Health and Management Implications of Regulating Consumer Product Compositions: a Case Study of d-Limonene

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ABSTRACT

Elizabeth Naess: Health and Management Implications of Regulating Consumer Product Compositions: a Case Study of d-Limonene
(Under the direction of Harvey Jeffries)

Volatile organic compounds (VOCs) are precursors for ozone formation. Although the major sources of VOC emissions are regulated, many areas in the United States seek further emissions reductions. As additional source categories are identified, regulations have focused more on consumer products. VOC emissions from consumer products, either applied outdoors or detrained outdoors from indoor applications, are believed to be an important anthropogenic VOC emissions source. Regulations are already in place limiting the reactivity, or ozone forming potential, of VOC content in consumer product formulations. These regulations may result in the substitution of some petrochemical solvents for biogenic solvents, due to their lower estimated reactivity. While this approach may help control localized outdoor air pollution issues, they may negatively impact indoor air quality.

This research examines the potential impacts of VOC consumer product regulations by focusing on d-limonene – a biogenic solvent with high estimated ozone forming reactivity. An orange was initially examined in an indoor environment, as it is the source for the d-limonene solvent. d-Limonene is then compared to a petrochemical solvent, both as in neat form and as an ingredient in a cleaning product formulation. These compounds and mixtures were injected into a chamber and cultured human epithelial lung cells were exposed to the gases and the particles, both before and after the chamber atmosphere was oxidized with

ozone, as a means of estimating the potential indoor respiratory toxicity of the systems. The inflammation and cytotoxicity induced from these exposures were examined and dose-response curves were generated to assess the risk and policy management implications of such substitutions. Overall, the results showed that decreasing estimated ozone forming reactivity of pure solvents and cleaning product formulations containing these solvents do not reduce the potential toxicity to human lung cells. Therefore, regulations implemented to decrease outdoor exposure to air pollutants may be increasing health risks indoors.

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ABBREVIATIONS

2BE	2-butoxy ethanol
AIHA	American Industrial Hygiene Association
AMCH	4-acetyl-1-methyl-cyclohexene
CAA	Clean Air Act
CalEPA	California Environmental Protection Agency
CARB	California Air Resources Board
EPA	Environmental Protection Agency (U.S.)
ECD	electron capture detector
FDACS	Florida Department of Agriculture and Consumer Services
FID	flame ionization detector
GC	gas chromatography
HC_{2BE}	household cleaning product containing 2-butoxy ethanol
HC_{Lim}	household cleaning product containing d-limonene
IL-8	Interleukin-8
IOPH	3-isopropenyl-6-oxoheptana
LD_{50}	dose that kills 50% of exposed population
LDH	lactate dehydrogenase
MIR	maximum incremental reactivity
MS	mass spectrometry
NAAQS	National Ambient Air Quality Standard
NICNAS	National Industrial Chemicals Notification and Assessment Scheme
NRC	National Research Council
NOx	nitrogen oxides (nitrogen oxide and nitrogen dioxide)
NOEL	No Observed Effect Level
OSHA	Occupational Safety and Health Administration
PM	particulate matter

ppb	parts per million
ppbV	parts per billion volume
ppm	parts per million
ppmV	parts per million volume
RD_{50}	50% reduction in respiratory rate
SIP	State Implementation Plan
SMPS	Scanning Mobility Particle Sizer ®
SO2	sulfur dioxide
SOA	secondary organic aerosol
THC	total hydrocarbon
TWA	time-weighted average
VOC	volatile organic compound
WHO	World Health Organization
WRPPN	Western Regional Pollution Prevention Network

1. Study Overview

The quality of the atmosphere has been in the spotlight since the 1970 Clean Air Act (CAA). Although the first laws to control air pollution were enacted in the 1880s, the 1970 CAA brought the issue into focus. The 1977 and 1990 CAA Amendments helped to further protect the quality of the air we breathe. Today the Environmental Protection Agency (EPA) regulates six designated criteria air pollutants and other hazardous air toxics. The criteria pollutants are: particulate matter (PM), ozone, sulfur dioxide (SO_2), nitrous oxides (NO_X), lead and carbon monoxide. SO_2 and NO_X, along with volatile organic compounds (VOCs) are precursor pollutants that react to form PM and ozone. Many research dollars have been allocated to characterize and identify sources of these major pollutants. The major source categories for these pollution emissions are electric generating units, industry and mobile sources. As these sources become more regulated, other sources of emissions are being identified

VOCs, although not a criteria pollutant, play an important role in ozone and $PM_{2.5}$ formation. The major sources of anthropogenic VOC emissions in the United States are industrial processes (44%), transportation (42%), and stationary fuel combustion (7%); 9% is designated as miscellaneous (NRC, 2004). Areas with high ozone and/or PM concentrations search for new control measures to lower the emissions of anthropogenic VOCs. Over the past several years, California has determined that consumer products are an important VOC source. Their state government has regulated VOCs contained in consumer product formulations based on their ozone forming reactivity, or ozone forming potential. While

there are specific regulations for products used outdoors, such as aerosol coatings, there are also regulations for over 100 categories of consumer products ranging from hair styling gel to multi-purpose solvents. These consumer product regulations do not take into consideration potential impacts to the indoor environment, where the products are actually used. Now the EPA is looking into regulating products based on estimated reactivity values, which would affect product formulations as well as the indoor air quality of consumers across the country.

