

Anonymous Referee #2

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Referee's comment:

The following should be addressed before publication should be considered.

1. Each experiment was only performed once. I strongly suggest that repeat experiments are performed to obtain information of the variability of the results presented here.

Authors' response:

While we understand and share the Referee's concerns about experimental reproducibility and variability, we disagree that the data presented here are inadequate to extend the findings of the previous study (Part 1) to the more-complex systems described herein.

The amount of systematic variability in our atmospheric ageing and in vitro exposure systems is illustrated by our control test results (included in this manuscript) as well as the exposures to the 'fresh' pollutant mixtures (exposures C and D). The amount of variability introduced to our results due to differences in meteorological conditions should not be considered exclusive of our ability to demonstrate the phenomena of interest. When the MOA was tested in clean air, we did not observe a biological response (exposure B). When we exposed cells to our aged test atmosphere without PM present, we saw no PM-only exposure response (exposure E, Fig 11). When a similarly-complex mixture was mixed with our non-toxic PM material in situ, the PM became toxic. Thus, the data are sufficient to test our hypothesis and demonstrate that the biological effects of the PM have been modified by the gas-phase toxic species.

Referee's comment:

2. p.7375, line 12: The compound mixture used here seems to be derived from studies performed some 25 years ago. Although it is mentioned later in the manuscript that the composition of urban VOC mixtures did not change over this time, no evidence for this statement is given. This should be added.

Authors' response:

Given that the mixture was created from an average of thousands of samples taken across an entire country, the mixture itself is not an attempt to model any one urban airshed. There are no exotic species included in SynUrb54 that are no longer in general useage. As such, the species present in the test mixture are currently found in urban airsheds nation-wide. Therefore, we find the Referee's criticism on this point to be of tangential importance to our ability to test our hypothesis. As stated several times in this and the previous manuscript, while we endeavour to use species relevant to current air quality, these studies are not an attempt to precisely model any specific location in the ambient environment. The focus of the reader should, therefore, remain on whether the mixture is sufficient to create an appropriately-complex ageing environment that provides the types of atmospheric ageing processes found in the ambient atmosphere, rather than focusing on the individual components of the mixture.

Moreover, and quite frankly, the mixture is based on studies from that period of time primarily because there is no data set that is more complete, and obtaining and analyzing thousands of canister samples from across the United States is a costly enterprise and no one in the current financial climate has voiced interest in funding or providing logistical support for an update of the mixture since the original study.

Referee's comment:

3. p. 7376, line 18: It is argued that the seed particles used here (MOA) are a good surrogate of ambient PM. This statement should be supported with data or additional arguments, also in this manuscript. Ambient PM is a highly complex compound mixture with a large number of mostly polar compounds. Thus, I am not sure the apolar composition of MOA can be called a "good surrogate for ambient PM".

Authors' response:

We are accustomed to publishing our results in journals that have strict restrictions on word-count. Our exclusion of the requested justification for MOA as a suitable surrogate was, therefore, intended to avoid unwarranted repetition of material between the companion papers. The material from the first companion paper read:

"Mineral oil aerosol (MOA) was used as a surrogate for organic-containing ambient PM (pharmaceutical grade, 100 %). Exposure to mineral oil elicits no acute biological effects from lung cells, making mineral oil an ideal PM material for this study. While most ambient PM has a mixture of elemental and organic carbon (EC/OC), the EC fraction is at the core of the particle and is (generally) completely surrounded by an OC layer. What this means is that any interactions between the PM and the gas phase happens via the OC fraction. In addition, mineral oil aerosolised with a large Collison nebuliser (May, 1973) has a size distribution similar to diesel exhaust particles observed in other chamber studies. For this study, MOA is a convenient and sufficiently complex surrogate for ambient PM that contains organic material."

Moreover, MOA is a good surrogate for fresh diesel exhaust, which is often used as an example of atmospherically-relevant PM. Atmospheric PM contains a mixture of polar and non-polar components, the ratios of which depend on any number of factors from PM source to the time of year. As stated above, our intention was not to recreate the ambient environment, rather to find a non-toxic PM material with which to challenge our cells. To fit our requirements, therefore, MOA doesn't have to be a perfect representation of ambient PM. We maintain that it is a sufficient surrogate with which to test our hypothesis.

