, D., Flagan, R., and Seinfeld, J.:
771 Formation of Organic Aerosols from the Oxidation of Biogenic Hydrocarbons, Journal of
772 Atmospheric Chemistry, 26, 189-222, 10.1023/A:1005734301837, 1997.

773 Huang, Y.-C. T., Ghio, A. J., Stonehuerner, J., McGee, J., Carter, J. D., Grambow, S. C., and
774 Devlin, R. B.: The role of soluble components in ambient fine particles-induced changes in
775 human lungs and blood, Inhal. Toxicol., 15, 327-342, 2003.

776 Jenkin, M. E., Saunders, S. M., Wagner, V., and Pilling, M. J.: Protocol for the development of
777 the Master Chemical Mechanism, MCM v3 (Part B): tropospheric degradation of aromatic
778 volatile organic compounds, Atmos. Chem. Phys., 3, 181-193, 10.5194/acp-3-181-2003, 2003.

779 Jia, C., and Batterman, S.: A Critical Review of Naphthalene Sources and Exposures Relevant to
780 Indoor and Outdoor Air, International Journal of Environmental Research and Public Health, 7,
781 2903-2939, 10.3390/ijerph7072903, 2010.

782 Jimenez, J. L., Canagaratna, M. R., Donahue, N. M., Prevot, A. S. H., Zhang, Q., Kroll, J. H.,
783 DeCarlo, P. F., Allan, J. D., Coe, H., Ng, N. L., Aiken, A. C., Docherty, K. S., Ulbrich, I. M.,
784 Grieshop, A. P., Robinson, A. L., Duplissy, J., Smith, J. D., Wilson, K. R., Lanz, V. A., Hueglin,
785 C., Sun, Y. L., Tian, J., Laaksonen, A., Raatikainen, T., Rautiainen, J., Vaattovaara, P., Ehn, M.,
786 Kulmala, M., Tomlinson, J. M., Collins, D. R., Cubison, M. J., Dunlea, J., Huffman, J. A.,
787 Onasch, T. B., Alfarra, M. R., Williams, P. I., Bower, K., Kondo, Y., Schneider, J., Drewnick, F.,
788 Borrmann, S., Weimer, S., Demerjian, K., Salcedo, D., Cottrell, L., Griffin, R., Takami, A.,
789 Miyoshi, T., Hatakeyama, S., Shimono, A., Sun, J. Y., Zhang, Y. M., Dzepina, K., Kimmel, J.
790 R., Sueper, D., Jayne, J. T., Herndon, S. C., Trimborn, A. M., Williams, L. R., Wood, E. C.,
791 Middlebrook, A. M., Kolb, C. E., Baltensperger, U., and Worsnop, D. R.: Evolution of Organic
792 Aerosols in the Atmosphere, Science, 326, 1525-1529, 10.1126/science.1180353, 2009.

793 Kamimura, D., Ishihara, K., and Hirano, T.: IL-6 signal transduction and its physiological roles:
794 the signal orchestration model, in: Reviews of Physiology, Biochemistry and Pharmacology,
795 Springer Berlin Heidelberg, Berlin, Heidelberg, 1-38, 2004.

796 Kautzman, K. E., Surratt, J. D., Chan, M. N., Chan, A. W. H., Hersey, S. P., Chhabra, P. S.,
797 Dalleska, N. F., Wennberg, P. O., Flagan, R. C., and Seinfeld, J. H.: Chemical Composition of
798 Gas- and Aerosol-Phase Products from the Photooxidation of Naphthalene, The Journal of
799 Physical Chemistry A, 114, 913-934, 10.1021/jp908530s, 2010.

800 Khmelinskaia, A., Ibarguren, M., de Almeida, R. F. M., López, D. J., Paixão, V. A., Ahyayauch,
801 H., Goñi, F. M., and Escribá, P. V.: Changes in Membrane Organization upon Spontaneous
802 Insertion of 2-Hydroxylated Unsaturated Fatty Acids in the Lipid Bilayer, Langmuir, 30, 2117-
803 2128, 10.1021/la403977f, 2014.