This research was designed to address the issue of whether policies meant to improve outdoor air quality could in fact negatively alter the quality of indoor air, potentially increasing health risks to people inside. To give direction to this work, a chemical was chosen that potentially would be replaced by the regulations described above. d-Limonene is a solvent that is derived from orange peels and has a high estimated ozone forming reactivity. This chemical has become a popular ingredient in cleaners and degreasers due to its favorable scent and solvent abilities. Chapter two provides background information on this chemical compound and chapters three through five layout the hypothesis and objectives of this study.

d-Limonene is derived from orange peels, therefore this research first examined the act of peeling an orange. From real-life observation, the strong scent emanating from a peeled orange is evidence that d-limonene is emitted during the peeling process. To better understand these emissions, the sixth chapter examines the gas and particle phase emissions from a peeled orange, both with and without ozone being present.

The seventh chapter assesses d-limonene's effects on gases, particles and cultured human lung cells from an actual cleaning product. For these experiments, a second petroleum-based solvent was compared to d-limonene. The solvent chosen for the

comparison was 2-butoxy ethanol (2BE), a petroleum-based solvent with an estimated ozone forming reactivity value lower than d-limonene. For these experiments, two general purpose household cleaners were used, one containing d-limonene, the other 2BE. Cultured human epithelial lung cells were exposed to the cleaners and the individual solvents, both before and after oxidation of the VOC mixtures with ozone. The cells were then analyzed for inflammation and cell death induced by the exposure. The purpose of these experiments was to determine whether (1) the atmospheric reactivity of a solvent was indicative of the potential health risks, and (2) if the individual solvents behave similarly when they are present in pure form and when they are in a mixture.

In chapter eight, the findings from these experiments will inform a discussion on the current framework for controlling air quality. The fragmented organization of the EPA will be examined, paying particular attention to the ability to capture potential risk in the decision making process. Dose-response information gathered in the consumer products experiments will provide a case study example of the potential risks associated with regulating consumer products based on estimated reactivity.

2. Background

2.1. d-Limonene

d-Limonene is a chemical obtained from orange peels during orange juice processing. The orange rinds are pressed and the oil from this initial process produces *food grade* d-limonene. A steam extraction process obtains additional oil, which becomes *technical grade* d-limonene (Florida Chemical, 2006). This technical grade is used as a solvent in many cleaning products. d-Limonene is also emitted from a variety of other sources: cleaning products, air fresheners, flavor and fragrance additives, resins and adhesives, industrial cleaners, personal cleaners and deodorants, certain trees and bushes, citrus peels, dill, and celery (NICNAS, 2002). It has been measured indoors at concentrations between 5-12 ppb (WHO, 1998; FL-DACS, 2004; Tamás, 2006). Outdoor levels have been measured between 0-2 ppb (WHO, 1998). Although the U.S. does not have an occupational exposure limit for inhalation of d-limonene at this time, the Occupational Safety and Health Administration (OSHA) has set the occupational time weighted average (TWA) for turpentine (which contains d-limonene) at 100 ppm (OSHA, 1992), and American Industrial Hygiene Association (AIHA) has a guideline exposure level 8-hour TWA of 30 ppm for d-limonene (AIHA, 2005). Sweden has an 8-hour TWA for d-limonene exposure of 150 mg/m^3 (27 ppm). Human sensory irritation has been measured at 80 ppm (Larsen, 2000) and indoor levels have been recommended to be around 30 ppm (Kasanen, 1999).

2.2. Chemistry of d-Limonene

2.2.1. Pure d-Limonene

Several experimental studies have looked at the chemistry of d-limonene in indoor environments. Fan's experiments determined that in a mixture of 23 VOCs, d-limonene and α-pinene reactions with ozone were responsible for the majority of particle mass and formaldehyde generated in a simulated indoor environment (Fan, 2003). The major products identified in Fan's ozone/terpene-only reactions were formaldehyde and acetaldehyde, and the reactions generated secondary particle mass of 190 $\mu g/m^3$. Reactions between 48 ppm d-limonene and 4 ppm ozone produced four major products: AMCH (1-methyl-4-acetylcyclohexene), IOPH (3-isopropenyl-6-oxoheptana), formaldehyde and formic acid. These reactions also generated a 2-fold increase in particles over the background concentration when d-limonene was added and 4-fold increase over background when ozone was added to d-limonene (Clausen, 2001). When 10 mL of d-limonene was injected into a 25 m^3 chamber at three different conditions of relative humidity, and 2-3 bursts of 60-100 ppb of ozone were added, a significant increase in particle growth was observed in the 0.1-0.2 μm size range, while growth in the 0.2-0.3 μm size range did not occur until the third injection of ozone (Wainman, 2000). Tamás found that particle concentrations were much higher in low ozone/high d-limonene situations than high ozone/low d-limonene (Tamás, 2006).

Weschler attempted to study particle generation in "real-life" scenarios with experiments conducted in two adjacent offices, first by emitting an unknown concentration of d-limonene in both offices and 330 ppb ozone in one of the offices. This initially resulted in a sharp increase in only the smaller particle size bins in the ozone room, but after 17 hours, almost all

the size bins were greater in the room with ozone. The difference in particle mass concentrations ranged from 60-95 $\mu g/m^3$ (Weschler, 1999). An unknown concentration of a commercial cleaner (containing a-pinene, d-limonene and α-terpinene) was injected into both offices with an ozone concentration of 250 ppb into one office. Some increase in particle generation was observed. For the final experiment, ozone was allowed to entrain into the two offices from the outdoors while an unknown amount of d-limonene was emitted in only one of the offices. The ozone concentration averaged 23-28 ppb in the room with d-limonene and 5-10 ppb higher in the room without d-limonene. Weschler observed an increase in particle generation. The difference in mass concentrations between two rooms ranged from 2.5-5.5 $\mu g/m^3$.

