Response to the comments on the manuscript acp-2021-666

Title: **Are reactive oxygen species (ROS) a suitable metric to predict toxicity of carbonaceous aerosol particles?**

Author(s):   Zhi-Hui Zhang et al.

We thank the reviewers for the insightful comments and valuable suggestions based on which we improved the quality of the manuscript significantly. Our point-by-point response to each of the comments is given below. The revisions made in the manuscript are marked in red and are also given below for easy reference. Please note that the line numbers that we mention in this response refer to our revised manuscript, which may be different from that in our original one due to the changes in texts.

Anonymous Referee #1

Major comments:

Comment 1: Page 1, line 29, "The SOA mass was condensed onto soot particles …". What is the major purpose to use soot particles? Will the soot particles also cause OP or cellular response? It would also be better to briefly introduce how soot particles are connected to SOA and OP in the abstract.

Response: As described in the introduction section (in lines 84-85), the oxidation of a complex mixture of AVOCs and BVOCs and exhaust emissions by vehicles (including SP) play a significant role for the formation of SOA and contribute prominently to the urban SOA burden (e.g., Gentner et al., 2017). Therefore, the purpose of using soot particles as seed in this study is to mimic the formation of SOA from the coating of VOCs on atmospheric SP originated from traffic emissions to simulate realistic urban carbonaceous particles. To clarify this point, the sentence in lines 29-31 has been revised as "*The SOA mass was condensed onto soot particles (SP) under varied atmospherically relevant conditions (photochemical aging and humidity) to mimic the SOA formation from a mixture of traffic-related carbonaceous primary aerosols and VOCs.*"

As described in lines 31-34, in this study, we analysed the ability of the aqueous extracts of the two aerosol types to induce ROS production, OP and cellular responses (cytotoxicity and cellular ROS production). We would like to clarify that we also quantified online and offline ROS, OP, and the cellular responses caused by aqueous extracts of fresh (i.e., not aged) and aged soot, respectively. We found that all soot particle extracts showed no detectable ROS concentrations, no significant OP, and no significant change in cellular responses compared to blank filters. This is described in lines 254-257 and 462-463. To clarify this, the sentences in lines 254-257 have been revised as "*In this study, we quantified online and offline ROS, and OP from the aqueous extracts of fresh (i.e., not aged in the PAM) and aged soot particles generated with the Mini-CAST soot generator but without addition of SOA. All the aqueous extracts of soot particles (fresh or aged and analysed online or offline) showed no detectable ROS concentrations and no significant OP compared to blank filters.*"

We did not analyse how the insoluble fraction of soot causes OP and cellular responses (which is clearly most of the total soot mass), but we have highlighted that this should be done in the future (see the last paragraph of the manuscript, lines 586-590). The texts are also given here for easy reference: "*Additionally, our findings that aqueous extracts of the soot particles (fresh, aged, analysed online or offline) showed no detectable ROS concentrations and no significant*

OP compared to blank filters do not necessarily mean that the insoluble part of soot particles are not a health-relevant part of ambient aerosol particles, but indicate that no significant water-soluble fraction exists in the soot produced here that lead to ROS production and OP. Therefore, more research is warranted to identify and quantity if and how the insoluble fraction of soot particles cause a change in cytotoxicity and cellular ROS production."

Comment 2: Page 5, line 134. It would be better to provide a simple explanation to calculate the carbon oxidation state of aerosol. The brief description of the post-process of AMS data would also be helpful.

Response: Thank you for your suggestion. We have provided additional information in the revised manuscript (see lines 133-138) as "*A high-resolution time-of-flight aerosol mass spectrometry (HR-TOF-AMS, Aerodyne Inc., Billerica, MA, USA) provided chemical aerosol mass loading information and aerosol elemental composition (e.g., O:C, H:C and N:C ratios) using the method described in Canagaratna et al. (2015), based on which the average carbon oxidation state ($\overline{OSc}$ = 2*O:C–H:C) was calculated following the method of Kroll et al., (2011)*."

Additional references:

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

Kroll, J. H., Donahue, N. M., Jimenez, J. L., Kessler, S. H., Canagaratna, M. R., Wilson, K. R., Altieri, K. E., Mazzoleni, L. R., Wozniak, A. S., Bluhm, H., Mysak, E. R., Smith, J. D., Kolb, C. E., and Worsnop, D. R.: Carbon oxidation state as a metric for describing the chemistry of atmospheric organic aerosol, Nat. Chem., 3, 133–139, 2011.

Comment 3: L163: The different precursor produces the different ROS which can have varying physicochemical properties. Naphthalene SOA and b-pinene SOA may have different solubility in water. Can Milli-Q water thoroughly extract the organic products on the filter?

