Referee # 1:

The paper presents data on ROS measured by the DCFH assay on fine particles collected from filters. Ambient data over a longer time period are presented, which the authors suggest make the paper unique. The use of integrated filters to assess ROS (reactive oxygen species), reactive being the key word, is somewhat suspect and never really addressed in the paper. Many very general statements are made without sufficient explanation or validation. This includes why the authors believe oxidants on the particle are much worse than gas species, or aerosol components that generate ROS in vivo (example transition metals, etc). It appears at times the authors equate what they refer to as exogenous and endogenous ROS, when discussing ROS modes of action or discussions referencing published work. Overall, a number of points should be clarified prior to publication.

Author Response: We thank the referee for the substantive comments on our manuscript. We have addressed each comment separately below and plan to make the stated changes to the manuscript after the end of the open discussion period. The use of sampling filters to assess ROS has been used by several groups to assess particulate ROS and peroxides, both in chamber studies as well as ambient air studies (Chen et al., 2011; See and Wang, 2007; Venkatachari et al., 2007; Docherty et al., 2005; Hung and Wang, 2001; as well as references in these papers). The highly volatile reactive oxygen species may decay during the sampling duration of 3 hours, but this approach presents a convenient way to assess the semi-volatile and non-volatile ROS. This is addressed in detail in the relevant comment below. We have also explained why particle-phase ROS may have greater physiological relevance. In addition, the terms 'endogenous' and 'exogenous', have been replaced. We hope that our responses clarify the text of the manuscript. We look forward to getting additional feedback if anything remains unclear.

Pg 5063 line28. Why are water-soluble ROS species more likely to cause oxidative stress and cell damage deep in the lung, as stated, versus if they were deposited in the upper airways (ie, by what mechanism)? What is special about deposition deep in the lung in terms of ROS?

Along these lines, the concentrations of gas phase oxidants appear to be much higher than the oxidants found on the particles measured by this assay. For example, the level or oxidants calculated by this assay is of the order of 0.01 to 4 nMolH2O2/m3 (see Abstract). O3 concentrations in this study are between 20 and 60 ppb (Fig 2), which is roughly 800 to 2500 nMoles O3/m3, orders of magnitude larger than the ROS measured. A similar calculation could be done for H2O2, say ambient levels are 1 ppb, or about 40 nMoles H2O2/m3, over an order of magnitude of the ROS measured. The authors seem to argue that transport of oxidants by particles deep in the lung make them much more toxic than gas phase oxidants, which are much more abundant, but may be deposited higher up in the respiratory system. Why is this?

Author Response: We did not mean to imply that ROS and gas phase oxidants have a lesser effect in the upper airways due to any inherent property. We wanted to make the point that since SOA are more likely to reach deep into the lungs, the ROS on the SOA are also more likely to reach there and lead to oxidative stress in the tissue. The text in the manuscript will be edited to make this clearer.

The underlying principle is based on previously published studies, a few of which are referenced in the paper and are summarized here. Morio et al., (2001) exposed rats to ammonium sulfate (as a model atmospheric aerosol) alone, H_2O_2 alone, or both together in order to investigate if particulate matter can

transport H_2O_2 into the lower lung and induce tissue injury. Their results demonstrated that H_2O_2 augmented the biological effects of particulate matter and these effects were greater in rats exposed to particles with H_2O_2 than those exposed to H_2O_2 alone for some biomarkers. Wexler and Sarangapani (1998) simulated heat and mass transport in the lungs to predict the deposition patterns of inhaled water-soluble vapors in the presence and absence of aerosols. Their results showed that compounds with Henry's law constants greater than 100 M/atm (such as H_2O_2) were mainly deposited in the upper branches of the airways, whereas these compounds could be carried to the lower airways by particles. They also commented that clearance in the upper airways is rapid and the epithelium is protected by a mucus layer, both of which make pollutant deposition in the upper airways less likely to be harmful than in the lower airways.

Page 5064 Line 3. What is the difference between the ROS being measured and what is referred to as endogenous ROS? Can the two be readily compared? Also, the meaning of line 6 is not clear, how does exogenous ROS influence production of endogenous ROS (by what mechanism)? (Really, the authors should define what exactly is meant by endogenous and exogenous ROS).

Author Response: The terms 'exogenous ROS' and 'endogenous ROS' were introduced into the text after Access Review to clarify the distinction between ROS associated with ambient particles (what was measured in this study) and ROS generated in the body in response to environmental influences. In light of both the referees' comments, these sentences will be clarified and the reference to endogenous and exogenous will be removed. The end of the paragraph will now read, "While it would be presumptuous to declare that ROS has a direct toxic mechanism in tissue injury, many *in vitro* (Oosting et al., 1990; Holm et al., 1991; Geiser et al., 2004; Crim and Longmore, 1995; LaCagnin et al., 1990) and some *in vivo* studies have drawn links between ROS generated in the body and cell injury, and have also established the involvement of ROS in different pathologies, such as oxygen toxicity disorder (Kehrer, 1993; Sanders et al., 1995; Bowler et al., 2002; Li et al., 2003; Li et al., 2008). It appears likely that external factors (such as ROS associated with ambient particles) can influence the production of ROS in the body and affect the disease process."

Page 5065. The sampling method is: _3hr sampling time, analysis 1hr after end of sampling. By definition, what's being measured is highly reactive. Are there any studies tested the viability of filter sampling of this type of ROS, or have the authors tested sensitivity of the measured ROS to sample integration time and delay between sampling and analysis?

It is possible that many of the chemical components making up ROS that are being measured in this study on filters are in the gas phase given the low levels of particle water (except under high RH conditions), H2O2 being an example. There did not appear to be any gas denuders used in this study. How do the authors know if much of the ROS is actually a positive artifact and not really associated with the particle?

Author Response: We have studied the decay of particulate ROS over a 24-period using the same method that was used in the study and have found particulate ROS to be fairly stable over 24 hours. In addition, Chen et al., (2011) demonstrated that ROS generated from mixtures of terpenes and ozone consists of volatile ROS, semi-volatile ROS, and non-volatile ROS. They showed that semi-volatile and non-volatile ROS were relatively stable over a 24-hour period, whereas the volatile component of ROS typically amounted to less than 25% of the sample and decayed over the course of a 24-hour period. Given that our sampling duration of 3 hours was significantly longer than their sampling duration of 30 minutes, any highly volatile reactive oxygen species have likely degraded prior to sample analysis. The particulate ROS that remains on the sampling filters appears to be relatively stable over at least a 24-hour period.

The reviewer raises a good point about the possibility of artifacts from gas-phase ROS influencing the results of particle-phase ROS concentrations. However, the use of denuders to remove gas-phase pollutants is not ideal (Zhang et al., 2013), especially given the reactivity of ROS. Furthermore, particles in the accumulation mode (0.1 μm – 2.5 μm) coagulate very slowly and have a relatively long lifetime in the atmosphere, giving them enough time to reach equilibrium with gas-phase pollutants. Studies typically assume that ambient particles are in equilibrium with gas-phase pollutants (Wexler and Sarangapani, 1998) and if we assume the same, then the likelihood of gas-phase pollutants adsorbing onto and desorbing from the collected ambient particles would be low.

Only a few studies have simultaneously measured gas-phase and particle-phase ROS. Hasson and Paulson (2003) used a helical coil collector to extract gas-phase hydroperoxides into aqueous phase (however, they used a different reagent than what we used in our study). They found that the particle-phase hydroperoxide concentrations were several times higher than the equilibrium concentrations predicted by Henry's Law and they suggested some possible reasons for this (higher effective Henry's Law constant, aqueous-phase photochemical production of H_2O_2 in the aerosol). This brings up an important point about how little we understand gas/liquid/particle partitioning of hydroperoxides (and other ROS). This is especially important if we consider the fact that the human respiratory system is a very humid environment. Inhaled particles are exposed to an environment with high, even supersaturated relative humidity, which causes rapid particle growth, subsequent dilution of the ROS in the particle, and is followed by condensation and evaporation of ROS (Sarangapani and Wexler, 1996). Given the complex dynamics, it is difficult to predict the concentration of ROS that our lungs are actually exposed to from particles that have ROS associated with them.

Page 5065 line 18. It is stated that the method used here was modified, but not explicitly stated what that modification was.

Author Response: The method has been adapted from previously published studies and the modification has been described in detail in another article by our group that is currently in press. A reference to that article will be included in the Materials and Methods section.