In all of these studies, formaldehyde and fine particle generation have been observed at higher concentrations when ozone was present. Therefore, the presence of ozone in indoor environments is an important factor to consider when assessing the impact of the presence of d-limonene indoors. Fan states that reducing the amounts of VOCs indoors may be an overwhelming and troublesome task and emphasizes the importance of reducing outdoor ozone concentrations and reducing its seepage indoors (Fan, 2003). This will help reduce indoor terpene/ozone reactions. The research described above focuses on the emission and oxidation of pure d-limonene, which is beneficial in understanding its chemistry. This information, however, does not help determine its effect indoors when emitted as part of a mixture.

2.2.2. d-Limonene in Cleaning Products

Research has been conducted to examine cleaning products themselves. Nazaroff et.al. oxidized three cleaning products: an all purpose cleaner, a degreaser and an air freshener in

clean air and measured their reaction products (Nazaroff et.al., 2006). These experiments used ozone concentrations ranging from 30-250 ppb; and d-limonene concentrations ranging between 1-30 ppb, 100-230 ppb and 580-740 ppb. Several oxidations products were observed: formaldehyde, acetaldehyde, acetone, glycolaldehyde, formic and acetic acid; formaldehyde was of most concern to the authors. Results from these experiments show that when d-limonene was the only VOC present in the mixture, the number of oxidation products dropped to three: formaldehyde and formic and acetic acid. A set of "real-use" experiments were conducted using ozone concentrations between 114-120 ppb with the following consumer product amounts: ~50 g all-purpose cleaner (1.2% d-limonene), ~4-7 g degreaser (25% d-limonene) and an air freshener (~1.3% d-limonene). Formaldehyde was present at 7-8 ppb averaged over a 12-hour period when ozone was not present and 13-20 ppb when ozone was present. Without ozone, the cleaners generated particle mass concentrations ranging from 1-5 $\mu g/m^3$; and when ozone was present, the fine particle generation grew to 30 $\mu g/m^3$, with one experiment reaching 90 $\mu g/m^3$. The number of particles generated without ozone ranged from 230-620 particles/cm^3, and when ozone was present, these numbers increased to 750-1550 particles/m^3 for the air freshener and between 350,000-440,000 particles/$m^3$for the cleaner and degreaser. Nazaroff et.al. state that the main concerns raised by these experiments are the formaldehyde production and fine particle mass generation resulting from these cleaners when used in the presence of ozone. This research also raises specific concerns about the presence of d-limonene, terpenes and 2-butoxy ethanol (a glycol ether) in cleaning products.

Other research has also examined consumer products. A lemon-scented air freshener was placed in a chamber and ozone concentrations between 50-110 ppb were injected four

times over approximately twelve hours. While particle generation in the 0.1-0.2 μm size range began soon after the ozone injection, growth in the 0.2-0.3 μm size range did not occur until after almost ten hours, immediately following the fourth ozone injection (Wainman, 2000). These experiments infer that cleaning products containing d-limonene may have similar oxidation products and particle generation characteristics when compared to results from d-limonene only experiments. No research has been completed, until now, to directly compare pure d-limonene as the sole VOC and d-limonene in a VOC solvent mixture. While these consumer product research endeavors look at the chemistry of product use, they do not explore how the secondary reaction products and particles may affect human health.

2.3. Observed Health Effects

2.3.1. Mice Exposures

Mice have been the focus for exploring the health effects of d-limonene. Mice exposed to a 16 second aged mixture of 48 ppm d-limonene and 4 ppm ozone experienced approximately a 35% reduction in respiratory rate, but only a 10% reduction when exposed to 44ppm of d-limonene or 0.19 ppm of formaldehyde (Clausen, 2001). Therefore, a reaction product other than formaldehyde was affecting the respiratory rate. Mice exposed to 61 ppm d-limonene for 30 minutes experienced a 10% reduction in respiratory rate, and those exposed to 48 ppm d-limonene and 4-6 ppm ozone for 30 minutes experienced a 30% reduction in respiratory rate. When exposed to 1,014 ppm d-limonene and 4-6 ppm ozone for 30 minutes mice would experience a 50% reduction in respiratory rate (RD_{50}) (Wolkoff, 2000). Wolkoff measured less than 0.02 ppm of formaldehyde as a result of the d-limonene/ozone reaction and noted that to correctly model mouse-human exposures, the concentration levels needed to be 10 times higher than the potential human exposure.

Wolkoff found that all the terpene/ozone exposures resulted in greater irritation than the terpenes alone, and irritation caused by the d-limonene exposures were below its RD_{50}.

Mice exposed to 3.4 ppm ozone and 47 ppm d-limonene experienced prominent sensory irritation and airflow limitation, which were reversible and did not persist past 6 hours (Rohr, 2002). Rohr found that after 45 minutes the irritation from the ozone/d-limonene exposure normalized to the irritation levels of a d-limonene only exposure, showing that the oxidative effects only persisted for 45 minutes. Additionally exposure to oxidation products did not enhance sensory irritation or airflow limitations when the mice were challenged with a d-limonene only exposure (Rohr, 2002). While d-limonene/ozone exposure appears to have an immediate sensory response, it appears to be acute, reversible and is short-lived.

Larson exposed mice to 197-1,599 ppm d-limonene and found a No Observed Effect Level (NOEL) for mice of 900-1,600 ppm (Larsen, 2000). He extrapolated to determine that the human sensory threshold should be about 50 ppm and the occupational exposure limit should be 30-45 ppm. Yet men exposed to 450, 225, and 10 mg/m^3 of d-limonene did not have any irritation or central nervous system related symptoms (Falk-Filipsson, 1993). Therefore, mouse models may not be an effective way to assess human effects from d-limonene exposure.

2.3.2. Human Exposures

There have been few studies directly looking at the impacts of human exposure to limonene. The majority of the research has been focused on subjects detecting the presence of limonene or its oxidative products. Human subjects noted that perceived air quality drastically decreased in a room with d-limonene and ozone compared to rooms containing only d-limonene or only ozone (Tamás, 2006). In addition, whole-body human exposures of

1.8, 40 and 81 ppm to d-limonene did not result in consistent sensory irritation (Cometto-Muniz, 1998). Out of 47 chemicals inhaled by test subjects, d-limonene was ranked 12th of having the least intensity when smelled (Doty, 1978).

2.3.3. Occupational Exposures

There has been research attempting to assess terpene and d-limonene exposure in an occupational setting. An exposure assessment to solvents during graffiti removal found that workers' long-term exposure limits never exceeded Sweden's permissible limits (Anundi, 2000). While there were a few short-term exposures that exceeded the allowable limits, these only occurred in confined spaces such as elevators; the d-limonene limit of 300 mg/m3/15 minutes was never exceeded. While looking at the toxicology of exposure to metal degreasing with aqueous cleaners, Lavoue observed occupational exposure levels of 0.9 and 6 mg/m^3 for d-limonene from a 6% d-limonene solution (Lavoue, 2003).

2.3.4. Other Exposures

Exposures of d-limonene have been conducted to assess health effects through routes other than inhalation, looking at dermal irritation, kidney and liver impacts. The dermal lethal dose for 50% of the study population (LD_{50}) found in rabbits was greater than 5,000 mg/kg and eye irritation has been observed in rabbits when d-limonene was instilled in the eyes (WHO, 1998). The EPA has determined that the critical endpoint for characterizing effects is not the kidney, but the liver (EPA TRED 1996). The NOEL in livers of male rats is 150 mg/kg/day and 300 mg/kg/day for Lowest Observed Effect Levels (Kanerva, 1987). "Limonene is not mutagenic or a developmental toxicant" (EPA RED, 1994) and is not a carcinogen; in fact there is work that shows d-limonene may have anti-carcinogenic effects

(NICNAS 2002). These studies touch on other effects of d-limonene exposure that are studied, but this proposed research only explores the potential respiratory effects.

3. Hypothesis

Regulating household consumer products based solely on their reactivity (ozone formation potential) and possible detrainment outdoors may replace currently used biogenic solvents with petrochemical solvents that, while feasibly decreasing the localized anthropogenic volatile organic compound emissions, could increase the risk for health effects for consumers indoors.

4. Approach

The approach of this study was to examine the potential health risks from using consumer products indoors. Two household spray-cleaning products were chosen, one with a biogenic solvent, and the other with a petroleum-based solvent. The individual solvents in these cleaning products, d-limonene (biogenic) and 2-butoxy ethanol (petrochemical) were also examined in pure form, to contrast their behavior both alone and with that mixture. Cultured human epithelial lung cells were exposed to these two mixtures and to the individual neat solvents both before and after oxidation with ozone. The cells were examined for inflammation and cell death induced by the exposures. Prior to this work, the emissions from peeled oranges, the source of d-limonene, were analyzed both before and after oxidation with ozone to examine reaction products and secondary organic aerosol formation. This research concluded with a policy management assessment examining the structure of government and its ability to capture risk in its decision-making process.

5. Objectives

The necessary steps to evaluate the hypothesis are outlined below:

1. To evaluate the reaction products and secondary aerosol formation potential of compounds emitted from a peeled orange, with and without the presence of ozone.

2. To assess the indoor inhalation health risks from exposure to unreacted and ozone-reacted household cleaners and their principle solvents, d-limonene and 2-butoxy ethanol, by measuring the inflammatory response and cytotoxicity induced in cultured human epithelial lung cells from exposure to these mixtures.

3. To examine the different types of risks addressed by air pollution controls and the potential problems that arise from a fractured environmental regulatory management system in the United States.

6. Major Oxidation Products and Particle Formation Potential of Peeling Oranges in an Indoor Environment

Manuscript to be submitted to Indoor Air
with Seth M. Ebersiller[1], Kenneth G. Sexton[2] and Harvey E. Jeffries

[1] Assistance with experiments and particle-phase analysis

[2] Assistance with experiments and gas-phase analysis

6.1. Abstract

There are many sources of indoor emissions of volatile organic compounds (VOCs) such as furniture, cleaning products, air fresheners, appliances, and citrus products. Both federal and state regulations exist to control VOC emissions, as they are precursors of ozone formation. Recently, d-limonene, a biogenic VOC, has come under scrutiny, due to its high estimated reactivity (photochemical ozone-forming potential). d-Limonene is used in many cleaners, due to its effective solvent abilities and friendly odor. It is biodegradable and, as it is extracted directly from orange peels, has great potential as an environmentally friendly solvent. As interest grows in this solvent's use – and in regulating its use in consumer products – the source of this chemical, and its behavior in the environment, become interesting considerations. This study assesses the emissions, in both the gas and particle phases, from peeled oranges with and without the presence of ozone. Experiments to assess the emissions and indoor chemistry of an orange were conducted in a 120 m^3 smog chamber and a 300 L box reactor. Oranges were peeled in environments with four levels of ozone: 0, 35, 85, and 250 ppb. An increase in particle formation was measured at the initial peeling of the orange, after which the particle number concentration decreased over time. In the presence of ozone, the mass concentration of the particles increased greatly. Both formaldehyde and secondary organic aerosol formation were limited by the amount of ozone present in the system. Therefore, oranges peeled in an ozone-rich environment can generate significant levels of formaldehyde and secondary organic aerosols, thereby negatively impacting indoor air quality.