Response: In this study, we measured the ROS from of the aqueous extracts (i.e., water-soluble fraction) of the two aerosol types. To clarify this, the sentence in line 164 has been revised as: "*In parallel to online ROS measurement, we quantified water-soluble ROS contents in the collected aerosol samples using an offline DCFH/HRP method, where we used the same concentrations of DCFH and HRP as for the online ROS measurement.*"

The reaction system we used to quantify ROS is DCFH/HRP. The HRP is an enzyme and it is thus not possible with this method to use, for example, an organic extraction solvent to characterise the ROS activity of particle components extracted in non-aqueous media. In the last paragraph of the manuscript, we addressed this issue and highlighted that more research is warranted to identify and quantity if and how other particle components (e.g., metals, soot) contribute to ROS production.

Comment 4: Page 9, line 254. In order to show that the ROS is caused mainly by the SOA coated on the soot particle but not the soot only, it would be valuable to add the ROS control for soot particle only in Figure 2.

Response: First, we would like to clarify that we found that all the aqueous extracts of soot particles (fresh, aged, analysed online or offline) showed no detectable ROS concentrations

and no significant OP compared to blank filters. Therefore, the sentence in lines 255-257 has been revised as "*All the aqueous extracts of soot particles (fresh, aged, analysed online or offline) showed no detectable ROS concentrations and no significant OP compared to blank filters.*"

Therefore, we think it is not necessary to add ROS control for the aqueous extracts of soot particles in Figure 2 due to no detectable ROS concentrations and no significant OP compared to blank filters. But we added a respective comment in the caption of Figure 2 for clarification: *"ROS was also quantified in uncoated soot (fresh and aged) but no ROS content was measured in the aqueous extracts of uncoated soot, see text for details."*

Comment 5: Page 9, line 263-265. Is there any evidence during the experiments about hygroscopic aerosol growth when RH increased from 40% to 70%? Both naphthalene SOA and beta-pinene SOA are relatively less polar in general (Chhabra et al., 2010; Chen et al., 2015). If water is less partitioned onto aerosols, the impact of humidity on the aerosol phase reaction will be little, and humidity will have a small impact on aerosols. However, for other types of SOA, such as isoprene oxygenated products, it is relatively polar, and their SOA formation, as well as OP, could be potentially impacted by RH. Can the authors conclude the humidity effect on the SOA by using less polar organic matter?

Response: Thank you very much for your suggestion. We did not investigate aerosol hygroscopic property. But, we fully agree with your comment, and we have provided additional information in the revised manuscript (see lines 272-274) as follows:

In addition, a change in RH from 40 to 70% showed marginal effects on ROS content in both aerosol types. This maybe because that naphthalene SOA and β-pinene SOA does not contain as highly oxidised compounds as other SOA types, which leads generally to a modest hygroscopicity (Chhabra et al., 2010; Chen et al., 2015) and thus the aerosol phase reactions might not be affected much by RH.

Two references below have also been added in the revised manuscript.

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, https://doi.org/10.5194/acp-10-4111-2010, 2010.

Chen, Q., Heald, C. L., Jimenez, J. L., Canagaratna, M. R., Zhang, Q., He, L. Y., Huang, X. F., Campuzano-Jost, P., Palm, B. B., Poulain, L., Kuwata, M., Martin, S. T., Abbatt, J. P. D., Lee, A. K. Y., and Liggio, J.: Elemental composition of organic aerosol: The gap between ambient and laboratory measurements, Geophys. Res. Lett., 42, 4182–4189, https://doi.org/10.1002/2015GL063693, 2015.

Comment 6: Page 11, line 313-319. Figure 3 shows that the carbon oxidation state for terpene SOA is different between the experiments under 40% RH and that under 70% RH (the highest number is 3 times different). Please provide the explanation for the higher carbon oxidation at the higher RH? Is it due to the high OH radical concentration at the higher RH? (high ozone production at the high RH). However, their observed ROS is very similar. It seems that the correlation between ROS and carbon oxidation state is only valid within the type of precursor but it is not sensitive to experimental conditions within the same precursor (i.e., SOA from different RH). This needs to be explained.

Response: As described in lines 131-132, we generated SOA with a photochemical aging equivalent to 2, 3, 4, 5 and 9 days at ambient OH concentration of 1 x 10^6 molecules/cm^3. OH concentrations were quantified using the D9-butanol approach as described in lines 129-131

and are therefore accounted for in the data presented here. Overlaying the data of Figure 3c and 3d into one graph (see Figure below) clearly indicates, that for β-pinene SOA the higher RH is promoting higher $\overline{OSc}$ and higher ROS concentrations, while this is not the case for naphthalene SOA.

