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

Response to anonymous referee #1 comments:

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

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

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

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

We have rephrased this section accordingly.

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

Since H$_2$O$_2$ uptake by inorganic seed particles was not observed (as shown by the blank results), it is unlikely that more H$_2$O$_2$ 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.
“Naphthalene exhibits a different, more distinct pattern compared to the rest of the SOA systems…”

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

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

Nevertheless, a significant correlation (p < 0.05) was observed…

…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:


