The Dissertation Committee for Shahana Sarfraz Khurshid Certifies that this is the approved version of the following dissertation:

PARTICULATE REACTIVE OXYGEN SPECIES IN INDOOR AND OUTDOOR ENVIRONMENTS: PREVALENCE AND HEALTH EFFECTS

Committee:

Kerry Kinney, Supervisor

Jeffrey Siegel

Richard Corsi

Atila Novoselac

J. Raymond Wells

PARTICULATE REACTIVE OXYGEN SPECIES IN INDOOR AND OUTDOOR ENVIRONMENTS: PREVALENCE AND HEALTH EFFECTS

by

Shahana Sarfraz Khurshid, B.S., M.S.E.

Dissertation

Presented to the Faculty of the Graduate School of

The University of Texas at Austin

in Partial Fulfillment

of the Requirements

for the Degree of

Doctor of Philosophy

The University of Texas at Austin

May, 2015

Dedication

To my children, who made this journey both difficult and hugely rewarding.

Acknowledgements

I would like to thank my advisors, Drs. Kerry Kinney and Jeffrey Siegel for their support and guidance throughout my doctoral research. Dr. Kinney introduced me to the world of indoor air research as it applies to human health and has provided all kinds of support to me since taking me on into her research group in 2009. Dr. Siegel has provided invaluable direction and aim in all aspects of my research. He is not listed as a co-supervisor on this dissertation as he has joined University of Toronto, but he provided input as a co-supervisor even after moving away from Austin. Not only this research, but also I, have benefitted greatly from their supervision.

The indoor air quality group has been pivotal in my growth as a researcher. It has been an honor to learn from professors like Drs. Richard Corsi and Atila Novoselac who have a wealth of information in their respective fields. I have also learned a lot from my peers and am grateful to several of them for their help during my research, including Drs. Elliott Gall, Brent Stephens, Andy Hoisington, Elena Nirlo, Marwa Zaatari, James Lo, Fernando Almada, and Juan Pedro Maestre. Dr. Neil Crain also provided valuable research expertise. Dori Eubank, who is one of the nicest people I have come across, always helped smooth out any last minute crises.

The NSF IGERT fellowship program not only provided funding for the early years of my research but also provided a great network for stimulating and exchanging ideas on indoor air science for which I am immensely grateful. It enabled me to conduct research at NIOSH which was a very insightful experience with Drs. Ray Wells and Stacey Anderson. I would also like to thank my other major funding sources which included the U.S. EPA STAR fellowship program, the Cockrell School of Engineering

Thrust fellowship, and a grant from NIOSH to the Southwest Center for Occupational and Environmental Health (SWCOEH). I also received support from the Air and Waste Management Association (A&WMA), American Association of University Women (AAUW), and American Society of Heating, Refrigerating and Air-conditioning Engineers (ASHRAE).

Finally, I would like to thank my family: my mother, who worked tirelessly to support me and provide me with all the opportunities to get me where I am today; my grandparents, for the love they gave me; my husband, for standing by me; my uncles and father, for their support and belief in me. I am who I am today because of all of you.

Particulate Reactive Oxygen Species in Indoor and Outdoor Environments: Prevalence and Health Effects

Shahana Sarfraz Khurshid, Ph.D.

The University of Texas at Austin, 2015

Supervisor: Kerry Kinney

Reactive Oxygen Species (ROS) are an important class of air pollutants generated from photochemical and ozone-initiated reactions in indoor and outdoor environments. Despite the fact that Americans spend nearly 90% of their time inside buildings and extended exposures to ROS can occur in indoor environments, ROS has received very little attention as an indoor pollutant. This is one of the first research studies to measure the concentration of particulate ROS (on $PM_{2.5}$ and TSP) in indoor environments. A significant fraction of indoor particulate ROS was found to exist on $PM_{2.5}$ (58±10%) which is important from a health perspective since $PM_{2.5}$ can carry ROS deep into the lungs. The indoor concentrations of ROS on $PM_{2.5}$ sampled in residential and commercial buildings were not significantly different from the outdoor concentrations. This result is intriguing because it implies that generation of ROS inside buildings and/or transport of outdoor ROS and precursors of ROS into buildings are important processes and can be as significant as ROS generation in outdoor environments. Controlled studies show that when outdoor ozone concentrations are relatively low, indoor concentrations of ROS are dominated by indoor sources of ROS rather than outdoor sources of ROS. However, when outdoor ozone concentrations are relatively high, indoor and outdoor sources of

ROS contribute almost equally to the indoor concentration of ROS. This study is also one of the first to assess seasonal variations in outdoor particulate ROS concentrations. Ambient sampling conducted over an 11-month period indicates that outdoor particulate ROS concentrations are influenced by the ozone concentration, solar radiation intensity and temperature. In order to understand the potential health effects of exposure to ROS, an *in vitro* exposure system of lung epithelial cells and differentiated lung tissue was also utilized. Results from these experiments indicate that exposure to products of limonene ozonolysis (which include ROS) can lead to a greater inflammatory response than exposure to either ozone or limonene. This highlights the need to include biologically relevant pollutants, such as ROS, in indoor air quality studies. Further work is warranted to better understand the parameters that drive indoor particulate ROS concentrations.

Table of Contents

List of Tables in Research Summary

List of Figures in Research Summary

Chapter 1: Introduction

Reactive oxygen species (ROS) are an important class of secondary pollutants and consist of free radicals (e.g. hydroxyl radical), molecules (e.g. peroxides), and ions (e.g. superoxide anion). It is widely understood that exposure to particulate matter (PM) has a detrimental effect on human health (Samet et al., 2000; Pope et al., 2002; Pope and Dockery, 2006). However, it is unclear what characteristics or components of PM are the main contributors to the adverse health effects observed. Instead of using PM mass as the only metric for measuring the level of particulate pollution, recent research efforts have turned towards using biologically active chemical species of PM, such as ROS, as better predictors of the health effects associated with PM. This chapter summarizes the motivation for assessing the prevalence of particulate ROS in different environments and understanding the potential health effects of secondary pollutants such as ROS. The objectives of this dissertation are outlined at the end of the chapter. A brief review of the background literature is then presented in Chapter 2.

1.1 MOTIVATION

Although theory suggests that hydrogen peroxide is formed as a result of chemical reactions in indoor environments (Nazaroff and Cass, 1986), it was not until Li et al. (2002) (office) and Fan et al. (2005) (simulated indoor conditions) that evidence of these mechanisms in indoor environments was found. These studies as well as chamber studies of ozone/terpene reactions (Docherty et al. 2005; Venkatachari & Hopke, 2008a; Chen & Hopke, 2009a; Chen & Hopke, 2009b; Chen & Hopke, 2010; Chen et al., 2011) have shown that secondary organic aerosols (SOA) are formed in conjunction with peroxides and other ROS. SOA can carry ROS into the lower respiratory tract where there is increased probability of health impacts. While gas phase ROS exists, it is likely to be

absorbed and removed by mucus in the upper airways because of its high water solubility and molecular diffusivity (Friedlander and Yeh, 1998). ROS on particles, on the other hand, can reach deep into the lungs, especially if the particles are in the respirable range. Because of its relevance to health, the focus of this dissertation will be on particulate phase ROS.

A substantial body of evidence links the human body's production of reactive oxygen radicals, and subsequently oxidative stress and damage, to the pathogenesis of age-related and chronic diseases, including cancer (Trush and Kensler, 1991; Witz, 1991; Guyton and Kensler, 1993). While it is not yet clear that ROS have a direct toxic mechanism in tissue injury, many *in vitro* and some *in vivo* studies have established the involvement of ROS in different pathologies, especially in many pulmonary diseases (Kehrer, 1993; Lansing et al., 1993; Sanders et al., 1995; Stevens et al., 1995; Bowler et al., 2002; Li et al., 2003; Li et al., 2008). Oxidative stress can arise from both endogenous sources (inside the body) and exogenous sources (from the environment) and, as a consequence, it seems logical that ROS from exogenous sources may cause the same health outcomes as endogenously generated ROS. This warrants further investigation of exogenous sources of ROS, especially since reduction of avoidable endogenous and exogenous causes of oxidative stress has been advised due to the ineffectiveness of antioxidant intervention strategies (Dreher et al., 1996). However, studies to assess air quality have focused on measuring the concentration of pollutants such as particulate matter and volatile organic compounds (VOCs). While these pollutants are linked to adverse health outcomes (e.g., DALYs for particulate matter exposure (Zelm et al., 2008) and sick building syndrome ailments for VOC exposure (e.g., Fisk et al., 1997)), the concentration of ROS is a biologically relevant property of PM that may be as important as PM mass, if not more important, when assessing the quality of air in an environment.

Despite the prevalence of ROS precursors and the potential health effects of ROS, previous research has focused almost exclusively on determining the concentration of these species in outdoor environments. Indeed, only one study has assessed the concentration of particulate ROS in an indoor environment (in a university building in Singapore: See et al., 2007). Given the large proportion of time people spend inside buildings, and the substantial differences that exist between different kinds of indoor environments, it is imperative to assess the concentration of particulate ROS in both residential and commercial buildings. These buildings differ in terms of air exchange rates, recirculation rates, source emission profiles, and precursor pollutant concentrations. A major source of indoor particulate ROS may be outdoor particulate ROS, especially in buildings with higher air exchange rates such as commercial buildings. Thus, it is also important to assess outdoor particulate ROS concentrations. Furthermore, it is necessary to investigate the sources that can contribute to indoor concentrations of particulate ROS.

1.2 OBJECTIVES

The goal of this research is to explore the prevalence and potential health effects of ROS as a biologically relevant property of PM. Broadly, the objectives of this research are to assess the concentration of particulate ROS in select indoor and outdoor environments, as well as to understand the potential health effects of exposure to ROS. Specifically, the main objectives are to determine:

- The relative concentrations of indoor and outdoor particulate ROS (on total suspended particles (TSP) and $PM_{2.5}$) in residential and commercial buildings,
- The effect of environmental factors on the indoor concentration of particulate ROS,
- The effect of selected sources, such as ozone and terpene concentrations, on the indoor concentration of particulate ROS through controlled studies,

- The seasonal variation in the outdoor concentration of particulate ROS and the environmental factors that influence this variation, and
- The potential health effects of exposure to ROS and products of ozone-initiated chemistry.

Results from this research will help provide a better understanding of the concentrations of particulate ROS in the places where we spend most of our time and also provide insight into the potential health effects of products of ozone-initiated reactions.

1.3 ORGANIZATION

This dissertation is divided into two parts. The first part is an executive summary containing the motivation behind the investigation into particulate ROS and its potential health effects, overview of methods, discussion of results and the overall conclusions of the research. The second part is made up of appendices, namely four research articles that result from this research as well as an appendix containing detail measurements taken during controlled studies at the Test House. Three of the research articles have already been published in peer-reviewed journals and one is under review. The five appendices are as follows:

- Appendix A: Khurshid, S. S., Siegel, J. A., Kinney, K. A., "Indoor Particulate Reactive Oxygen Species Concentrations". Published in Environmental Research (2014), 132, 46-53.
- Appendix B: Khurshid, S. S., Siegel, J. A., Kinney, K. A., "Particulate Reactive Oxygen Species Concentrations and their association with Environmental Conditions in an Urban, Subtropical Climate". Published in Atmospheric Chemistry and Physics (2014), 14, 6777-6784.
- Appendix C: Khurshid, S. S., Siegel, J. A., Kinney, K. A., "Particulate Reactive Oxygen Species on Total Suspended Particles – Measurements in

Residences in Austin, Texas". Submitted to Building and Environment as an invited article.

- Appendix D: Anderson, S. E., Khurshid, S. S., Meade, B. J., Lukomska, E., Wells, J. R., "Toxicological Analysis of Limonene Reaction Products using an *in vitro* Exposure System". Published in Toxicology in Vitro (2013), 27, 721-730.
- Appendix E: Concentrations of Volatile Organic Compounds in Test House during Controlled ROS Studies.

Chapter 2: Background

This chapter presents a brief review of the background literature in support of the research objectives outlined at the end of Chapter 1. It describes pathways for the formation of particulate ROS in indoor and outdoor environments, typical concentrations of ROS reported in the literature, potential health effects of ROS, and exposure models that have been used to assess the health effects of these kinds of pollutants.

2.1 Particulate Reactive Oxygen Species

ROS, such as hydrogen peroxide, are formed in the atmosphere through photochemical reactions involving ozone, NO_x, carbon monoxide (CO), formaldehyde and volatile organic compounds (VOCs) (Gunz and Hoffman, 1990; Finlayson-Pitts and Pitts, 2000; Seinfeld and Pandis, 2006). In indoor environments, ozone-initiated reactions with certain chemicals (such as terpenes) can be an important pathway for ROS formation (Weschler, 2006; Venkatachari et al., 2007; Paulson & Orlando, 1996; Wayne et al., 1991). Unsaturated hydrocarbons, such as terpenes, are prevalent inside buildings (Brown et al., 1994; Wallace et al., 1987 & 1991) and are emitted from sources such as cleaning products (Zhu et al., 2001), air fresheners (Singer et al., 2006a & 2006b; Steinemann, 2009; Steinemann et al., 2011), and wood products (Hodgson et al., 2000). These types of consumer products are ubiquitous in indoor environments; for instance, the U.S. Federal Register (2007) reports that air fresheners are used in approximately 70% of U.S. homes.

Reactions between ozone and unsaturated hydrocarbons produce a variety of compounds ranging from short-lived species – such as ozonides, Criegee bi-radicals, and radicals such as nitrate (NO_3•), hydroxyl (•OH), hydroperoxy (HOO•), organic peroxy (ROO•), and alkoxy (RO•) radicals – to stable gases – such as aldehydes, ketones, carboxylic acids (COOH), hydroperoxides (ROOH), nitric acid (HNO_3), and nitrous acid

(HONO) – and organic aerosols that condense or self-nucleate from low vapor pressure gases and contribute to the growth of secondary organic aerosols (SOA) (Weschler and Shields, 1997 & 1999; Weschler, 2000; Weschler, 2003; Wells, 2005; Destaillats et al., 2006; Sarwar et al., 2007; Coleman et al., 2008; Forester et al., 2009; Weschler, 2009). Ozone can react with organic compounds at fast enough rates that the reaction products can accumulate indoors despite removal by air exchange processes (Weschler, 2006). Several products of ozone-initiated reactions contain ROS or can generate ROS. ROS include free radicals such as the hydroxyl (•OH), hydroperoxy (HOO•), and alkyl peroxy radicals (ROO•), molecules such as hydrogen peroxide (H_2O_2) and organic peroxides (ROOR'), and ions such as the hypochlorite ion (OCl^-) and the superoxide anion (O_2^-). Recent research indicates that hydroxyl radical (which is one of the most important oxidants) can not only be formed in indoor air via ozonolysis of alkenes as was previously thought, but also by photolysis of nitrous acid (HONO) with direct solar irradiation filtering into a room through windows (Alvarez et al., 2013). With an increased understanding of the indoor pathways of hydroxyl radical generation, the formation of secondary species, including ROS, in indoor environments gains importance.

People can be exposed to gas-phase or particulate ROS. Gas-phase ROS may constitute as little as 10% of the total ROS (Hung et al., 2001) or up to 85% of the total ROS (Huang et al., 2005) depending on the source of the ROS. However, due to its high water solubility and molecular diffusivity, most gas phase ROS will likely be removed by the wet mucus lining in the upper airways (Friedlander & Yeh, 1998; Wexler and Sarangapani, 1998; Sarangapani and Wexler, 2000). Particulate ROS, on the other hand, can reach deep into the lungs, especially if the particles are in the respirable range. Results from studies on particle deposition due to impaction indicate that particles

smaller than 3 μm are more likely to deposit in the deep lungs (Carvalho et al., 2011). SOA are among the reaction products of ozone-initiated reactions with terpenes. SOA can also form by condensation of low vapor pressure gases and subsequently grow in size as more matter adsorbs onto the particles. They typically range on the order of 1 nm – 300 nm which makes it very likely for them to reach the smallest air passages in the lungs without being removed in the upper lungs by gravitational settling, interception or impaction. Gas-phase ROS and other soluble pollutants, which may typically be removed in the upper regions of the respiratory tract, can reach the lower lungs once they sorb onto SOA. Indoor SOA formation has been reported to increase with lower air exchange rates, higher indoor VOC emission rates, lower indoor temperature, higher outdoor ozone concentrations, and higher outdoor particle concentrations (Sarwar et al., 2003, 2004 & 2007). Studies have found that ozone-initiated reactions with terpenes lead to the co-formation of peroxides and particles, which can increase the likelihood of generating particulate ROS (Li et al. 2002; Docherty et al. 2005; Fan et al. 2005; Venkatachari & Hopke, 2008a; Chen & Hopke, 2009a; Chen & Hopke, 2009b; Chen & Hopke, 2010; Chen et al., 2011).

2.2 HEALTH EFFECTS OF EXPOSURE TO ROS

Exposure to fine particulate matter (PM) is linked with lung cancer and cardiopulmonary mortality (Samet et al., 2000; Pope et al., 2002 & 2004; Pope and Dockery, 2006). The fact that even relatively low concentrations of ambient PM can lead to apparent health effects, has spurred additional research on PM, including trying to identify the components of PM that can lead to respiratory (Pope et al., 1991; Pope and Dockery, 1992) and cardiovascular (Pope et al., 2004) illness, and other adverse health effects. While the pathways linking exposure to PM with cardiopulmonary illnesses have not been fully understood, PM-mediated generation of ROS in the human body has been

proposed as a contributing factor in the adverse health effects related to exposure to PM (Li et al., 2008; Shen et al., 2011). ROS-induced pulmonary and systemic oxidative stress has been implicated as an important molecular mechanism of PM-mediated toxicity in a rat exposure study (Gurgueira et al., 2002). Exposure to ROS on fine PM has been shown to augment the biological effects of exposure to fine PM in rats (Morio et al., 2001). ROS can alter the production of inflammatory mediators in alveolar macrophages and lung epithelial cells (Morio et al., 2001; Anderson et al., 2013).

Under normal conditions, ROS are generated in the body to defend against foreign organisms and other environmental challenges such as diesel exhaust particles (Kenyon and Liu, 2011; Riedl & Diaz-Sanchez, 2005). In addition, cells have a range of defenses, including several anti-oxidants, to prevent oxidative damage to DNA, proteins, and lipids. However, when homeostatic mechanisms fail to keep pace with excessive ROS generation and exposure, detrimental effects of ROS can become evident (Kehrer et al., 1993). An improved understanding of the role of free radicals in the functioning of the immune system would help define their precise role in the immune system, but the present literature certainly suggests that free radicals and ROS may be important factors in modulating how an organism ultimately responds to injury and disease (Kehrer et al., 1993).

Several *in vitro* and some *in vivo* studies have established the involvement of intracellular ROS in different pathologies. In particular, ROS has been implicated as a central agent in many pulmonary diseases, as well as in oxygen toxicity disorder (Kehrer et al., 1993). ROS likely play a role in chronic airway inflammation in people with asthma, as demonstrated by the presence of H_2O_2, CO and nitric oxide (NO) in the exhaled breath of these people. While it could be argued that ROS production is the consequence of airway inflammation, there is good evidence that ROS is one of the

primary causes of pulmonary inflammation, e.g. O_2^- generation has been demonstrated at sites of allergen challenge in the human lung (Li et al., 2003; Bowler et al., 2002; Sanders et al., 1995; Stevens et al., 1995; Lansing et al., 1993). Furthermore, ROS generated chemically or enzymatically has been shown to oxidatively modify DNA in both *in vivo* and *in vitro* studies (Klaunig & Kamendulis, 2004). It has been suggested that increased concentrations of active oxygen, organic peroxides and radicals can promote initiated cells to uncontrolled growth, such as in a tumor (Cerutti et al., 1985). Peroxynitrites and nitrogen oxides have also been implicated in cancer formation (Klaunig & Kamendulis, 2004).

It should be noted that studies on particulate ROS in the environment (in the literature as well as the present study) are motivated by the observed health effects of *intracellular* ROS. Epidemiological studies have mainly demonstrated that increased exposure to PM is associated with increased respiratory, cardiovascular, and malignant lung disease (Samet et al., 2000; Pope et al., 2002; Bell et al., 2004). The components of PM that mediate progression of these diseases have not been determined. The present study captures a biologically relevant property of PM (i.e. ROS on PM) in an effort to address the research objectives and contribute towards developing a better understanding of the components of PM that mediate the adverse health effects of PM.

2.3 Studies on Indoor and Outdoor Concentrations of ROS

Despite their prevalence and potential health effects, ROS have mainly been studied in outdoor environments and only one study has assessed the concentration of particulate ROS in an indoor environment (in a university building in Singapore: See et al., 2007). Several studies have assessed the factors that influence the formation of ROS under controlled conditions in chambers (Chen et al., 2011; Chen et al., 2009a; Chen et al., 2009b; Chen et al., 2010; Docherty et al., 2005). However, indoor environments are

much more complex in that several ROS precursors are present and there is the possibility of unfiltered outdoor particulate ROS and ROS precursors to penetrate indoors. Furthermore, residential buildings constructed in the last two decades tend to be tighter than dwellings constructed prior to the 1970s (Weisel et al., 2005; Persily et al., 2010), even though the same is not true for commercial buildings (Persily, 1999). Tighter buildings can trap indoor pollutants and their reaction products. Given that Americans stay indoors for 87% of the time and inside cars for 6% of the time (Klepeis et al., 1996 & 2001), it is crucial to determine indoor particulate ROS concentrations and determine the principle factors that influence these concentrations. The importance of assessing indoor particulate ROS concentrations is further reinforced by the work of Lai et al. (2000) who found that the population inhalation transfer factor (also known as intake fraction) for an indoor emission source can be up to five orders of magnitude higher than for an outdoor emission source (10^{-3} to 10^{-1} indoors as compared to 10^{-6} to 10^{-3} outdoors). This implies that exposure to indoor particulate ROS may be more significant than exposure to outdoor particulate ROS.

Given that there are substantial differences in the HVAC systems, and operation and ventilation strategies used in residential and commercial buildings, it is necessary to determine the particulate ROS concentrations in both types of buildings. Residential buildings generally do not have outdoor air intakes and rely on infiltration for air exchange with the outside. However, commercial buildings generally have dedicated outdoor air intakes which make them much more susceptible to outdoor pollutants (Chao and Chan, 2001; Bennett et al., 2012). The differences between residential and commercial buildings can lead to very different exposure profiles and makes it important to sample in both types of buildings.

While ROS in indoor environments has not received much attention, several studies have measured the concentrations of H_2O_2 and other ROS in the outdoor environment since the late nineteenth century (Schone, 1874), but mainly in rainwater, snow and gas-phase in the troposphere (Singh et al., 1986; Gunz and Hoffman, 1990 and references within; Ayers et al., 1992; Dollard and Davies, 1992; Yamada et al., 2002; Liu et al., 2003; Zhang et al., 2012). Fewer studies have measured the concentration of *particulate* ROS or H_2O_2 in outdoor environments (Hewitt and Kok, 1991; Hung and Wang, 2001; Hasson and Paulson, 2003; Venkatachari et al., 2005; Arellanes et al., 2006; Venkatachari et al., 2007; See et al., 2007; Wang et al., 2010; Shen et al., 2011). The majority of these studies collected particle samples over short periods of time and were not able to assess seasonal variations in particulate ROS concentrations. A few studies have measured H_2O_2 (Shen et al., 2011), •OH (Vidrio et al., 2009) and ROS (Baulig et al., 2004) generated from particles collected in different seasons, but their study objectives were slightly different in that they assessed the effect either in lung epithelial cells or in surrogate lung fluid. Furthermore, they generally did not measure a range of ambient environmental conditions during PM sampling. It is important to understand how the ROS concentration on respirable PM varies as environmental conditions change, not only to better understand the driving forces behind this pollutant but also because outdoor particulate ROS concentrations can directly influence indoor particulate ROS concentrations by infiltrating through the building envelope.

Several studies which have sought to measure the overall outdoor particulate ROS concentration, as opposed to individual ROS concentrations, have reported high background values for blank filters (22-75% of field samples) (Hung and Wang, 2001; Venkatachari et al., 2005; Venkatachari et al., 2007). Assessing the overall concentration of particulate ROS helps in developing a more accurate understanding of the oxidative

potential of PM. However, the high background values reported for blank filters highlights the need to optimize the analytical method for assessing particulate ROS concentrations. This is essential to ensure the reliability and sensitivity of the results.

2.4 EXPOSURE STUDIES

In addition to measuring the concentration of particulate ROS, there is a need to better understand the potential health effects of secondary pollutants such as ROS. Indoor oxidation reactions produce a range of oxygenated species including free radicals, secondary ozonides, epoxides, aldehydes, ketones, acids, diacids, dicarbonyls, and other oxygenated species (Weschler 2000 & 2006). These reaction products have been shown to produce respiratory and eye irritation in acute exposures over relatively short time periods mostly in animal models (Clausen et al., 2001; Rohr et al., 2002 & 2003; Wolkoff et al., 1999, 2000 & 2012). In studies done in humans, eye blink frequency has been shown to increase upon 20-minute exposure to high concentrations (one to two orders of magnitude higher than mean indoor concentrations) of VOCs and ozone (Kleno et al., 2004). However, short exposures (2 hours) to acute concentrations of VOCs (ppm) and ambient concentrations of ozone (40 ppb) have not been found to increase symptoms in humans (Fiedler et al., 2005; Laumbach et al., 2005).

Given the discrepancy in the results from different models and the need to assess health effects of longer exposures, other exposure models need to be explored. For instance, the health effects of several nanoparticulate aerosols have been studied *in vitro* with human lung epithelial, and human and murine alveolar macrophage cell lines (Soto et al., 2005, 2006, 2007, & 2008) and *in vivo* with animal models (Lam et al., 2004). Both cell and animal studies have found deleterious health effects including cytotoxicity, lung lining inflammation and dermal inflammation in response to exposure to nanoparticles. However, there are currently no established guidelines for determining the potential

toxicity of particles in the lung or any other organ, which has led to a wide range of methods, cell types, animal models and endpoints being used in these studies (Card et al., 2008). Continued investigation into the mechanisms underlying the adverse *in vitro* and *in vivo* effects is needed in order to develop a better understanding of the potential health hazards associated with exposure to different pollutants.

Animal studies are complicated to perform and cannot be used as an accurate representation of the response in humans. *In vitro* models cannot simulate the full range of physiological processes that influence a pollutant inside the human body, and that the pollutant in turn affects. However, because they are more convenient to use, researchers are able to test several experimental conditions with them. In addition, recently developed *in vitro* models are coming closer to simulating a subset of *in vivo* conditions (The Engineer, 2013) which makes their results more physiologically relevant.

CULTEX and Vitrocell are commercially developed cell exposure chambers that have been designed to expose pollutants to lung cells at the air-liquid interface to mimic exposure in the human lungs. CULTEX has been referenced in the literature since 1999 and has been used to assess the effects of a variety of air pollutants including cigarette smoke, VOCs and carbonyl compounds (Aufderheide et al., 1999 & 2000; Pariselli et al., 2009; Okuwa et al., 2010). Vitrocell has been used more recently in two studies (Gminski et al., 2010; Anderson et al., 2010). Gminski et al. (2010) assessed the cytotoxicity and genotoxicity of VOCs emitted from pine boards and oriented strand boards and found that 1-hour exposures did not produce any detectable response in the lung cells. Anderson et al. (2010) evaluated changes in inflammatory cytokine expression of lung epithelial cells after exposure to dicarbonyls that are produced from ozone-initiated reactions: diacetyl, 4-oxopentanal (4-OPA), glyoxal, methyl glyoxal and glutaraldehyde. They found that exposure to 4-OPA produced the greatest response with significantly elevated

levels of all inflammatory cytokines tested (IL-8, IL-6, GM-CSF, TNF-α). Exposure to the other dicarbonyls also increased inflammatory cytokine expression, especially IL-8 and IL-6. These *in vitro* exposure models enable researchers to compare the relative inflammatory effects of different pollutants and can be used to compare the inflammatory potential of products of ozone-initiated reactions (including ROS) with the inflammatory potential of precursor pollutants. Furthermore, recent advances in cell culture have led to the development of conglomerate lung tissue comprised of several cell types (basal, goblet and ciliated cells) that can be used in these exposure models to better represent the human respiratory epithelium as compared to cell monocultures (Anderson et al., 2013).

The overall goal of this research is to develop a better understanding of ROS as a biologically relevant property of PM that mediates the adverse health effects of PM. A review of the literature shows that very little is known about particulate ROS in indoor environments. However, extended exposures to ROS can occur inside buildings and several precursors of ROS are present indoors which makes it important to study ROS in indoor environments. Furthermore, the potential health effects of products from ozone-initiated reactions, such as ROS, are not well understood and need further study. The following chapter describes the sets of experiments conducted to address the specific research objectives.

Chapter 3: Methods

This chapter summarizes the rationale for the analytical method selected to assess particulate ROS and briefly describes the analytical methods for each set of experiments. Specifically, the experiments conducted in the method development phase of the research project are described, followed by a description of the sampling protocol for particulate ROS in residential and commercial buildings. After that, a controlled set of experiments to determine the influence of different sources on the indoor concentration of particulate ROS concentrations is described. The sampling protocol for particulate ROS in outdoor air over the course of a year is described next. Finally, methods are presented for the toxicological analysis of ozone-initiated reaction products that include ROS. For detailed study methods, please refer to Appendices A-D.

3.1 Method for Measuring ROS

The most common method to measure particulate ROS includes capturing particle phase ROS on a filter and using a fluorogenic indicator to determine the concentration of ROS on the filter. Fluorogenic indicators are popular as they are relatively easy to use and provide rapid response times. 2',7'-dichlorofluorescin diacetate (DCF-DA) is probably the most commonly used fluorescent reagent for detecting ROS species because of its non-specificity for ROS species (LeBel et al., 1992). DCF-DA is a cell-permeable, sensitive indicator of most reactive oxygen species (ROS). DCF-DA becomes fluorescent in the presence of a wide variety of ROS including, but not limited to, hydrogen peroxide (H_2O_2), peroxyl (ROO•) and hydroxyl (•OH) radicals and the peroxynitrite anion (ONOO-) (Zhu et al., 1994; Kooy et al., 1997). As such, ROS is an operationally defined quantity determined by the conversion of a non-fluorescent compound to a fluorescent one.

DCF-DA carries two acetate groups. After hydrolysis of the diacetate groups by cytosolic esterases or base-catalyzed cleavage of the diacetate groups, 2',7'-dichlorofluorescin (DCFH) is oxidized by reactive oxygen species (if present) to the highly fluorescent product 2',7'-dichlorofluorescein (DCF). Various studies have analyzed the oxidation pathways of DCFH (Zhu et al., 1994; Kooy et al., 1997) and the proposed mechanism of reactions is depicted in Figure 3-1. Formation of DCF can be

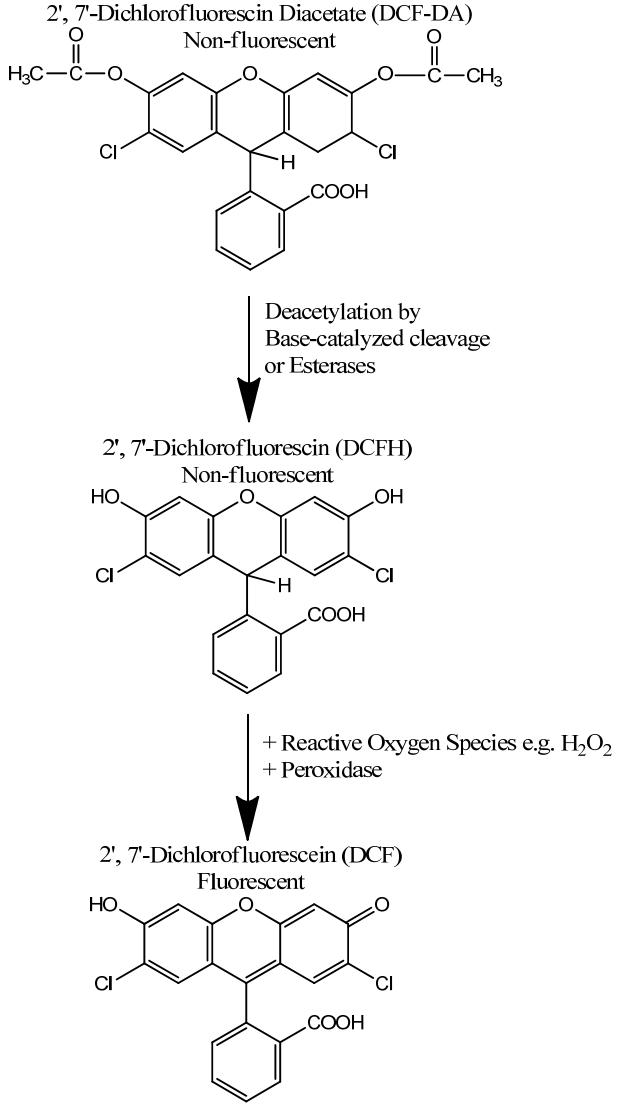

Figure 3-1: The proposed mechanism of reactions DCF-DA undergoes to form the fluorescent compound DCF (adapted from Bass et al., 1983).

monitored by fluorescence spectroscopy with excitation at 485 nm and emission read at 530 nm. Additionally, DCF can also be monitored with absorbance spectroscopy at 500 nm (ε = 79,500 M^{-1} cm^{-1}). The overall method was initially developed by Cathcart et al. (1983) and Bass et al. (1983). LeBel et al. (1992) improved the method with the use of Fe^{2+} or horseradish peroxidase (HRP) as the catalyst.

DCF-DA has been used as a measure for antioxidants in food extracts (Adom et al., 2005), ROS in ambient aerosols (Hung and Wang, 2001; Venkatachari et al., 2005 & 2007; See et al., 2007), as well as – most popularly – for the degree of overall oxidative stress in cells, including physiologically sensitive cells such as brain neurons and other cells (Scott et al., 1988; LeBel et al., 1989, 1990, 1991 & 1992; Bondy et al., 1990 & 1991; Rosenkranz et al., 1992; Oyama et al., 1994; Baulig et al., 2004).

Two studies have comparatively assessed different methods for measuring ROS (Venkatachari & Hopke, 2008b; Molecular Probes product sheet, 2005). Venkatachari and Hopke (2008b) evaluated three methods for their response to specific oxidants and the linearity of response: (i) the method of reduction of oxygen by dithiothreitol (DTT) (Cho et al., 2005), (ii) the peroxidase enzyme catalyzed reaction of hydroperoxides with *p*-hydroxyphenylacetic acid (POHPAA) (Li et al., 2002; Fan et al., 2005), and (iii) the DCFH method described above. The Molecular Probes product sheet (2005) compared aminophenyl fluorescein (APF) and hydroxyphenyl fluorescein (HPF) to DCFH. APF and HPF were developed to be more resistant to light-induced autooxidation than DCF-DA and are useful for quantifying certain types of ROS (Setsukinai et al., 2003). The relative fluorescence of APF, HPF, and DCFH in response to different ROS is given in Table 3-1. Both Venkatachari and Hopke (2008b) and the Molecular Probes product sheet (2005) demonstrated that DCFH provides the broadest response to oxidants, making it the best bulk measure of ROS currently available.

Table 3-1: Fluorescence response of three ROS indicators – DCF-DA, APF, HPF – to various reactive oxygen species. 10 μM of APF, HPF, or DCFH were added to sodium phosphate buffer and each of the ROS species listed. Fluorescence was measured using excitation/emission wavelengths of 490/515 nm for APF and HPF, and 500/520 nm for DCFH (adapted from Molecular Probes product sheet for products A36003 and H36004).

ROS species	**ROS Generation Method**	**APF**	**HPF**	**DCFH**
•OH	100 μM of ferrous perchlorate (II) and 1 mM of H_2O_2	1200	730	7400
ONOO-	3 μM of ONOO-	560	120	6600
-OCl	3 μM of -OCl	3600	6	86
O_2	100 μM of 3-(1,4-dihydro-1,4-epidioxy-1-naphthyl)propionic acid	9	5	26
$\bullet O_2^-$	100 μM of KO_2	6	8	67
H_2O_2	100 μM of H_2O_2	<1	2	190
NO	100 μM of 1-hydroxy-2-oxo-3-(3-aminopropyl)-3-methyl-1-triazene	<1	6	150
ROO•	100 μM of 2,2'-azobis(2-amidinopropane), dihydrochloride (AAPH)	2	17	710
Auto-oxidation	2.5 hrs exposure to fluorescent light source	<1	<1	2000

3.2 ANALYTICAL PROTOCOL

The method for assessing particulate ROS using DCF-DA (Hung and Wang, 2001; Huang et al., 2005; Venkatachari et al., 2005; Venkatachari et al., 2007; See et al., 2007; Chen and Hopke, 2009) was modified to reduce background signal in the samples of particulate ROS. A reagent was prepared by incubating 0.5 ml of 1 mM 2',7'-dichlorofluorescin diacetate (DCF-DA, Cayman Chemical, MI, USA) in ethanol with 2 ml of 0.01 N NaOH at room temperature for 30 mins in the dark to cleave off the acetate groups. After the 30 min incubation period, the 2',7'-dichlorofluorescin (DCFH) solution was neutralized with 10 ml sodium phosphate buffer (pH 7.2) and the solution was kept on ice in the dark till needed. Each sampling filter was sonicated in 5 ml sodium phosphate buffer for 10 minutes. Horseradish peroxidase (HRP, ThermoScientific, IL,

USA) in sodium phosphate buffer (pH 7.0) and DCFH were then added to the solution to yield a final volume of 10 ml with a concentration of 5 μM of DCFH and 1 unit/ml of HRP. After incubation at 37˚C for 15 mins, 0.1 ml aliquots were placed in triplicate in a 96-well plate and the fluorescence intensity was read at 530 nm with excitation at 485 nm (Synergy HT, Biotek, VT, USA).

3.3 METHOD DEVELOPMENT AND QUALITY ASSURANCE

Sonication of Sample Filters

Sonication of DCFH may cause auto-oxidation of the reagent into the fluorescent compound, dichlorofluorescein (DCF). This can lead to high fluorescence intensities being detected for blank filters (Hasson and Paulson, 2003). In order to determine the influence of sonication times on the fluorescence intensity generated by blank filters, PTFE sampling filters (TF1000, 1μm pore size, 37 mm, Pall, NY, USA) were sonicated in (i) 10 ml DCFH-HRP solution for 10 minutes, (ii) 10 ml of DCFH-HRP solution for 5 minutes, and (iii) 5 ml buffer for 10 minutes followed by addition of 5 ml reagent to achieve the same final concentration of DCFH-HRP as in (i) and (ii). The rest of the protocol was followed as described above.

Impact of Filter Selection

The background fluorescence of several types of particle sampling filters used in Personal Environmental Monitors (PEMs) (SKC, PA, USA) was assessed in order to select a suitable filter for sampling. Each filter was sonicated in 5 ml buffer, as described in the protocol, followed by addition of DCFH-HRP. Based on their low background fluorescence, PTFE filters were selected for sampling in the commercial buildings. In order to ensure that background fluorescence was minimized to enhance the sensitivity of the measurements, different protocols for washing glassware were also compared. Five

ml buffer was added to empty beakers, which were then sonicated, followed by addition of 5 ml DCFH-HRP. The beakers were then incubated at 37˚C for 15 mins, after which the fluorescence was read.

Degradation of ROS

To assess the degradation of ROS after collection, total suspended particles (TSP) were collected at an outdoor sampling location on a lawn at the University of Texas at Austin campus, 0.6 miles from a major highway. Six filter holders (SKC, PA, USA) were used to sample TSP at 10 L/min on two days in October 2012 for 3±0.25 hours between 11am and 2pm. The concentration of ROS on three filters was assessed right after sampling and the remaining three filters were analyzed after 24 hours of storage at room temperature.

3.4 PARTICULATE ROS IN RESIDENCES AND COMMERCIAL BUILDINGS

An extensive field sampling campaign was conducted to measure the concentration of particulate ROS on TSP and $PM_{2.5}$ in different kinds of buildings. The residential sampling was conducted in two phases. In the first phase, total suspended particles (TSP) were collected at eight homes on PTFE filters using filter holders (SKC, PA, USA) on different days in October 2012. Sampling was conducted for 3±0.25 hours between 11am and 2pm using air sampling pumps at 10 L/min. All pumps were calibrated before sampling with a mini-Buck Calibrator M-30 (A.P.Buck, Orlando, FL; accuracy ±0.5%). Triplicate samplers were placed 1m above the ground outside and in a central room inside the homes. Some deviations in the sampling protocol caused by occupants are described in Appendix C. At six of the eight homes where TSP was collected, indoor $PM_{2.5}$ was also collected using triplicate Personal Environmental Monitors (PEM, SKC, PA, USA) to compare relative concentrations of particulate ROS

on TSP to that on $PM_{2.5}$. Teflon tape was wrapped around the edges of the support screen in the PEMs to ensure a proper seal of the thin Teflon filters inside the PEMs. In the second phase of the residential sampling effort, indoor and outdoor $PM_{2.5}$ was collected in a different set of twelve homes using duplicate PEMs from March to August 2012 (for details on $PM_{2.5}$ sampling, see Appendix A). All homes were located in Austin, Texas. Field blanks were periodically used to check that there was no significant difference in fluorescence between unsampled filters and field blanks. All sampling filters were transported to the lab and assessed with the same method within 1 hour of collection.

The sampling in commercial buildings was conducted in institutional buildings and retail stores. For the sampling in institutional buildings, indoor and outdoor samples of $PM_{2.5}$ were collected at six buildings located on the University of Texas at Austin campus using PEMs during March and July 2012. For the sampling in retail stores, indoor and outdoor samples of $PM_{2.5}$ were collected at five retail stores in Austin, Texas using PEMs during January-April 2012. Sampling was conducted in the same way at all buildings. The main exception to this is that at retail sites 1-3, indoor and outdoor sampling was not conducted simultaneously but on consecutive days, due to the availability of a single sampler. Sampling was repeated on two or more days at selected sites for each type of building (namely, at three homes, one institutional building, and all of the five retail stores).

The concentration of ROS on the sampled filters was expressed in terms of H_2O_2 per volume of air sampled (rather than per mass of particles) because this describes exposure to ROS as it occurs in the lungs (Boogaard et al., 2012). To prepare the standards, 0.1 ml aliquots of appropriate H_2O_2 concentrations were added to 3 ml of DCFH-HRP reagent in glass tubes to yield 0, 1.0, 2.0, 3.0, and 4.0×10^{-7} M H_2O_2 in the final solutions. These tubes were incubated at 37°C for 15 minutes and fluorescence was

measured. All glassware used in the experiments was scrubbed with soap, followed by immersion in a 10% nitric acid bath and subsequent 7× rinse with deionized (dI) water. The Method Detection Limit (EPA, 2011) of the analytical procedure was 1.2 nmoles H_2O_2/l, which converts to 0.01 nmoles/m^3 assuming a 3-hour sample at 10 l/min.

Graphical representations of the data and Shapiro-Wilk tests for normality indicated that most of the datasets were not normally distributed. Thus, the non-parametric Spearman Rank Correlation Coefficient test was used to determine the strength (ρ) and significance ($p<0.05$) of any relationships between the concentration of ROS and environmental factors. Bonferroni adjustments were generally not used as the purpose of this study was to provide a baseline assessment of indoor ROS. The Wilcoxon matched-pairs signed-ranks test was used to assess differences between the indoor and outdoor ROS datasets at the buildings.

Indoor and outdoor air quality parameters (including $PM_{2.5}$ concentration, ozone concentration, total VOC concentration, temperature, and relative humidity) were measured at all buildings during sampling. In some cases, additional parameters were measured, as in the case of the retail buildings and experiments at the test house. Appendices A-C contain details on the instruments used to collect all air quality measurements. The overall uncertainty for each measurement was calculated using standard error propagation to include variance in the measured readings and the uncertainty of the instrument itself.

3.5 Controlled Studies to Study Sources of Indoor Particulate ROS

While ROS formation has been studied in atmospheric contexts, the pathways for ROS formation in indoor environments have not been studied. Indoor conditions present the potential for very different kinds of reactions because of different surface area to volume ratios, light intensities, seed particle concentrations, and source emission profiles.

Controlled experiments were conducted at an unoccupied manufactured house (UTest House) to explore some of the fundamental mechanisms that influence indoor particulate ROS concentrations. The influence of select sources (namely ozone and terpene concentrations) on indoor particulate ROS concentrations was assessed in these experiments. Four sets of indoor conditions were tested: (i) low ozone and low terpene (ii) low ozone and high terpene, (iii) high ozone and low terpene, and (iv) high ozone and high terpene. Each of these four indoor conditions was tested on low and high outdoor ozone days to assess the influence of outdoor ozone concentrations on the indoor conditions. Each condition was tested on three separate days. Indoor and outdoor samples of ROS on TSP were collected and assessed in triplicate on each sampling day. Sampling was conducted in January and July-September, 2014, on 12 days when outdoor ozone concentrations during the 3 hours of sampling were below 40ppb (categorized as low outdoor ozone days) and another 12 days when the outdoor ozone concentrations were above 40 ppb (categorized as high outdoor ozone days). An ozone generator was used to elevate and maintain the indoor ozone concentration at 75-100 ppb for the high indoor ozone cases. For the high terpene concentration cases, 6-7 ml Pine-Sol® (a household cleaner) was applied with a moistened rag on the floor in two rooms of the house which elevated VOC concentrations to 400-500 ppb. VOC samples were collected using sorbent tubes filled with a minimum of 0.11 mg of Tenax GR during the sampling events in July-September, 2014 – the detailed VOC speciation is presented in Appendix E. The sorbent tubes were analyzed using thermal desorption followed by gas chromatograph and mass spectrometry (TD/GCMS). The air exchange rate was measured during all sampling events with the tracer gas method using carbon dioxide (CO_2). Indoor and outdoor particle concentrations, temperature and relative humidity were also measured, details of which are given in Appendix C.

3.6 PARTICULATE ROS IN OUTDOOR AIR

A major source of indoor particulate ROS may be outdoor particulate ROS, which makes it important to assess outdoor particulate ROS concentrations. Furthermore, seasonal variations in particulate ROS concentrations are not well understood. Samples of $PM_{2.5}$ were collected in an open area on the University of Texas at Austin campus using a PEM on 40 randomly selected days during November 2011 and September 2012. Two to five replicate samples were taken on 20 of these days to determine the average covariance in ROS concentration between multiple samplers. Sampling was conducted for 3±0.5 hours between 10am and 3pm using air sampling pumps at 10 l/min. The samples were assessed in the same way as the samples collected inside buildings. Ambient environmental conditions were mainly obtained from the nearest Texas Commission on Environmental Quality (TCEQ) sampling stations (located 6 to 17 miles from the ROS sampling location depending on the environmental parameter).

3.7 TOXICOLOGICAL ANALYSIS OF PRODUCTS OF OZONE-INITIATED REACTIONS

This part of the research was conducted at the National Institute for Occupational Safety and Health (NIOSH) laboratories in Morgantown, West Virginia, led by Dr. Ray Wells and Dr. Stacey Anderson. *In vitro* exposure models enable researchers to compare the relative inflammatory effects of different pollutants and can be used to compare the inflammatory potential of products of ozone-initiated reactions (including ROS) with the inflammatory potential of precursor pollutants. In this research, human alveolar epithelial cells (A549) were exposed to different pollutant mixtures in Vitrocell® exposure chambers. Cells were incubated at 37 °C with 5% CO_2 in F12 K medium (Kaighn's Modification of Ham's F-12 with L-Glutamine, ATCC, VA, USA) supplemented with 10% heat inactivated fetal bovine serum (FBS) and 0.05 mg/ml of Gentamycin. Cells were propagated in sterile cell culture flasks after which they were harvested, counted

and seeded on Costar 24 mm (0.4 μm) transwell inserts and placed in 6-well tissue culture treated plates. Twenty-four hours prior to exposure, the culture medium (which included 10% FBS) was removed and replaced with serum-free medium to synchronize the cells. Immediately before exposures, the culture medium was completely removed from the apical side of the inserts and cells were washed twice with sterile phosphate buffered saline (PBS) and then the inserts were transferred into the Vitrocell® PT-CF exposure system (Vitrocell, Waldkirch, Germany). During exposure, cells were immersed in serum-free medium on the basal surface, allowing cells to be nourished from below while being exposed to gas at the air-liquid interface above the cells. Cells were exposed to clean air, 20 ppm limonene, 4 ppm ozone, or a mixture of 20 ppm limonene and 4 ppm ozone via trumpets raised 0.5 cm above the cell layer at a constant air flow of 3 ml/min. Details on the preparation of the pollutant mixtures as well as details on the complete list of exposure scenarios tested are described in Appendix D. Exposures lasted 1 or 4 hours, after which the cell inserts were transferred to regular 6-well plates with medium containing 10% FBS added on both apical and basal sides. Cells were allowed to recover in the incubator and the concentrations of inflammatory cytokines (IL-8 and MCP-1) in the combined apical and basal culture supernatants were assessed 10-24 hours post-exposure using commercially available ELISA kits according to the manufacturer's instructions.

Similar exposures were conducted with MucilAir™ tissue samples which are human airway epithelium tissue consisting of primary human cells isolated from the nasal cavity, trachea, and bronchus. Commercially available transwell inserts with MucilAir™ epithelium were purchased from Epithelix (Geneva, Switzerland). They were stored in 24-well tissue culture plates containing 0.8 ml of serum free MucilAir™ Culture Medium (Geneva, Switzerland) which did not exceed the air/liquid interface. Similar to the A549

cells, cultures were maintained at 37 °C in a 5% CO_2 incubator, with media being changed every 2-3 days. For exposures of MucilAir™ tissue, the inserts were transferred directly into the Vitrocell® PT-CF exposure system. MucilAir™ inserts were exposed for 1 hour/day, 5 days/weeks for a total of 4 weeks. MucilAir™ inserts were exposed to limonene (500 ppb) or a mixture of limonene (500 ppb) and ozone (100 ppb) via trumpets at a constant air flow of 2 ml/min. Once a week, a washing step was performed using MucilAir™ culture medium to remove accumulated mucus produced by the differentiated tissue. Immediately after each exposure, inserts were transferred to a 24-well plate and fresh MucilAir™ culture medium was added on the basal side. Culture supernatants were collected at 10-12 hours post-exposure and 72 hours after the last exposure of each week for 4 weeks. The concentrations of IL-8, IL-6, MCP-1, and GM-CSF were measured from basal supernatants of MucilAir™ tissues.

Inserts containing unexposed cells (n=3) were included in every experiment to evaluate cellular integrity. These controls were treated in the same way as the experimental cells except for the fact that they were retained in the incubator while the other cells were exposed in the Vitrocell® chambers.

A two-tailed unpaired *t*-test was used to compare inflammatory cytokine production from cells for every pair of pollutants, at each specified time point. Cytokine levels are based on the mean of triplicate samples from each biological replicate at each time point. Linear trend analysis was performed to determine if the test articles had exposure duration-related effects for the specified end-points.

Chapter 4: Results and Discussion

This chapter summarizes the main results from the investigation of particulate ROS in select indoor and outdoor environments. The experimental results are presented in the order in which the experimental methods were described in Chapter 3. Namely, the results of the method development phase of the research study are described first, followed by the results of the particulate ROS sampling campaigns in residential, institutional and retail buildings. The results of the controlled experiments on the influence of ozone and terpene concentrations on indoor particulate ROS concentrations are discussed next. This is followed by results of the year-long sampling campaign for ambient particulate ROS concentrations. The chapter ends with results from the *in vitro* exposure experiments of products of ozone-initiated reactions. For further discussion of the results and the corresponding graphs, please refer to Appendices A-E.

4.1 Method Development and Quality Assurance

Several modifications to the established analytical methods were investigated during the method development phase to reduce background fluorescence of blank filters and improve the sensitivity of the assay. As postulated by Hasson and Paulson (2003), the results of this study indicate that sonication of the DCFH reagent causes auto-oxidation of the fluorescent reagent, leading to high and variable background fluorescence intensities. While sonication helps to suspend particles captured on the sampling filter into the reagent, it was found that sonication in buffer, followed by addition of reagent produced the same net effect but achieved consistently lower background fluorescence readings for blank filters. In order to select the type of sampling filter that produced the lowest background fluorescence, the background fluorescence of eight types of filters typically used in PEMs and filter holders was assessed. PTFE filters were selected as sampling filters because they produced the lowest background signal and were

mechanically resilient. Based on results of the effect of the cleaning protocol on the background fluorescence produced by laboratory glassware, all glassware used in the analytical experiments was first passed through an overnight soak in an acid bath followed by a 7× rinse to remove all traces of contaminants. Details of results pertaining to method development are presented in Appendix A.

As a result of these modifications, the fluorescence intensity of field blanks in this study was lowered below that of previous work on outdoor particulate ROS in which field blanks were reported to have a background fluorescence of 25-75% (Hung and Wang, 2001), 22-56% (Venkatachari et al., 2005) and 28-60% (Venkatachari et al., 2007) of the field samples. The background fluorescence of unsampled filters in this study was 20% of the sampled filters on average (with a range of 7-50%); after correcting for background fluorescence of blank water and reagents, this represents less than 8% of the ROS concentration measured on the sampled filters. The reduced background was beneficial in increasing the reliability and sensitivity of the results obtained in this study.

The results of the degradation studies of particulate ROS indicate that ambient particulate ROS in Austin collected over a 3-hour period remain relatively stable for 24 hours. Previous studies which collected samples over a few minutes (e.g. Chen et al., 2011 and Antonini et al., 1998, from VOC ozonolysis in environmental chambers and welding fumes, respectively) are likely to measure higher degradation rates because their samples contain many more short-lived species than studies which collect samples over a few hours (e.g. the present study). In the present study, volatile species likely decomposed during the 3-hour sampling period and 1-hour post-sampling period when the filters were brought to the lab and prepared for reagent addition. The ROS that remained on the filters were likely more stable (such as peroxides), which is why significant degradation was not observed over the next 24 hours. These results suggest

that the sampling methodology used in this study detects relatively stable species of ROS that are likely to persist in the indoor environment for several hours.

4.2 PARTICULATE ROS IN INDOOR ENVIRONMENTS

Particle samples were collected inside and outside twenty homes, six institutional buildings, and five retail stores in order to determine the concentration of particulate ROS in different kinds of indoor environments.

Particulate ROS in Residential Buildings

TSP samples were collected at eight homes and $PM_{2.5}$ samples were collected at twelve homes. The mean (± s.e.) indoor concentration of ROS on TSP sampled at eight homes (labeled H1-H8) was 1.59 ± 0.33 nmoles/m^3 and the mean outdoor concentration was 2.35 ± 0.57 nmoles/m^3. The indoor and outdoor concentrations of ROS on TSP (Figure 4-1) were significantly different from each other (Wilcoxon matched-pairs signed-ranks test, p=0.049). The indoor concentration of ROS on TSP was, on average, about 75% of the outdoor concentration of ROS on TSP. The fact that ROS on TSP was higher outside than inside may be due to the fact that outdoor environments typically have a higher concentration of coarse particles than indoor residential environments (Jones et al., 2000), and gas-phase and fine particulate ROS can adsorb onto these particles leading to a higher outdoor concentration of ROS on TSP than indoor concentration.

The mean (± s.e.) indoor concentration of ROS on $PM_{2.5}$ sampled at twelve residential homes (labeled R1-R12) was 1.37 ± 0.30 nmoles/m^3 and the mean outdoor concentration was 1.41 ± 0.25 nmoles/m^3. The indoor and outdoor concentrations of ROS on $PM_{2.5}$ were not significantly different (Wilcoxon matched-pairs signed-ranks test, p=0.959). Greater uncertainty in the $PM_{2.5}$ dataset (which was one of the reasons which

led to the inclusion of TSP samples in the study) could have masked differences in the indoor and outdoor $PM_{2.5}$ datasets or it might be that the concentration of ROS on $PM_{2.5}$ inside and outside homes is truly similar. This is an intriguing result because it suggests that transport of outdoor ROS into the buildings or generation of ROS inside the buildings may be as important as photochemical processes generating ROS in outdoor environments. Furthermore, given that people spend the majority of their time at home, the cumulative exposure to particulate ROS in these environments can be considerable. The concentrations of ROS on $PM_{2.5}$ are displayed in Figure 4-2 for all twelve homes as well as for repeated measurements conducted on different days at three of these homes. Details on the results from the repeated measurements at homes are given in Appendix C.

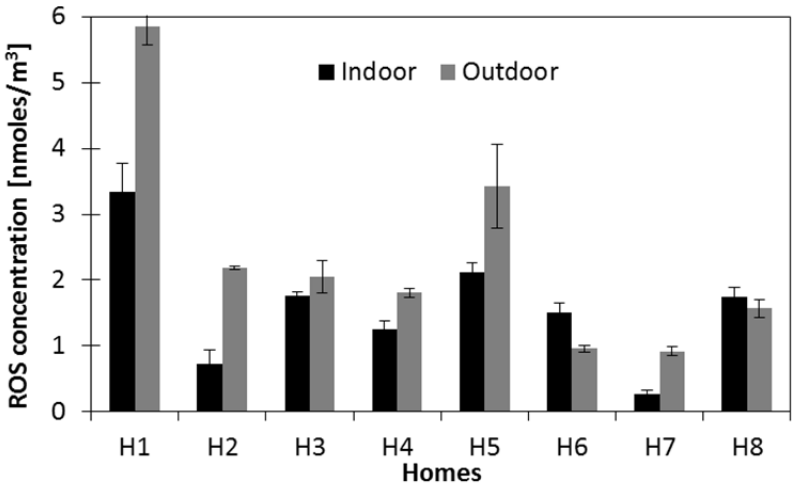

Figure 4-1: Indoor and outdoor concentrations of ROS on total suspended particles (TSP) sampled at eight residential homes. The error bars represent standard error of triplicate samples.

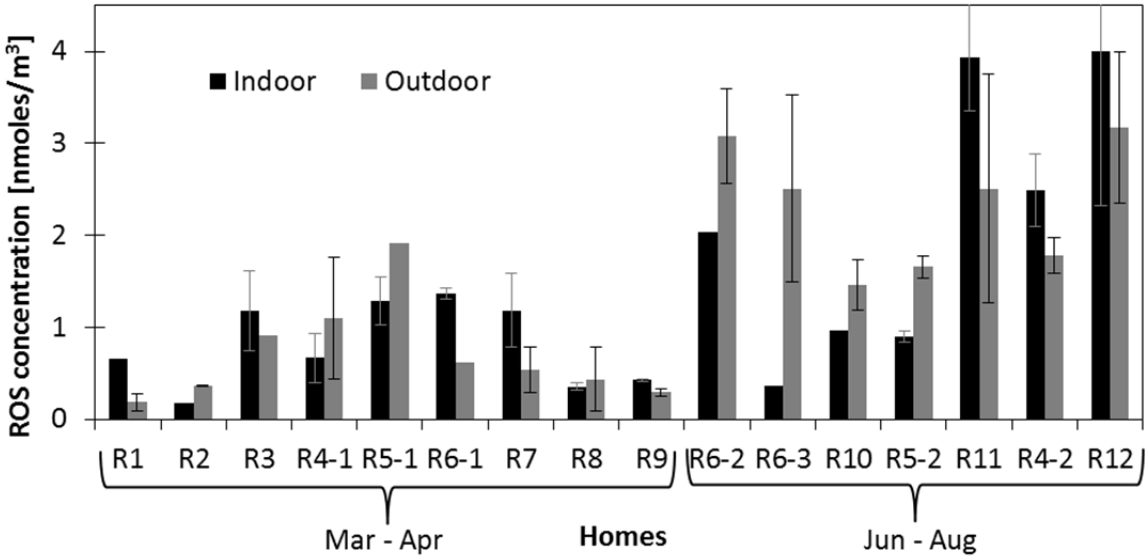

Figure 4-2: Indoor and outdoor concentrations of ROS on $PM_{2.5}$ sampled at twelve residential homes. Repeat sampling was conducted at R4, R5, and R6 under different conditions and a number is appended to these labels to differentiate between multiple visits to the same home. The error bars represent standard error of duplicate samples.

This is one of the first studies to simultaneously assess the indoor and outdoor concentration of particulate ROS. The only other study that the author is aware of that reports the ROS concentration in an indoor environment is See et al. (2007) which recorded a concentration of 3 nmoles/m^3 on $PM_{2.5}$ inside a university building in Singapore. No simultaneous outdoor measurement was made in that study. Other than the indoor study by See et al. (2007), research has mostly focused on ROS in outdoor air. Studies on particulate ROS in outdoor air have reported concentrations ranging from 0.61 nmoles/m^3 in Taipei, Taiwan for PM_{10} (0.54 nmoles/m^3 for $PM_{3.2}$), to 6.11 nmoles/m^3 near Los Angeles around midday during summer for TSP (4.95 nmoles/m^3 for $PM_{2.5}$) (Hung and Wang, 2001; Venkatachari et al., 2005; Venkatachari et al., 2007; See et al., 2007). Our indoor and outdoor measurements either fall in or below the range of outdoor

concentrations reported in these studies. Most of the concentrations we measured were below 3 nmoles/m^3.

Results from the Spearman Rank Correlation Coefficient test show that indoor particulate ROS is associated with outdoor particulate ROS for both ROS on TSP and ROS on $PM_{2.5}$, although the former was only marginally significant (ROS on TSP: ρ = 0.69, p=0.05; ROS on $PM_{2.5}$: ρ = 0.66, p=0.006). This suggests that a link might exist between the indoor and outdoor concentrations of particulate ROS, although the distinction between ROS precursors and ROS itself is still unresolved.

Indoor $PM_{2.5}$ samples were also collected in six of the eight homes where TSP samples were collected. In these homes, the mean indoor concentration of ROS on TSP was 1.72 ± 0.36 nmoles/m^3 and the mean indoor concentration of ROS on $PM_{2.5}$ was 0.90 ± 0.16 nmoles/m^3 (Figure 4-3). The indoor concentrations of ROS on TSP and ROS on

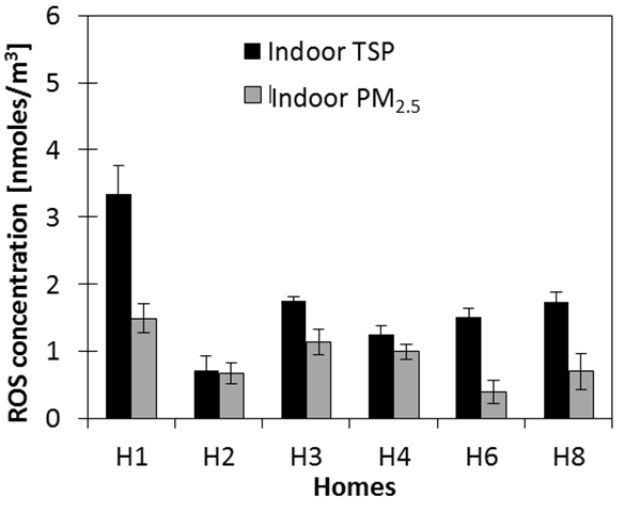

Figure 4-3: Indoor concentrations of ROS on $PM_{2.5}$ and total suspended particles (TSP) sampled at six residential homes. The error bars represent standard error of triplicate samples.

$PM_{2.5}$ were significantly different (Wilcoxon matched-pairs signed-ranks test, p=0.028), indicating that the amount of ROS on particles varies with the size of the particles. Several studies of particulate ROS in outdoor air (Hung & Wang, 2001; Venkatachari et al., 2005; Venkatachari et al., 2007) and in cigarette smoke (Huang et al., 2005) have found that ROS on $PM_{2.5}$ constitutes the majority of the ROS on TSP (44-95 % for outdoor air, 58-96% for cigarette smoke). The ratio of indoor ROS on $PM_{2.5}$ to indoor ROS on TSP determined in the current study was 58±10% which is closer to the lower ratios reported in the literature. These results imply that the majority of particulate ROS in indoor environments can be found on $PM_{2.5}$ similar to that in outdoor environments.

Indoor and outdoor air quality parameters (indoor and outdoor $PM_{2.5}$ concentrations, temperature, and RH, as well as indoor VOC concentration and outdoor ozone concentration) were measured at all homes. The measured parameters and other building characteristics recorded did not appear to have a distinct influence on indoor ROS concentrations which is discussed in Appendices A and C.

Particulate ROS in Commercial Buildings

Commercial buildings typically have higher air exchange rates than residential buildings, which increases the likelihood of ROS and ROS precursors bring brought in from the outside. In order to assess the concentration of particulate ROS in commercial buildings, $PM_{2.5}$ samples were collected at six institutional buildings and five retail stores. The mean (± s.e.) indoor concentration of ROS on $PM_{2.5}$ sampled at six institutional buildings (labeled I1-I6 in Figure 4-4) was 1.16 ± 0.14 nmoles/m^3 and the outdoor concentration was 1.68 ± 0.48 nmoles/m^3. The indoor and outdoor concentrations were not significantly different (Wilcoxon matched-pairs signed-ranks test, p=0.40). The two highest indoor and outdoor particulate ROS concentrations were measured at I2 and I4 which corresponded to some of the highest measurements of indoor and outdoor $PM_{2.5}$

as well as the highest measurements of outdoor ozone. The lowest indoor concentration of ROS was measured at I6, when the outdoor concentration of $PM_{2.5}$ was the lowest in this dataset.