Unfortunately, the current set of experiments does not allow to investigate the mechanisms driving the higher $\overline{OSc}$ and higher ROS concentrations of b-pinene SOA at higher RH. This would be interesting to investigate but would be clearly beyond the focus of the current study.
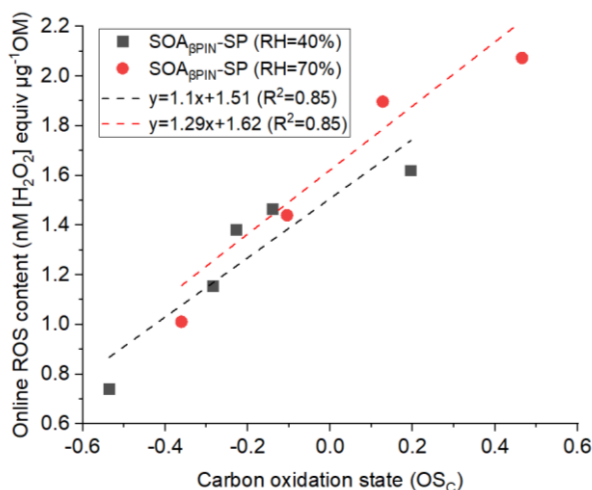
Figure caption: Combined data of Figures 3c and 3d into one graph, indicating that the relationship between $\overline{OSc}$ and ROS is reasonably constant across the RH range investigated.

Comment 7: Page 12, line 333-line 343. The filter samples were stored at -20°C for about 6 months. Can the short-lived ROS decay during this period and cause the uncertainties in the analysis? If SOA products are semi-volatile compounds, how can the one understand whether these species are reduced due to decay or by evaporation? There is no clear definition between short-live and long-live species in this manuscript. What is the expected lifetime of short-live species? Is it in second or minutes magnitude? The comparison of the samples between real-time samples and samples after 6 months is too much long time gap.

Response: The answer to each of your questions and comments is given below:

Q1: Can the short-lived ROS decay during this period and cause the uncertainties in the analysis?

Response: As described in lines 361-364, a pervious study in our group showed that approximately 75% of the total ROS has a half-life of a few minutes when we compared offline and online ROS analyses in SOA formed from ozonolysis of oleic acid aerosol in a flow tube (Fuller et al., 2014). We have published other studies where we quantified the lifetimes of ROS components in different SOA systems in the order of minutes to a few hours (Gallimore et al., Atmos. Chem. Phys., 17, 9853–9868, 2017 and Steimer et al., Atmos. Chem. Phys., 18, 10973–10983, 2018) Similarly, Wang et al. (2011) reported that the H_2O_2 yield (a significant component of ROS) from both α-pinene and β-pinene SOA was about 70% smaller after storing the filter samples in petri-dishes in the dark at room temperature for 20 hrs. Our current study found that more than 90% of all ROS components decay in both SOA types when we compared offline and online ROS analyses. All these studies together, clearly indicate that for conventional offline ROS analysis method, significant amount of ROS will be lost during sampling and filter storage. We argue that offline analysis is severely underestimating true ROS

concentrations, even if there is only a delay of a few minutes or hours between aerosol sampling and analysis.

That is the main reason why we developed our online ROS instrument, i.e., to capture the most or all of the total ROS components, including short- and long-lived ROS. These key points are described in the manuscript (e.g., lines 339-341, lines 371-373).

Q2: If SOA products are semi-volatile compounds, how can the one understand whether these species are reduced due to decay or by evaporation?

Response: Because ROS is reactive compounds, we believe that decomposition is the main pathway although we can not rule out the possible evaporation. Samples were stored at -20°C, thus evaporation during storage period is likely not a major process.

Q3: There is no clear definition between short-live and long-live species in this manuscript. What is the expected lifetime of short-live species? Is it in second or minutes magnitude?

Response: The decomposition behaviour of ROS under different time intervals has been studied previously and are beyond the scope of our current study (references above in our answer to Q1). However, in current study, we defined the long-lived ROS as the amount of ROS measured from quartz filter with a lifetime of longer than 6 months at -20°C. This is described in the first paragraph of section 3.2 (see lines 341-346).

Q4: The comparison of the samples between real-time samples and samples after 6 months is too much long time gap.

Response: Based on previous studies, people usually store the samples for several days to months before they conduct chemical analysis. We showed in our previous studies (references given above in Q1) that the short-lived ROS has a lifetime of minutes to hours. Thus, if there is a delay between sampling and analysis of only a day, all the short-lived ROS already decayed and only stable ROS compounds are left. Therefore, we think that the delay between sampling and offline analysis of 6 months is appropriate to capture the stable, long-lived ROS fraction.

