



Short communication

A comparison of hydroxyl radical and hydrogen peroxide generation in ambient particle extracts and laboratory metal solutions

Huiyun Shen, Cort Anastasio*

Department of Land, Air and Water Resources, University of California, Davis, One Shields Avenue, Davis, CA 95616, USA

ARTICLE INFO

Article history:

Received 25 May 2011

Received in revised form

16 September 2011

Accepted 3 October 2011

Keywords:

Particulate matter (PM)

Reactive oxygen species (ROS)

Transition metals

Copper

Iron

ABSTRACT

Generation of reactive oxygen species (ROS) – including superoxide (O_2^-), hydrogen peroxide (HOOH), and hydroxyl radical (OH^\bullet) – has been suggested as one mechanism underlying the adverse health effects caused by ambient particulate matter (PM). In this study we compare HOOH and OH^\bullet production from fine and coarse PM collected at an urban (Fresno) and rural (Westside) site in the San Joaquin Valley (SJV) of California, as well as from laboratory solutions containing dissolved copper or iron. Samples were extracted in a cell-free, phosphate-buffered saline (PBS) solution containing $50 \mu\text{M}$ ascorbate (Asc). In our laboratory solutions we find that Cu is a potent source of both HOOH and OH^\bullet , with approximately 90% of the electrons that can be donated from Asc ending up in HOOH and OH^\bullet after 4 h. In contrast, in Fe solutions there is no measurable HOOH and only a modest production of OH^\bullet . Soluble Cu in the SJV PM samples is also a dominant source of HOOH and OH^\bullet . In both laboratory copper solutions and extracts of ambient particles we find much more production of HOOH compared to OH^\bullet : e.g., HOOH generation is approximately 30–60 times faster than OH^\bullet generation. The formation of HOOH and OH^\bullet are positively correlated, with roughly 3% and 8% of HOOH converted to OH^\bullet after 4 and 24 h of extraction, respectively. Although the SJV PM produce much more HOOH than OH^\bullet , since OH^\bullet is a much stronger oxidant it is unclear which species might be more important for oxidant-mediated toxicity from PM inhalation.

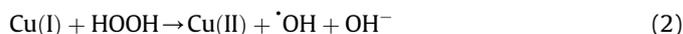
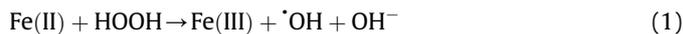
© 2011 Elsevier Ltd. All rights reserved.

1. Introduction

One suggested mechanism by which ambient particulate matter (PM) causes adverse health effects is PM-mediated oxidative stress and cell damage through the generation of reactive oxygen species (ROS) such as superoxide (O_2^-), hydrogen peroxide (HOOH), and hydroxyl radical (OH^\bullet) (Donaldson et al., 2003; Gonzalez-Flecha, 2004; Li et al., 2008; Valavanidis et al., 2008). ROS can be chemically generated by redox-active transition metals (TM) such as iron (Fe) and copper (Cu), which shuttle electrons from reductants such as ascorbate (Asc) onto dissolved oxygen (Shen et al., 2011). Multiple *in vitro* and *in vivo* studies have shown that HOOH can induce cytotoxicity (Hyslop et al., 1988; Oosting et al., 1990), while the highly reactive OH^\bullet can react with carbohydrates, lipids, proteins, and nucleic acids, resulting in cell death and disease (Valavanidis et al., 2008; Kell, 2010).

Previous studies have shown that Fe and Cu are the most important TMs in chemically generating ROS from ambient

particles (Donaldson et al., 1997; Shi et al., 2003; Vidrio et al., 2008, 2009; DiStefano et al., 2009; Wang et al., 2010; Shen et al., 2011). These metals are also important in converting HOOH to OH^\bullet via the Fenton, or Fenton-like, reaction (Valko et al., 2005):



A number of groups have measured the chemical generation of OH^\bullet or HOOH from particles extracted in cell-free buffer solutions (Hasson and Paulson, 2003; Shi et al., 2003; Baulig et al., 2004; Arellanes et al., 2006; Jung et al., 2006; Kunzli et al., 2006; Alaghmand and Blough, 2007; DiStefano et al., 2009; Vidrio et al., 2009; Wang et al., 2010). We recently reported the generation of HOOH and, separately, OH^\bullet by fine and coarse PM collected at an urban and rural site in the San Joaquin Valley (SJV) of California (Shen and Anastasio, 2011; Shen et al., 2011). In order to take advantage of this unique data set, the first where both of these ROS species were measured on the same particle extracts, here we compare our HOOH and OH^\bullet results in order to examine the relative amounts formed and the efficiency with which HOOH is converted to OH^\bullet .

* Corresponding author. Tel.: +1 530 754 6095; fax: +1 530 752 1552.
E-mail address: canastasio@ucdavis.edu (C. Anastasio).

2. Materials and methods

Below we briefly describe our experimental techniques; details are available in Shen et al. (2011) and Shen and Anastasio (2011). All experiments were performed in a chelex-100-treated, cell-free surrogate lung fluid (SLF) solution that contained 114 mM NaCl, 7.8 mM Na₂HPO₄ and 2.2 mM KH₂PO₄ to buffer the solution at pH 7.2–7.4, and 10 mM sodium benzoate (for [•]OH measurements).

Fine (PM_{2.5}) and coarse (PM_{2.5–10} or PM_{cf}) particle samples were collected at an urban (Fresno) and rural (Westside) site in California's SJV during summer and winter between 2006 and 2009. The urban site has abundant vehicular emissions, while the rural site is largely agricultural. For ROS measurements, a punch of PM_{2.5} filter or a piece of PM_{cf} foil was placed in a Teflon vial containing SLF with 50 μM Asc (an important endogenous antioxidant in lung lining fluid) and shaken in the dark at room temperature for up to 4 h (for HOOH) or 24 h (for [•]OH). The median extracted PM masses in each vial were 56 and 67 μg for PM_{2.5} and PM_{cf}, respectively. We performed similar measurements using laboratory metal solutions of CuSO₄ or FeSO₄ in 4 mL of SLF with 50 μM Asc. HOOH was analyzed using HPLC (Kok et al., 1995), while [•]OH was determined with a benzoate probe by HPLC (Jung et al., 2006). The detection limits for HOOH and [•]OH measurements were approximately 20 and 30 nM, respectively. Cu and Fe in the filtered PM extracts were analyzed by ICP-MS.

Two parameters were determined for each ROS species in each sample: (1) the initial rate of formation, calculated using the 0 and 1 h time points, and (2) the maximum formed during the extraction time (i.e., the highest concentration of HOOH measured within 4 h and the total amount of [•]OH formed after 24 h). PM results are normalized by the sampled air volume. Air-volume-normalized Cu concentrations in PM can be converted to aqueous-extract concentrations using

$$\text{Cu(nM)} = \text{Cu}(\text{nmol/m}^3 - \text{air}) \times V_{\text{air}}/V_{\text{SLF}} \quad (3)$$

where V_{air} is the sampled air volume corresponding to the filter punch or foil piece (2.346 m³ for PM_{2.5} and 21.444 m³ for PM_{cf}) and V_{SLF} is the extract volume (0.004 L for HOOH and 0.006 L for [•]OH). ROS and metal results are adjusted for the volume difference in the HOOH and [•]OH extracts. Data are presented as means ± SD.

3. Results and discussion

3.1. Generation of HOOH and [•]OH from dissolved Cu and Fe

We first measured HOOH and [•]OH formation in laboratory solutions containing Cu(II) (e.g., Fig. S1) or Fe(II). For copper solutions, HOOH generation is approximately 20–60 times faster than [•]OH generation (Fig. 1A), and the maximum concentration of HOOH formed is 7–13 times higher than the total [•]OH formed over 24 h (Fig. 1B). The maxima for both HOOH and [•]OH plateau at higher Cu(II) concentrations (Fig. 1), probably because of depletion of ascorbate. Assuming that all 50 μM Asc is depleted in the 600 nM Cu solution, we can calculate an “electron balance” based on the knowledge that Asc can donate 2 e⁻ and that it requires 2 and 3 e⁻, respectively, to form HOOH and [•]OH from dissolved O₂. Thus the 41.5 μM of HOOH formed at 4 h from 600 nM Cu (Fig. 1B) represents 83% of the electrons from Asc while the 1.9 μM of [•]OH formed at 4 h (data not shown) corresponds to 6% of the electrons from Asc. Thus ROS formation at high copper concentrations is very efficient, with approximately 90% of the Asc electrons ending up in HOOH and [•]OH after 4 h.

In contrast, Fe(II) generates very low concentrations of [•]OH, with an initial rate approximately 20 times lower than for Cu, and makes

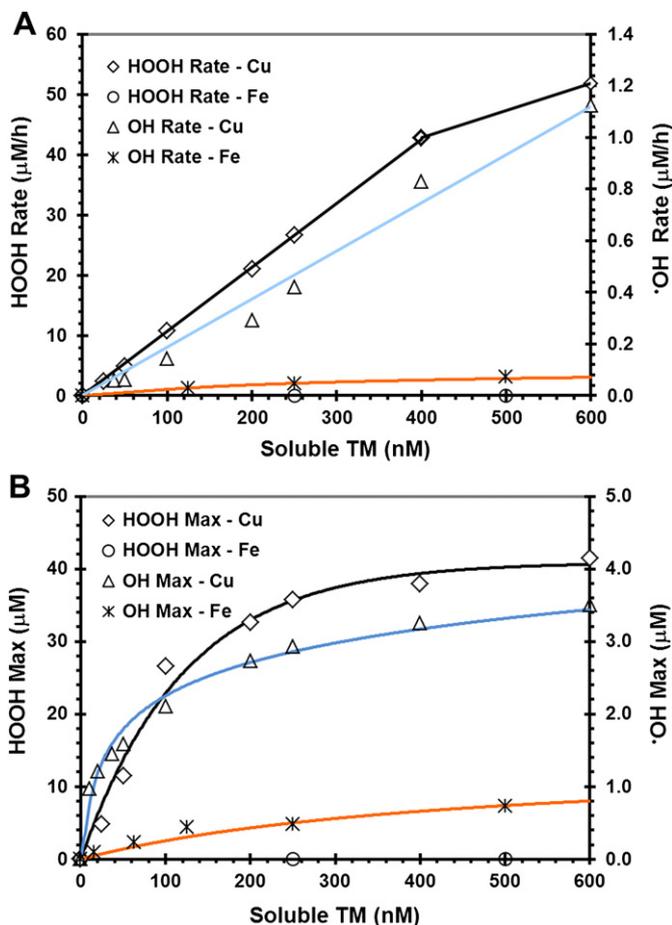


Fig. 1. Initial rate of ROS formation (panel A) and maximum ROS formed (panel B) as a function of soluble metal concentration in SLF with 50 μM ascorbate. The HOOH data for copper is from Shen et al. (2011). Regression equations are given in the Supplementary data.

no measurable HOOH (Fig. 1). By 4 h the [•]OH concentration from 500 nM Fe(II) is 0.16 μM (data not shown), representing only 0.5% of the initial ascorbate electrons.

However, while Cu is a much more potent source of HOOH and [•]OH than Fe in these laboratory solutions, the reactivity of both of these metals depends strongly on the composition of the surrogate lung fluid. In the SLF used in our current experiments, with Asc as the only antioxidant, Cu is much more efficient than Fe at making [•]OH (Vidrio et al., 2008; Charrier and Anastasio, in press). However, the presence of other physiologically important compounds can both suppress [•]OH generation from Cu and enhance [•]OH from Fe (Vidrio et al., 2008; Charrier and Anastasio, in press).

3.2. Generation of HOOH and [•]OH from ambient particles

The initial rates of formation of HOOH and [•]OH in the Fresno particle extracts are both strongly linearly correlated with the SLF-soluble Cu concentrations (Fig. 2). In these ambient PM extracts, HOOH generation is approximately 30 times faster than [•]OH generation at a given copper level (Fig. 2), in general agreement with our laboratory Cu solution results (Fig. 1A). The maximum amounts of HOOH and [•]OH generated in the particle extracts are also strongly, though non-linearly, related to soluble copper, with approximately 10 times more HOOH formed than [•]OH (Fig. S2). That is, over the 24 h of extraction, approximately 10% of the HOOH