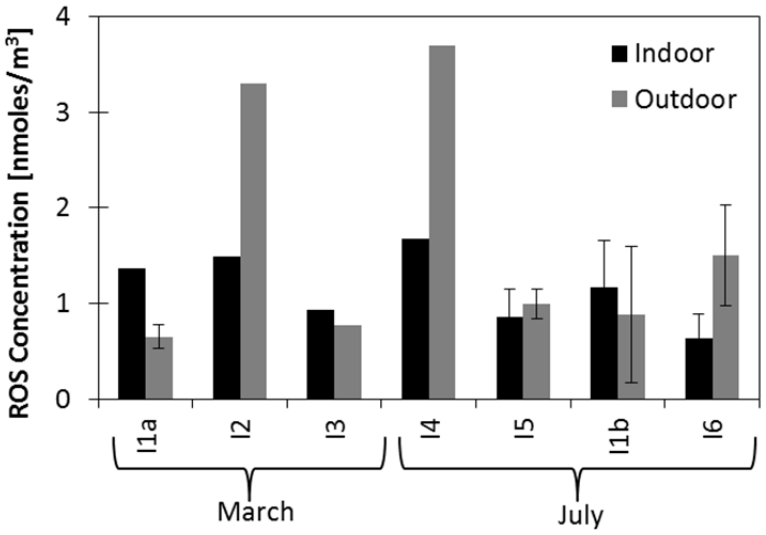

Figure 4-4: Indoor and outdoor concentrations of ROS on $PM_{2.5}$ at six institutional buildings. The error bars represent standard error of duplicate samples when applicable. Repeat sampling was conducted at I1 under different conditions.

The mean (± s.e.) indoor concentration of ROS on $PM_{2.5}$ sampled at five retail stores was 1.09 ± 0.25 nmoles/m^3 and the outdoor concentration was 1.12 ± 0.36 nmoles/m^3 (Figure 4-5). These stores included grocery (Store 1), general merchandise (Stores 2, 4, 5) and furniture (Store 3) stores. The indoor and outdoor concentrations were not significantly different (Wilcoxon matched-pairs signed-ranks test, p=0.35) even if only those measurements that were simultaneously taken inside and outside are considered. Uncertainty in the single-sample measurements could have masked differences in the indoor and outdoor datasets or it might be that the indoor and outdoor concentration of ROS is closely related in these types of buildings as well. If the concentration of ROS is calculated on a mass basis (i.e. nanomoles ROS / μg $PM_{2.5}$ rather

than nanomoles ROS / m^3 air sampled), then indoor ROS concentrations are actually found to be much higher than outdoor ROS concentrations. This is because indoor particle concentrations are typically lower than outdoor particle concentrations (even for fine particles, in most cases). The fact that the concentration of ROS on indoor particles can be much higher than outdoor particles points to the importance of measuring this pollutant in indoor environments.

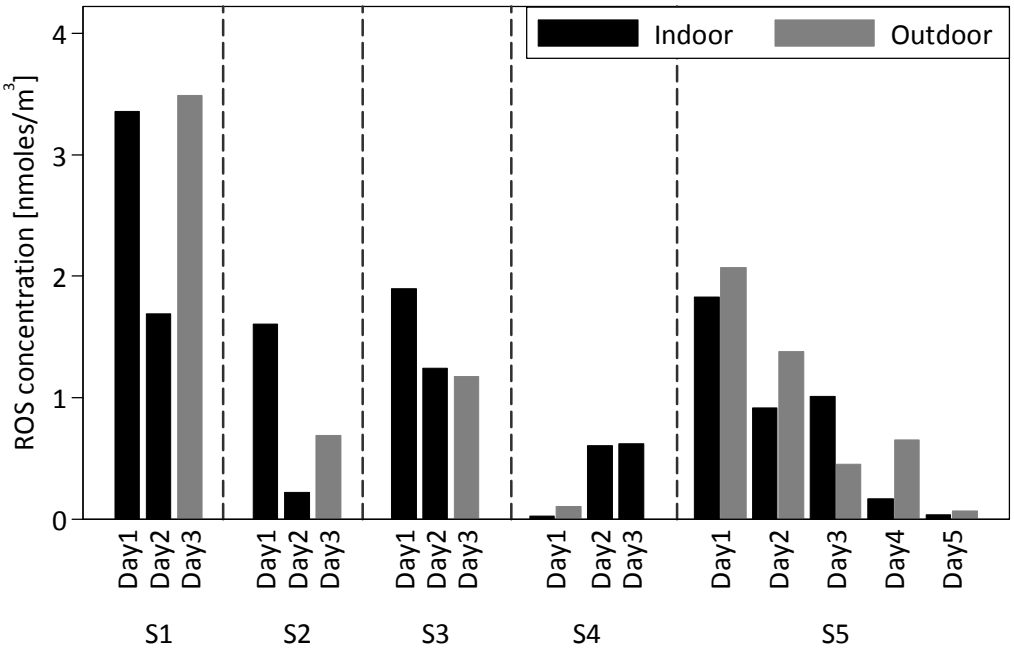

Figure 4-5: Indoor and outdoor concentrations of ROS on $PM_{2.5}$ sampled at five retail stores. A single sampler was used to measure either indoor or outdoor concentrations at Stores 1, 2, 3, and 4 (Days 2 and 3 only). Two samplers were used to simultaneously measure indoor and outdoor concentrations at Store 4 (Day 1) and Store 5 (all days).

Several indoor and outdoor air quality parameters (indoor and outdoor particle concentrations, indoor and outdoor ozone concentrations, indoor and outdoor total VOC concentrations, air exchange rates) were measured during sampling at the retail stores and details are provided in Appendix A. No statistical correlations were found between ROS

and the air quality parameters measured at the retail stores. While this was a limited dataset, the absence of a clear relationship between the concentration of ROS and any specific pollutant may be because the chemistry of ROS formation is quite complex. This has been cited in the atmospheric chemistry literature as a reason for weak or moderate correlations between peroxide concentrations and certain atmospheric conditions (such as ambient ozone concentration) that are thought to influence peroxide concentrations (Logan et al., 1981; Jackson and Hewitt, 1999; Largiuni et al., 2002; Venkatachari et al., 2007). The absence of direct correlations between particulate ROS concentrations and pollutant concentrations also indicates the need to include particulate ROS measurements in indoor air quality studies.

4.3 Controlled Experiments to Study Sources of Indoor Particulate ROS

The results obtained during the field sampling in buildings prompted a search to better understand the origins of indoor particulate ROS. Is indoor particulate ROS mostly derived from outdoor sources where photochemical processes dominate, or are there significant indoor sources that generate particulate ROS? While pathways for ROS formation in outdoor environments have been (and are still being) studied, little is known about ROS formation in indoor environments. Some reaction pathways have been studied in indoor environments but mainly in the context of SOA formation. Chamber studies have sought to address specific questions about the fundamentals of ROS and SOA formation from terpene ozonolysis (Docherty et al., 2005; Chen and Hopke, 2009a; Chen and Hopke, 2009b; Chen and Hopke, 2010; Chen et al., 2011) but the controlled conditions in these chambers are very artificial compared to the actual conditions inside buildings. A whole house presents different surface to volume, source emission, deposition, and air circulation characteristics than an experimental chamber. As a result, a few sets of experiments were conducted at an unoccupied house (UTest House) to

explore some of the fundamental mechanisms that influence indoor particulate ROS concentrations.

The influence of outdoor ozone concentration on indoor generation of particulate ROS was assessed for different indoor conditions (of indoor ozone and terpene concentrations). Mopping the floor of the test house with Pine-Sol® elevated the concentration of several volatile organic compounds (the detailed speciation is given in Appendix E) including some terpene hydrocarbons, such as α-pinene, α-terpineol, β-phellandrene, β-pinene camphene, eucalyptol, which have been shown to be elevated in other indoor experiments with cleaning products (Singer et al., 2006a). While the elevated ozone concentration (75-100 ppb) in the high indoor ozone cases was only realistic of indoor environments which have active ozone generation sources (such as printers or strong ozone-emitting air purifiers), the terpene concentrations were quite realistic of indoor environments where chemical cleaners or other scented consumer products, such as air fresheners, have been used.

Based on the field sampling results discussed in section 4.2, one of the main factors that can likely influence indoor particulate ROS concentrations are outdoor particulate ROS concentrations. As such, it is useful to consider the indoor to outdoor (I/O) ratio of particulate ROS concentrations when comparing particulate ROS concentrations across different indoor and outdoor conditions. The I/O ratio was found to be highest after the floor of the test house had been cleaned with Pine-Sol® and a relatively high concentration of indoor ozone was present (75-100 ppb) (Table 4-1). This was true when outdoor ozone concentrations were low (< 40 ppb) or high (> 40 ppb). The presence of either high indoor ozone concentrations or high indoor terpene concentrations did not elevate the I/O ratio of particulate ROS above the I/O ratio in the base case of low indoor ozone and terpene concentrations. Pine-Sol® contains several hydrocarbons, many

of which are unsaturated and readily react with ozone to form oxygenated organic products including SOA and ROS. The formation of SOA was evident by the increase in indoor particle concentrations measured during these sampling events. Appendix C lists air quality parameters ($PM_{2.5}$ and PM_{10} concentrations, temperature, relative humidity, and ozone concentration) measured inside and outside the test house during the sampling events.

Table 4-1: Indoor to outdoor ratio of particulate ROS concentrations measured at the UTest House under different indoor (low/high ozone concentration, low/high terpene concentration) and outdoor (low/high ozone concentration) conditions. Each condition was tested in triplicate and means ± standard error are reported.

Indoor conditions at UTest House	In/Out Ratio of Particulate ROS	
	Low Outdoor O_3	High Outdoor O_3
Low O_3, Low Terpene	1.50 ± 0.26	0.77 ± 0.19
Low O_3, High Terpene	0.74 ± 0.05	0.96 ± 0.26
High O_3, Low Terpene	0.99 ± 0.22	0.93 ± 0.20
High O_3, High Terpene	4.39 ± 1.11	1.23 ± 0.55

In an effort to better understand the influence of outdoor sources on indoor particulate ROS concentrations, outdoor sources were compared to total (indoor and outdoor) sources in each condition. The effective indoor emission of particulate ROS was estimated using a simple time-averaged mass balance,

$$\frac{E}{V} = \lambda C + \beta C - p\lambda C_{out} \qquad (1)$$

where C represents the indoor concentration of particulate ROS, p is the penetration factor for particulate ROS, C_{out} is the outdoor concentration of particulate ROS, E is the indoor emission rate of ROS, V is the volume of the house, λ is the air exchange rate, β is the deposition loss rate. Since the heating ventilation and air conditioning (HVAC) system was turned off during sampling events, the loss term due to

filtration could be neglected. The fraction of outdoor sources to total (indoor and outdoor sources) was calculated with,

$$\text{Fraction of outdoor sources to total sources} = \frac{p\lambda C_{out}}{\frac{E}{V} + p\lambda C_{out}} \qquad (2)$$

Using typical values for the penetration factor and deposition loss rate (details of which are given in Appendix C), the percentage of outdoor sources to total sources of indoor particulate ROS was calculated for each experimental condition (Table 4-2). When the outdoor ozone concentration was low, the outdoor source term ($p\lambda C_{out}$) contributed 34% of the total sources in the low indoor ozone and low indoor terpene case, whereas it contributed only 16% of the total sources in the high indoor ozone and high indoor terpene case. This implies that indoor sources may contribute a major portion of the indoor particulate ROS concentrations under some conditions.

Table 4-2: Outdoor sources as a percentage of total (indoor and outdoor) sources of indoor particulate ROS for each of the different indoor and outdoor conditions tested at the UTest House. Data for each condition was collected on three separate days, and means ± standard error are reported.

Indoor conditions at UTest House	Outdoor Sources as % of Total Sources	
	Low Outdoor O_3	High Outdoor O_3
Low O_3, Low Terpene	34% ± 7%	51% ± 9%
Low O_3, High Terpene	62% ± 3%	44% ± 12%
High O_3, Low Terpene	47% ± 8%	48% ± 9%
High O_3, High Terpene	16% ± 6%	41% ± 20%

However, when the outdoor ozone concentration was high (>40ppb), the average fraction of outdoor sources to total sources ranged 41-51% for the different indoor conditions and no clear pattern was observed when the indoor ozone and terpene concentrations were varied (right column in Table 4-2). One potential reason for this observation could be that the outdoor conditions, especially outdoor ozone concentration,

play a significant role in the amount of ROS and precursors to ROS that penetrate into buildings from outdoors. As an illustration of this point, it should be noted that indoor PM levels were found to be higher on the days with high outdoor ozone. The atmospheric conditions during the high outdoor ozone days (which fell in the July-September sampling period) were quite different from the atmospheric conditions on the low outdoor ozone days (which mostly fell in the January sampling period). During sampling events on the high outdoor ozone days, the mean outdoor temperature was 32°C and the mean outdoor ozone concentration was 47 ppb, whereas during sampling events on low outdoor ozone days, the mean outdoor temperature was 17°C and the mean outdoor ozone concentration was 27 ppb. Outdoor conditions, such as ozone concentration, likely influence the amount of ROS and precursors to ROS that penetrate into buildings. It is also interesting to note that the highest contribution of outdoor sources to total sources of indoor particulate ROS occurred on the day corresponding to the highest outdoor ozone concentration (61 ppb) and one of the highest outdoor $PM_{2.5}$ (49 $\mu g/m^3$) and PM_{10} (53 $\mu g/m^3$) concentrations.

The concentration of VOCs and terpenoids inside the UTest House (see Tables C.S1 and C.S2 in Appendix C) were highest in the low indoor ozone/high indoor terpene case when PineSol® had been applied suggesting that the indoor chemistry was ozone limited. When ozone was also introduced (in the high indoor ozone/ high indoor terpene case), the concentration of VOCs and terpenoids reduced slightly, likely because reactions between unsaturated hydrocarbons and ozone had depleted some of the unsaturated hydrocarbons. The terpenoid concentrations in the two high terpene cases described above were obviously higher than the two low terpene cases, but the same effect was observed when ozone was introduced. When no supplemental VOCs were introduced into the indoor environment (the low indoor ozone/low indoor terpene case),

the indoor concentration of terpenoids was approximately 15-20 ppb, indicating that the building materials themselves provided a source of terpenes. However, when the indoor concentration of ozone was increased without supplemental VOC introduction (i.e., high ozone/low terpene case) the concentration of terpenoids decreased to 5-7 ppb indicating that the ozone had again depleted some of the unsaturated hydrocarbons.

Regardless of the experimental condition, it appears that indoor generation of particulate ROS contributes substantially to indoor particulate ROS concentration. The contribution of indoor sources to total sources can be calculated from Table 4-2 and ranges from 38% to 84%. Indoor generation of particulate ROS is likely heavily influenced by the influx of precursors to ROS into buildings. Nonetheless, this highlights an important point that buildings have active chemical processes going on inside them, including particulate ROS formation. Tracking methods, such as tracking specific species of ROS from outdoor to indoor environments, could help identify some of the sources of indoor particulate ROS. However, the present state of the art for speciation of ROS is limited and new analytical techniques are needed to adequately address these questions.

4.4 PARTICULATE ROS IN OUTDOOR AIR

It is important to assess how outdoor particulate ROS varies, not only to better understand the driving forces behind this pollutant but also because outdoor particulate ROS concentrations can influence indoor particulate ROS concentrations by infiltrating through the building envelope. The mean (± s.e.) concentration of ROS on $PM_{2.5}$ samples collected over 3 hours around midday at a fixed location on the University of Texas at Austin campus on 40 random days between November 2011 and September 2012 was 1.25 ± 0.17 nmoles/m^3, (standard deviation of 1.08 nmoles/m^3) ranging from 0.02 nmoles/m^3 measured on December 23 to 3.81 nmoles/m^3 on September 20. The concentrations on each sampled day are depicted in Figure 4-6 with the error bars

depicting the average standard error of replicate samples taken on 20 of the 40 sampling days. During the sampling periods on the 40 days, the ozone concentrations ranged from 8 to 72 ppb, $PM_{2.5}$ concentrations ranged from 1 to 22 μg/m^3, and solar radiation ranged from 23 to 928 W/m^2. The temperature during the sampling periods ranged from 37 to 95°F, relative humidity ranged from 21 to 95%, precipitation ranged from 0 to 80 mm, and wind direction varied between 8 to 326 degrees compass. The winds prevailed from

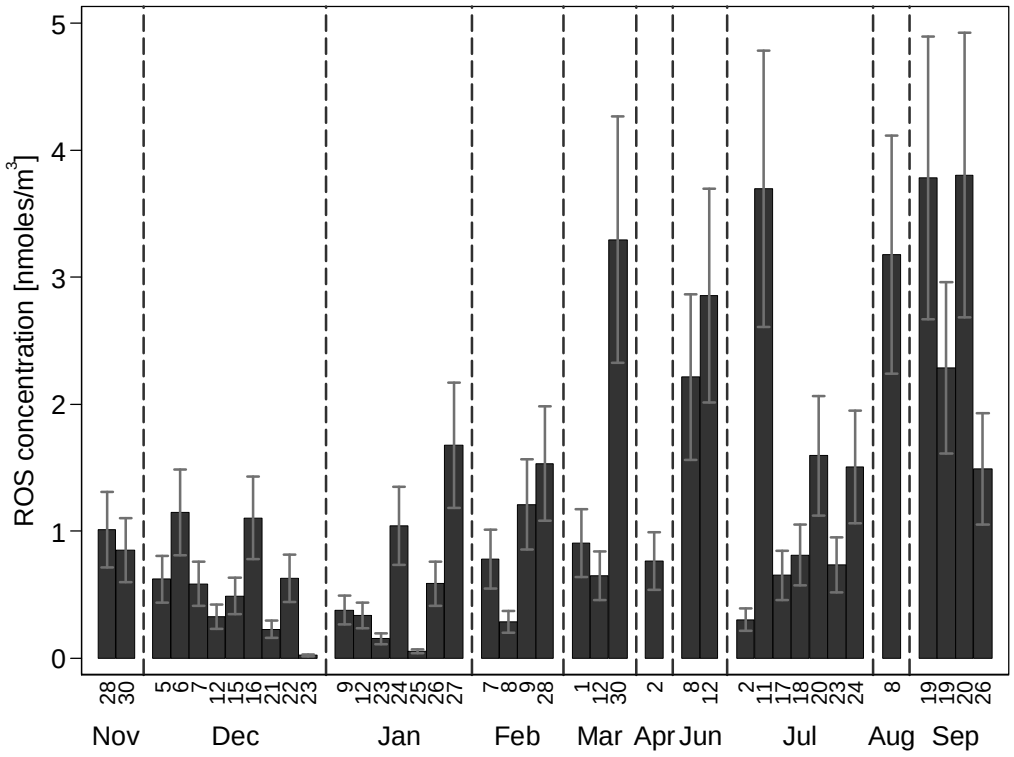

Figure 4-6: Concentration of ROS on $PM_{2.5}$ sampled at an outdoor location away from point sources in Austin, Texas. The error bars represent the average standard error of replicate samples taken on 20 of the 40 sampling days.

the east-southeast on 27 of the 40 sampling days, potentially bringing pollutants from upwind sources including Houston located 165 miles east-southeast of Austin. The mean (± s. d.) wind speed during the sampling periods was 6.0 ± 2.9 miles/hour. The data shows that particulate ROS concentrations tend to be higher in the warmer months than in

the colder months, implying that particulate ROS follows trends similar to gas-phase and rainwater H_2O_2 in the atmosphere.

Spearman Rank Correlation tests were conducted between particulate ROS concentrations and the recorded environmental conditions (namely, ozone and $PM_{2.5}$ concentrations, temperature, relative humidity, precipitation, solar radiation and wind direction). The results are shown in Table 4-3. The concentration of ROS on $PM_{2.5}$ was found to be statistically significantly correlated with the ozone concentration (ρ=0.61, p=0.0000), temperature (ρ=0.56, p=0.0002) and solar radiation (ρ=0.61, p=0.0000). The concentration of ROS on $PM_{2.5}$ was also found to be statistically significantly correlated with winds blowing from the east-southeast (ρ=0.36, p=0.0244). Winds blowing from the east-southeast were significantly correlated with ozone concentration (ρ=0.37, p=0.0177) and $PM_{2.5}$ concentration (ρ=0.5446, p=0.0003) indicating that they might be bringing pollutants from upwind sources including petrochemical and other industries in Houston. The concentration of ROS on $PM_{2.5}$ was also found to be statistically significantly correlated with winds blowing from the north (ρ=0.35, p=0.0253) indicating that some sources might be blowing from the direction of Dallas.

Several studies have also found moderate correlations between particulate ROS concentrations and ozone (Hung and Wang, 2001; Venkatachari et al., 2005; Venkatachari et al., 2007) and gas-phase H_2O_2 and ozone (Liu et al., 2003). Complexities in the chemistry of formation of ROS have been cited as the reason for the relatively moderate correlations with ozone (Venkatachari et al., 2007), since meteorological conditions, such as solar radiation, water vapor concentration, temperature and pressure, are thought to also influence the atmospheric concentration of H_2O_2 (Logan et al., 1981). However, very few studies have assessed the relationship between particulate ROS concentrations and meteorological conditions other than ozone concentration.

Venkatachari et al., (2007) had found a weak, but statistically significant, correlation between particulate ROS and estimated secondary organic carbon concentrations in the atmosphere. The present study provides some additional information on the environmental conditions that can influence particulate ROS concentrations. The significant correlation between particulate ROS and solar radiation provides additional evidence for ROS being photochemically driven.

Table 4-3: Spearman's rank correlation coefficients between the concentration of ROS on $PM_{2.5}$ (ROS), ozone concentration (O_3), $PM_{2.5}$ concentration, temperature (T), relative humidity (RH), precipitation (ppt), solar radiation measured at the sampling site (Solar Rad), and solar radiation measured at the nearest TCEQ site (Solar Rad-TCEQ). Significant relationships are in bold ($p<0.05$) and * denotes significance at $p<0.001$.

	ROS on $PM_{2.5}$	**O_3**	**$PM_{2.5}$**	**T**	**RH**	**ppt**	**Solar Rad**
O_3	**0.61***						
$PM_{2.5}$	0.27	0.03					
T	**0.56***	**0.52***	**0.36**				
RH	-0.17	**-0.53***	0.19	**-0.32**			
ppt	-0.15	**-0.38**	0.08	0.26	**0.53***		
Solar Rad	0.46	**0.74***	0.20	**0.70**	**-0.54**	-0.42	
Solar Rad-TCEQ	**0.61***	**0.69***	0.11	**0.78***	**-0.50***	**-0.54***	**0.78***

To date, seven studies have reported ROS measurements in ambient aerosols (this does not include studies on hydroxyl radical generation by PM which use a completely different analytical approach involving electron paramagnetic resonance). The outdoor ROS concentration on $PM_{2.5}$ reported in the literature ranges from 0.80-0.97 nmoles/m^3 at a location 14 km west of Manhattan during winter (Venkatachari et al., 2007), and 4.37-4.98 nmoles/m^3 close to highway traffic during Los Angeles basin inversion conditions in summer (Venkatachari et al., 2005), to 5.71 nmoles/m^3 in Singapore during December (See et al., 2007). A study in Taiwan reported a concentration of 0.54 nmoles/m^3 on $PM_{3.2}$ on an urban sidewalk during summer (Hung and Wang, 2001). Additionally, a few studies use a different reagent (*para*-hydroxyphenyl acetic acid, POHPAA) (specifically,

to measure peroxides) and report hydroperoxide concentrations on TSP ranging from 0-0.38 nmoles/m^3 in summer in west Los Angeles (Hasson and Paulson, 2003), 0-0.24 nmoles/m^3 in summer at Niwot Ridge, CO (Hewitt and Kok, 1991), to 0.1-1.6 nmoles/m^3 during various parts of the year in west and downtown Los Angeles (Arellanes et al., 2006). Also with POHPAA, hydroperoxide concentrations on coarse particles (2.5-10 μm) were reported to be about 0.01-0.04 nmoles/m^3 upwind and downwind of major freeways in summer in Riverside, CA (Wang et al., 2010). In the present study, we measured ROS concentrations on $PM_{2.5}$ in the 0.02-3.81 nmoles/m^3 range during November 2011 – September 2012 in Austin, Texas. The winter concentrations measured in this study are comparable to winter concentrations measured near Manhattan and summer concentrations in Taiwan. The summer concentrations measured in this study are lower than summer concentrations measured during basin inversion conditions in LA and winter concentrations in Singapore. In comparison, ROS concentrations on TSP in mainstream cigarette smoke (4-16 μmol/m^3 for three different brands of cigarettes; Huang et al., 2005) are 3-4 orders of magnitude higher than all ambient particulate ROS concentrations reported in the literature.

4.5 Toxicological Analysis of Products of Ozone-Initiated Reactions

In addition to measuring the concentration of particulate ROS, there is a need to better understand the potential health effects of secondary pollutants such as ROS. Previous results from *in vivo* studies suggest that more severe health effects can potentially occur following exposure to ozone/limonene reaction products compared to the individual parent compounds (Wolkoff et al., 2012). Motivated by the developments in *in vitro* exposure systems, a series of experiments were conducted at NIOSH in collaboration with NIOSH researchers to test the potential health effects of products from the ozone/limonene reaction in an *in vitro* exposure system for the first time. The

ozone/limonene reaction was used as a prototypical indoor ozone-initiated reaction in these experiments. Exposures were conducted in Vitrocell® exposure chambers (shown in Figure 4-7). Some of the key results are presented in Figures 4-8 and 4-9, while the complete set of results are located in Appendix D. The results from this study help characterize the relative toxicity of secondary products as compared to the toxicity of their parent compounds, and also shed light on the importance of method development and validation for *in vitro* exposure models, as discussed further in Appendix D.

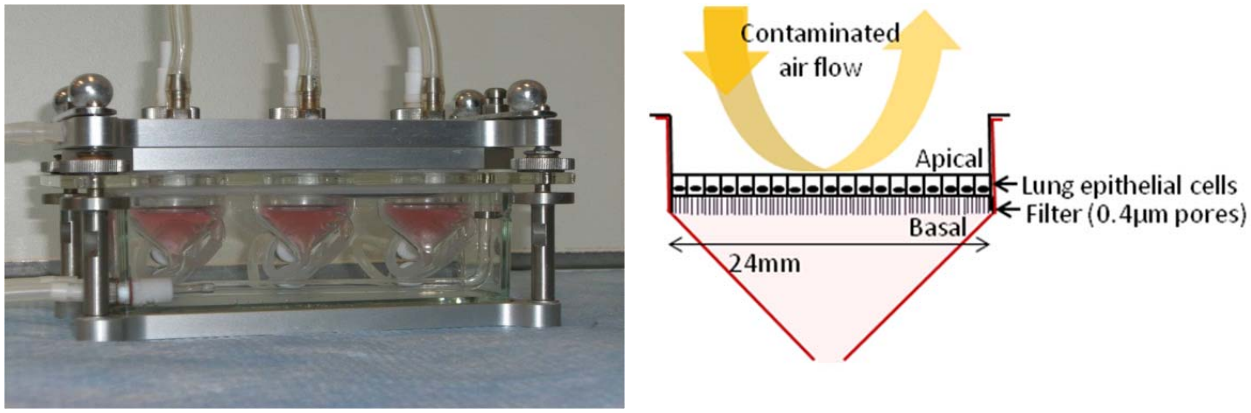

Figure 4-7: Photograph (left) showing the Vitrocell exposure chamber with three cell inserts containing A549 cells (photograph courtesy Anderson et al., 2010). Diagram (right) shows an enlargement of the air flow in a cell insert.

An assessment of exposures to individual parent compounds shows that exposure to ozone does not significantly influence cytokine production in A549 (human lung epithelial) cells whereas exposure to limonene does. Exposure to ozone at 4 ppm for 4 hours did not lead to a change in IL-8 and MCP-1 production (Figure 4-8C and D). However, both 1-hour (Figure 4 of Appendix D) and 4-hour (Figure 4-8A and B) exposures to limonene at 20 ppm resulted in significant increases in IL-8 and MCP-1 at 24 hours post exposure compared to the clean air control.

An assessment of exposures to secondary products from limonene ozonolysis as compared to limonene alone indicates that the secondary products can significantly influence cytokine production in A549 cells. A 4-hour exposure to limonene (20 ppm) and ozone (4 ppm) reaction products was shown to augment pro inflammatory cytokine production in A549 cells. A significant increase in IL-8 cytokine production was observed in these cells following exposure to limonene + ozone (12 hours post-exposure) when compared to limonene alone (Figure 4-8E). The data presented are the best representation of three separate studies. Exposure to limonene + ozone for 1 hour resulted in modified cytokine expression when compared to limonene alone and led to a decreased production of MCP-1 at the 10 and 24 hour post exposure time points with no effect on IL-8 production (Figure 4 of Appendix D). No change in cytokine production was observed when the A549 cells were exposed to lower chemical concentrations of limonene (500 ppb) and limonene (500 ppb) + ozone (100 ppb) for 1 hour (Figure 6 in Appendix D).

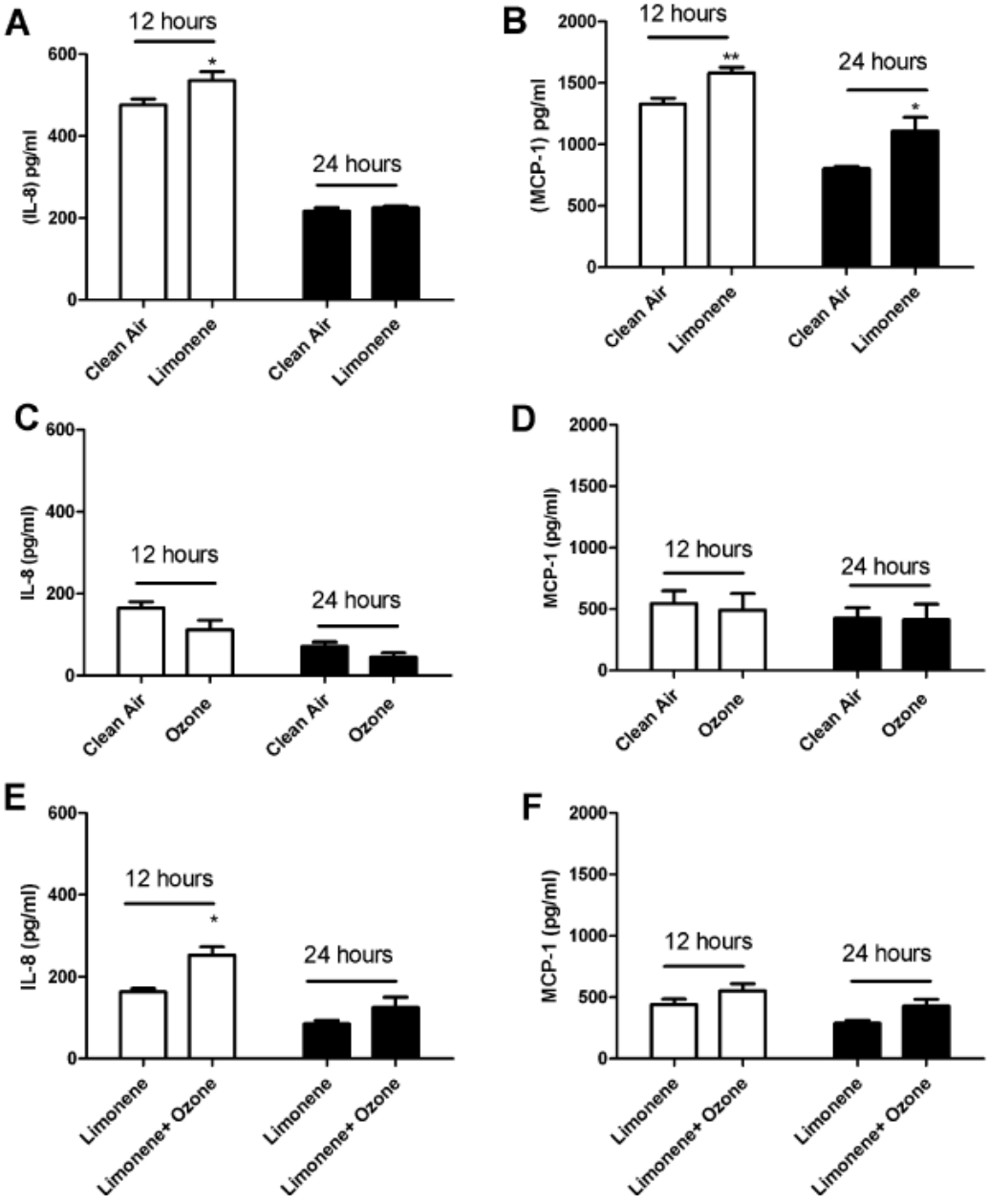

Figure 4-8: The Effect of limonene and limonene + Ozone Reaction Products on A549 cells following a 4 hour Exposure. Cells were evaluated for IL-8 and MCP-1 protein production at 12 and 24 hours post-exposure. Comparisons were made for (A and B) clean air vs. limonene (20 ppm), (C and D) clean air vs. ozone (4 ppm) and (E and F) limonene (20 ppm) vs. limonene (20 ppm)/ozone (4 ppm). Bars represent the mean ± SE. Significant differences are designated with * ($p < 0.05$). (Figure reproduced from Anderson et al., 2013)

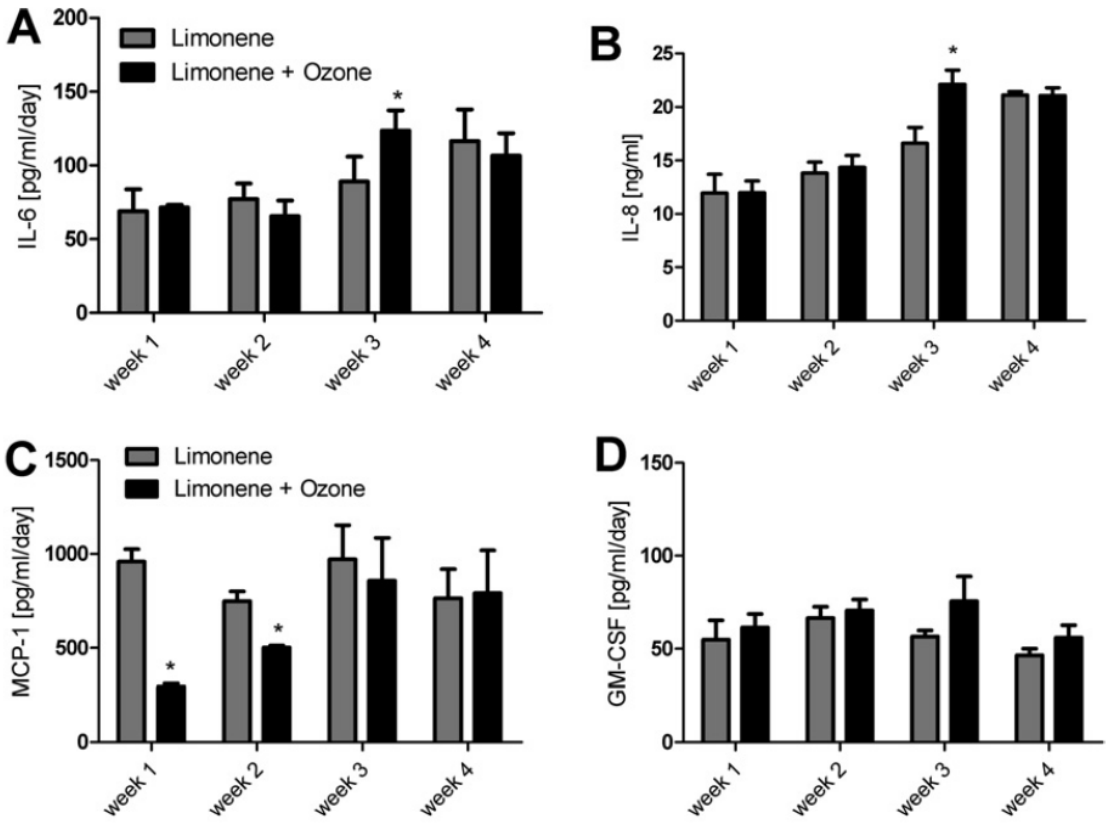

Figure 4-9: The Effect of limonene and limonene + Ozone Reaction Products on MucilAir™ Tissue following a repeated dose exposure routine of 1 hour/day, 5 days/week for 4 weeks. 72 hours following the final weekly exposure, supernatant was evaluated for IL-8 (A), IL-6 (B), MCP-1 (C), and GM-CSF (D) protein production. Comparisons were made for unexposed vs. limonene (500 ppb) and limonene (500 ppb) vs. limonene (500 ppb)/ozone (100 ppb). Bars represent the mean ± SE. Significant differences are designated with * ($p \leq 0.05$). (Figure reproduced from Anderson et al., 2013)

While A549 cells have been used in several studies to assess the response of lung epithelial cells to pollutants, these cultured cells represent very simplified living systems and do not possess the complexity of integrated functioning tissues. The use of differentiated tissue helps to overcome some of these issues. MucilAir™ tissues are made of primary human cells isolated from the nasal cavity, the trachea and the bronchus to

better mimic the human respiratory epithelium. Furthermore, these tissue samples are functional for more than one year, according to the manufacturer, and can therefore be used for long term and/or repeated dose exposures. Cell lines such as A549, in comparison, are often limited to a single acute exposure due to growth requirements (as was the case in the present study). The MucilAir™ model allowed for repeated exposures over 4 weeks testing concentrations of limonene (500 ppb) and limonene (500 ppb) + ozone (100 ppb) that are related to indoor environments. The effects of repeated dose exposures on pro inflammatory as well as proliferative responses were able to be assessed with this *in vitro* model. Statistically significant increases in IL-8 and IL-6 cytokine production were observed for the limonene + ozone exposure group when compared to the limonene exposure group at week three (Figure 4-9A and B). Similar to the A549 exposures, statistically significant decreases in MCP-1 were observed for limonene + ozone when compared to limonene at weeks one and two (Figure 4-10C). Thus, the lower dose MucilAir™ exposure studies induced a similar pattern of cytokine modulation as seen in the A549 cells after a high dose single exposure. In addition, increases in cytokine production were observed for limonene (IL-6) and limonene + ozone (IL-6 and IL-8) over the 4 week exposure period (Linear Trend Test $p<0.05$).

The data from these *in vitro* exposure studies suggests that exposure to reactions products of ozone-initiated reactions can induce a greater inflammatory response than exposure to the parent compounds. This observation is consistent with results from animal studies which have demonstrated increased respiratory distress in animals exposed to reaction products compared to parent compounds.

4.6 Particulate ROS as a Metric for Adverse Health Effects of PM

The results from this doctoral research indicate that the concentration of particulate ROS varies with environmental conditions. Outdoor particulate ROS

concentrations are influenced by ozone concentration, solar radiation intensity and temperature. Indoor particulate ROS formation is more complex and appears to be influenced by several factors including outdoor particulate ROS concentrations, indoor terpene concentrations, and indoor and outdoor ozone concentrations. While the mass concentration of PM can be significantly different in indoor and outdoor environments, it is interesting to note that the indoor and outdoor concentrations of particulate ROS on respirable PM were quite similar across a range of indoor environments, including residential and commercial buildings. Results from *in vitro* exposure studies indicate that exposure to products of ozone-initiated reactions, such as ROS, can induce a greater inflammatory response than exposure to precursor compounds. The results indicate that particulate ROS is a biologically relevant property of PM that may well play a part in mediating the adverse health effects of PM. Given that indoor environments represent an important exposure route for particulate ROS, the concentration of particulate ROS should be included as a metric in indoor air quality studies.

Chapter 5: Conclusions

Given that $PM_{2.5}$ can carry ROS deep into the lungs where ROS can cause oxidative stress and cell damage, it is important to determine typical concentrations of ROS on $PM_{2.5}$ and the conditions that influence the indoor and outdoor concentrations of this pollutant. The indoor concentrations of ROS on $PM_{2.5}$ in the buildings sampled in this study were not significantly different from the outdoor concentrations of ROS on $PM_{2.5}$. This result is especially intriguing because photochemical activity (which is one of the main pathways for ROS formation in outdoor environments) is generally absent inside buildings. This implies that: (1) transport of outdoor ROS into the buildings or generation of ROS inside the buildings are important; (2) human exposure to ROS is likely dominated by exposure in indoor environments, since Americans spend 87% of their time indoors (Klepeis et al., 2001). Furthermore, the concentration of ROS on $PM_{2.5}$ in commercial and residential buildings appears to be similar. The concentration of ROS on $PM_{2.5}$ in institutional (1.16 ± 0.14 nmoles/m^3 indoors and 1.68 ± 0.48 nmoles/m^3 outdoors) and retail (1.09 ± 0.25 nmoles/m^3 indoors and 1.12 ± 0.36 nmoles/m^3 outdoors) buildings was similar to the concentration of ROS on $PM_{2.5}$ in a sample of homes (1.37 ± 0.30 nmoles/m^3 indoors and 1.41 ± 0.25 nmoles/m^3 outdoors). About 58% of the indoor particulate ROS was present on $PM_{2.5}$, which is important from a health point of view, since it appears that the majority of particulate ROS occurs on respirable particles. For the first time, controlled ROS studies were conducted in a test house to better understand some of the driving factors for indoor particulate ROS. These studies indicate that when outdoor ozone concentrations are low, indoor concentrations of terpenes and ozone are influential in indoor generation of particulate ROS. However, when outdoor ozone concentrations are high, changing the indoor conditions does not substantially change the indoor generation of particulate ROS possibly because ROS precursors are already

present inside the house regardless of the indoor experimental condition. Overall, this research represents one of the first studies to assess particulate ROS concentrations in indoor environments. Given the similarity in particulate ROS concentrations between different indoor environments, new analytical techniques should be developed to better understand the sources of indoor particulate ROS.

This study found that the ambient ROS concentration on $PM_{2.5}$ in an urban, semi-arid environment varies over the course of a year, with a minimum during the winter and a maximum during the summer. This is similar to observations made by studies on ambient H_2O_2 concentrations in gas-phase and rainwater. This research represents one of the first times that seasonal variation in ambient particulate ROS concentrations was assessed. The results show that ambient particulate ROS concentrations are influenced by ozone concentration, solar radiation intensity and temperature.

In vitro exposure models of lung epithelial cells (A549 cells) and differentiated lung tissue (MucilAir™ tissue) were used to understand the potential health effects of secondary pollutants such as particulate ROS. The results indicate that exposure to secondary pollutants formed from ozone-initiated reactions can induce alterations in inflammatory responses that are greater than those induced by exposure to the individual parent compounds. This observation is consistent with results from animal studies which have demonstrated increased respiratory distress in animals exposed to reaction products compared to parent compounds. The results from this study suggest that exposure to ozone-initiated reaction products, which include ROS, may lead to more adverse health effects than their parent compounds. Overall, the results from this doctoral research provide a baseline assessment of particulate ROS and lay the foundation for particulate ROS to be used as a metric in indoor air quality studies.

Appendix A: Indoor Particulate Reactive Oxygen Species Concentrations

Indoor Particulate Reactive Oxygen Species Concentrations

Shahana S. Khurshid*[1], Jeffrey A. Siegel[2], Kerry A. Kinney[1]

Published in *Environmental Research*, 132, 45-53.

[1]Department of Civil, Architectural and Environmental Engineering, The University of Texas at Austin, Austin, Texas, USA.
[2]Department of Civil Engineering, The University of Toronto, Toronto, Ontario, Canada.

Abstract

Despite the fact that precursors to reactive oxygen species (ROS) are prevalent indoors, the concentration of ROS inside buildings is unknown. ROS on $PM_{2.5}$ was measured inside and outside twelve residential buildings and eleven institutional and retail buildings. The mean (±s.d.) concentration of ROS on $PM_{2.5}$ inside homes (1.37±1.2 nmoles/m^3) was not significantly different from the outdoor concentration (1.41±1.0 nmoles/m^3). Similarly, the indoor and outdoor concentrations of ROS on $PM_{2.5}$ at institutional buildings (1.16±0.38 nmoles/m^3 indoors and 1.68±1.3 nmoles/m^3 outdoors) and retail stores (1.09±0.93 nmoles/m^3 indoors and 1.12±1.1 nmoles/m^3 outdoors) were not significantly different and were comparable to those in residential buildings. The indoor concentration of particulate ROS cannot be predicted based on the measurement of other common indoor pollutants, indicating that it is important to separately assess the concentration of particulate ROS in air quality studies. Daytime indoor occupational and residential exposure to particulate ROS dominates daytime outdoor exposure to particulate ROS. These findings highlight the need for further study of ROS in indoor microenvironments.

Keywords: $PM_{2.5}$, ozone, air quality, residential, commercial

1. Introduction

Although kinetic modeling suggests that hydrogen peroxide (a reactive oxygen species) is formed as a result of chemical reactions in indoor environments (Nazaroff and Cass, 1986), it was not until studies by Li et al. (2002) (office) and Fan et al. (2005) (simulated indoor conditions) that evidence of these mechanisms in indoor environments was found. These studies as well as chamber studies of ozone/terpene reactions (Docherty et al. 2005; Venkatachari & Hopke, 2008; Chen & Hopke, 2009; Chen et al., 2011) have shown that secondary organic aerosols (SOA) are formed in conjunction with peroxides and other reactive oxygen species (ROS). Particles, especially $PM_{2.5}$, can carry ROS into the lower respiratory tract where there is increased probability of health impacts, whereas gas phase ROS (which have high solubility and diffusivity) are likely absorbed and removed by mucus in the upper airways (Friedlander and Yeh, 1998). ROS include hydroperoxides, organic peroxides (ROOR'), hypochlorite ions (OCl^-), hydroxyl (•OH) radicals, and alkyl peroxyl radicals (ROO•). They can be formed through photochemical reactions (with NO_x, carbon monoxide, formaldehyde and volatile organic compounds (VOCs)) (Gunz and Hoffman, 1990; Finlayson-Pitts and Pitts, 2000) and via ozone-initiated reactions (Paulson & Orlando, 1996; Weschler, 2006; Venkatachari et al., 2007).

A substantial body of evidence links the endogenous production of reactive oxygen radicals, and subsequently oxidative stress and damage, to the pathogenesis of age-related and chronic diseases including cancer (Trush and Kensler, 1991; Witz, 1991; Guyton and Kensler, 1993; Klaunig & Kamendulis, 2004). Many *in vitro* and some *in vivo* studies have established the involvement of ROS in different pathologies, especially in many pulmonary diseases (Kehrer, 1993; Lansing et al., 1993; Sanders et al., 1995; Stevens et al., 1995; Bowler et al., 2002; Li et al., 2003; Li et al., 2008). Exposure to

exogenous sources can influence endogenous ROS production (such as greater generation of peroxynitrite anion (Lang F., 2010)), which can lead to oxidative stress and damage (Klaunig and Kamendulis, 2004). This warrants further investigation of exogenous sources of ROS. However, studies to assess air quality have focused on measuring pollutants such as particle and VOC concentrations. While these pollutants are linked to adverse health outcomes (e.g., DALYs for particulate matter exposure (Zelm et al., 2008) and sick building syndrome symptoms for VOC exposure (e.g., Fisk et al., 1997)), the concentration of ROS is a metric that may be as important for assessing the quality of air in an environment. Reducing exposure to exogenous sources of ROS may reduce the likelihood of oxidative stress and subsequent disease formation (Churg, 2003).

Despite their potential health effects, ROS have mainly been studied in outdoor environments and only one study has assessed the concentration of ROS in an indoor environment (in a university building in Singapore: See et al., 2007). Unsaturated hydrocarbons, which can react with ozone to produce ROS, are prevalent inside buildings (Wallace et al., 1987 & 1991; Brown et al., 1994) and are emitted from sources such as cleaning products (Zhu et al., 2001), air fresheners (Steinemann, 2009; Steinemann et al., 2011), and wood products (Hodgson et al., 2000). A few studies have studied the factors that influence the formation of ROS under controlled conditions in chambers (Docherty et al., 2005; Chen and Hopke, 2009; Chen et al., 2011). However, indoor environments are much more complex in that several ROS precursors are present and there is the possibility that unfiltered outdoor ROS and precursors penetrate indoors.

Given that Americans spend more than 85% of their time inside buildings (Klepeis et al., 2001), it is crucial to determine actual indoor concentrations of ROS. Residential environments have the greatest potential for exposure because people spend almost 70% of their time at home (Klepeis et al., 2001). Exposure to pollutants in

commercial buildings can be very different from that in residential buildings because commercial buildings have higher air exchange rates (Chao and Chan, 2001; Bennett et al., 2012), higher recirculation rates (Thornburg et al., 2001; Bennett et al., 2012), and different operation and ventilation strategies. Employed Americans spend 8.8 hours on average working on weekdays (U.S. BLS, 2011a), a major portion of which may be spent in office buildings. Retail stores are frequented by a large section of the population and 7.6 million Americans work as retail salespeople and cashiers (U.S. BLS, 2011b and c). In this study, samples of $PM_{2.5}$ were collected at twelve homes, six institutional buildings and five retail stores in Austin, Texas to compare the indoor and outdoor concentrations of particulate ROS, and to determine the influence of environmental factors on particulate ROS concentrations. Because several studies have reported high background ROS values for blank filters (22-75% of field samples) (Hung and Wang, 2001; Venkatachari et al., 2005; Venkatachari et al., 2007), steps were taken in this study to improve the analytical method before collecting field samples.

2. Methods

Sample Collection at Homes, Institutional Buildings and Retail Stores

$PM_{2.5}$ was collected inside and outside twelve homes during March and August 2012 on Teflon filters (TF-1000, 1μm pore size, 37 mm, Pall, NY, USA) using Personal Environmental Monitors (PEM, SKC, PA, USA). Similarly, indoor and outdoor samples of $PM_{2.5}$ were collected at seven institutional buildings located on the University of Texas at Austin campus on different days in March and July 2012, and at five retail stores during January-April 2012. Teflon tape was wrapped around the edges of the support screen in the PEMs to ensure a proper seal of the thin Teflon filters inside the PEMs. Sampling was conducted for 3±0.25 hours between 11 am and 2 pm using air sampling

pumps at 10 L/min. All pumps were calibrated before sampling with a mini-Buck Calibrator M-30 (A.P.Buck, Orlando, FL; accuracy ±0.5%). Duplicate samplers were placed 1m above the ground outside and in a central location inside the buildings (variations from this protocol are described in the next paragraph). All buildings were located in Austin, Texas. Field blanks were periodically used to check that there was no significant difference in fluorescence between laboratory blanks and field blanks. The background fluorescence intensity produced by an unsampled filter was subtracted from the samples. All sampling filters were transported to the lab and assessed with the fluorescence assay described below within 1 hour of collection.

For the institutional buildings, indoor sampling was conducted in an office at street level except for I2 (where the sampling room was on the 3rd floor), I3 (2nd floor), I4 (6th floor), and I1 (where the sampling room was a classroom on the 7th floor). Replicate samples were collected for 10 out of the 14 measurements. For the retail buildings, single samplers were used both indoors and outdoors. At retail sites 1-3, indoor and outdoor sampling was not conducted simultaneously, but rather on consecutive days.

ROS concentrations measured inside or outside the buildings that were greater than 3.5 times the median absolute deviation (MAD) away from the median were considered outliers (5 out of 48 samples for the commercial buildings and 6 out of 64 samples for the residential buildings), based on the Iglewicz and Hoaglin method (NIST, 2010).

Environmental Factors Measured

Indoor and outdoor air quality parameters were measured and building characteristics were recorded at all buildings. Indoor and outdoor temperature and relative humidity were measured with a HOBO U10 (Onset, Bourne, MA) with an uncertainty of ± 0.35 ºC in temperature and ± 2.5% in relative humidity (RH). A photo-

ionization detector (PID, Geotechnical Services, Tustin, CA) calibrated with isobutylene was used to measure the indoor concentration of total volatile organic carbon (TVOC), with an uncertainty of the greater of ± 20 ppb or 10% of the reading. A DustTrak 8520 Aerosol Monitor with a size-selective aerosol conditioner (TSI, Shoreview, MN; uncertainty 1 $\mu g/m^3$) was used to measure indoor $PM_{2.5}$ concentration. The DustTrak was calibrated against a Tapered Element Oscillating Microbalance (TEOM) 1405D (Thermo Environmental Instruments, Franklin, MA) resulting in a gain of 0.9 and an offset of -5.3. In nine of the homes (R1-R9), a SidePak Personal Aerosol Monitor AM510 (TSI, Shoreview, MN) was used to measure indoor $PM_{2.5}$ concentrations instead of the DustTrak. The SidePak was calibrated against a TEOM resulting in a gain of 3 and an uncertainty of ±3.2 $\mu g/m^3$ for measurements below 3 $\mu g/m^3$. Outdoor ozone and $PM_{2.5}$ concentrations were obtained from Texas Commission on Environmental Quality's (TCEQ) nearest sampling station (# 484530014) located within 11 km of the buildings. Overall uncertainty for each measurement was calculated using standard error propagation techniques to include variance in the measured readings and the uncertainty of the instrument itself.

Additional air quality measurements were made at the retail stores using several instruments. A SidePak Personal Aerosol Monitor AM510 (TSI, Shoreview, MN), calibrated against the TEOM, was used to measure indoor $PM_{2.5}$ concentrations. The DustTrak 8520 with a size-selective aerosol conditioner, calibrated against the TEOM, was used to measure indoor PM_{10} concentrations. An Aerocet-531 Mass Particle Counter / Dust Monitor (Met One Instruments, Grants Pass, OR), calibrated against gravimetric measurements of $PM_{2.5}$ and PM_{10} with PEMs in retail stores, was used to measure outdoor $PM_{2.5}$ and PM_{10} concentrations. The air exchange rate was measured at all retail sites by measuring the decay of sulfur hexafluoride (SF_6) over a four-hour period on one

of the sampling days. Measurement of four-hour average VOC concentrations (with Summa canisters and sorbent tubes) and light aldehyde concentrations (with dinitrophenylhydrazine (DNPH) tubes) were also made during this period. Summa canisters are more reliable for quantifying low molecular weight compounds, whereas the sorbent tubes used (indoor and outdoor) in this study were more adapted to quantify high molecular weight compounds. A PID was used to measure the indoor TVOC concentration during all ROS sampling events. Indoor and outdoor concentrations of ozone were measured using a UV-absorbance ozone monitor (2B Technologies model 202, uncertainty of ± 1.5 ppb or 2% of reading, lower detection limit 2 ppb). At Sites 1-3, the outdoor ozone concentration was obtained from the nearest TCEQ sampling station. Details about the instrument calibrations and the methods for air exchange rate and VOC measurements at the retail sites are given in the ASHRAE RP-1596 report (Siegel et al., 2013). For comparison with data in the RP-1596 report, it should be noted that retail sites 1-5 in this study are labeled GeT2, MbT3, FfT2, MbT4, MiT, respectively, in the report.

Graphical representations of the data and Shapiro-Wilk tests for normality indicated that the indoor and outdoor ROS concentrations were generally not normally distributed. The Spearman Rank Correlation Coefficient test was used to determine the strength (ρ) and significance ($p<0.05$) of any relationships between the concentration of ROS and environmental factors with Stata version 11.2. Bonferroni adjustments were generally not used as the purpose of this study was to provide a baseline assessment of indoor ROS. The Wilcoxon matched-pairs signed-ranks test was used to assess differences between the indoor and outdoor ROS datasets at the buildings.

Method Development for Measuring ROS Concentration

The reagent used to quantify ROS, 2',7'-dichlorofluorescin diacetate (DCF-DA), is a non-specific indicator for ROS (Venkatachari and Hopke, 2008). It becomes

fluorescent in the presence of a wide variety of ROS including, but not limited to, hydrogen peroxide (H_2O_2), peroxyl (ROO•) and hydroxyl (•OH) radicals and the peroxynitrite anion ($ONOO^-$) (Zhu et al., 1994; Kooy et al., 1997). Several studies in the last decade or so have used DCF-DA as a bulk measure of ROS (Hung and Wang, 2001; Huang et al., 2005; Venkatachari et al., 2005; Venkatachari et al., 2007; See et al., 2007; Chen and Hopke, 2009). Steps were taken to reduce the high background values reported by these studies. Sonication of the activated form of DCF-DA may cause auto-oxidation of the reagent into the fluorescent compound, dichlorofluorescein (DCF). This can lead to high fluorescence intensities being detected for blank filters (Hasson and Paulson, 2003). In order to determine the influence of sonication times on the fluorescence intensity generated by blank filters, PTFE filters (Pall TF1000) were sonicated in (i) 10 ml DCFH-HRP solution for 10 minutes (see below for description of reagent), (ii) 10 ml of DCFH-HRP solution for 5 minutes, and (iii) 5 ml buffer for 10 minutes followed by addition of 5 ml reagent to achieve the same final concentration of DCFH-HRP as in (i) and (ii). As described in the results, the fluorescence was lowest when the filter was sonicated in buffer and the reagent was not sonicated. Other steps for reducing the background fluorescence are described in SI.

Based on the results of the method development tests, the following protocol was developed for measuring the concentration of particulate ROS. The reagent was prepared by incubating 0.5 ml of 1 mM DCF-DA (Cayman Chemical, MI, USA) in ethanol with 2 ml of 0.01 N NaOH at room temperature for 30 mins in the dark to cleave off the acetate groups. After the 30 mins incubation period, the 2',7'-dichlorofluorescin (DCFH) solution was neutralized with 10 ml sodium phosphate buffer (pH 7.2) and the solution was kept on ice in the dark till needed. Each filter was sonicated in 5 ml sodium phosphate buffer for 10 minutes. Horseradish peroxidase (HRP, ThermoScientific, IL,

USA) in sodium phosphate buffer (pH 7.0) and DCFH were then added to the solution to yield a final volume of 10 ml with a concentration of 5 μM of DCFH and 1 unit/ml of HRP. After incubation at 37˚C for 15 mins, 0.1 ml aliquots were placed in triplicate in a 96-well plate and the fluorescence intensity was read at 530 nm with excitation at 485 nm (Synergy HT, Biotek, VT, USA).

The concentration of ROS on the sampled filters was expressed in terms of H_2O_2 per volume of air sampled (rather than per mass of particles) because this describes exposure to ROS as it occurs in the lungs (Boogaard et al., 2012). To prepare the standards, 0.1 ml aliquots of appropriate H_2O_2 concentrations were added to 3 ml of DCFH-HRP reagent in glass tubes to yield 0, 1.0, 2.0, 3.0, and 4.0×10^{-7} M H_2O_2 in the final solutions. These tubes were incubated at 37°C for 15 minutes and fluorescence was measured. All glassware used in the experiments was cleaned in a 10% nitric acid bath. The Method Detection Limit (EPA, 2011) of the analytical procedure was 1.2 nmoles H_2O_2/l, which converts to 0.01 nmoles/m^3 assuming a 3-hour sample at 10 l/min.

Exhaust air from sampling pumps may carry pollutants, particularly ultrafine particles, from the pump's internal machinery. Tests were conducted to verify that the exhaust air from sampling pumps didn't influence the concentration of particulate ROS collected on the sampling filters. The indoor concentration of ROS detected by duplicate samplers placed close to two sampling pumps was not found to be significantly different from the concentration of ROS detected by duplicate samplers placed far away from the sampling pumps (Wilcoxon Signed-Rank Test, p=0.18).

3. Results and Discussion

Method Development

High background values have been reported in the literature using the existing DCFH method (Hung and Wang, 2001; Venkatachari et al., 2005; Venkatachari et al., 2007). In this study, several steps were taken to try to reduce the background in order to increase the sensitivity and accuracy of the reported ROS concentrations. Sonication of blank filters in DCFH was seen to influence the background fluorescence of these filters (Figure A.1). Hasson and Paulson (2003) had postulated that high blank levels measured by Hung and Wang (2001) may have been the result of using sonication to extract their samples. This may indeed be the case, as our results show that sonication of blank filters in DCFH reagent increases the variability in background fluorescence of the filters. The lowest and most consistent background fluorescence was observed when the filters were sonicated in buffer alone, followed by the addition of DCFH and incubation at 37°C.

Other methods to reduce the background included selection of filters that produce low backgrounds. These are described further in SI. As a result of these modifications, we were able to achieve lower fluorescence intensity for field blanks than that reported in previous studies. Hung and Wang (2001) had reported that field blanks had a background fluorescence of 25-75% of the field samples, Venkatachari et al. (2005) had reported it as 22-56% and Venkatachari et al. (2007) had reported it as 28-60%. The mean background fluorescence of unsampled filters in this study was 20% of the sampled filters (with a range of 7-35%); after correcting for background fluorescence of blank water and reagents, this represents less than 8% of the ROS concentration measured on the sampled filters.

Indoor and Outdoor ROS Concentrations at the Sampled Buildings

Residences

The mean (± s.d.) indoor concentration of ROS on $PM_{2.5}$ sampled at twelve homes (labeled R1-R12) was 1.37 ± 1.2 nmoles/m^3 and the mean outdoor concentration was 1.41 ± 1.0 nmoles/m^3. The indoor and outdoor concentrations of ROS on $PM_{2.5}$ were not significantly different (Wilcoxon matched-pairs signed-ranks test, p=0.959). The concentrations are displayed in Figure A.2 for all twelve homes as well as for repeated measurements conducted on different days at three of these homes. The first nine homes were assessed in March-April, while the remaining sampling events were conducted in June-August when outdoor temperatures were higher. All homes had operating heating and cooling (HAC) units, except R1 which did not have an HAC unit and R7 and R8 where the HAC had been turned off because of favorable weather. Indoor ROS on $PM_{2.5}$ ranged from 0.18 to 4.01 nmoles/m^3 whereas outdoor ROS on $PM_{2.5}$ ranged from 0.19 to 3.18 nmoles/m^3.

Indoor and outdoor air quality parameters (indoor and outdoor $PM_{2.5}$ concentrations, temperature, and RH, as well as indoor VOC concentration and outdoor ozone concentration) were collected at the homes and are given in Table A.S1 along with the building characteristics. Indoor $PM_{2.5}$ concentrations ranged from 0.0 to 9.0 μg/m^3, except at R12 where the indoor VOC concentration was the highest and the indoor $PM_{2.5}$ concentration was 22.9 μg/m^3. Outdoor $PM_{2.5}$ concentrations ranged from 7.4 to 22.8 μg/m3. Outdoor ozone concentrations ranged between 23 and 63 ppb. The Spearman Rank Correlation Coefficient test was used to determine the strength (ρ) and significance (p) of any relationships that exist between particulate ROS concentrations and these air quality parameters. There appear to be few correlations between particulate ROS concentrations and air quality parameters, especially if the Bonferroni correction is

applied. The indoor concentration of ROS on $PM_{2.5}$ was significantly correlated with the outdoor concentration of ROS on $PM_{2.5}$ ($\rho = 0.66$, $p=0.006$) (though not if the Bonferroni correction is applied) which suggests that a link might exist between the indoor and outdoor concentrations of particulate ROS, although the distinction between ROS precursors and ROS itself is still unresolved. Five of the homes had wood as the dominant floor type and four homes had carpet as the dominant floor type. Even though carpet is known to react readily with ozone which can lead to a lower concentration of indoor ozone (Morrison and Nazaroff, 2002), indoor ROS concentrations were not found to be necessarily lower in homes where carpet was the dominant floor type. In a similar way, Avol et al., (1998) did not find any correlation between indoor ozone concentrations and the presence of carpet. Brick exteriors can decrease the penetration of ozone into buildings (Stephens et al., 2012a; Liu and Nazaroff, 2001), however, the presence of brick alone did not appear to influence indoor ROS concentrations. Older homes tend to be leakier than newer homes (Persily et al., 2010) which can lead to greater penetration of ozone and particles through the building envelope (Stephens et al., 2012a and 2012b), however, no obvious trend was observed between the ROS concentration and the age of the building. The homes were built between 1953 and 2008. Collectively, these results at most suggest that indoor particulate ROS concentrations may be influenced by the outdoor particulate ROS concentration, and it does not seem likely that indoor particulate ROS concentrations are directly linked in a simple fashion with the other air quality parameters or building characteristics recorded in this study. However, it may be that there is not sufficient resolution and variation in the data to observe the presence of a relationship.

Previous studies suggest that season may affect ambient particulate ROS formation. For instance, the highest outdoor particulate ROS concentration reported in

previous studies occurred during the summer (Venkatachari et al., 2005), whereas one of the lowest reported concentrations occurred during a winter sampling study (Venkatachari et al., 2007). In order to investigate the effect of season on the indoor to outdoor ratio of particulate ROS in the current study, sampling was conducted at two houses (R4 and R5) in both spring (March) and summer (July). While the indoor concentration of ROS on $PM_{2.5}$ was higher than the outdoor concentration during the summer at R4, it was lower than the outdoor concentration during the summer at R5. Examination of the air quality measurements for these sites (Table A.1 and Table A.S2) shows that the higher indoor concentration of ROS on $PM_{2.5}$ at R4 during the summer coincided with the highest outdoor particle concentrations recorded in this dataset. These preliminary studies indicate that season alone may not be a good indicator of the concentrations of ROS, and under certain conditions, (such as high outdoor particle concentrations) indoor ROS can even be higher than outdoor ROS.