Comment 8: Page 15, line 378. When were OP determined by using the filter samples? Were these samples measured right after sampling or stored for 6 months before analysis?

Response: The OP was determined from filter samples that stored for 6 months before analysis. The storage duration is the same as that for offline ROS analysis. The additional information has been added in the revised manuscript as (see line 390)"*OP was evaluated from offline filter samples after storage of about 6 months at -20°C based on the mass-normalized DTT decay rate (OP-DTT), with a higher DTT decay rate indicating a higher OP.*"

Comment 9: Page 15, line 382. What is the possible reason for the higher OP at 40% RH than that at 70% RH? Does this observation happen in NAP SOA but not in terpene SOA?

Response: As can be seen from Figure 6, although SOA_{NAP}-SP seems to show a higher OP at 40% RH under 5 and 9 aging days compared to 70% RH, the standard deviation of these data overlap. Therefore, there is no clear statistical evidence that the OP of SOA_{NAP}-SP at 40% HR is higher than that at 70% HR. In the manuscript we did not suggest such a conclusion.

Comment 10: Section 3.4. The cell studies were performed with the filter extractions. Then, were the most ROS species used for cell studies long-live products? Can the cell studies using filter extraction be same with the results of online ROS measurement?

Response: Yes, we investigated the cell responses of the water-soluble long-lived ROS components, because aerosols collected on filters were used. In contrast, the online ROS

measurement included both short-lived and long-lived ROS compounds. In addition to the online ROS measurements, we also determined the long-lived ROS fraction only as discussed above, which we compared directly with the cell responses.

In a recent accompanying study, Offer et al. (2021), we used the same aerosols as discussed in this study to expose lung cells to particles directly at the air-liquid interface (ALI) without prior particle collection on filters and extraction. In the study by Offer et al., we compared the online ROS measurement directly with cell exposure results. These results indicated that SOA$_{NAP}$-SP causes higher negative biological responses than SOA$_{βPIN}$-SP (i.e., higher DNA damage, and higher secretion of malondialdehyde and interleukin-8). The consistent results obtained from these different particle exposure methods (ALI cell exposure vs. filter extracts) may indicate that both types of cell exposure studies are indicative of particle toxicity, although more aerosol systems and multiple biological responses should be investigated to confirm these conclusions. The above information is summarised in lines 567-575.

Comment 11: Page 23, line 527. Will the ROS products slowly decay in the cell medium in the absence of cell cultures? It may also be useful to test how SOA products decay in the cell culture buffer without cells within 24 hrs.

Response: The purpose of cell exposure study is to investigate how water-soluble organic fraction causes cell responses. How ROS decay in the cell medium would be interesting to investigate but is beyond the scope of this study.

Comment 12: Table 1: Author increased the concentration of both soot particles and VOC, and Table 1 shows the changes in the oxidative characteristics of particles generated form the different concentration of soot particles and VOC. If there is a change in the concentration of VOC or soot particle only, are those oxidative characteristics influenced? Which one mainly cause this difference in oxidative characteristics?

Response: In this study, we generated SOA with a photochemical aging equivalent to 2, 3, 4, 5 and 9 days at ambient OH concentration of 1×10^6 molecules/cm^3. The number of OH radicals per VOC molecule available is lower at the higher VOC concentration, which lead to a lower overall degree of oxidation and thus a difference in ROS. Thus, we argue that oxidative characteristics of particles are mainly affected by VOC concentration. As for soot particles, they serve as seed for oxidised VOC reaction products during SOA formation, the change of which could also affect the participation of some ROS components from gas phase into particle phase. The explanation can be found in the first paragraph of section 3.4 (see lines 449-457).

Comment 13: Table 1: Carbon oxidation state of particle from SOA$_{bpin}$-SP are negative values. What does the negative values of carbon oxidation state of particle mean?

Response: As explained in section 2.1 (lines 133-137), the elemental ratios (O:C, H:C, and N:C) of SOA were determined using an AMS. Average aerosol carbon oxidation state values were calculated using the equation below:

$$\overline{OS}c = 2*\text{O:C} - \text{H:C}$$

Negative values of $\overline{OS}c$ mean the compounds contain a lower O/C ratio and/or a higher H/C ratio. See Kroll et al., (Nat. Chem., 3, 133–139, 2011) who developed this method for more details.

Comment 14: Is there possible impact of vapor-wall loss on the oxidative characteristics of particles produced in the reactor or sampling lines?

Response: Wall losses of gaseous organic oxidation products of the two VOC systems investigated here probably did occur, as it is the case for any laboratory SOA experiment. Effects of gaseous wall losses were not further investigated here but it seems not very likely the ROS components were selectively lost to reactor walls and thus a preferential ROS- specific wall loss effect is probably not significant.