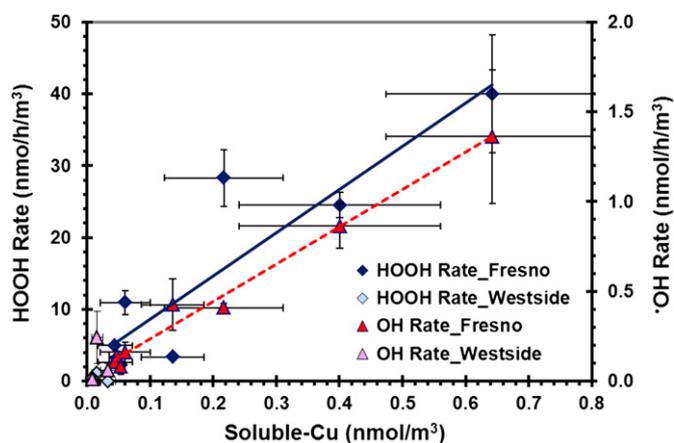


Fig. 2. Correlations between the initial rates of HOOH and $\cdot\text{OH}$ formation and soluble Cu in SJV PM extracts. Regressions: $y = 60x + 2.7$, $R^2 = 0.82$ for HOOH (Shen et al., 2011) and $y = 2.1x + 0.03$, $R^2 = 0.98$ for $\cdot\text{OH}$ (Shen and Anastasio, 2011). See Equation (3) for converting the air-volume-normalized Cu concentrations in PM to aqueous extract concentrations.

formed was converted to $\cdot\text{OH}$. In contrast to these strong correlations for the Fresno particles, we found no correlation between Cu and ROS formation by Westside PM.

The initial rates of HOOH and $\cdot\text{OH}$ formation in the Fresno particle extracts are well correlated, with approximately 3% of HOOH being converted to $\cdot\text{OH}$ at short times (Fig. 3). The maximum amounts of HOOH and $\cdot\text{OH}$ in the Fresno PM extracts are also positively correlated, with approximately 8% of HOOH converted to $\cdot\text{OH}$ over 24 h (Fig. S3).

3.3. Factors influencing the $\cdot\text{OH}/\text{HOOH}$ ratio

In this last section we examine whether the relative amounts of $\cdot\text{OH}$ and HOOH generated by laboratory solutions and ambient particle extracts depend on the amounts of soluble copper and/or iron. As our diagnostic parameters, we use the ratio of the initial rates of $\cdot\text{OH}$ and HOOH formation (“rate ratio”) and the ratio of the maximum amounts formed (“max ratio”), both expressed as $\cdot\text{OH}/\text{HOOH}$.

Fig. 4 shows the relationship between the $\cdot\text{OH}/\text{HOOH}$ ratios versus the SLF-soluble Cu concentration in both lab solutions and

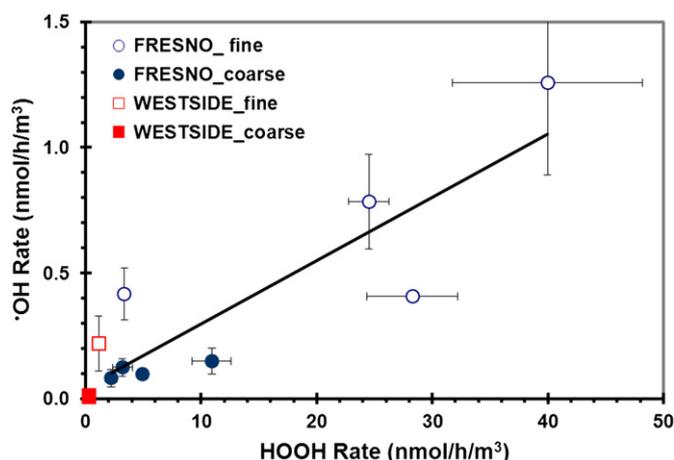


Fig. 3. Correlation between the initial rates of $\cdot\text{OH}$ and HOOH formation from SJV PM. The linear correlation for the Fresno PM is $y = 0.025x + 0.046$, $R^2 = 0.75$.