804 Kishimoto, T.: Interleukin-6, The cytokine handbook, 4, 281-304, 2003.

805 Kleinman, M. T., Hamade, A., Meacher, D., Oldham, M., Sioutas, C., Chakrabarti, B., Stram, D.,
806 Froines, J. R., and Cho, A. K.: Inhalation of concentrated ambient particulate matter near a
807 heavily trafficked road stimulates antigen-induced airway responses in mice, Journal of the Air
808 & Waste Management Association, 55, 1277-1288, 2005.

809 Kramer, A. J., Rattanavaraha, W., Zhang, Z., Gold, A., Surratt, J. D., and Lin, Y.-H.: Assessing
810 the oxidative potential of isoprene-derived epoxides and secondary organic aerosol, Atmos.
811 Environ., http://dx.doi.org/10.1016/j.atmosenv.2015.10.018, 2016.

812 Kroll, J. H., Ng, N. L., Murphy, S. M., Flagan, R. C., and Seinfeld, J. H.: Secondary organic
813 aerosol formation from isoprene photooxidation under high-NOx conditions, Geophysical
814 Research Letters, 32, n/a-n/a, 10.1029/2005GL023637, 2005.

815 Kroll, J. H., Donahue, N. M., Jimenez, J. L., Kessler, S. H., Canagaratna, M. R., Wilson, K. R.,
816 Altieri, K. E., Mazzoleni, L. R., Wozniak, A. S., Bluhm, H., Mysak, E. R., Smith, J. D., Kolb, C.
817 E., and Worsnop, D. R.: Carbon oxidation state as a metric for describing the chemistry of
818 atmospheric organic aerosol, Nat Chem, 3, 133-139,
819 http://www.nature.com/nchem/journal/v3/n2/abs/nchem.948.html#supplementary-information,
820 2011.

821 Kumagai, Y., Koide, S., Taguchi, K., Endo, A., Nakai, Y., Yoshikawa, T., and Shimojo, N.:
822 Oxidation of Proximal Protein Sulfhydryls by Phenanthraquinone, a Component of Diesel
823 Exhaust Particles, Chemical Research in Toxicology, 15, 483-489, 10.1021/tx0100993, 2002.

824 Lambe, A. T., Onasch, T. B., Massoli, P., Croasdale, D. R., Wright, J. P., Ahern, A. T.,
825 Williams, L. R., Worsnop, D. R., Brune, W. H., and Davidovits, P.: Laboratory studies of the
826 chemical composition and cloud condensation nuclei (CCN) activity of secondary organic
827 aerosol (SOA) and oxidized primary organic aerosol (OPOA), Atmos. Chem. Phys., 11, 8913-
828 8928, 10.5194/acp-11-8913-2011, 2011.

829 Landreman, A. P., Shafer, M. M., Hemming, J. C., Hannigan, M. P., and Schauer, J. J.: A
830 macrophage-based method for the assessment of the reactive oxygen species (ROS) activity of
831 atmospheric particulate matter (PM) and application to routine (daily-24 h) aerosol monitoring
832 studies, Aerosol Sci. Technol., 42, 946-957, 10.1080/02786820802363819, 2008.

833 Li, N., Hao, M. Q., Phalen, R. F., Hinds, W. C., and Nel, A. E.: Particulate air pollutants and
834 asthma - A paradigm for the role of oxidative stress in PM-induced adverse health effects,
835 Clinical Immunology, 109, 250-265, 10.1016/j.clim.2003.08.006, 2003a.

836 Li, N., Sioutas, C., Cho, A., Schmitz, D., Misra, C., Sempf, J., Wang, M. Y., Oberley, T.,
837 Froines, J., and Nel, A.: Ultrafine particulate pollutants induce oxidative stress and mitochondrial
838 damage, Environmental Health Perspectives, 111, 455-460, 10.1289/ehp.6000, 2003b.

839 Li, N., Xia, T., and Nel, A. E.: The role of oxidative stress in ambient particulate matter-induced
840 lung diseases and its implications in the toxicity of engineered nanoparticles, Free Radical
841 Biology and Medicine, 44, 1689-1699, 10.1016/j.freeradbiomed.2008.01.028, 2008.