Referee's comment:

4. The most puzzling result is that in experiment F (aged with MOA) the "gas-only" exposure generates the most pronounced result (Figure 10a, 11a). Very vague arguments are given on p. 7391: Line 11: Why should carbonyls explain this observation? They should be present in both experiments E and F. Line 13: The same is true for "undetected species".

Authors' response:

The Referee's comment has completely ignored our justification of carbonyls as important species (with appropriate referenced literature; p. 7372, LL 4) as well as an extensive discussion that covers the entirety of p. 7390 and carries on to p. 7391. In particular: "These experimental physical condition differences (e.g., less sunlight) resulted in both chemical differences (see Table 3 VOCs for Exp. E versus VOCs for Exp. F, where parent and daughter VOCs in the longer day were 20% lower) and gas-phase biological results differences (see Figs. 10a and 11a – Exp. E vs. Exp. F – where higher levels of gas-phase carbonyls that remained unreacted during the shorter day are effective in generating inflammation and cell damage)" (p. 7390 line 27 – p. 7391 line 3).

Referee's comment:

Two possible alternative explanations: A. The two exposure systems do not separate gases and particles so clearly as it is argued. B. Ozone/OH radicals present in the smog chamber in experiments E and F could alter the composition of MOA (generating an oxidized MOA). This could possibly partly explain why experiment F shows the most pronounced effects in the "particle-only" exposures. Generally I suggest that data of more experiments (repeats) and more control experiments should be shown.

Authors' response:

To address point A: While we understand the Referee's concern about the exposure systems, we have shown numerous times in peer-reviewed journal articles that the separation occurs.

To address point B: The following text is already included in the manuscript: "In addition, PM was never introduced to the chamber in the presence of sunlight in any of the experiments presented here, meaning that the particles themselves were never subjected to the possibility of having their toxicity or chemical composition altered by direct reaction with the sun. Further, the PM composition is a mixture of alkanes that do not react with ozone and, in the dark, there is no source of hydroxyl radicals. Thus, any increase in the toxicity of the particle phase was most likely due to its uptake of gas-phase toxics" (p. 7388, LL 15).

In addition, empirically speaking, after the MOA was added to the chamber, the ozone concentration did not decrease more quickly than the dilution tracer – which indicates that ozone was not reacting with MOA or anything else to any appreciable degree. This is not a surprise, given the absence of light and compounds that could react with ozone (such as alkenes). Moreover, even if a source of OH radicals escaped our detection, the Referee's hypothesized reaction of the radicals with the PM would simply be an alternate iteration of a gas-phase species modifying the toxicity of the PM present in the airborne mixture – thereby illustrating another mechanism for 'effect modification'.

Referee's comment:

5. Section 4.3: This is not a comparison with other work. Are there no other studies investigating particle effects with online deposition chambers?

Authors' response:

This appears to be a misunderstanding of what we meant by a 'comparison'. This section was not intended to be a lengthy review of existing technologies for online PM exposures, but a comparison of the demonstrated capabilities of online PM exposure methods with a posited inability to replicate our findings using the limited capabilities of resuspension exposure methods. Rather, while we do not make direct comparisons of quantitative response measurements, in this section we hypothesize what we think to be a likely cause for the inability of traditional PM toxicity assessments (such as those using PM that has been extracted from filter media) to observe the effects seen in this and the previous study. Moreover, a lengthy review of existing exposure technologies would diverge from the goal of this study, the demonstration of 'effect modification' in complex systems created in situ.

Referee's comment:

6. Section 4.4: I suggest merging the "summary" with the following conclusion section

Authors' response:

We defer to the judgement of the editor in regard to whether to make the suggested edit to the text.

Referee's comment:

7. Figure 8: Typos: The same experiments are labelled with "SE: ::" and "ST: ::". I strongly suggest re-labelling the experiments and simply number them 1, 2, 3, etc. This would strongly simplify reading the paper.

Authors' response:

Thank you for noticing this error, we have corrected it in the manuscript.

Referee's comment:

8. Figure 12 is hardly discussed. Considering that this is one of the main Figures (besides Figure 10 and 11) it should be either discussed or omitted

Authors' response:

Readers process information in different ways. As such, Figure 12 is a visual representation of the information presented in Table 7, and is included as an attempt to present data in multiple ways to ease comprehension by a wider range of readers. In response to the Referee's comments, we have inserted additional references to Figure 12 in the text.