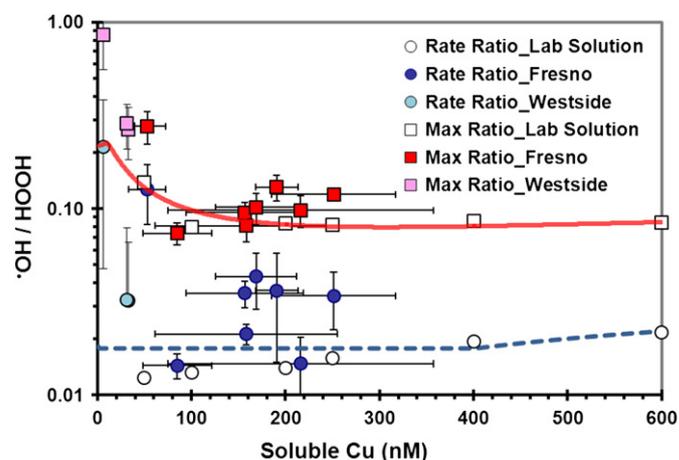


Fig. 4. The ratio of $\cdot\text{OH}$ formation over HOOH formation as a function of soluble Cu. The rate ratio and max ratio from both the lab solutions and the SJV PM extracts are shown. The two lines show the calculated rate ratio (dashed) and max ratio (solid) based on the lab solution data (Fig. 1).

PM extracts. The two lines show the calculated rate ratio (dashed) and max ratio (solid), respectively, based on our Cu solution data. In these lab solutions the rate ratios (0.018–0.022), measured within the first hour of extraction, are much lower than the max ratios (0.080–0.225), measured later, reflecting the fact that HOOH generally peaks within a few hours during extraction (Shen et al., 2011), while the total $\cdot\text{OH}$ increases throughout the 24 h of extraction (Shen and Anastasio, 2011). Compared to the laboratory copper solutions, the $\cdot\text{OH}/\text{HOOH}$ ratios in the SJV PM extracts are higher by average factors of 3.1 (for rate ratio) and 1.8 (for max ratio). This suggests that species in ambient particles enhance the conversion of HOOH to $\cdot\text{OH}$. In addition, the max ratios for $\cdot\text{OH}/\text{HOOH}$ are higher when the soluble Cu concentration is lower (below 100 nM), suggesting more efficient conversion of HOOH to $\cdot\text{OH}$ at lower copper concentrations (Fig. 4). We calculate that the typical Cu concentration in human lung lining fluid from inhalation of Fresno PM is 100 nM (73% from $\text{PM}_{2.5}$), based on an average adult lung lining fluid volume of 25 mL, an inhaled air volume of 20 m^3 per day, assuming 30% and 70% of inhaled fine and coarse PM deposit in the lung, respectively, and using our Fresno median Cu mass concentrations of 19.5 and 3.2 ng m^{-3} for $\text{PM}_{2.5}$ and PM_{co} , respectively (Shen et al., 2011). The Westside particles, with low Cu concentrations, generally have higher $\cdot\text{OH}/\text{HOOH}$ values (Fig. 4), although they generate much lower absolute amounts of $\cdot\text{OH}$ and HOOH than the Fresno PM (Fig. 2).

Fig. 5 shows that a higher soluble Fe/Cu ratio is also associated with a higher $\cdot\text{OH}/\text{HOOH}$ ratio, i.e., with more efficient conversion of HOOH to $\cdot\text{OH}$. Part of this relationship is probably due to higher $\cdot\text{OH}/\text{HOOH}$ ratios at lower copper amounts (Figs. 4, S4A, and S4C). However, while the SLF-soluble Fe amount does not seem to affect $\cdot\text{OH}/\text{HOOH}$ directly (Figs. S4B and S4D), higher $\cdot\text{OH}/\text{HOOH}$ values at higher Fe/Cu ratios (Fig. 5) is consistent with the fact that Fe(II) converts HOOH to $\cdot\text{OH}$ via the Fenton reaction (R1).

4. Conclusion

In cell-free solutions containing ascorbate, Cu is a very efficient source of HOOH and $\cdot\text{OH}$, while Fe is a modest source of $\cdot\text{OH}$. Both Cu solutions and urban (Fresno, CA) particles generate much more HOOH than $\cdot\text{OH}$, and levels of these ROS are positively correlated. The conversion of HOOH to $\cdot\text{OH}$ is generally more efficient at lower Cu concentrations and higher Fe/Cu ratios.