Comment 15: QC/QA: How many data points were used for the QC/QA? This information can improve the reliability of the QC/QA.

Response: For the online ROS measurement, the ROS concentration under each test condition was continually monitored for about one hour. The additional information has been added in revised manuscript as (see lines 157-158) "The ROS concentration under each test condition was continually monitored for about one hour."

For the offline ROS and OP analysis, the additional sentence has been added in lines 179-180 & 206-207, respectively, as "For each sample, three replicate analyses were conducted."

For cellular ROS analysis, each sample was tested in six replicate wells. The information can be found in lines 216-217.

Comment 16: Figure 2, Figure 4, Figure 5, Figure 6, Figure 8, and Figure 9: Please explain the calculation of the error bars?

Response: As explained in lines 251-252, "*with the exception of $\overline{OSc}$ of the particles, all other reported values are presented as mean $\pm$ SD (standard deviations).*"

Minor comments:

Comment 1: Page 9, line 276. What is "photothermal aging"? Should it be "photochemical aging"?

Response: Thank you for pointing out this typographical error, which has been rectified.

Comment 2: Page 9, line 277, "highest ROS formation" -> "the highest ROS formation".

Response: The typographical error has been rectified.

Comment 3: Figure 3 and 7. It would be better to add order number (e.g., a, b, c, and d) for each sub figures.

Response: The order number has been added in Figures 3 & 7. Meanwhile, the sentence in lines 427-428 has been revised as "As can be seen from Figures 7(a) and (b), we further found that OP and offline ROS measurements for SOA_NAP-SP are clearly positively correlated, while there is a weaker correlation between OP and offline ROS for SOAβPIN-SP (Figures 7(c) and (d))."

Comment 4: It would be also useful for readers to organize the experimental conditions in a Table (in main content or SI) for the experiments described in Section 3.1-3.3.

Response: The experiments described in Section 3.1-3.3 include different types of tests, each of which has its unique experimental parameters. Therefore, a table would become very large and not straight forward to read. We think that all experimental conditions are clearly described and can be easily found in the main text, and we therefore would prefer not to summarise them in an additional table.

Anonymous Referee #2

Comment 1: Page 11, line 303: The authors attributed the differences in the content of organic peroxides vs. total ROS in naphthalene vs. pinene-derived SOA to the oxidation regimes. My 1st question: isn't there much more in the total ROS than simply the organic peroxide? And, if so, is this comparison valid? My 2nd question: if it is attributed to the oxidation conditions, then which regime is more atmospherically relevant (photo-oxidation vs. ozonolysis)?

Response: We agree that not only peroxides but likely also other compound classes contribute to ROS measured with the HRP/DCFH assay used here. But hydroperoxides, peroxy acids and H_2O_2 are some of the few compounds which are specifically known to react with HRP and which are also known to be abundant oxidation products of any organic SOA precursor. In section 3.1 we pointed out that quinones and semi-quinones in naphthalene-derived SOA also contribute to ROS. The sentence in lines 315-317 has been revised as *"These differences might be explained by the oxidation regimes used (i.e. photo-oxidation vs. ozonolysis) in different studies, and also the different contributions of other known and unknown ROS species in SOA."*

In this study, we did not compare the ROS yield from photo-oxidation vs. ozonolysis. In our another recent study (paper is under preparation), we found that ROS generated from both biogenic and anthropogenic VOCs are affected by oxidation regime. For example, we found that SOA produced from the photooxidation of carene has a higher ROS/SOA than SOA generated from ozonolysis of carene.

Comment 2: Page 12, Line 333: Do you really have to store the filters for 6 months? It is kind of expected that most of the particle-bound ROS will be lost in that time-frame. A more relevant experiment could have been analysing the filters after couple of days (which is equivalent to ambient filter sampling for days), so that the effect of the integrated filter sampling, could have been better captured.

Response: As described in detail above in our replies to Anonymous Referee #1 (see the response to comment 7) and in lines 361-364, previous studies in our group showed that approximately 75% of the total ROS has a half-life of a few minutes to a few hours (Fuller et al., 2014; Gallimore et al., 201; Steimer et al., 2018). Similarly, Wang et al. (2011) reported that the H_2O_2 yield (a significant component of ROS) from both α-pinene and β-pinene SOA was about 70% smaller after storing the filter samples in petri-dishes in the dark at room temperature for 20 hrs. Based on these results, we believe that most particle-bound ROS has a very short life (mins-hrs). Therefore, analysing the filters after couple of days could not capture these short-lived ROS.