842 Lim, S. S., Vos, T., Flaxman, A. D., Danaei, G., Shibuya, K., Adair-Rohani, H., AlMazroa, M.
843 A., Amann, M., Anderson, H. R., Andrews, K. G., Aryee, M., Atkinson, C., Bacchus, L. J.,
844 Bahalim, A. N., Balakrishnan, K., Balmes, J., Barker-Collo, S., Baxter, A., Bell, M. L., Blore, J.
845 D., Blyth, F., Bonner, C., Borges, G., Bourne, R., Boussinesq, M., Brauer, M., Brooks, P., Bruce,
846 N. G., Brunekreef, B., Bryan-Hancock, C., Bucello, C., Buchbinder, R., Bull, F., Burnett, R. T.,
847 Byers, T. E., Calabria, B., Carapetis, J., Carnahan, E., Chafe, Z., Charlson, F., Chen, H., Chen, J.
848 S., Cheng, A. T.-A., Child, J. C., Cohen, A., Colson, K. E., Cowie, B. C., Darby, S., Darling, S.,
849 Davis, A., Degenhardt, L., Dentener, F., Des Jarlais, D. C., Devries, K., Dherani, M., Ding, E. L.,
850 Dorsey, E. R., Driscoll, T., Edmond, K., Ali, S. E., Engell, R. E., Erwin, P. J., Fahimi, S., Falder,
851 G., Farzadfar, F., Ferrari, A., Finucane, M. M., Flaxman, S., Fowkes, F. G. R., Freedman, G.,

Freeman, M. K., Gakidou, E., Ghosh, S., Giovannucci, E., Gmel, G., Graham, K., Grainger, R., Grant, B., Gunnell, D., Gutierrez, H. R., Hall, W., Hoek, H. W., Hogan, A., Hosgood, H. D., III, Hoy, D., Hu, H., Hubbell, B. J., Hutchings, S. J., Ibeanusi, S. E., Jacklyn, G. L., Jasrasaria, R., Jonas, J. B., Kan, H., Kanis, J. A., Kassebaum, N., Kawakami, N., Khang, Y.-H., Khatibzadeh, S., Khoo, J.-P., Kok, C., Laden, F., Lalloo, R., Lan, Q., Lathlean, T., Leasher, J. L., Leigh, J., Li, Y., Lin, J. K., Lipshultz, S. E., London, S., Lozano, R., Lu, Y., Mak, J., Malekzadeh, R., Mallinger, L., Marcenes, W., March, L., Marks, R., Martin, R., McGale, P., McGrath, J., Mehta, S., Memish, Z. A., Mensah, G. A., Merriman, T. R., Micha, R., Michaud, C., Mishra, V., Hanafiah, K. M., Mokdad, A. A., Morawska, L., Mozaffarian, D., Murphy, T., Naghavi, M., Neal, B., Nelson, P. K., Nolla, J. M., Norman, R., Olives, C., Omer, S. B., Orchard, J., Osborne, R., Ostro, B., Page, A., Pandey, K. D., Parry, C. D. H., Passmore, E., Patra, J., Pearce, N., Pelizzari, P. M., Petzold, M., Phillips, M. R., Pope, D., Pope, C. A., III, Powles, J., Rao, M., Razavi, H., Rehfuess, E. A., Rehm, J. T., Ritz, B., Rivara, F. P., Roberts, T., Robinson, C., Rodriguez-Portales, J. A., Romieu, I., Room, R., Rosenfeld, L. C., Roy, A., Rushton, L., Salomon, J. A., Sampson, U., Sanchez-Riera, L., Sanman, E., Sapkota, A., Seedat, S., Shi, P., Shield, K., Shivakoti, R., Singh, G. M., Sleet, D. A., Smith, E., Smith, K. R., Stapelberg, N. J. C., Steenland, K., Stöckl, H., Stovner, L. J., Straif, K., Straney, L., Thurston, G. D., Tran, J. H., Van Dingenen, R., van Donkelaar, A., Veerman, J. L., Vijayakumar, L., Weintraub, R., Weissman, M. M., White, R. A., Whiteford, H., Wiersma, S. T., Wilkinson, J. D., Williams, H. C., Williams, W., Wilson, N., Woolf, A. D., Yip, P., Zielinski, J. M., Lopez, A. D., Murray, C. J. L., and Ezzati, M.: A comparative risk assessment of burden of disease and injury attributable to 67 risk factors and risk factor clusters in 21 regions, 1990–2010: a systematic analysis for the Global Burden of Disease Study 2010, The Lancet, 380, 2224-2260, 10.1016/S0140-6736(12)61766-8, 2012.