Additional experiments were conducted at the UTest House (R6), a $120m^2$ manufactured home at the Pickle Research Campus, to assess the effect of different HAC filtration practices on the indoor concentration of particulate ROS. Sampling was conducted at the UTest House on similar days in June when the HAC system was running without any filter (R6-2 in Figure A.2 and Table A.S1) and with a filter with a Minimum Efficiency Reporting Value (MERV) from ASHRAE Standard 52.2 (ASHRAE, 2012) of 16 (R6-3 in Figure A.2 and Table A.S1). As expected, the use of the MERV 16 filter dropped the concentration of indoor $PM_{2.5}$ substantially (by 91%) as compared to when no filter was used. A substantial decrease was also noted in the concentration of particulate ROS on $PM_{2.5}$ (82%). However, the indoor ROS results are based on a single indoor measurement for each sampling event and additional testing should be conducted

to further validate the effect of high efficiency filters on reducing the concentration of particulate ROS.

Institutional Buildings

The mean (± s.d.) indoor concentration of ROS on $PM_{2.5}$ sampled at six institutional buildings (labeled I1-I6) was 1.16 ± 0.38 nmoles/m^3 and the outdoor concentration was 1.68 ± 1.3 nmoles/m^3. The indoor and outdoor concentrations were not significantly different (Wilcoxon matched-pairs signed-ranks test, p=0.40). Average indoor concentrations ranged from 0.63 to 1.68 nmoles/m^3 and average outdoor concentrations ranged from 0.65 to 3.70 nmoles/m^3 (Figure A.3). The average standard error of the concentration of ROS on replicate samples of $PM_{2.5}$ (taken for 10 out of the 14 measurements at institutional buildings) was 0.36 nmoles/m^3 which is fairly similar to the standard error of the concentration of ROS on replicate $PM_{2.5}$ samples in the residential samples (0.41 nmoles/m^3).

Indoor and outdoor air quality parameters (indoor and outdoor $PM_{2.5}$, indoor VOC concentration, outdoor ozone concentration, indoor and outdoor temperature and RH) measured during sampling are listed in Table A.S2. Indoor $PM_{2.5}$ concentrations ranged from 0.0 to 4.7 μg/m^3 and outdoor $PM_{2.5}$ concentrations ranged from 3.5 to 12.2 μg/m^3 Outdoor ozone concentrations ranged between 20.0 and 48.3 ppb. The two highest indoor and outdoor particulate ROS concentrations were measured at I2 and I4 which corresponded to some of the highest measurements of indoor and outdoor $PM_{2.5}$ as well as the highest measurements of outdoor ozone. The lowest indoor concentration of ROS was measured at I6, when the outdoor concentration of $PM_{2.5}$ was the lowest in this dataset. These observations are consistent with the fact that many commercial buildings have higher outdoor air intake fractions than residential buildings (Chao and Chan, 2001; Bennett et al., 2012) which allows greater penetration of outdoor pollutants.

Repeat measurements taken at I1 when the indoor total VOC concentrations were quite different produced similar particulate ROS concentrations. The indoor VOC concentration at I1a was 1-2 orders of magnitude higher than those at all other sites because a small portion of the sampled room had been painted a few hours prior to sampling. However, total VOC concentration measured with a PID is not likely a good indicator of the comparative concentrations of unsaturated organic compounds that can generate ROS. Another factor to note is that outdoor particle and ozone concentrations were fairly similar during both sampling events.

Retail Buildings

Sampling was conducted at different types of retail stores, including grocery (Store 1), general merchandise (Stores 2, 4, 5) and furniture (Store 3) stores. The mean (± s.d.) indoor concentration of ROS on $PM_{2.5}$ sampled at five retail stores was 1.09 ± 0.93 nmoles/m^3 and the outdoor concentration was 1.12 ± 1.1 nmoles/m^3. The indoor and outdoor concentrations were not significantly different (Wilcoxon matched-pairs signed-ranks test, p=0.35) even if only simultaneously collected indoor and outdoor measurements are considered. Indoor concentrations ranged from 0.02 to 3.36 nmoles/m^3 and outdoor concentrations ranged from 0.07 to 3.49 nmoles/m^3 (Figure A.4). While replicate measurements were not conducted at the retail stores, uncertainty in these measurements is likely similar to the uncertainty in replicate ROS measurements taken at residential buildings (average standard error of 29%) and institutional buildings (average standard error of 26%).

Indoor and outdoor air quality parameters ($PM_{2.5}$, $PM_{10,}$ ozone concentrations, and VOC concentrations) measured during sampling at the retail stores are summarized in Table A.S3. The indoor concentration of $PM_{2.5}$ at the retail sites ranged from 0.1 to 10.9 μg/m^3 and the outdoor concentration ranged from 4.1 to 116.7 μg/m^3. The ozone

concentration inside the retail sites ranged from 1.7 to 9.4 ppb, while the outdoor concentration ranged from 10.0 to 55.8 ppb. The indoor air quality measurements at the retail stores indicate that, in general, the stores had relatively clean environments. The volume of the stores and their air exchange rates are given in Table A.2. Statistical correlations of ROS were conducted with all air quality parameters measured at the retail stores. However, similar to the residential and institutional building datasets, indoor particulate ROS did not correlate with any of the measured air quality parameters. This was true even if indoor particulate ROS concentrations were calculated on a per mass basis (i.e. nmoles H_2O_2 / μg $PM_{2.5}$) rather than on a volume of air basis (i.e. nmoles H_2O_2 / m^3). The absence of a correlation between indoor ROS concentrations and the air exchange rate at the stores indicates that the formation and removal of indoor particulate ROS is influenced by several factors. One such factor may be better removal of indoor particulate pollutants due to a higher recirculation rate in retail buildings. Ozonolysis of unsaturated hydrocarbons is known to generate ROS. However, total VOC concentration measured with PID or select VOC concentrations measured with sorbent tubes and suma canisters were not found to be correlated with the concentration of ROS. While photolysis of formaldehyde is one of the sources of H_2O_2 in the atmosphere (Bufalini et al., 1972; Largiuni et al., 2002), the concentration of ROS was not found to be correlated with formaldehyde concentrations inside the retail stores. Similarly, a significant correlation was not observed between ROS concentrations and the concentrations of particles and ozone.

On the other hand, all trends between air quality parameters at the stores (particle, VOC, and ozone concentrations) were consistent with observed trends in the literature. For instance, the indoor concentrations of ozone and PM_{10} were correlated with the air

exchange rate since commercial buildings have relatively high air exchange rates and are more susceptible to outdoor pollutants than residential buildings.

The absence of a clear relationship between the concentration of ROS and other pollutants may be due to the fact that the chemistry of formation of ROS is quite complex. This has also been cited in the atmospheric chemistry literature as a reason for weak or moderate correlations between peroxide concentrations and certain atmospheric conditions (such as ambient ozone concentration) that are thought to influence peroxide concentrations (Logan et al., 1981; Jackson and Hewitt, 1999; Largiuni et al., 2002; Venkatachari et al., 2007). The absence of direct correlations between particulate ROS concentrations and pollutant concentrations indicates the need to separately assess indoor concentrations of particulate ROS to better understand the oxidative potential of the indoor environment. In the same way, some researchers have concluded that the oxidative activity of PM needs to be measured to capture a toxicologically relevant feature of PM because no other PM characteristic is a reliable surrogate for it. They have measured the ability of ambient PM to generate •OH or measured its capacity to deplete antioxidants in simulated lung-lining fluid, and have found that the oxidative activity of PM is not related to PM mass concentration or PM characteristics, such as the mass concentration of chemical elements on PM, including sulfur, silicon, aluminum, iron, zinc, and lead (Shi et al., 2003; Kunzli et al., 2006).

This is one of the first studies to simultaneously assess the indoor and outdoor concentration of particulate ROS. The only other study that the authors are aware of that reports the ROS concentration in an indoor environment is See et al. (2007) which recorded a concentration of 3 nmoles/m^3 on $PM_{2.5}$ inside a university building in Singapore. No simultaneous outdoor measurement was made in that study. Other than that, research has mostly focused on ROS in outdoor air. Studies on particulate ROS in

outdoor air have reported concentrations ranging from 0.54 nmoles/m^3 for $PM_{3.2}$ in Taipei, Taiwan (0.61 nmoles/m^3 for PM_{10}), to 4.95 nmoles/m^3 for $PM_{2.5}$ near Los Angeles around midday during summer (6.11 nmoles/m^3 for TSP) (Hung and Wang, 2001; Venkatachari et al., 2005; Venkatachari et al., 2007; See et al., 2007). The indoor and outdoor particulate ROS concentrations measured in our study either fall in or below the range of outdoor concentrations reported in these studies.

In this study, the mean concentration of ROS on $PM_{2.5}$ in each dataset (residential, institutional and retail buildings) ranges between 1 and 1.5 nmoles/m^3. The highest particulate ROS concentrations (3-5 nmoles/m^3) in the residential and institutional building datasets were generally recorded when the outdoor ozone concentration was close to 50 ppb. In certain conditions, high particulate ROS concentrations also coincided with high outdoor particle concentrations. Despite these observations, this study found that indoor particulate ROS concentrations are not correlated with other air quality parameters or building characteristics. However, the absence of variation and sufficient resolution in the data may have led to this result. The size of a particle partially determines where it is deposited in the respiratory tract (Yeh et al., 1996) and $PM_{2.5}$ was chosen for sampling in this study because it has a high likelihood of reaching the alveoli and triggering a health outcome. Given that indoor concentrations of ROS on $PM_{2.5}$ can be similar to outdoor concentrations, it is important to investigate the sources of indoor particulate ROS and try to reduce exposure to this pollutant.

Exposure to Particulate ROS

A simplified model, assuming steady state concentrations of ROS, was developed to estimate exposure to particulate ROS in the sampled environments. Exposure to ROS was calculated using the following principle: $E = H \times BR \times C$, where E denotes the exposure to ROS during a particular activity, H denotes the average number of hours

people are involved in that activity, BR denotes the average breathing rate of people, and C denotes the concentration of ROS in the location where the activity is taking place. An inhalation rate of 8.4 m^3/day (corresponding to 0.35 m^3/hr), which is representative of inhalation rates for children and female adults, was used to estimate ROS intake (Layton, 1993). The length of time people spend in different activities was obtained from the American Time Use Survey (ATUS) at the Bureau of Labor Statistics (U.S. BLS, 2013), a human activity data set (Klepeis et al., 2001), and a research study on retail workers (Retail Action Project, 2012). Occupational exposures to particulate ROS were estimated for office workers (working full time 40.5 hours/week) and retail workers (60% working part time for 26 hours/week, 40% working full time 39 hours/week). Other short-term exposures during time spent outdoors, doing housework at home and shopping in retail stores was also estimated. The model parameters as well as the estimated ROS intake for each activity are presented in Table A.3.

The model results indicate that occupational exposure to particulate ROS can be one of the largest sources of exposure to particulate ROS, several times greater than exposure to ROS during spending time outside. In this model, the length of time individuals spend at each location drives the extent of their exposure. This is because the inhalation rate was assumed to remain constant; however, it should be noted that the inhalation rate depends greatly on the level of activity. In particular, it may be almost 5 times higher during intense exercise (such as riding a bicycle) as compared to during sedate activities (Panis et al., 2010). The breathing rate is likely going to be much higher during housework than during sleeping, leading to higher rates of exposures to ROS during cleaning than while sitting quietly. This model has several additional limitations. The concentration of outdoor particulate ROS tends to peak around midday and falls to its minimum at night, which may be at least 15-30% lower than midday concentrations

(Venkatachari et al., 2005; Venkatachari et al., 2007). The indoor concentration of ROS was only measured around midday in this study, which is why nighttime exposure to ROS (during sleeping) cannot be accurately predicted. If the concentration of ROS in homes is similar during the day and night, then sleep-time exposure to ROS can be higher than occupational exposure to ROS because of the duration of exposure. In addition, it was assumed that the concentration of particulate ROS did not vary significantly during the day. The model also assumes that the particulate ROS concentration in most buildings in the U.S. is similar to the concentrations measured in the buildings sampled in this study. This is justifiable only for urban areas which have indoor and outdoor conditions similar to that in Austin, TX. Variations to the model include some occupational groups, such as cleaning personnel, who may be exposed to localized sources of high particulate ROS concentrations. Despite these shortcomings, this model gives a rough estimate of the types of exposure people may face in different types of environments. More targeted studies would have to be conducted to determine the exposures for specific groups of workers.

4. Conclusions

This study advanced methods for measurement of particulate ROS and applied these methods to measure ROS in residential buildings, institutional buildings and retail stores. After taking steps to minimize background, 40 sampling events were conducted at 23 residential and commercial buildings to measure indoor and outdoor ROS on $PM_{2.5}$ concentrations. The most important conclusion drawn was that the indoor concentrations of particulate ROS in the residential, institutional and retail buildings sampled in this study were not significantly different from the outdoor concentrations of particulate ROS ($n=40$, $p=0.48$). Secondly, the concentrations of particulate ROS inside these different types of buildings were not significantly different from each other ($p=0.09$ for

institutional and residential buildings, p=0.25 for institutional and retail buildings, p=0.65 for retail and residential buildings). The indoor to outdoor ratio of ROS on $PM_{2.5}$ at the retail and institutional buildings (0.80 ± 0.75 and 1.02 ± 0.55, respectively) was not significantly different from that at residential buildings (1.22 ± 0.85). These are important results in two ways: (1) they imply that transport of outdoor ROS into the buildings or generation of ROS inside the buildings are important enough to compete with photochemical processes generating ROS in outdoor environments; (2) occupational exposure to particulate ROS can be one of the largest sources of exposure to particulate ROS, several times greater than exposure to ROS during time spent outside. Given the similarity between different indoor environments, the objective of future studies should be to elucidate the possible sources of indoor particulate ROS and appropriate strategies to reduce indoor exposures.

Acknowledgements

S.S.K. was funded by the National Science Foundation (IGERT Award DGE 0549428), US EPA (STAR Fellowship FP-91747501-0), and Cockrell School of Engineering Thrust Fellowship. Funding for this research was partially supported by Grant No. 5T42OH008421 from the National Institute for Occupational Safety and Health (NIOSH) / Centers for Disease Control and Prevention (CDC) to the Southwest Center for Occupational and Environmental Health (SWCOEH), a NIOSH Education and Research Center. Access to and measurements of the air quality parameters at the retail stores were measured as part of research project RP-1596 funded by the American Society of Heating, Refrigerating, and Air-Conditioning Engineers. We thank all the residents and building owners who volunteered their buildings for testing. We also thank Brent Stephens and Marwa Zaatari who collected the calibration data for the DustTrak and SidePak.

Tables and Figures

Table A.1: The indoor and outdoor concentration of ROS on $PM_{2.5}$, indoor and outdoor concentration of $PM_{2.5}$, indoor concentration of total VOCs, and outdoor concentration of ozone during sampling at two houses in March (R4-1 and R5-1) and July (R4-2, R5-2).

Site	ROS [nmoles/m^3]		$PM_{2.5}$ [$\mu g/m^3$]		VOC [ppb]	Ozone [ppb]
	In	Out	In	Out	In	Out
R4-1 (March)	0.67 ± 0.3	1.10 ± 0.66	0.0 ± 3.2	8.7 ± 2.2	91 ± 22	25.3 ± 3.8
R4-2 (July)	2.49 ± 0.39	1.78 ± 0.19	2.1 ± 1.0	22.8 ± 2.6	120 ± 21	26.8 ± 3.9
R5-1 (March)	1.29 ± 0.27	1.91 ± 0.0	3.9 ± 3.2	9.0 ± 2.2	185 ± 21	23.7 ± 1.6
R5-2 (July)	0.90 ± 0.06	1.66 ± 0.13	4.6 ± 1.0	9.2 ± 2.5	156 ± 20	37.7 ± 1.8

Table A.2: The volume of the retail stores and the air exchange rate at each site measured over a four-hour period on one of the sampling days.

Retail Store	Volume [m^3]	ACH [1/hr]	Store Type
S1	14,900	1.14 ± 0.25	Grocery
S2	61,200	0.42 ± 0.10	General Merchandise
S3	19,800	0.30 ± 0.03	Furniture
S4	61,200	0.49 ± 0.10	General Merchandise
S5-1[a]	55,200	0.68 ± 0.28	General Merchandise
S5-2[a]	55,200	0.48 ± 0.14	General Merchandise

[a]The air exchange rate at Store 5 was raised during Week 1 (5-1) for the purpose of a complementary study and brought back down during Week 2 (5-2).

Table A.3: Estimated Occupational and Casual Exposures to Particulate ROS

Activity	Average % Population Engaged	Average Hours/Day Engaged	Breathing Rate During Activity [m^3/hr]	Activity Location	Particulate ROS Concentration [nmoles/m^3]	Dose [nmoles/ day]
Occupational Exposures	44					
Office Worker	-	8.1	0.35	Institutional	1.12	3.2
Retail Worker	2.6	4.5	0.35	Retail	1.09	1.7
Casual Exposures						
Purchasing Consumer Goods	38	0.9	0.35	Retail	1.09	0.3
Housework	35	1.7	0.35	Residential	1.38	0.8
Spending Time Outside	-	1.8	0.35	Outdoor	1.34	0.8

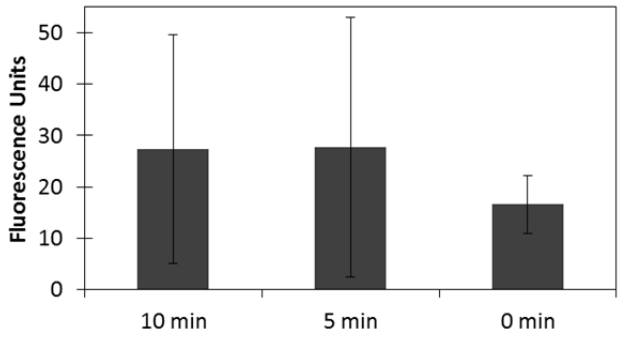

Figure A.1: Background fluorescence of blank PTFE filters sonicated for various durations in DCFH-HRP reagent. L to R: Sonication in 10 ml DCFH-HRP solution for 10 minutes or 5 minutes and sonication in 5 ml buffer for 10 minutes prior to addition of DCFH-HRP ("0 min"). The error bars denote standard deviation from five samples.

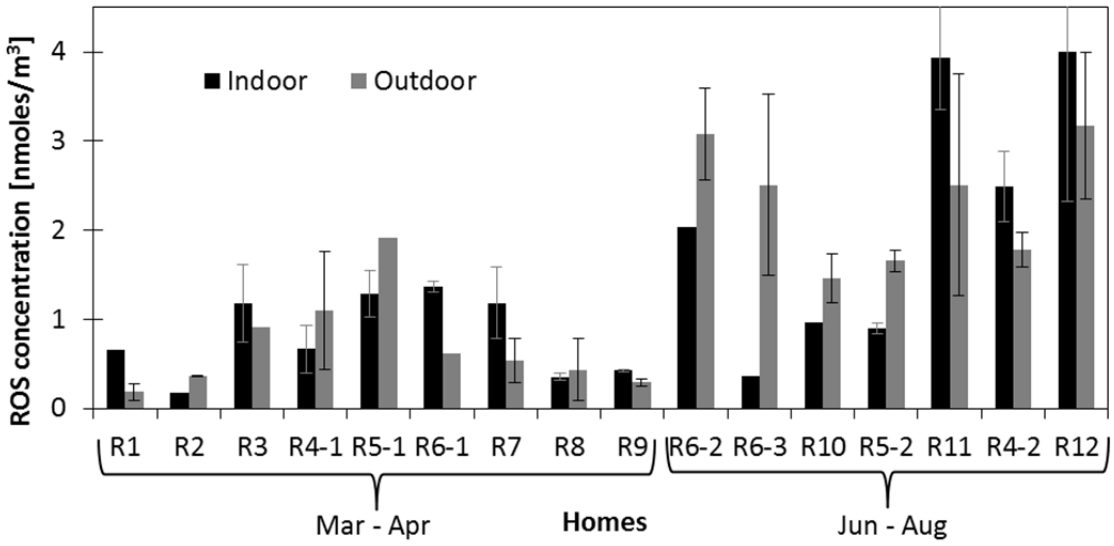

Figure A.2: Indoor and outdoor concentrations of ROS on PM2.5 at twelve residential buildings. The error bars represent standard error of duplicate samples. Repeat sampling was conducted at R4, R5, and R6 under different conditions and a number is appended to these labels to differentiate between multiple visits to the same house.

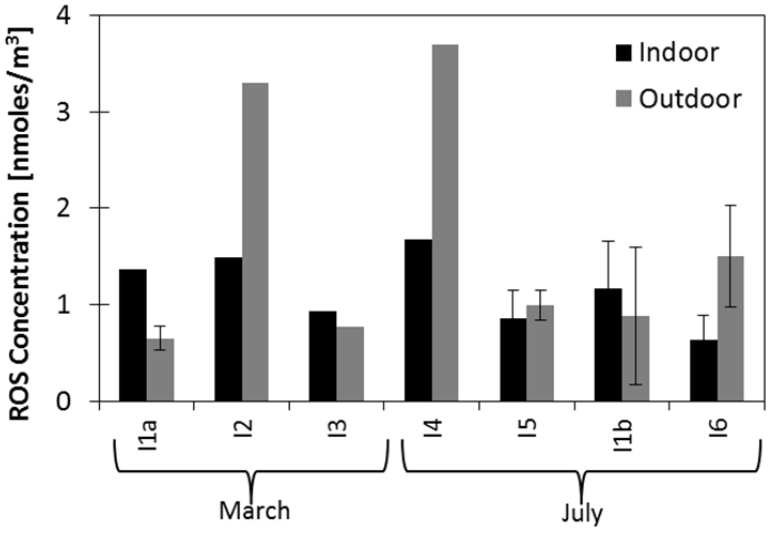

Figure A.3: Indoor and outdoor concentrations of ROS on $PM_{2.5}$ sampled at six institutional buildings. The error bars represent standard error of duplicate samples when applicable. Repeat sampling was conducted at I1 under different conditions.

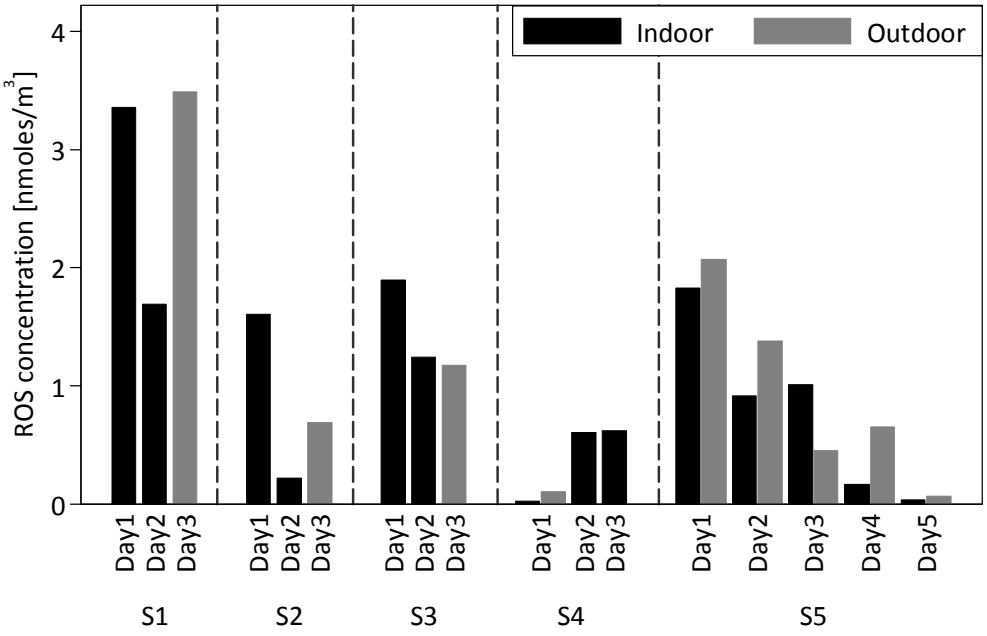

Figure A.4: Indoor and outdoor concentrations of ROS on $PM_{2.5}$ sampled at five retail stores. One sampler was used either indoors or outdoors at Stores 1, 2, 3, and 4 (Days 2 and 3 only). Two samplers were used to take simultaneously indoor and outdoor measurements at Store 4 (Day 1) and Store 5 (all days).

References

Avol, E. L.; Navidi, W. C.; Colome, S. D. Modeling ozone levels in and around Southern California homes. *Environ. Sci. Technol.*, 1998, 32, 463–468.

Bennett, D. H., Fisk, W., Apte, M. G., Wu, X., Trout, A., Faulkner, D., Sullivan, D. Ventilation, Temperature, and HVAC Characteristics in Small and Medium Commercial Buildings in California. *Indoor Air*, 2012, 22, 309-320.

Boogaard, H., Janssen, N. A. H., Fischer, P. H., Kos, G. P. A., Weijers, E. P., Cassee, F. R., van der Zee, S. C., de Hartog, J. J., Brunekreef, B., Hoek, G. (2012) Contrasts in Oxidative Potential and Other Particulate Matter Characteristics Collected Near Major Streets and Background Locations. *Environ Health Persp*, 2012, 120, 2, 185-191.

Bowler, R. P., Crapo, J. D. Oxidative Stress in Allergic Respiratory Diseases. *Journal of Allergy and Clinical Immunology*, 2002, 110, 3, 349-356.

Brown, S. K.; Sim, M. R.; Abramson, M. J.; Gray, C. N. Concentrations of Volatile Organic Compounds in Indoor Air – A Review. *Indoor Air*, 1994, 4, 2, 123-134.

Bufalini, J. J., Gay, B. W., Brubaker, K. L. Hydrogen Peroxide Formation from Formaldehyde Photooxidation and its Presence in Urban Atmospheres. *Environ. Sci. Technol.*, 1972, 6, 9, 816-821.

Chao, C. Y. and Chan, G. Y. Quantification of Indoor VOCs in Twenty Mechanically Ventilated Buildings in Hong Kong. *Atmos. Environ.*, 2001, 35, 5895-5913.

Chen, X. and Hopke, P. K. Secondary organic aerosol from α-pinene ozonolysis in dynamic chamber system. *Indoor Air*, 2009, 19, 335-345.

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.

Churg, A. Interactions of Exogenous or Evoked Agents and Particles: The Role of Reactive Oxygen Species. *Free Radical Biology & Medicine*, 2003, 34, 10, 1230-1235.

Docherty, K. S., Wu, W., Lim, Y. B., Ziemann, P. J. Contributions of Organic Peroxides to Secondary Aerosol formed from Reactions of Monoterpenes with O_3. *Environ. Sci. Technol.*, 2005, 39, 4049-4059.

EPA 40CFR, Part 136, 2011 – Guidelines Establishing Test Procedures for the Analysis of Pollutants, Appendix B – Definition and Procedure for the Determination of the Method Detection Limit, Revision 1.11. Accessed 4/2/2013 at http://www.gpo.gov/fdsys/pkg/CFR-2011-title40-vol23/pdf/CFR-2011-title40-vol23-part136-appB.pdf

Fan, Z., Weschler, C. J., Han, I-K., Zhang, J. Co-formation of hydroperoxides and ultra-fine particles during the reactions of ozone with a complex VOC mixture under simulated indoor conditions. *Atmos. Environ.*, 2005, 39, 5171-5182.

Finlayson-Pitts, B. J.; Pitts, J. N. Chemistry of the Upper and Lower Atmosphere; Academic Press, San Diego, CA, 2000.

Fisk, W. J. and Rosenfeld, A. H. Estimates of Improved Productivity and Health from Better Indoor Environments, *Indoor Air*, 1997, 7, 158-172.

Friedlander, S. K. and Yeh, E. K. The Submicron Atmospheric Aerosol as a Carrier of Reactive Chemical Species: Case of Peroxides. *Appl. Occup. Environ. Hyg.*, 1998, 13, 6, 416-420.

Gunz, D. W.; Hoffman, M. R. Atmospheric Chemistry of Peroxides: A Review. *Atmos. Environ.*, 1990, 24A, 7, 1601-1633.

Guyton, K. Z., and Kensler, T. W. Oxidative Mechanisms in Carcinogenesis, *Brit. Med. Bull.*, 1993, 49, 3, 523-544.

Hasson, A. S. and Paulson, S. E. An Investigation of the Relationship between Gas-Phase and Aerosol-Borne Hydroperoxides in Urban Air, *J. Aerosol Sci.*, 2003, 34, 459-468.

Huang, M-F., Lin, W-L., Ma, Y-C. A Study of Reactive Oxygen Species in Mainstream of Cigarette. *Indoor Air*, 2005, 15, 135-140.

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

Hodgson, A. T.; Rudd, A. F.; Beal, D.; Chandra, S. Volatile Organic Compound Concentrations and Emission Rates in New Manufactured and Site-Built Houses. *Indoor Air*, 2000, 10: 178-192.

Jackson, A. V. and Hewitt, C. N., Atmosphere Hydrogen Peroxide and Organic Hydroperoxides: A Review. *Crit. Rev. Env. Sci. Tech.*, 1999, 29, 2, 175-228.

Kehrer, J. P. Free Radicals as Mediators of Tissue Injury and Disease. *Crit. Rev. Toxicol.*, 1993, 23, 1, 21-48.

Klaunig, J. E., Kamendulis, L. M. The role of oxidative stress in carcinogenesis. *Annu. Rev. Pharmacol.*, 2004, 44, 239-267.

Klepeis, N. E., Nelson, W. C., Ott, W. R., Robinson, J. P., Tsang, A. M., Switzer, P., Behar, J. V., Hern, S. C., Engelmann, W. H. The National Human Activity Pattern Survey (NHAPS): A Resource for assessing exposure to environmental pollutants, *J. Expo. Anal. Env. Epid.*, 2001, 11, 231-252.

Kooy, N. W., Royall, J. A., Ischiropoulos, H. Oxidation of 2',7'-Dichlorofluorescin by Peroxynitrite. *Free Radical Res.*, 1997, 27,3: 245-254.

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. A. Comparison of Oxidative Properties, Light Absorbance, and Total and Elemental Mass Concentration of Ambient $PM_{2.5}$ Collected at 20 European Sites. *Environ. Health Persp.,* 2006, 114, 5, 684-690.

Lang, F.; Gulbins, E.; Lang, P. A.; Zapulla, D.; Foller, M. Ceramide in Suicidal Death of Erythrocytes. *Cellular Physiology and Biochemistry*, 2010, 26, 1, 21-28.

Lansing, M. W., Ahmed, A., Cortes, A., Sielczak, M. W., Wanner, A., Abraham, W. M. Oxygen radicals contribute to antigen-induced airway hyperresponsiveness in conscious sheep. *Am. Rev. Respir. Dis.*, 1993, 147, 2, 321-326.

Largiuni, O., Giacomelli, M. C., Piccardi, G. Concentration of Peroxides and Formaldehyde in Air and Rain and Gas-Rain Partitioning. *J. Atmos. Chem.,* 2002, 41, 1-20.

Layton, D. W. Metabolically consistent breathing rates for use in dose assessments. *Health Phys.*, 1993, 64, 1, 23-36.

Li, N., Xia, T., Nel, A. E. The role of oxidative stress in ambient particulate matter-induced lung diseases and its implictions in the toxicity of engineered nanoparticles. *Free Radical Bio. Med.*, 2008, 44, 1689-1699.

Li, N., Hao, M., Phalen, R. F., Hinds, W. C., Nel, A. E. Particulate air pollutants and asthma – A paradigm for the role of oxidative stress in PM-induced adverse health effects. *Clin. Immunol*, 2003, 109, 250-265.

Li, T-H., Turpin, B. J., Shields, H. C., Weschler, C. J. Indoor Hydrogen Peroxide Derived from Ozone/*d*-Limonene Reactions. *Environ. Sci. Technol.*, 2002, 36, 3295-3302.

Liu, D-L; Nazaroff, W. W. Modeling Pollutant Penetration across building envelope. *Atmos. Environ.*, 2001, 35, 4451-4462.

Logan, J. A., Prather, M. J., Wofsy, S. C., McElroy, M. B. Tropospheric Chemistry: A Global Perspective. *J. Geophys. Res.* 1981, 86, 7210-7254.

Morrison, G. C.; Nazaroff, W. W. The rate of ozone uptake on carpet: mathematical modeling. *Atmos. Environ.*, 2002, 36, 1749-1756.

Nazaroff, W. W.; Cass, G. R. Mathematical Modeling of Chemically Reactive Pollutants in Indoor Air. *Environ. Sci. Technol.*, 1986, 20, 924-934.

NIST e-Handbook of Statistical Methods, 2010. Detection of Outliers, 1.3.5.17. http://www.itl.nist.gov/div898/handbook/, accessed 11/1/2012

Panis, L. I., Geus, B., Vandenbulcke, G., Willems, H., Degraeuwe, B., Bleux, N., Mishra, V., Thomas, I., Meeusen, R. Exposure to Particulate Matter in Traffic: A Comparison of Cyclists and Car Passengers. *Atmos. Environ.*, 2010, 44, 2263-2270.

Paulson, S. E.; Orlando, J. J. The reactions of ozone with alkenes: An important source of HO_x in the boundary layer. *Geophys. Res. Lett.*, 1996, 23, 25, 3727-3730.

Persily, A.; Musser, A.; Emmerich, S. J. Modeled infiltration rate distributions for U.S. housing. *Indoor Air*, 2010, 20, 473-485.

Retail Action Project, 2012. Discounted Jobs: How Retailers Sell Workers Short. http://retailactionproject.org/2012/01/discounted-jobs-how-retailers-sell-workers-short-executive-summary/, accessed 9/10/2013.

Sanders, S. P., Zweier, J. L., Harrison, S. J., Trush, M. A., Rembish, S. J., Liu, M. C. Spontaneous oxygen radical production at sites of antigen challenge in allergic subjects. *Am. J. Resp. Crit. Care*, 1995, 151, 6, 1725-1733.

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.

Siegel, J. A., Srebric, J., Crain, N., Nirlo, E., Zaatari, M., Hoisington, A., Urquidi, J., Shu, S., Kim, Y-S., Jareemit, D. (2013) Ventilation and Indoor Air Quality in Retail Stores, ASHRAE Retail Project-1596, American Society of Heating, Refrigerating and Air-Conditioning Engineers.

Shi, T., Knaapen, A. M., Begerow, J., Birmili, W., Borm, P. J. A., Schins, R. P. F. Temporal Variation of Hydroxyl Radical Generation and 8-hydroxy-2'-deoxyguanosine formation by Coarse and Fine Particulate Matter. *Occup. Environ. Med.*, 2003, 60, 315-321.

Steinemann, A. C. Fragranced consumer products and undisclosed ingredients. *Environ. Impact Asses.*, 2009, 29, 32-38.

Steinemann, A. C.; MacGregor, I. C.; Gordon S. M.; Gallagher, L. G.; Davis, A. L.; Ribeiro, D. S.; Wallace, L. A. Fragranced consumer products: Chemicals emitted, ingredients unlisted. *Environ. Impact Asses.*, 2011, 31, 328-333.

Stephens, B.; Gall, E. T.; Siegel, J. A. Measuring the Penetration of Ambient Ozone into Residential Buildings. *Environ. Sci. Technol.*, 2012a, 46, 929-936.

Stephens, B.; Siegel, J. A. Penetration of ambient submicron particles into single-family residences and associations with building characteristics. *Indoor Air*, 2012b, 22, 501-513.

Stevens, W. H., Inman, M. D., Wattie, J., O'Byrne, P. M. Allergen-induced oxygen radical release from Bronchoalveolar Lavage cells and Airway Hyperresponsiveness in dogs. *Am. J. Resp. Crit. Care*, 1995, 151, 5: 1526-1531.

Thornburg, J., Ensor, D. S., Rodes, C. E., Lawless, P. A., Sparks, L. E., Mosley, R. B. Penetration of Particles into Buildings and Associated Physical Factors, Part I: Model Development and Computer Simulations, *Aerosol Sci. Technol.*, 2001, 34, 284-296.

Trush, M. A., and Kensler, T. W. An Overview of the Relationship between Oxidative Stress and Chemical Carcinogenesis, *Free Radical Bio. Med.*, 1991, 10, 201-209.

U. S. Department of Labor, Bureau of Labor Statistics, American Time Use Survey (ATUS), 2011a. Accessed 4/1/13. http://www.bls.gov/tus/charts/

U.S. Department of Labor, Bureau of Labor Statistics, Occupational Employment Statistics Tables, National Cross-Industry 2011b. Accessed 4/24/12. http://www.bls.gov/oes/current/oes_nat.htm#41-0000

U. S. Department of Labor, Bureau of Labor Statistics, News Release, American Time Use Survey (ATUS), 2011c, Table 1. Accessed 4/1/13. http://www.bls.gov/news.release/pdf/atus.pdf

U. S. Department of Labor, Bureau of Labor Statistics, News Release, American Time Use Survey (ATUS), 2013, Table 1. Accessed 4/1/13. http://www.bls.gov/news.release/pdf/atus.pdf

Venkatachari, P., Hopke, P. K., Grover, B. D., Eatough, D. J. Measurement of Particle-Bound Reactive Oxygen Species in Rubidoux Aerosols. *J. Atmos. Chem.*, 2005, 50: 49-58.

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.

Venkatachari, P., Hopke, P. K. Development and Laboratory Testing of an Automated Monitor for the Measurement of Atmospheric Particle-bound Reactive Oxygen Species (ROS), *Aerosol Sci. Technol.*, 2008, *42*, 629–635.

Wallace, L. A.; Pellizzari, E.; Leaderer, B.; Hartwell, T.; Perritt, R.; Zelon, H.; Sheldon, I. Emissions of volatile organic compounds from building materials and consumer products. *Atmos. Environ.*, 1987, 21(22), 385-393.

Wallace, L. A.; Pellizzari, E.; Wendel, C. Total volatile organic concentrations in 2700 personal, indoor, and outdoor air samples collected in US EPA TEAM Studies. *Indoor Air*, 1991, 4, 465-477.

Weschler, C. J. Ozone's Impact on Public Health: Contributions from Indoor Exposures to Ozone and Products of Ozone-Initiated Chemistry. *Environ. Health Persp.*, 2006, 114, 10, 1489-1496.

Witz, G. Active Oxygen Species as Factors in Multistage Carcinogenesis, *P. Soc. Exp. Biol. Med.*, 1991, 198, 2, 675-682.

Yeh, H-C., Cuddihy, R. G., Phalen, R. F., Chang, I-Y. Comparisons of Calculated Respiratory Tract Deposition of Particles Based on the Proposed NCRP Model and the new ICRP66 Model, *Aerosol Sci. Tech.*, 1996, 25, 2, 134-140.

Zelm, R. V., Huijbregts, M. A. J., Hollander, H. A. D., Jaarsveld, H. A. V., Sauter, F. J., Struijs, J., Wijnen, H. J. V., Meent, D. V. European Characterization Factors for

Human Health Damage of PM_{10} and Ozone in Life Cycle Impact Assessment, *Atmos. Environ.*, 2008, 42, 441-453.

Zhu, H., Bannenberg, G. L., Moldeus, P., Shertzer, H. G. Oxidation pathways for the intracellular probe 2',7'-dichlorofluorescin. *Arch. Toxicol.*, 1994, 68: 582-587.

Zhu, J.; Cao, X. L.; Beauchamp, R. Determination of 2-butoxyethanol emissions from selected consumer products and its application in assessment of inhalation exposure associated with cleaning tasks. *Environ. Int.*, 2001, 26, 589-597.

Supplementary Information

Optimization of Methods

The background fluorescence of several types of particle sampling filters used in Personal Environmental Monitors (PEMs) (SKC, PA, USA) was assessed in order to select a suitable filter for sampling that produced the lowest fluorescence intensity. Each filter was sonicated in 5 ml buffer, followed by addition of DCFH-HRP. Based on their low background fluorescence, PTFE filters (TF1000, 1μm pore size, 37 mm, Pall, NY, USA) were selected for sampling in the buildings. These filters produced a 70-95% lower background signal with the reagent than other 37 mm particle sampling filters used in PEMs (Figure A.S1). Glass-fiber based filters (Pall 2: Emfab filter; W: Whatman glass fiber filter) and filters with polymethylpentene (PMP) support rings (Pall 1: Teflo membrane; SKC: PTFE filter with PMP support ring) are frequently used for particle sampling, but produce high background fluorescence with the DCFH assay. Other filters were fragile and would rupture (Pall 5: GLA5000 PVC membrane) or break (Pall Tissuquartz™ filter, not shown on graph) during handling. Polytetrafluoroethylene (PTFE) filters offer mechanical resistance and are supposed to have low chemical backgrounds, but the filter support material was seen to influence the background fluorescence of the filters in the DCFH assay (Pall 3: Zefluor™ membrane; Pall 4: Zylon™ membrane; Pall 6: TF1000). Pall TF1000 (Pall 6, a PTFE membrane with a

polypropylene support) was chosen for sampling in this study because of its mechanical resistance and low background fluorescence in the DCFH assay, which was further reduced by rinsing the filter overnight in dI water followed by complete air-drying (Pall 6R).

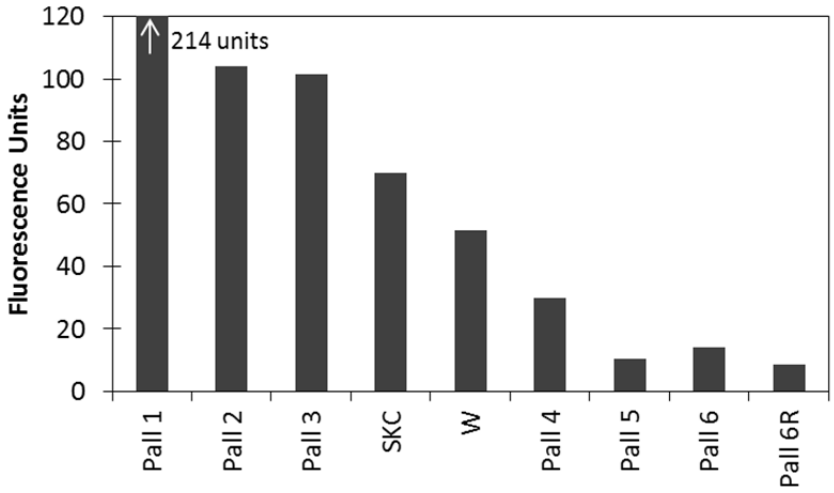

Figure A.S1: Background fluorescence of filters of different brands and materials. From left to right: Pall 1 = Pall Teflo, PTFE with PMP ring; Pall 2 = Pall Emfab; Pall 3 = Pall Zefluor, PTFE with PTFE support; SKC = SKC PTFE filter with PMP support ring; W = Whatman Glass Fiber Filter; Pall 4 = Pall Zylon, unsupported PTFE; Pall 5 = Pall GLA5000, low ash, PVC membrane; Pall 6 = Pall TF1000, PTFE filter on polypropylene support; Pall 6R = Pall TF1000 rinsed overnight.

In addition, we found that the background fluorescence generated by glassware that had been soaked overnight in a 10% nitric acid bath was 32% lower than that of glassware that had been washed with soap alone. As a result, all experiments were conducted with acid-cleaned glassware. As a result of these modifications, we were able to achieve lower fluorescence intensity of field blanks than that reported by previous studies. The mean background fluorescence of unsampled filters in this study was 20% of the sampled filters (with a range of 7-35%), which represents less than 8% of the ROS

concentration measured on the sampled filters after correcting for background fluorescence of blank water.

Air Quality Data

Tables A.S1, A.S2 and A.S3 contain air quality data collected at the residential, institutional and retail buildings during sampling.

Table A.S2: Air quality parameters measured during sampling at six institutional buildings.

Site	$PM_{2.5}$ [$\mu g/m^3$]		VOC Conc. [ppb]	Ozone Conc.[a] [ppb]	Temperature [°C]		Relative Humidity [%]	
	Indoor	Outdoor[a]	Indoor	Outdoor	Indoor	Outdoor	Indoor	Outdoor
I1a	na	7.0 ± 2	4738 ± 6530	37.7 ± 2.6	na	24.5 ± 1.7[a]	na	na
I2	na	9.0 ± 2	na	47.3 ± 3.9	na	27.2 ± 0.8[a]	na	na
I3	na	8.2 ± 4	na	45.2 ± 8.2	na	26.1 ± 1.5[a]	na	na
I4	4.7 ± 2	10.0 ± 3	298 ± 20	48.3 ± 4.1	23.2 ± 0.4	30.4 ± 0.8	56 ± 1	63 ± 3
I5	0.0 ± 1	5.5 ± 2	10 ± 20	37.8 ± 1.8	24.2 ± 0.4	39.3 ± 6.4	56 ± 1	47 ± 10
I1b	0.0 ± 1	8.7 ± 3	19 ± 20	30.3 ± 1.9[b]	22.7 ± 0.3	36.3 ± 0.7	54 ± 2	40 ± 2
I6	0.4 ± 3	3.5 ± 2	50 ± 20	34.0 ± 1.9	23.0 ± 0.4	35.2 ± 0.6	49 ± 1	44 ± 3

na Data not collected.
[a] Data from CAMS3 located 8 km away from the institutional buildings.
[b] Data from CAMS38 (next closest TCEQ site) because of instrument error at CAMS3.

Table A.S1: The indoor and outdoor concentration of ROS on $PM_{2.5}$, air quality parameters collected during sampling and building characteristics for sixteen sampling events at twelve homes.

Site	ROS Conc [nmoles/m^3]		$PM_{2.5}$ [$\mu g/m^3$]		Temperature [°C]		Relative Humidity [%]		VOC [ppb]	Ozone [ppb]	Dist to major Road [m]	Year Built	Area [m^2]	Floor	Building Exterior	People
	In	Out	In	Out	In	Out	In	Out	In	Out						
R1	0.66 [d]	0.19	6.0 ± 3.2	11.2 ± 2.6	na	na	na	na	0 ± 20	23.2 ± 4.9	1931	1953	220	Wood	Vinyl siding	1
R2	0.18 [d]	0.37	6.0 ± 3.2	10.8 ± 2.2	na	na	na	na	257 ± 46	23.6 ± 3.0	805	1996	201	Carpet 80%, wood 20%	Brick	0
R3	1.18	0.91 *	0.0 ± 3.2	10.5 ± 2.2	na	na	na	na	506 ± 111	25.3 ± 4.9	805	1961	74	Tile	Wood siding	D
R4-1	0.67	1.10	0.0 ± 3.2	8.7 ± 2.2	na	na	na	na	91 ± 22	25.3 ± 3.8	1448	1985	183	Wood 60%, carpet 40%	Stone, Painted wood siding	0
R5-1	1.29	1.91 *	3.9 ± 3.2	9.0 ± 2.2	na	na	na	na	185 ± 21	23.7 ± 1.6	161	1969	177	Wood 75%, carpet 25%	Brick	4
R6-1	1.37	0.62 *	3.6 ± 3.2	8.6 ± 2.1	na	na	na	na	73 ± 23	25.8 ± 3.4	483	2008	121	Linoleum	Painted fiber cement siding	0
R7	1.19	0.54	9.0 ± 3.0	err	28.2 ± 1.4	34.9 ± 2.1	41.1 ± 4.1	25.0 ± 1.5	176 ± 24	57.0 ± 3.5	483	2005	111	Carpet 50%, Tile 50%	Painted wood siding	D
R8	0.36	0.44	3.3 ± 3.2	err	24.4 ± 0.6	31.2 ± 4.3	52.8 ± 2.1	44.6 ± 10.0	34 ± 20	57.8 ± 6.5	644	2004	251	Carpet 33%, Wood 67%	Painted wood siding	1
R9	0.43	0.29	1.5 ± 3.2	err	25.1 ± 0.4	30.4 ± 4.6	56.4 ± 1.7	37.7 ± 8.7	133 ± 21	62.5 ± 4.7	644	1960	111	Linoleum	Red Brick	2
R6-2	2.03 *	3.08	3.4 ± 2.8	9.0 ± 2.0	25.6 ± 0.4	34.7 ± 0.8	65.5 ± 1.9	43.4 ± 2.3	na	32.3 ± 1.6	483	2008	121	Linoleum	Painted fiber cement siding	0
R6-3	0.36 *	2.51	0.3 ± 2.1	8.3 ± 2.1	25.7 ± 0.4	33.4 ± 0.7	61.2 ± 1.9	43.0 ± 3.1	na	52.0 ± 2.3	483	2008	121	Linoleum	Painted fiber cement siding	0
R10	0.97 *	1.46	8.7 ± 1.0	15.0 ± 2.2	27.6 ± 0.9	33.6 ± 1.0	52.6 ± 1.4	44.0 ± 4.3	360 ± 36	27.0 ± 3.4	1931	2003	121	Carpet 95%, Tile 5%	Concrete	1 + D
R5-2	0.90	1.66	4.6 ± 1.0	9.2 ± 2.5	28.6 ± 0.4	42.5 ± 6.8	46.7 ± 1.6	25.4 ± 7.4	156 ± 20	37.7 ± 1.8	161	1969	177	Wood 75%, carpet 25%	Brick	4
R11	3.93	2.51	1.6 ± 1.0	7.4 ± 2.5	25.9 ± 0.7	38.6 ± 6.2	51.9 ± 2.1	43.0 ± 1.1	2100 ± 210	60.6 ± 4.7	805	1979	111	Carpet 85%, wood 15%	Concrete	1 + C
R4-2	2.49	1.78	2.1 ± 1.0	22.8 ± 2.6	25.3 ± 0.4	32.6 ± 3.6	72.9 ± 2.7	58.7 ± 10.9	120 ± 21	26.8 ± 3.9	1448	1985	183	Wood 60%, carpet 40%	Stone, Painted wood siding	0
R12	4.01	3.18	22.9 ± 1.0	8.8 ± 2.8	27.6 ± 0.4	41.3 ± 2.2	41.6 ± 1.3	28.2 ± 3.0	2873 ± 326	59.4 ± 2.6	1931	1972	60	Wood 60%, carpet 40%	Concrete	0

All ROS measurements are averages of replicate samples, except when the replicate measurement was excluded because it was below the detection limit ([d]) or was an outlier (*). Temperature, RH and VOC concentrations were not measured at some homes, denoted na. The outdoor $PM_{2.5}$ concentration is not available on a few days due to an error at the TCEQ sampling station, denoted err. If a house pet remained indoors during the length of the sampling, a D (dog) or C (cat) was included in the People column.

Table A.S3: Air quality measurements inside and outside the retail stores during ROS sampling.

Site	$PM_{2.5}$ Conc. [µg/m^3]		PM_{10} Conc. [µg/m^3]		VOC Concentration [ppb]					Ozone Conc. [ppb]	
					Sorbent Tubes		PID	Suma Canister	DNPH		
	Indoor	Outdoor	Indoor	Outdoor	Indoor	Outdoor	Indoor	Indoor	Indoor	Indoor	Outdoor
Site1-Day1	7.2 ± 2.9	4.1 ± 2.9	20.7 ± 4.5	12.0 ± 2.9	-	-	792 ± 108	-	-	9.2 ± 1.9	26.3 ± 1.5[a]
-Day2	10.9 ± 1.5	7.4 ± 4.9	20.4 ± 6.0	15.6 ± 2.7	18.4 ± 13.2	7.7 ± 2.2	762 ± 85	1975 ± 1115	56.5 ± 14.8	6.3 ± 1.7	21.7 ± 2.1[a]
-Day3	-	22.3 ± 2.6	-	21.5 ± 3.6	-	-	-	-	-	-	28.7 ± 5.1[a]
Site2-Day1	4.0 ± 1.8	19.1 ± 10.8	10.9 ± 1.6	18.5 ± 7.9	-	-	103 ± 20	-	-	4.6 ± 1.6	31.3 ± 1.5[a]
-Day2	3.2 ± 1.2	5.8 ± 2.9	7.8 ± 2.1	5.6 ± 1.9	67.5 ± 17.1	16.8 ± 12.2	-	274 ± 98	17.4 ± 1.7	3.5 ± 1.6	28.8 ± 7.8[a]
-Day3	9.3 ± 1.0	116.7 ± 27.1	13.0 ± 1.6	60.2 ± 12.2	-	-	167 ± 22	-	-	4.5 ± 1.8	10.0 ± 3.2[a]
Site3-Day1	0.8 ± 1.7	9.3 ± 4.4	-	13.7 ± 4.6	22.4 ± 4.8	62.1 ± 21.0	-	169 ± 39	26.9 ± 3.3	1.9 ± 1.6	22.5 ± 12.4[a]
-Day2	3.0 ± 1.0	-	5.0 ± 1.4	-	-	-	94 ± 20	-	-	1.7 ± 1.6	16.8 ± 10.2[a]
-Day3	-	13.9 ± 4.6	-	17.6 ± 4.9	-	-	88 ± 22	-	-	2.2 ± 1.9	20.0 ± 3.4[a]
Site4-Day1	3.0 ± 1.0	-	20.2 ± 1.4	-	-	-	1000 ± 103	-	-	7.4 ± 1.7	-
-Day2	0.1 ± 1.1	22.2 ± 5.6	17.4 ± 1.4	25.8 ± 7.1	118.6 ± 27.2	113.2 ± 25.6	644 ± 64	757 ± 282	24.1 ± 2.3	3.7 ± 2.0	41.8 ± 9.6
-Day3	-	-	-	-	-	-	479 ± 49	-	-	-	-
Site5-Day1	3.3 ± 1.3	-	-	-	-	-	299 ± 163	-	-	3.0 ± 1.6	-
-Day2	10.1 ± 1.4	57.2 ± 10.9	21.0 ± 3.6	51.4 ± 9.3	76.3 ± 20.5	20.4 ± 4.5	278 ± 62	133 ± 29	22.3 ± 2.8	8.0 ± 2.3	33.4 ± 4.6
-Day3	6.6 ± 1.8	-	3.4 ± 1.6	-	64.6 ± 17.1	16.3 ± 4.3	70 ± 58	160 ± 34	24.2 ± 3.0	6.4 ± 2.4	-
-Day4	3.6 ± 1.6	9.3 ± 2.9	14.5 ± 2.3	14.2 ± 2.9	144.4 ± 58.4	7.9 ± 2.9	246 ± 36	291 ± 101	33.5 ± 4.1	9.4 ± 2.5	55.8 ± 4.1
-Day5	3.8 ± 1.8	-	8.7 ± 1.5	-	128.0 ± 50.7	8.1 ± 2.6	177 ± 21	343 ± 110	32.8 ± 4.1	6.1 ± 2.5	-

- Data not collected during ROS sampling.

[a] Data from CAMS3 located within 4 miles of the retail stores.

Appendix B: Particulate Reactive Oxygen Species Concentrations and their association with Environmental Conditions in an Urban, Subtropical Climate

Technical Note: Particulate Reactive Oxygen Species Concentrations and their Association with Environmental Conditions in an Urban, Subtropical Climate

Shahana S. Khurshid*[1], Jeffrey A. Siegel[1,2], Kerry A. Kinney[1]

Published in *Atmospheric Chemistry and Physics*, 14, 6777-6784.

[1]Department of Civil, Architectural and Environmental Engineering, The University of Texas at Austin, Austin, Texas, USA.
[2]Department of Civil Engineering, The University of Toronto, Toronto, Ontario, Canada.

Abstract

Reactions between hydrocarbons and ozone or hydroxyl radicals lead to the formation of oxidized species, including reactive oxygen species (ROS), and secondary organic aerosol (SOA) in the troposphere. ROS can be carried deep into the lungs by small aerodynamic particles where they can cause oxidative stress and cell damage. While environmental studies have focused on ROS in the gas-phase and rainwater, it is also important to determine concentrations of ROS on respirable particles. Samples of $PM_{2.5}$ collected over three hours at midday on 40 days during November 2011 and September 2012 show that the particulate ROS concentration in Austin, Texas ranged from a minimum value of 0.02 nmoles H_2O_2/m^3 air in December to 3.81 nmoles H_2O_2/m^3 air in September. Results from correlation tests and linear regression analysis on particulate ROS concentrations and environmental conditions (which included ozone and $PM_{2.5}$ concentrations, temperature, relative humidity, precipitation, wind direction, and solar radiation) indicate that ambient particulate ROS is significantly influenced by the ambient ozone concentration, temperature and incident solar radiation. Particulate ROS concentrations measured in this study were in the range reported by other studies in the U.S., Taiwan and Singapore. This study is one of the first to assess seasonal variations in particulate ROS concentrations and helps explain the influence of environmental conditions on particulate ROS concentrations.

Keywords:

$PM_{2.5}$, O_3, Ozone-initiated reactions, Solar Radiation, Temperature, Reactive Oxygen Species

1. Introduction

Peroxides are generated in ambient air from alkene ozonolysis and photochemical reactions with VOCs and NO_x (Seinfeld and Pandis, 2006). As an example, hydrogen peroxide (H_2O_2) and hydroperoxyl radical (HOO•) are produced from the photooxidation of formaldehyde (Bufalini et al., 1972; Gay and Bufalini, 1972; Largiuni et al., 2002). Photochemical models suggest that peroxides can be present in both polluted and clean air (Kleinman, 1986; Heikes et al., 1996) which is confirmed by measurements (Walker et al., 2006; Snow et al., 2007). H_2O_2 is an important species in photochemical smog as a chain terminator. Its concentration in rainwater and snow has been measured since the late nineteenth century (Schöne, 1874) and studies have found strong seasonal and diurnal variations in the concentrations of H_2O_2 and other reactive oxygen species (ROS) in rainwater, water vapor, and air in gas-phase (Singh et al., 1986; Gunz and Hoffman, 1990 and references within; Ayers et al., 1992; Dollard and Davies, 1992; Lee et al., 2000; Yamada et al., 2002; Liu et al., 2003; Zhang et al., 2012). However, data on peroxide and ROS concentrations in the aerosol phase are limited.

It can be suggested that the concentration of peroxides in water associated with atmospheric aerosols can be estimated using gas-phase concentrations of peroxides. Hydroperoxides are thought to partition between the gas-phase and liquid water according to their Henry's law constants (e.g. H_{H2O2} is 0.7 - 1.1 × 10^5 M/atm at 298K; Hwang and Dasgupta, 1985; Staffelbach and Kok, 1993; Lind and Kok, 1994; Huang and Chen, 2010). Following this reasoning, an ambient gas-phase H_2O_2 concentration of 10 ppb would lead to a 1 mM concentration in liquid water associated with aerosols. However, studies on gas-phase ROS have found that urban hydroperoxide levels within aerosols are at least an order of magnitude higher than concentrations predicted by Henry's law (Arellanes et al., 2006; Hasson and Paulson, 2003;

Hewitt and Kok, 1991). The Henry's law constant in aerosols may be different from that in liquid water (Hasson and Paulson, 2003) which makes it important to assess the concentration of peroxides and ROS in aerosols.

Reactions between hydrocarbons and ozone or hydroxyl radicals (generated during the photolysis of ozone and in catalytic cycles in the troposphere) lead to the formation of oxidized species and secondary organic aerosol (SOA). Highly soluble gases of oxidized species (such as H_2O_2) will be removed by the wet mucus lining in the upper airways when they are inhaled (Wexler and Sarangapani, 1998; Sarangapani and Wexler, 2000). However, SOA are more likely to reach deep into the lungs due to their physical properties, and the ROS associated with these aerosols can, thus, reach the deeper parts of the lung and lead to oxidative stress in the tissue (Morio et al., 2001; Wexler and Sarangapani, 1998). 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.

In this study we use a bulk measure to assess the concentration of ROS on ambient particulate matter (PM). 2',7'-dichlorofluorescin diacetate (DCF-DA) is a non-specific fluorescent reagent for detecting ROS, such as hydrogen peroxide (H_2O_2), and hydroxyl radical (•OH). Ambient concentrations of particulate ROS (Hung and Wang, 2001; Venkatachari et al., 2005; Venkatachari et al., 2007; See et al., 2007) and peroxides in aerosols (Hewitt and Kok, 1991; Hasson and Paulson, 2003; Arellanes et al., 2006; Wang et al., 2010) have been measured previously. However, these studies have been conducted over short periods of time, a few months at most, and do not allow an assessment of seasonal variations in particulate ROS. Ambient particulate matter collected in different seasons has been used in studies to determine

the generation of selected oxidative species in lung epithelial cells and surrogate lung fluid (Shen et al., 2011; Vidrio et al., 2009; Baulig et al., 2004). But these seasonal studies generally did not measure the ambient environmental conditions during PM sampling. It is important to understand how the ROS concentration on respirable PM varies as environmental conditions change. Thus, the main objectives of the current study are to (1) determine the concentration of ROS on $PM_{2.5}$ in a semi-arid urban environment over a year, and (2) assess the influence of environmental conditions on these particulate ROS concentrations.

2. Materials and Methods

Samples of $PM_{2.5}$ were collected in an open area on the University of Texas at Austin campus using a Personal Environmental Monitor (PEM, SKC, PA, USA) on 40 days between November 2011 and September 2012. Two to five replicate samples were taken on 20 of these days to determine the average covariance in ROS concentration between multiple samplers. Sampling was conducted for 3±0.5 hours between 10am and 3pm using air sampling pumps at 10 l/min. Pumps were calibrated before sampling with a mini-Buck Calibrator M-30 (A. P. Buck, Orlando, FL; accuracy ±0.5%). Samplers were placed 1 m above the ground. Teflon tape was wrapped around the edges of the support screen in the PEMs to ensure a proper seal of the thin PTFE filters inside the PEMs. All sampling filters were assessed within 1 hour of collection. This methodology assesses the persistent species in ROS. Highly volatile species are likely to degrade on the order of hours (and may even degrade prior to sample analysis), but the more persistent components of ROS degrade on the order of days – our control studies indicate that the majority of particulate ROS captured on sampling filters remains stable over a day.

DCF-DA has been used as a bulk measure of ROS (Hung and Wang, 2001; Venkatachari et al., 2005; Venkatachari et al., 2007; See et al., 2007) since it becomes fluorescent in the presence of a wide variety of ROS including, but not limited to, hydrogen peroxide (H_2O_2), organic peroxyl (ROO•) and hydroxyl (•OH) radicals and the peroxynitrite anion ($ONOO^-$) (Zhu et al., 1994; Kooy et al., 1997). The use of a bulk measure enables a better understanding of the

overall toxicity potential of the PM. For instance, H_2O_2 is generally considered to be less toxic than hydroxyl radicals (Valavanidis et al., 2008), but H_2O_2 likely has significant indirect biological effects since it can diffuse across membranes easily because of its lack of charge (LaCagnin et al., 1990). The method for quantifying ROS with DCF-DA was modified slightly from previous studies (Hung and Wang, 2001; Venkatachari et al., 2005; Venkatachari et al., 2007; See et al., 2007) to help reduce high fluorescence intensity of field blanks. The method development is described in detail elsewhere (Khurshid et al., 2014). Briefly, 0.5 ml of 1 mM DCF-DA (Cayman Chemical, MI, USA) in ethanol was incubated with 2 ml of 0.01 N NaOH at room temperature for 30 mins in the dark to cleave off the acetate groups. The 2',7'-dichlorofluorescin (DCFH) solution was neutralized with 10 ml sodium phosphate buffer (pH 7.2) and the solution was kept on ice in the dark till needed. Each sampled filter was sonicated in 5 ml sodium phosphate buffer for 10 minutes. Horseradish peroxidase (HRP, ThermoScientific, IL, USA) in sodium phosphate buffer (pH 7.0) was mixed with the DCFH solution and added to the sampled filter in the dark to yield a final volume of 10 ml with a concentration of 5 μM of DCFH and 1 unit/ml of HRP. The sample was then incubated in the dark at 37˚C for 15 mins, after which 0.1 ml aliquots were placed in triplicate in a 96-well plate and the fluorescence intensity was read at 530 nm with excitation at 485 nm (Synergy HT, Biotek, VT, USA). The concentration of ROS on the sampled filters was expressed in terms of H_2O_2 per volume of air sampled (rather than per mass of particles) because this describes exposure to ROS as it occurs in the lungs (Boogaard et al., 2012). The background fluorescence intensity produced by an unsampled filter was subtracted from the samples.

Standards were prepared with hydrogen peroxide (H_2O_2). To prepare the standards, aliquots of 0.1 ml of appropriate H_2O_2 concentration were added to 3 ml of DCFH-HRP reagent to get 0, 1.0, 2.0, 3.0, and 4.0 x 10^{-7} M H_2O_2 in final solutions. These solutions were incubated at 37°C for 15 minutes and fluorescence was measured. All glassware used in the experiments was scrubbed with soap, followed by immersion in a 10% nitric acid bath and subsequent 7x rinsing with deionized water.

The Method Detection Limit of the analytical procedure, as determined using U.S. EPA's guidelines (EPA, 2011) is 1.2 nmoles H_2O_2/l, which converts to 0.01 nmoles/m^3 assuming a 3-hour sample at 10 l/min. Outliers were excluded using a more conservative approach than the Iglewicz and Hoaglin method (NIST, 2010) in that only sample concentrations with an absolute modified Z-score value greater than 10 (instead of 3.5, as recommended by the method) were excluded as outliers. This was done in order to not exclude any real data resulting from variations in outdoor conditions.