Comment 3: Section 3.4: I think the relevant discussion of this section actually starts from line 444. The discussion above that line does not fit under the heading of this section. Some rearrangement is warranted in this section.

Response: Thank you for your comment. The first paragraph of section 3.4 provided basic information on (1) how to generate SOA for biological studies, and (2) the relationship of SOA that used for chemical and biological studies, respectively. The paragraph serves as a connecting link between the chemical and biological analysis. We thus think the text is in the right place. However, the sentence in line 447-449 has been revised as below to make them fit well the text.

We also evaluated the oxidative characteristics of aerosols produced from the mixture of 1 mg/m^3 soot particles and 4 mg/m^3 precursor VOCs under 3 day-equivalent photochemical aging at 40% RH for the biological studies discussed here.

Comment 4: Lines 450: The insignificant toxicity of fresh or aged-soot particles is surprising and inconsistent with the previous studies. I think it is related with water-insolubility of the soot particles. Did the authors make sure that soot particles remained suspended and are not lost?

Response: We would like to clarify that we studied exclusively and specifically only the toxicity of aqueous extraction (i.e., water-soluble fraction) of fresh or aged-soot particles and of soot-SOA mixtures. We did not study the toxicity of soot particle, and we have highlighted the texts below, which can be found in the last paragraph of the manuscript (see lines 584-588):

"Our findings that aqueous extracts of the soot particles (fresh, aged, analysed online or offline) showed no detectable ROS concentrations and no significant OP compared to blank filters do not necessarily mean that the insoluble part of soot particles are not a health-relevant part of ambient aerosol particles, but indicate that no significant water-soluble fraction exists in the soot produced here that lead to ROS production and OP. Therefore, more research is warranted to identify and quantity if and how the insoluble fraction of soot particles cause a change in cytotoxicity and cellular ROS production."

Comment 5: The trend of carbon oxidation state vs. ROS content does not match in Table 1 vs. Figure 3. Figure 3 shows an increase in the ROS content with the carbon oxidation state while Table 1 shows the reverse trend (see top two rows). Can the authors provide an explanation?

Response: In Figure 3, the SOA generated from 0.25 mg/m^3 soot + 1 mg/m^3 precursor VOCs are given. We investigated how the ROS content in SOA are affected by aging conditions, and we found that the ROS content in the SOA increased with an increase in aging time. However, in Table 1, we compared the oxidative characteristics of SOA generated from different concentrations of soot and precursor VOCs under the same photooxidation aging condition. We have clearly explained the reasons of the discrepancy of the two SOA in the first paragraph of section 3.4.

Comment 6: Line 533: Since the authors didn't measure different ROS components (and just hypothesized), I don't think this sentence is well supported from the authors' results.

Response: Thank you for pointing this out. The sentence has been deleted.

Anonymous Referee #3

Major comments:

Comment 1: Please specify what ROS species can be detected by the DCFH/HRP assay? OH, O_2^- etc? also organic peroxides can react with the assay but organic peroxides are not ROS.

Response: Currently, there is no analytical techniques available to identify and quantify all ROS components equally on a molecular level. Therefore, it is not possible to provide accurate information on what ROS species can be detected by the DCFH/HRP assay. However, based on our and other previous studies, DCFH/HRP assay is sensitive to H_2O_2, organic peroxides, hyperoxides, which have been reacted individually with HRP/DCFH and are thus included here in the definition of ROS (e.g., Fuller et al., 2014; Wragg et al., 2016; Zhou et al., 2018).

A number of authors include organic and inorganic radicals and peroxides in their definition of ROS, e.g. Dellinger 2001; Donaldson 2003; Kelly 2012; Cassee 2013; Lakey 2016.

References:

Cassee F.R., Heroix M., Gerlofs-Nijland M.E., Kelly F.J., Particulate matter beyond mass: recent health evidence on the role of fractions, chemical constituents and sources of emission, Inhal. Toxicol., 25, 802-812, 2013.

Dellinger B., Pryor W.A., Cueto R., Squadrito G.l., Hegde V., and Deutsch W.A., Role of Free Radicals in the Toxicity of Airborne Fine Particulate Matter, Chem. Res. Toxicol. 14, 1371-1377, 2001.

Donaldson K., Stone V., Borm P.J.A., Jimenez L.A., Gilmour P.S., Schins R.P.F., Knaapen M., Rahman I., Faux S.P., Brown D.M., Nee W.M., Oxidative stress andcalcium signalling in the adverse effects of environmental particles (PM10), Free Radical Biology & Medicine, Vol. 34, No. 11, pp. 1369–1382, 2003.