Lin, P., and Yu, J. Z.: Generation of Reactive Oxygen Species Mediated by Humic-like Substances in Atmospheric Aerosols, Environmental Science & Technology, 45, 10362-10368, 10.1021/es2028229, 2011.

Lin, Y.-H., Zhang, Z., Docherty, K. S., Zhang, H., Budisulistiorini, S. H., Rubitschun, C. L., Shaw, S. L., Knipping, E. M., Edgerton, E. S., Kleindienst, T. E., Gold, A., and Surratt, J. D.: Isoprene Epoxydiols as Precursors to Secondary Organic Aerosol Formation: Acid-Catalyzed Reactive Uptake Studies with Authentic Compounds, Environmental science & technology, 46, 250-258, 10.1021/es202554c, 2012.

Lin, Y.-H., Arashiro, M., Martin, E., Chen, Y., Zhang, Z., Sexton, K. G., Gold, A., Jaspers, I., Fry, R. C., and Surratt, J. D.: Isoprene-Derived Secondary Organic Aerosol Induces the Expression of Oxidative Stress Response Genes in Human Lung Cells, Environmental Science & Technology Letters, 3, 250-254, 10.1021/acs.estlett.6b00151, 2016.

Lin, Y.-H., Arashiro, M., Clapp, P. W., Cui, T., Sexton, K. G., Vizuete, W., Gold, A., Jaspers, I., Fry, R. C., and Surratt, J. D.: Gene Expression Profiling in Human Lung Cells Exposed to Isoprene-Derived Secondary Organic Aerosol, Environmental Science & Technology, 10.1021/acs.est.7b01967, 2017.

Lorentzen, R. J., Lesko, S. A., McDonald, K., and Ts'o, P. O. P.: Toxicity of Metabolic Benzo(a)pyrenediones to Cultured Cells and the Dependence upon Molecular Oxygen , Cancer Research, 39, 3194-3198, 1979.

Loza, C. L., Craven, J. S., Yee, L. D., Coggon, M. M., Schwantes, R. H., Shiraiwa, M., Zhang, X., Schilling, K. A., Ng, N. L., Canagaratna, M. R., Ziemann, P. J., Flagan, R. C., and Seinfeld, J. H.: Secondary organic aerosol yields of 12-carbon alkanes, Atmos. Chem. Phys., 14, 1423-1439, 10.5194/acp-14-1423-2014, 2014.

Lund, A. K., Doyle-Eisele, M., Lin, Y. H., Arashiro, M., Surratt, J. D., Holmes, T., Schilling, K. A., Seinfeld, J. H., Rohr, A. C., Knipping, E. M., and McDonald, J. D.: The effects of alpha-pinene versus toluene-derived secondary organic aerosol exposure on the expression of markers associated with vascular disease, Inhal. Toxicol., 25, 309-324, 10.3109/08958378.2013.782080, 2013.

Matsunaga, K., Klein, T. W., Friedman, H., and Yamamoto, Y.: Involvement of Nicotinic Acetylcholine Receptors in Suppression of Antimicrobial Activity and Cytokine Responses of Alveolar Macrophages to Legionella pneumophila Infection by Nicotine, The Journal of Immunology, 167, 6518-6524, 10.4049/jimmunol.167.11.6518, 2001.

Mbawuike, I. N., and Herscowitz, H. B.: MH-S, a murine alveolar macrophage cell line: morphological, cytochemical, and functional characteristics, Journal of Leukocyte Biology, 46, 119-127, 1989.