Hourly averages of outdoor ozone and $PM_{2.5}$ concentrations, temperature, solar radiation, relative humidity (RH), and wind direction were obtained from the nearest Texas Commission on Environmental Quality's (TCEQ) sampling stations to report the data (located within 7 miles of the ROS sampling site, except for solar radiation which was obtained from a site 17 miles from the sampling site). Global horizontal (GH) solar radiation data was also measured during January – June 2012 on top of a 9-storey building located next to the sampling site using a rotating shadowband radiometer with a data logger (Campbell Scientific, Logan, UT). These GH measurements were taken every minute and averaged over the sampling duration. Daily precipitation data for Austin was obtained from Weather Underground (Weather Underground, 2013). Overall uncertainty for each measurement was calculated using standard error propagation to include variance in the measured readings and the uncertainty of the instrument when it was known. Graphical representations of the data and Shapiro-Wilk tests for normality indicated that all the datasets, except for ozone concentration, either followed lognormal distributions or did not follow normal or lognormal distributions. This led to the selection of the non-parametric Spearman Rank Correlation Coefficient test to determine the strength (ρ) and significance ($p<0.01$) of any relationships between the concentration of particulate ROS and environmental factors. Bonferroni correction was applied to these tests. Simple linear regression analysis was also performed between particulate ROS concentrations and each environmental condition measured. All statistics were done with Stata version 11.2.

3. Results and Discussion

The mean (± s.d.) concentration of ROS on $PM_{2.5}$ samples collected over three hours around midday in Austin, Texas on 40 days between November 2011 and September 2012 was 1.25 ± 1.1 nmoles/m^3. The concentrations ranged from 0.02 nmoles/m^3 measured on December 23 to 3.81 nmoles/m^3 on September 20. The concentrations on each sampled day are depicted in Figure B.1 with the error bars depicting the average standard error of replicate samples taken on 20 of the 40 sampling days. The sampling site was located away from any point sources, at a distance of about 0.7 miles from an interstate highway. Austin has a transitional, semi-arid climate, characterized by hot summers and mild winters. This is evidenced by the fact that the mean monthly temperature in November-February was 13°C whereas during June-September it was 28°C. Ambient environmental conditions measured at the nearest TCEQ sampling sites during the ROS sampling are given in Table B.S1. During the sampling periods on the 40 days, the ozone concentration ranged from 8 to 72 ppb, $PM_{2.5}$ concentration ranged from 1 to 22 μg/m^3, temperature ranged from 3 to 35°C, relative humidity ranged from 21 to 95%, precipitation ranged from 0 to 80 mm, solar radiation ranged from 23 to 928 W/m^2, and the wind direction varied from 8 to 326 degrees compass.

Studies have found strong seasonal and diurnal variations in the concentrations of H_2O_2 in air, rainwater and water vapor, typically with higher concentrations measured during the summer than the winter (references within Gunz and Hoffman, 1990; references within Sakugawa et al., 1990; references within Lee et al., 2000; Yamada et al., 2002; Liu et al., 2003). However, other studies have found that some ROS species, e.g. peroxyacetyl nitrates (RCO_2ONO_2) and methyl hydroperoxide (CH_3OOH), follow the opposite trend because of greater sensitivity to NO_x precursor pollutants (Singh et al., 1986; Zhang et al., 2012). In this study, we found that particulate ROS concentrations tend to be higher in the warmer months than in the colder months, implying that particulate ROS follows trends similar to gas-phase and rainwater H_2O_2 in the atmosphere.

Table B.1 displays the results of the Spearman Rank Correlation tests between particulate ROS concentrations and measured environmental conditions (ozone and $PM_{2.5}$ concentrations, temperature, relative humidity, precipitation and solar radiation). The concentration of ROS on $PM_{2.5}$ was statistically significantly correlated with ozone concentration (ρ=0.61, p=0.0000), temperature (ρ=0.56, p=0.0002) and solar radiation (ρ=0.61, p=0.0000). Several studies have assessed the correlation between particulate ROS concentrations and ozone (Hung and Wang, 2001; Venkatachari et al., 2005; Venkatachari et al., 2007) and also between gas-phase H_2O_2 and ozone (Liu et al., 2003). These studies have found moderate correlations between the concentrations of ROS and ozone, with the strongest correlations occurring around midday. Complexities in the chemistry of formation of ROS have been cited as the reason for the relatively moderate correlations with ozone (Venkatachari et al., 2007). Meteorological conditions, such as solar radiation, water vapor concentration, temperature and pressure, are thought to influence the atmospheric concentration of H_2O_2 and peroxides (Logan et al., 1981; Jackson and Hewitt, 1999). Only a few studies have assessed the relationship between particulate ROS concentrations and meteorological conditions other than ozone concentration. Venkatachari et al., (2007) had found a weak, but statistically significant, correlation between particulate ROS and estimated secondary organic carbon concentrations in the atmosphere. Given the evidence for ROS being photochemically driven, one of the objectives of this study was to study the relationship between particulate ROS and certain meteorological conditions that influence photochemical reactions.

The correlations drawn from this data on particulate ROS (between particulate ROS concentrations and ambient air quality parameters) are fairly similar to correlations drawn from studies on gas-phase ROS (between gas-phase ROS/H_2O_2 concentrations and ambient air quality parameters). Yamada et al., (2002) found that gas-phase H_2O_2 was positively correlated with solar radiation, UV radiation and temperature, while it was negatively correlated with relative humidity. Liu et al., (2003) found that gas-phase H_2O_2 was positively correlated with ozone, and negatively correlated with NOx. Similar to Liu et al., (2003), we did not find a discernible

correlation between ROS and relative humidity. While not significant, the inverse relationship between particulate ROS and daily precipitation could be because of the reduction in gas-phase H_2O_2 during rain events (Gunz and Hoffman, 1990) which could lead to reduction in adsorption of gas-phase ROS onto atmospheric aerosols.

The nearest TCEQ sampling site to measure solar radiation was located 17 miles from the ROS sampling site (all other environmental conditions were obtained from TCEQ sites within 7 miles of the ROS sampling site). In order to ensure that the conditions at the ROS sampling site were similar to those at the TCEQ sampling site, solar radiation was measured next to the ROS sampling site during January to June 2012. The solar radiation data from the two sources was seen to match well (ρ=0.78, p=0.0002), and data from the TCEQ site were used for analysis over the entire study period. In addition, other environmental conditions were correlated with each other in ways that were expected. When solar radiation during the sampling event was strong, ozone concentrations and temperatures also tended to be high, as indicated by significant correlations between these parameters. In contrast, solar radiation was lower on days when it rained or had high RH, as indicated by the significant inverse correlations between solar radiation and daily precipitation / RH. Ozone concentrations were also inversely correlated with RH and daily precipitation. Higher temperatures tended to increase the concentration of $PM_{2.5}$, as indicated by a significant correlation between temperature and $PM_{2.5}$ concentration, possibly due to an increase in reaction rates leading to SOA formation.

Regression analysis between particulate ROS concentrations and environmental conditions shows that linear regression models between particulate ROS concentrations and ozone concentrations, temperature, and solar radiation are significant ($p<0.001$ for the F-test on the model) but with R^2 values ranging from 0.29 to 0.56. These regression models are shown in Figure B.2. t-tests on the regression coefficients for these linear regression models are also significant ($p<0.001$). A multiple regression model of particulate ROS concentration with ozone concentration, temperature and solar radiation is also significant (p=0.0000) with an R^2 value of 0.6 which means that 60% of the variance of particulate ROS concentrations is accounted for by

the model. Standardized coefficients for the multiple regression model are given in supplementary information (SI). It should be noted that the predictor variables (ozone concentration, temperature and solar radiation) for the multiple regression analysis are correlated which limits the conclusions that can be derived from the model. Linear regression models between particulate ROS concentrations and $PM_{2.5}$ concentrations, relative humidity and precipitation were not significant and are displayed in Figure B.S1.The regression results indicate that ambient particulate ROS is likely a function of the ambient ozone concentration, temperature and incident solar radiation. Some other contributing factors to particulate ROS concentrations may include ambient particle concentrations, relative humidity and wind direction, as well as parameters that were not measured in this study, such as the concentration of VOCs, NO_x, hydroxyl and other radical species.

The concentration of ROS on $PM_{2.5}$ was found to be statistically significantly correlated with winds blowing into Austin from the east-southeast (ρ=0.36, p=0.0244). Winds blowing from the east-southeast were also significantly correlated with ozone concentration (ρ=0.37, p=0.0177) and $PM_{2.5}$ concentration (ρ=0.5446, p=0.0003) indicating that they might be bringing pollutants from upwind sources including petrochemical and other industries in Houston. The concentration of ROS on $PM_{2.5}$ was also found to be statistically significantly correlated with winds blowing from the north (ρ=0.35, p=0.0253) indicating that some sources might be bringing particulate ROS from the direction of Dallas.

The ROS concentration on $PM_{2.5}$ reported in the literature ranges from 0.80-0.97 nmoles/m^3 at a location 14 km west of Manhattan during winter (Venkatachari et al., 2007), and 4.37-4.98 nmoles/m^3 close to highway traffic during Los Angeles basin inversion conditions in summer (Venkatachari et al., 2005), to 5.71 nmoles/m^3 in Singapore during December (See et al., 2007). A study in Taiwan reported a concentration of 0.54 nmoles/m^3 on $PM_{3.2}$ on an urban sidewalk during summer (Hung and Wang, 2001). Some other studies use a different analytical method and report ROS concentrations on TSP ranging from 0-0.38 nmoles/m^3 in summer in west Los Angeles (Hasson and Paulson, 2003) to 0-0.24 nmoles/m^3 in summer at Niwot Ridge, CO

(Hewitt and Kok, 1991). In the present study, we measured ROS concentrations on $PM_{2.5}$ in the 0.02-3.81 nmoles/m^3 range during November 2011 – September 2012 in Austin, Texas. The winter concentrations measured in this study are comparable to winter concentrations measured near Manhattan and summer concentrations in Taiwan. The summer concentrations measured in this study are lower than summer concentrations measured during basin inversion conditions in LA and winter concentrations in Singapore. In comparison, ROS concentrations on TSP in mainstream cigarette smoke (4-16 μmol/m^3 for three different brands of cigarettes; Huang et al., 2005) are 3-4 orders of magnitude higher than all ambient particulate ROS concentrations reported in the literature.

4. Conclusions

It is important to measure biologically relevant characteristics of PM to understand the association between PM and adverse health effects including respiratory and cardiovascular illnesses (Samet et al., 2000; Pope et al., 2002; Bell et al., 2004). In this study, we measured the concentration of ROS associated with $PM_{2.5}$ in an urban, semi-arid environment over the course of a year. We found that the minimum concentration occurred during the winter while the maximum concentration occurred during the summer, which was similar to the results reported in studies on ambient H_2O_2 concentrations in gas-phase and rainwater. Given that $PM_{2.5}$ can carry ROS deep into the lungs where the particulate ROS can potentially cause oxidative stress and cell damage, it is important to better understand the environmental conditions that influence the concentrations of ROS on $PM_{2.5}$. Results from correlation tests and linear regression analysis of particulate ROS concentrations and environmental conditions (which included ozone and $PM_{2.5}$ concentrations, temperature, relative humidity, precipitation and solar radiation) indicate that ROS associated with ambient particles is significantly influenced by the ambient ozone concentration, temperature and incident solar radiation. Particulate ROS concentrations measured in this study were within the range 0.0-5.7 nmoles/m^3 reported by other studies in the U.S., Taiwan and Singapore (Hewitt and Kok, 1991; Hung and Wang, 2001; Hasson and

Paulson, 2003; Venkatachari et al., 2005; Venkatachari et al., 2007; See et al., 2007). This study is one of the first to assess seasonal variations in particulate ROS concentrations and helps delineate the principle factors which influence this pollutant.

Acknowledgements

S.S.K. was funded by the National Science Foundation (IGERT Award DGE 0549428), US EPA (STAR Fellowship FP-91747501-0), and Cockrell School of Engineering Thrust Fellowship. We thank Dr. Mack Grady for collecting solar radiation data at the University of Texas at Austin campus.

Tables and Figures

Table B.1: Spearman's rank correlation coefficients between the concentration of ROS on $PM_{2.5}$ (ROS), ozone concentration (O_3), $PM_{2.5}$ concentration, temperature (T), relative humidity (RH), precipitation (ppt), and solar radiation measured at the nearest TCEQ site (Solar Rad). Significant relationships at $p<0.01$ are in bold and those at $p<0.001$ are further denoted with *.

	ROS on $PM_{2.5}$	**O_3**	**$PM_{2.5}$**	**T**	**RH**	**ppt**
O_3	**0.61***					
$PM_{2.5}$	0.27	0.03				
T	**0.56**	**0.52**	0.36			
RH	-0.17	**-0.53**	0.19	-0.32		
ppt	-0.15	-0.38	0.08	0.26	**0.53**	
Solar Rad	**0.61***	**0.69***	0.11	**0.78***	-0.50	**-0.54**

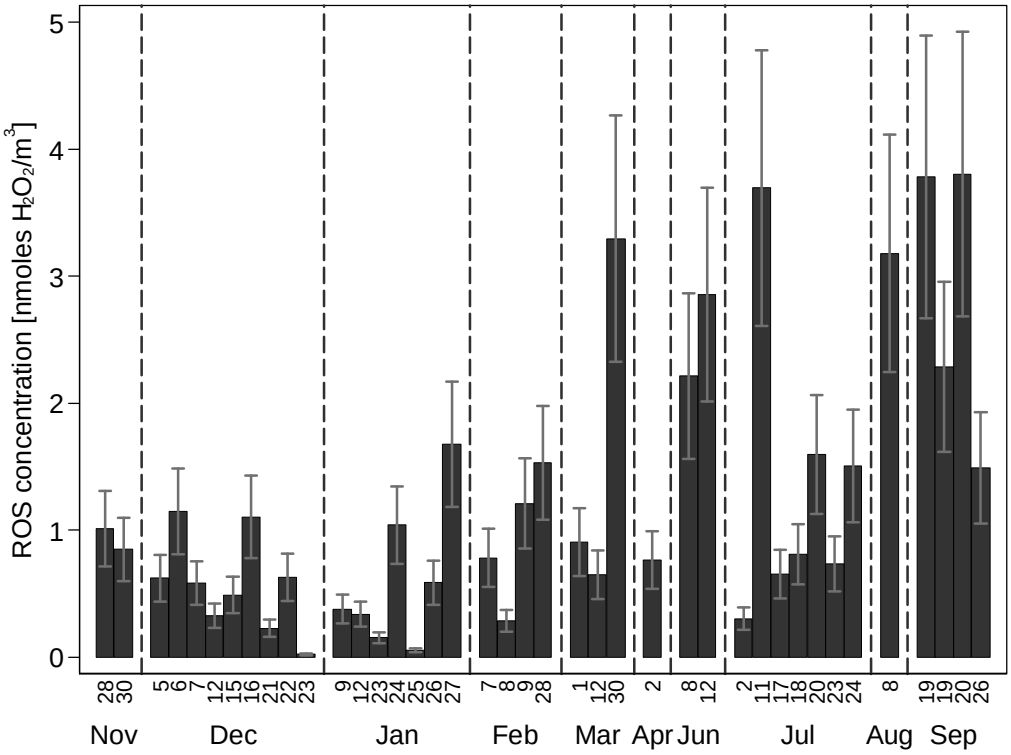

Figure B.1: Concentration of ROS on $PM_{2.5}$ sampled at an outdoor location away from point sources in Austin, Texas. The error bars represent the average standard error of replicate samples taken on 20 of the 40 sampling days.

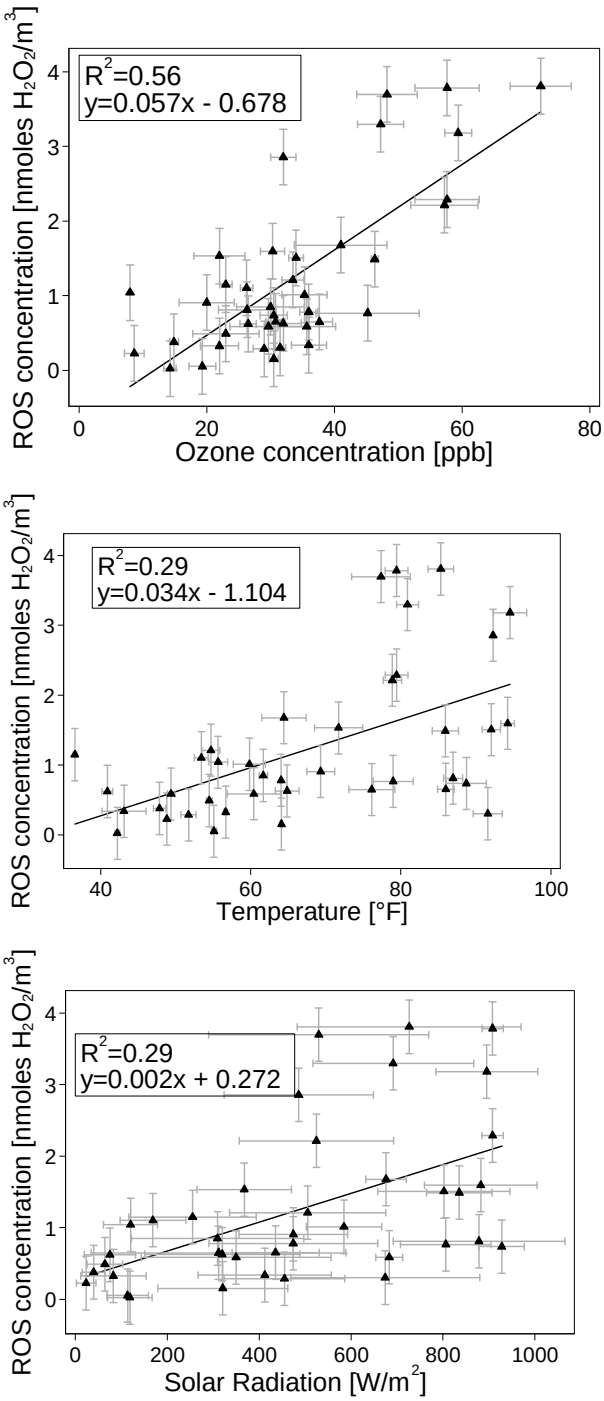

Figure B.2: Linear regression graphs showing significant relationships between particulate ROS concentrations and O3 concentration, temperature, and solar radiation. Error bars for ROS concentration represent the average standard error of replicate ROS samples. Error bars for environmental conditions represent the variance in the measurements during the 3-hour sampling period.

References

Arellanes, C., Paulson, S. E., Fine, P. M., and Sioutas, C.: Exceeding of Henry's Law by Hydrogen Peroxide Associated with Urban Aerosols. Environ Sci Technol, 40, 4859-4866, 2006.

Ayers, G. P., Penkett, S. A., Gillett, R. W., Bandy, B., Galbally, I. E., Meyer, C. P., Elsworth, C. M., Bentley, S. T., and Forgan, B. W.: Evidence for Photochemical Control of Ozone Concentrations in Unpolluted Marine Air. Nature, 360, 6403, 446-449, 1992.

Baulig, A., Poirault, J-J., Ausset, P., Schins, R., Shi, T., Baralle, D., Dorlhene, P., Meyer, M., Lefevre, R., Baeza-Squiban, A., and Marano, F.: Physicochemical Characteristics and Biological Activities of Seasonal Atmospheric Particulate Matter Sampling in Two Locations of Paris. Environ Sci Technol, 38, 5985-5992, 2004.

Bell, M. L., Samet, J. M., and Dominici, F.: Time-Series Studies of Particulate Matter. Annu Rev Publ Health, 25, 247-280, 2004.

Boogaard, H., Janssen, N. A. H., Fischer, P. H., Kos, G. P. A., Weijers, E. P., Cassee, F. R., van der Zee, S. C., de Hartog, J. J., Brunekreef, B., and Hoek, G.: Contrasts in Oxidative Potential and Other Particulate Matter Characteristics Collected Near Major Streets and Background Locations, Environ Health Persp, 120, 2, 185-191, 2012.

Bowler, R. P., and Crapo, J. D.: Oxidative Stress in Allergic Respiratory Diseases, J Allergy Clin Immunol, 110, 349-356, 2002.

Bufalini, J. J., Gay, B. W., and Brubaker, K. L.: Hydrogen Peroxide Formation from Formaldehyde Photooxidation and its Presence in Urban Atmospheres. Environ Sci Technol, 6, 9, 816-821, 1972.

Crim, C., and Longmore, W. J.: Sublethal Hydrogen Peroxide Inhibits Alveolar Type II Cell Surfactant Phospholipid Biosynthetic Enzymes, Am J Physiol –Lung C, L129-L135, 1995.

Dollard, G. J., and Davies, T. J.: Observations of H_2O_2 and PAN in a Rural Atmosphere. Environ Pollut, 75, 1, 45-52, 1992.

EPA 40CFR, Part 136, 2011 – Guidelines Establishing Test Procedures for the Analysis of Pollutants, Appendix B – Definition and Procedure for the Determination of the Method Detection Limit, Revision 1.11. http://www.gpo.gov/fdsys/pkg/CFR-2011-title40-vol23/pdf/CFR-2011-title40-vol23-part136-appB.pdf, last accessed 2 April 2013.

Gay, B. W., and Bufalini, J. J.: Hydrogen Peroxide in the Urban Atmosphere. Adv Chem Ser, 113, 255-264, 1972.

Geiser, T., Ishigaki, M., van Leer, C., Matthay, M. A., and Broaddus, V. C.: H_2O_2 inhibits alveolar epithelial wound repair in vitro by induction of apoptosis, Am J Physiol-Lung C, 287, L448-L453, 2004.

Gunz, D. W., and Hoffman, M. R.: Atmospheric Chemistry of Peroxides: A Review. Atmos Environ, 24A, 7, 1601-1633, 1990.

Hasson, A. S. and Paulson, S. E.: An Investigation of the Relationship between Gas-Phase and Aerosol-Borne Hydroperoxides in Urban Air, J Aerosol Sci, 34, 459-468, 2003.

Heikes, B., Lee, M., Jacob, D., Talbot, R., Bradshaw, J., Singh, H., Blake, D., Anderson, B., Fuelberg, H., and Thompson, A. M.: Ozone, hydroperoxides, oxides of nitrogen, and hydrocarbon budgets in the marine boundary layer over the South Atlantic. J Geophys Res, 101, 24221-24234, 1996.

Hewitt, C. N., and Kok, G. L.: Formation and Occurrence of Organic Hydroperoxides in the Troposphere: Laboratory and Field Observations, J Atmos Chem, 12, 181-194, 1991.

Holm, B. A., Hudak, B. B., Keicher, L., Cavanaugh, C., Baker, R. R., Hu, P., and Matalon, S.: Mechanisms of H2O2 –mediated Injury to Type-II Cell Surfactant Metabolism and Protection with PEG-Catalase, Am J Physiol, 261, C751-C757, 1991.

Huang, D. M., and Chen, Z. M.: Reinvestigation of the Henry's Law Constant for Hydrogen Peroxide with Temperature and Acidity Variation, J Environ Sci-China, 22, 570-574, doi: 10.1016/S1001-0742(09)60147-9, 2010.

Huang, M-F., Lin, W-L., and Ma, Y-C.: A Study of Reactive Oxygen Species in Mainstream of Cigarette. Indoor Air, 15, 135-140, 2005.

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

Hwang, H. and Dasgupta, P. K.: Thermodynamics of the Hydrogen Peroxide-Water System, Environ Sci Technol, 19, 255-258, 1985.

Jackson, A. V. and Hewitt, C. N.: Atmosphere Hydrogen Peroxide and Organic Hydroperoxides: A Review, Crit Rev Env Sci Tec, 29, 2, 175-228, 1999.

Kehrer, J. P.: Free Radicals as Mediators of Tissue Injury and Disease, CRC Cr Rev Toxicol, 23, 1, 21-48, 1993.

Khurshid, S. S., Siegel, J. A., and Kinney, K. A.: Indoor Particulate Reactive Oxygen Species Concentrations. Environ. Res., 132, 46-53, 2014.

Kleinman, L. I.: Photochemical formation of peroxides in the Boundary Layer, J Geophys Res, 91, 10889-10904, 1986.

Kooy, N. W., Royall, J. A., and Ischiropoulos, H.: Oxidation of 2',7'-Dichlorofluorescin by Peroxynitrite, Free Radical Res., 27, 3, 245-254, 1997.

LaCagnin, L. B., Bowman, L., Ma, J. Y. C., and Miles, P. R.: Metabolic Changes in Alveolar Type II Cells after Exposure to Hydrogen Peroxide, Am J Physiol, 259, L57-L65, 1990.

Largiuni, O., Giacomelli, M. C., and Piccardi, G.: Concentration of Peroxides and Formaldehyde in Air and Rain and Gas-Rain Partitioning, J Atmos Chem, 41, 1-20, 2002.

Lee, M., Heikes, B. G., and O'Sullivan, D. W.: Hydrogen Peroxide and Organic Hydroperoxide in the Troposphere: A Review, Atmos Environ, 34, 3475-3494, 2000.

Li, N., Hao, M., Phalen, R. F., Hinds, W. C., and Nel, A. E.: Particulate Air Pollutants and Asthma – A Paradigm for the Role of Oxidative Stress in PM-induced Adverse Health Effects, Cl Immunol, 109, 250-265, 2003.

Li, N., Xia, T., and Nel, A. E.: The Role of Oxidative Stress in Ambient Particulate Matter-Induced Lung Diseases and Its Implications in the Toxicity of Engineered Nanoparticles, Free Radical Bio Med, 44, 1689-1699, 2008.

Lind, J. A., and Kok, G. L.: Henry's Law Determinations for Aqueous Solutions of Hydrogen Peroxide, Methylhydroperoxide, and Peroxyacetic Acid, J Geophys Res-Atmos, 99, 21119, 1994.

Liu, J., Steinberg, S. M., and Johnson, B. J.: A High Performance Liquid Chromatography Method for Determination of Gas-Phase Hydrogen Peroxide in Ambient Air using Fenton's Chemistry. Chemosphere, 52, 5, 815-823, 2003.

Logan, J. A., Prather, M. J., Wofsy, S. C., and McElroy, M. B.: Tropospheric Chemistry: A Global Perspective, J Geophys Res, 86, 7210-7254, 1981.

Morio, L. A., Hooper, K. A., Brittingham, J., Li, T.-H., Gordon, R. E., Turpin, B. J., and Laskin, D. L.: Tissue Injury following Inhalation of Fine Particulate Matter and Hydrogen Peroxide is associated with altered Production of Inflammatory Mediators and Antioxidants by Alveolar Macrophages, Toxicol Appl Pharm, 177, 188-199, 2001.

NIST e-Handbook of Statistical Methods, 2010. Detection of Outliers, 1.3.5.17. http://www.itl.nist.gov/div898/handbook/, last accessed 1 November 2012.

Oosting, R. S., Vanbree, L., Vaniwaarden, J. F., Vangolde, L. M. G., and Verhoef, J.: Impairment of Phagocytic Functions of Alveolar Macrophages by Hydrogen Peroxide, Am J Physiol, 259, L87-L94, 1990.

Pope III, C.A., Burnett, R.T., Thun, M.J., Calle, E.E., Krewski, D., Ito, K., and Thurston, G.D.: Lung Cancer, Cardiopulmonary Mortality, and Long-term Exposure to Fine Particulate Air Pollution, JAMA-J Am Med Assoc, 287, 9, 1132–1141, 2002.

Sakugawa, H., Kaplan, I. R., Tsai, W., and Cohen, Y.: Atmospheric Hydrogen Peroxide. Environ Sci Technol, 24, 10, 1452-1462, 1990.

Samet, J. M., Dominici, F., Curriero, F. C., Coursac, I., and Zeger, S. L.: Fine Particulate Air Pollution and Mortality in 20 U.S. Cities, 1987-1994. New Engl J Med, 343, 1742-1749, 2000.

Sanders, S. P., Zweier, J. L., Harrison, S. J., Trush, M. A., Rembish, S. J., and Liu, M. C.: Spontaneous Oxygen Radical Production at Sites of Antigen Challenge in Allergic Subjects, Am J Resp Crit Care, 151, 1725-1733, 1995.

Sarangapani, R., and Wexler, A. S.: The Role of Dispersion in Particle Deposition in Human Airways, Toxicol Sci, 54, 229-236, 2000.

Schöne, E.: Ueber das atmosphärische Wasserstoffhyperoxyd, Berichte der deutschen chemischen Gesellschaft, 7, 1693–1708, 1874.

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

Seinfeld J. H., Pandis S. N.: Atmospheric Chemistry and Physics: From Air Pollution to Climate Change (2nd edition), Wiley Publishers, Hoboken, NJ, 2006.

Shen, H., Barakat, A. I., and Anastasio, C.: Generation of Hydrogen Peroxide from San Joaquin Valley Particles in a Cell-free Solution, Atmos Chem Phys, 11, 753-765, 2011.

Singh, H. B., Salas, L. J., and Viezee, W.: Global Distribution of Peroxyacetyl Nitrate. Nature, 321, 6070, 588-591, 1986.

Snow, J. A., Heikes, B. G., Shen, H., O'Sullivan, D. W., Fried, A., and Walega, J.: Hydrogen Peroxide, Methyl Hydroperoxide, and Formaldehyde over North America and the North Atlantic, J Geophys Res, 112, D12S07, 2007.

Staffelbach, T. A., and Kok, G. L.: Henry Law Constants for Aqueous Solutions of Hydrogen Peroxide and Hydroxymethyl Hydroperoxide, J Geophys Res-Atmos, 98, 12713-12717, doi: 10.1029/93JD01022, 1993.

Valavanidis, A., Fiotakis, K., and Vlachogianni, T.: Airborne Particulate Matter and Human Health: Toxicological Assessment and Importance of Size and Composition of Particles for Oxidative Damage and Carcinogenic Mechanisms, J Environ Sci Heal C, 26, 4, 339-362, 2008.

Venkatachari, P., Hopke, P. K., Grover, B. D., and Eatough, D. J.: Measurement of Particle-Bound Reactive Oxygen Species in Rubidoux Aerosols, J Atmos Chem, 50: 49-58, 2005.

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

Vidrio, E., Phuah, C. H., Dillner, A. M., and Anastasio, C.: Generation of Hydroxyl Radicals from Ambient Fine Particles in a Surrogate Lung Fluid Solution, Environ Sci Technol, 43, 922-927, 2009.

Wang, Y., Arellanes, C., Curtis, D. B., and Paulson, S. E.: Probing the Source of Hydrogen Peroxide Associated with Coarse Mode Aerosol Particles in Southern California, Environ Sci Technol, 44, 11, 4070-4075, 2010.

Walker, S. J., Evans, M. J., Jackson, A. V., Steinbacher, M., Zellweger, C., and McQuaid, J. B.: Processes Controlling the Concentration of Hydroperoxides at Jungfraujoch Observatory, Switzerland, Atmos Chem Phys, 6, 5525-5536, 2006.

Weather Underground, Inc.: www.wunderground.com, last accessed 17 December 2013.

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

Yamada, E., Tomozawa, K., Nakanishi, Y., and Fuse, Y.: Behavior of Hydrogen Peroxide in the Atmosphere and Rainwater in Kyoto, and its Effect on the Oxidation of SO_2 in Rainwater, B Chem Soc Jpn, 75, 6, 1385-1391, 2002.

Zhang, X., He, S. Z., Chen, Z. M., Zhao, Y., and Hua, W.: Methyl hydroperoxide (CH3OOH) in Urban, Suburban, and Rural Atmosphere: Ambient Concentration, Budget, and Contribution to the Atmospheric Oxidizing Capacity, Atmos Chem Phys, 12, 8951-8962, 2012.

Zhu, H., Bannenberg, G. L., Moldeus, P., and Shertzer, H. G.: Oxidation pathways for the intracellular probe 2',7'-dichlorofluorescin, Arch. Toxicol., 68, 582-587, 1994.

Supplementary Information

Table B.S1: Outdoor environmental conditions during ROS sampling, November 2011 – September 2012.

Date	O_3 Conc. [ppb][a]	$PM_{2.5}$ Conc. [$\mu g/m^3$][a]	Temperature [°C][a]	RH %[b]	Precip. [mm][c]	Solar Radiation [W/m^2][d]	Wind Dir. [°][a]
28-Nov	35.3 ± 3.5	1.4 ± 2.0	15.5 ± 1.2	20.6 ± 2.4	0.0	584.6 ± 82.0	168 ± 74
30-Nov	30.0 ± 5.3	6.5 ± 4.7	16.5 ± 0.3	31.4 ± 0.9	0.0	309.6 ± 187.4	149 ± 21
5-Dec	26.5 ± 1.3	2.1 ± 2.0	4.9 ± 0.4	83.0 ± 0.4	16.3	75.5 ± 55.5	333 ± 2
6-Dec	23.0 ± 1.0	4.2 ± 2.0	2.5 ± 0.1	56.0 ± 1.2	0.0	255.4 ± 138.7	312 ± 5
7-Dec	35.7 ± 4.5	1.5 ± 2.3	9.7 ± 0.3	36.1 ± 1.9	0.0	349.9 ± 206.7	248 ± 45
12-Dec	22.0 ± 3.0	21.9 ± 2.1	13.7 ± 0.1	72.8 ± 1.0	0.0	83.2 ± 71.6	118 ± 2
15-Dec	23.0 ± 5.2	3.7 ± 2.2	12.5 ± 0..2	95.5 ± 1.6	18.0	64.7 ± 38.6	8 ± 6
16-Dec	26.3 ± 1.0	5.3 ± 2.2	11.9 ± 0.5	74.8 ± 1.3	0.0	169.1 ± 71.4	266 ± 176
21-Dec	8.7 ± 1.5	13.4 ± 2.4	9.4 ± 0.0	56.7 ± 1.6	9.1	23.5 ± 21.3	168 ± 13
22-Dec	32.0 ± 2.8	9.0 ± 2.1	18.3 ± 0.9	45.2 ± 6.6	18.0	319.6 ± 168.5	192 ± 148
23-Dec	14.3 ± 1.0	9.4 ± 3.0	5.7 ± 0.2	77.4 ± 2.1	0.0	118.9 ± 48.3	121 ± 204
9-Jan	14.9 ± 0.7	2.8 ± 2.5[e]	8.8 ± 0.5	92.6 ± 1.1	22.1	40.2 ± 24.9	
12-Jan	36.0 ± 2.8	0.8 ± 2.1[e]	6.2 ± 1.6	22.8 ± 4.9	0.0	412.2 ± 144.8	326 ± 3
23-Jan	30.5 ± 0.7	2.7 ± 2.9[e]	17.8 ± 0.1	36.3 ± 1.1	0.0	321.1 ± 141.5	43 ± 5
24-Jan	8.0 ± 0.5	13.8 ± 2.5	13.2 ± 0.7	81.3 ± 2.2	10.2	120.5 ± 58.6	46 ± 22
25-Jan	19.3 ± 2.1	2.5 ± 2.4	12.8 ± 0.2	86.5 ± 2.9	79.8	114.0 ± 45.3	
26-Jan	29.7 ± 6.0	2.3 ± 2.6	15.8 ± 2.0	44.4 ± 6.7	0.0	683.1 ± 29.1	289 ± 20
27-Jan	41.0 ± 7.3	3.8 ± 2.4	18.0 ± 1.6	46.3 ± 1.5	0.0	676.0 ± 44.3	177 ± 8
7-Feb	36.0 ± 1.4	6.2 ± 2.0	17.8 ± 0.0	46.3 ± 0.6	0.0	474.4 ± 183.5	134 ± 128
8-Feb	29.5 ± 1.7	6.7 ± 2.1	11.0 ± 0.6	63.3 ± 2.1	0.0	455.2 ± 130.9	107 ± 168
9-Feb	33.5 ± 1.7	8.9 ± 2.5	12.6 ± 0.7	58.9 ± 2.6	0.0	505.5 ± 169.6	145 ± 5
28-Feb	22.0 ± 4.0	7.3 ± 2.4	22.1 ± 1.8	74.9 ± 8.0	1.0	367.7 ± 102.6	199 ± 18
1-Mar	20.0 ± 4.3	12.3 ± 2.5	20.7 ± 1.0	89.8 ± 1.9	0.0	474.5 ± 118.1	206 ± 143
12-Mar	37.7 ± 2.1	7.0 ± 2.2	24.5 ± 1.7	73.9 ± 6.1	0.0	311.7 ± 277.2	170 ± 15
30-Mar	47.3 ± 3.6	9.0 ± 2.3	27.2 ± 0.8	60.5 ± 4.1	0.0	691.5 ± 175.0	172 ± 11
2-Apr	45.2 ± 8.1	8.2 ± 3.6	26.1 ± 1.5	57.5 ± 4.9	0.0	806.2 ± 98.5	162 ± 13
8-Jun	57.3 ± 5.3	16.5 ± 3.3	26.0 ± 0.7	66.6 ± 3.4	0.3	524.5 ± 167.9	96 ± 173
12-Jun	32.0 ± 2.0	15.3 ± 2.1	33.5 ± 0.1	44.0 ± 1.7	0.8	486.3 ± 162.4	148 ± 13
2-Jul	31.4 ± 0.9	19.6 ± 3.3	33.1 ± 1.0	38.5 ± 5.6	0.0	674.3 ± 205.4	153 ± 4
11-Jul	48.3 ± 4.7	11.0 ± 2.2	25.2 ± 2.2	87.9 ± 3.9	3.1	529.6 ± 239.6	60 ± 30
17-Jul	30.8 ± 1.5	7.2 ± 2.5	30.0 ± 0.6	62.6 ± 2.5	0.0	435.8 ± 94.9	143 ± 4
18-Jul	26.3 ± 4.5	6.0 ± 3.3	30.5 ± 0.7	62.5 ± 2.8	0.0	878.2 ± 186.5	175 ± 12
20-Jul	30.3 ± 1.9[e]	8.7 ± 3.1	34.6 ± 0.5	42.2 ± 2.0	0.0	882.1 ± 122.4	171 ± 4
23-Jul	30.5 ± 2.1	9.4 ± 3.5	31.5 ± 1.5	50.0 ± 7.2	0.0	927.7 ± 49.4	166 ± 15
24-Jul	34.0 ± 1.2	3.5 ± 2.4	33.4 ± 0.7	44.7 ± 2.4	0.0	801.9 ± 143.7	167 ± 6
8-Aug	59.4 ± 2.1	8.8 ± 2.8	34.8 ± 1.2	36.2 ± 4.2	0.0	895.2 ± 110.4	130 ± 14
19-Sep	57.7 ± 5.0	7.3 ± 2.1	26.4 ± 0.8	39.6 ± 4.5	0.0	907.9 ± 22.9	62 ± 55
20-Sep	71.5 ± 4.8	12.0 ± 2.4	29.6 ± 0.9	43.9 ± 5.5	0.0	726.4 ± 243.5	169 ± 15
26-Sep	46.3 ± 0.6	3.0 ± 2.9[e]	30.0 ± 1.0	39.9 ± 5.6	0.0	835.4 ± 71.0	174 ± 5

[a] Data from TCEQ site CAMS3, located 5 miles from ROS sampling site. MDL for ozone measurements is 5 ppb and for $PM_{2.5}$ measurements is 2 $\mu g/m^3$. Wind direction is measured in degrees compass, measured clockwise from the north.

[b] Relative humidity data from CAMS5003 (nearest TCEQ site to take this measurement).

[c] Daily precipitation data from Weather Underground.

[d] Solar Radiation data from TCEQ site CAMS38.

[e] Data from CAMS38 (next closest TCEQ site to measure these parameters) because of instrument error at CAMS3.

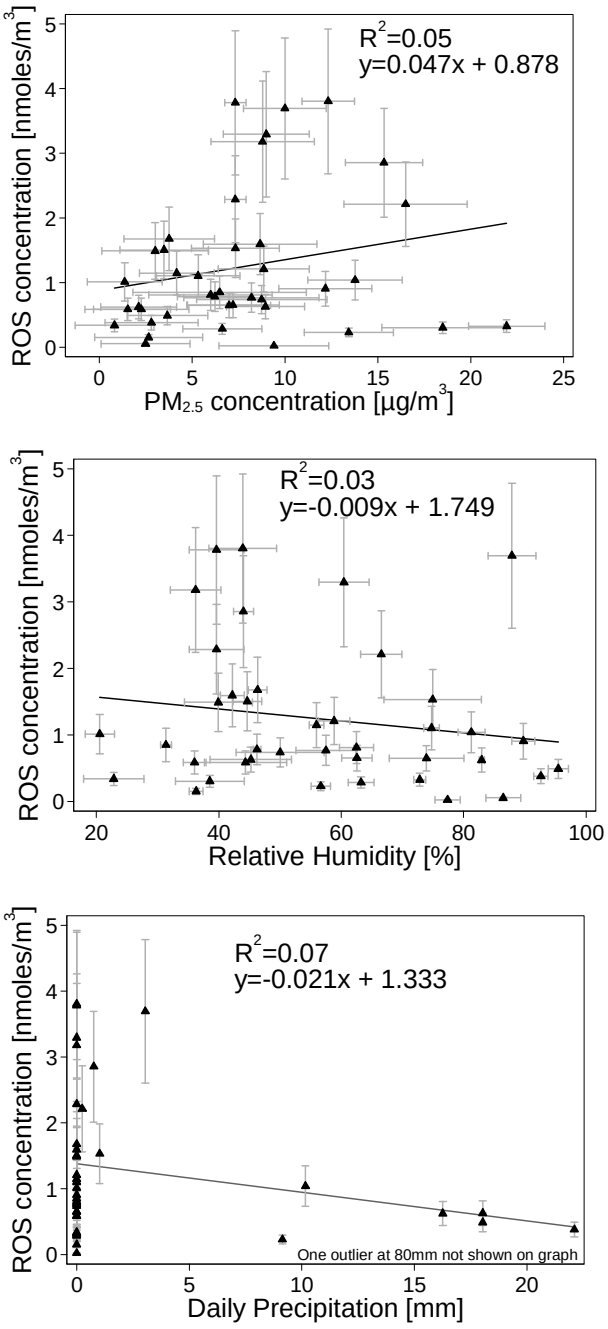

Figure B.S1: Particulate ROS concentrations depicted with respect to $PM_{2.5}$ concentration, relative humidity, and precipitation. Linear regression analysis indicates that these relationships are not significant. Error bars for ROS concentration represent the average standard error of replicate ROS samples. Error bars for environmental conditions represent the variance in the measurements during the 3-hour sampling period.

ROS on $PM_{2.5}$ and Wind Direction – Methodology and Results

The resultant wind direction was obtained from TCEQ's nearest sampling station located 6 miles from the sampling site. The resultant wind direction is the direction of the vector obtained from combining the wind speed and direction over an hour. Wind direction is recorded in degrees compass, starting from 0° for winds blowing from the north, progressing clockwise to 360°. For instance, winds blowing from the west have a wind direction of 270°. Average wind direction during each sampling period was categorized into eight sectors. Each sector was ranked on a scale of 1-3, 3 being the direction which was linked with higher particulate ROS measurements in Austin (e.g. Figure B.S2). Different combinations of sector rankings were tested to determine the wind directions which correlated significantly with higher particulate ROS concentrations at the sampling site.

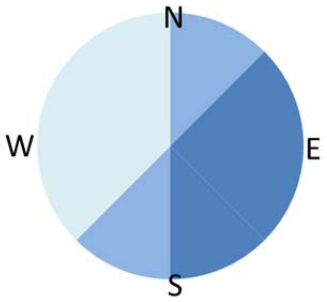

Figure B.S2: Wind direction was categorized into eight sections. Each section was ranked on a scale of 1-3, 3 being the direction most likely to bring ROS and 1 being the least likely to bring ROS. In the example shown above, three sections in the east-southeast direction (45°-180°) were ranked highest, followed by the N-NE and S-SW sections, and the west-northwest sections.

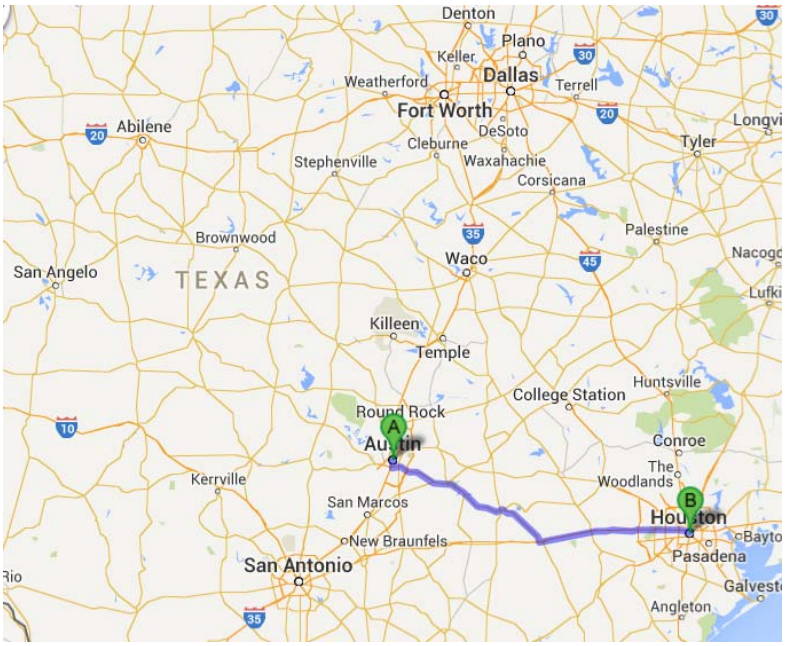

Figure B.S3: Map showing the location of Austin in central Texas, located 165 miles west of Houston and 195 miles south of Dallas.

Winds blowing from Houston to Austin in the east-southeast direction (45°-180°) were found to be statistically significantly correlated with higher concentrations of ROS on $PM_{2.5}$. Winds blew from the east-southeast during 27 of the 40 sampling periods. No significant correlations were found between the concentration of ROS on $PM_{2.5}$ and winds blowing only from the east, i.e. 45°-135°. Other combinations of sector rankings were also tried and the only other significant correlation was with winds blowing from the north (315°-45°). Winds blew from the north on 6 of the 40 sampling periods.

Appendix C: Particulate Reactive Oxygen Species on Total Suspended Particles – Measurements in Residences in Austin, Texas

Particulate Reactive Oxygen Species on Total Suspended Particles – Measurements in Residences in Austin, Texas

Shahana S. Khurshid[a], Jeffrey A. Siegel[a,b], Kerry A. Kinney[a]

Submitted for publication

[a]Department of Civil, Architectural and Environmental Engineering, The University of Texas at Austin, 301 E Dean Keeton St., MS C1786, Austin, Texas, 78712, USA.
[b]Department of Civil Engineering, The University of Toronto, 35 St. George St., Toronto, Ontario, M5S 1A4, Canada.

Abstract

Very little work has been done on assessing biologically relevant characteristics of particulate matter (PM) in homes. The concentration of particulate reactive oxygen species (pROS) on TSP was assessed in eight homes and was found to be significantly lower inside (mean±s.e. =1.59±0.33 nmol/m^3) than outside (2.35±0.57 nmol/m^3). Indoor pROS concentrations were substantive despite the absence of photochemical activity. A majority of indoor pROS existed on $PM_{2.5}$ (58±10%) which is important from a health perspective since $PM_{2.5}$ can carry ROS deep into the lungs. No obvious relationships were evident between select building characteristics and indoor pROS concentrations, but this observation would need to be verified by larger, controlled studies. Controlled experiments conducted at a test house to elucidate the influence of terpene and ozone concentrations on indoor pROS concentrations suggest that outdoor conditions play an important role in the penetration of ROS and ROS precursors into a house. Indoor ozone and terpene concentrations appeared to substantively influence indoor pROS concentrations when outdoor ozone concentrations were low, but they had a weaker influence on indoor pROS concentrations when outdoor ozone concentrations were high. Further work is warranted to assess other key parameters that drive indoor pROS concentrations.

Keywords: Particles; SOA; Ozone; Terpenes; Indoor Air Quality; Homes.

Highlights

- Very little work has been done to assess and understand ROS formation in homes.
- First study to assess ROS on TSP in indoor environments (8 homes and a test house).
- Indoor particulate ROS conc. are substantive despite absence of photochemistry.
- Majority of indoor particulate ROS exists on $PM_{2.5}$ (58±10%).
- Outdoor source term analysis conducted with controlled experiments at test house.

Practical Implications

Biologically active chemical species on PM, such as reactive oxygen species (ROS), may serve as better predictors of health effects associated with PM than PM mass. Knowledge of indoor pROS concentrations in homes and the factors that drive their concentrations is important because people spend extended periods of time at home and several potential pathways exist for ROS formation indoors. Indoor concentrations of ROS on TSP were about 75% of outdoor concentrations of ROS on TSP in the measured homes which indicates that indoor levels of ROS may not be much lower than outdoor levels despite the absence of sunlight. On average, about 58% of the indoor pROS exists on respirable particles ($PM_{2.5}$) which is important to consider in exposure analysis studies on ROS. This study contributes to developing an understanding of the parameters necessary for modeling ROS generation in real indoor environments.

1. Introduction

It is widely understood that exposure to particulate matter (PM) has a detrimental effect on human health (Samet et al., 2000; Pope et al., 2002; Pope and Dockery, 2006). The dramatic increases in morbidity and mortality observed after extreme air pollution episodes helped establish the link between very high concentrations of PM and cardiopulmonary disease (Ciocco and Thompson,1961; Bell and Davis, 2001; Nemery et al., 2001; Bell et al., 2004). Over the last two decades, epidemiological studies have reported associations between daily changes in PM and daily mortality in several cities (Schwartz, 1991; Dockery et al., 1992; Pope et al., 1992;

Schwartz and Dockery, 1992; Zmirou et al., 1998; Samet et al., 2000; Schwartz et al., 2001) and have found that even low-to-moderate particle concentrations are linked to adverse health effects. The fact that even relatively low concentrations of ambient PM can lead to apparent health effects, has spurred additional research in PM, including trying to identify the components of PM that are causing respiratory (Pope et al., 1991; Pope and Dockery, 1992) and cardiovascular illness (Pope et al., 2004) and other adverse health effects.

Recent efforts have turned towards using biologically active chemical species of PM, such as reactive oxygen species (ROS), as better predictors of the health effects associated with PM than PM mass. ROS include molecules such as hydroperoxides and organic peroxides (ROOR'), ions such as hypochlorite ion (OCl^-) and peroxynitrite anion ($ONOO^-$), and radicals such as hydroxyl (•OH) radical and alkyl peroxyl radicals (ROO•). They can be formed through photochemical reactions (with NO_x, carbon monoxide, formaldehyde and volatile organic compounds (VOCs)) (Gunz and Hoffman, 1990; Finlayson-Pitts and Pitts, 2000) and via ozone-initiated reactions (Paulson and Orlando, 1996; Weschler, 2006; Venkatachari et al., 2007). ROS in the environment may occur in the gas-phase in which case they can occur freely as a gaseous compound or can adsorb onto particles. Depending on their degree of oxidation and vapor pressure, ROS can also nucleate into particles or condense onto existing particles. ROS may also dissolve in water associated with particles, due to their polar and hydrophilic nature. While gas-phase ROS are likely to be absorbed in the mucus of the upper airways (and removed out of the respiratory tract), ROS on particles can be carried into the lower lungs (Friedlander and Yeh, 1998) where the particles can come into direct contact with the lung tissue and can transfer into the bloodstream and reach secondary organs (Bailey et al., 1985; Snipes, 1989; Semmler et al., 2004). The body's anti-oxidant defense mechanism can counteract foreign sources of ROS (since ROS generation and neutralization is part of basic cellular processes) but it is unknown to what extent and for how long the body is able to sustain this defense, and what the subsequent health effects may be.

ROS on outdoor particles have been studied in a few cities (Hung and Wang, 2001; Venkatachari et al., 2005; Venkatachari et al., 2007; See et al., 2007; Khurshid et al., 2014a), but very little has been done to assess particulate ROS in indoor environments. Given that Americans spend almost 70% of their time in residential environments (Klepeis et al., 2001), it is important to determine indoor concentrations of ROS. The indoor concentration of ROS on $PM_{2.5}$ had been measured in a university building in Singapore (without a simultaneous outdoor measurement being made) (See et al., 2007). Khurshid et al., (2014b) conducted a larger survey of the concentration of ROS on $PM_{2.5}$ at twelve residential buildings and eleven commercial buildings (with simultaneous indoor and outdoor measurements). However, ROS on TSP has not been assessed in indoor environments as yet. The focus of this study is to measure the concentration of ROS on TSP in residential homes, to compare the level of ROS on $PM_{2.5}$ with the level of ROS on TSP to determine the fraction of particulate ROS that exists on $PM_{2.5}$, and also to explore selected sources that may contribute to indoor particulate ROS. This information will help in developing an understanding of the parameters necessary for modeling ROS generation in real indoor environments and determining exposure to indoor ROS.

2. Materials and Methods

2.1 Indoor and Outdoor ROS on TSPs and $PM_{2.5}$

Total suspended particles (TSP) were collected at eight homes in Austin, Texas on Teflon filters (TF-1000, 1μm pore size, 37 mm, Pall, NY, USA) using filter holders (SKC, PA, USA) on different days in October 2012. Sampling was conducted for 3±0.25 hours around midday when ambient ROS concentrations are at their highest [21, 27], between 11am and 2pm using air sampling pumps at 10 L/min. Samples taken over shorter sampling periods would have the advantage of capturing very reactive species but would also result in reduction of signal, while samples taken over longer periods might lead to loss of some reactive species due to degradation and may also lead to some samples being too concentrated. All pumps were calibrated before sampling with a mini-Buck Calibrator M-30 (A. P. Buck, Orlando, FL; accuracy ±0.5%).

Triplicate samplers were placed 1m above the ground outside and in a central room inside the homes. Some deviations in the sampling protocol caused by occupants are described in the SI. At six of the eight homes where TSP was collected, indoor $PM_{2.5}$ was also collected using triplicate Personal Environmental Monitors (PEM, SKC, PA, USA) to compare relative concentrations of particulate ROS on TSP to ROS on $PM_{2.5}$. Teflon tape was wrapped around the edges of the support screen in the PEMs to ensure a proper seal of the thin Teflon filters inside the PEMs. Field blanks were periodically used to check that there was no significant difference in fluorescence between unsampled filters and field blanks. All sampling filters were transported to the lab and assessed within 1 hour of collection.

The method for quantifying ROS was adapted from Black & Brandt (1974). Important modifications made to the method to reduce high background levels reported in previous studies are described in detail elsewhere (Khurshid, et al., 2014b). This method uses 2',7'-dichlorofluorescin diacetate (DCF-DA) which is a non-specific indicator for reactive oxygen species. It becomes fluorescent in the presence of a wide variety of ROS including, but not limited to, hydrogen peroxide (H_2O_2), peroxyl (ROO•) and hydroxyl (•OH) radicals and the peroxynitrite anion (ONOO-) (Zhu et al., 1994; Kooy et al., 1997). As such, ROS is an operationally defined quantity determined by the conversion of a non-fluorescent compound to a fluorescent one. Briefly, 0.5 ml of 1 mM 2',7'-dichlorofluorescin diacetate (DCF-DA, Cayman Chemical, MI, USA) in ethanol was incubated with 2 ml of 0.01 N NaOH at room temperature for 30 mins in the dark to cleave off the acetate groups. After the 30 mins incubation period, the 2',7'-dichlorofluorescin (DCFH) solution was neutralized with 10 ml sodium phosphate buffer (pH 7.2) and the solution was kept on ice in the dark till needed. Each sampled filter was sonicated in 5 ml sodium phosphate buffer in an acid-cleaned 50-ml beaker for 10 minutes. Horseradish peroxidase (HRP, ThermoScientific, IL, USA) in sodium phosphate buffer (pH 7.0) was mixed with the DCFH solution and added to the beakers in the dark to yield a final volume of 10 ml with a concentration of 5 μM of DCFH and 1 unit/ml of HRP. The beaker was then incubated in the dark at 37˚C for 15 mins, after which 0.1 ml aliquots from each beaker were

placed in triplicate in a 96-well plate and the fluorescence intensity was read at 530 nm with excitation at 485 nm (Synergy HT, Biotek, VT, USA). The concentration of ROS on the sampled filters was expressed in terms of H_2O_2 per volume of air sampled (rather than per mass of particles) because this describes exposure to ROS as it occurs in the lungs (Boogard et al., 2012). The background fluorescence intensity produced by an unsampled filter was subtracted from the sample.

Standards were prepared with hydrogen peroxide (H_2O_2). To prepare the standards, aliquots of 0.1 ml of appropriate H_2O_2 concentration were added to 3 ml of DCFH-HRP reagent in glass tubes to get 0, 1.0, 2.0, 3.0, and 4.0 x 10^{-7} M H_2O_2 in final solutions. These tubes were incubated at 37°C for 15 minutes and fluorescence was measured. All glassware used in the experiments was scrubbed with soap, followed by immersion in a 10% nitric acid bath and subsequent 7x rinsing with deionized water.

2.2 Environmental Measurements at Homes

Estimates of indoor and outdoor air quality parameters were collected at all study homes during the 3-hr sampling period. Indoor and outdoor temperature and relative humidity were measured with a HOBO U12 (Onset, Bourne, MA) with an uncertainty of ± 0.6 ºF in temperature and ± 2.5% in relative humidity (RH). A photo-ionization detector (PID, Geotechnical Services, Tustin, CA) calibrated with isobutylene was used to measure the indoor concentration of total volatile organic carbon (TVOC), with an uncertainty of the greater of ± 20 ppb or 10% of the reading. Hourly outdoor ozone and $PM_{2.5}$ concentrations were obtained from Texas Commission on Environmental Quality's (TCEQ) nearest sampling station (# 484530014) located within 7 miles of all the sampled houses. A DustTrak 8520 Aerosol Monitor with a size-selective aerosol conditioner (TSI, Shoreview, MN; uncertainty 1 $\mu g/m^3$) was used to measure indoor $PM_{2.5}$ concentration at the sampled homes. The DustTrak was calibrated against a Tapered Element Oscillating Microbalance (TEOM) 1405D (Thermo Environmental Instruments, Franklin, MA) resulting in a gain of 0.9 and an offset of -5.3. Uncertainty for each

measurement was calculated using standard error propagation techniques to include variance in the measured readings and the uncertainty of the instrument itself.

The influence of each of the recorded air quality parameters on ROS and the relationship between indoor and outdoor ROS was analyzed using non-parametric statistical analyses with Stata version 11.2. Results were deemed significant if the statistical test had a p-value lower than 0.05. Bonferroni adjustments were not used as the purpose of this initial study was to provide a baseline assessment of indoor ROS in homes.

2.3 UTest House Experiments to Study Sources of Indoor Particulate ROS

Controlled experiments were conducted at an unoccupied manufactured house (UTest House) to assess the influence of ozone and terpene concentrations on indoor particulate ROS concentrations. Similar to the field testing, TSP samples were collected in triplicate, inside and outside the test house. Four sets of indoor conditions were tested: (i) low ozone/low terpene (ii) low ozone/high terpene, (iii) high ozone/low terpene, and (iv) high ozone/high terpene. Each of these four indoor conditions was tested on low and high outdoor ozone days to assess the influence of outdoor ozone concentrations. Each condition was tested on three separate days. Sampling was conducted in January and July-September, 2014, on 12 days when outdoor ozone concentrations during the 3 hours of sampling were below 40ppb (categorized as low outdoor ozone days) and another 12 days when the outdoor ozone concentrations were above 40ppb (categorized as high outdoor ozone days). An ozone generator (Odor-Free, model Hotel 350, Tallahassee, Florida) was used to elevate and maintain the indoor ozone concentration at 75-100 ppb for the high indoor ozone cases. For the high terpene concentration cases, 6-7ml Pine-Sol® (a household cleaning solvent) was applied with a moistened rag on the floor in two rooms of the house which elevated VOC concentrations to 400-500 ppb as measured by the PID; the VOC concentration was allowed to naturally decay over the 3-hour sampling period (it was approximately 100 ppb at the end of the sampling period).

The air exchange rate was measured during all sampling events by measuring the decay of carbon dioxide (CO_2) with the tracer gas method. CO_2 concentrations were elevated by releasing CO_2 from a cylinder in two locations of the house and allowing it to mix in all rooms to more than 500 ppm above background, and then measurements were taken in the central living room every minute with an infrared absorption CO_2 monitor (Telaire Model 7001) connected to a data acquisition system (instruNet model 100). Indoor and outdoor ozone concentrations were monitored with a UV-absorbance ozone monitor (2B Technologies model 202). Indoor $PM_{2.5}$ and PM_{10} concentrations were measured at the Test House with a Tapered Element Oscillating Microbalance (TEOM) 1405D (Thermo Environmental Instruments, Franklin, MA). Outdoor $PM_{2.5}$ and PM_{10} concentrations were measured with a SidePak Personal Aerosol Monitor AM510 (TSI, Shoreview, MN) and a DustTrak 8520 Aerosol Monitor (TSI, Shoreview, MN), respectively, with size-selective aerosol conditioners. The SidePak was calibrated against the TEOM resulting in a gain of 1.02, which is in the range 0.55-1.08 reported by Jiang et al., (2011). For outdoor PM_{10} concentrations, the DustTrak is known to read well below measurements taken by gravimetric samplers (Watson et al., 2011). A gain of 2.08 was applied to the DustTrak measurements based on the average calibration factor calculated from data reported by Park et al., (2009). There is inherent uncertainty in the calibrated measurements from the DustTrak and SidePak, particularly because there can be a different impact on low and high concentrations. Nonetheless these measurements help identify variations in outdoor particle concentrations on different days. Indoor temperature and indoor and outdoor relative humidity were measured as before. Outdoor temperature was obtained from Texas Commission on Environmental Quality's (TCEQ) nearest sampling station (# 484530014) located 3.5 miles from the UTest House.

During the July-September sampling, VOC samples were collected inside the test house using glass sorbent tubes filled with a minimum of 0.11 mg of Tenax GR. Air was sampled at 20 ml/min and the sorbent tubes were stored in an air-tight protective casing at room temperature until they were analyzed (which was typically on the following day). The sorbent tubes were

analyzed using thermal desorption followed by gas chromatograph and mass spectrometry (TD/GCMS, Hewlett Packard 5890 Series II Gas Chromatograph). Individual VOCs were statistically identified and quantified using a Library Compound Search (LCS), which identifies the most probable VOC for an unknown analyte using a statistical comparison of the ions produced by the unknown analyte to a library developed and maintained by the National Institute for Science and Technology (NIST): NIST 98 Compound Library. The mass of compounds identified by a LCS was estimated using an internal standard (IS), 4-Bromoflourobenzene (BFB), and a response ratio of one was used. The uncertainty associated with the mass of compounds identified and quantified using a LCS is typically assumed to be ±100%. The mass of each compound was converted to number of moles of each compound and were summed across all compounds. The total number of moles of VOCs was used to calculate the average concentration of VOCs (including terpenoids) in the test house during the sampling period.

3. Results and Discussion

3.1 Comparison of Indoor and Outdoor ROS Concentrations

The mean (± s.e.) indoor concentration of ROS on TSP sampled at eight homes (labeled H1-H8) was 1.59 ± 0.33 nmol/m^3 and the mean outdoor concentration was 2.35 ± 0.57 nmol/m^3. The indoor and outdoor concentrations of ROS on TSP (Figure C.1) were significantly different (Wilcoxon matched-pairs signed-ranks test, p=0.049). All homes in this dataset had central heating and air conditioning (HAC) except H6. Operating HAC systems tend to increase the infiltration of outdoor contaminants as well as promote heterogeneous ozone reactions because of increased mixing. The highest indoor and outdoor ROS on TSP concentrations were recorded at H1, where two workers were doing minor indoor renovation work (drywall mudding) near an open window which happened to be close to the outdoor sampling location.

This is one of the first studies to simultaneously assess the indoor and outdoor concentration of ROS on TSP. Two studies have reported on the concentration of ROS on $PM_{2.5}$ in indoor environments. See et al. (2007) recorded a concentration of 3 nmol/m^3 of ROS on

$PM_{2.5}$ inside a university building in Singapore. No simultaneous outdoor measurement was made. In a previous study done by our group (Khurshid et al., 2014b), the concentration of ROS on $PM_{2.5}$ was measured at twelve residential buildings (during March-April and June-August, 2012, the same year as the current study) and eleven commercial buildings (institutional buildings during March and July, 2012, and retail buildings during January-April, 2012) in Austin, Texas. The concentration of ROS on $PM_{2.5}$ inside and outside the buildings was not found to be significantly different (mean ± s.e. at homes: 1.37±0.30 nmol/m^3 inside and 1.41±0.25 nmol/m^3 outside; at institutional buildings: 1.16±0.14 nmol/m^3 inside and 1.68±0.48 nmol/m^3 outside; and at retail stores 1.09±0.25 nmol/m^3 inside and 1.12±0.36 nmol/m^3 outside). Unlike indoor and outdoor concentrations of ROS on $PM_{2.5}$, the concentrations of ROS on TSP were found to be higher outside than inside. This may be due to the fact that outdoor environments typically have a higher concentration of coarse particles than indoor residential environments (Jones et al., 2000), and gas-phase and fine particulate ROS can adsorb onto these particles leading to a higher outdoor concentration of ROS on TSP than indoor concentration.