Fuller, S. J., Wragg, F. P. H., Nutter, J., and Kalberer, M.: Comparison of on-line and off-line methods to quantify reactive oxygen species (ROS) in atmospheric aerosols, Atmos. Environ., 92, 97–103, https://doi.org/10.1016/j.atmosenv.2014.04.006, 2014.

Kelly F.J., Fussell J.C., Size, source and chemical composition as determinants of toxicity attributable to ambient particulate matter, Atmos. Environ. 60:504–26, 2012.

Lakey P.S.J., Berkemeier T., Tong H., Arangio A.M., Lucas K., Poschl U., Shiraiwa M., Chemical exposure response relationship between air pollutants and reactive oxygen species in the human respiratory tract, Scientific Reports, 6, 32916, 2016.

Wragg, F. P. H., Fuller, S. J., Freshwater, R., Green, D. C., Kelly, F. J., and Kalberer, M.: An automated online instrument to quantify aerosol-bound reactive oxygen species (ROS) for ambient measurement and health-relevant aerosol studies, Atmos. Meas. Tech., 9, 4891–4900, https://doi.org/10.5194/amt-9-4891-2016, 2016.

Zhou, J., Bruns, E. A., Zotter, P., Stefenelli, G., Prévôt, A. S. H., Baltensperger, U., El-Haddad, I., and Dommen, J.: Development, characterization and first deployment of an improved online reactive oxygen species analyzer, Atmos. Meas. Tech., 11, 65–80, https://doi.org/10.5194/amt-11-65-2018, 2018.

Comment 2: The authors find that naphthalene SOA has a higher ROS content than the biogenic SOA, and it is likely due to quinones and semiquinones in naphthalene SOA that forms superoxide radicals. Is DCFH assay known to be sensitive to superoxide? Semiquinone

radical can oxidize DCF radical to form DCF which yields superoxide (Rota et al., provided below) and superoxide forms H_2O_2. How do the authors tell whether the ROS signal is from quinones-DCF chemistry or SOA aqueous chemistry?

Rota C, Chignell CF, Mason RP. Evidence for free radical formation during the oxidation of 2'-7'-dichlorofluorescin to the fluorescent dye 2'-7'-dichlorofluorescein by horseradish peroxidase: possible implications for oxidative stress measurements. Free Radic Biol Med. 1999 Oct;27(7-8):873-81. doi: 10.1016/s0891-5849(99)00137-9. PMID: 10515592.

Response: Thank you for your comment. We cannot clearly distinguish if the ROS quantified with our online ROS instrument is due to direct quinones-DCF chemistry or due to SOA aqueous chemistry as both are possible (and likely) chemical pathways. As we are aiming to capture an integrated proxy signal for total ROS activity and toxicity of aerosols with our online ROS instrument, it is actually an advantage if we quantify any direct aerosol components as well as secondary chemistry in the aqueous phase, as both pathways are also likely relevant in the aqueous layer of the lung epithelial surface.

Comment 3: The analytical methods used in the work (DCFH/HRP, DTT, and cellular DCFH assay) are known to be sensitive to H_2O_2 and/or organic peroxides. Is it possible that peroxides are essentially what the authors are measuring which explains the strong correlations between acellular and cellular ROS?

Response: As explained in this study, SOA generated from β-pinene contains a substantial fraction of peroxides. However, naphthalene-derived SOA contains peroxides and quinones, both of which could generate ROS and thus could contribute to acellular and cellular ROS formation (see question and our reply above). Therefore, we believe that peroxides contribute to the ROS measured here, but that our instrument can measure more general proxy of oxidising particle components as explained in the response to comment 1.

Comment 4: section 2.2, details are in Fuller et al. (2014) and Wragg et al., (2016) but it would be useful to briefly discuss what the differences are between online and offline ROS measurements? Are the online extracts filtered? How did you quantify losses inside the denuders? Some descriptions about the DCFH/HRP methods are mentioned in section 2.3, if the online system uses the same method, maybe should move the related method description up to section 2.2.

Response: In the first paragraph of section 3.2, we provided a brief description of the differences between online and offline ROS analysis. To clarify this, the sentence in lines 154-155 has been revised as *"A detailed description of the ROS analyser can be found in Fuller et al. (2014) and Wragg et al. (2016), and are briefly summarized in section 3.2."*

In addition, we pointed out the similarity of the two methods in several places. For example, in line 164-165, *"In parallel to online ROS measurement, we quantified water-soluble ROS contents in the collected aerosol samples using an offline DCFH/HRP method, where we used the same concentrations of DCFH and HRP as for the online ROS measurement."* In lines 171-172, we highlighted that *"This mixing dilutes both reactants to the same concentrations of DCFH (5 μM) and HRP (0.5 unit ml^{-1}) as used in the online method."*

We qualified the loss of particle counts inside the denuders by simultaneously monitoring the particle number concentrations prior to and after the denuders. Based on that, we corrected the particle and ROS loss inside the denuders. The information has been added in lines 161-162 as *"The particle loss inside the denuders was quantified by simultaneously monitoring the particle number concentrations prior to and after the denuders, and results presented here were corrected for these losses."*

Comment 5: line 327, "…ROS components react with HRP seconds after the particles enter the instrument." Some ROS have lifetimes in a range of ns. It would be useful to specify what ROS can the authors capture with the method.