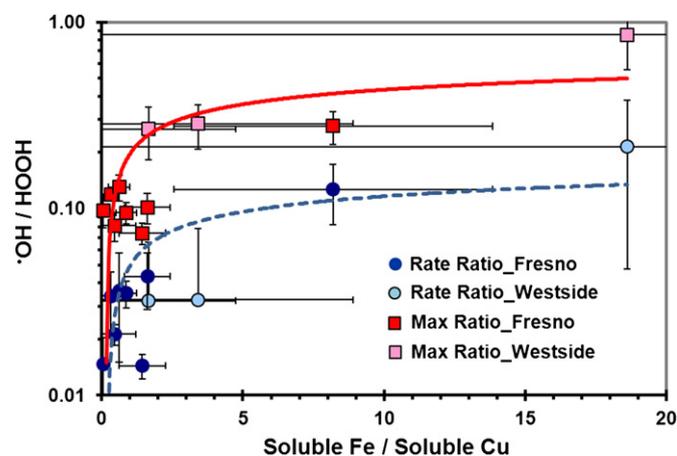


Fig. 5. Ratios of $\cdot\text{OH}$ over HO_2H as a function of the soluble Fe/Cu ratio in PM extracts. The regression equations are: $y = 0.029\ln(x) + 0.050$, $R^2 = 0.57$ for the rate ratio (dashed line) and $y = 0.103\ln(x) + 0.197$, $R^2 = 0.52$ for the max ratio (solid line).

Acknowledgments

We thank Yongjing Zhao, Walter Ham, Mike Kleeman, Chris Ruehl, Norman Kado, and Yuee Pan for PM samples and Jessie Charrier for HO_2H measurements from Fe. This research was funded by the U.S. EPA (RD-83241401-0) through the San Joaquin Valley Aerosol Health Effects Research Center at UC Davis. Additional funding was provided by the California Agricultural Experiment Station (Project CA-D*-LAW-6403-RR) and the NIEHS (P42ES004699).

Appendix. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.atmosenv.2011.10.006.

References

- Alaghmand, M., Blough, N.V., 2007. Source-dependent variation in hydroxyl radical production by airborne particulate matter. *Environmental Science & Technology* 41, 2364–2370.
- Arellanes, C., Paulson, S.E., Fine, P.M., Sioutas, C., 2006. Exceeding of Henry's law by hydrogen peroxide associated with urban aerosols. *Environmental Science & Technology* 40, 4859–4866.
- Baulig, A., Poirault, J.J., Ausset, P., Schins, R., Shi, T.M., Baralle, D., Dorlhene, P., Meyer, M., Lefevre, R., Baeza-Squiban, A., Marano, F., 2004. Physicochemical characteristics and biological activities of seasonal atmospheric particulate matter sampling in two locations of Paris. *Environmental Science & Technology* 38, 5985–5992.
- Charrier, J.G., Anastasio, C. Impacts of antioxidants on hydroxyl radical production from individual and mixed transition metals in a surrogate lung fluid. *Atmospheric Environment*, in press.
- DiStefano, E., Eiguren-Fernandez, A., Delfino, R.J., Sioutas, C., Froines, J.R., Cho, A.K., 2009. Determination of metal-based hydroxyl radical generating capacity of ambient and diesel exhaust particles. *Inhalation Toxicology* 21, 731–738.