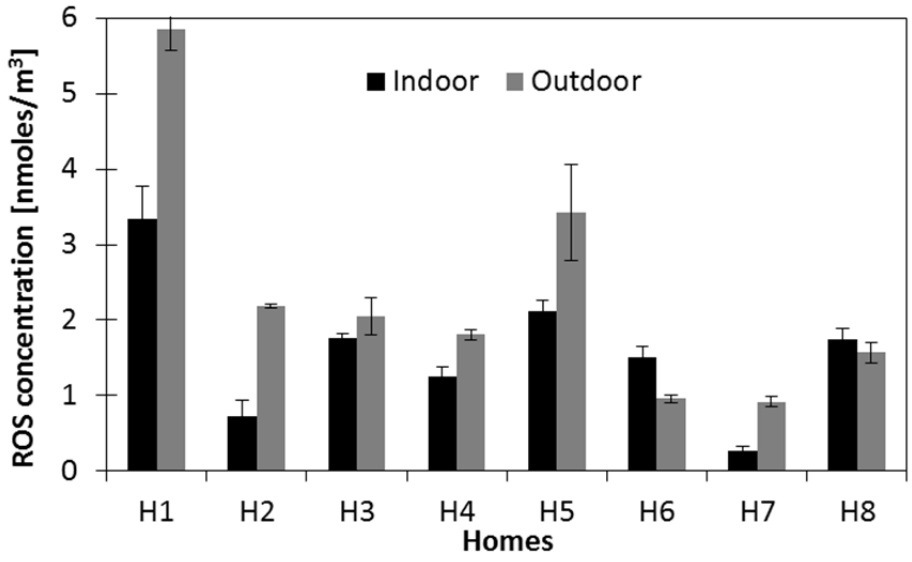

Figure C.1: Indoor and outdoor concentrations of ROS on total suspended particles (TSP) sampled at eight residential homes. The error bars represent standard error of triplicate samples.

Other studies of particulate ROS have measured ambient concentrations in outdoor environments in a few cities or have measured ROS generation in chambers from terpene ozonolysis (Docherty et al., 2005; Venkatachari and Hopke, 2008; Chen and Hopke, 2009; Chen et al., 2011). Studies of particulate ROS in outdoor air have reported concentrations ranging from 0.61 nmol/m^3 in Taipei, Taiwan for PM_{10} (0.54 nmol/m^3 for $PM_{3.2}$), to 6.11 nmol/m^3 near Los Angeles around midday during summer for TSP (4.95 nmol/m^3 for $PM_{2.5}$) (Hung and Wang, 2001; Venkatachari et al., 2005; See et al., 2007; Venkatachari et al., 2007). Our indoor and outdoor measurements either fall in or below the range of outdoor concentrations reported in these studies.

3.2 Comparison of Indoor Concentrations of ROS on TSP and ROS on $PM_{2.5}$

In the six homes where both $PM_{2.5}$ and TSP were collected, the mean indoor concentration of ROS on TSP was 1.72 ± 0.36 nmol/m^3 and the mean indoor concentration of ROS on $PM_{2.5}$ was 0.90 ± 0.16 nmol/m^3. Indoor ROS on TSP in these six homes ranged from 0.72 to 3.35 nmol/m^3 and indoor ROS on $PM_{2.5}$ ranged from 0.40 to 1.50 nmol/m^3 (Figure C.2). The indoor concentrations of ROS on TSP and ROS on $PM_{2.5}$ were significantly different (Wilcoxon matched-pairs signed-ranks test, p=0.028), indicating that the amount of ROS on particles varies with the size of the particles.

Several studies of particulate ROS in outdoor air (Hung and Wang, 2001; Venkatachari et al., 2005; Venkatachari et al., 2007) and in cigarette smoke (Huang et al., 2005) have found that ROS on $PM_{2.5}$ constitutes the majority of the ROS on TSP (44-95 % for outdoor air, 58-96% for cigarette smoke). The percentage of ROS on indoor $PM_{2.5}$ as a fraction of ROS on indoor TSP determined in the current study ranged from 26 to 93% with a mean (± s.e.) of 58±10% which is closer to the lower ratios reported in the literature. These results imply that the majority of indoor ROS is on $PM_{2.5,}$ similar to that in outdoor environments. It is interesting to note that the ratio of ROS on $PM_{2.5}$ to ROS on TSP was lowest in H6, which did not have a central heating and cooling system (26%). However, the duty cycles of HAC systems in the homes were not

recorded in this study, so the potential impact of HAC systems (which can increase the infiltration of outdoor contaminants, remove ROS and precursors with filtration and reactions in the system, and/or promote heterogeneous ozone reactions because of increased mixing) on the indoor concentration of ROS cannot be explicitly ascertained.

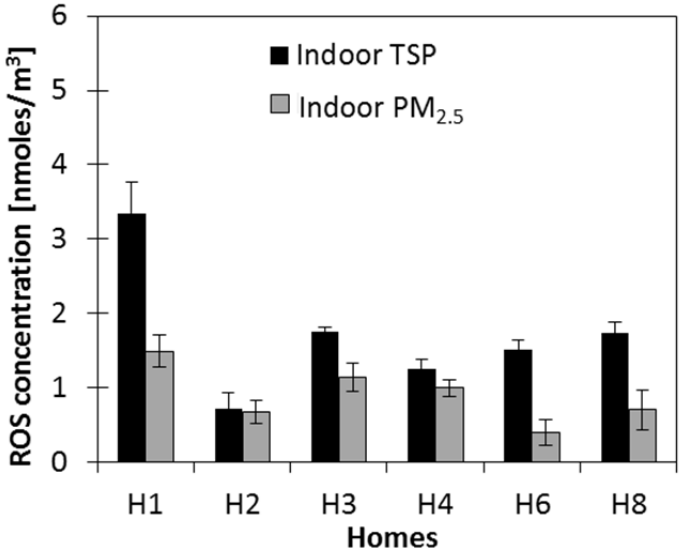

Figure C.2: Indoor concentrations of ROS on $PM_{2.5}$ and total suspended particles (TSP) sampled at six residential homes. The error bars represent standard error of triplicate samples.

3.3 Influence of Environmental Factors on Indoor ROS

Indoor and outdoor air quality parameters (indoor and outdoor $PM_{2.5}$ concentrations, temperature, and RH, as well as indoor VOC concentration and outdoor ozone concentration) measured during TSP sampling at eight homes are given in Table C.1. The Spearman Rank Correlation Coefficient test was used to determine the strength (ρ) and significance (p) of any relationships that exist between ROS on TSP and these air quality parameters. Though only marginally significant, the indoor concentration of ROS on TSP showed some correlation with the outdoor concentration of ROS on TSP (ρ = 0.69, p=0.05). This implies that ROS concentrations in the outdoor environment can influence indoor ROS concentrations, although the distinction between ROS precursors and ROS itself is still unresolved. As expected, the outdoor concentrations of $PM_{2.5}$ and ozone were correlated (ρ = 0.81, p=0.015), indicating the

influence of ozone-initiated reactions on the generation of $PM_{2.5}$. If H1, where drywall mudding work was being done in one room during sampling is excluded from the dataset, indoor VOC and indoor $PM_{2.5}$ concentrations were correlated ($\rho = 0.79$, $p=0.034$).

Table C.1: Air quality parameters during sampling at eight homes where the concentration of ROS on total suspended particles (TSP) was measured.

	$PM_{2.5}$ Conc. [$\mu g/m^3$]		Temperature [°C]		Relative Humidity %		VOC Conc. [ppb]	Ozone Conc. [ppb]
Home	Indoor	Outdoor	Indoor	Outdoor	Indoor	Outdoor	Indoor	Outdoor
H1	7.8 ± 10.6	8.0 ± 2.0	23.5 ± 0.6	31.1 ± 3.9	56 ± 2	35 ± 7	19 ± 3	46 ± 2
H2	0.0 ± 1.0	9.3 ± 2.1	26.0 ± 0.4	25.3 ± 1.2	51 ± 2	48 ± 3	50 ± 3	61 ± 3
H3	1.2 ± 1.2	10.3 ± 2.1	28.5 ± 1.2	29.9 ± 3.0	54 ± 3	55 ± 4	301 ± 64	50 ± 1
H4	0.0 ± 1.0	6.0 ± 2.2	28.8 ± 0.4	28.7 ± 1.2	47 ± 3	61 ± 5	208 ± 14	47 ± 3
H5	0.0 ± 1.0	3.3 ± 2.1	22.0 ± 0.6	21.2 ± 1.4	50 ± 1	34 ± 6	185 ± 5	44 ± 4
H6	0.0 ± 1.0	3.0 ± 4.2	28.2 ± 0.6	31.3 ± 1.1	67 ± 2	57 ± 5	69 ± 10	30 ± 2
H7	0.0 ± 1.0	5.8 ± 2.2	24.6 ± 0.4	25.6 ± 1.2	49 ± 4	74 ± 5	117 ± 4	31 ± 6
H8	1.1 ± 1.0	6.5 ± 2.4	19.2 ± 0.5	17.1 ± 1.3	44 ± 1	*	656 ± 28	53 ± 8

* Instrument error.

Building characteristics of the eight homes where indoor and outdoor ROS on TSP was measured are listed in Table C.2. The sample size in the current study is too small to fully assess the impact of different building components on indoor concentrations of particulate ROS.

Table C.2: Building characteristics of eight homes where the concentration of ROS on total suspended particles (TSP) was measured.

Home	Distance to major Road [m]	Year Built	Area [m^2]	Floor	Building Exterior
H1	966	1920	216	Wood 90%, carpet 10%	Painted wood siding
H2	1448	1985	183	Wood 60%, carpet 40%	Stone, Painted wood siding
H3	805	2009	102	Wood 60%, carpet 40%	Brick
H4	161	1969	177	Wood 80%, carpet 20%	Brick
H5	805	1996	201	Carpet 80%, wood 20%	Brick
H6	322	1945	83	Wood 70%, linoleum 20%, Tile 10%	Unpainted wood siding
H7	483	1963	65	Carpet 80%, linoleum 20%	Brick
H8	2736	1984	236	Wood 60%, carpet 25%, Tile 15%	Brick

Nonetheless, due to the limited work done in the field of indoor ROS, an attempt was made to see if any obvious trends appear to exist. In six of the eight homes where ROS on TSP was assessed, wood was the dominant floor type and in the remaining two homes, carpet was the dominant floor type. While carpet can react with ozone to lower indoor concentrations of ozone (Morrison and Nazaroff, 2002), indoor particulate ROS concentrations were not found to be necessarily lower in homes where carpet was the dominant floor type (indoor ROS was low in H7 but relatively high in H5). One possible reason for this could be that ozone reactions with carpet may lead to ROS formation. Both these homes (along with three others) had brick exteriors which would likely decrease the penetration of ozone into these buildings (Stephens et al., 2012; Liu and Nazaroff, 2001). However, the presence of brick also did not appear to influence indoor particulate ROS. In addition, the age of the building did not appear to influence the concentration of ROS, even though older homes tend to have higher penetration of outdoor ozone (Stephens et al., 2012) and particles (Stephens and Siegel, 2012) because of leaks in the building envelope (Persily et al., 2010). The year in which the eight homes were built ranged from 1920 to 2009.

3.4 Controlled Experiments at UTest House

Terpenes are readily oxidized to oxygenated products (including ROS), many of which have low enough vapor pressures that they can condense into secondary organic aerosols (SOA) (Docherty et al., 2005; Venkatachari and Hopke, 2008; Chen and Hopke, 2009; Chen et al., 2011). Unsaturated hydrocarbons, such as terpenes, are emitted from building materials such as wood, and consumer products such as air fresheners and cleaning solvents (Wallace et al., 1987; Brown et al., 1994; Nazaroff and Weschler, 2004; Steinmann et al., 2011). Given the prevalence of unsaturated hydrocarbons in indoor environments, it is important to assess the influence of high concentrations of unsaturated hydrocarbons on indoor particulate ROS concentrations. A routine indoor activity which elevates the concentration of unsaturated hydrocarbons is cleaning with chemical solvents, such as Pine-Sol®. A few studies have assessed the generation of ROS

from monoterpene ozonolysis (α-pinene, β-pinene, Δ^3-carene, linalool, limonene, sabinene) under controlled conditions in chambers (Docherty et al., 2005; Chen and Hopke, 2009; Chen et al., 2011) but a whole house presents different surface to volume, deposition, and air circulation characteristics which was the motivation behind the set of experiments we conducted at the UTest House. These are the first studies of their kind to try to assess the driving factors for indoor ROS in a house.

Based on our field testing of particulate ROS in homes (in this study and Khurshid et al., 2014b), one of the main factors that can likely influence indoor particulate ROS concentrations are outdoor particulate ROS concentrations. As such, it is useful to consider the indoor to outdoor (I/O) ratio of particulate ROS concentrations when comparing particulate ROS concentrations across different indoor and outdoor conditions. The I/O ratio was found to be highest after the floor of the test house had been cleaned with Pine-Sol® and a relatively high concentration of indoor ozone was present (75-100 ppb) (Table C.3). This was true when outdoor ozone concentrations were low (< 40 ppb) or high (> 40 ppb). The presence of either high indoor ozone concentrations or high indoor terpene concentrations did not elevate the I/O ratio of particulate ROS above the I/O ratio in the base case of low indoor ozone and terpene concentrations. Pine-Sol® contains several VOCs, many of which are unsaturated (such as α-pinene) and readily react with ozone to form oxygenated organic products including SOA and ROS. The formation of SOA was evident by the increase in indoor particle concentrations measured during these sampling events (Table C.S1 and C.S2). Table C.S1 and C.S2 list air quality parameters ($PM_{2.5}$ and PM_{10} concentrations, temperature, relative humidity, VOC concentration, Terpenoid concentration, and ozone concentration) measured inside and outside the test house during the sampling events.

In an effort to better understand the influence of outdoor sources on indoor particulate ROS concentrations, outdoor sources were compared to total (indoor and outdoor) sources in each condition. The effective indoor emission of particulate ROS was estimated using a simple time-averaged mass balance,

$$\frac{E}{V} = \lambda C + \beta C - p\lambda C_{out} \quad (1)$$

where C represents the indoor concentration of particulate ROS, p is the penetration factor for

Table C.3: Indoor to outdoor ratio of particulate ROS concentrations measured at the UTest House under different indoor (low/high ozone concentration, low/high terpene concentration) and outdoor (low/high ozone concentration) conditions. Each condition was tested in triplicate and means ± standard error are reported.

Indoor conditions at UTest House	In/Out Ratio of Particulate ROS	
	Low Outdoor O_3	High Outdoor O_3
Low O_3, Low Terpene	1.50 ± 0.26	0.77 ± 0.19
Low O_3, High Terpene	0.74 ± 0.05	0.96 ± 0.26
High O_3, Low Terpene	0.99 ± 0.22	0.93 ± 0.20
High O_3, High Terpene	4.39 ± 1.11	1.23 ± 0.55

particulate ROS (assumed to be 1), C_{out} is the outdoor concentration of particulate ROS, E is the indoor emission rate of ROS, V is the volume of the house, λ is the air exchange rate, β is the deposition loss rate. Since the HAC system was turned off during sampling events, the loss term due to filtration could be neglected. The fraction of outdoor sources to total (indoor and outdoor sources) was calculated with,

$$\text{Fraction of outdoor sources to total sources} = \frac{p\lambda C_{out}}{\frac{E}{V} + p\lambda C_{out}} \quad (2)$$

The deposition loss rate varies based on the size of particles from 0.04/hr for particles 0.1μm in diameter to about 2/hr for particles 10μm in diameter (Riley et al., 2002). Table C.4 lists the ratio of outdoor sources to total sources of indoor particulate ROS for each of the conditions using a β value of 0.5/hr (corresponding to particles 2.5μm in diameter). This data is also displayed in Figure C.S1. When the outdoor ozone concentration was low (left column in Table C.4), the outdoor source term ($p\lambda C_{out}$) contributed 34% of the total sources in the low indoor ozone/low indoor terpene case, whereas it contributed only 16% of the total sources in the high indoor ozone/high indoor terpene case. Similarly, for other values of β, a smaller fraction of indoor ROS appears to come from outdoors when high ozone and terpenes are present inside the house. The outdoor source contribution in the high indoor ozone/high indoor terpene case was statistically

significantly different from both the high indoor ozone/low indoor terpene and the low indoor ozone/high indoor terpene cases ($p=0.0495$ for each using Wilcoxon rank-sum test for unmatched data). This indicates that modulating the indoor conditions significantly influences the outdoor contribution of indoor particulate ROS concentrations when outdoor ozone concentrations are low. This also shows that indoor sources can contribute a major portion of indoor particulate ROS concentrations.

Table C.4: Outdoor sources as a percentage of total (indoor and outdoor) sources of indoor particulate ROS for each of the different indoor and outdoor conditions tested at the UTest House. Data for each condition was collected on three separate days, and means ± standard error are reported.

Indoor conditions at UTest House	Outdoor Sources as % of Total Sources	
	Low Outdoor O_3	High Outdoor O_3
Low O_3, Low Terpene	34% ± 7%	51% ± 9%
Low O_3, High Terpene	62% ± 3%	44% ± 12%
High O_3, Low Terpene	47% ± 8%	48% ± 9%
High O_3, High Terpene	16% ± 6%	41% ± 20%

On the other hand, when the outdoor ozone concentration was high (>40ppb), the average fraction of outdoor sources to total sources ranged 41-51% for the different indoor conditions and no clear pattern was observed when the indoor ozone and terpene concentrations were varied (right column in Table C.4). The outdoor source contribution was not significantly different between any of the indoor conditions. One reason for this observation may be that the outdoor conditions, especially outdoor ozone concentrations, play a significant role in the amount of ROS and precursors to ROS that penetrate into buildings from outdoors. Outdoor conditions can thus modulate the outdoor source contribution of indoor particulate ROS concentrations. As an illustration of this point, it should be noted that indoor PM levels were found to be higher on the days with high outdoor ozone. The atmospheric conditions during the high outdoor ozone days (which fell in the July-September sampling period) were quite different from the atmospheric conditions on the low outdoor ozone days (which mostly fell in the January sampling period). During sampling events on the high outdoor ozone days, the mean outdoor temperature was

32°C and the mean outdoor ozone concentration was 46 ppb, whereas during sampling events on low outdoor ozone days, the mean outdoor temperature was 17°C and the mean outdoor ozone concentration was 27 ppb. Outdoor conditions such as temperature, ozone concentration, and incident solar radiation are known to influence outdoor particulate ROS concentrations (Khurshid et al., 2014a) but they may also influence the amount of ROS and precursors to ROS that penetrate into buildings. It is also interesting to note that the highest contribution of outdoor sources to total sources of indoor particulate ROS occurred on the day corresponding to the highest outdoor ozone concentration (61 ppb) and one of the highest outdoor $PM_{2.5}$ (49 $\mu g/m^3$) and PM_{10} (53 $\mu g/m^3$) concentrations (Table C.S2).

Another contributing factor for indoor particulate ROS formation may be relative humidity levels, which has not been explored in this study. Indoor relative humidity levels were much higher during sampling events on high outdoor ozone days (mean = 49%) than on low outdoor ozone days (mean = 24%), which may also contribute to differences observed in the source term analysis.

During sampling in January, the air exchange rate at the test house ranged 0.28 – 0.99 /hr (mean 0.5 /hr) and in July – September it ranged 0.16 – 0.42 /hr (mean 0.3 /hr). The air exchange rate was higher in the winter due to the larger indoor-outdoor temperatures and the stack effect. Outdoor $PM_{2.5}$ concentrations were higher during the summer, which is generally consistent with other studies (Parkhurst et al., 1999; Bari et al., 2003).

While the ozone concentration in the high indoor ozone cases was only realistic of indoor environments which have active ozone generation sources (such as printers or ozone-emitting air purifiers), the terpene concentrations were similar to levels reported in indoor environments (Brown et al., 1994). Elevated indoor concentrations of VOCs, including terpenes, can especially be found when chemical cleaners or other scented consumer products, such as air fresheners, have been used. As displayed in tables S1 and S2, the concentration of VOCs and terpenoids inside the UTest House were found to be the highest in the low indoor ozone/high indoor terpene case when PineSol® had been applied suggesting that the indoor chemistry was ozone limited.

When ozone was also introduced (in the high indoor ozone/ high indoor terpene case), the concentration of VOCs and terpenoids reduced slightly, likely because reactions between unsaturated hydrocarbons and ozone had depleted some of the unsaturated hydrocarbons. The terpenoid concentrations in the two high terpene cases described above were obviously higher than the two low terpene cases, but the same effect was observed when ozone was introduced. When no supplemental VOCs were introduced into the indoor environment (the low indoor ozone/low indoor terpene case), the indoor concentration of terpenoids was approximately 15-20 ppb, indicating that the building materials themselves provided a source of terpenes. However, when the indoor concentration of ozone was increased without supplemental VOC introduction (i.e., high ozone/low terpene case) the concentration of terpenoids decreased to 5-7 ppb indicating that the ozone had again depleted some of the unsaturated hydrocarbons.

From the results of the controlled experiments at the UTest House, it appears that indoor generation of particulate ROS contributes substantially to indoor particulate ROS concentrations regardless of the experimental conditions. The contribution of indoor sources to total sources can be calculated from Table C.4 and ranges from 38% to 84%. This highlights an important point that buildings have active chemical processes going on inside them, including particulate ROS formation. The results from these experiments also indicate that indoor generation of particulate ROS is likely heavily influenced by the influx of precursors to ROS into buildings.

4. Conclusions

There are several factors that likely cause the adverse health effects that result from exposure to particulate matter. Given the role of ROS in pulmonary diseases, oxygen toxicity disorder, and tumor formation, the ROS on particles may be contributing to the adverse health effects caused by exposure to PM (Kehrer, 1993; Sanders et al., 1995; Bowler and Crapo, 2002; Li et al., 2003; Klaunig and Kamendulis, 2004; Li et al., 2008; Anderson et al., 2013). Several ROS precursors are present in homes which makes it important to determine typical concentrations of ROS that people are exposed to in their homes. The main objectives of this

study were to measure the indoor and outdoor concentrations of particulate ROS in a sample of homes and to study possible sources of indoor particulate ROS by running controlled experiments at a test house. The indoor concentration of ROS on TSP measured in the homes in this study was about 75% of the outdoor concentration of ROS on TSP. It is interesting to see that indoor particulate ROS concentrations are significant despite the absence of photochemical activity (which is one of the main pathways for ROS formation in outdoor environments). About 58% of the indoor particulate ROS was present on $PM_{2.5}$, which are particles small enough to reach the lower lungs and potentially lead to adverse health effects. Two pathways for indoor particulate ROS are: (1) substantial penetration of outdoor ROS into homes, or (2) substantial production of ROS inside homes. The results from controlled experiments at the test house imply that, when outdoor ozone concentrations are low, indoor concentrations of terpenes and ozone are influential in indoor generation of particulate ROS. Indoor activities (such as cleaning with chemical solvents) can be significant contributors of indoor particulate ROS in this case. However, when outdoor ozone concentrations are high, indoor activities play a smaller role in influencing indoor particulate ROS concentrations. Further work is warranted to better understand the formation of particulate ROS in indoor environments and to assess other key parameters that drive indoor particulate ROS concentrations. A speciated comparison of indoor and outdoor ROS would help in developing a better understanding of the fraction and components of indoor ROS that penetrate into buildings from outdoors.

Acknowledgements

S.S.K. was funded by the National Science Foundation (IGERT Award DGE 0549428), US EPA (STAR Fellowship FP-91747501-0), and Cockrell School of Engineering Thrust Fellowship. We thank all the residents who volunteered their homes for testing. We also thank Dr. Neil Crain for running the TD/GCMS analysis of the sorbent tubes collected during sampling at the UTest House.

References

Anderson, S. E., Khurshid, S. S., Meade, B. J., Lukomska, E., Wells, J. R. (2013) Toxicological Analysis of Limonene Reaction Products using an in vitro Exposure System. *Toxicol. In Vitro*, 27, 721-730.

Bailey, M. R., Fry, F. A., James, A. C. (1985) Long-term Retention of Particles in the Human Respiratory Tract. *J. Aerosol Sci.*, 16, 4, 295-305.

Bari, A., Ferraro, V., Wilson, L. R., Luttinger, D., Husain, L. (2003) Measurements of Gaseous HONO, HNO_3, SO_2, HCL, NH_3, particulate sulfate, and $PM_{2.5}$ in New York, NY. *Atmos. Environ.*, 37, 2825-2835.

Bell, M. L., Davis, D. L. (2001) Reassessment of the Lethal London Fog of 1952: Novel Indicators of Acute and Chronic Consequences of Acute Exposure to Air Pollution. *Environ. Health Persp*. 109, 389-394.

Bell, M.L., Davis, D. L., Fletcher, T. (2004) A Retrospective Assessment of Mortality from the London Smog Episode of 1952: The Role of Influenza and Pollution. *Environ. Health Perspect.* 112, 6-8.

Black, M. J. and Brandt, R. B. (1974) Spectrofluorometric Analysis of Hydrogen Peroxide. *Anal. Chem.*, 58, 246-254.

Boogaard, H., Janssen, N. A. H., Fischer, P. H., Kos, G. P. A., Weijers, E. P., Cassee, F. R., van der Zee, S. C., de Hartog, J. J., Brunekreef, B., Hoek, G. (2012) Contrasts in Oxidative Potential and Other Particulate Matter Characteristics Collected Near Major Streets and Background Locations. *Environ. Health Perspect.*, 120, 2, 185-191.

Bowler, R. P., and Crapo, J. D.(2002) Oxidative Stress in Allergic Respiratory Diseases. *J. Allergy Clin. Immun.*, 110, 3, 349-356.

Brown, S. K., Sim, M. R., Abramson, M. J., Gray, C. N. (1994) Concentrations of Volatile Organic Compounds in Indoor Air – A Review. *Indoor Air*, 4, 123-134.

Chen, X., and Hopke, P. K. (2009) Secondary organic aerosol from α-pinene ozonolysis in dynamic chamber system. *Indoor Air*, 19, 335-345.

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

Ciocco, A., Thompson, D. J. A Follow-Up of Donora Ten Years After: Methodology and Findings. *Am. J. Public Health*, 1961, 51, 155-164.

Docherty, K. S., Wu, W., Lim, Y. B., Ziemann, P. J. (2005) Contributions of Organic Peroxides to Secondary Aerosol formed from Reactions of Monoterpenes with O_3. *Environ. Sci. Technol.*, 39, 4049-4059.

Dockery, D. W., Schwartz, J., Spengler, J. D. (1992) Air Pollution and Daily Mortality: Associations with Particulates and Acid Aerosols. *Environ.Res.*, 59, 362-373.

Finlayson-Pitts, B. J. and Pitts, J. N. (2000) *Chemistry of the Upper and Lower Atmosphere,* Academic Press, San Diego, CA.

Friedlander, S. K., Yeh, E. K. (1998) The Submicron Atmospheric Aerosol as a Carrier of Reactive Chemical Species: Case of Peroxides. *Appl. Occup. Environ. Hyg.*, 13, 6, 416-420.

Gunz, D. W. and Hoffman, M. R. (1990) Atmospheric Chemistry of Peroxides: A Review. *Atmos. Environ.*, 24A, 7, 1601-1633.

Huang, M-F., Lin, W-L., Ma, Y-C. (2005) A Study of Reactive Oxygen Species in Mainstream of Cigarette. *Indoor Air*, 15, 135-140.

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

Jiang, R.-T., Acevedo-Bolton, V., Cheng, K.-C., Klepeis, N. E., Ott, W. R., Hildemann, L. M. (2011) Determination of response of real-time SidePak AM510 monitor to secondhand smoke, other common indoor aerosols, and outdoor aerosol. *J. Environ. Monitor.*, 13, 6, 1695–1702.

Jones, N. C., Thornton, C. A., Mark, D., Harrison, R. M. (2000) Indoor/outdoor Relationships of Particulate Matter in Domestic Homes with Roadside, Urban, and Rural Locations. *Atmos. Environ.*, 34, 2603-2612.

Kehrer, J. P. (1993) Free Radicals as Mediators of Tissue Injury and Disease. *Crit. Rev. Toxicol.*, 23, 1, 21-48.

Khurshid, S. S., Siegel, J. A., Kinney, K. A. (2014a) Particulate Reactive Oxygen Species Concentrations and their association with Environmental Conditions in an Urban, Subtropical Climate. *Atmos. Chem. Phys.*, 14, 6777-6784.

Khurshid, S. S., Siegel, J. A., Kinney, K. A. (2014b) Indoor Particulate Reactive Oxygen Species Concentrations. *Environ. Res.*, 132, 46-53.

Klaunig, J. E., and Kamendulis, L. M. (2004) The role of oxidative stress in carcinogenesis. *Annu. Rev. Pharmacol. Toxicol.*, 44, 239-267.

Klepeis, N. E., Nelson, W. C., Ott, W. R., Robinson, J. P., Tsang, A. M., Switzer, P., Behar, J. V., Hern, S. C., Engelmann, W. H. (2001) The National Human Activity Pattern Survey (NHAPS): A Resource for assessing exposure to environmental pollutants. *J. Expo. Anal. Env. Epid.*, 11, 231-252.

Kooy, N. W., Royall, J. A., Ischiropoulos, H. (1997) Oxidation of 2',7'-Dichlorofluorescin by Peroxynitrite. *Free Radical Res.*, 27, 3, 245-254.

Li, N., Hao, M., Phalen, R. F., Hinds, W. C., Nel, A. E. (2003) Particulate air pollutants and asthma – A paradigm for the role of oxidative stress in PM-induced adverse health effects. *Clin. Immunol.*, 109, 250-265.

Li, N., Xia, T., Nel, A. E. (2008) The role of oxidative stress in ambient particulate matter-induced lung diseases and its implictions in the toxicity of engineered nanoparticles. *Free Radical Biol. Med.*, 44, 1689-1699.

Liu, D-L, and Nazaroff, W. W. (2001) Modeling Pollutant Penetration across building envelope. *Atmos. Environ.*, 35, 4451-4462.

Morrison, G. C., and Nazaroff, W. W. (2002) The rate of ozone uptake on carpet: mathematical modeling. *Atmos. Environ.*, 36, 1749-1756.

Nazaroff, W. W., and Weschler, C. J. (2004) Cleaning Products and Air Fresheners: Exposure to Primary and Secondary Air Pollutants. *Atmos. Environ.*, 38, 2841-2865.

Nemery, B., Hoet, P. H. M., Nemmar, A. (2001) The Meuse Valley Fog of 1930: An Air Pollution Disaster. *Lancet*, 357, 704-708.

Park, J.-M., Rock, J. C., Wang, L., Seo, Y.-C., Bhatnagar, A., Kim, S. (2009) Performance evaluation of six different aerosol samplers in a particulate matter generation chamber. *Atmos. Environ.*, 43, 280–289.

Parkhurst, W. J., Tanner, R. L., Weatherford, F. P., Valente, R. J., Meagher, J. F. (1999) Historic $PM_{2.5}/PM_{10}$ concentrations in the southeastern United States-Potential implications of the revised particulate matter standard. *J. Air Waste Manag. Assoc.,* 49, 1060–1067.

Paulson, S. E. and Orlando, J. J. (1996) The reactions of ozone with alkenes: An important source of HO_x in the boundary layer. *Geophys. Res. Lett.*, 23, 25, 3727-3730.

Persily, A., Musser, A., Emmerich, S. J. (2010) Modeled infiltration rate distributions for U.S. housing. *Indoor Air*, 20, 473-485.

Pope III, C. A., Dockery, D. W., Spengler, J. D., Raizenne, M. E. (1991) Respiratory Health and PM_{10} Pollution: A Daily Time Series Analysis. *Am. Rev. Respir. Dis.,* 144, 668-674.

Pope III, C. A., Dockery, D. W. (1992) Acute Health Effects of PM_{10} Pollution on Symptomatic and Asymptomatic Children. *Am. Rev. Respir. Dis.*, 145, 1123-1128.

Pope III, C. A., Schwartz, J., Ransom, M. R. (1992) Daily Mortality and PM_{10} Pollution in Utah Valley. *Arch. Environ. Health*, 47, 211-217.

Pope III, C. A., Burnett, R. T., Thun, M. J., Calle, E. E., Krewski, D., Ito, K., Thurston, G. D. (2002) Lung cancer, cardio-pulmonary mortality, and long-term exposure to fine particulate air pollution, *J. Am. Med. Assoc.*, 287, 1132–1141.

Pope III, C. A., Burnett, R. T., Thurston, G. D., Thun, M. J., Calle, E. E., Krewski, D., Godleski, J. J. (2004) Cardiovascular Mortality and Long-Term Exposure to Particulate Air Pollution. *Circulation*, 109, 71-77.

Pope III, C. A., and Dockery, D. W. (2006) Health Effects of Fine Particulate Air Pollution: Lines that Connect. *J. Air Waste Manage. Assoc.*, 56, 6, 709-742.

Riley, W. J., McKone, T. E., Lai, A. C. K., Nazaroff, W. W. (2002) Indoor Particulate Matter of Outdoor Origin: Importance of Size-Dependent Removal Mechanisms. *Environ. Sci. Technol.*, 36, 200-207.

Samet, J. M., Dominici, F., Curriero, F. C., Coursac, I., and Zeger, S. L. (2000) Fine Particulate Air Pollution and Mortality in 20 U.S. Cities, 1987–1994, *New Engl. J. Med.*, 343, 1742–1749.

Sanders, S. P., Zweier, J. L., Harrison, S. J., Trush, M. A., Rembish, S. J., Liu, M. C. (1995) Spontaneous oxygen radical production at sites of antigen challenge in allergic subjects. *Am. J. Resp. Crit. Care*, 151, 6, 1725-1733.

Schwartz, J. (1991) Particulate Air Pollution and Daily Mortality in Detroit. *Environ. Res.*, 56, 204-213.

Schwartz, J., Dockery, D. W. (1992) Increased Mortality in Philadelphia Associated with Daily Air Pollution Concentrations. *Am. Rev. Respir. Dis.,* 145, 600-604.

Schwartz, J., Ballester, F., Saez, M., Perez-Hoyos, S., Bellido, J., Cambra, K., Arribas, F., Canada, A., Perez-Boillos, M. J., Sunyer, J. (2001) The Concentration-Response Relation between Air Pollution and Daily Deaths. *Environ. Health Perspect.*, 109, 10, 1001-1006.

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

Semmler, M., Seitz, J., Erbe, F., Mayer, P., Heyder, J. (2004) Long-term Clearance Kinetics of Inhaled Ultrafine Insoluble Iridium Particles from the Rat Lung, including Transient Translocation into Secondary Organs. *Inhal. Toxicol.*, 16, 453-459.

Snipes, M. B. (1989) Long-Term Retention and Clearance of Particles Inhaled by Mammalian Species. *Crit. Rev. Toxicol.*, 20, 3, 175-211.

Steinemann, A. C., MacGregor, I. C., Gordon S. M., Gallagher, L. G., Davis, A. L., Ribeiro, D. S., Wallace, L. A. (2011) Fragranced consumer products: Chemicals emitted, ingredients unlisted. *Environ. Impact Asses.*, 31, 328-333.

Stephens, B., Gall, E. T., Siegel, J. A. (2012) Measuring the Penetration of Ambient Ozone into Residential Buildings. *Environ. Sci. Technol.*, 46, 929-936.

Stephens, B., and Siegel, J. A. (2012) Penetration of ambient submicron particles into single-family residences and associations with building characteristics. *Indoor Air*, 22, 501-513.

Venkatachari, P., Hopke, P. K., Grover, B. D., Eatough, D. J. (2005) Measurement of Particle-Bound Reactive Oxygen Species in Rubidoux Aerosols. *J. Atmos. Chem.*, 50: 49-58.

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

Venkatachari, P., and Hopke, P. K. (2008) Characterization of products formed in the reaction of ozone with α-pinene: case for organic peroxides. *J. Environ. Monitor.*, 10, 966-974.

Wallace, L. A., Pellizzari, E., Leaderer, B., Hartwell, T., Perritt, R., Zelon, H., Sheldon, I. (1987) Emissions of volatile organic compounds from building materials and consumer products. *Atmos. Environ.*, 21(22), 385-393.

Watson, J. G., Chow, J. C., Chen, L., Wang, X., Merrifield, T. M., Fine, P. M., Barker, K. (2011) Measurement system evaluation for upwind/downwind sampling of fugitive dust emissions. *Aerosol Air Qual. Res.*, 11, 331–350.

Weschler, C. J. (2006) Ozone's Impact on Public Health: Contributions from Indoor Exposures to Ozone and Products of Ozone-Initiated Chemistry. *Environ. Health Perspect.*, 114, 10, 1489-1496.

Zhu, H., Bannenberg, G. L., Moldeus, P., Shertzer, H. G. (1994) Oxidation pathways for the intracellular probe 2',7'-dichlorofluorescin. *Arch. Toxicol.*, 68, 582-587.

Zmirou, D., Schwartz, J., Saez, M., Zanobetti, A., Wojtyniak, B., Touloumi, G., Spix, C., de Leon, A. P., Le Moullec, Y., Bacharova, L., Schouten, J., Ponka, A., Katsouyanno, K. (1998) Time- Series Analysis of Air Pollution and Cause-Specific Mortality. *Epidemiology*, 9, 5, 495-503.

Supplementary Information

Additional Details on Study Homes

The homes were unoccupied during the sampling events except H1, which had two occupants, and H3 and H4, which each had one occupant present during sampling. Indoor sampling was conducted in a central room in all homes except H3 and H4 where the indoor sampling was conducted in a closed bedroom (which was connected to the central HAC unit) to minimize disturbance to the occupant. All homes were detached houses except H3 and H7 which were second floor apartments. Windows in all the homes were closed during sampling except H6 which didn't have HAC (40% of the windows were open), and H1 where renovation work was taking place in the room next to the front patio (the windows were only open in the room where renovation work was ongoing). The front patio was selected as the outdoor sampling location at H1 because it was one of the few places around the house that provided shelter for the instruments and had an outdoor power socket to plug in the instruments. The open window may have caused the outdoor sample at H1 to be influenced by indoor activities.

Normality Tests on Data Distributions

The Shapiro-Wilk test for normality was used to determine if the measured ROS and air quality datasets followed an underlying distribution. Datasets were deemed to fit a normal or lognormal distribution if $p>0.05$, and the best fit was determined by the larger value of the Shapiro-Wilk test statistic, W, and by visualization of q-q plots.

In the sample of eight homes, the indoor concentration of ROS on TSP (W= 0.96, p=0.77) was normally distributed and the outdoor concentration of ROS on TSP followed a lognormal distribution (W= 0.95, p=0.67) and had a geometric mean of 1.97 nmol/m^3 (GSD = 1.85). The

indoor concentration of ROS on $PM_{2.5}$ collected in six of the eight homes where TSP sampling was conducted was normally distributed (W= 0.98, p=0.93). Most air quality parameters measured in the homes followed either a normal or a lognormal distribution, but some did not follow either. The fact that the majority of datasets were not normally distributed was an important factor in the choice of non-parametric statistics to analyze the data.

Outdoor Source Contribution during Controlled ROS Experiments at UTest House

The outdoor source contribution to indoor particulate ROS concentrations is given in Table C.4 in section 3.4 and is displayed here in Figure C.S1. When the outdoor ozone concentration was low, outdoor sources of particulate ROS contributed 34% of the total sources of indoor particulate ROS concentrations in the low indoor ozone/low indoor terpene case, whereas they contributed only 16% of the total sources in the high indoor ozone/high indoor terpene case. Outdoor sources as a percentage of total sources were significantly higher (62%) for the low indoor ozone/high indoor terpene case, but the increase in outdoor source contribution was likely influenced by the fact that two of the three sampling days for this case occurred in the July – September sampling period when outdoor temperatures and $PM_{2.5}$ concentrations were higher than in the January sampling period. All other sampling on low outdoor ozone concentration days was conducted in the January sampling period. The higher outdoor temperature and $PM_{2.5}$ concentration likely contributed to the increase in outdoor source contribution.

The introduction of VOCs into the UTest House with the use of PineSol® may also lead to the generation of ROS species that have a poorer response to the bulk ROS indicator used in this study (DCFH). For instance, DCFH has a much stronger response to •OH and ONOO- than to other ROS species such as NO and H_2O_2. This may appear to increase the outdoor source contribution when in fact indoor sources may be generating ROS that is left undetected with DCFH. A better understanding of ROS speciation during each of the indoor conditions would

help to more fully interpret the results of the outdoor source contribution for each of the indoor conditions.

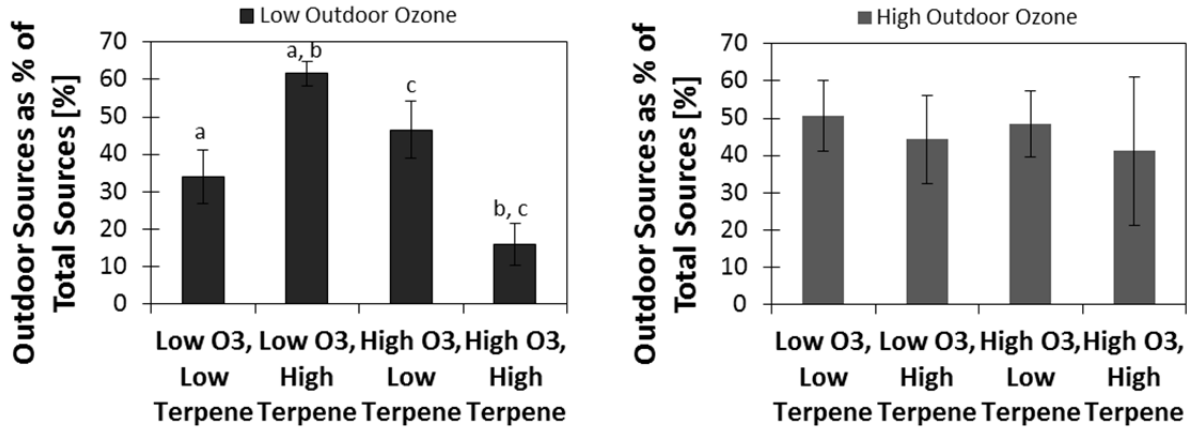

Figure C.S1: The contribution of outdoor sources to indoor particulate ROS concentrations as a percentage of total indoor and outdoor sources when outdoor ozone concentrations are low (left) and high (right). Pairs of statistically significant differences ($p<0.05$) are marked with matching letters.

Air Quality Data at UTest House

Tables C.S1 and C.S2 contain air quality data collected inside and outside the test house during the controlled experiments conducted there.

Table C.S1: Indoor and outdoor $PM_{2.5}$ and PM_{10} concentrations, temperature, relative humidity and ozone concentrations during the sampling events conducted at the UTest House when outdoor ozone concentrations were low (<40 ppb). Indoor VOC concentrations were measured during some sampling events.

Indoor Conditions at Utest House		Indoor		Outdoor		Indoor					Outdoor		
		$PM_{2.5}$ [μg/m^3]	PM_{10} [μg/m^3]	$PM_{2.5}$ [μg/m^3]	PM_{10} [μg/m^3]	Temperature [°C]	RH [%]	Ozone [ppb]	VOC [ppb]	Terp [ppb]	Temperature^T [°C]	RH [%]	Ozone [ppb]
Low O_3, Low Terpene	Day1	5.6 ± 1.3	7.5 ± 2.4	6.6 ± 1.2	22.9 ± 2.1	30.8 ± 0.2	27.5 ± 0.2	9.1 ± 3.0			17.1 ± 0.7	85.7 ± 6.4	22.4 ± 5.0
	Day2	2.1 ± 0.7	2.3 ± 1.6	2.5 ± 1.2	45.9 ± 3.5	29.6 ± 0.9	30.8 ± 6.0	1.6 ± 1.5			16.7 ± 1.1	15.4 ± 2.0	29.5 ± 11.9
	Day3	1.2 ± 1.0	2.2 ± 1.6	6.5 ± 1.5	47.7 ± 3.5	27.0 ± 0.9	12.4 ± 0.1	2.9 ± 1.8			0.5 ± 1.3	46.6 ± 8.1	22.9 ± 2.4
Low O_3, High Terpene	Day1	1.4 ± 0.4	2.4 ± 0.5	3.6 ± 1.2	50.0 ± 3.5	30.4 ± 0.1	14.9 ± 0.3	4.4 ± 1.5			17.3 ± 0.7	20.5 ± 7.2	22.5 ± 13.3
	Day2	5.0 ± 1.1	7.7 ± 1.9	36.0 ± 10.6	48.4 ± 7.7	29.5 ± 1.2	51.7 ± 0.8	3.3 ± 1.8	173	28	34.1 ± 1.6	18.5 ± 3.8	22.9 ± 3.0
	Day3	7.0 ± 1.9	11.9 ± 3.2	63.5 ± 26.1	47.3 ± 8.3	29.9 ± 1.4	50.5 ± 0.1	2.4 ± 2.1	223	41	36.0 ± 0.1	17.1 ± 7.5	27.3 ± 3.6
High O_3, Low Terpene	Day1	8.7 ± 3.8	11.0 ± 3.3	3.2 ± 1.0	52.7 ± 4.4	30.5 ± 0.3	20.0 ± 0.6	109.3 ± 4.0			24.5 ± 1.4	31.6 ± 10.8	38.7 ± 3.8
	Day2	2.7 ± 1.7	4.4 ± 2.6	3.1 ± 1.2	48.8 ± 3.5	29.6 ± 0.1	14.8 ± 0.2	107.2 ± 3.0			17.0 ± 1.3	20.8 ± 4.0	30.0 ± 5.1
	Day3	4.3 ± 3.7	6.3 ± 3.4	10.4 ± 4.1	66.9 ± 5.4	30.2 ± 0.1	18.9 ± 0.2	106.3 ± 6.7			18.3 ± 1.1	12.8 ± 2.2	27.0 ± 2.7
High O_3, High Terpene	Day1	12.4 ± 3.3	16.2 ± 2.9	5.7 ± 1.2	41.3 ± 4.4	27.9 ± 0.5	14.7 ± 0.4	104.8 ± 19.9			5.4 ± 0.5	26.8 ± 0.7	22.8 ± 2.4
	Day2	8.8 ± 2.4	10.7 ± 1.6	3.8 ± 1.2	48.8 ± 2.7	28.8 ± 0.4	10.9 ± 0.2	103.4 ± 16.0			4.8 ± 1.8	15.1 ± 3.9	23.9 ± 6.0
	Day3	12.6 ± 4.7	15.6 ± 2.7	2.3 ± 1.2	34.2 ± 12.1	30.5 ± 0.1	24.2 ± 0.2	92.0 ± 10.7			22.2 ± 1.4	42.9 ± 8.8	34.7 ± 6.6

Table C.S2: Indoor and outdoor $PM_{2.5}$ and PM_{10} concentrations, temperature, relative humidity and ozone concentrations during the sampling events conducted at the UTest House when outdoor ozone concentrations were high. Indoor VOC concentrations were measured during some sampling events.

Indoor Conditions at Utest House		Indoor		Outdoor		Indoor					Outdoor		
		$PM_{2.5}$ [μg/m^3]	PM_{10} [μg/m^3]	$PM_{2.5}$ [μg/m^3]	PM_{10} [μg/m^3]	Temperature [°C]	RH [%]	Ozone [ppb]	VOC [ppb]	Terp [ppb]	Temperature^T [°C]	RH [%]	Ozone [ppb]
Low O_3, Low Terpene	Day1	8.4 ± 1.6	11.2 ± 1.8	25.5 ± 11.3	39.8 ± 18.8	30.2 ± 1.3	53.0 ± 0.8	2.0 ± 1.5			33.1 ± 0.8	32.3 ± 9.1	40.9 ± 1.9
	Day2	7.5 ± 1.6	10.4 ± 2.2	12.3 ± 6.8	20.6 ± 12.5	28.8 ± 1.0	48.1 ± 0.4	2.8 ± 1.6	148	15	31.3 ± 0.4	41.6 ± 14.1	51.6 ± 19.9
	Day3	6.6 ± 1.3	8.2 ± 2.3	8.7 ± 7.0	18.3 ± 13.5	-	-	4.4 ± 2.7	162	20	27.0 ± 0.7	-	47.0 ± 6.3
Low O_3, High Terpene	Day1	7.1 ± 2.0	10.2 ± 2.5	36.2 ± 23.3	38.8 ± 13.8	29.5 ± 1.3	50.6 ± 0.3	2.7 ± 2.2			33.9 ± 0.7	21.6 ± 10.3	45.9 ± 2.7
	Day2	8.0 ± 1.0	10.8 ± 1.7	35.3 ± 12.7	42.9 ± 19.8	31.7 ± 1.9	43.0 ± 2.0	3.0 ± 2.0	280	79	33.7 ± 0.3	22.2 ± 8.8	47.8 ± 7.2
	Day3	6.9 ± 1.1	10.5 ± 3.0	3.2 ± 1.9	8.1 ± 2.5	-	-	2.4 ± 2.1	221	45	26.4 ± 0.7	-	44.7 ± 3.3
High O_3, Low Terpene	Day1	6.2 ± 2.5	8.1 ± 2.5	40.9 ± 13.9	44.4 ± 11.0	31.5 ± 1.7	45.6 ± 0.9	81.3 ± 10.1	121	5	32.6 ± 0.5	24.0 ± 4.7	42.1 ± 6.7
	Day2	7.3 ± 2.8	9.4 ± 3.0	58.9 ± 16.8	51.1 ± 9.8	28.5 ± 0.6	51.4 ± 0.4	69.7 ± 4.8	178	7	33.8 ± 0.5	15.5 ± 3.9	41.3 ± 3.2
	Day3	9.6 ± 2.0	13.0 ± 1.5	11.8 ± 12.0	21.9 ± 17.9	29.2 ± 0.8	48.9 ± 0.3	92.0 ± 17.8			33.5 ± 1.1	31.0 ± 12.0	30.9 ± 4.8
High O_3, High Terpene	Day1	10.4 ± 4.2	13.1 ± 4.2	49.1 ± 13.0	52.9 ± 7.3	29.3 ± 1.0	51.2 ± 0.5	80.2 ± 4.6	207	22	33.2 ± 0.2	16.7 ± 2.9	60.6 ± 1.5
	Day2	12.0 ± 5.5	15.7 ± 4.7	22.8 ± 9.2	32.1 ± 21.7	29.5 ± 1.1	49.8 ± 0.3	86.4 ± 10.2	240	24	33.6 ± 0.5	26.3 ± 9.9	40.6 ± 2.3
	Day3	13.4 ± 3.6	16.8 ± 2.9	26.7 ± 7.0	37.9 ± 16.7	-	-	87.0 ± 8.0	246	26	27.2 ± 0.1	-	47.2 ± 5.8

Indoor particle measurements were taken with a TEOM. Outdoor $PM_{2.5}$ and PM_{10} concentrations were measured with a SidePak and DustTrak, respectively, and scaled with corresponding calibration factors. Ozone concentrations were measured with an ozone monitor. VOC samples were taken with a sorbent tube during some sampling events. 'Terp' refers to the concentration of terpenoids, calculated from the VOC samples. Temperature and relative humidity were measured with HOBOs. "-" indicates that the measurement was not taken due to an instrument error. ^{T} indicates that the measurement was obtained from the TCEQ sampling site.

Appendix D: Toxicological Analysis of Limonene Reaction Products Using an *in vitro* Exposure System

Toxicological Analysis of Limonene Reaction Products using an In Vitro Exposure System

Stacey E. Anderson[1], Shahana S. Khurshid[2], B. Jean Meade[3], Ewa Lukomska[1], and J.R. Wells[1]

Published in *Toxicology in Vitro*, 27, 721-730.

[1]Health Effects Laboratory Division, National Institute for Occupational Safety and Health, Morgantown, WV
[2]Department of Civil, Architectural and Environmental Engineering, The University of Texas at Austin, Austin, Texas
[3] Office of the Director, National Institute for Occupational Safety and Health, Atlanta, Ga

Abstract

Epidemiological investigations suggest a link between exposure to indoor air chemicals and adverse health effects. Consumer products contain reactive chemicals which can form secondary pollutants which may contribute to these effects. The reaction of limonene and ozone is a well characterized example of this type of indoor air chemistry. The studies described here characterize an *in vitro* model using an epithelial cell line (A549) or differentiated epithelial tissue (MucilAir™). The model is used to investigate adverse effects following exposure to combinations of limonene and ozone. In A549 cells, exposure to both the parent compounds and reaction products resulted in alterations in inflammatory cytokine production. A one hour exposure to limonene + ozone resulted in decreased proliferation when compared to cells exposed to limonene alone. Repeated dose exposures of limonene or limonene + ozone were conducted on MucilAir™ tissue. No change in proliferation was observed but increases in cytokine production were observed for both the parent compounds and reaction products. Factors such as exposure duration, chemical concentration, and sampling time point were identified to influence result outcome. These findings suggest that exposure to reaction products may produce more severe effects compared to the parent compound.

Key Words: indoor air, limonene, ozone, secondary oxidation products

Abbreviations:

A549- alveolar epithelia cells

Fetal Bovine Serum- FBS

Reactive Oxygen Species- ROS

Granulocyte-macrophage colony-stimulating factor- GM-CSF

Glutathione- GSH

Tumor Necrosis Factor alpha-TNF-α

VOC- volatile organic compounds

IL-8- Interleukin 8

IL-6- Interleukin 6

MCP-1- monocyte chemoattractant protein 1

1. Introduction

Exposure to the indoor air environment has the potential for a wide range of effects on human health and it has been estimated that indoor air quality-related health issues cost businesses $20-70 billion annually due to lost productivity, decreased performance, and sick absences (Mendell *et al*., 2002). Investigations have ascribed these effects (Arif and Shah, 2007; Jang *et al*., 2007), in part, to volatile organic compounds (VOCs) emitted from building materials and furnishings and application of chemicals (paints, cleaners, pesticides, glues and adhesives). (Singer et al., 2006; Weschler, 2004). In addition, the secondary pollutants resulting from reactive indoor air chemistry (e.g. ozonolysis of VOCs) may also be responsible for some of the health effects associated with indoor air exposures. Consumer cleaning products and air fresheners contain large amounts of VOCs which can react with OH• (hydroxyl radicals), ozone, and/or NO_3• (nitrate radicals) to form secondary oxidation products or secondary pollutants not detected with conventional sampling methods. These secondary pollutants include oxygenated organic chemicals, such as aldehydes, ketones, carboxylic acids and dicarbonyls (Forester et al., 2007; Ham et al., 2006; Harrison et al., 2007; Wells, 2005) which can be formed into thousands of chemical compounds. The potential toxicity of

these chemicals, either individually or as mixtures, is poorly understood. Although many of these secondary pollutants have been observed from simulated indoor air chemistry, they are not routinely detected with conventional sampling methods which may lead to inaccurate exposure assessments of indoor environments.

The respiratory tract plays a protective role against xenobiotics and invading microorganisms and also plays a significant role in immune surveillance. Epithelial cells are a major contact point for atmospheric pollutants since they are needed for gaseous exchange, mucous secretion, and protection. Disorders of the respiratory tract following chemical exposure include: disruption of the barrier functions including the mucociliary clearance, irritation, coughing, acute injury, altered gas exchange and decreased immune function. Due to the complexity of chemical-respiratory tract interactions, several *in vitro* methods using relevant airway cells, or tissues and implementation of target specific endpoints have been developed for toxicity assessment (Lambre, 1996). However, a lack of standardization among methods has made data interpretation and extrapolation challenging (Ritter et al., 2001). Complicating factors include: lack of complexity, differences in exposure method, chemical exposure concentration, flow and duration of exposure, experimental model and endpoints selected for analysis (Bakand et al., 2005). More primitive exposure systems include the addition of the chemical or compound of interest directly to the media in a closed flask (static environment). While the main benefits of these types of exposure studies include reduced costs and large sample number, they do have limited sensitivity and provide an unrealistic environment due to chemical-media interactions. (Fischader et al., 2008). Recent advances in the field include the development of air/cell interface exposure systems such as those produced by

companies including Vitrocell® Systems (Waldkirch, Germany) and Cultex Laboratories (Hannover, Germany). These exposure systems allow for direct exposure (flowing system) of the apical surface of the cell line or tissue with the aerosolized compound of interest, eliminating the potential for chemical/media interactions (Anderson et al., 2010; Persoz et al., 2010; Schmalz et al., 2011). While these systems are highly efficient and sensitive they are often expensive and most do not easily allow for dose response studies.

In addition to exposure system, selection of the experimental model is another potential for variability. Different models can be utilized depending on the health effect of interest (Verstraelen et al., 2008a). Inflammation and irritation of the lower respiratory tract is often evaluated in bronchial epithelial cells (NHBE, BEAS-2B) (Pichavant et al., 2005) (Persoz et al., 2012) or alveolar epithelial cells (A549) (Krakauer, 2000) while respiratory sensitization is often evaluated in monocyte/macrophage (Mono-Mac-6, THP-1) cell lines (Elms et al., 2001; Verstraelen et al., 2008b). Other advances in the field also include the use of primary cell lines and the development of highly differentiated three dimensional human airway tissue samples, such as (EpiAirway™ Tissue Model (Mattek, Ashland, MA) and MucilAir™ Epithelix (Geneva, Switzerland). To a lesser extent, cellular co-cultures consisting of epithelial cells, human blood monocyte-derived macrophages and dendritic cells have been used for investigational purposes (Lehmann et al., 2011). The selection of relevant endpoints is often based on the cell line or tissue selected for use and include but are not limited to: inflammatory cytokines [Interleukin 8 (IL-8), Interleukin 6 (IL-6), monocyte chemoattractant protein 1 (MCP-1)], cell proliferation, cytotoxicity (measurements of metabolic activity and cell membrane integrity), oxidative stress [glutathione (GSH), cellular markers (HO-1, SOD-1, GSTP1,

PTGS2, DUSP1)], reactive oxygen species (ROS), signaling pathways (NF-kB and MAP kinase), and genotoxicity (DNA damage). Differences in cell culture technique, use of cell stimulation with agents such as tumor necrosis factor alpha (THF-α) as surrogates for cellular signaling, and time point for experimental sampling are also potential sources of variability.

The majority of research in the field of indoor air has focused on the parent compounds, or the chemicals most widely recognized as indoor air pollutants including chlorobenzene, styrene, m-xylene, formaldehyde, toluene, terpenes, and aldehydes. Research has shown that exposure of TNF-α stimulated A549 cells (Static/20 hours) to chlorobenzene, styrene or m-xylene (within the indoor relevant concentration range 1-25,000 mg/m^3) increased MCP-1 production while higher concentrations increased IL-8 production (Fischader et al., 2008). Mixtures of the 3 VOCs produced similar results. In addition to alternations in IL-8 and MCP-1 production, increased IL-13 levels were observed when supernatants of chlorobenzene exposed A549 cells (Static/20 hours) were incubated with human peripheral blood mononuclear cells (Lehmann et al., 2008). Expression of cellular markers for oxidative stress, such as HO-1, GSTP1, SOD-1, prostaglandin-PTGS2 and DUSP1, were also found to be elevated in the presence of chlorobenzene (10^2-10^4 mg/m^3 for 24 hours) along with intracellular ROS. However, in the presence of antioxidants chlorobenzene-induced alterations were suppressed (Feltens et al., 2010). Exposure of A549 cells (0.2 ppmv for 1 hour/Cultex®) to toluene and benzene, but not formaldehyde, increased IL-8 production and cytotoxicity following exposure. The ratio of reduced to oxidized glutathione was increased for benzene treated cells and decreased for formaldehyde treated cells (Pariselli et al., 2009). However, cells

pre-stimulated with TNF-α prior to formaldehyde (50 mg/m^3 for 30 minutes) exposure, resulted in enhanced IL-8 expression (Persoz et al., 2010). Gminski et al., (2010) demonstrated that the aldehydes 2-heptenal and 2-octenal (main VOC constituents emitted from pine wood) caused genotoxic effects in A549 cells following exposure (15-65 ppm; Vitrocell® for 1 hour) to concentrations exceeding 100 mg/m^3 and 40 mg/m^3, respectively (Gminski et al., 2010). *In vitro* investigations into the specific health effects associated with exposure to secondary pollutants in the indoor environment are limited. One study conducted by Anderson et al. (2010) demonstrated that exposure of A549 cells (Vitrocell® for 4 hours) to structurally similar terpene ozonolysis reaction products (dicarbonyl compounds) resulted in an increased pro-inflammatory response suggesting the potential for toxicity of secondary pollutants. The differences in exposure techniques and endpoints among the above mentioned studies emphasize the need for the standardization of this type of model.

The ozone-initiated reaction of limonene, an abundant VOC that provides a citrus smell to many cleaning supplies and personal care products, is a well characterized chemistry model for the identification of secondary pollutants and the evaluation of indoor air mixtures. Currently no *in vitro* work has evaluated the potential health effects following exposure to ozone/limonene reaction products. Therefore, this study used the prototypical indoor air reaction of limonene + ozone to begin to characterize if secondary products are more toxic than their parent compounds and to emphasize the importance of method development and validation for these types of *in vitro* exposure models.

2. Experimental Methods

2.1 Teflon Chamber Preparation

Teflon chambers (FEP 500, American Durafilm, Hollston, MA) were constructed and filled with treated air (described below) to facilitate cell exposure to gas-phase chemicals via the Vitrocell® apparatus. Compressed air from the National Institute for Occupational Safety and Health (NIOSH) facility was passed through anhydrous $CaSO_4$ and molecular sieves (Drierite, Xenia, OH) to remove both moisture and organic contaminants. The resultant dry air (less than 5% relative humidity) was humidified to 50% relative humidity to simulate average indoor environment conditions. R(+)-Limonene (99% purity) was injected into a 50% relative humidity air stream through a heated ¼ inch stainless steel tee into the 60 liter teflon chambers. Lower target concentrations (500 ppb (1.2×10^{13} molecule cm^{-3})) of limonene in both limonene and limonene/ozone chambers were used for the MucilAir™ exposures while higher concentrations of approximately 20 ppm (5×10^{14} molecule cm^{-3}) was used for A549 exposures. For the reaction product experiments, ozone was produced by photolyzing air with a mercury pen lamp (Jelight, Irvine, CA) in a separate Teflon chamber. Ozone concentrations were measured with a UV photometric ozone analyzer (model 49C or 49i, Thermo Fisher Scientific, Inc., Waltham, MA). Ozone concentrations of either 100 ppb (2.5×10^{12} molecule cm^{-3} for MucilAir™ exposure) or 4 ppm (1×10^{14} molecule cm^{-3} for A549 exposure) were achieved by transferring large volumes (2 liters) from the separate high concentration (~120 ppm) ozone chamber using a gas-tight syringe or an additional smaller Teflon chamber. Ozone was injected into the respective Teflon chamber containing ~500 ppb (1.2×10^{13} molecule cm^{-3}) or ~ 20 ppm (5×10^{14} molecule cm^{-3}) limonene 15 to 30

minutes prior to the Vitrocell® exposure. Previous gas-phase VOC experiments indicated the sample preparation method above provides multi-hour concentration stability (Forester and Wells, 2009).

2.2 Chemical Characterization

Sampling for monitoring chamber contents was performed using a 65 μm polydimethylsiloxane/divinylbenzene (PDMS/DVB) solid phase micro-extraction (SPME) fiber (Supelco, Milwaukee, WI) assembly which was inserted into a 6.4-mm Swagelok (Solon, OH) fitting attached to the Teflon®-film chambers (described above). The chamber contents were sampled for 5 minutes then the SPME was inserted through a Merlin Microseal (Half Moon Bay, CA) and into the heated injector of an Agilent (Wilmington, DE) 6890 gas chromatograph with a 5975 mass selective detector (GC/MS) and Agilent ChemStation software. Compound separation was achieved by a J&W Scientific (Folsom, CA) HP-5MS (0.25 mm i.d., 30-m long, 0.25 μm film thickness) column and the following GC oven parameters: injection port was set to 250 °C, and oven temperature began at 40 °C for 2 minutes and was ramped 20 °C min^{-1} to 130 °C then ramped 40 °C min^{-1} to 240 °C and held for 2 minutes.

2.3 A549 Cell Culture

Human alveolar epithelial cells (A549) were purchased from American Type Culture Collection (ATCC No.CCL-185). For each set of experiments cell culture was initiated from an A549 stock ($1x10^6$ cells/ml) prepared from early passages. Cells were incubated at 37 °C with 5% CO_2 in F12K medium (Kaighn's Modification of Ham's F-12 with L-Glutamine, ATCC, VA, USA) supplemented with 10% heat inactivated fetal bovine

serum (FBS) and 0.05mg/ml of Gentamycin. Cells were propagated in sterile and vented 75cm^2 cell culture flasks until desired number of cells was reached then harvested, counted and seeded on Costar 24mm (0.4 μm) transwell inserts and placed in 6-well tissue culture treated plates. To determine the optimal growth of A549 cells on inserts, a range of 1.25 x 10^5 - 5 x 10^5 cells per insert and incubation times of 24 to 48 hours were tested. During this incubation period complete culture medium (with 10% FBS) was added to the apical (1.5 ml) and basolateral (2.5 ml) tissue surfaces. Twenty-four hours prior to exposure the complete culture medium was removed and replaced with serum-free medium to synchronize the cells. To test modulation of the production of pro-inflammatory mediators in exposed cells, recombinant human TNF- α (Invivogen, San Diego, CA) at a final concentration of 2 ng/ml was added to serum-free medium for pre-sensitization in select experiments. Inserts containing unexposed cells (n = 3) were included in every experiment to evaluate cellular integrity. These controls were treated exactly the same as the experimental cells except they remained in the incubator while the other cells were exposed in the Vitrocell® chambers.