Response: As explained in the response to comment 1, it is not possible to give an exhaustive list of compounds reacting in our online ROS instrument. Inorganic and organic peroxides, intrinsically present in the particles or generated in the aqueous phase after extraction, are compound classes that contribute to the overall ROS signal.

ROS components with a lifetime of ns will most likely not be captured with this technique, but also not with any other technique or method currently used to estimate particle-bound or particle-generated ROS.

Comment 6: I am also confused by the authors' use of "particle-bound" and "Short-lived ROS" to describe ROS formation from SOA. My understanding of the online system is that SOA are collected into liquid and then mix with the DCFH probe. Some ROS lifetime can be very short that by the time the samples react with DCFH probe, they might be gone. Particle-bound ROS refer to ROS on the SOA particle. However, the method not only captures the particle-bound ROS with a lifetime longer than the time it takes to travel from PAM to mix with the probe, but also the ROS formed through SOA aqueous chemistry.

Response: Please see our replies to comments 1, 2, 3 and 5 above. As pointed out there we do indeed capture ROS components present in SOA as well as ROS generated in the aqueous extract. We described this in line 45: *"particle-bound or particle-induced reactive oxygen species (ROS)"*

Minor comments:

Comment 1: Offer et al. (2021) are cited many times throughout the manuscript, but according to the reference list, it is a paper under review. Please specify in the main text.

Response: Thank you for your suggestion. The information has been added in the flowing sentence where Offer et al. (2021) appeared in this main text for the first time.

In the lines 109-110, "The setup is explained here only briefly, but more details can be found in Offer et al. (2021, under review)."

Comment 2: line 125, could you explain why O_3 are removed prior to online measurement and filter collection?

Response: O_3 can react with the HRP/DCFH assay. To rule out such gas phase artifacts, we removed ozone prior to conducting online composition measurements and offline filter samples collection. To clarify this, the sentence in lines 124-127 has been revised as *"A KNO_2-coated ceramic ozone denuder (BCE Special Ceramics GmbH, Germany) was installed downstream of the PAM, where an ozone analyzer (model APOA 350E, Horiba, Japan) monitored the ozone concentration. The purpose is to assure that almost all ozone formed in the PAM was removed prior to conducting online composition measurements and offline filter samples collection to rule out any sampling artifacts caused by O_3."*

Comment 3: line 387, "To the best of our knowledge, the OP of $SOA_{\beta PIN}$-SP from this study is the first reported in the literature." Tong et al., EST 2018 paper has provided OP of $SOA_{\beta PIN}$.

Response: Tong et al., (2018) investigated radicals (e.g., OH) of SOA generated from ozonolysis of β-pinene using electron paramagnetic resonance spectrometer method. While, in

our study, we observed particle-bound ROS from the SOA formed from the photooxidation of β-pinene using DCFH/HRP assay. Therefore, our current study is different from that of Tong et al, (2008). To clarity this, the sentence has been revised as (see lines 399-400) *"To the best of our knowledge, the OP of SOA$_{βPIN}$-SP generated from photooxidation of β-pinene as observed in this study is the first reported in the literature"*.

Comment 4: line 404, "Compared to naphthalene-derived SOA, β-pinene SOA are expected to contain a negligible amount of quinones but peroxides are suggested to contribute significantly to the OP of β-pinene SOA." The authors cited Wang et al., 2018 and Jiang and Jing, 2018, but these two studies did not use β-pinene SOA. Please correct.

Response: Wang et al., (2018) and Jiang & Jing (2018) found that SOA formed from photooxidation of α-pinene consist of substantial fraction of organic hydroperoxides. β-pinene has a very similar molecular structure as α-pinene. We therefore believe that SOA generated from β-pinene also contain a substantial fraction of organic hydroperoxides. The sentence has been revised as (in lines 418-419) *"Compared to naphthalene-derived SOA, β-pinene SOA are expected to contain a negligible amount of quinones but peroxides are suggested to contribute significantly to the OP of β-pinene SOA. Previous studies found that SOA formed from photooxidation of α-pinene consist of substantial fraction of organic hydroperoxides (Wang et al, 2018; Jiang and Jing, 2018)."*