Page 5069, paragraph starting on line 6. The discussion here is on correlations between gas phase H2O2 and particle phase ROS? Is gas phase H2O2 correlated with particle phase H2O2, which in turn could be correlated with particle ROS. The logic on which this discussion is based is not clear.

Author Response: The objective of this paragraph is to compare the correlations drawn from our study (between particulate ROS concentrations, ozone concentrations, temperature and solar radiation) with the correlations drawn from other studies on gas-phase ROS (between gas-phase ROS/H_2O_2 concentrations, ozone concentrations, NOx concentrations, temperature, solar radiation, and UV radiation). The introductory line of the paragraph will be edited to make this clearer.

While it was out of scope of this paper, future work should include simultaneous measurements of gas-phase and particle-phase ROS in order to compare the two concentrations (as was done in Hasson and Paulson, 2003 with a different reagent).

Page 5070 line 16. The regression results, as far as I can tell, do not prove that ROS is a function of O3, T, and solar radiation since O3, T and solar radiation are correlated with each other (Table 1), they are not independent variables in the regression model.

Author Response: The referee makes a very valid observation that the predictor variables for the multiple regression analysis are correlated. The discussion of the multiple linear regression model had been expanded slightly after the Access Review in light of a referee's comment asking for analysis of the standardized coefficients of the multiple regression model. However, as the current referee points out, the fact that the predictor variables for the multiple regression analysis are correlated limits the conclusions that can be derived from the multiple regression model and a caveat will be added in the text to emphasize this.

First line of conclusions. Does this assay really measure the oxidative capacity of PM? It appears to measure the concentration of oxidants associated with the particle, which is not the same thing.

Author Response: The objective of the sentence was to make a general statement on the importance of measuring a biologically relevant metric of PM. However, in light of the referee's comment, we will edit the line and make it more specific to ROS.

Referee # 2:

The authors addressed all comments raised during the initial manuscript evaluation. I only have one comment related to endogenous and exogenous ROS. The authors introduce the term "endogenous" on line 3/p. 5064 without prior explanation. The authors should provide more explanation on what they mean by endogenous and exogenous ROS production. This may not be clear to all readers.

Author Response: We thank the referee for reviewing our responses in the initial manuscript evaluation and for providing further feedback. We will edit the text from page 5063 line 29 to page 5064 line 7, remove the terms 'endogenous' and 'exogenous', and replace them with self-explanatory phrases.

References:

Chen, X., Hopke, P. K., Carter, W. P. L. Secondary Organic Aerosol from Ozonolysis of Biogenic Volatile Organic Compounds: Chamber Studies of Particle and Reactive Oxygen Species Formation. *Environ. Sci. Technol.*, 2011, 45, 276-282.

Docherty, K. S., Wu, W., Lim, Y. B., Ziemann, P. J. Contributions of Organic Peroxides to Secondary Aerosol formed from Reactions of Monoterpenes with O3. *Environ. Sci. Technol.*, 2005, 39, 4049-4059.

Hung, H-F. and Wang, C-S. Experimental determination of reactive oxygen species in Taipei aerosols. *J Aerosol Sci*, 2001, 32, 1201-1211.

Sarangapani, R. and Wexler. A. S. Growth and neutralization of sulfate aerosols in human airways. J. *Appl. Physiol.*, 1996, 81, 480-490.

See, S. W., Wang, Y. H., Balasubramanian, R. Contrasting Reactive Oxygen Species and Transition Metal Concentrations in Combustion Aerosols. *Environ. Res.*, 2007, 103, 317-324.

Venkatachari, P., Hopke, P. K., Brune, W. H., Ren, X., Lesher, R., Mao, J., Mitchell, M. Characterization of Wintertime Reactive Oxygen Species Concentrations in Flushing, New York. *Aerosol Sci. Technol.*, 2007, 41, 97-111.

Wexler, A. S., and Sarangapani, R. Particles do not Increase Vapor Deposition in Human Airways, J Aerosol Sci, 1998, 29, 197-204.

Zhang, J., Fan, X., Graham, L., Chan, T. W., Brook, J. R., Evaluation of an annular denuder system for carbonaceous aerosol sampling of diesel engine emission. J Air Waste Manag Assoc., 2013, 63, 1: 87-99.