2.4 MucilAir™ Tissue Culture

MucilAir™ tissue samples are 3D models of highly differential human airway epithelium consisting of primary human cells isolated from the nasal cavity, the trachea and the bronchus. The manufacturer claims that these samples are functional for more than 1 year and can therefore be used for long term and/or repeated dose exposures. Commercially available transwell inserts with MucilAir™ epithelium were purchased from Epithelix (Geneva, Switzerland). Upon arrival inserts were transferred into 24- well tissue culture plates containing 0.8 ml of serum free MucilAir^{TM} Culture Medium

(Geneva, Switzerland) which did not exceed the air/liquid interface. Cultures were maintained at 37 °C in a humidified 5% CO_2 incubator. Media were changed every 2-3 days. Unexposed inserts (n = 3) were included in the 28-day exposure experiment to evaluate cellular integrity. These controls were treated exactly the same as the experimental cells except they remained in the incubator while the other inserts were exposed in the Vitrocell® chambers.

2.5 Vitrocell Exposures

For A549 cells, immediately before exposures culture medium was completely removed from the apical side of the inserts, cells were washed twice with sterile phosphate buffered saline then transferred into the Vitrocell® PT-CF exposure system (Vitrocell, Waldkirch, Germany). For the exposures of MucilAir™ tissue, the inserts were transferred directly into the Vitrocell® PT-CF exposure system. Once a week, a washing step (3 times within 1 hour using MucilAir™ culture medium) was performed to remove accumulated mucus produced by fully differentiated and functional MucilAir™ tissue. Exposures were conducted as previously described (Anderson et al., 2010). In brief, 2 separate exposure modules, each accommodating 3 inserts were used for parallel exposures to control and test atmospheres. During exposure cells were immersed in serum-free medium on the basal surface, allowing cells to be nourished from the bottom while being exposed to gas on air/liquid interface from the top. To minimize mechanical stress and maintain cell viability, the test atmosphere was delivered via trumpets raised 0.5 cm above the cell layer at an optimal constant air flow of 3 ml/minute (A549 cells) or 2 ml/minute (MucilAir). A single exposure (1-4 hours) was tested for A549 cells. MucilAir™ inserts were exposed for 1 hour per day, 5 days a week for a total of 4 weeks.

Immediately after the exposure, inserts were transferred to regular 6-well (A549) or 24-well (MucilAir™) plates. Complete medium with 10% FBS (A549) or MucilAir^{TM} Culture Medium was added on both (apical and basolateral) or basolateral side respectively. Cells were allowed to recover in a 37 °C, 5% CO_2 incubator. Culture supernatants were collected at 10-12 hours post-exposure and then again at 24 hours (A549) or at 72 hours after the last exposure of each week for 4 weeks (MucilAir™). Supernatants were stored at -20 °C for subsequent analysis. Following exposure, cells and tissues were analyzed for cell proliferation and supernatants were evaluated for cytokine production.

2.6 XTT Proliferation Assay

Cell proliferation of A549 cells and MucilAir™ tissue samples was determined using Cell Proliferation Kit II -XTT, (Roche Diagnostics, Mannheim, Germany) according to the manufacturer's protocol with slight modifications. In brief, in order to minimize the loss of cells during the trypsinization process the reaction was performed directly on the transwell inserts in a 6-well (A549) or a 24-well plate (MucilAir™). For A549 cells 24 hours post exposure both top and bottom culture supernatants were removed. Cells were washed once with F-12K Medium supplemented with 10% of heat inactivated fetal bovine serum. Fresh culture medium (1 ml) pre warmed to 37 °C was added to each insert. For MucilAir™ samples basal supernatants were removed after the last exposure (at the end of 4 weeks). Inserts were rinsed once with MucilAir^{TM} Culture Medium and 125 µl of fresh warm medium was added on top of each insert. Reaction reagents were thawed immediately prior to use. A XTT labeling mixture was prepared by mixing 5 ml of XTT labeling reagent and 100 µl of electron-coupling reagent. The mixture was then

added to each insert (0.5 ml per A549 or 125 µl per MucilAir™ insert) to obtain a final concentration of XTT 0.3 mg/ml. To ensure even distribution of the dye on top of the inserts, the plate was swirled in a circular motion and incubated for 2 hours in a humidified atmosphere (37 °C, 5% CO_2 incubator). Following the 2 hour incubation period, 100 µl aliquots from each sample were transferred into a 96-well, flat bottom plate and the absorbance was determined using a Spectramax Vmax plate reader (Molecular Devices, Sunnyvale, CA) at 492 nm.

2.7 Cytokine Detection

Levels of IL-8 and MCP-1 were measured in the combined apical and basal culture supernatants of A549 cells (10-24 hours post exposure) and IL-8, IL-6, MCP-1, and granulocyte-macrophage colony-stimulating factor (GM-CSF) were measured from combined supernatants of MucilAir™ tissues collected post exposure using commercially available ELISA kits (OptEIA™, BD Biosciences, San Jose, CA) according to the manufacturer's instructions.

2.8 Statistics

To determine statistically significant differences in cell proliferation or concentrations of inflammatory proteins, a 2-tailed unpaired t-test was used to compare clean air or limonene exposed to limonene or limonene + ozone exposed samples for each specified time point. Cytokine levels are based on the mean of triplicate samples from 3 cultures for each treatment group at each time point. Analysis of cell proliferation is based on the mean of triplicate samples for each treatment group. Linear trend analysis was performed to determine if the test articles

had exposure duration-related effects for the specified endpoints. Significant differences between control and experimental groups are designated with ** ($p \leq 0.01$) or * ($p \leq 0.05$).

3. Results

3.1 Generation of Limonene and Ozone Reaction Products

Figure D.1 shows the overlaid chromatograms following SPME sampling of the chamber contents connected to the Vitrocell® apparatus used for the exposures in the above mentioned studies. The dashed lines show the limonene peak before and after addition of ozone in the limonene + ozone chamber while the solid line (shifted by +0.05 minutes) shows the limonene peak for the limonene alone chamber. Because the peak areas are proportional to concentration it can be observed that prior to ozone addition the 2 chambers contained the same limonene concentration. After addition of ozone to the limonene + ozone chamber, the decrease in the limonene chromatographic peak area demonstrates the reaction of limonene with ozone. The ozone is consumed completely by the limonene + ozone reaction because there is significantly more limonene than ozone (data not shown).

3.2 A 4 hour exposure to limonene and ozone reaction products augments pro inflammatory cytokine production in A549 cells

To determine if exposure to indoor air reaction products alters the pro inflammatory response, pulmonary epithelial cells were exposed to clean air, ozone (4 ppm), limonene (20 ppm), or limonene (20 ppm) + ozone (4 ppm) (Figure D.2) for 4 hours. Statistically significant increases in IL-8 (12 hours post-exposure) and MCP-1 (12 and 24 hours post-

exposure) were observed following exposure to limonene when compared to clean air (Figure D.2A and B). No significant differences in cytokine production were observed following exposure to ozone when compared to clean air (Figure D.2C and D) which suggests that ozone alone does not influence the inflammatory cytokine response. However, a significant increase in IL-8 cytokine production was observed following exposure to limonene + ozone (12 hours post-exposure) when compared to limonene alone (Figure D.2E). Although not statistically significant, a modest increase in cytokine production was observed at 24 hours post-exposure. There were no statistically significant increases for MCP-1 cytokine production at 12 or 24 hours post-exposure, however an increasing trend was observed at both time points (Figure D.2F). The data presented are the best representation of 3 separate studies. Literature searches have identified that exposure times vary for research utilizing similar types of exposure models. To mimic a realistic indoor air environment an extended exposure duration is desired. To determine if exposure duration influences cell proliferation, A549 cells were exposed to clean air for durations of 1 and 4 hours (Figure D.3). A statistically significant reduction in the metabolic state of the cells, indicating decreased proliferation, was observed following a 4 hour exposure to clean air when compared to unexposed controls. This result was not observed following the 1 hour exposure to clean air.

3.3 A 1 hour exposure to limonene and ozone reaction products augments pro inflammatory cytokine production in unstimulated A549 cells

Due to the potential toxicity induced by exposure duration, subsequent exposures were reduced to 1 hour. To determine if a 1 hour exposure to indoor air reaction products alters cell proliferation or the pro inflammatory responses, pulmonary epithelial cells

were exposed to clean air, limonene (20 ppm), or limonene (20 ppm) + ozone (4 ppm). Consistent with the 4 hour exposure duration (Figure D.2), exposure to limonene resulted in significant increases in IL-8 and MCP-1 at 24 hours post exposure when compared to the clean air control (Figure D.4A and B). Exposure to limonene + ozone resulted in a decreased production of MCP-1 at the 10 and 24 hour post-exposure time points when compared to limonene (Figure D.3D). No change was observed in IL-8 production. No changes in cell viability were observed following exposure to limonene (Figure D.4E) when compared to the clean air control. However, a statistically significant decrease in cellular metabolism/proliferation was observed following limonene + ozone exposure (Figure D.4F). The data presented are the best representation of 3 separate studies. Similar exposure models described in the literature have elected to stimulate cells prior to exposure. Therefore, the effect of pre stimulation on cellular proliferation and pro inflammatory cytokine production was explored following exposure to limonene, or limonene + ozone (Figure D.5). In comparison to unstimulated cells, no changes in cell proliferation or cytokine production were observed. Lower, exposure concentrations of limonene and ozone were used to explore the influence of exposure concentration on the pro inflammatory and proliferative responses of A549 cells. Pulmonary epithelial cells were exposed to clean air, limonene (500 ppb), or limonene (500 ppb) + ozone (100 ppb). In contrast to previous studies that used higher concentrations of limonene and ozone (Figure D.2), no changes in proliferation or cytokine production were observed following exposure (Figure D.6).

3.4 Repeated dose exposure to limonene and ozone reaction products augments pro inflammatory cytokine production in MucilAir™ Tissue

MucilAir™ tissue samples were tested in the Vitrocell® system to evaluate the effects of repeated dose exposure (1 hour per day/5 days per week/4 weeks) on pro inflammatory and proliferative responses. MucilAir™ samples were exposed to limonene (500 ppb), or limonene (500 ppb) + ozone (100 ppb). Increases (Linear Trend Test $p < 0.05$) in cytokine production were observed for limonene (IL-6) and limonene + ozone (IL-6 and IL-8) over the 4 week exposure period. Statistically significant increases in IL-8 and IL-6 cytokine production were observed for the limonene + ozone exposure group when compared to the limonene exposure group at week 3 (Figure D.7A and B). Although at week 3 it appears there is an increase in GM-CSF production for limonene + ozone compared to limonene, it was not statistically significant. Similar to the A549 exposures, statistically significant decreases in MCP-1 were observed for limonene + ozone when compared to limonene at weeks 1 and 2 (Figure D.7C). There were no modulations in cytokine levels [IL-6 (70 ± 8 pg/ml), IL-8 (15 ± 1 pg/ml), MCP-1 (837 ± 263 pg/ml), and GM-CSF (59 ± 9 pg/ml)] at 1 week for limonene compared to unexposed tissues. No differences in metabolic activity for the limonene or limonene + ozone exposure groups compared to the unexposed tissues were observed following the 4 week exposure period (Figure D.7E).

4. Discussion

The studies described in this manuscript have utilized an *in vitro* exposure system to evaluate the toxicity associated with exposure to secondary pollutants generated from the reaction of limonene and ozone using both an isolated epithelial cell line (A549 cells) and

highly differentiated epithelial tissue (MucilAir™). The data suggest that exposure to either the parent compound (limonene) or secondary pollutants (reactions of limonene + ozone) can induce alternations in inflammatory responses in A549 cells and MucilAir™ tissue. At higher concentrations exposure to secondary pollutants resulted in greater toxicity as observed in a decrease in cell proliferation in A549 cells. In most cases where alterations in MCP-1, IL-8 or IL-6 cytokine expression occurred in either A549 or MucilAir™ tissue, a greater response was observed following exposure to limonene + ozone as compared to limonene alone. These data are consistent with results from animal studies which have demonstrated increased respiratory distress in animals exposed to reaction products compared to parent compounds. Wolkoff et al. (2012) showed that when mice were exposed to air, limonene (52 ppm/289 mg/m^3); ozone (0.1 ppm/0.2mg/m^3); or a reaction mixture of limonene (52 ± 8 ppm) and ozone (0.5, 2.5 and 3.9 ppm) 1 hour per day for 10 consecutive days increases in sensory irritation and airflow limitations and a concentration-dependent decrease in respiratory rate developed for the limonene + ozone groups compared to the controls. However, in contrast to the data presented here where there was a trend toward an increase in inflammatory cytokine expression over the 4 week exposure period with MucilAir™ tissue, the severity of the effects observed in the animals did not change with increasing number of exposures. Other studies have demonstrated significant increases in upper airway irritation and airflow limitations in mice exposed for 1 hour to reaction products compared to mice exposed to the reactants separately (Rohr et al., 2003; Wilkins et al., 2003). These results support the hypothesis that reaction products or secondary pollutants may yield health effects that are more severe than those resulting from exposure to the parent compounds

raising the concern that exposure assessments may be overlooking the most toxic components of indoor air contaminants.

In vitro models play an important role in understanding the biological effects of indoor air pollutants, however standardization of these models will be important in order to interpret data and compare results between laboratories. Culture conditions such as media, number of cells used and growth duration can contribute to variability between studies and may affect result outcomes (Anderson et al., 2010; Feltens et al., 2010; Fischader et al., 2008; Gminski et al., 2010). Heterogeneity in culture conditions can result in different growth characteristics and even phenotypes. For example, in these studies it was determined that the number of cells seeded on the insert and the growth duration prior to exposure can affect cytokine production. Lower cell concentration (250,000) and an increased growth period (48 hours following addition to insert) were identified to produce the greatest cytokine production and provide optimal conditions for the detection of cytokine modulation. This is demonstrated in Figures D.2 and D.4 where basal levels of cytokine production were much higher in Figures D.4A and B (48 hours) compared to Figure D.2 A and B (24 hours) for clean air and limonene.

It is critical to identify the conditions that will be sensitive enough to predict alterations, yet robust enough to be applied to various systems and across chemicals because the sample requirements often limit analysis to a single endpoint. Based on their relationship to human disease there are several endpoints which have been chosen for analysis. The airway epithelium is a complex physicochemical barrier that plays a pivotal role in host defense and is a rich source of modulatory compounds including cytokines which have been shown to play an import role in the etiology of airway disease (Mills et

al., 1999). The development of specific epithelial cell culture techniques has enabled investigators to examine differences that exist in the airway between health and disease states. Soluble inflammatory cytokines such as, IL-6, IL-8 and MCP-1 are often described in the literature as markers for the analysis of adverse outcomes induced by chemical exposure in cell lines such as A549; these can be collected directly from the supernatant and analyzed easily using methods such as ELISA or flow cytometry (Fischader et al., 2008; Persoz et al., 2010). The choice of these markers is supported by studies using primary cultures of human nasal epithelial cells from atopic individual with and without rhinitis (Calderon et al., 1997). In general, nasal epithelial cells from atopic individuals release significantly greater amounts of MCP-1, IL-8, TNF-α, and GM-CSF compared to cells collected from non-atopic, non-rhinitic individuals. Additionally, IL-8 levels have been shown to be increased in asthmatics and MCP-1 has been implicated in the pathogenesis of diseases characterized by monocytic infiltrates (Wood et al., 2012; Bafadhel et al., 2012). However, there is not a consensus in the literature with regards to the need or impact of cell stimulation prior to chemical exposure in the evaluation of cytokine modulation. For example, Persoz et al., (2010) found that pre-stimulation was required to detect changes in IL-8 production following formaldehyde exposure. In the studies presented here contradictory results were obtained when TNF-α was used for pre-stimulation in the A549 studies. Although following exposure to limonene reaction products cytokine levels in TNF-α stimulated cells were increased for both IL-8 and MCP-1 compared to unstimulated cultures, there was no longer a statistically significant increase in cytokine production between exposure groups and controls as was seen with

unstimulated cells (Figs D.4 and D.5). This points out the need for optimization such that there is room on the dose response curve to observe both up and down regulatory events.

One important shortcoming of more primitive *in vitro* methods is the lack of complexity. Cells in culture represent very simplified living systems; they do not possess the complexity of integrated functioning tissues. The use of differentiated tissue such as MucilAir™ helps to overcome some of these issues. MucilAir™ tissues are made of primary human cells isolated from the nasal cavity, the trachea and the bronchus to mimics the human respiratory epithelium. They contain basal, goblet, ciliated cells, and mucus and have features such as cilia beating, tight junctions, active ion transport, metabolic activity / detoxification (CYP450), and cytokine / chemokine / metalloproteinase release. Due to growth requirements, cell lines such as A549 are often limited to a single acute exposure and high doses representing cumulative exposure are frequently tested. The MucilAir™ model allows for repeated exposures and the studies presented here demonstrate the use of this more complex *in vitro* model to evaluate repeated exposures testing chemical concentrations closely related to indoor environments for up to 4 weeks. Due to growth requirements, cell lines are often limited to a single exposure. In these A549 cells studies, a high dose single exposure induced a similar pattern of cytokine modulation as seen in the lower dose MucilAir™ studies.

In summary, these studies suggest that secondary reaction products may be a significant contributor to adverse health effects associated with contaminated indoor air exposure. A combined approach using representative cell cultures as a screening tool, followed when appropriate, with more complex tissues including engineered tissues or lung slices may provide a valuable tool in investigating the role of indoor contaminants in

respiratory disease. Further development, standardization, and validation of these *in vitro* test methods could play a significant role in understanding the cellular, biochemical, and molecular mechanisms underlying the pulmonary toxicity resulting from exposure to indoor environments.

Acknowledgements

This work was supported in part by the Inter-Agency Agreement NIEHS Y1-ES0001-06. The findings and conclusions in this report are those of the authors and do not necessarily represent the views of the National Institute for Occupational Safety and Health, Centers for Disease Control and Prevention.

Figures

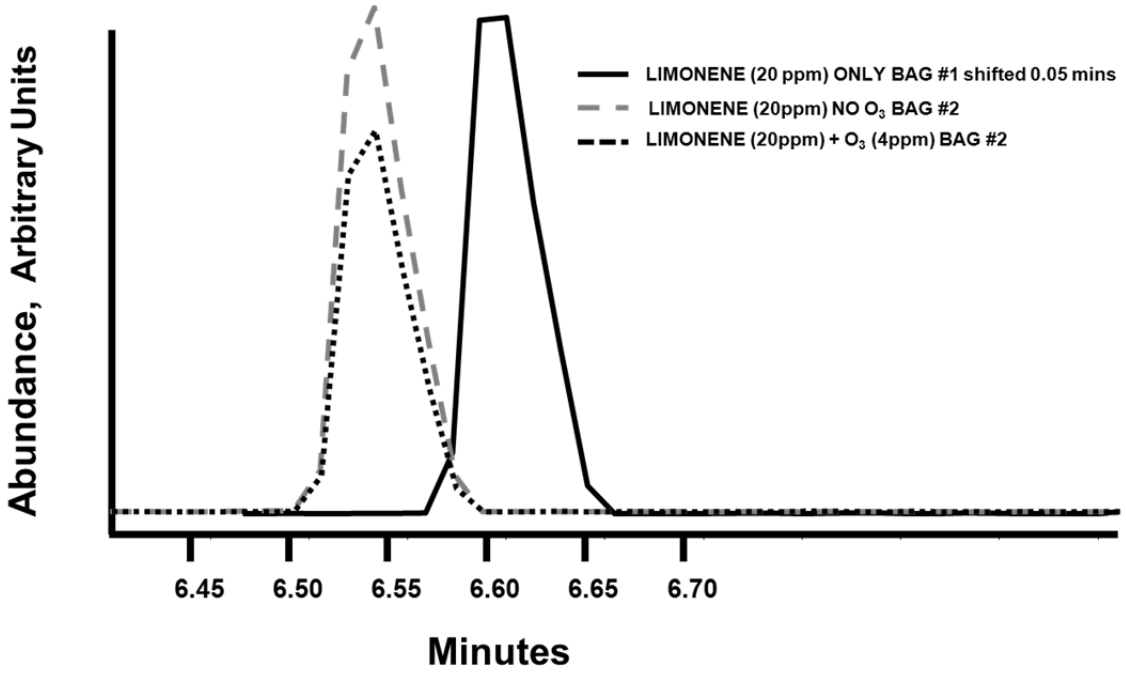

Figure D.1. GC/MS chromatogram of SPME sampled limonene and limonene/ozone chambers. Peak areas are proportional to concentration and all peaks are on the same y-axis scale. Solid line is the chromatographic peak of limonene only chamber (shifted by +0.05 minutes for clarity). Gray dashed line is the chromatographic peak of limonene (20 ppm) in limonene/ozone chamber prior to addition of ozone while black dashed line is limonene peak in limonene/ozone chamber after ozone (4 ppm) addition.

Figure D.2: Caption on following page.

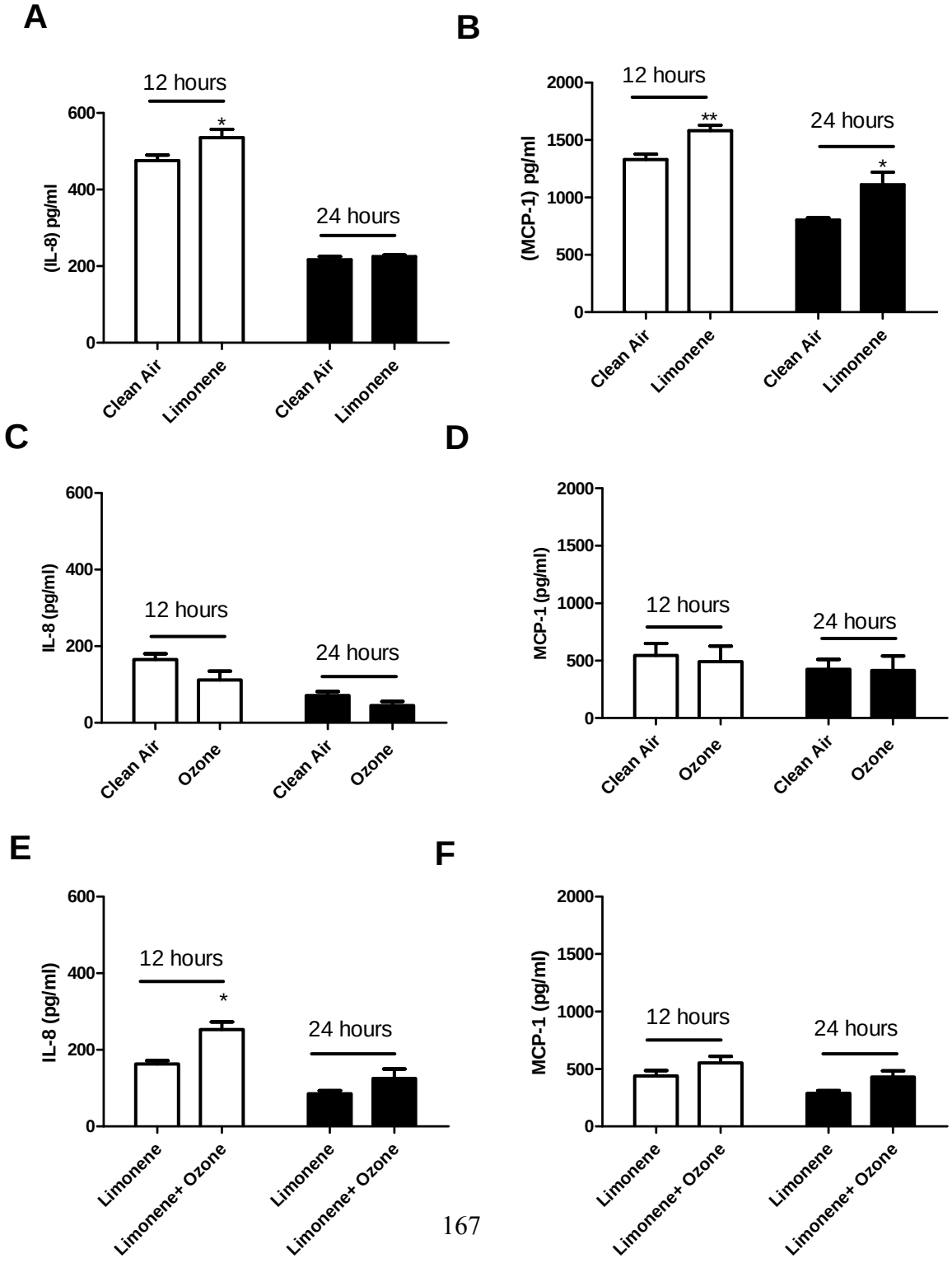

Figure D.2. The Effect of Limonene and Limonene + Ozone Reaction Products on A549 Cells Following a 4 Hour Exposure. A549 cells (250,000) were incubated for 24 hours on transwell inserts prior to exposure. Following exposure, cells were evaluated for IL-8 and MCP-1 protein production at 12 and 24 hours post-exposure. Comparisons were made for (A and B) clean air vs. limonene (20 ppm), (C and D) clean air vs. ozone (4 ppm) and (E and F) limonene (20 ppm) vs. limonene (20 ppm)/ozone (4 ppm). Bars represent the mean ± SE from three independent biological replicates per exposure group. Significant differences are designated with *$p < 0.05$.

Figure 3

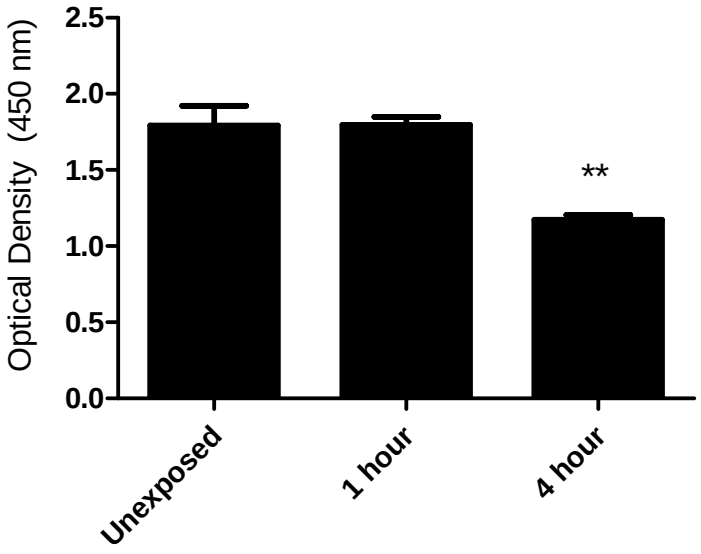

Figure D.3. The Effect of Exposure Duration on Proliferation of A549 Cells. A549 cells (250,000) were incubated for 48 hours on transwell insert prior to exposure. Unexposed cells remained in incubator (37^0C, 5% CO_2) while clean air was delivered to exposed cells for 1 or 4 hours. Cell proliferation was evaluation 24 hours post exposure. Bars represent the mean ± SE from three independent biological replicates per exposure group. Significant differences are designated with ** $p \leq 0.01$.

Figure D.4: Caption on following page.

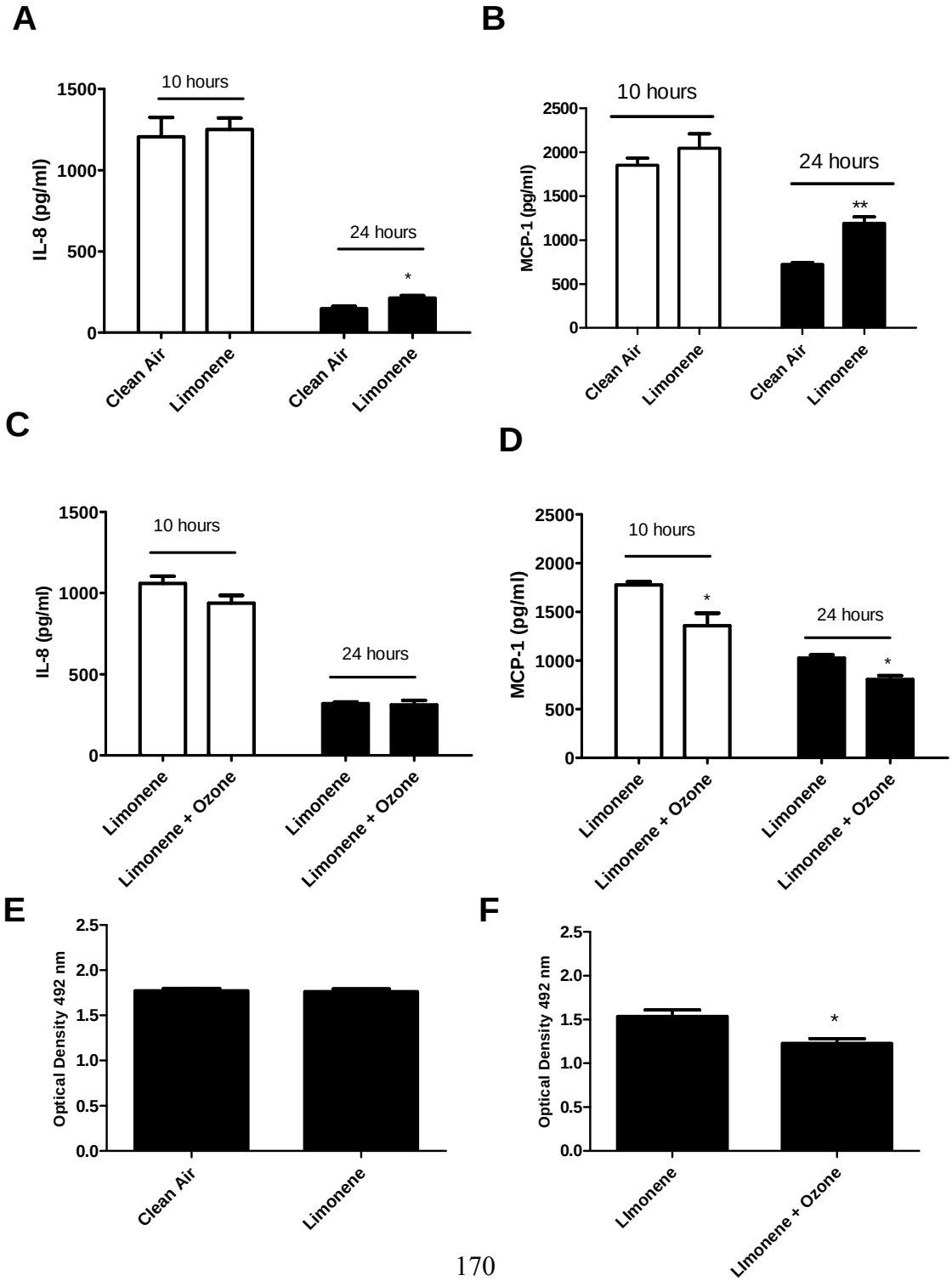

Figure D.4. The Effect of Limonene and Limonene + Ozone Reaction Products on A549 cells Following a 1 hour Exposure. A549 cells (250,000) were incubated for 48 hours on transwell insert prior to exposure. Following exposure, cells were evaluated for IL-8 (A and B) and MCP-1 (C and D) protein production at 10 and 24 hours post-exposure and cell proliferation (E and F) at 24 hours post exposure. Comparisons were made for clean air vs. limonene (20 ppm) and limonene (20 ppm) vs. limonene (20 ppm)/ozone (4 ppm). Bars represent the mean ± SE from three independent biological replicates per exposure group. Significant differences are designated with $^{**}p \leq 0.01$ or $^{*}p \leq 0.05$.

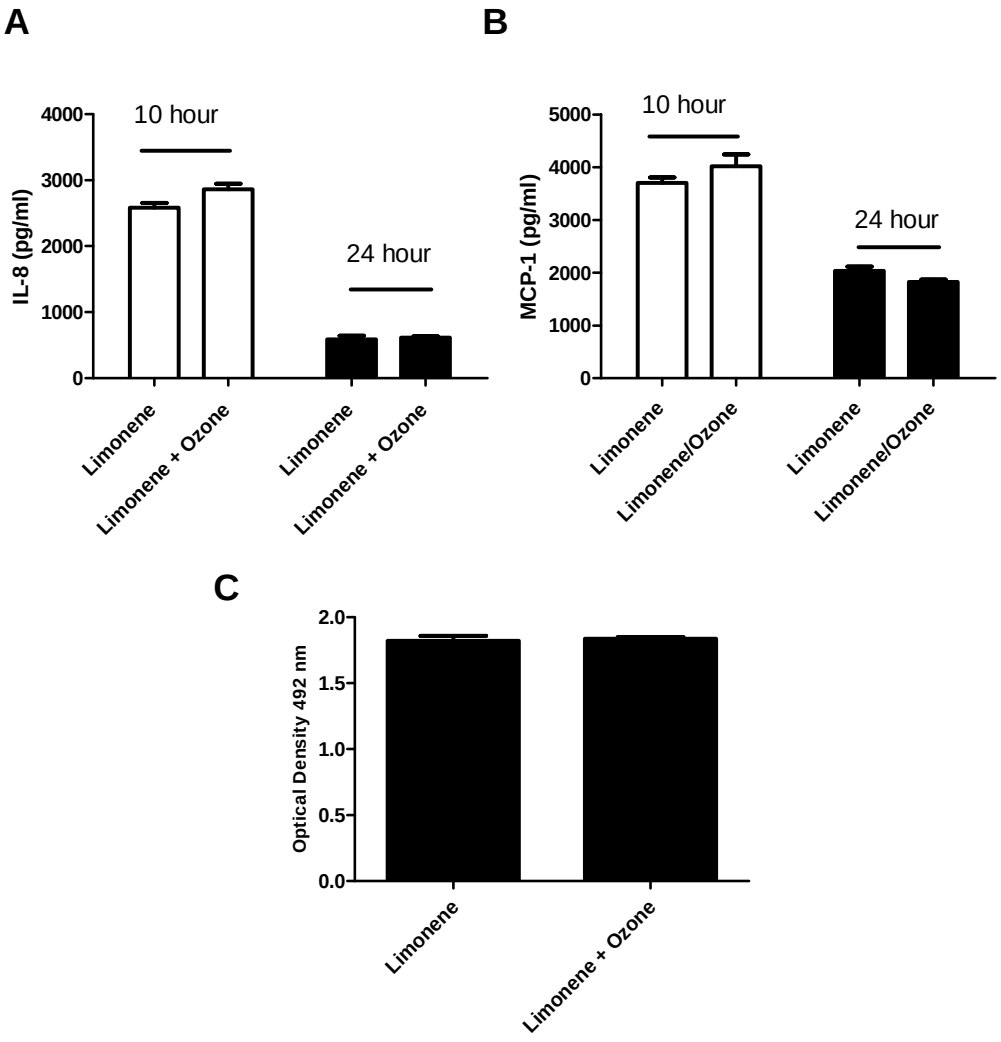

Figure D.5. The Effect of Limonene and Limonene + Ozone Reaction Products on Stimulated A549 Cells Following a 1 hour Exposure. A549 cells (250,000) were incubated for 48 hours on transwell insert prior to exposure. Following exposure, TNF-α stimulated cells (2 ng/ml) were evaluated for IL-8 (A) and MCP-1 (B) protein production at 10 and 24 hours post-exposure and cell proliferation (C) at 24 hours post exposure. Comparisons were made between limonene (20 ppm) vs. limonene (20 ppm)/ozone (4 ppm). Bars represent the mean ± SE from three independent biological replicates per exposure group.

Figure D.6: Caption on following page.

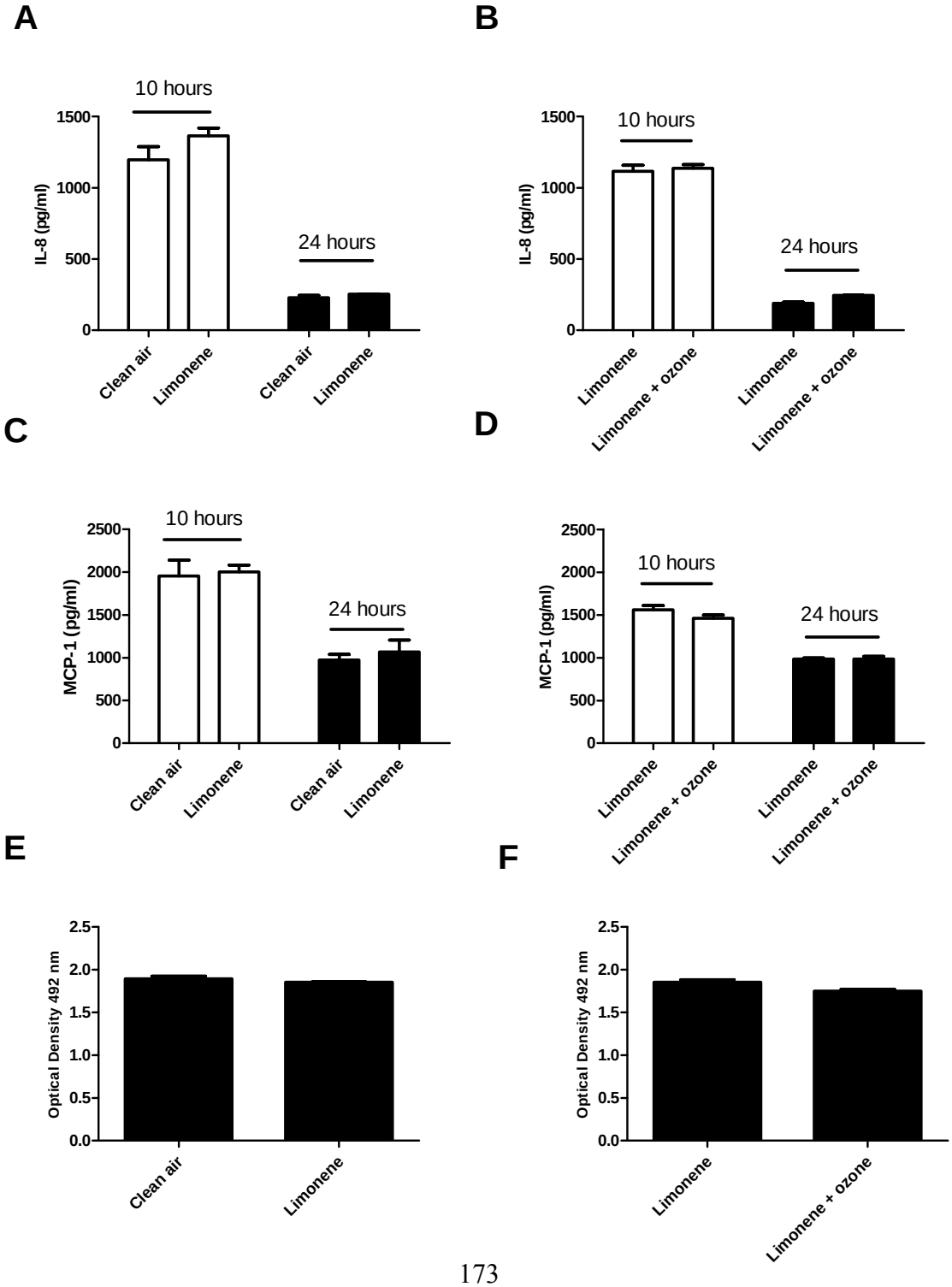

Figure D.6. The Effect of Exposure Concentration on A549 Cells Following a 1 hour Exposure. A549 cells (250,000) were incubated for 48 hours on transwell insert prior to exposure. Following exposure, cells were evaluated for IL-8 (A and B) and MCP-1 (C and D) protein production at 10 and 24 hours post-exposure and cell proliferation (E and F) at 24 hours post exposure. Comparisons were made for clean air vs. limonene (500 ppb) and limonene (500 ppb) vs. limonene (500 ppb)/ozone (100 ppb). Bars represent the mean ± SE from three independent biological replicates per exposure group. Significant differences are designated with $**p \leq 0.01$ or $*p \leq 0.05$.

Figure D.7: Caption on following page.

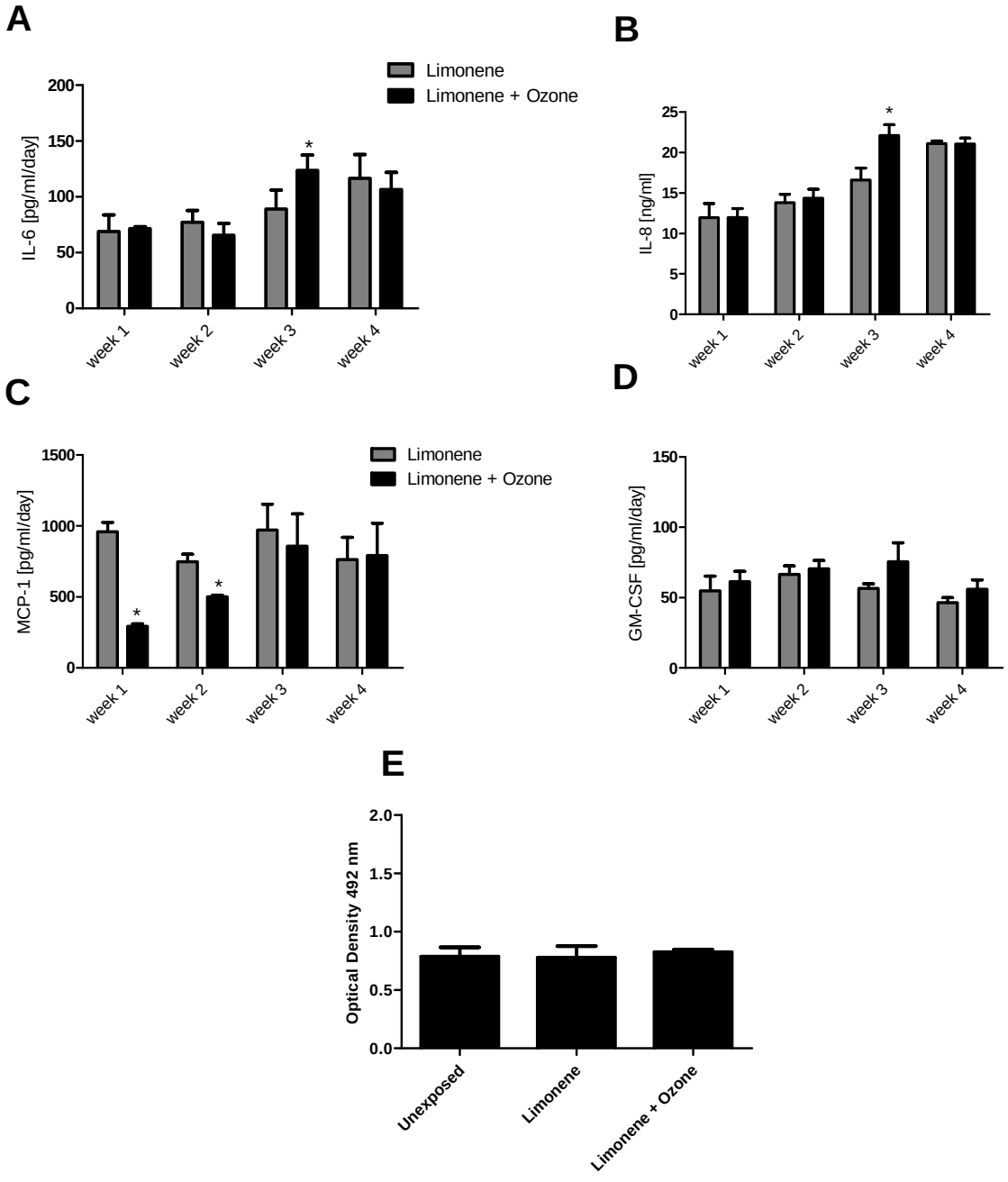

Figure D.7. The Effect of Limonene and Limonene + Ozone Reaction Products on MucilAir™ Tissue Following a Repeated Dose Exposure. MucilAir™ tissue was exposed to limonene (500 ppb) vs. limonene (500 ppb)/ozone (100 ppb) for 1 hour per day/5 days per week/4 weeks. 72 hours following the final weekly exposure, supernatant was evaluated for IL-8 (A), IL-6 (B), MCP-1 (C), and GM-CSF (D) protein production. Cell proliferation was evaluated 72 hours following the final experimental exposure (E). Comparisons were made for unexposed vs. limonene (500 ppb) and limonene (500 ppb) vs. limonene (500 ppb)/ozone (100 ppb). Basal cytokine levels for unexposed tissues are as follows: IL-6 (70 ± 8 pg/ml), IL-8 (15 ± 1 pg/ml), MCP-1 (837 ± 263 pg/ml), and GM-CSF (59 ± 9 pg/ml). Bars represent the mean ± SE from three independent biological replicates per exposure group. Significant differences are designated with $*p \leq 0.05$.

References

Anderson, S.E., Jackson, L.G., Franko, J., and Wells, J.R. (2010). Evaluation of dicarbonyls generated in a simulated indoor air environment using an in vitro exposure system. Toxicol Sci *115*, 453-461.

Arif, A.A., and Shah, S.M. (2007). Association between personal exposure to volatile organic compounds and asthma among US adult population. Int Arch Occup Environ Health *80*, 711-719.

Bafadhel, M., McCormick, M., Saha, S., McKenna, S., Shelley, M., Hargadon, B., Mistry, V., Reid, C., Parker, D., Dodson, P.*, et al.* (2012). Profiling of sputum inflammatory mediators in asthma and chronic obstructive pulmonary disease. Respiration *83*, 36-44.

Bakand, S., Winder, C., Khalil, C., and Hayes, A. (2005). Toxicity assessment of industrial chemicals and airborne contaminants: transition from in vivo to in vitro test methods: a review. Inhalation toxicology *17*, 775-787.

Calderon, M.A., Devalia, J.L., Prior, A.J., Sapsford, R.J., and Davies, R.J. (1997). A comparison of cytokine release from epithelial cells cultured from nasal biopsy specimens of atopic patients with and without rhinitis and nonatopic subjects without rhinitis. The Journal of allergy and clinical immunology *99*, 65-76.

Elms, J., Beckett, P.N., Griffin, P., and Curran, A.D. (2001). Mechanisms of isocyanate sensitisation. An in vitro approach. Toxicology in vitro : an international journal published in association with BIBRA *15*, 631-634.

Feltens, R., Mogel, I., Roder-Stolinski, C., Simon, J.C., Herberth, G., and Lehmann, I. (2010). Chlorobenzene induces oxidative stress in human lung epithelial cells in vitro. Toxicol Appl Pharmacol *242*, 100-108.

Fischader, G., Roder-Stolinski, C., Wichmann, G., Nieber, K., and Lehmann, I. (2008). Release of MCP-1 and IL-8 from lung epithelial cells exposed to volatile organic compounds. Toxicology in vitro : an international journal published in association with BIBRA *22*, 359-366.

Forester, C.D., Ham, J.E., and Wells, J.R. (2007). Geraniol (2,6-dimethyl-2,6-octadien-8-ol) reactions with ozone and OH radical: Rate constants and gas-phase products. Atmospheric Environment *41*, 1188-1199.

Forester, C.D., and Wells, J.R. (2009). Yields of Carbonyl Products from Gas-Phase Reactions of Fragrance Compounds with OH Radical and Ozone. Environmental Science & Technology *43*, 3561-3568.

Gminski, R., Tang, T., and Mersch-Sundermann, V. (2010). Cytotoxicity and genotoxicity in human lung epithelial A549 cells caused by airborne volatile organic compounds emitted from pine wood and oriented strand boards. Toxicol Lett *196*, 33-41.

Ham, J.E., Proper, S.P., and Wells, J.R. (2006). The gas-phase chemistry of citronellol with ozone and OH radical: Rate constants and products. Atmos Environ *40*, 726-735.

Harrison, J.C., Ham, J.E., and Wells, J.R. (2007). Citronellal reactions with ozone and OH radical: Rate constants and gas-phase products detected using PFBHA derivatization. Atmospheric Environment *41*, 4482-4491.

Jang, A.S., Choi, I.S., Koh, Y.I., and Park, C.S. (2007). Volatile organic compounds contribute to airway hyperresponsiveness. Korean J Intern Med *22*, 8-12.

Krakauer, T. (2000). Pentoxifylline inhibits ICAM-1 expression and chemokine production induced by proinflammatory cytokines in human pulmonary epithelial cells. Immunopharmacology *46*, 253-261.

Lambre, C.R., Auftherheide, M., Bolton, R.E., Rubini, B., Haagsman, H.P., Hext, P.M., Jorissen, M., Landry, Y., Morin, J.P., Nemery, B., Nettesheim, P., Pauluhn, J., Richards, R.J., Vickers, A.E.M., and We, R. (1996). In vitro test for respiratory toxicity, The report and recommemdations of ECVAM working 18. Altern Lab Anim> J *24*, 671-681.

Lehmann, A.D., Daum, N., Bur, M., Lehr, C.M., Gehr, P., and Rothen-Rutishauser, B.M. (2011). An in vitro triple cell co-culture model with primary cells mimicking the human alveolar epithelial barrier. Eur J Pharm Biopharm *77*, 398-406.

Lehmann, I., Roder-Stolinski, C., Nieber, K., and Fischader, G. (2008). In vitro models for the assessment of inflammatory and immuno-modulatory effects of the volatile organic compound chlorobenzene. Experimental and toxicologic pathology : official journal of the Gesellschaft fur Toxikologische Pathologie *60*, 185-193.

Mendell, M.J., Fisk, W.J., Kreiss, K., Levin, H., Alexander, D., Cain, W.S., Girman, J.R., Hines, C.J., Jensen, P.A., Milton, D.K., *et al.* (2002). Improving the health of workers in indoor environments: Priority research needs for a national occupational research agenda. Amer J Public Health *92*, 1430-1440.

Mills, P.R., Davies, R.J., and Devalia, J.L. (1999). Airway epithelial cells, cytokines, and pollutants. American journal of respiratory and critical care medicine *160*, S38-43.

Pariselli, F., Sacco, M.G., and Rembges, D. (2009). An optimized method for in vitro exposure of human derived lung cells to volatile chemicals. Experimental and toxicologic pathology : official journal of the Gesellschaft fur Toxikologische Pathologie *61*, 33-39.

Persoz, C., Achard, S., Leleu, C., Momas, I., and Seta, N. (2010). An in vitro model to evaluate the inflammatory response after gaseous formaldehyde exposure of lung epithelial cells. Toxicol Lett *195*, 99-105.

Persoz, C., Achard, S., Momas, I., and Seta, N. (2012). Inflammatory response modulation of airway epithelial cells exposed to formaldehyde. Toxicol Lett.

Pichavant, M., Charbonnier, A.S., Taront, S., Brichet, A., Wallaert, B., Pestel, J., Tonnel, A.B., and Gosset, P. (2005). Asthmatic bronchial epithelium activated by the proteolytic allergen Der p 1 increases selective dendritic cell recruitment. The Journal of allergy and clinical immunology *115*, 771-778.

Ritter, D., Knebel, J.W., and Aufderheide, M. (2001). In vitro exposure of isolated cells to native gaseous compounds--development and validation of an optimized system for human lung cells. Experimental and toxicologic pathology : official journal of the Gesellschaft fur Toxikologische Pathologie *53*, 373-386.

Rohr, A.C., Shore, S.A., and Spengler, J.D. (2003). Repeated exposure to isoprene oxidation products causes enhanced respiratory tract effects in multiple murine strains. Inhal Toxicol *15*, 1191-1207.

Scheers, E.M., Ekwall, B., and Dierickx, P.J. (2001). In vitro long-term cytotoxicity testing of 27 MEIC chemicals on Hep G2 cells and comparison with acute human toxicity data. Toxicology in vitro : an international journal published in association with BIBRA *15*, 153-161.

Schmalz, C., Wunderlich, H.G., Heinze, R., Frimmel, F.H., Zwiener, C., and Grummt, T. (2011). Application of an optimized system for the well-defined exposure of human lung cells to trichloramine and indoor pool air. J Water Health *9*, 586-596.

Singer, B.C., Destaillats, H., Hodgson, A.T., and Nazaroff, W.W. (2006). Cleaning products and air fresheners: emissions and resulting concentrations of glycol ethers and terpenoids. Indoor Air *16*, 179-191.

Verstraelen, S., Bloemen, K., Nelissen, I., Witters, H., Schoeters, G., and Van Den Heuvel, R. (2008a). Cell types involved in allergic asthma and their use in in vitro models to assess respiratory sensitization. Toxicology in vitro : an international journal published in association with BIBRA *22*, 1419-1431.

Verstraelen, S., Wens, B., Hooyberghs, J., Nelissen, I., Witters, H., Schoeters, G., Cauwenberge, P.V., and Heuvel, R.V. (2008b). Gene expression profiling of in

vitro cultured macrophages after exposure to the respiratory sensitizer hexamethylene diisocyanate. Toxicology in vitro : an international journal published in association with BIBRA *22*, 1107-1114.

Wells, J.R. (2005). Gas-phase chemistry of alpha-terpineol with ozone and OH radical: Rate constants and products. Environ Sci Technol *39*, 6937-6943.

Weschler, C.J. (2004). Chemical reactions among indoor pollutants: what we've learned in the new millennium. Indoor Air *14*, 184-194.

Wilkins, C.K., Wolkoff, P., Clausen, P.A., Hammer, M., and Nielsen, G.D. (2003). Upper airway irritation of terpene/ozone oxidation products (TOPS). Dependence on reaction time, relative humidity and initial ozone concentration. Toxicol Lett *143*, 109-114.

Wolkoff, P., Clausen, P.A., Larsen, S.T., Hammer, M., and Nielsen, G.D. (2012). Airway effects of repeated exposures to ozone-initiated limonene oxidation products as model of indoor air mixtures. Toxicol Lett *209*, 166-172.

Wood, L.G., Baines, K.J., Fu, J., Scott, H.A., and Gibson, P.G. (2012). The neutrophilic inflammatory phenotype is associated with systemic inflammation in asthma. Chest.

Yoon, H. I., Hong, Y. C., Cho, S. H., Kim, H., Kim, Y. H., Sohn, J. R., Kwon, M., Park, S. H., Cho, M. H., Cheong, H. K., (2010). Exposure to volatile organic compounds and loss of pulmonary function in the elderly. Eur. Respir. J.: Official J. Eur. Soc. Clin. Respi. Physiol. 36, 1270–1276.

Appendix E: Concentrations of Volatile Organic Compounds in Test House during Controlled Studies of ROS

Tables of VOCs

The following pages list the compounds detected by TD/GCMS on sorbent tubes samples collected on select days during controlled experiments at the Test House. Individual VOCs were statistically identified and quantified using a Library Compound Search (LCS). All compounds that were detected in excess of 2.5 ng on the sorbent tube are listed. The uncertainty associated with the mass of compounds identified and quantified using a LCS is typically assumed to be ±100%. The names of the compounds are given as reported by the TD/GCMS and CAS numbers are given when available. The compound names are formatted such that the last part of the name is given first followed by a comma and the first part of the name, e.g. 5-methyl-2-(1-methylethyl)-Cyclohexanone is written as "Cyclohexanone, 5-methyl-2-(1-methylehtyl)". Terpenes are hydrocarbons that comprise of several isoprene units (C_5H_8); terpenoids are modified terpenes and can contain additional moieties such as oxygen atoms. Terpenoids can be used as an umbrella term to include both terpenes and modified terpenes. All terpenoids identified in the lists have been highlighted in yellow. The indoor experimental condition at the Test House is given above each sample list. Sorbent tube samples collected as part of the QA/QC of the study are listed at the end, which included:

1. Sample blanks assessed on 5 days.
2. A sample of PineSol® which was prepared by injecting 5 μl of PineSol® into a 10 liter Teflon bag and drawing a sorbent tube sample from the bag at a flowrate of 25 ml/min for 30 minutes.
3. An outdoor sample collected on July 30, 2014, and indoor and outdoor samples collected simultaneously on August 25, 2014.