- Donaldson, K., Brown, D.M., Mitchell, C., Dineva, M., Beswick, P.H., Gilmour, P., MacNee, W., 1997. Free radical activity of PM_{10} : iron-mediated generation of hydroxyl radicals. *Environmental Health Perspectives* 105, 1285–1289.
- Donaldson, K., Stone, V., Borm, P.J., Jimenez, L.A., Gilmour, P.S., Schins, R.P., Knaapen, A.M., Rahman, I., Faux, S.P., Brown, D.M., MacNee, W., 2003. Oxidative stress and calcium signaling in the adverse effects of environmental particles (PM_{10}). *Free Radical Biology and Medicine* 34, 1369–1382.
- Gonzalez-Flecha, B., 2004. Oxidant mechanisms in response to ambient air particles. *Molecular Aspects of Medicine* 25, 169–182.
- Hasson, A.S., Paulson, S.E., 2003. An investigation of the relationship between gas-phase and aerosol-borne hydroperoxides in urban air. *Journal of Aerosol Science* 34, 459–468.
- Hyslop, P.A., Hinshaw, D.B., Halsey Jr., W.A., Schraufstatter, I.U., Sauerheber, R.D., Spragg, R.G., Jackson, J.H., Cochrane, C.G., 1988. Mechanisms of oxidant-mediated cell injury. The glycolytic and mitochondrial pathways of ADP phosphorylation are major intracellular targets inactivated by hydrogen peroxide. *The Journal of Biological Chemistry* 263, 1665–1675.
- Jung, H., Guo, B., Anastasio, C., Kennedy, I.M., 2006. Quantitative measurements of the generation of hydroxyl radicals by soot particles in a surrogate lung fluid. *Atmospheric Environment* 40, 1043–1052.
- Kell, D.B., 2010. Towards a unifying, systems biology understanding of large-scale cellular death and destruction caused by poorly liganded iron: Parkinson's, Huntington's, Alzheimer's, prions, bactericides, chemical toxicology and others as examples. *Archives of Toxicology* 84, 825–889.
- Kok, G.L., McLaren, S.E., Staffelbach, T.A., 1995. HPLC determination of atmospheric organic hydroperoxides. *Journal of Atmospheric and Oceanic Technology* 12, 282–289.
- Kunzli, N., Mudway, I.S., Gotschi, T., Shi, T., Kelly, F.J., Cook, S., Burney, P., Forsberg, B., Gauderman, J.W., Hazenkamp, M.E., Heinrich, J., Jarvis, D., Norback, D., Payo-Losa, F., Poli, A., Sunyer, J., Borm, P.J., 2006. Comparison of oxidative properties, light absorbance, total and elemental mass concentration of ambient $\text{PM}_{2.5}$ collected at 20 European sites. *Environmental Health Perspectives* 114, 684–690.
- Li, N., Xia, T., Nel, A.E., 2008. The role of oxidative stress in ambient particulate matter-induced lung diseases and its implications in the toxicity of engineered nanoparticles. *Free Radical Biology and Medicine* 44, 1689–1699.
- Oosting, R.S., van Bree, L., van Iwaarden, J.F., van Golde, L.M., Verhoef, J., 1990. Impairment of phagocytic functions of alveolar macrophages by hydrogen peroxide. *American Journal of Physiology* 259, L87–L94.
- Shen, H., Anastasio, C., 2011. Formation of hydroxyl radical from San Joaquin Valley particles extracted in a cell-free surrogate lung fluid. *Atmospheric Chemistry and Physics* 11, 9671–9682.
- Shen, H., Barakat, A.I., Anastasio, C., 2011. Generation of hydrogen peroxide from San Joaquin Valley particles in a cell-free solution. *Atmospheric Chemistry and Physics* 11, 753–765.
- Shi, T., Knaapen, A.M., Begerow, J., Birmili, W., Borm, P.J., Schins, R.P., 2003. Temporal variation of hydroxyl radical generation and 8-hydroxy-2'-deoxyguanosine formation by coarse and fine particulate matter. *Occupational and Environmental Medicine* 60, 315–321.
- Valavanidis, A., Fiotakis, K., Vlachogianni, T., 2008. Airborne particulate matter and human health: toxicological assessment and importance of size and composition of particles for oxidative damage and carcinogenic mechanisms. *Journal of Environmental Science and Health, Part C: Environmental Carcinogenesis and Ecotoxicology Reviews* 26, 339–362.
- Valko, M., Morris, H., Cronin, M.T.D., 2005. Metals, toxicity and oxidative stress. *Current Medicinal Chemistry* 12, 1161–1208.
- Vidrio, E., Jung, H., Anastasio, C., 2008. Generation of hydroxyl radicals from dissolved transition metals in surrogate lung fluid solutions. *Atmospheric Environment* 42, 4369–4379.
- Vidrio, E., Phuath, C.H., Dillner, A.M., Anastasio, C., 2009. Generation of hydroxyl radicals from ambient fine particles in a surrogate lung fluid solution. *Environmental Science & Technology* 43, 922–927.
- Wang, Y., Arellanes, C., Curtis, D.B., Paulson, S.E., 2010. Probing the source of hydrogen peroxide associated with coarse mode aerosol particles in Southern California. *Environmental Science & Technology* 44, 4070–4075.