McDonald, J. D., Doyle-Eisele, M., Campen, M. J., Seagrave, J., Holmes, T., Lund, A., Surratt, J. D., Seinfeld, J. H., Rohr, A. C., and Knipping, E. M.: Cardiopulmonary response to inhalation of biogenic secondary organic aerosol, Inhal. Toxicol., 22, 253-265, 10.3109/08958370903148114, 2010.

McDonald, J. D., Doyle-Eisele, M., Kracko, D., Lund, A., Surratt, J. D., Hersey, S. P., Seinfeld, J. H., Rohr, A. C., and Knipping, E. M.: Cardiopulmonary response to inhalation of secondary organic aerosol derived from gas-phase oxidation of toluene, Inhal. Toxicol., 24, 689-697, 10.3109/08958378.2012.712164, 2012.

McWhinney, R. D., Badali, K., Liggio, J., Li, S.-M., and Abbatt, J. P. D.: Filterable Redox Cycling Activity: A Comparison between Diesel Exhaust Particles and Secondary Organic Aerosol Constituents, Environmental Science & Technology, 47, 3362-3369, 10.1021/es304676x, 2013a.

McWhinney, R. D., Zhou, S., and Abbatt, J. P. D.: Naphthalene SOA: redox activity and naphthoquinone gas-particle partitioning, Atmos. Chem. Phys., 13, 9731-9744, 10.5194/acp-13-9731-2013, 2013b.

Mohr, C., Huffman, J. A., Cubison, M. J., Aiken, A. C., Docherty, K. S., Kimmel, J. R., Ulbrich, I. M., Hannigan, M., and Jimenez, J. L.: Characterization of Primary Organic Aerosol Emissions from Meat Cooking, Trash Burning, and Motor Vehicles with High-Resolution Aerosol Mass Spectrometry and Comparison with Ambient and Chamber Observations, Environmental Science & Technology, 43, 2443-2449, 10.1021/es8011518, 2009.

Ng, N. L., Chhabra, P. S., Chan, A. W. H., Surratt, J. D., Kroll, J. H., Kwan, A. J., McCabe, D. C., Wennberg, P. O., Sorooshian, A., Murphy, S. M., Dalleska, N. F., Flagan, R. C., and Seinfeld, J. H.: Effect of NOx level on secondary organic aerosol (SOA) formation from the photooxidation of terpenes, Atmos. Chem. Phys., 7, 5159-5174, 10.5194/acp-7-5159-2007, 2007a.

Ng, N. L., Kroll, J. H., Chan, A. W. H., Chhabra, P. S., Flagan, R. C., and Seinfeld, J. H.: Secondary organic aerosol formation from m-xylene, toluene, and benzene, Atmos. Chem. Phys., 7, 3909-3922, 10.5194/acp-7-3909-2007, 2007b.

Ng, N. L., Canagaratna, M. R., Zhang, Q., Jimenez, J. L., Tian, J., Ulbrich, I. M., Kroll, J. H., Docherty, K. S., Chhabra, P. S., Bahreini, R., Murphy, S. M., Seinfeld, J. H., Hildebrandt, L., Donahue, N. M., DeCarlo, P. F., Lanz, V. A., Prévôt, A. S. H., Dinar, E., Rudich, Y., and Worsnop, D. R.: Organic aerosol components observed in Northern Hemispheric datasets from Aerosol Mass Spectrometry, Atmos. Chem. Phys., 10, 4625-4641, 10.5194/acp-10-4625-2010, 2010.

Ng, N. L., Canagaratna, M. R., Jimenez, J. L., Chhabra, P. S., Seinfeld, J. H., and Worsnop, D. R.: Changes in organic aerosol composition with aging inferred from aerosol mass spectra, Atmos. Chem. Phys., 11, 6465-6474, 10.5194/acp-11-6465-2011, 2011.

Nguyen, T. B., Roach, P. J., Laskin, J., Laskin, A., and Nizkorodov, S. A.: Effect of humidity on the composition of isoprene photooxidation secondary organic aerosol, Atmos. Chem. Phys., 11, 6931-6944, 10.5194/acp-11-6931-2011, 2011.