6.2. Introduction

d-Limonene is produced naturally from the oil in orange peels. It is collected during the orange juice-making process and used to produce technical and food-grade limonene products (FL Chemical, 2006). Over the past decade, regulations potentially controlling this chemical for its use as a solvent in consumer products have been increasing in number (EPA, 2007; CARB 2007). These regulations are based on concerns that using highly reactive volatile organic compounds (VOCs) indoors, such as the solvents in consumer cleaning products, are adding to outdoor ozone attainment problems. VOCs are precursors to ozone formation and ozone forming reactivity scales are employed to estimate the ozone-forming potential of VOCs. Regulators are concerned that gases from consumer products detrain outdoors, increasing local anthropogenic VOC emissions and, subsequently, local ozone production. As requirements are implemented for formulators to decrease the VOC-reactivity of their products, solvents with higher estimated reactivity values will be replaced with less-reactive compounds. d-Limonene is becoming a popular focus for these concerns, and may eventually be regulated out of these products completely.

To begin exploring the potential impact of this chemical on indoor air quality, the source of the chemical was investigated – namely an orange. Since oranges can be a major source of d-limonene indoors, they are an interesting focal point for further exploration of d-limonene's impact indoors. At this time, little work has been done to assess the VOC emissions from oranges. Outdoor emissions of d-limonene, α-pinene, β-pinene, sabinene, β-caryophyllene, and linalool have been measured above and around citrus groves in Spain (Cicciolo, 1999; Darmais 2000). These individual VOCs have been studied closely, but not as direct emissions from an orange.

To demonstrate ozone oxidation in the classroom, the exercise of putting an orange in a box with ozone has been in practice for many years. This classroom demonstration is usually comprised of injecting a high concentration of ozone into a clear box, and introducing orange peels to the box. Typically, a light is shown through the box, or the box is placed on an over-head projector. Observers are able to the see the secondary organic aerosols (SOAs) generated from the immediate oxidation of the d-limonene emitted from the orange peels. Here, we will go a step further by measuring exactly what is occurring in the "box."

This research examines the potential indoor impacts of peeling an orange, with and without the presence of ozone. Our purpose is to monitor the VOC emissions, secondary gas-phase products, and secondary organic aerosol formation from oxidation of a peeled orange at ozone levels potentially found indoors. Experiments were performed in two different reaction chambers, a 300 L Teflon-lined box reactor and a 120 m^3 Teflon outdoor smog chamber. The oranges were peeled inside these chambers with varying levels of ozone. The chamber air was sampled, and gas and particle-phase analyses were completed. The oranges used in these experiments were naval oranges grown for eating and obtained from a local grocery store. Due to the use of different chambers and protocols, the instruments, methods and results of the 300 L box reactor experiments will be described first, followed by the instruments, methods and results of the 120 m^3 chamber experiments.

6.3. 300 L Box Reactor Experiments

A 300 L box reactor was built to examine the primary and secondary products formed while peeling an orange in two different ozone concentrations.

6.3.1. Methods

To create an airtight environment in which an orange could be peeled, a 300 L box reactor was constructed. A cardboard box was lined on the bottom and the sides with Fluorinated Ethylene Propylene (FEP) Teflon film, and sealed with Teflon tape. A lid of Teflon film was taped across the top of the box, with one corner taped in such a way that it could be opened and resealed with minimal effort. Two holes were cut into the side of the box, and lab gloves with Teflon sleeves were mounted in the openings with Teflon tape, to allow an orange to be peeled inside the sealed chamber (Figure 6.1). A short Teflon sample line was mounted on the side of the reactor opposite the gloves. The box was then filled with ozone to condition the inside of the chamber.

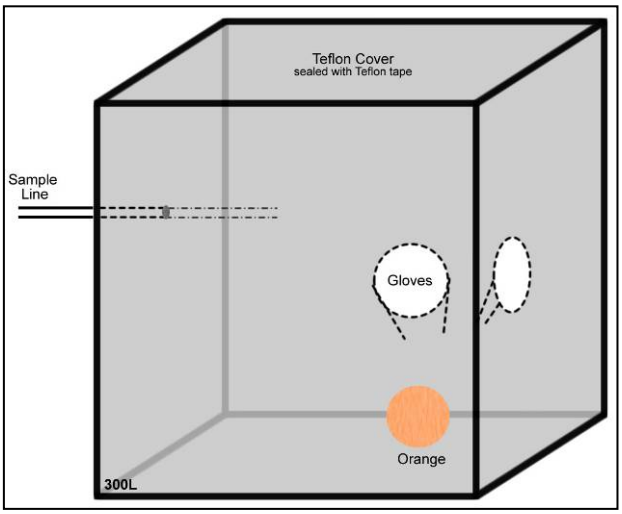


Figure 6.1 – 300 L Teflon-lined box reactor

Two experiments were conducted with this reactor, one with 35 ppb ozone (rural ambient ozone in Pittsboro, NC) and the other with 85 ppb ozone (a higher, urban ambient ozone concentration). To remove the possibility of photochemical reactions, and thereby better mimic indoor conditions, these experiments were performed in the laboratory. For the experiments, the reactor was sealed and then flushed with clean air before ozone was injected and its concentration confirmed. After initial background measurements were made with all the instruments (described in the next section), a small corner of the Teflon lid was opened for less than two seconds, just wide enough to get an orange into the reactor. Once the orange was in the reactor, the lid was quickly resealed with Teflon tape. The orange was

immediately peeled, and both the orange and its peelings were left inside the reactor. The reactor air was sampled with all instruments (both particle and gas phase analysis) over the next hour. During the experiment at the lower ozone concentration, additional samples were taken during the second hour. Mixing was achieved by hand movement during the peeling of the oranges, uniformly mixing the air in the reactor.