Low Outdoor Ozone -- Low indoor Ozone, High indoor VOC: Day 2								
9/3	Compound	Mol. Weight	Mol. Formula	CAS #	Mass [ng]	nanomoles	ppb	µg/m^3
1	Acetic acid	60	$C_2H_4O_2$	000064-19-7	67.18	1.12	9.9	24.0
2	Cyclobutanol	72	C_4H_8O	002919-23-5	18.63	0.26	2.3	6.7
3	1-Pentene	70	C_5H_{10}	000109-67-1	13.94	0.20	1.8	5.0
4	Hexanal	100	$C_6H_{12}O$	000066-25-1	87.87	0.88	7.8	31.4
5	Furfural	96	$C_5H_4O_2$	000098-01-1	89.07	0.93	8.2	31.8
6	Heptanal	114	$C_7H_{14}O$	000111-71-7	29.33	0.26	2.3	10.5
7	1S-.alpha.-Pinene	136	$C_{10}H_{16}$	007785-26-4	81.10	0.60	5.3	29.0
8	Camphene	136	$C_{10}H_{16}$	000079-92-5	25.62	0.19	1.7	9.2
9	.beta.-Pinene	136	$C_{10}H_{16}$	000127-91-3	39.52	0.29	2.6	14.1
10	Benzaldehyde	106	C_7H_6O	000100-52-7	53.29	0.50	4.4	19.0
11	Octanal	128	$C_8H_{16}O$	000124-13-0	66.69	0.52	4.6	23.8
12	d-limonene	136	$C_{10}H_{16}$	005989-27-5	98.80	0.73	6.4	35.3
13	Eucalyptol	154	$C_{10}H_{18}O$	000470-82-6	29.32	0.19	1.7	10.5
14	Phenol	94	C_6H_6O	000108-95-2	22.96	0.24	2.2	8.2
15	Cyclohexene, 1-methyl-4-(1-methylet	136	$C_{10}H_{16}$	000586-62-9	18.29	0.13	1.2	6.5
16	7-Octen-2-ol, 2,6-dimethyl-	156	$C_{10}H_{20}O$	018479-58-8	102.48	0.66	5.8	36.6
17	Benzoic acid, 2-[(trimethylsilyl)ox	282	$C_{13}H_{22}O_3Si_2$	003789-85-3	77.81	0.28	2.4	27.8
18	12-Oxabicyclo[9.1.0]dodeca-3,7-dien	220	$C_{15}H_{24}O$	019888-34-7	20.40	0.09	0.8	7.3
19	Acetophenone	120	C_8H_8O	000098-86-2	14.51	0.12	1.1	5.2
20	Nonanal	142	$C_9H_{18}O$	000124-19-6	102.62	0.72	6.4	36.7
21	Fenchol, exo-	154	$C_{10}H_{18}O$	022627-95-8	33.73	0.22	1.9	12.0
22	Camphor	152	$C_{10}H_{16}O$	000076-22-2	24.24	0.16	1.4	8.7
23	Borneol	154	$C_{10}H_{18}O$	010385-78-1	72.75	0.47	4.2	26.0
24	Borneol	154	$C_{10}H_{18}O$	010385-78-1	52.00	0.34	3.0	18.6
25	3-Cyclohexene-1-methanol, alpha.,.	154	$C_{10}H_{18}O$	000098-55-5	38.45	0.25	2.2	13.7
26	Bicyclo[2.2.1]heptan-2-ol, 1,7,7-tr	196	$C_{12}H_{20}O_2$	005655-61-8	185.76	0.95	8.4	66.3
27	Tetradecane	198	$C_{14}H_{30}$	000629-59-4	439.20	2.22	19.6	156.9
28	Benzene, (1-methylheptyl)-	190	$C_{14}H_{22}$	000777-22-0	23.31	0.12	1.1	8.3
29	Butanoic acid, butyl ester	144	$C_8H_{16}O_2$	000109-21-7	75.77	0.53	4.6	27.1
30	Benzene, (1-butylheptyl)-	232	$C_{17}H_{28}$	004537-15-9	26.18	0.11	1.0	9.4
31	4-Phenylnonane	204	$C_{15}H_{24}$	065185-83-3	31.14	0.15	1.3	11.1
32	Pentadecane	212	$C_{15}H_{32}$	000629-62-9	201.44	0.95	8.4	71.9
33	Benzene, (1-butylhexyl)-	218	$C_{16}H_{26}$	004537-11-5	21.94	0.10	0.9	7.8
34	Benzene, (1-butylhexyl)-	218	$C_{16}H_{26}$	004537-11-5	37.55	0.17	1.5	13.4
35	Benzene, (1-propylheptyl)-	218	$C_{16}H_{26}$	004537-12-6	13.66	0.06	0.6	4.9
36	N-(2-Bromophenyl)-N'-(4-methylbenzy	374	$C_{18}H_{19}BrN_2O_2$	1000225-36-1	21.67	0.06	0.5	7.7
37	Naphthalene, 2,6-dimethyl-	156	$C_{12}H_{12}$	028804-88-8	35.82	0.23	2.0	12.8
38	Benzene, (1-butylhexyl)-	218	$C_{16}H_{26}$	004537-11-5	112.04	0.51	4.5	40.0
39	Benzene, (1-propylheptyl)-	218	$C_{16}H_{26}$	004537-12-6	37.23	0.17	1.5	13.3
40	Hexadecane	226	$C_{16}H_{34}$	000544-76-3	55.87	0.25	2.2	20.0
41	Benzene, (1-methylnonyl)-	218	$C_{16}H_{26}$	004537-13-7	21.12	0.10	0.9	7.5
42	Butyric acid, thio-, S-decyl ester	244	$C_{14}H_{28}OS$	002432-55-5	190.51	0.78	6.9	68.0
43	Benzene, (1-propyloctyl)-	232	$C_{17}H_{28}$	004536-86-1	29.19	0.13	1.1	10.4
44	Benzene, (1-ethylnonyl)-	232	$C_{17}H_{28}$	004536-87-2	36.26	0.16	1.4	13.0
45	Hexane, 3-ethyl-4-methyl-	128	C_9H_{20}	003074-77-9	137.81	1.08	9.5	49.2
46	Benzene, (1-pentylheptyl)-	246	$C_{18}H_{30}$	002719-62-2	30.93	0.13	1.1	11.0
47	Benzene, (1-butyloctyl)-	246	$C_{18}H_{30}$	002719-63-3	26.63	0.11	1.0	9.5
48	Benzene, (1-propylnonyl)-	246	$C_{18}H_{30}$	002719-64-4	21.28	0.09	0.8	7.6
49	Benzene, (1-ethyldecyl)-	246	$C_{18}H_{30}$	002400-00-2	21.55	0.09	0.8	7.7
50	Benzene, (1-methylundecyl)-	246	$C_{18}H_{30}$	002719-61-1	17.89	0.07	0.6	6.4
	Terpenoids				457.1	3.2	28.1	163.2
	Total				3132.4	19.6	173.3	1118.7

Low Outdoor Ozone -- Low indoor Ozone, High indoor VOC: Day 3								
7/27	Compound	Mol. Weight	Mol. Formula	CAS #	Mass [ng]	nanomoles	ppb	µg/m^3
1	Acetic acid	60	$C_2H_4O_2$	000064-19-7	70.88	1.18	12.5	30.5
2	1-Butanol	74	$C_4H_{10}O$	000071-36-3	18.22	0.25	2.6	7.8
3	Pentanal	86	$C_5H_{12}O$	000110-62-3	30.95	0.36	3.8	13.3
4	1-Pentene	70	C_5H_{10}	000109-67-1	35.32	0.50	5.4	15.2
5	Hexanal	100	$C_6H_{12}O$	000066-25-1	120.02	1.20	12.8	51.6
6	Furfural	96	$C_5H_4O_2$	000098-01-1	84.04	0.88	9.3	36.1
7	Heptanal	114	$C_7H_{14}O$	000111-71-7	40.44	0.35	3.8	17.4
8	1S-.alpha.-Pinene	136	$C_{10}H_{16}$	007785-26-4	142.01	1.04	11.1	61.1
9	Camphene	136	$C_{10}H_{16}$	000079-92-5	37.80	0.28	3.0	16.3
10	.beta.-Pinene	136	$C_{10}H_{16}$	000127-91-3	57.31	0.42	4.5	24.6
11	Benzaldehyde	106	C_7H_6O	000100-52-7	47.68	0.45	4.8	20.5
12	Octanal	128	$C_8H_{16}O$	000124-13-0	80.77	0.63	6.7	34.7
13	d-limonene	136	$C_{10}H_{16}$	005989-27-5	113.58	0.84	8.9	48.9
14	.beta.-Phellandrene	136	$C_{10}H_{16}$	000555-10-2	18.38	0.14	1.4	7.9
15	Eucalyptol	154	$C_{10}H_{18}O$	000470-82-6	34.38	0.22	2.4	14.8
16	1-Hexanol, 2-ethyl-	130	$C_8H_{18}O$	000104-76-7	21.85	0.17	1.8	9.4
17	Phenol	94	C_6H_6O	000108-95-2	19.65	0.21	2.2	8.5
18	Bicyclo[4.1.0]hept-2-ene, 3,7,7-tri	136	$C_{10}H_{16}$	000554-61-0	21.67	0.16	1.7	9.3
19	7-Octen-2-ol, 2,6-dimethyl-	156	$C_{10}H_{20}O$	018479-58-8	115.87	0.74	7.9	49.8
20	Nonanal	142	$C_9H_{18}O$	000124-19-6	107.66	0.76	8.1	46.3
21	Fenchol, exo-	154	$C_{10}H_{18}O$	022627-95-8	33.00	0.21	2.3	14.2
22	Cyclopentane, propyl-	112	C_8H_{16}	002040-96-2	35.20	0.31	3.3	15.1
23	Borneol	154	$C_{10}H_{18}O$	010385-78-1	78.63	0.51	5.4	33.8
24	Borneol	154	$C_{10}H_{18}O$	010385-78-1	59.87	0.39	4.1	25.8
25	Tricyclo[2.2.1.02,6]heptane, 1,7,7-	136	$C_{10}H_{16}$	000508-32-7	25.41	0.19	2.0	10.9
26	Cyclodecane	140	$C_{10}H_{20}$	000293-96-9	20.56	0.15	1.6	8.8
27	Bicyclo[2.2.1]heptan-2-ol, 1,7,7-tr	196	$C_{12}H_{20}O_2$	005655-61-8	171.48	0.87	9.3	73.8
28	Tetradecane	198	$C_{14}H_{30}$	000629-59-4	438.86	2.22	23.5	188.8
29	Cyclohexanemethanol, 4-hydroxy-.alp	172	$C_{10}H_{20}O_2$	000080-53-5	21.62	0.13	1.3	9.3
30	Benzene, (1-methylheptyl)-	190	$C_{14}H_{22}$	000777-22-0	24.51	0.13	1.4	10.5
31	Propanoic acid, 2-methyl-, 2,2-dime	216	$C_{12}H_{24}O_3$	074367-33-2	99.63	0.46	4.9	42.9
32	Benzene, (1-butylpentyl)-	204	$C_{15}H_{24}$	020216-88-0	21.60	0.11	1.1	9.3
33	4-Phenylnonane	204	$C_{15}H_{24}$	065185-83-3	29.29	0.14	1.5	12.6
34	Pentadecane	212	$C_{15}H_{32}$	000629-62-9	202.11	0.95	10.1	86.9
35	Benzene, (1-butylhexyl)-	218	$C_{16}H_{26}$	004537-11-5	21.13	0.10	1.0	9.1
36	Benzene, (1-butylhexyl)-	218	$C_{16}H_{26}$	004537-11-5	34.52	0.16	1.7	14.8
37	Benzene, (1-ethyl-1-methylbutyl)-	176	$C_{13}H_{20}$	002132-86-7	34.35	0.20	2.1	14.8
38	Naphthalene, 2,7-dimethyl-	156	$C_{12}H_{12}$	000582-16-1	30.97	0.20	2.1	13.3
39	Benzene, (1-butylhexyl)-	218	$C_{16}H_{26}$	004537-11-5	113.62	0.52	5.5	48.9
40	Benzene, (1-propylheptyl)-	218	$C_{16}H_{26}$	004537-12-6	47.53	0.22	2.3	20.4
41	Hexadecane	226	$C_{16}H_{34}$	000544-76-3	55.09	0.24	2.6	23.7
42	Benzene, (1-methylnonyl)-	218	$C_{16}H_{26}$	004537-13-7	20.11	0.09	1.0	8.6
43	Butyric acid, thio-, S-decyl ester	244	$C_{14}H_{28}OS$	002432-55-5	201.10	0.82	8.8	86.5
44	Benzene, (1-propyloctyl)-	232	$C_{17}H_{28}$	004536-86-1	26.87	0.12	1.2	11.6
45	Benzene, (1-ethylnonyl)-	232	$C_{17}H_{28}$	004536-87-2	35.99	0.16	1.6	15.5
46	Benzene, (1-methyldecyl)-	232	$C_{17}H_{28}$	004536-88-3	58.16	0.25	2.7	25.0
47	Benzene, (1-pentylheptyl)-	246	$C_{18}H_{30}$	002719-62-2	27.44	0.11	1.2	11.8
48	Benzene, (1-butyloctyl)-	246	$C_{18}H_{30}$	002719-63-3	28.96	0.12	1.3	12.5
49	Benzene, (1-propylnonyl)-	246	$C_{18}H_{30}$	002719-64-4	20.36	0.08	0.9	8.8
50	Benzene, (1-ethyldecyl)-	246	$C_{18}H_{30}$	002400-00-2	20.16	0.08	0.9	8.7
	Terpenoids				542.0	3.8	40.8	233.1
	Total				3256.6	21.0	223.2	1400.7

High Outdoor Ozone -- Low indoor Ozone, Low indoor VOC: Day 2								
7/24	Compound	Mol. Weight	Mol. Formula	CAS #	Mass [ng]	nanomoles	ppb	µg/m^3
1	Acetic acid	60	$C_2H_4O_2$	000064-19-7	157.31	2.62	15.2	37.0
2	1-Butanol	74	$C_4H_{10}O$	000071-36-3	15.36	0.21	1.2	3.6
3	Pentanal	86	$C_5H_{10}O$	000110-62-3	51.98	0.60	3.5	12.2
4	Toluene	92	C_7H_8	000108-88-3	15.61	0.17	1.0	3.7
5	1-Pentanol	88	$C_5H_{12}O$	000071-41-0	40.24	0.46	2.7	9.5
6	Hexanal	100	$C_6H_{12}O$	000066-25-1	193.01	1.93	11.2	45.4
7	Furfural	96	$C_5H_4O_2$	000098-01-1	103.44	1.08	6.3	24.3
8	Styrene	104	C_8H_8	000100-42-5	11.96	0.12	0.7	2.8
9	2-Heptanone	114	$C_7H_{14}O$	000110-43-0	15.42	0.14	0.8	3.6
10	Heptanal	114	$C_7H_{14}O$	000111-71-7	52.08	0.46	2.7	12.3
11	1S-.alpha.-Pinene	136	$C_{10}H_{16}$	007785-26-4	211.66	1.56	9.0	49.8
12	Bicyclo[3.1.1]heptane, 6,6-dimethyl	136	$C_{10}H_{16}$	018172-67-3	104.39	0.77	4.5	24.6
13	Heptanol	116	$C_7H_{16}O$	053535-33-4	23.70	0.20	1.2	5.6
14	2,3-Octanedione	142	$C_8H_{14}O_2$	000585-25-1	12.85	0.09	0.5	3.0
15	Benzaldehyde	106	C_7H_6O	000100-52-7	79.73	0.75	4.4	18.8
16	Octanal	128	$C_8H_{16}O$	000124-13-0	77.95	0.61	3.5	18.3
17	d-limonene	136	$C_{10}H_{16}$	005989-27-5	112.79	0.83	4.8	26.5
18	.beta.-Phellandrene	136	$C_{10}H_{16}$	000555-10-2	33.58	0.25	1.4	7.9
19	1-Hexanol, 2-ethyl-	130	$C_8H_{18}O$	000104-76-7	35.68	0.27	1.6	8.4
20	Phenol	94	C_6H_6O	000108-95-2	26.51	0.28	1.6	6.2
21	1-Octanol	130	$C_8H_{18}O$	00011187-5	68.60	0.53	3.1	16.1
22	2-Nonanone	142	$C_9H_{18}O$	000821-55-6	11.50	0.08	0.5	2.7
23	Nonanal	142	$C_9H_{18}O$	000124-19-6	112.87	0.79	4.6	26.6
24	Acetic Acid, octyl ester	172	$C_{10}H_{20}O_2$	000112-14-1	19.91	0.12	0.7	4.7
25	2-Undecene, 4,5-dimethyl-, [R,S*-(	182	$C_{13}H_{26}$	055170-93-9	27.42	0.15	0.9	6.5
26	Decanal	156	$C_{10}H_{20}O$	000112-31-2	40.93	0.26	1.5	9.6
27	Cyclobutane, 1-butyl-2-ethyl-	140	$C_{10}H_{20}$	1000150-67-3	19.31	0.14	0.8	4.5
28	Bornyl acetate	196	$C_{12}H_{20}O_2$	000076-49-3	13.56	0.07	0.4	3.2
29	Tetradecane	198	$C_{14}H_{30}$	000629-59-4	611.88	3.09	18.0	144.0
30	Benzene, (1-methylheptyl)-	190	$C_{14}H_{22}$	000777-22-0	30.70	0.16	0.9	7.2
31	Propanoic Acid, 2-methyl-, 2,2-dime	216	$C_{12}H_{24}O_3$	074367-33-2	143.29	0.66	3.9	33.7
32	Benzene, (1-butylpentyl)-	204	$C_{15}H_{24}$	020216-88-0	31.51	0.15	0.9	7.4
33	4-Phenylnonane	204	$C_{15}H_{24}$	065185-83-3	36.44	0.18	1.0	8.6
34	Pentadecane	212	$C_{15}H_{32}$	000629-62-9	269.15	1.27	7.4	63.3
35	Benzene, (1-butylhexyl)-	218	$C_{16}H_{26}$	004537-11-5	52.21	0.24	1.4	12.3
36	Benzene, (1-butylhexyl)-	218	$C_{16}H_{26}$	004537-11-5	64.36	0.30	1.7	15.1
37	Benzene, (1-propylnonyl)-	246	$C_{18}H_{30}$	002719-64-4	26.34	0.11	0.6	6.2
38	Benzene, (1-methylundecyl)-	246	$C_{18}H_{30}$	002719-61-1	40.94	0.17	1.0	9.6
39	Benzene, (1-ethyldecyl)-	246	$C_{18}H_{30}$	002400-00-2	42.51	0.17	1.0	10.0
40	Benzene, (1-butylhexyl)-	218	$C_{16}H_{26}$	004537-11-5	141.43	0.65	3.8	33.3
41	Benzene, (1-propylheptyl)-	218	$C_{16}H_{26}$	004537-12-6	46.16	0.21	1.2	10.9
42	Hexadecane	226	$C_{16}H_{34}$	000544-76-3	72.96	0.32	1.9	17.2
43	Benzene, (1-methylnonyl)-	218	$C_{16}H_{26}$	004537-13-7	27.47	0.13	0.7	6.5
44	Butyric acid, thio-, S-decyl ester	244	$C_{14}H_{28}OS$	002432-55-5	214.91	0.88	5.1	50.6
45	Benzene, (1-propyloctyl)-	232	$C_{17}H_{28}$	004536-86-1	39.82	0.17	1.0	9.4
46	Benzene, (1-ethylnonyl)-	232	$C_{17}H_{28}$	004536-87-2	46.37	0.20	1.2	10.9
47	Benzene, (1,1-dimethylnonyl)-	232	$C_{17}H_{28}$		12.78	0.06	0.3	3.0
48	3-Benzoyl-2-t-butyl-4-isopropyloxaz	289	$C_{17}H_{23}NO_3$		54.25	0.19	1.1	12.8
49	Benzene, (1-pentylheptyl)-	246	$C_{18}H_{30}$	002719-62-2	34.81	0.14	0.8	8.2
50	Benzene, (1-butyloctyl)-	246	$C_{18}H_{30}$	002719-63-3	34.43	0.14	0.8	8.1
51	Benzene, (1-propylnonyl)-	246	$C_{18}H_{30}$	002719-64-4	25.27	0.10	0.6	5.9
52	Benzene, (1-ethyldecyl)-	246	$C_{18}H_{30}$	002400-00-2	24.75	0.10	0.6	5.8
53	Benzene, (1-methylundecyl)-	246	$C_{18}H_{30}$	002719-61-1	18.96	0.08	0.4	4.5
	Terpenoids				358.03	2.6	15.3	84.2
	Total				3833.05	25.4	147.6	901.9

High Outdoor Ozone -- Low indoor Ozone, Low indoor VOC: Day 3								
9/26	Compound	Mol. Weight	Mol. Formula	CAS #	Mass [ng]	nanomoles	ppb	µg/m^3
1	Acetic acid	60	$C_2H_4O_2$	000064-19-7	187.84	3.13	18.3	44.5
2	1-Butanol	74	$C_4H_{10}O$	000071-36-3	18.12	0.24	1.4	4.3
3	Butanal, 3-methyl-	86	$C_5H_{10}O$	000590-86-3	46.29	0.54	3.2	11.0
4	1-Pentanol	88	$C_5H_{12}O$	000071-41-0	47.72	0.54	3.2	11.3
5	Hexanal	100	$C_6H_{12}O$	000066-25-1	186.35	1.86	10.9	44.2
6	Furfural	96	$C_5H_4O_2$	000098-01-1	95.06	0.99	5.8	22.5
7	2-Heptanone	114	$C_7H_{14}O$	000110-43-0	16.49	0.14	0.8	3.9
8	Heptanal	114	$C_7H_{14}O$	000111-71-7	46.5	0.41	2.4	11.0
9	1R-.alpha.-Pinene	136	$C_{10}H_{16}$	007785-70-8	240.16	1.77	10.3	56.9
10	Bicyclo[3.1.1]heptane, 6,6-dimethyl	136	$C_{10}H_{16}$	018172-67-3	107.81	0.79	4.6	25.5
11	Formic acid, heptyl ester	144	$C_8H_{16}O_2$	000112-23-2	18.28	0.13	0.7	4.3
12	Benzaldehyde	106	C_7H_6O	000100-52-7	71.49	0.67	3.9	16.9
13	Octanal	128	$C_8H_{16}O$	000124-13-0	92.54	0.72	4.2	21.9
14	d-limonene	136	$C_{10}H_{16}$	005989-27-5	121.6	0.89	5.2	28.8
15	.beta.-Phellandrene	136	$C_{10}H_{16}$	000555-10-2	26.51	0.19	1.1	6.3
16	Eucalyptol	154	$C_{10}H_{18}O$	000470-82-6	18.7	0.12	0.7	4.4
17	1-Hexanol, 2-ethyl-	130	$C_8H_{18}O$	000104-76-7	31.58	0.24	1.4	7.5
18	Phenol	94	C_6H_6O	000108-95-2	28.33	0.30	1.8	6.7
19	Cyclodecane, methyl-	154	$C_{11}H_{22}$	013151-43-4	76.79	0.50	2.9	18.2
20	Nonanal	142	$C_9H_{18}O$	000124-19-6	130.61	0.92	5.4	31.0
21	Bicyclo[2.2.1]heptan-2-ol, 1,3,3-tr	154	$C_{10}H_{18}O$	001632-73-1	16.17	0.11	0.6	3.8
22	1-Tridecene	182	$C_{13}H_{26}$	002437-56-1	36.66	0.20	1.2	8.7
23	Borneol	154	$C_{10}H_{18}O$	010385-78-1	61.92	0.40	2.4	14.7
24	3,4-Dimethylcyclohexanol	128	$C_8H_{16}O$	005715-23-1	87.37	0.68	4.0	20.7
25	(-)-.alpha.-Terpineol (p-menth-1-en	154	$C_{10}H_{18}O$	1000151-92-4	17.7	0.11	0.7	4.2
26	3-Octene, (E)-	112	C_8H_{16}	014919-01-8	23.03	0.21	1.2	5.5
27	Isobornyl acetate	196	$C_{12}H_{20}O_2$	000125-12-2	82.31	0.42	2.5	19.5
28	Tetradecane	198	$C_{14}H_{30}$	000629-59-4	729.87	3.69	21.6	173.0
29	Benzene, (1-methylheptyl)-	190	$C_{14}H_{22}$	000777-22-0	35.87	0.19	1.1	8.5
30	Benzene, (1-butylpentyl)-	204	$C_{15}H_{24}$	020216-88-0	35.22	0.17	1.0	8.3
31	4-Phenylnonane	204	$C_{15}H_{24}$	065185-83-3	44.58	0.22	1.3	10.6
32	Pentadecane	212	$C_{15}H_{32}$	000629-62-9	289.84	1.37	8.0	68.7
33	Benzene, (1-pentylhexyl)-	232	$C_{17}H_{28}$	004537-14-8	29.2	0.13	0.7	6.9
34	Benzene, (1-butylhexyl)-	218	$C_{16}H_{26}$	004537-11-5	53.91	0.25	1.4	12.8
35	Benzene, (1-propylheptyl)-	218	$C_{16}H_{26}$	004537-12-6	17.91	0.08	0.5	4.2
36	Benzene, (1-methylnonyl)-	218	$C_{16}H_{26}$	004537-13-7	32.42	0.15	0.9	7.7
37	Naphthalene, 1,5-dimethyl-	156	$C_{12}H_{12}$	000571-61-9	47.03	0.30	1.8	11.1
38	Benzene, (1-butylhexyl)-	218	$C_{16}H_{26}$	004537-11-5	154.82	0.71	4.2	36.7
39	Benzene,(1-propylheptyl)-	218	$C_{16}H_{26}$	004537-12-6	70.01	0.32	1.9	16.6
40	Hexadecane	226	$C_{16}H_{34}$	000544-76-3	74.66	0.33	1.9	17.7
41	Benzene, (1-ethyldecyl)-	232	$C_{17}H_{28}$	002400-00-2	29.4	0.13	0.7	7.0
42	2,2,4-Trimethyl-1,3-pentanediol dii	286	$C_{16}H_{30}O_4$	006846-50-0	266.94	0.93	5.5	63.3
43	Benzene, (1-propyloctyl)-	232	$C_{17}H_{28}$	004536-86-1	34.16	0.15	0.9	8.1
44	Benzene, (1-ethylnonyl)-	232	$C_{17}H_{28}$	004536-87-2	47.04	0.20	1.2	11.1
45	Nonanoic acid, isopentyl ester	228	$C_{14}H_{28}O_2$		105.28	0.46	2.7	24.9
46	Benzene, (1-pentylheptyl)-	246	$C_{18}H_{30}$	002719-62-2	38.35	0.16	0.9	9.1
47	Benzene, (1-butyloctyl)-	246	$C_{18}H_{30}$	002719-63-3	34.04	0.14	0.8	8.1
48	Benzene, (1-propylnonyl)-	246	$C_{18}H_{30}$	002719-64-4	25.73	0.10	0.6	6.1
49	Benzene, (1-ethyldecyl)-	246	$C_{18}H_{30}$	002400-00-2	25.65	0.10	0.6	6.1
50	Benzene, (1-methylundecyl)-	246	$C_{18}H_{30}$	002719-61-1	18.66	0.08	0.4	4.4
	Terpenoids				486.6	3.5	20.4	115.3
	Total				4174.5	27.6	161.5	989.2

High Outdoor Ozone -- Low indoor Ozone, High indoor VOC: Day 2								
8/27	Compound	Mol. Weight	Mol. Formula	CAS #	Mass [ng]	nanomoles	ppb	$\mu g/m^3$
1	Pentane	72	C_2H_{12}	000109-66-0	4.01	0.06	3.4	10.0
2	Acetic Acid	60	$C_2H_4O_2$	000064-19-7	7.19	0.12	7.4	18.0
3	Acetaldehyde	44	C_2H_4O	000075-07-0	5.59	0.13	7.8	14.0
4	Butanoic Acid, 2-[(phenylmethoxy)im	279	C14H21NO3Si	055520-91-7	4.42	0.02	1.0	11.1
5	Hexanal	100	$C_6H_{12}O$	000066-25-1	16.77	0.17	10.4	41.9
6	Furfural	96	$C_5H_4O_2$	000098-01-1	13.23	0.14	8.5	33.1
7	3-Carene	136	$C_{10}H_{16}$	013466-78-9	7.65	0.06	3.5	19.1
8	1S-.alpha.-Pinene	136	$C_{10}H_{16}$	007785-26-4	43.11	0.32	19.6	107.8
9	Camphene	136	$C_{10}H_{16}$	000079-92-5	24.43	0.18	11.1	61.1
10	.beta.-Pinene	136	$C_{10}H_{16}$	000127-91-3	15.57	0.11	7.1	38.9
11	Benzaldehyde	106	C_7H_6O	000100-52-7	11.69	0.11	6.8	29.2
12	7-Oxabicyclo[2.2.1]heptane, 1-methy	154	$C_{10}H_{18}O$	000470-67-7	8.4	0.05	3.4	21.0
13	Octanal	128	$C_8H_{16}O$	000124-13-0	8.82	0.07	4.3	22.1
14	d-limonene	136	$C_{10}H_{16}$	005989-27-5	34.68	0.26	15.7	86.7
15	Eucalyptol	154	$C_{10}H_{18}O$	000470-82-6	19.93	0.13	8.0	49.8
16	4-Carene	136	$C_{10}H_{16}$	029050-33-7	9.01	0.07	4.1	22.5
17	7-Octen-2-ol, 2,6-dimethyl-	156	$C_{10}H_{20}O$	018479-58-8	47.01	0.30	18.6	117.5
18	3-Cyclohexen-1-carboxaldehyde	138	$C_9H_{14}O$	1000131-99-4	5.18	0.04	2.3	13.0
19	Nonanal	142	$C_9H_{18}O$	000124-19-6	19.37	0.14	8.4	48.4
20	Fenchol	154	$C_{10}H_{18}O$	1000150-75-8	26.01	0.17	10.4	65.0
21	Camphor	152	$C_{10}H_{16}O$	000076-22-2	8.86	0.06	3.6	22.2
22	Borneol	154	$C_{10}H_{18}O$	010385-78-1	42.36	0.28	17.0	105.9
23	Borneol	114	$C_{10}H_{18}O$	010385-78-1	21.13	0.19	11.4	52.8
24	3-Cyclohexene-1-methanol, .alpha.,.	114	$C_{10}H_{18}O$	000098-55-5	27.12	0.24	14.7	67.8
25	Morphinan-6-ol, 7,8-didehydro-4,l-e	341	$C_{20}H_{23}NO_4$	006703-27-1	4.43	0.01	0.8	11.1
26	Isobornyl Acetate	196	$C_{12}H_{20}O_2$	000125-12-2	109.97	0.56	34.6	274.9
27	Cyclohexanol, 4-(1,1-dimethylethyl)	156	$C_{10}H_{20}O$	000098-52-2	3.15	0.02	1.2	7.9
28	Tetradecane	198	$C_{14}H_{30}$	000629-59-4	62.27	0.31	19.4	155.7
29	Propanenitrile, 3-(dimethylamino)-	98	C5H10N2	001738-25-6	7.69	0.08	4.8	19.2
30	Benzene, (1-methylheptyl)-	190	$C_{14}H_{22}$	000777-22-0	3.78	0.02	1.2	9.5
31	Propanoic acid, 2-methyl-, 2,2-dime	216	$C_{12}H_{24}O_3$	074367-33-2	5.86	0.03	1.7	14.7
32	4-Phenylnonane	204	$C_{15}H_{24}$	065185-83-3	9.91	0.05	3.0	24.8
33	Pentadecane	212	$C_{15}H_{32}$	000629-62-9	31.12	0.15	9.1	77.8
34	Benzene, (1-butylhexyl)-	218	$C_{16}H_{26}$	004537-11-5	3.27	0.02	0.9	8.2
35	Benzene, (1-butylhexyl)-	218	$C_{16}H_{26}$	004537-11-5	5.78	0.03	1.6	14.5
36	Benzene, (1-propylheptyl)	218	$C_{16}H_{26}$	004537-12-6	3.45	0.02	1.0	8.6
37	Benzene, (1-methyltridecyl)-	274	$C_{20}H_{34}$	004534-59-2	3.93	0.01	0.9	9.8
38	Naphthalene, 2,7-dimethyl-	156	$C_{12}H_{12}$	000582-16-1	5.95	0.04	2.4	14.9
39	Benzene, (1-butylhexyl)-	218	$C_{16}H_{26}$	004537-11-5	17.56	0.08	5.0	43.9
40	Benzene, (1-propylheptadecyl)-	358	$C_{26}H_{46}$	002400-03-5	6.48	0.02	1.1	16.2
41	Hexadecane	226	$C_{16}H_{34}$	000544-76-3	9.58	0.04	2.6	24.0
42	Benzene, (1-methylnonyl)-	218	$C_{16}H_{26}$	004537-13-7	3.03	0.01	0.9	7.6
43	Propanoic acid, 2-methyl-, 1-(1,1-d	286	$C_{16}H_{30}O_4$		24.39	0.09	5.3	61.0
44	Benzene, (1-propyloctyl)-	232	$C_{17}H_{28}$	004536-86-1	3.99	0.02	1.1	10.0
45	Benzene, (1-ethylnonyl)-	232	$C_{17}H_{28}$	004536-87-2	5.5	0.02	1.5	13.8
46	2-Hexene, 2,5,5-trimethyl-	126	C_9H_{18}	040467-04-7	30.29	0.24	14.8	75.7
47	Benzene, (1-pentylheptyl)-	246	$C_{18}H_{30}$	002719-62-2	3.82	0.02	1.0	9.6
48	Benzene, (1-butyloctyl)-	246	$C_{18}H_{30}$	002719-64-4	2.99	0.01	0.8	7.5
49	Benzene, (1-propylnonyl)-	246	$C_{18}H_{30}$	002719-64-4	3.8	0.02	1.0	9.5
50	Benzene, (1-ethyldecyl)-	246	$C_{18}H_{30}$	002400-00-2	3.38	0.01	0.8	8.5
	Terpenoids				279.9	2.0	126.2	699.7
	Total				806.9	5.3	326.9	2017.3

** This was a short sample taken over a period of 20 minutes only. The mass of compounds collected was low and, as a result, all other samples were taken over longer periods lasting 1.5 to 3 hours. This data point was adjusted with the average mass of compounds detected on sample blanks for the manuscript submitted to Building and Environment. Unadjusted values are given here to maintain consistency with the rest of the tables in this section.*

High Outdoor Ozone -- Low indoor Ozone, High indoor VOC: Day 3								
9/25	Compound	Mol. Weight	Mol. Formula	CAS #	Mass [ng]	nanomoles	ppb	µg/m^3
1	Acetic acid	60	$C_2H_4O_2$	000064-19-7	130.12	2.17	14.1	34.2
2	Pentanal	86	$C_5H_{10}O$	000110-62-3	46.83	0.54	3.5	12.3
3	1-Pentanol	88	$C_5H_{12}O$	000071-41-0	42.61	0.48	3.1	11.2
4	Hexanal	100	$C_6H_{12}O$	000066-25-1	186.45	1.86	12.1	49.1
5	Furfural	96	$C_5H_4O_2$	000098-01-1	89.67	0.93	6.1	23.6
6	Heptanal	114	$C_7H_{14}O$	000111-71-7	49.53	0.43	2.8	13.0
7	1S-.alpha.-Pinene	136	$C_{10}H_{16}$	007785-26-4	200.54	1.47	9.6	52.8
8	Camphene	136	$C_{10}H_{16}$	000079-92-5	53.61	0.39	2.6	14.1
9	Bicyclo[3.1.1]heptane, 6,6-dimethyl	136	$C_{10}H_{16}$	018172-67-3	92	0.68	4.4	24.2
10	Benzaldehyde	106	C_7H_6O	000100-52-7	76.18	0.72	4.7	20.0
11	7-Oxabicyclo[2.2.1]heptane, 1-methy	154	$C_{10}H_{18}O$	000470-67-7	44.34	0.29	1.9	11.7
12	Octanal	128	$C_8H_{16}O$	000124-13-0	65.88	0.51	3.3	17.3
13	d-limonene	136	$C_{10}H_{16}$	005989-27-5	240.39	1.77	11.5	63.3
14	Eucalyptol	154	$C_{10}H_{18}O$	000470-82-6	79.59	0.52	3.4	20.9
15	Phenol	94	C_6H_6O	000108-95-2	24.76	0.26	1.7	6.5
16	(+)-4-Carene	136	$C_{10}H_{16}$	029050-33-7	37.14	0.27	1.8	9.8
17	7-Octen-2-ol, 2,6-dimethyl-	156	$C_{10}H_{20}O$	018479-58-8	295.78	1.90	12.3	77.8
18	3-Cyclohexen-1-carboxaldehyde, 3,4-	138	$C_9H_{14}O$	1000131-99-4	33.3	0.24	1.6	8.8
19	Nonanal	142	$C_9H_{18}O$	000124-19-6	115.49	0.81	5.3	30.4
20	Bicyclo[2.2.1]heptan-2-ol, 1,3,3-tr	154	$C_{10}H_{18}O$	001632-73-1	104.41	0.68	4.4	27.5
21	Camphor	152	$C_{10}H_{16}O$	000076-22-2	66.12	0.44	2.8	17.4
22	Borneol	154	$C_{10}H_{18}O$	010385-78-1	222.11	1.44	9.4	58.5
23	Bicyclo[2.2.1]heptan-2-ol, 1,7,7-tr	154	$C_{10}H_{18}O$	000464-45-9	130.8	0.85	5.5	34.4
24	(+)-.alpha.-Terpineol (p-menth-1-en	154	$C_{10}H_{18}O$	1000157-89-9	88.04	0.57	3.7	23.2
25	Cyclohexanol, 4-(1,1-dimethylethyl)	156	$C_{10}H_{20}O$	000098-52-2	32.92	0.21	1.4	8.7
26	Cyclooctane	112	C_8H_{16}	000292-64-8	54.98	0.49	3.2	14.5
27	Bicyclo[2.2.1]heptan-2-ol, 1,7,7-tr	196	$C_{12}H_{20}O_2$	005655-61-8	533.54	2.72	17.7	140.4
28	Tetradecane	198	$C_{14}H_{30}$	000629-59-4	625.23	3.16	20.5	164.5
29	Cyclohexanemethanol, 4-hydroxy-.alp	172	$C_{10}H_{20}O_2$	000080-53-5	47.48	0.28	1.8	12.5
30	Benzene, (1-methylheptyl)-	190	$C_{14}H_{22}$	000777-22-0	36.44	0.19	1.2	9.6
31	Benzene, (1-butylpentyl)-	204	$C_{15}H_{24}$	020216-88-0	28.52	0.14	0.9	7.5
32	4-Phenylnonane	204	$C_{15}H_{24}$	065185-83-3	44.28	0.22	1.4	11.7
33	Pentadecane	212	$C_{15}H_{32}$	000629-62-9	291.17	1.37	8.9	76.6
34	Benzene, (1-butylhexyl)-	218	$C_{16}H_{26}$	004537-11-5	57.05	0.26	1.7	15.0
35	Benzene, (1-butylhexyl)-	218	$C_{16}H_{26}$	004537-11-5	74.04	0.34	2.2	19.5
36	Benzene, (1-propylheptadecyl)-	358	$C_{26}H_{46}$	002400-03-5	32.43	0.09	0.6	8.5
37	Benzene, (1-methylnonyl)-	218	$C_{16}H_{26}$	004537-13-7	46.99	0.22	1.4	12.4
38	Naphthalene, 1,3-dimethyl-	156	$C_{12}H_{12}$	000575-41-7	50.48	0.32	2.1	13.3
39	Benzene, (1-butylhexyl)-	218	$C_{16}H_{26}$	004537-11-5	156.9	0.72	4.7	41.3
40	Benzene,(1-propylheptyl)-	218	$C_{16}H_{26}$	004537-12-6	68.41	0.31	2.0	18.0
41	Hexadecane	226	$C_{16}H_{34}$	000544-76-3	77.85	0.34	2.2	20.5
42	Benzene, (1-methylnonyl)-	218	$C_{16}H_{26}$	004537-13-7	27.43	0.13	0.8	7.2
43	2,2,4-Trimethyl-1,3-pentanediol dii	286	$C_{16}H_{30}O_4$	006846-50-0	258.76	0.90	5.9	68.1
44	Benzene, (1-propyloctyl)-	232	$C_{17}H_{28}$	004536-86-1	32.85	0.14	0.9	8.6
45	Benzene, (1-ethylnonyl)-	232	$C_{17}H_{28}$	004536-87-2	47.53	0.20	1.3	12.5
46	Nonanoic acid, isopentyl ester	228	$C_{14}H_{28}O_2$		96.03	0.42	2.7	25.3
47	Benzene, (1-pentylheptyl)-	246	$C_{18}H_{30}$	002719-62-2	37.02	0.15	1.0	9.7
48	Benzene, (1-butyloctyl)-	246	$C_{18}H_{30}$	002719-63-3	34.86	0.14	0.9	9.2
49	Benzene, (1-propylnonyl)-	246	$C_{18}H_{30}$	002719-64-4	27.87	0.11	0.7	7.3
50	Benzene, (1-ethyldecyl)-	246	$C_{18}H_{30}$	002400-00-2	25.33	0.10	0.7	6.7
	Terpenoids				987.5	6.9	44.7	259.9
	Total				5362.1	33.9	220.6	1411.1

High Outdoor Ozone -- High indoor Ozone, Low indoor VOC: Day 1								
8/28	Compound	Mol. Weight	Mol. Formula	CAS #	Mass [ng]	nanomoles	ppb	µg/m^3
1	Acetic acid	60	$C_2H_4O_2$	000064-19-7	87.53	1.46	8.8	21.3
2	Pentanal	86	$C_5H_{10}O$	000110-62-3	47.34	0.55	3.3	11.5
3	Cyclotrisiloxane, hexamethyl-	222	C6H18O3Si3	000541-05-9	13.58	0.06	0.4	3.3
4	Hexanal	100	$C_6H_{12}O$	000066-25-1	151.43	1.51	9.1	36.9
5	Furfural	96	$C_5H_4O_2$	000098-01-1	112.8	1.18	7.1	27.5
6	2-Heptanone	114	$C_7H_{14}O$	000110-43-0	15.01	0.13	0.8	3.7
7	2-Butanone	72	C_4H_8O	000078-93-3	17.57	0.24	1.5	4.3
8	Heptanal	114	$C_7H_{14}O$	000111-71-7	57.15	0.50	3.0	13.9
9	1S-.alpha.-Pinene	136	$C_{10}H_{16}$	007785-26-4	47.98	0.35	2.1	11.7
10	.beta.-Pinene	136	$C_{10}H_{16}$	000127-91-3	39.11	0.29	1.7	9.5
11	Benzaldehyde	106	C_7H_6O	000100-52-7	55.46	0.52	3.2	13.5
12	Octanal	128	$C_8H_{16}O$	000124-13-0	90.03	0.70	4.2	22.0
13	Benzene, 1-methyl-4-(1-methylethyl)	134	$C_{10}H_{14}$	000099-87-6	13.3	0.10	0.6	3.2
14	Eucalyptol	154	$C_{10}H_{18}O$	000470-82-6	16.57	0.11	0.6	4.0
15	Phenol	94	C_6H_6O	000108-95-2	23.87	0.25	1.5	5.8
16	7-Octen-2-ol, 2,6-dimethyl-	156	$C_{10}H_{20}O$	018479-58-8	14.36	0.09	0.6	3.5
17	Benzaldehyde, 4-hydroxy-	122	$C_7H_6O_2$	000123-08-0	21.39	0.18	1.1	5.2
18	2-Nonanone	142	$C_9H_{18}O$	000821-55-6	10.62	0.07	0.5	2.6
19	Acetophenone	120	C_8H_8O	000098-86-2	10.97	0.09	0.6	2.7
20	Nonanal	142	$C_9H_{18}O$	000124-19-6	174.17	1.23	7.4	42.5
21	Cyclooctane	112	C_8H_{16}	000292-64-8	9.09	0.08	0.5	2.2
22	Acetic acid, octyl ester	172	$C_{10}H_{20}O_2$	000112-14-1	13.98	0.08	0.5	3.4
23	Decanal	156	$C_{10}H_{20}O$	000112-31-2	53.97	0.35	2.1	13.2
24	Bicyclo[2.2.1]heptan-2-ol, 1,7,7-tr	196	$C_{12}H_{20}O_2$	005655-61-8	50.85	0.26	1.6	12.4
25	Cyclooctane, methoxy-	142	$C_9H_{18}O$	013213-32-6	11.85	0.08	0.5	2.9
26	2-Cyclohexen-1-one, 3,5-dimethyl-,	153	$C_9H_{15}NO$	056336-06-2	14.33	0.09	0.6	3.5
27	Tetradecane	198	$C_{14}H_{30}$	000629-59-4	583.11	2.95	17.7	142.2
28	Benzene, (1-methylheptyl)-	190	$C_{14}H_{22}$	000777-22-0	30.26	0.16	1.0	7.4
29	Propanoic acid, 2-methyl-, 2,2-dime	216	$C_{12}H_{24}O_3$	074367-33-2	47.78	0.22	1.3	11.7
30	Benzene, (1-butylpentyl)-	204	$C_{15}H_{24}$	020216-88-0	37.62	0.18	1.1	9.2
31	4-Phenylnonane	204	$C_{15}H_{24}$	065185-83-3	42.51	0.21	1.3	10.4
32	Pentadecane	212	$C_{15}H_{32}$	000629-62-9	262.79	1.24	7.5	64.1
33	Benzene, (1-butylhexyl)-	218	$C_{16}H_{26}$	004537-11-5	29.09	0.13	0.8	7.1
34	Benzene, (1-butylhexyl)-	218	$C_{16}H_{26}$	004537-11-5	47.54	0.22	1.3	11.6
35	Benzene, (1-methylnonyl)-	218	$C_{16}H_{26}$	004537-13-7	47.94	0.22	1.3	11.7
36	Naphthalene, 1,6-dimethyl-	156	$C_{12}H_{12}$	000575-43-9	40.85	0.26	1.6	10.0
37	Benzene, (1-butylhexyl)-	218	$C_{16}H_{26}$	004537-11-5	146.53	0.67	4.0	35.7
38	Benzene, (1-propylheptyl)-	218	$C_{16}H_{26}$	004537-12-6	65.25	0.30	1.8	15.9
39	Hexadecane	226	$C_{16}H_{34}$	000544-76-3	72.11	0.32	1.9	17.6
40	Benzene, (1-methylnonyl)-	218	$C_{16}H_{26}$	004537-13-7	29.68	0.14	0.8	7.2
41	Butyric acid, thio-, S-decyl ester	244	$C_{14}H_{28}OS$	002432-55-5	226.13	0.93	5.6	55.2
42	Benzene, (1-propyloctyl)-	232	$C_{17}H_{28}$	004536-86-1	31.94	0.14	0.8	7.8
43	Benzene, (1-ethylnonyl)-	232	$C_{17}H_{28}$	004536-87-2	47.14	0.20	1.2	11.5
44	Benzene, (1,1-dimethyldecyl)-	246	$C_{18}H_{30}$		12.02	0.05	0.3	2.9
45	Benzene, (1-methyldecyl)-	232	$C_{17}H_{28}$	004536-88-3	94.84	0.41	2.5	23.1
46	Benzene, (1-pentylheptyl)-	246	$C_{18}H_{30}$	002719-62-2	35.21	0.14	0.9	8.6
47	Benzene, (1-butyloctyl)-	246	$C_{18}H_{30}$	002719-63-3	38.05	0.15	0.9	9.3
48	Benzene, (1-propylnonyl)-	246	$C_{18}H_{30}$	002719-64-4	24.96	0.10	0.6	6.1
49	Benzene, (1-ethyldecyl)-	246	$C_{18}H_{30}$	002400-00-2	25.65	0.10	0.6	6.3
50	Benzene, (1-methylundecyl)-	246	$C_{18}H_{30}$	002719-61-1	19.26	0.08	0.5	4.7
	Terpenoids				103.7	0.7	4.5	25.3
	Total				3212.6	20.1	121.1	783.6

High Outdoor Ozone -- High indoor Ozone, Low indoor VOC: Day 2								
9/6	Compound	Mol. Weight	Mol. Formula	CAS #	Mass [ng]	nanomoles	ppb	µg/m^3
1	Acetic acid	60	$C_2H_4O_2$	000064-19-7	98.82	1.65	10.6	25.9
2	1-Butanol	74	$C_4H_{10}O$	000071-36-3	16.97	0.23	1.5	4.4
3	Pentanal	86	$C_5H_{10}O$	000110-62-3	37.7	0.44	2.8	9.9
4	1-Pentene	70	C_5H_{10}	000109-67-1	41.59	0.59	3.8	10.9
5	Hexanal	100	$C_6H_{12}O$	000066-25-1	103.92	1.04	6.7	27.2
6	Furfural	96	$C_5H_4O_2$	000098-01-1	126.47	1.32	8.5	33.1
7	2-Heptanone	114	$C_7H_{14}O$	000110-43-0	17.66	0.15	1.0	4.6
8	2-Butanone	72	C_4H_8O	000078-93-3	11.85	0.16	1.1	3.1
9	Heptanal	114	$C_7H_{14}O$	000111-71-7	40.48	0.36	2.3	10.6
10	1S-.alpha.-Pinene	136	$C_{10}H_{16}$	007785-26-4	78.79	0.58	3.7	20.6
11	.beta.Pinene	136	$C_{10}H_{16}$	000127-91-3	51.72	0.38	2.5	13.5
12	Heptanol	116	$C_7H_{16}O$	053535-33-4	16.96	0.15	0.9	4.4
13	Benzaldehyde	106	C_7H_6O	000100-52-7	66.52	0.63	4.1	17.4
14	Octanal	128	$C_8H_{16}O$	000124-13-0	134.25	1.05	6.8	35.1
15	d-limonene	136	$C_{10}H_{16}$	000138-86-3	11.55	0.08	0.5	3.0
16	Benzene, 1-methyl-4-(1-methylethyl)	134	$C_{10}H_{14}$	000099-87-6	15.51	0.12	0.7	4.1
17	.alpha.-Phellandrene	136	$C_{10}H_{16}$	000099-83-2	13.44	0.10	0.6	3.5
18	1-Hexanol, 2-ethyl-	130	$C_8H_{18}O$	000104-76-7	35.58	0.27	1.8	9.3
19	Phenol	94	C_6H_6O	000108-95-2	28.88	0.31	2.0	7.6
20	Formic acid, octyl ester	158	$C_9H_{18}O_2$	000112-32-3	64.17	0.41	2.6	16.8
21	2-Nonanone	142	$C_9H_{18}O$	000821-55-6	13.54	0.10	0.6	3.5
22	Acetophenone	120	C_8H_8O	000098-86-2	17.61	0.15	0.9	4.6
23	Nonanal	142	$C_9H_{18}O$	000124-19-6	217.83	1.53	9.9	57.0
24	1-Tetradecene	196	$C_{14}H_{28}$	001120-36-1	28.99	0.15	1.0	7.6
25	Bicyclo[2.2.1]heptane, 2-methoxy-1	168	$C_{11}H_{20}O$	004443-51-0	50.52	0.30	1.9	13.2
26	Decanal	156	$C_{10}H_{20}O$	000112-31-2	98.48	0.63	4.1	25.8
27	Tridecane	184	$C_{13}H_{28}$	000629-50-5	12.79	0.07	0.4	3.3
28	3-Tetradecene, (E)-	196	$C_{14}H_{28}$	041446-68-8	13.27	0.07	0.4	3.5
29	Acetic acid, 1,7,7-trimethyl-bicycl	196	$C_{12}H_{20}O_2$	092618-89-8	41.13	0.21	1.4	10.8
30	Tridecane, 6-methyl-	198	$C_{14}H_{30}$	013287-21-3	30.58	0.15	1.0	8.0
31	Tetradecane	198	$C_{14}H_{30}$	000629-59-4	576.39	2.91	18.8	150.9
32	Benzene, (1-methylheptyl)-	190	$C_{14}H_{22}$	000777-22-0	118.95	0.63	4.0	31.1
33	Butanoic acid, butyl ester	144	$C_8H_{16}O_2$	000109-21-7	67.94	0.47	3.1	17.8
34	Benzene, (1-butylpentyl)-	204	$C_{15}H_{24}$	020216-88-0	25.04	0.12	0.8	6.6
35	4-Phenylnonane	204	$C_{15}H_{24}$	065185-83-3	28.3	0.14	0.9	7.4
36	Bacchotricuneatin c	342	$C_{20}H_{22}O_5$	066563-30-2	414.13	1.21	7.8	108.4
37	Benzene, (1-ethyloctyl)-	218	$C_{16}H_{26}$	004621-36-7	44.52	0.20	1.3	11.7
38	Benzene, (1,3,3-trimethylnonyl)-	246	$C_{18}H_{30}$	054986-44-6	268.93	1.09	7.1	70.4
39	Benzene, (1-butylhexyl)-	218	$C_{16}H_{26}$	004537-11-5	105.59	0.48	3.1	27.6
40	Benzene, (1-propylheptyl)-	218	$C_{16}H_{26}$	004537-12-6	129.77	0.60	3.8	34.0
41	Decane, 5-propyl-	184	$C_{13}H_{28}$	017312-62-8	64.21	0.35	2.3	16.8
42	Eicosane	282	$C_{20}H_{42}$	000112-95-8	645.86	2.29	14.8	169.1
43	Pentanoic acid, 2,2,4-trimethyl-3-c	286	$C_{16}H_{30}O_4$		183.24	0.64	4.1	48.0
44	Benzene, (1-propyloctyl)-	232	$C_{17}H_{28}$	004536-86-1	25.55	0.11	0.7	6.7
45	Heneicosane	296	$C_{21}H_{44}$	000629-94-7	670.97	2.27	14.7	175.6
46	2-Hexene, 3,5,5-trimethyl-	126	C_9H_{18}	026456-76-8	54.07	0.43	2.8	14.2
47	Benzene, (1-pentylheptyl)-	246	$C_{18}H_{30}$	002719-62-2	17.14	0.07	0.5	4.5
48	Benzene, (1-propylnonyl)-	246	$C_{18}H_{30}$	002719-64-4	29.71	0.12	0.8	7.8
49	Benzene, (1-ethyldecyl)-	246	$C_{18}H_{30}$	002400-00-2	22.72	0.09	0.6	5.9
	Terpenoids				155.5	1.1	7.4	40.7
	Total				5030.6	27.6	178.4	1316.9

High Outdoor Ozone -- High indoor Ozone, High indoor VOC: Day 1								
9/8	Compound	Mol. Weight	Mol. Formula	CAS #	Mass [ng]	nanomoles	ppb	µg/m^3
1	Acetic acid	60	$C_2H_4O_2$	000064-19-7	89.5	1.49	17.2	41.8
2	1-Butanol	74	$C_4H_{10}O$	000071-36-3	14.94	0.20	2.3	7.0
3	Pentanal	86	$C_5H_{10}O$	000110-62-3	27.83	0.32	3.7	13.0
4	1-Pentene	70	C_5H_{10}	000109-67-1	27.81	0.40	4.6	13.0
5	Hexanal	100	$C_6H_{12}O$	000066-25-1	91.6	0.92	10.6	42.8
6	Furfural	96	$C_5H_4O_2$	000098-01-1	66.4	0.69	8.0	31.0
7	2-Heptanone	114	$C_7H_{14}O$	000110-43-0	10.61	0.09	1.1	5.0
8	2-Butanone	72	C_4H_8O	000078-93-3	14.7	0.20	2.4	6.9
9	Heptanal	114	$C_7H_{14}O$	000111-71-7	35.91	0.32	3.6	16.8
10	1S-.alpha.-Pinene	136	$C_{10}H_{16}$	007785-26-4	60.81	0.45	5.2	28.4
11	Camphene	136	$C_{10}H_{16}$	000079-92-5	38.94	0.29	3.3	18.2
12	Bicyclo[3.1.1]heptane, 6,6-dimethyl	136	$C_{10}H_{16}$	018172-67-3	35.5	0.26	3.0	16.6
13	Benzaldehyde	106	C_7H_6O	000100-52-7	43.09	0.41	4.7	20.1
14	Octanal	128	$C_8H_{16}O$	000124-13-0	87.18	0.68	7.9	40.7
15	d-limonene	136	$C_{10}H_{16}$	000138-86-3	14.22	0.10	1.2	6.6
16	Benzene, 1-methyl-4-(1-methylethyl)	134	$C_{10}H_{14}$	000099-87-6	19.52	0.15	1.7	9.1
17	Eucalyptol	154	$C_{10}H_{18}O$	000470-82-6	37.65	0.24	2.8	17.6
18	1-Hexanol, 2-ethyl-	130	$C_8H_{18}O$	000104-76-7	16.22	0.12	1.4	7.6
19	Phenol	94	C_6H_6O	000108-95-2	15.56	0.17	1.9	7.3
20	7-Octen-2-ol, 2,6-dimethyl	156	$C_{10}H_{20}O$	018479-58-8	106.44	0.68	7.9	49.7
21	Nonanal	142	$C_9H_{18}O$	000124-19-6	147.95	1.04	12.0	69.1
22	Pentanedioic acid, dimethyl ester	160	$C_7H_{12}O_4$	001119-40-0	10.77	0.07	0.8	5.0
23	Bicyclo[2.2.1]heptan-2-ol, 1,3,3-tr	154	$C_{10}H_{18}O$	001632-73-1	43.44	0.28	3.3	20.3
24	Camphor	152	$C_{10}H_{16}O$	000076-22-2	35.51	0.23	2.7	16.6
25	Isoborneol	154	$C_{10}H_{18}O$	000124-76-5	92.41	0.60	6.9	43.2
26	Cyclooctane	112	C_8H_{16}	000292-64-8	23.73	0.21	2.4	11.1
27	Bicyclo[2.2.1]heptan-2-ol, 1,7,7-tr	196	$C_{12}H_{20}O_2$	005655-61-8	221.49	1.13	13.0	103.5
28	Cyclohexanol, 4-(1,1-dimethylethyl)	156	$C_{10}H_{20}O$	000937-05-3	13.67	0.09	1.0	6.4
29	Tetradecane	198	$C_{14}H_{30}$	000629-59-4	318.82	1.61	18.6	149.0
30	Terpin Hydrate	172	$C_{10}H_{20}O_2$	002451-01-6	21.33	0.12	1.4	10.0
31	Benzene, (1-methylheptyl)-	190	$C_{14}H_{22}$	000777-22-0	19.51	0.10	1.2	9.1
32	Propanoic acid, 2-methyl-, 2,2-dime	216	$C_{12}H_{24}O_3$	074367-33-2	73.02	0.34	3.9	34.1
33	Benzene, (1-butylpentyl)-	204	$C_{15}H_{24}$	020216-88-0	16.88	0.08	1.0	7.9
34	4-Phenylnonane	204	$C_{15}H_{24}$	065185-83-3	22.93	0.11	1.3	10.7
35	Pentadecane	212	$C_{15}H_{32}$	000629-62-9	146.6	0.69	8.0	68.5
36	Benzene, (1-butylhexyl)-	218	$C_{16}H_{26}$	004537-11-5	12.96	0.06	0.7	6.1
37	Benzene, (1-butylhexyl)-	218	$C_{16}H_{26}$	004537-11-5	26.54	0.12	1.4	12.4
38	Benzene, (1-methylnonyl)-	218	$C_{16}H_{26}$	004537-13-7	16.04	0.07	0.8	7.5
39	Naphthalene, 1,4-dimethyl-	156	$C_{12}H_{12}$	000571-58-4	22.31	0.14	1.7	10.4
40	Benzene, (1-butylhexyl)-	218	$C_{16}H_{26}$	004537-11-5	84.27	0.39	4.5	39.4
41	Benzene, (1-propylheptyl)-	218	$C_{16}H_{26}$	004537-12-6	35.46	0.16	1.9	16.6
42	Hexadecane	226	$C_{16}H_{34}$	000544-76-3	40.72	0.18	2.1	19.0
43	Benzene, (1-methylnonyl)-	218	$C_{16}H_{26}$	004537-13-7	16.4	0.08	0.9	7.7
44	Propanoic acid, 2-methyl-, 1-(1,1-d	286	$C_{16}H_{30}O_4$		130.03	0.45	5.2	60.8
45	Benzene, (1-propyloctyl)-	232	$C_{17}H_{28}$	004536-86-1	17.78	0.08	0.9	8.3
46	Benzene, (1-ethylnonyl)-	232	$C_{17}H_{28}$	004536-87-2	28.68	0.12	1.4	13.4
47	Hexane, 3-ethyl-4-methyl-	128	C_9H_{20}	003074-77-9	115.45	0.90	10.4	53.9
48	Benzene, (1-pentylheptyl)-	246	$C_{18}H_{30}$	002719-62-2	20.08	0.08	0.9	9.4
49	Benzene, (1-propylnonyl)-	246	$C_{18}H_{30}$	002719-64-4	27.32	0.11	1.3	12.8
50	Benzene, (1-ethyldecyl)-	246	$C_{18}H_{30}$	002400-00-2	15.32	0.06	0.7	7.2
	Terpenoids				279.5	1.9	22.1	130.6
	Total				2671.9	17.9	206.8	1248.5

High Outdoor Ozone -- High indoor Ozone, High indoor VOC: Day 2								
9/9	Compound	Mol. Weight	Mol. Formula	CAS #	Mass [ng]	nanomoles	ppb	µg/m^3
1	Acetic acid	60	$C_2H_4O_2$	000064-19-7	143.03	2.38	22.6	55.0
2	1-Butanol	74	$C_4H_{10}O$	000071-36-3	17.69	0.24	2.3	6.8
3	Pentanal	86	$C_5H_{10}O$	000110-62-3	36.82	0.43	4.1	14.2
4	1-Pentanol	88	$C_5H_{12}O$	000071-41-0	35.63	0.40	3.8	13.7
5	Hexanal	100	$C_6H_{12}O$	000066-25-1	111.28	1.11	10.6	42.8
6	Furfural	96	$C_5H_4O_2$	000098-01-1	72.71	0.76	7.2	28.0
7	2-Butanone	72	C_4H_8O	000078-93-3	32.81	0.46	4.3	12.6
8	Heptanal	114	$C_7H_{14}O$	000111-71-7	46.56	0.41	3.9	17.9
9	1S-.alpha.-Pinene	136	$C_{10}H_{16}$	007785-26-4	61.79	0.45	4.3	23.8
10	Camphene	136	$C_{10}H_{16}$	000079-92-5	50.14	0.37	3.5	19.3
11	.beta.-Pinene	136	$C_{10}H_{16}$	000127-91-3	39.26	0.29	2.7	15.1
12	1-Heptanol	116	$C_7H_{16}O$	000111-70-6	16.84	0.15	1.4	6.5
13	Benzaldehyde	106	C_7H_6O	000100-52-7	56.23	0.53	5.0	21.6
14	Octanal	128	$C_8H_{16}O$	000124-13-0	124.4	0.97	9.2	47.8
15	Benzene, 1-methyl-3-(1-methylethyl)	134	$C_{10}H_{14}$	000535-77-3	37.2	0.28	2.6	14.3
16	Eucalyptol	154	$C_{10}H_{18}O$	000470-82-6	50.55	0.33	3.1	19.4
17	1-Hexanol, 2-ethyl-	130	$C_8H_{18}O$	000104-76-7	18.82	0.14	1.4	7.2
18	Phenol	94	C_6H_6O	000108-95-2	21.9	0.23	2.2	8.4
19	7-Octen-2-ol, 2,6-dimethyl	156	$C_{10}H_{20}O$	018479-58-8	123.02	0.79	7.5	47.3
20	Nonanal	142	$C_9H_{18}O$	000124-19-6	214.1	1.51	14.3	82.3
21	Bicyclo[2.2.1]heptan-2-ol, 1,3,3-tr	154	$C_{10}H_{18}O$	001632-73-1	60.24	0.39	3.7	23.2
22	Camphor	152	$C_{10}H_{16}O$	000076-22-2	46.56	0.31	2.9	17.9
23	Borneol	154	$C_{10}H_{18}O$	010385-78-1	128.09	0.83	7.9	49.3
24	Cyclohexanol, 4-(1,1-dimethylethyl)	156	$C_{10}H_{20}O$	000098-52-2	16.85	0.11	1.0	6.5
25	Cyclodecane	140	$C_{10}H_{20}$	000293-96-9	34.97	0.25	2.4	13.5
26	Bicyclo[3.3.1]nonane	124	C_9H_{16}	000280-65-9	18.08	0.15	1.4	7.0
27	Bicyclo[2.2.1]heptan-2-ol, 1,7,7-tr	196	$C_{12}H_{20}O_2$	005655-61-8	304.75	1.55	14.8	117.2
28	Cyclohexanol, 4-(1,1-dimethylethyl)	156	$C_{10}H_{20}O$	000937-05-3	17.09	0.11	1.0	6.6
29	Tetradecane	198	$C_{14}H_{30}$	000629-59-4	431.35	2.18	20.7	165.9
30	Cyclohexanemethanol, 4-hydroxy-.alp	172	$C_{10}H_{20}O_2$	000080-53-5	39.8	0.23	2.2	15.3
31	Benzene, (1-methylheptyl)-	190	$C_{14}H_{22}$	000777-22-0	28.76	0.15	1.4	11.1
32	Propanoic acid, 2-methyl-, 2-methyl	144	$C_8H_{16}O_2$	000097-85-8	93.56	0.65	6.2	36.0
33	Benzene, (1-butylpentyl)-	204	$C_{15}H_{24}$	020216-88-0	24.23	0.12	1.1	9.3
34	4-Phenylnonane	204	$C_{15}H_{24}$	065185-83-3	30.16	0.15	1.4	11.6
35	Pentadecane	212	$C_{15}H_{32}$	000629-62-9	195.25	0.92	8.7	75.1
36	Benzene, (1-butylhexyl)-	218	$C_{16}H_{26}$	004537-11-5	18.07	0.08	0.8	7.0
37	Benzene, (1-butylhexyl)-	218	$C_{16}H_{26}$	004537-11-5	37.81	0.17	1.6	14.5
38	Bicyclo[4.2.0]oct-5-ene-2,3-dicarbo	206	$C_{12}H_{14}O_3$		21.69	0.11	1.0	8.3
39	Naphthalene, 2,3-dimethyl-	156	$C_{12}H_{12}$	000581-40-8	36.23	0.23	2.2	13.9
40	Benzene, (1-butylhexyl)-	218	$C_{16}H_{26}$	004537-11-5	116.04	0.53	5.1	44.6
41	Benzene,(1-propylheptyl)-	218	$C_{16}H_{26}$	004537-12-6	45.87	0.21	2.0	17.6
42	Hexadecane	226	$C_{16}H_{34}$	000544-76-3	50.88	0.23	2.1	19.6
43	Benzene, (1-methylnonyl)-	218	$C_{16}H_{26}$	004537-13-7	22.03	0.10	1.0	8.5
44	Pentanoic acid, 2,2,4-trimethyl-3-c	286	$C_{16}H_{30}O_4$		195.77	0.68	6.5	75.3
45	Benzene, (1-propyloctyl)-	232	$C_{17}H_{28}$	004536-86-1	21.88	0.09	0.9	8.4
46	Benzene, (1-ethylnonyl)-	232	$C_{17}H_{28}$	004536-87-2	38.1	0.16	1.6	14.7
47	Hexane, 3-ethyl-4-methyl-	128	C_9H_{20}	003074-77-9	246.81	1.93	18.3	94.9
48	Benzene, (1-pentylheptyl)-	246	$C_{18}H_{30}$	002719-62-2	31.2	0.13	1.2	12.0
49	Benzene, (1-propylnonyl)-	246	$C_{18}H_{30}$	002719-64-4	42.35	0.17	1.6	16.3
50	Benzene, (1-ethyldecyl)-	246	$C_{18}H_{30}$	002400-00-2	20.62	0.08	0.8	7.9
	Terpenoids				376.4	2.6	24.5	144.8
	Total				3725.9	25.2	239.8	1433.0

High Outdoor Ozone -- High indoor Ozone, High indoor VOC: Day 3								
9/23	Compound	Mol. Weight	Mol. Formula	CAS #	Mass [ng]	nanomoles	ppb	µg/m^3
1	Acetic acid	60	$C_2H_4O_2$	000064-19-7	147.22	2.45	26.3	64.0
2	1-Butanol	74	$C_4H_{10}O$	000071-36-3	18.98	0.26	2.8	8.3
3	Pentanal	86	$C_5H_{10}O$	000110-62-3	37.81	0.44	4.7	16.4
4	1-Pentanol	88	$C_5H_{12}O$	000071-41-0	30.58	0.35	3.7	13.3
5	Hexanal	100	$C_6H_{12}O$	000066-25-1	110.82	1.11	11.9	48.2
6	Furfural	96	$C_5H_4O_2$	000098-01-1	60.62	0.63	6.8	26.4
7	2-Butanone	72	C_4H_8O	000078-93-3	18.1	0.25	2.7	7.9
8	Heptanal	114	$C_7H_{14}O$	000111-71-7	44.29	0.39	4.2	19.3
9	1S-.alpha.-Pinene	136	$C_{10}H_{16}$	007785-26-4	77.92	0.57	6.2	33.9
10	Camphene	136	$C_{10}H_{16}$	000079-92-5	70.07	0.52	5.5	30.5
11	Bicyclo[3.1.1]heptane, 6,6-dimethyl	136	$C_{10}H_{16}$	018172-67-3	43.81	0.32	3.5	19.0
12	Benzaldehyde	106	C_7H_6O	000100-52-7	56.37	0.53	5.7	24.5
13	Octanal	128	$C_8H_{16}O$	000124-13-0	91.67	0.72	7.7	39.9
14	Benzene, 1-methyl-4-(1-methylethyl	134	$C_{10}H_{14}$	000099-87-6	32.86	0.25	2.6	14.3
15	Eucalyptol	154	$C_{10}H_{18}O$	000470-82-6	57.1	0.37	4.0	24.8
16	1-Hexanol, 2-ethyl-	130	$C_8H_{18}O$	000104-76-7	17.04	0.13	1.4	7.4
17	Phenol	94	C_6H_6O	000108-95-2	17.85	0.19	2.0	7.8
18	7-Octen-2-ol, 2,6-dimethyl-	156	$C_{10}H_{20}O$	018479-58-8	137.08	0.88	9.4	59.6
19	Nonanal	142	$C_9H_{18}O$	000124-19-6	149.036	1.05	11.3	64.8
20	Bicyclo[2.2.1]heptan-2-ol, 1,3,3-tr	154	$C_{10}H_{18}O$	001632-73-1	66.73	0.43	4.7	29.0
21	Bicyclo[2.2.1]heptan-2-one, 1,7,7-t	152	$C_{10}H_{16}O$	000464-49-3	51.6	0.34	3.6	22.4
22	Isoborneol	154	$C_{10}H_{18}O$	000124-76-5	147.1	0.96	10.3	64.0
23	6-Hepten-1-ol, 2-methyl-	128	$C_8H_{16}O$	1000132-12-0	149.02	1.16	12.5	64.8
24	7-Octen-2-ol, 2,6-dimethyl-	156	$C_{10}H_{20}O$	018479-58-8	15.9	0.10	1.1	6.9
25	Cyclohexanol, 4-(1,1-dimethylethyl)	156	$C_{10}H_{20}O$	000098-52-2	16.84	0.11	1.2	7.3
26	Cyclodecane	140	$C_{10}H_{20}$	000293-96-9	27.88	0.20	2.1	12.1
27	Isobornyl Acetate	196	$C_{12}H_{20}O_2$	000125-12-2	338.73	1.73	18.6	147.3
28	4-tert-Butylcyclohexyl acetate	198	$C_{12}H_{22}O_2$	032210-23-4	17.37	0.09	0.9	7.6
29	Tetradecane	198	$C_{14}H_{30}$	000629-59-4	379.39	1.92	20.6	165.0
30	Cyclohexanemethanol, 4-hydroxy-.alp	172	$C_{10}H_{20}O_2$	000080-53-5	27.77	0.16	1.7	12.1
31	Benzene, (1-methylheptyl)-	190	$C_{14}H_{22}$	000777-22-0	21.83	0.11	1.2	9.5
32	Benzene, (1-butylpentyl)-	204	$C_{15}H_{24}$	020216-88-0	18.04	0.09	0.9	7.8
33	4-Phenylnonane	204	$C_{15}H_{24}$	065185-83-3	25.88	0.13	1.4	11.3
34	Pentadecane	212	$C_{15}H_{32}$	000629-62-9	174.81	0.82	8.9	76.0
35	Benzene, (1-pentylhexyl)-	232	$C_{17}H_{28}$	004537-14-8	15.52	0.07	0.7	6.7
36	Benzene, (1-butylhexyl)-	218	$C_{16}H_{26}$	004537-11-5	30.83	0.14	1.5	13.4
37	Benzene, (1,2-dimethylpropyl)-	148	$C_{11}H_{16}$	004481-30-5	19.08	0.13	1.4	8.3
38	Naphthalene, 1,3-dimethyl-	156	$C_{12}H_{12}$	000575-41-7	29.93	0.19	2.1	13.0
39	Benzene, (1-butylhexyl)-	218	$C_{16}H_{26}$	004537-11-5	94.73	0.43	4.7	41.2
40	Benzene,(1-propylheptyl)-	218	$C_{16}H_{26}$	004537-12-6	29.23	0.13	1.4	12.7
41	Hexadecane	226	$C_{16}H_{34}$	000544-76-3	43.76	0.19	2.1	19.0
42	Benzene, (1-methylnonyl)-	218	$C_{16}H_{26}$	004537-13-7	15.74	0.07	0.8	6.8
43	Butyric acid, thio-, S-decyl ester	244	$C_{14}H_{28}OS$	002432-55-5	154.9	0.63	6.8	67.3
44	Benzene, (1-propyloctyl)-	232	$C_{17}H_{28}$	004536-86-1	19.98	0.09	0.9	8.7
45	Benzene, (1-ethylnonyl)-	232	$C_{17}H_{28}$	004536-87-2	27.02	0.12	1.3	11.7
46	Hexane, 3-ethyl-4-methyl-	128	C_9H_{20}	003074-77-9	80.89	0.63	6.8	35.2
47	Benzene, (1-pentylheptyl)-	246	$C_{18}H_{30}$	002719-62-2	22.46	0.09	1.0	9.8
48	Benzene, (1-butyloctyl)-	246	$C_{18}H_{30}$	002719-63-3	22.04	0.09	1.0	9.6
49	Benzene, (1-propylnonyl)-	246	$C_{18}H_{30}$	002719-64-4	16.2	0.07	0.7	7.0
50	Benzene, (1-ethyldecyl)-	246	$C_{18}H_{30}$	002400-00-2	15	0.06	0.7	6.5
	Terpenoids				352.2	2.4	25.9	153.1
	Total				3335.4	22.9	245.8	1450.2

Sample Blank: Low Outdoor Ozone - Low indoor Ozone, High indoor VOC: Day 2						
9/3	Compound	Mol. Weight	Mol. Formula	CAS #	Mass [ng]	nanomoles
1	1-Pentene, 2-methoxy-	100	$C_6H_{12}O$	053119-70-3	3.98	0.04
2	Acetic acid	60	$C_2H_4O_2$	000064-19-7	28.98	0.48
3	Acetic acid, 1-methylethyl ester	102	$C_5H_{10}O_2$	000108-21-4	2.9	0.03
4	Hexanal	100	$C_6H_{12}O$	000066-25-1	6.26	0.06
5	Benzaldehyde	106	C_7H_6O	000100-52-7	6.11	0.06
6	Octanal	128	$C_8H_{16}O$	000124-13-0	7.46	0.06
7	Nonanal	142	$C_9H_{18}O$	000124-19-6	28.45	0.20
8	Decanal	156	$C_{10}H_{20}O$	000112-31-2	20.64	0.13
9	1,2-15,16-Diepoxyhexadecane	254	$C_{16}H_{30}O_2$	1000192-65-0	3.68	0.01
10	Tetradecane	198	$C_{14}H_{30}$	000629-59-4	2.79	0.01
11	9-Octadecene, 1,1-dimethoxy-, (Z)-	312	$C_{20}H_{40}O_2$	015677-71-1	2.64	0.01
12	1,3,7-Octatriene, 3,7-dimethyl-	136	$C_{10}H_{16}$	000502-99-8	10.74	0.08
13	Benzene, (1-butylhexyl)-	218	$C_{16}H_{26}$	004537-11-5	2.96	0.01
14	1-Decanol, 2-methyl-	172	$C_{11}H_{26}O$	018675-24-6	2.9	0.02
15	1H-Inden-2-amine, N,N-dimethyl-	159	$C_{11}H_{13}N$	035336-08-4	5.1	0.03
	Total				135.6	1.2

Sample Blank: High Outdoor Ozone - Low indoor Ozone, High indoor VOC: Day 2						
8/27	Compound	Mol. Weight	Mol. Formula	CAS #	Mass [ng]	nanomoles
1	Acetic acid, [(phenylmethoxy)imino]	251	$C_{12}H_{17}NO_3Si$	055494-08-1	6.78	0.03
2	Propylene Glycol	76	$C_3H_8O_2$	000057-55-6	6.38	0.08
3	Hexanal	100	$C_6H_{12}O$	000066-25-1	4.57	0.05
4	Benzaldehyde	106	C_7H_6O	000100-52-7	4.75	0.04
5	Octanal	128	$C_8H_{16}O$	000124-13-0	3.28	0.03
6	Acetophenone	120	C_8H_8O	000098-86-2	3.35	0.03
7	Nonanal	142	$C_9H_{18}O$	000124-19-6	6.13	0.04
8	Decanal	156	$C_{10}H_{20}O$	000112-31-2	5.05	0.03
9	Acetic acid, 1,7,7-trimethyl-bicycl	196	$C_{12}H_{20}O_2$	092618-89-8	2.72	0.01
	Total				43.0	0.3

Sample Blank: High Outdoor Ozone - High indoor Ozone, Low indoor VOC: Day 1						
8/28	Compound	Mol. Weight	Mol. Formula	CAS #	Mass [ng]	nanomoles
1	Acetamide, N-(3-methyl-2-oxobutyl)-	143	$C_7H_{13}NO_2$	082479-25-2	3.98	0.03
2	Ethanamide, 2-(methylthio)-	91	C3H9NS	018542-42-2	6.56	0.07
3	Hexanal	100	$C_6H_{12}O$	000066-25-1	2.58	0.03
4	Benzaldehyde	106	C_7H_6O	000100-52-7	3.14	0.03
5	2-Nonen-1-ol	142	$C_9H_{18}O$	022104-79-6	3.66	0.03
6	Decanal	156	$C_{10}H_{20}O$	000112-31-2	3.00	0.02
	Total				22.9	0.2

Sample Blank: High Outdoor Ozone - High indoor Ozone, Low indoor VOC: Day 2						
9/6	Compound	Mol. Weight	Mol. Formula	CAS #	Mass [ng]	nanomoles
1	2-Butanone	72	C_4H_8O	000078-93-3	7.28	0.10
2	Acetic acid	60	$C_2H_4O_2$	000064-19-7	5.27	0.09
3	Pentanal	86	$C_5H_{10}O$	000110-62-3	2.66	0.03
4	Hydrazinecarbothioamide	91	CH_5N_3S	000079-19-6	11.27	0.12
5	Cyclotrisiloxane, hexamethyl-	222	$C_6H_{18}O_3Si_3$	000541-05-9	4.83	0.02
6	Hexanal	100	$C_6H_{12}O$	000066-25-1	7.92	0.08
7	Furfural	96	$C_5H_4O_2$	000098-01-1	2.99	0.03
8	2-Butanone	72	C_4H_8O	000078-93-3	2.74	0.04
9	Hexanal, 5-methyl-	114	$C_7H_{14}O$	001860-39-5	3.33	0.03
10	1S-.alpha.-Pinene	136	$C_{10}H_{16}$	007785-26-4	5.41	0.04
11	Benzaldehyde	106	C_7H_6O	000100-52-7	5.53	0.05
12	Octanal	128	$C_8H_{16}O$	000124-13-0	4.67	0.04
13	Nonanal	142	$C_9H_{18}O$	000124-19-6	13.31	0.09
14	Decanal	156	$C_{10}H_{20}O$	000112-31-2	12.53	0.08
15	Tetradecane	198	$C_{14}H_{30}$	000629-59-4	3.65	0.02
16	Pentatriacontane	493	$C_{35}H_{72}$	000630-07-9	19.54	0.04
17	1H-Inden-2-amine, N,N-dimethyl-	159	$C_{11}H_{13}N$	035336-08-4	8.59	0.05
	Total				121.5	1.0

Sample Blank (on August 25, 2014)*						
8/25	Compound	Mol. Weight	Mol. Formula	CAS #	Mass [ng]	nanomoles
1	Butanoic acid, 3-methyl-2-[(phenylm	293	$C_{15}H_{23}NO_3Si$	055520-96-2	23.95	0.08
2	Cyclotrisiloxane, hexamethyl-	222	$C_6H_{18}O_3Si_3$	000541-05-9	7.30	0.03
3	Hexanal	100	$C_6H_{12}O$	000066-25-1	13.41	0.13
4	Benzaldehyde	106	C_7H_6O	000100-52-7	4.51	0.04
5	Octanal	128	$C_8H_{16}O$	000124-13-0	4.15	0.03
6	1-Octanol, 2-butyl-	186	$C_{12}H_{26}O$	003913-02-8	2.57	0.01
7	Nonanal	142	$C_9H_{18}O$	000124-19-6	13.94	0.10
8	1,2-Cyclohexanedione	112	$C_6H_8O_2$	000765-87-7	13.51	0.12
9	Benzoselenazole, 5-methoxy-2-methyl	227	C9H9NOSe	002946-17-0	8.25	0.04
10	Piperidine, 3-isopropyl-	127	$C_8H_{17}N$	1000197-57-3	4.01	0.03
11	Hexane, 3-ethyl-4-methyl	128	C_9H_{20}	003074-77-9	55.62	0.43
	Total				151.2	1.1
	* Nothing was injected inside the Test House on this day.					