Oberdörster, G., Ferin, J., Gelein, R., Soderholm, S. C., and Finkelstein, J.: Role of the alveolar macrophage in lung injury: studies with ultrafine particles, Environmental Health Perspectives, 97, 193-199, 1992.

Oberdörster, G.: Lung Dosimetry: Pulmonary Clearance of Inhaled Particles, Aerosol Sci. Technol., 18, 279-289, 10.1080/02786829308959605, 1993.

Pan, Q., and Shimizu, I.: Imputation Variance Estimation by Multiple Imputation Method for the National Hospital Discharge Survey, 2009.

Pardo, M., Shafer, M. M., Rudich, A., Schauer, J. J., and Rudich, Y.: Single Exposure to near Roadway Particulate Matter Leads to Confined Inflammatory and Defense Responses: Possible Role of Metals, Environmental Science & Technology, 49, 8777-8785, 10.1021/acs.est.5b01449, 2015.

Philip, M., Rowley, D. A., and Schreiber, H.: Inflammation as a tumor promoter in cancer induction, Seminars in Cancer Biology, 14, 433-439, http://dx.doi.org/10.1016/j.semcancer.2004.06.006, 2004.

Piccot, S. D., Watson, J. J., and Jones, J. W.: A global inventory of volatile organic compound emissions from anthropogenic sources, Journal of Geophysical Research: Atmospheres, 97, 9897-9912, 10.1029/92JD00682, 1992.

Platt, S. M., Haddad, I. E., Pieber, S. M., Huang, R. J., Zardini, A. A., Clairotte, M., Suarez-Bertoa, R., Barmet, P., Pfaffenberger, L., Wolf, R., Slowik, J. G., Fuller, S. J., Kalberer, M., Chirico, R., Dommen, J., Astorga, C., Zimmermann, R., Marchand, N., Hellebust, S., Temime-Roussel, B., Baltensperger, U., and Prévôt, A. S. H.: Two-stroke scooters are a dominant source of air pollution in many cities, Nature Communications, 5, 3749, 10.1038/ncomms4749

http://www.nature.com/articles/ncomms4749#supplementary-information, 2014.

Pope, C. A., Burnett, R. T., Thun, M. J., Calle, E. E., Krewski, D., Ito, K., and Thurston, G. D.: Lung cancer, cardiopulmonary mortality, and long-term exposure to fine particulate air pollution,

977 Jama-Journal of the American Medical Association, 287, 1132-1141, 10.1001/jama.287.9.1132,
978 2002.

979 Pope III, C. A., and Dockery, D. W.: Health effects of fine particulate air pollution: Lines that
980 connect, Journal of the Air and Waste Management Association, 56, 709-742, 2006.

981 Rattanavaraha, W., Rosen, E., Zhang, H., Li, Q., Pantong, K., and Kamens, R. M.: The reactive
982 oxidant potential of different types of aged atmospheric particles: An outdoor chamber study,
983 Atmos. Environ., 45, 3848-3855, http://dx.doi.org/10.1016/j.atmosenv.2011.04.002, 2011.

984 Robinson, A. L., Donahue, N. M., Shrivastava, M. K., Weitkamp, E. A., Sage, A. M., Grieshop,
985 A. P., Lane, T. E., Pierce, J. R., and Pandis, S. N.: Rethinking Organic Aerosols: Semivolatile
986 Emissions and Photochemical Aging, Science, 315, 1259-1262, 10.1126/science.1133061, 2007.

987 Rogge, W. F., Hildemann, L. M., Mazurek, M. A., Cass, G. R., and Simoneit, B. R. T.: Sources
988 of fine organic aerosol. 1. Charbroilers and meat cooking operations, Environmental Science &
989 Technology, 25, 1112-1125, 10.1021/es00018a015, 1991.

990 Saffari, A., Hasheminassab, S., Wang, D., Shafer, M. M., Schauer, J. J., and Sioutas, C.: Impact
991 of primary and secondary organic sources on the oxidative potential of quasi-ultrafine particles
992 (PM0.25) at three contrasting locations in the Los Angeles Basin, Atmos. Environ., 120, 286-
993 296, http://dx.doi.org/10.1016/j.atmosenv.2015.09.022, 2015.