6.3.2. Instrumentation and Chemical Analysis

A TSI Scanning Mobility Particle Sizer™ (SMPS) Spectrometer was used to measure the size distributions and mass yields of the SOA. Mass concentrations assume a density of 1.0 g/ml. The parameters on the SMPS were optimized for particles with diameters in the 20-890 nm range. The SMPS was connected directly to the 300 L box reactor with Teflon tubing approximately two feet in length and sampled at various times throughout the experiments for 15-minute intervals. Formaldehyde was measured intermittently, using the automated Dasgupta-diffusion-tube sampler (Dasgupta 1988). Ozone was measured using an EPA standard reference method based on photometry with a Thermo Environmental Instruments Inc., Model 49 monitor.

Several instruments were used to measure the d-limonene and reaction products. A Varian capillary column (DB1 phase, 60 meter, 0.32 micron id, 1 micron film) STAR 3400 gas chromatograph with flame-ionization detector (FID) and Saturn 2000 ion trap mass spectrometer (GCMS) instrument system operating in electron-impact ionization mode and equipped with a liquid-nitrogen cryotrap for direct gas sample injection was used to measure chemical composition of the gas phase in the reactor. There was no derivatization of the compounds prior to analysis with the GCMS. The 300 L reactor was sampled periodically throughout the two experiments. For the 300 L reactor, an additional columnless GC-FID

system (Carle, Inc., Chandler Engineering, Tulsa, OK), was used to measure the total hydrocarbon (THC) concentration in the reactor.

6.3.3. Particle-phase results

85 ppb of ozone was used to conduct the first experiment. Prior to the introduction of the orange to the reactor, the background particle number concentration was low (3.5 x 10^9 particles/m^3, see Figure 6.2). After the orange was peeled in the box, there was a rapid increase in particle generation, resulting in a four hundred-fold increase in particle number concentration (14.4 x 10^{11} particles/m^3). This increase in particle number resulted in a rise in the particle mass concentration – an increase of 134 μg/m^3 within an hour of the orange being peeled (see Figure 6.3). The success of the classroom experiment described above depends on this immediate generation of SOA.