PineSol® Sample								
7/23	Compound	Mol. Weight	Mol. Formula	CAS #	Mass [ng]	Nanomoles	ppb	µg/m^3
1	Tricyclo[2.2.1.02,6]heptane, 1,7,7-	136	$C_{10}H_{16}$	000508-32-7	30.37	0.22	11.0	60.7
2	1S-.alpha.-Pinene	136	$C_{10}H_{16}$	007785-26-4	28.20	0.21	10.2	56.4
3	Camphene	136	$C_{10}H_{16}$	000079-92-5	128.47	0.94	46.7	256.9
4	Decane	142	$C_{10}H_{22}$	000124-18-5	13.90	0.10	4.8	27.8
5	3-Carene	136	$C_{10}H_{16}$	013466-78-9	26.52	0.20	9.6	53.0
6	7-Oxabicyclo[2.2.1]heptane, 1-methy	154	$C_{10}H_{18}O$	000470-67-7	104.31	0.68	33.5	208.6
7	d-limonene	136	$C_{10}H_{16}$	005989-27-5	210.59	1.55	76.5	421.2
8	Eucalyptol	154	$C_{10}H_{18}O$	000470-82-6	174.71	1.13	56.0	349.4
9	1,4-Cyclohexadiene, 1-methyl-4	136	$C_{10}H_{16}$	000099-85-4	20.67	0.15	7.5	41.3
10	Phenol	94	C_6H_6O	000108-95-2	36.80	0.39	19.3	73.6
11	2-Propanol, 1,1'-oxybis-	134	$C_6H_{14}O_3$	000110-98-5	83.25	0.62	30.7	166.5
12	Cyclohexene, 1-methyl-4-(1-methylet	136	$C_{10}H_{16}$	000586-62-9	129.00	0.95	46.9	258.0
13	7-Octen-2-ol, 2,6-dimethyl-	156	$C_{10}H_{20}O$	018479-58-8	722.01	4.63	228.6	1444.0
14	1-Propanol, 2-(2-hydroxypropoxy)-	134	$C_6H_{14}O_3$	000106-62-7	46.68	0.35	17.2	93.4
15	2-Methoxy-5-methylphenol	138	$C_8H_{10}O_2$	001195-09-1	90.38	0.65	32.4	180.8
16	Acetophenone	120	C_8H_8O	000098-86-2	20.37	0.17	8.4	40.7
17	Nonanal	142	$C_9H_{18}O$	000124-19-6	15.12	0.11	5.3	30.2
18	Cyclohexanone, 2-(1-methylethyliden	138	$C_9H_{14}O$	013747-73-4	21.86	0.16	7.8	43.7
19	Bicyclo[2.2.1]heptan-2-ol, 1,3,3-tr	154	$C_{10}H_{18}O$	001632-73-1	432.95	2.81	138.9	865.9
20	Cyclohexanemethanol, .alpha.,.alpha	156	$C_{10}H_{20}O$	000498-81-7	18.46	0.12	5.8	36.9
21	Cyclohexanone, 5-methyl-2-(1-methyl	154	$C_{10}H_{18}O$	010458-14-7	35.13	0.23	11.3	70.3
22	Camphor	152	$C_{10}H_{16}O$	000076-22-2	77.30	0.51	25.1	154.6
23	Isoborneol	154	$C_{10}H_{18}O$	000124-76-5	763.86	4.96	245.0	1527.7
24	Bicyclo[2.2.1]heptan-2-ol, 1,7,7-tr	154	$C_{10}H_{18}O$	000464-45-9	411.43	2.67	132.0	822.9
25	Cyclohexanol, 4-(1,1-dimethylethyl)	156	$C_{10}H_{20}O$	000937-05-3	64.71	0.41	20.5	129.4
26	Cyclohexanol, 4-(1,1-dimethylethyl)	156	$C_{10}H_{20}O$	000098-52-2	162.57	1.04	51.5	325.1
27	2,3-Diethylpyrazine	136	$C_8H_{12}N_2$	015707-24-1	23.86	0.18	8.7	47.7
28	Cyclooctane	112	C_8H_{16}	000292-64-8	154.63	1.38	68.2	309.3
29	2-Undecanone	170	$C_{11}H_{22}O$	000112-12-9	36.85	0.22	10.7	73.7
30	Bicyclo[2.2.1]heptan-2-ol, 1,7,7-tr	196	$C_{12}H_{20}O$	005655-61-8	1665.69	8.50	419.8	3331.4
31	Dicyclopentenyl alcohol	150	$C_{10}H_{14}O$	027137-33-3	83.81	0.56	27.6	167.6
32	4-tert-Butylcyclohexyl acetate	198	$C_{12}H_{22}O_2$	032210-23-4	88.51	0.45	22.1	177.0
33	Undecanal, 2-methyl-	184	$C_{12}H_{24}O$	000110-41-8	74.59	0.41	20.0	149.2
34	4-tert-Butylcyclohexyl acetate	198	$C_{12}H_{22}O_2$	032210-23-4	17.15	0.09	4.3	34.3
35	Diphenyl ether	170	$C_{12}H_{10}O$	000101-84-8	27.63	0.16	8.0	55.3
36	Benzene, 1-methyl-3-(1-methylethyl)	134	$C_{10}H_{14}$	000535-77-3	12.82	0.10	4.7	25.6
37	p-Menthane, 2,3-d bromo-8-phenyl-	372	$C_{16}H_{22}Br_2$		25.17	0.07	3.3	50.3
38	3-Tetradecanol	214	$C_{14}H_{30}O$	001653-32-3	10.19	0.05	2.4	20.4
39	Butylated Hydroxytoluene	220	$C_{15}H_{24}O$	000128-37-0	23.22	0.11	5.2	46.4
40	Tricyclopentadiene	198	$C_{15}H_{18}$		25.40	0.13	6.3	50.8
41	2-Undecene, 2,5-dimethyl-	182	$C_{13}H_{26}$	049622-16-4	61.02	0.34	16.6	122.0
	Terpenoids				1383.13	9.3	459.6	2766.3
	Total				6200.2	38.7	1910.5	15166.6

Outdoor Sample (on July 30, 2014)								
7/30	Compound	Mol. Weight	Mol. Formula	CAS #	Mass [ng]	nanomoles	ppb	µg/m^3
1	Butanal	72	C_4H_8O	000123-72-8	3.70	0.05	0.7	2.0
2	Butanoic acid, 3-methyl-2-[(phenylm	293	$C_{15}H_{23}NO_3Si$	055520-96-2	17.03	0.06	0.8	9.1
3	Octane	114	C_8H_{18}	000111-65-9	3.20	0.03	0.4	1.7
4	Cyclotrisiloxane, hexamethyl-	222	$C_6H_{18}O_3Si_3$	000541-05-9	8.96	0.04	0.5	4.8
5	Hexanal	100	$C_6H_{12}O$	000066-25-1	13.96	0.14	1.8	7.4
6	Furfural	96	$C_5H_4O_2$	000098-01-1	2.92	0.03	0.4	1.6
7	N-Heptanal N-methyl-N-formylhydraz	170	$C_9H_{18}N_2O$	061748-12-7	3.26	0.02	0.3	1.7
8	Phenylethyne	102	C_8H_6	000536-74-3	4.34	0.04	0.6	2.3
9	Styrene	104	C_8H_8	000100-42-5	2.47	0.02	0.3	1.3
10	Cyclopentanol, 2-methyl-, acetate,	142	$C_8H_{14}O_2$	040991-94-4	3.69	0.03	0.3	2.0
11	2-Butanone	72	C_4H_8O	000078-93-3	8.75	0.12	1.6	4.7
12	Heptanal	114	$C_7H_{14}O$	000111-71-7	6.87	0.06	0.8	3.7
13	1R-.alpha.-Pinene	136	$C_{10}H_{16}$	007785-70-8	3.47	0.03	0.3	1.9
14	Cyclotetrasiloxane, octamethyl-	296	$C_8H_{24}O_4Si_4$	000556-67-2	4.81	0.02	0.2	2.6
15	Trisiloxane, 1,1,3,3,5,5-hexamethyl	208	$C_6H_{20}O_2Si_3$	001189-93-1	2.59	0.01	0.2	1.4
16	Benzaldehyde	106	C_7H_6O	000100-52-7	49.60	0.47	6.2	26.5
17	Octanal	128	$C_8H_{16}O$	000124-13-0	14.90	0.12	1.5	7.9
18	1-Hexanol, 2-ethyl-	130	$C_8H_{18}O$	000104-76-7	2.89	0.02	0.3	1.5
19	Phenol	94	C_6H_6O	000108-95-2	12.49	0.13	1.8	6.7
20	Benzene, propyl-	120	C_9H_{12}	000103-65-1	11.13	0.09	1.2	5.9
21	Acetophenone	120	C_8H_8O	000098-86-2	44.89	0.37	4.9	23.9
22	Nonanal	142	$C_9H_{18}O$	000124-19-6	48.81	0.34	4.5	26.0
23	2-Furanone, 2,5-dihydro-3,5-dimethy	112	$C_6H_8O_2$	1000196-88-1	3.03	0.03	0.4	1.6
24	Decanal	156	$C_{10}H_{20}O$	000112-31-2	31.00	0.20	2.6	16.5
25	Fluoren-9-ol, 3,6-dimethoxy-9-(2-ph	342	$C_{23}H_{18}O_3$	1000217-31-2	3.73	0.01	0.1	2.0
26	Z-10-Pentadecen-1-ol	226	$C_{15}H_{30}O$	1000131-00-6	2.76	0.01	0.2	1.5
27	Tetradecanal	212	$C_{14}H_{28}O$	000124-25-4	5.56	0.03	0.3	3.0
28	Tetradecane	198	$C_{14}H_{30}$	000629-59-4	7.57	0.04	0.5	4.0
29	Tetradecanal	212	$C_{14}H_{28}O$	000124-25-4	5.07	0.02	0.3	2.7
30	Decane, 2,3,5,8-tetramethyl-	198	$C_{14}H_{30}$	1000149-58-9	3.71	0.02	0.2	2.0
31	Tridecanal	198	$C_{13}H_{26}O$	010486-19-8	3.19	0.02	0.2	1.7
32	1-Hexene, 3,5,5-trimethyl-	126	C_9H_{18}	004316-65-8	120.24	0.95	12.6	64.1
33	Benzophenone	182	$C_{13}H_{10}O$	000119-61-9	4.80	0.03	0.3	2.6
34	Propane, 2-isothiocyanato-2-methyl-	115	C_5H_9NS	000590-42-1	4.20	0.04	0.5	2.2
35	Oxirane, (Z)-2-acetoxy-2,3-diphenyl	254	$C_{16}H_{14}O_3$	135455-96-8	3.46	0.01	0.2	1.8
	Terpenoids				3.5	0.0	0.3	1.9
	Total				473.1	3.6	48.1	252.3

Indoor Sample (on August 25, 2014)*								
8/25	Compound	Mol. Weight	Mol. Formula	CAS #	Mass [ng]	nanomoles	ppb	µg/m^3
1	Acetic acid	60	$C_2H_4O_2$	000064-19-7	29.10	0.49	3.1	7.5
2	1-Butanol	74	$C_4H_{10}O$	000071-36-3	8.78	0.12	0.8	2.3
3	Pentanal	86	$C_5H_{12}O$	000110-62-3	31.46	0.37	2.3	8.1
4	Toluene	92	C_7H_8	000108-88-3	8.27	0.09	0.6	2.1
5	1-Pentanol	88	$C_5H_{12}O$	000071-41-0	25.52	0.29	1.8	6.5
6	Hexanal	100	$C_6H_{12}O$	000066-25-1	127.81	1.28	8.1	32.8
7	Furfural	96	$C_5H_4O_2$	000098-01-1	95.35	0.99	6.3	24.4
8	2-Heptanone	114	$C_7H_{14}O$	000110-43-0	11.40	0.10	0.6	2.9
9	Heptanal	114	$C_7H_{14}O$	000111-71-7	39.44	0.35	2.2	10.1
10	1S-.alpha.-Pinene	136	$C_{10}H_{16}$	007785-26-4	130.90	0.96	6.1	33.6
11	Bicyclo[3.1.1]heptane, 6,6-dimethyl	136	$C_{10}H_{16}$	018172-67-3	59.55	0.44	2.8	15.3
12	Cyclopentane, 1,3-dimethyl-	98	C_7H_{14}	002453-00-1	11.45	0.12	0.7	2.9
13	1-Hexene, 4-ethyl-	112	C_8H_{16}	016746-85-3	9.53	0.09	0.5	2.4
14	Benzaldehyde	106	C_7H_6O	000100-52-7	56.68	0.53	3.4	14.5
15	Octanal	128	$C_8H_{16}O$	000124-13-0	59.10	0.46	2.9	15.2
16	d-Limonene	136	$C_{10}H_{16}$	005989-27-5	59.32	0.44	2.8	15.2
17	Bicyclo[3.1.0]hex-2-ene, 4-methyl-1	136	$C_{10}H_{16}$	028634-89-1	21.17	0.16	1.0	5.4
18	1-Hexanol, 2-ethyl-	130	$C_8H_{18}O$	000104-76-7	23.19	0.18	1.1	5.9
19	Phenol	94	C_6H_6O	000108-95-2	20.48	0.22	1.4	5.3
20	1-Octanol	130	$C_8H_{18}O$	000111-87-5	30.79	0.24	1.5	7.9
21	Nonanal	142	$C_9H_{18}O$	000124-19-6	109.03	0.77	4.9	28.0
22	Acetic acid, nonyl ester	186	$C_{11}H_{22}O_2$	000143-13-5	12.98	0.07	0.4	3.3
23	2-Decanone	156	$C_{10}H_{20}O$	000693-54-9	12.32	0.08	0.5	3.2
24	Decanal	156	$C_{10}H_{20}O$	000112-31-2	27.31	0.18	1.1	7.0
25	Bicyclo[3.1.1]hept-3-en-2-one, 4,6,	150	$C_{10}H_{14}O$	000080-57-9	9.40	0.06	0.4	2.4
26	Isobornyl acetate	196	$C_{12}H_{20}O_2$	000125-12-2	14.15	0.07	0.5	3.6
27	Tetradecane	198	$C_{14}H_{30}$	000629-59-4	450.79	2.28	14.4	115.6
28	Benzene, (1-methylheptyl)-	190	$C_{14}H_{22}$	000777-22-0	23.13	0.12	0.8	5.9
29	Propanoic acid, 2-methyl-, 2,2-dime	216	$C_{12}H_{24}O_3$	074367-33-2	89.73	0.42	2.6	23.0
30	Benzene, (1-butylpentyl)-	204	$C_{15}H_{24}$	020216-88-0	24.61	0.12	0.8	6.3
31	4-Phenylnonane	204	$C_{15}H_{24}$	065185-83-3	29.19	0.14	0.9	7.5
32	Pentadecane	212	$C_{15}H_{32}$	000629-62-9	186.79	0.88	5.6	47.9
33	Benzene, (1-butylhexyl)-	218	$C_{16}H_{26}$	004537-11-5	19.21	0.09	0.6	4.9
34	Benzene, (1-butylhexyl)-	218	$C_{16}H_{26}$	004537-11-5	36.84	0.17	1.1	9.4
35	Benzene, (1-propyloctyl)-	232	$C_{17}H_{28}$	004536-86-1	11.84	0.05	0.3	3.0
36	1H-Cycloprop[e]azulene, decahydro-1	204	$C_{15}H_{24}$	025246-27-9	19.74	0.10	0.6	5.1
37	Naphthalene, 2,7-dimethyl-	156	$C_{12}H_{12}$	000582-16-1	29.24	0.19	1.2	7.5
38	Benzene, (1-butylhexyl)-	218	$C_{16}H_{26}$	004537-11-5	105.15	0.48	3.1	27.0
39	Benzene, (1-propylheptyl)-	218	$C_{16}H_{26}$	004537-12-6	45.39	0.21	1.3	11.6
40	Hexadecane	226	$C_{16}H_{34}$	000544-76-3	48.33	0.21	1.4	12.4
41	Benzene, (1-methylnonyl)-	218	$C_{16}H_{26}$	004537-13-7	20.94	0.10	0.6	5.4
42	Propanoic acid, 2-methyl-, 1-(1,1-d	286	$C_{16}H_{30}O_4$		146.32	0.51	3.2	37.5
43	Benzene, (1-propyloctyl)-	232	$C_{17}H_{28}$	004536-86-1	23.07	0.10	0.6	5.9
44	Benzene, (1-ethylnonyl)-	232	$C_{17}H_{28}$	004536-87-2	32.28	0.14	0.9	8.3
45	2-Octene, 2,6-dimethyl-	140	$C_{10}H_{20}$	004057-42-5	31.37	0.22	1.4	8.0
46	Benzene, (1-pentylheptyl)-	246	$C_{18}H_{30}$	002719-62-2	22.74	0.09	0.6	5.8
47	Benzene, (1-butyloctyl)-	246	$C_{18}H_{30}$	002719-63-3	24.08	0.10	0.6	6.2
48	Benzene, (1-propylnonyl)-	246	$C_{18}H_{30}$	002719-64-4	18.10	0.07	0.5	4.6
49	Benzene, (1-ethyldecyl)-	246	$C_{18}H_{30}$	002400-00-2	18.73	0.08	0.5	4.8
50	Benzene, (1-methylundecyl)-	246	$C_{18}H_{30}$	002719-61-1	13.11	0.05	0.3	3.4
	Terpenoids				59.3	0.4	2.8	15.2
	Total				2515.2	16.0	101.6	644.9
	* Nothing was injected inside the Test House on this day.							

Outdoor Sample (on August 25, 2014)								
8/25	Compound	Mol. Weight	Mol. Formula	CAS #	Mass [ng]	nanomoles	ppb	µg/m^3
1	Acetic acid	60	$C_2H_4O_2$	000064-19-7	8.16	0.14	0.9	2.1
2	Propanal	58	C_3H_6O	000123-38-6	3.78	0.07	0.4	1.0
3	Butanoic acid, 2-[(phenylmethoxy)im	279	$C_{14}H_{21}NO_3Si$	055520-91-7	3.2	0.01	0.1	0.8
4	Toluene	92	C_7H_8	000108-88-3	3.71	0.04	0.3	1.0
5	Pentanol, 5-amino-	103	$C_5H_{13}NO$	002508-29-4	2.64	0.03	0.2	0.7
6	Heptane, 2,4-dimethyl-	128	C_9H_2O	002213-23-2	3.42	0.03	0.2	0.9
7	Cyclotrisiloxane, hexamethyl-	222	$C_6H_{18}O_3Si_3$	000541-05-9	2.49	0.01	0.1	0.6
8	Hexanal	100	$C_6H_{12}O$	000066-25-1	13.82	0.14	0.9	3.5
9	Acetic acid, fluoro-, ethyl ester	106	$C_4H_7FO_2$	000459-72-3	2.47	0.02	0.1	0.6
10	Nonane	128	C_9H_{20}	000111-84-2	3	0.02	0.1	0.8
11	Phenylethyne	102	C_8H_6	000536-74-3	8.56	0.08	0.5	2.2
12	Cyclopentanol, 2-methyl-, acetate,	142	$C_8H_{14}O_2$	040991-94-4	3.02	0.02	0.1	0.8
13	2-Propenoic acid, 3-phenyl-, 2-meth	202	$C_{13}H_{14}O_2$	054889-46-2	3.42	0.02	0.1	0.9
14	Heptanal	114	$C_7H_{14}O$	000111-71-7	5.68	0.05	0.3	1.5
15	Benzaldehyde	106	C_7H_6O	000100-52-7	146.97	1.39	8.8	37.7
16	Octanal	128	$C_8H_{16}O$	000124-13-0	12.99	0.10	0.6	3.3
17	Tricyclo[3.1.0.0(2,4)]hex-3-ene-3-c	103	C_7H_5N	103495-51-8	2.91	0.03	0.2	0.7
18	Phenol	94	C_6H_6O	000108-95-2	40.79	0.43	2.7	10.5
19	Decane, 2,5,6-trimethyl-	184	$C_{13}H_{28}$	062108-23-0	4.74	0.03	0.2	1.2
20	Benzeneacetaldehyde	120	C_8H_8O	000122-78-1	23.76	0.20	1.3	6.1
21	Acetophenone	120	C_8H_8O	000098-86-2	141.89	1.18	7.5	36.4
22	Nonanal	142	$C_9H_{18}O$	000124-19-6	48.74	0.34	2.2	12.5
23	2,5-Dimethylanisole	136	$C_9H_{12}O$	001706-11-2	13.22	0.10	0.6	3.4
24	3-Tetradecene, (E)-	196	$C_{14}H_{28}$	041446-68-8	2.54	0.01	0.1	0.7
25	Benzenepropanoic acid	150	$C_9H_{10}O_2$	000501-52-0	3.47	0.02	0.1	0.9
26	Decanal	156	$C_{10}H_{20}O$	000112-31-2	23.67	0.15	1.0	6.1
27	Benzoic acid, 2-butoxy-, methyl est	208	$C_{12}H_{16}O_3$	005446-96-8	2.53	0.01	0.1	0.6
28	2-Naphthalenol	144	$C_{10}H_8O$	000135-19-3	3.88	0.03	0.2	1.0
29	1-Tetralone, 8-hydroxy-	162	$C_{10}H_{10}O_2$	1000161-08-8	3.63	0.02	0.1	0.9
30	Octadecanal	268	$C_{18}H_{36}O$	000638-66-4	4.57	0.02	0.1	1.2
31	Tetradecane	198	$C_{14}H_{30}$	000629-59-4	11.03	0.06	0.4	2.8
32	2-Propanone, 2-propenylhydrazone	112	$C_6H_{12}N_2$	019031-79-9	3.54	0.03	0.2	0.9
33	Phthalic anhydride	148	$C_8H_4O_3$	000085-44-9	8.69	0.06	0.4	2.2
34	Pentadecane	212	$C_{15}H_{32}$	000629-62-9	5.73	0.03	0.2	1.5
35	Benzenebutanoic acid, .gamma.-oxo,	192	$C_{11}H_{12}O_3$	025333-24-8	3.79	0.02	0.1	1.0
36	Propanoic acid, 2-methyl-, 1-(1,1-d	286	$C_{16}H_{30}O_4$	074381-40-1	3.42	0.01	0.1	0.9
37	Phenylmaleic anhydride	174	$C_{10}H_6O_3$	036122-35-7	20.77	0.12	0.8	5.3
38	2-Hexene, 3,5,5-trimethyl-	126	C_9H_{18}	026456-76-8	73.91	0.59	3.7	19.0
39	Benzophenone	182	$C_{13}H_{10}O$	000119-61-9	6.62	0.04	0.2	1.7
40	Benzoic acid, phenyl ester	198	$C_{13}H_{10}O_2$	000093-99-2	8.14	0.04	0.3	2.1
41	Methyl .alpha.-1-arabinofuranoside	476	$C_{27}H_{24}O_8$		5.83	0.01	0.1	1.5
	Terpenoids				-	-	-	-
	Total				699.1	5.7	36.3	179.3

Appendix F: Decay of Particulate ROS

Decay of Ambient Particulate ROS -- Methodology and Results

Outdoor total suspended particles (TSP) were collected over a 3-hour period in the early afternoon on two separate days in October 2012. Three samples were assessed straight away and another three were reserved in the dark at room temperature for assessment one day later. Figure F.1 shows that the particulate ROS did not decay significantly over a day.

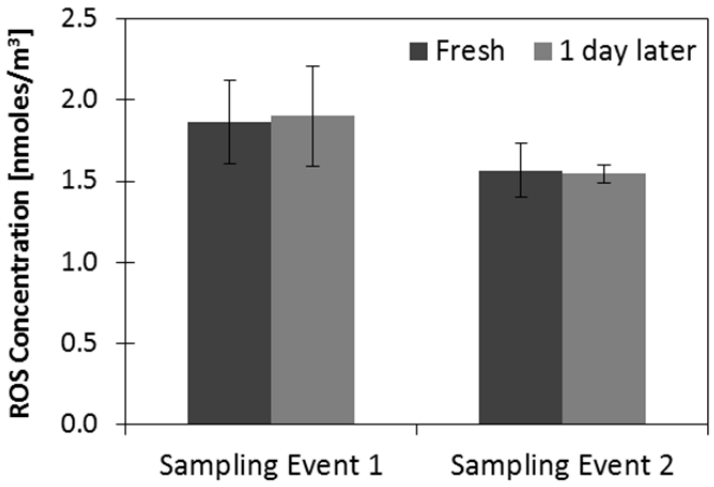

Figure F.1: Concentration of ROS on ambient TSP measured immediately after sampling and after a day of storage at room temperature.

Outdoor particulate ROS may be generated locally close to the time of sampling or may be transported over long distances, having already 'aged' before it reaches the sampler. While the above result indicates that outdoor particulate ROS is relatively stable over a day when collected and assessed in the manner described in Chapter 3 of this dissertation, this study has not ascertained whether the rate of decay of particulate ROS varies as a function of season, and outdoor air quality and meteorological conditions.

In earlier stages of this project, samples collected inside buildings were not seen to decay substantially over a day. However, no replicates were used in those studies so no statistically significant conclusion can be reached on the rate of decay of indoor particulate ROS.

References

Adom, K. K., Liu, R. H., (2005). Rapid Peroxyl Radical Scavenging Capacity (PSC) Assay for Assessing both Hydrophilic and Lipophilic Antioxidants. *Journal of Agricultural and Food Chemistry*, 53: 6572-6580.

Alvarez, E. G., Amedro, D., Afif, C., Gligorovski, S., Schoemaecker, C., Fittschen, C., Doussin, J-F., Wortham, H., (2013). Unexpectedly High Indoor Hydroxyl Radical Concentrations Associated with Nitrous Acid. *Proceedings of the National Academy of Sciences*, 110, 33: 13294-13299.

Anderson, S. E., Jackson, L. G., Franko, J., Wells, J. R., (2010). Evaluation of Dicarbonyls Generated in a Simulated Indoor Air Environment using an in vitro Exposure System. *Toxicological Sciences*, 115, 2: 453-461.

Anderson, S. E., Khurshid, S. S., Meade, B. J., Lukomska, E., Wells, J. R.., (2013). Toxicological Analysis of Limonene Reaction Products using an *in vitro* Exposure System. Toxicology in Vitro, 27, 721-730.

Antonini, J. M., Clarke, R. W., Murthy, G. G. K., Sreekanthan, P., Jenkins, N., Eagar, T. W., Brain, J. D. (1998). Freshly generated stainless steel welding fume induced greater lung inflammation in rats as compared to aged fume. *Toxicology Letters*, 98: 77-86.

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 and Technology, 40, 16, 4859-4866.

Aufderheide M., Mohr U., (1999). CULTEX – A new system and technique for the cultivation and exposure of cells at the air/liquid interface. *Experimental and Toxicologic Pathology*, 51, 6: 489-490.

Aufderheide M., Mohr U., (2000). CULTEX – An Alternative Technique for Cultivation and Exposure of cells of the respiratory tract to airborne pollutants at the air / liquid interface. *Experimental and Toxicologic Pathology*, 52, 3: 265-270.

Ayers, G. P., Penkett, S. A., Gillett, R. W., Bandy, B., Galbally, I. E., Meyer, C. P., Elsworth, C. M., Bentley, S. T., Forgan, B. W., (1992). Evidence for Photochemical Control of Ozone Concentrations in Unpolluted Marine Air. Nature, 360, 6403, 446-449.

Bass DA., Parce JW., Dechatelet LR., Szejda P., Seeds MC., Thomas M., (1983). Flow cytometric studies of oxidative product formation by neutrophils: A graded response to membrane stimulation. *Journal of Immunology*, 130: 1910-1917.

Baulig, A., Poirault, J-J., Ausset, P., Schins, R., Shi, T., 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.

Bennett, D. H., Fisk, W., Apte, M. G., Wu, X., Trout, A., Faulkner, D., Sullivan, D. (2012) Ventilation, Temperature, and HVAC Characteristics in Small and Medium Commercial Buildings in California. *Indoor Air*, 22, 309-320.

Bell, M. L., Samet, J. M., Dominici, F., (2004). Time-Series Studies of Particulate Matter. Annual Review of Public Health, 25, 247-280.

Bondy, S. C., McKee, M., LeBel, C. P., (1990). Changes in synaptosomal pH and rates of oxygen radical formation induced by chlordecone. *Molecular and Chemical Neuropathology*, 13: 95-106.

Bondy, S. C., LeBel, C. P., (1991). Oxygen radical generation as an index of neurotoxic damage. *Biomedical and Environmental Sciences*, 4: 217-223.

Boogaard, H., Janssen, N. A. H., Fischer, P. H., Kos, G. P. A., Weijers, E. P., Cassee, F. R., van der Zee, S. C., de Hartog, J. J., Brunekreef, B., Hoek, G. (2012) Contrasts in Oxidative Potential and Other Particulate Matter Characteristics Collected Near Major Streets and Background Locations. *Environmental Health Perspectives*, 120, 2, 185-191.

Bowler, R. P., Crapo, J. D. (2002) Oxidative Stress in Allergic Respiratory Diseases. *Journal of Allergy and Clinical Immunology*, 110, 3, 349-356.

Brown, S. K.; Sim, M. R.; Abramson, M. J.; Gray, C. N., (1994). Concentrations of Volatile Organic Compounds in Indoor Air – A Review. *Indoor Air*, 2, 123-134.

Card JW., Zeldin DC., Bonner JC., Nestmann ER., (2008). Pulmonary applications and toxicity of engineered nanoparticles. *American Journal of Physiology – Lung Cellular and Molecular Physiology*, 295: L400-L411.

Carvalho TC., Peters JI., Williams III RO., (2011). Influence of particle size on regional lung deposition – What evidence is there? *International Journal of Pharmaceutics*, 406: 1-10.

Cerutti PA., (1985). Prooxidant States and Tumor Promotion. *Science*, 227, 4685: 375-381.

Chao, C. Y. and Chan, G. Y. (2001) Quantification of Indoor VOCs in Twenty Mechanically Ventilated Buildings in Hong Kong. *Atmospheric Environment*, 35, 5895-5913.

Chen, X.; Hopke, P. K., (2009a). Secondary organic aerosol from α-pinene ozonolysis in dynamic chamber system. *Indoor Air*, 19, 335-345.

Chen, X.; Hopke, P. K., (2009b). A chamber study of secondary organic aerosol formation by linalool ozonolysis. *Atmospheric Environment*, 43, 3935-3940.

Chen, X.; Hopke, P. K., (2010). A chamber study of secondary organic aerosol formation by limonene ozonolysis. *Indoor Air*, 20, 320-328.

Chen, X.; Hopke, P. K.; Carter, W. P. L., (2011). Secondary Organic Aerosol from Ozonolysis of Biogenic Volatile Organic Compounds: Chamber Studies of Particle and Reactive Oxygen Species Formation. *Environmental Science and Technology*, 45, 276-282.

Cho A K., Sioutas C., Miguel AH., Kumagai Y., Schmitz DA., Singh M., Eiguren-Fernandez A., Froines JR., (2005). Redox Activity of airborne particulate matter at different sites in the Los Angeles Basin. *Environmental Research,* 99: 40–47.

Clausen PA., Wilkins CK., Wolkoff P., Nielsen GD., (2001). Chemical and Biological Evaluation of a Reaction Mixture of R-(+)-Limonene/Ozone – Formation of Strong Airway Irritants. *Environment International*, 26, 7-8: 511-522.

Coleman BK., Lunden MM., Destaillats H., Nazaroff WW., (2008). Secondary Organic Aerosol from Ozone-Initiated Reactions with Terpene-Rich Household Products. *Atmospheric Environment*, 42, 35: 8234-8245.

Destaillats H., Lunden MM., Singer BC., Coleman BK., Hodgson AT., Weschler CJ., Nazaroff WW., (2006). Indoor Secondary Pollutants from Household Product Emissions in the Presence of Ozone: A Bench-Scale Chamber Study. *Environmental Science and Technology*, 40, 14: 4421-4428.

Docherty, K. S.; Wu, W.; Lim, Y. B.; Ziemann, P. J., (2005). Contributions of Organic Peroxides to Secondary Aerosol formed from Reactions of Monoterpenes with O_3. *Environmental Science & Technology*, 39, 4049-4059.

Dollard, G. J., Davies, T. J., (1992). Observations of H_2O_2 and PAN in a Rural Atmosphere. Environmental Pollution, 75, 1, 45-52.

Dreher, D. and Junod, A. F. (1996) Role of oxygen free radicals in cancer development, *European Journal of Cancer*, 32, 1, 30-38.

EPA 40CFR, Part 136, 2011 – Guidelines Establishing Test Procedures for the Analysis of Pollutants, Appendix B – Definition and Procedure for the Determination of the Method Detection Limit, Revision 1.11. Accessed 4/2/2013 at http://www.gpo.gov/fdsys/pkg/CFR-2011-title40-vol23/pdf/CFR-2011-title40-vol23-part136-appB.pdf

Fan, Z.; Weschler, C. J.; Han, I-K.; Zhang, J., (2005). Co-formation of hydroperoxides and ultra-fine particles during the reactions of ozone with a complex VOC mixture under simulated indoor conditions. *Atmospheric Environment*, 39, 5171-5182.

Fiedler N., Laumbach R., Kelly-McNeil K., Lioy P., Fan ZH., Zhang JF., Ottenweller J., Ohman-Strickland P., Kipen H., (2005). Health Effects of a mixture of indoor air volatile organics, their ozone oxidation products, and stress. *Environmental Health Perspectives*, 113: 1542-1548.

Finlayson-Pitts, B. J.; Pitts, J. N. Chemistry of the Upper and Lower Atmosphere; Academic Press, San Diego, CA, 2000.

Fisk, W. J. and Rosenfeld, A. H. (1997) Estimates of Improved Productivity and Health from Better Indoor Environments, *Indoor Air*, 7, 158-172.

Forester CD., Wells JR., (2009). Yields of Carbonyl Products from Gas-Phase Reactions of Fragrance Compounds with OH Radical and Ozone. *Environmental Science and Technology*, 43: 3561-3568.

Friedlander, S. K. and Yeh, E. K. (1998) The Submicron Atmospheric Aerosol as a Carrier of Reactive Chemical Species: Case of Peroxides. *Applied Occupational and Environmental Hygiene*, 13, 6, 416-420.

Gminski R., Tang T., Mersch-Sundermann V., (2010). Cytotoxicity and Genotoxicity in Human Lung Epithelial A549 cells caused by airborne Volatile Organic Compounds emitted from Pine Wood and Oriented Strand Boards. *Toxicology Letters*, 196, 1: 33-41.

Gurgueira SA., Lawrence J., Coull B., Murthy GGK., Gonzalez-Flecha B., (2002). Rapid increases in the steady state concentration of reactive oxygen species in the lungs and heart after particulate air pollution inhalation. *Environmental Health Perspectives*, 110, 749–755.

Gunz, D. W.; Hoffman, M. R., (1990) Atmospheric Chemistry of Peroxides: A Review. *Atmospheric Environment*, 24A, 7, 1601-1633.

Guyton, K. Z., and Kensler, T. W. (1993) Oxidative Mechanisms in Carcinogenesis, *British Medical Bulletin*, 49, 3, 523-544.

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.

Hewitt, C. N., Kok, G. L., (1991). Formation and Occurrence of Organic Hydroperoxides in the Troposphere: Laboratory and Field Observations. Journal of Atmospheric Chemistry, 12, 181-194.

Hodgson, A. T.; Rudd, A. F.; Beal, D.; Chandra, S., (2000). Volatile Organic Compound Concentrations and Emission Rates in New Manufactured and Site-Built Houses. *Indoor Air*, 10: 178-192.

Huang, M-F., Lin, W-L., Ma, Y-C., (2005) A Study of Reactive Oxygen Species in Mainstream of Cigarette. *Indoor Air*, 15, 135-140.

Hung H-F., Wang C-S., (2001). Experimental determination of reactive oxygen species in Taipei aerosols. *Aerosol Science*, 32: 1201-1211.

Jackson, A. V., Hewitt, C. N., (1999). Atmosphere Hydrogen Peroxide and Organic Hydroperoxides: A Review. Critical Reviews in Environmental Science and Technology, 29, 2, 175-228.

Jones, N. C., Thornton, C. A., Mark, D., Harrison, R. M., (2000). Indoor/outdoor Relationships of Particulate Matter in Domestic Homes with Roadside, Urban, and Rural Locations. Atmospheric Environment, 34, 2603-2612.

Kehrer, J. P., (1993). Free Radicals as Mediators of Tissue Injury and Disease. *Critical Reviews in Toxicology*, 23, 1, 21-48.

Kenyon, N.; Liu, F. T., (2011). Pulmonary Effects of Diesel Exhaust: Neutrophil Inflammation, Oxidative Injury, and Asthma. *The American Journal of Pathology*, 179, 6, 2678-2682.

Klaunig, J. E., Kamendulis, L. M. (2004) The role of oxidative stress in carcinogenesis. *Annual Review of Pharmacology and Toxicology*, 44, 239-267.

Kleno J., Wolkoff P., (2004). Changes in eye blink frequency as a measure of trigeminal stimulation by exposure to limonene oxidation products, isoprene oxidation products and nitrate radicals.

Klepeis N., Tsang A., Behar JV., (1996). Analysis of the National Human Activity Pattern Survey (NHAPS) Respondents from a Standpoint of Exposure Assessment. Las Vegas NV: National Exposure Research Laboratory, *U.S. Environmental Protection Agency* (EPA-600-R-96/074).

Klepeis NE., Nelson WC., Ott WR., Robinson JP., Tsang AM., Switzer P., Behar JV., Hern SC., Engelmann WH., (2001). The National Human Activity Pattern Survey (NHAPS): A Resource for assessing exposure to environmental pollutants. *Journal of Exposure Analysis and Environmental Epidemiology*, 11: 231-252.

Kooy, N. W., Royall, J. A., Ischiropoulos, H. (1997). Oxidation of 2',7'-Dichlorofluorescin by Peroxynitrite. *Free Radical Research*, 27,3: 245-254.

Lai ACK., Thatcher TL., Nazaroff WW., (2000). Inhalation Transfer Factors for Air Pollution Health-Risk Assessments. *Journal of the Air & Waste Management Association*, 50, 1688-1699.

Lam CW., James JT., McCluskey R., Hunter RL., (2004). Pulmonary toxicity of single-wall carbon nanotubes in mice 7 and 90 days after intratracheal instillation. *Toxicological Sciences*, 77, 1: 126-134.

Lansing, M. W., Ahmed, A., Cortes, A., Sielczak, M. W., Wanner, A., Abraham, W. M., (1993) Oxygen radicals contribute to antigen-induced airway hyperresponsiveness in conscious sheep. *American Review of Respiratory Disease*, 147, 2, 321-326.

Largiuni, O., Giacomelli, M. C., Piccardi, G., (2002). Concentration of Peroxides and Formaldehyde in Air and Rain and Gas-Rain Partitioning. Journal of Atmospheric Chemistry, 41, 1-20.

Laumbach RJ., Fiedler N., Gardner CR., Laskin DL., Fan ZH., Zhang JF., Weschler CJ., Lioy PJ., Devlin RB., Ohman-Strickland P., Kelly-McNeil K., Kipen HM., (2005). Nasal effects of a mixture of Volatile Organic Compounds and their Ozone Oxidation Products. *Journal of Occupational and Environmental Medicine*, 47, 11: 1182-1189.

LeBel CP., Odunze IN., Adams JD Jr., Bondy SC., (1989). Perturbations in cerebral oxygen radical formation and membrane order following vitamin E deficiency. *Biochemical and Biophysical Research Communications*, 163:860-866.

LeBel CP., Ali SF., McKee M., Bondy SC., (1990). Organometal-induced increases in oxygen reactive species: The potential of 2',7'-dichlorofluorescin diacetate as an index of neurotoxic damage. *Toxicology and Applied Pharmacology*, 104: 17-24.

LeBel CP., Bondy SC., (1991). Oxygen radicals: Common mediators of neurotoxicity. *Neurotoxicology and Teratology*, 13: 341-346.

LeBel CP., Ischiropoulos H., Bondy SC., (1992). Evaluation of the Probe 2',7'-Dichlorofluorescin as an Indicator of Reactive Oxygen Species Formation and Oxidative Stress. *Chemical Research in Toxicology*, 5: 227-231.

Li, N.; Hao, M.; Phalen, R. F.; Hinds, W. C.; Nel, A. E., (2003). Particulate air pollutants and asthma – A paradigm for the role of oxidative stress in PM-induced adverse health effects. *Clinical Immunology*, 109, 250-265.

Li, N.; Xia, T.; Nel, A. E., (2008). The role of oxidative stress in ambient particulate matter-induced lung diseases and its implictions in the toxicity of engineered nanoparticles. *Free Radical Biology & Medicine*, 44, 1689-1699.

Li, T-H.; Turpin, B. J.; Shields, H. C.; Weschler, C. J., (2002). Indoor Hydrogen Peroxide Derived from Ozone/*d*-Limonene Reactions. *Environmental Science & Technology*, 36, 3295-3302.

Liu, J., Steinberg, S. M., Johnson, B. J., (2003). A High Performance Liquid Chromatography Method for Determination of Gas-Phase Hydrogen Peroxide in Ambient Air using Fenton's Chemistry. Chemosphere, 52, 5, 815-823.

Logan, J. A., Prather, M. J., Wofsy, S. C., McElroy, M. B., (1981). Tropospheric Chemistry: A Global Perspective. Journal of Geophysical Research, 86, 7210-7254.

Molecular Probes product sheet, (2005). Indicators for Highly Reactive Oxygen Species. Revised 9 May 2005. Retrieved June, 2011 from http://corefacilities.systemsbiology.net/imaging/documents/apf.pdf

Morio, L. A., Hooper, K. A., Brittingham, J., Li, T.-H., Gordon, R. E., Turpin, B. J., Laskin, D. L., (2001). Tissue Injury following Inhalation of Fine Particulate Matter and Hydrogen Peroxide is associated with altered Production of Inflammatory Mediators and Antioxidants by Alveolar Macrophages. Toxicology and Applied Pharmacology, 177, 188-199.

Nazaroff, W. W.; Cass, G. R., (1986). Mathematical Modeling of Chemically Reactive Pollutants in Indoor Air. *Environ. Sci. Technol.*, 20, 924-934.

Okuwa, K., Tanaka, M., Fukano, Y., Nara, H., Nishijima, Y., Nishino, T., (2010). In Vitro Micronucleus Assay for Cigarette Smoke using a Whole Smoke Exposure System: A Comparison of Smoking Regimens. *Experimental and Toxicologic Pathology*, 62, 4: 433-440.

Oyama, Y., Hayashi, A., Ueha, T., Maekawa, K., (1994). Characterization of 2',7'-dichlorofluorescin fluorescence in dissociated mammalian brain neurons: estimation on intracellular content of hydrogen peroxide. *Brain Research*, 635: 113-117.

Pariselli F., Sacco MG., Rembges D., (2009). An Optimized Method for in vitro Exposure of Human Derives Lung Cells to Volatile Chemicals. *Experimental and Toxicologic Pathology*, 61, 1: 33-39.

Paulson, S. E.; Orlando, J. J., (1996). The reactions of ozone with alkenes: An important source of HO_x in the boundary layer. *Geophysical Research Letters*, 23, 25, 3727-3730.

Persily AK, (1999). Myths about building envelopes. *ASHRAE Journal*, 41, 39–47.

Persily, A.; Musser, A.; Emmerich, S. J., (2010). Modeled infiltration rate distributions for U.S. housing. *Indoor Air*, 20, 473-485.

Pope III, C. A., Burnett, R.T., Thun, M.J., Calle, E.E., Krewski, D., Ito, K., Thurston, G.D., (2002). Lung Cancer, Cardiopulmonary Mortality, and Long-term Exposure to Fine Particulate Air Pollution. Journal of the American Medical Association, 287, 9, 1132–1141.

Pope III, C. A., Burnett, R. T., Thurston, G. D., Thun, M. J., Calle, E. E., Krewski, D., Godleski, J. J., (2004). Cardiovascular Mortality and Long-Term Exposure to Particulate Air Pollution: Epidemiological Evidence of General Pathophysiological Pathways of Disease. Circulation, 109, 71-77.

Pope III, C. A., Dockery, D. W., (2006). Health Effects of Fine Particulate Air Pollution: Lines that Connect. Journal of Air & Waste Management Association, 56, 709-742.

Riedl, M.; Diaz-Sanchez, D., (2005). Biology of Diesel Exhaust Effects on Respiratory Function. *Journal of Allergy and Clinical Immunology*, 115, 2, 221-228.

Rohr AC., Wilkins CK., Clausen PA., Hammer M., Nielsen GD., Wolkoff P., Spengler JD., (2002). Upper airway and pulmonary effects of oxidation products of (+)-α-pinene, *d*-limonene, and isoprene in BALB/c mice. *Inhalation Toxicology*, 14,7: 663-684..

Rohr AC., Shore SA., Spengler JD., (2003). Repeated Exposure to Isoprene Oxidation Products causes Enhanced Respiratory Tract Effects in Multiple Murine Strains. *Inhalation Toxicology*, 15, 12: 1191-1207.

Rosenkranz, A. R., Schmaldienst, S., Stuhlmeier, K. M., Chen, W., Knapp, W., Zlabinger, G. J., (1992). A microplate assay for the detection of oxidative products using 2',7'-dichlorofluorescin-diacetate. *Journal of Immunological Methods*, 156: 39-45.

Samet, J. M., Dominici, F., Curriero, F. C., Coursac, I., Zeger, S. L., (2000). Fine Particulate Air Pollution and Mortality in 20 U.S. Cities, 1987-1994. New England Journal of Medicine, 343, 1742-1749.

Sanders, S. P., Zweier, J. L., Harrison, S. J., Trush, M. A., Rembish, S. J., Liu, M. C. (1995) Spontaneous oxygen radical production at sites of antigen challenge in allergic subjects. *American Journal of Respiratory and Critical Care Medicine*, 151, 6, 1725-1733.

Sarangapani, R., Wexler, A. S., (2000). The Role of Dispersion in Particle Deposition in Human Airways. Toxicological Sciences, 54, 229-236.

Sarwar G., Corsi R., Allen D., Weschler C., (2003). The Significance of Secondary Organic Aerosol Formation and Growth in Buildings: Experimental and Computational Evidence. *Atmospheric Environment*, 37: 1365-1381.

Sarwar G., Olson DA., Corsi RL., Weschler CJ., (2004). Indoor Fine Particles: The Role of Terpene Emissions from Consumer Products. *Journal of the Air & Waste Management Association*, 54: 367-377.

Sarwar G., Corsi R., (2007). The Effects of Ozone/Limonene reactions on indoor secondary organic aerosols. *Atmospheric Environment*, 41: 959-973.

Schöne, E., (1874). Ueber das atmosphärische Wasserstoffhyperoxyd. Berichte der deutschen chemischen Gesellschaft, 7, 1693–1708.

Scott, J. A., Homcy, C. J., Khaw, B., Rabito, C. A., (1988). Quantitation of Intracellular Oxidation in a Renal Epithelial Cell Line. *Free Radical Biology & Medicine*, 4: 79-83.

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

Seinfeld J. H., Pandis S. N., 2006. Atmospheric Chemistry and Physics: From Air Pollution to Climate Change (2nd edition), Wiley Publishers, Hoboken, NJ.

Setsukinai K., Urano Y., Kakinuma K., Majima HJ., Nagano T., (2003). Development of Novel Fluorescence Probes that can reliably detect Reactive Oxygen Species and distinguish specific species. *Journal of Biological Chemistry*, 278, 5: 3170-3175.

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.

Singh, H. B., Salas, L. J., Viezee, W., (1986). Global Distribution of Peroxyacetyl Nitrate. Nature, 321, 6070, 588-591.

Stephens, B., Siegel, J. A., Novoselac, A. (2011) Operational Characteristics of Residential and Light-Commercial Air-conditioning Systems in a Hot and Humid Climate Zone, *Building and Environment*, 46, 1972-1983.

Singer BC., Destaillats H., Hodgson AT., Nazaroff WW., (2006a). Cleaning Products and Air Fresheners: Emissions and Resulting Concentrations of Glycol Ethers and Terpenoids. *Indoor Air*, 16: 179-191.

Singer BC., Coleman BK., Destaillats H., Hodgson AT., Lunden MM., Weschler CJ., Nazaroff WW., (2006b). Indoor Secondary Pollutants from Cleaning Product and Air Freshener use in the presence of Ozone. *Atmospheric Environment*, 40: 6696-6710.

Soto KF., Carrasco A., Powell TG., Garza KM., Murr LE., (2005). Comparative in vitro cytotoxicity assessment of some manufactured nanoparticulate materials characterized by transmission electron microscopy. *Journal of Nanoparticulate Research*, 7: 145-169.

Soto KF., Carrasco A., Powell TG., Murr LE., Garza KM., (2006). Biological effects of Nanoparticulate Materials. *Materials Science and Engineering C*, 26: 1421-1427.

Soto K., Garza KM., Murr., (2007). Cytotoxic effects of aggregated nanomaterials. *Acta Biomaterialia*, 3: 351-358.

Soto KF., Garza KM., Shi Y., Murr LE., (2008). Direct contact cytotoxicity assays for filter-collected, carbonaceous (soot) nanoparticulate material and observations of lung cell response. *Atmospheric Environment*, 42: 1970-1982.

Steinemann, A. C., (2009) Fragranced consumer products and undisclosed ingredients. *Environmental Impact Assessment Review*, 29, 32-38.

Steinemann, A. C.; MacGregor, I. C.; Gordon S. M.; Gallagher, L. G.; Davis, A. L.; Ribeiro, D. S.; Wallace, L. A., (2011). Fragranced consumer products: Chemicals emitted, ingredients unlisted. *Environmental Impact Assessment Review*, 31, 328-333.

Stevens, W. H., Inman, M. D., Wattie, J., O'Byrne, P. M. (1995) Allergen-induced oxygen radical release from Bronchoalveolar Lavage cells and Airway Hyperresponsiveness in dogs. *American Journal of Respiratory and Critical Care Medicine*, 151, 5: 1526-1531.

The Engineer (2013, Feb 27). Lung-on-a-Chip Device could Reduce Animal Drug Testing. Retrieved March 1, 2013, from http://www.theengineer.co.uk/sectors/medical-and-healthcare/news/lung-on-a-chip-device-could-reduce-animal-drug-testing/1015647.article

Thornburg, J., Ensor, D. S., Rodes, C. E., Lawless, P. A., Sparks, L. E., Mosley, R. B. (2001) Penetration of Particles into Buildings and Associated Physical Factors, Part I: Model Development and Computer Simulations, *Aerosol Science and Technology*, 34, 284-296.

Trush, M. A., and Kensler, T. W. (1991) An Overview of the Relationship between Oxidative Stress and Chemical Carcinogenesis, *Free Radical Biology and Medicine*, 10, 201-209.

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 & Ecotoxicology Reviews, 26, 4, 339-362.

Venkatachari, P., Hopke, P. K., Grover, B. D., Eatough, D. J. (2005) Measurement of Particle-Bound Reactive Oxygen Species in Rubidoux Aerosols. *Journal of Atmospheric Chemistry*, 50: 49-58.

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

Venkatachari, P.; Hopke, P. K., (2008a) Characterization of products formed in the reaction of ozone with α-pinene: case for organic peroxides. *Journal of Environmental Monitoring*, 10, 966-974.

Venkatachari, P., Hopke, P. K. (2008b) Development and Laboratory Testing of an Automated Monitor for the Measurement of Atmospheric Particle-bound Reactive Oxygen Species (ROS), *Aerosol Sci. Technol.*, *42*, 629–635.

Vidrio, E., Phuah, C. H., Dillner, A. M., Anastasio, C., (2009). Generation of Hydroxyl Radicals from Ambient Fine Particles in a Surrogate Lung Fluid Solution. Environmental Science and Technology, 43, 922-927.

Wallace LA., (1987). The TEAM Study: summary and analysis: vol. 1. US EPA, Washington, DC, EPA 600/6-87/002a, NTIS, PB 88-100060, 1987.

Wallace LA., (1991). Personal Exposure to 25 Volatile Organic Compounds EPAs 1987 TEAM Study in Los-Angeles, California. *Toxicology and Industrial Health*, 7, 5-6: 203-208.

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 and Technology, 44, 11, 4070-4075.

Waring, M. S., Siegel, J. A. (2008) Particle Loading Rates for HVAC Filters, Heat Exchangers, and Ducts, *Indoor Air*, 18, 209-224.

Wayne, R. P., Barnes, I., Biggs, P., Burrows, J. P., Canosa-Mas, C. E., Hjorth, J., Le Bras, G., Moortgat, G. K., Perner, D., Poulet, G., Restelli, G., Sidebottom, H., (1991). The Nitrate Radical: Physics, Chemistry and the Atmosphere. *Atmospheric Environment*, 25A, 1, 1-203.

Weisel C., Zhang J., Turpin B., Morandi M., Colome S., Stock T., Spektor D., (2005). Relationships of Indoor, Outdoor, and Personal Air (RIOPA): Part 1. Collection Methods and Descriptive Analyses. Health Effects Institute, Boston, MA, p. 127.

Wells, J. R., (2005). Gas-Phase Chemistry of α-Terpineol with Ozone and OH Radical: Rate Constants and Products. *Environmental Science and Technology*, 39: 6937-6943.

Weschler, C. J., Shields HC., (1997). Potential reactions among indoor pollutants. *Atmospheric Environment*, 31, 3487–3495.

Weschler, C. J., Shields HC., (1999). Indoor ozone/terpene reactions as a source of indoor particles. *Atmospheric Environment*, 33, 2301–2312.

Weschler, C. J., (2000). Ozone in Indoor Environments: Concentration and Chemistry. *Indoor Air*, 10: 269-288.

Weschler, C. J., (2003). Indoor chemistry as a source of particles. In: Morawska, L., Salthammer, T. (Eds.), Indoor Environment: Airborne Particles and Settled Dust. Wiley-VCH, Weinheim, pp. 167–189.

Weschler, C. J., (2006). Ozone's Impact on Public Health: Contributions from Indoor Exposures to Ozone and Products of Ozone-Initiated Chemistry. *Environmental Health Perspectives*, 114, 10: 1489-1496.

Weschler, C. J., (2009). Changes in Indoor Pollutants since the 1950s. *Atmospheric Environment*, 43, 153-169.

Wexler, A. S., Sarangapani, R., (1998). Particles do not Increase Vapor Deposition in Human Airways. Journal of Aerosol Science, 29, 197-204.

Witz, G. (1991) Active Oxygen Species as Factors in Multistage Carcinogenesis, *Proceedings of the Society for Experimental Biology and Medicine*, 198, 2, 675-682.

Wolkoff P., Clausen PA., Wilkins CK., Hougaard KS., Nielsen GD., (1999). Formation of strong airway irritants in a model mixture of (+)-α-pinene/ozone. *Atmospheric Environment*, 33, 5: 693-698.

Wolkoff P., Clausen PA., Wilkins CK., Nielsen GD., (2000). Formation of strong airway irritants in terpene/ozone mixtures. *Indoor Air*, 10, 2: 82-91.

Wolkoff, P., Clausen, P.A., Larsen, S.T., Hammer, M., Nielsen, G.D., (2012). Airway effects of repeated exposures to ozone-initiated limonene oxidation products as model of indoor air mixtures. *Toxicology Letters,* 209, 166–172.

Yamada, E., Tomozawa, K., Nakanishi, Y., Fuse, Y., (2002). Behavior of Hydrogen Peroxide in the Atmosphere and Rainwater in Kyoto, and its Effect on the Oxidation of SO_2 in Rainwater. Bulletin of the Chemical Society of Japan, 75, 6, 1385-1391.

Zhang, X., He, S. Z., Chen, Z. M., Zhao, Y., Hua, W., (2012). Methyl hydroperoxide (CH3OOH) in Urban, Suburban, and Rural Atmosphere: Ambient Concentration, Budget, and Contribution to the Atmospheric Oxidizing Capacity. Atmospheric Chemistry and Physics, 12, 8951-8962.

Zelm, R. V., Huijbregts, M. A. J., Hollander, H. A. D., Jaarsveld, H. A. V., Sauter, F. J., Struijs, J., Wijnen, H. J. V., Meent, D. V. (2008) European Characterization Factors for Human Health Damage of PM_{10} and Ozone in Life Cycle Impact Assessment, *Atmospheric Environment*, 42, 441-453.

Zhu, H., Bannenberg, G. L., Moldeus, P., Shertzer, H. G. (1994). Oxidation pathways for the intracellular probe 2',7'-dichlorofluorescin. *Archives of Toxicology*, 68: 582-587.

Zhu, J.; Cao, X. L.; Beauchamp, R., (2001). Determination of 2-butoxyethanol emissions from selected consumer products and its application in assessment of inhalation exposure associated with cleaning tasks. *Environment International*, 26, 589-597.

Vita

Shahana Sarfraz Khurshid grew up in Karachi, Pakistan before moving to the U.S. to pursue higher education. She received her B.S. in Environmental Engineering from MIT in 2003 graduating in the top 5% of her class, and M.S. in Biomedical Engineering from UT Austin in 2008. She has worked in the environmental consulting field and has completed several research projects in environmental and biomedical engineering in academic as well as corporate settings.

Permanent address: shahana@alum.mit.edu

This dissertation was typed by Shahana Sarfraz Khurshid.