994 Sankaran, K., and Herscowitz, H. B.: Phenotypic and functional heterogeneity of the murine
995 alveolar macrophage-derived cell line MH-S, Journal of Leukocyte Biology, 57, 562-568, 1995.

996 Spector, A. A., and Yorek, M. A.: Membrane lipid composition and cellular function, Journal of
997 Lipid Research, 26, 1015-1035, 1985.

998 Stirnweis, L., Marcolli, C., Dommen, J., Barmet, P., Frege, C., Platt, S. M., Bruns, E. A., Krapf,
999 M., Slowik, J. G., Wolf, R., Prévôt, A. S. H., El-Haddad, I., and Baltensperger, U.: α-Pinene
1000 secondary organic aerosol yields increase at higher relative humidity and low NOx conditions,
1001 Atmos. Chem. Phys. Discuss., 2016, 1-41, 10.5194/acp-2016-717, 2016.

1002 Surratt, J. D., Chan, A. W. H., Eddingsaas, N. C., Chan, M., Loza, C. L., Kwan, A. J., Hersey, S.
1003 P., Flagan, R. C., Wennberg, P. O., and Seinfeld, J. H.: Reactive intermediates revealed in
1004 secondary organic aerosol formation from isoprene, Proceedings of the National Academy of
1005 Sciences, 107, 6640-6645, 10.1073/pnas.0911114107, 2010.

1006 Tao, F., Gonzalez-Flecha, B., and Kobzik, L.: Reactive oxygen species in pulmonary
1007 inflammation by ambient particulates, Free Radical Biology and Medicine, 35, 327-340,
1008 http://dx.doi.org/10.1016/S0891-5849(03)00280-6, 2003.

1009 Tasoglou, A., and Pandis, S. N.: Formation and chemical aging of secondary organic aerosol
1010 during the β-caryophyllene oxidation, Atmos. Chem. Phys., 15, 6035-6046, 10.5194/acp-15-
1011 6035-2015, 2015.

1012 Terada, H.: Uncouplers of oxidative phosphorylation, Environmental Health Perspectives, 87,
1013 213-218, 1990.

1014 Tuet, W. Y., Fok, S., Verma, V., Tagle Rodriguez, M. S., Grosberg, A., Champion, J. A., and
1015 Ng, N. L.: Dose-dependent intracellular reactive oxygen and nitrogen species (ROS/RNS)
1016 production from particulate matter exposure: comparison to oxidative potential and chemical

composition, Atmos. Environ., 144, 335-344, http://dx.doi.org/10.1016/j.atmosenv.2016.09.005, 2016.

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, 10.5194/acp-17-839-2017, 2017.

Turner, J., Hernandez, M., Snawder, J. E., Handorean, A., and McCabe, K. M.: A Toxicology Suite Adapted for Comparing Parallel Toxicity Responses of Model Human Lung Cells to Diesel Exhaust Particles and Their Extracts, Aerosol Sci. Technol., 49, 599-610, 10.1080/02786826.2015.1053559, 2015.

Van Krevelen, D.: Graphical-statistical method for the study of structure and reaction processes of coal, Fuel, 29, 269-284, 1950.

Verma, V., Shafer, M. M., Schauer, J. J., and Sioutas, C.: Contribution of transition metals in the reactive oxygen species activity of PM emissions from retrofitted heavy-duty vehicles, Atmos. Environ., 44, 5165-5173, 10.1016/j.atmosenv.2010.08.052, 2010.

Verma, V., Rico-Martinez, R., Kotra, N., King, L., Liu, J. M., Snell, T. W., and Weber, R. J.: Contribution of Water-Soluble and Insoluble Components and Their Hydrophobic/Hydrophilic Subfractions to the Reactive Oxygen Species-Generating Potential of Fine Ambient Aerosols, Environmental Science & Technology, 46, 11384-11392, 10.1021/es302484r, 2012.

Verma, V., Fang, T., Xu, L., Peltier, R. E., Russell, A. G., Ng, N. L., and Weber, R. J.: Organic Aerosols Associated with the Generation of Reactive Oxygen Species (ROS) by Water-Soluble PM2.5, Environmental Science & Technology, 49, 4646-4656, 10.1021/es505577w, 2015a.