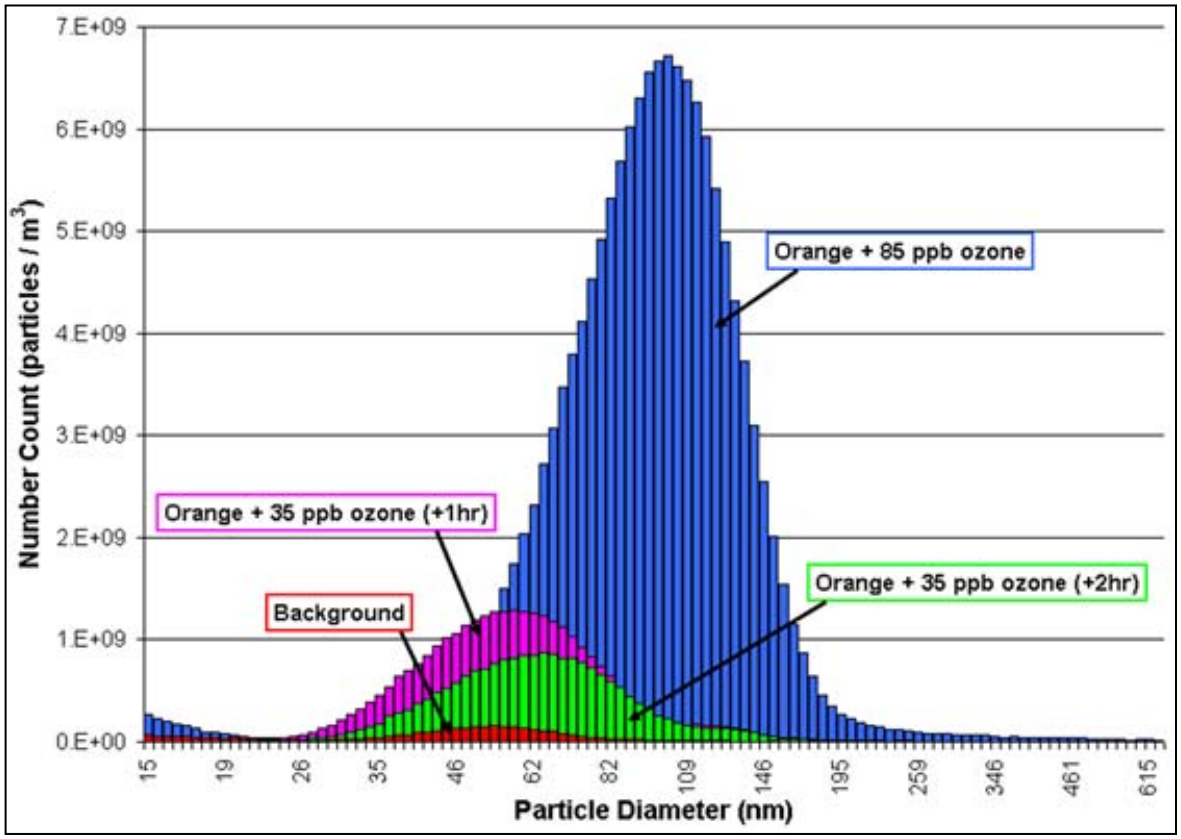


Figure 6.2 – Particle Diameter and Number Count

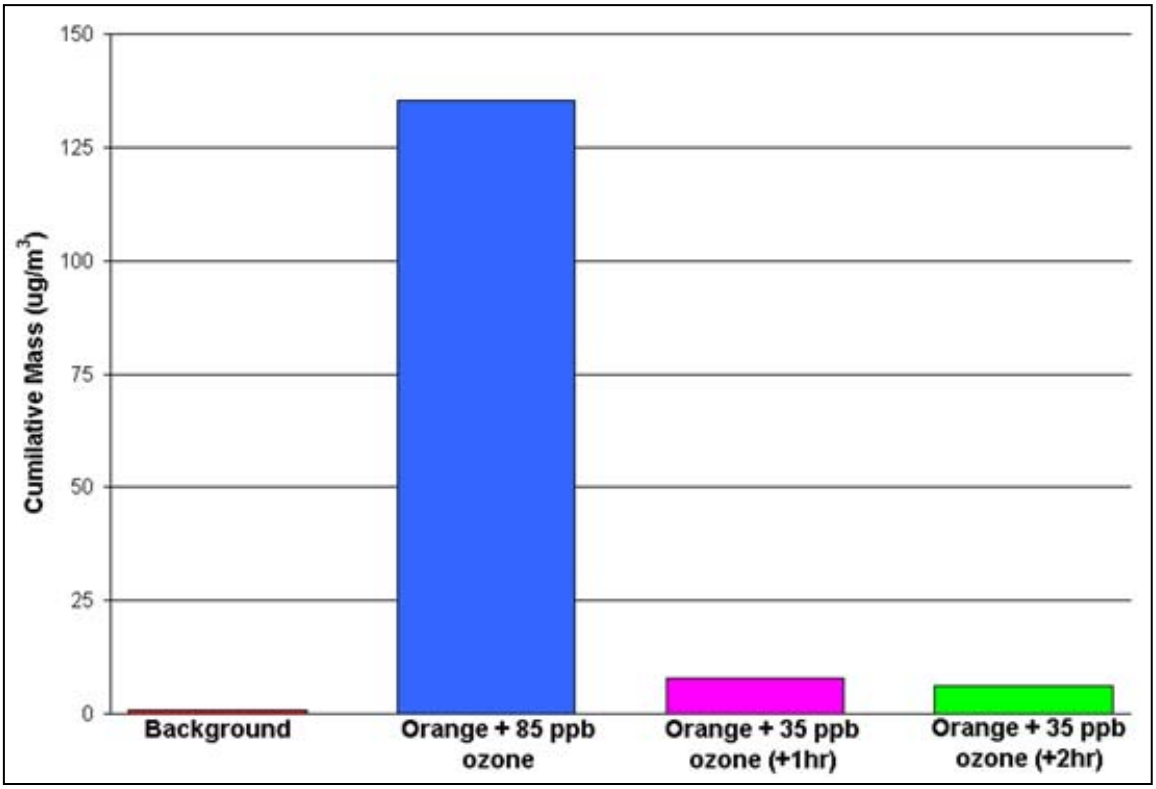


Figure 6.3 – Cumulative Mass

After the first experiment, the orange and peels were removed from the reactor, the insides were wiped down and the reactor was again flushed with clean air. As can be seen in Figure 6.2, a relatively high particle number concentration remained in the reactor after the clean-air flush (Exp 2 - Background). This could be due to ambient ozone reacting with d-limonene off-gassing from lingering orange debris in the reactor, resulting in the formation of very small particles.

The second reactor experiment used a lower ozone concentration of 35 ppb, as 85 ppb is not a concentration typically found indoors except in some more-polluted urban areas. Even with a background mass concentration of 9 $\mu g/m^3$ (compared to the nearly-clean background particle concentration in the first experiment (see "Exp 1 - Background" in Figure 6.2), the mass of the particles generated in this second experiment was far less than that formed when

the orange was peeled in 85 ppb ozone (see Figure 6.3). The particles were sampled approximately one and two hours after the orange was peeled, during which time the particle count deceased from 31,000 particles/cm^3 to 20,000 particles/cm^3 (see Figure 6.2). Figure 6.2 also shows that the average size of the particles was increasing as well, though not getting as large as the particles in the first experiment. The mass generated during this second lower-ozone experiment did not approach the levels seen in the first experiment, measuring only 8 $\mu g/m^3$ in the first hour and 6 $\mu g/m^3$ in the second. The decreased number count and mass concentration in the second experiment implies that ozone is the limiting factor in particle growth. To examine this assertion further, the gas-phase reactions will be analyzed.

6.3.4. 300 L box reactor gas-phase results

One hour after the orange was peeled in the presence of 85 ppb ozone, the d-limonene concentration was measured as 1,400 ppmC (140 ppmV) using the Carle GC-FID system. The d-limonene concentration in the reactor an hour after the second orange was peeled (in only 35 ppb ozone) was 1,700 ppmC (170 ppmV). Since the same size and type of orange was peeled in both experiments, it was assumed that equal amounts of d-limonene were emitted into the reactor. This higher concentration of d-limonene measured in the presence of lower ozone levels supports the assertion that ozone is the limiting factor in this reaction system. A second reading was taken during the lower-ozone experiment, and the d-limonene concentration had grown to 1,900 ppmC (190 ppmV). This increase in concentration was due to the continued emission of d-limonene from the orange peels, which were still sealed in the reactor. As no ozone remained with which to react, the d-limonene did not have a concentration sink, and therefore continued to grow in concentration with time.