Verma, V., Wang, Y., El-Afifi, R., Fang, T., Rowland, J., Russell, A. G., and Weber, R. J.: Fractionating ambient humic-like substances (HULIS) for their reactive oxygen species activity – Assessing the importance of quinones and atmospheric aging, Atmos. Environ., 120, 351-359, http://dx.doi.org/10.1016/j.atmosenv.2015.09.010, 2015b.

Vivanco, M. G., and Santiago, M.: Secondary Organic Aerosol Formation from the Oxidation of a Mixture of Organic Gases in a Chamber, Air Quality, Ashok Kumar (Ed.), InTech, DOI: 10.5772/9761. Available from: http://www.intechopen.com/books/air-quality/secondary-organic-aerosols-experiments-in-an-outdoor-chamber-, 2010.

Wang, H., Czura, C., and Tracey, K.: Tumor necrosis factor, The cytokine handbook, 4, 837-860, 2003.

Wen, Y., Gu, J., Chakrabarti, S. K., Aylor, K., Marshall, J., Takahashi, Y., Yoshimoto, T., and Nadler, J. L.: The Role of 12/15-Lipoxygenase in the Expression of Interleukin-6 and Tumor Necrosis Factor-α in Macrophages, Endocrinology, 148, 1313-1322, 10.1210/en.2006-0665, 2007.

Wiseman, H., and Halliwell, B.: Damage to DNA by reactive oxygen and nitrogen species: role in inflammatory disease and progression to cancer, Biochem. J., 313, 17-29, 1996.

Witkamp, R., and Monshouwer, M.: Signal transduction in inflammatory processes, current and future therapeutic targets: A mini review, Veterinary Quarterly, 22, 11-16, 10.1080/01652176.2000.9695016, 2000.

Wong, J. P. S., Lee, A. K. Y., and Abbatt, J. P. D.: Impacts of Sulfate Seed Acidity and Water Content on Isoprene Secondary Organic Aerosol Formation, Environmental Science & Technology, 49, 13215-13221, 10.1021/acs.est.5b02686, 2015.

Xu, L., Kollman, M. S., Song, C., Shilling, J. E., and Ng, N. L.: Effects of NOx on the Volatility of Secondary Organic Aerosol from Isoprene Photooxidation, Environmental Science & Technology, 48, 2253-2262, 10.1021/es404842g, 2014.

Xu, L., Guo, H., Boyd, C. M., Klein, M., Bougiatioti, A., Cerully, K. M., Hite, J. R., Isaacman-VanWertz, G., Kreisberg, N. M., Knote, C., Olson, K., Koss, A., Goldstein, A. H., Hering, S. V., de Gouw, J., Baumann, K., Lee, S.-H., Nenes, A., Weber, R. J., and Ng, N. L.: Effects of anthropogenic emissions on aerosol formation from isoprene and monoterpenes in the southeastern United States, Proceedings of the National Academy of Sciences, 112, 37-42, 10.1073/pnas.1417609112, 2015.

Zhang, Q., Jimenez, J. L., Canagaratna, M. R., Allan, J. D., Coe, H., Ulbrich, I., Alfarra, M. R., Takami, A., Middlebrook, A. M., Sun, Y. L., Dzepina, K., Dunlea, E., Docherty, K., DeCarlo, P. F., Salcedo, D., Onasch, T., Jayne, J. T., Miyoshi, T., Shimono, A., Hatakeyama, S., Takegawa, N., Kondo, Y., Schneider, J., Drewnick, F., Borrmann, S., Weimer, S., Demerjian, K., Williams, P., Bower, K., Bahreini, R., Cottrell, L., Griffin, R. J., Rautiainen, J., Sun, J. Y., Zhang, Y. M., and Worsnop, D. R.: Ubiquity and dominance of oxygenated species in organic aerosols in anthropogenically-influenced Northern Hemisphere midlatitudes, Geophysical Research Letters, 34, n/a-n/a, 10.1029/2007GL029979, 2007.