The reaction of ozone with the orange peel emissions produced formaldehyde. The higher-ozone concentration experiment produced 14 ppb of formaldehyde during the first hour, while the lower-ozone concentration experiment produced only 3 ppb of formaldehyde over the same amount of time. This provides further support for ozone as the limiting factor in this reaction system – not only in SOA production, but also in formaldehyde production. However, measurements taken during the 35 ppb ozone experiment two hours after the orange was peeled indicated that, although the ozone/d-limonene reaction seemed to have slowed (if not stopped altogether), the formaldehyde concentration increased 2 ppb throughout that second hour, indicating that ozone may not be the only source of formaldehyde in the system.

Several other reaction products have been identified from d-limonene's oxidation with ozone, such as limonaldehyde and 4-acetyl-1-methyl-cyclohexene (AMCH) (Atkinson 2003). AMCH was identified in the chromatograms, though not quantified, and limonaldehyde was unable to be identified.

6.4. 120 m^3 Outdoor Smog Chamber Experiments

6.4.1. Methods

Once the results from the 300 L box reactor were examined, it was decided that the experiment should be repeated in the larger chamber for three reasons: (1) to increase the volume to surface ratio, (2) to decrease potential wall-loss, and (3) to collect continuous measurements of the particle and gas phases. The 120 m^3 outdoor smog chamber used in this study is located on the UNC campus in Chapel Hill, NC (see Figure 6.4). It is lined with Fluorinated Ethylene Propylene (FEP) Teflon film. The dilution rate was measured, and is between 1-2% per hour. A two-part experiment was conducted in the chamber: (Part 1) an

orange was peeled in the chamber in the absence of ozone and (Part 2) 250 ppb ozone was injected into the chamber and allowed to react for 50 minutes. Particle- and gas-phase samples were taken continuously with instruments described in the next section. Though this chamber is outdoors, the experiment was performed at night to simulate indoor conditions by removing the possibility of photochemical reactions. Since the previous experiments demonstrated that ozone was the limiting factor in those reactions, a very high concentration of ozone was chosen to increase the concentration of oxidation products and SOA formation.

Figure 6.4 – 120 m^3 Outdoor Smog Chamber

During the afternoon before the experiment, the chamber was vented with a clean air generator, closed and dehumidified to prevent condensation on the chamber walls during the experiment. After sundown, an access door in the floor of the chamber was held open just wide enough to get the orange and two hands into the chamber, allowing the orange to be peeled directly in the chamber. The orange was peeled on a paper towel in approximately 1.5 minutes. The door was closed immediately after the orange was peeled and the peels were left in the chamber. Continuous measurements were taken of the chamber air for 35 minutes, after which the chamber door was opened slightly and the paper towel containing the orange and peelings was removed. 250 ppb of ozone was then injected into the chamber at 10 ppb/minute for 25 minutes. After the completion of the ozone injection, the chamber air was

allowed to react for an additional 25 minutes. For those 25 minutes of ozone injection, two mixing fans in the chamber were turned on to provide uniform mixing of the air within the chamber.

6.4.2. Instrumentation and Chemical Analysis

The same model SMPS described for the 300 L reactor experiments was connected to the 120 m^3 chamber with twelve feet of grounded, stainless steel tubing and carbon-impregnated silicone tubing to minimize particle loss between the chamber and the instrument. During the experiment in the large chamber, the SMPS sampled continuously. Formaldehyde was measured continuously, using the automated Dasgupta-diffusion-tube sampler (Dasgupta 1988). Ozone was measured using an EPA standard reference method based on photometry with a Thermo Environmental Instruments Inc., Model 49 monitor.

The direct injection GCMS-FID described for the 300 L reactor experiments was also used for the 120 m^3 chamber experiment. There was no derivatization of the compounds prior to analysis with this GCMS. Teflon tubing connects the GCMS to the 120 m^3 chamber. Air was drawn continuously from the chamber, and was analyzed approximately every 60 minutes throughout the experiment. Two additional GCs were used for the chamber. The first was a Varian packed-column (10%TCEP on 100/120 mesh Chromosorb PAW, 6 ft by 1/8 inch ID stainless steel) STAR 3800 gas chromatograph with a flame-ionization detector (FID). The second was equipped with an electron-capture detector (ECD). All GCs were located in the chamber lab, directly below the chamber – allowing short sample lines to go directly from the chamber, through the roof, and to the instruments for analysis. Calibrations were performed with standards prepared from pure compounds.

6.4.3. Particle–phase results

With the SMPS connected directly to the chamber, the particle phase could continuously be monitored. This provided a more complete record of the dynamic processes as they happened, and therefore more significant insight into the SOA formation occurring in the chamber. The dramatic increase in particle count resulting from the orange being peeled is represented by the first peak in Figure 6.5. This particle generation occurred very quickly, and peaked at 4.2 x 10^{11} partcles/m^3. Over the next hour the number declined, but the median particle diameter increased from 37 nm to 82 nm (see Figure 6.6). This increase in particle diameter resulted in an increase in mass concentration from 30 µg/m^3 to 60 µg/m^3 (see Figure 6.7). Note that the background mass in the chamber before the orange was peeled was 3 µg/m^3.

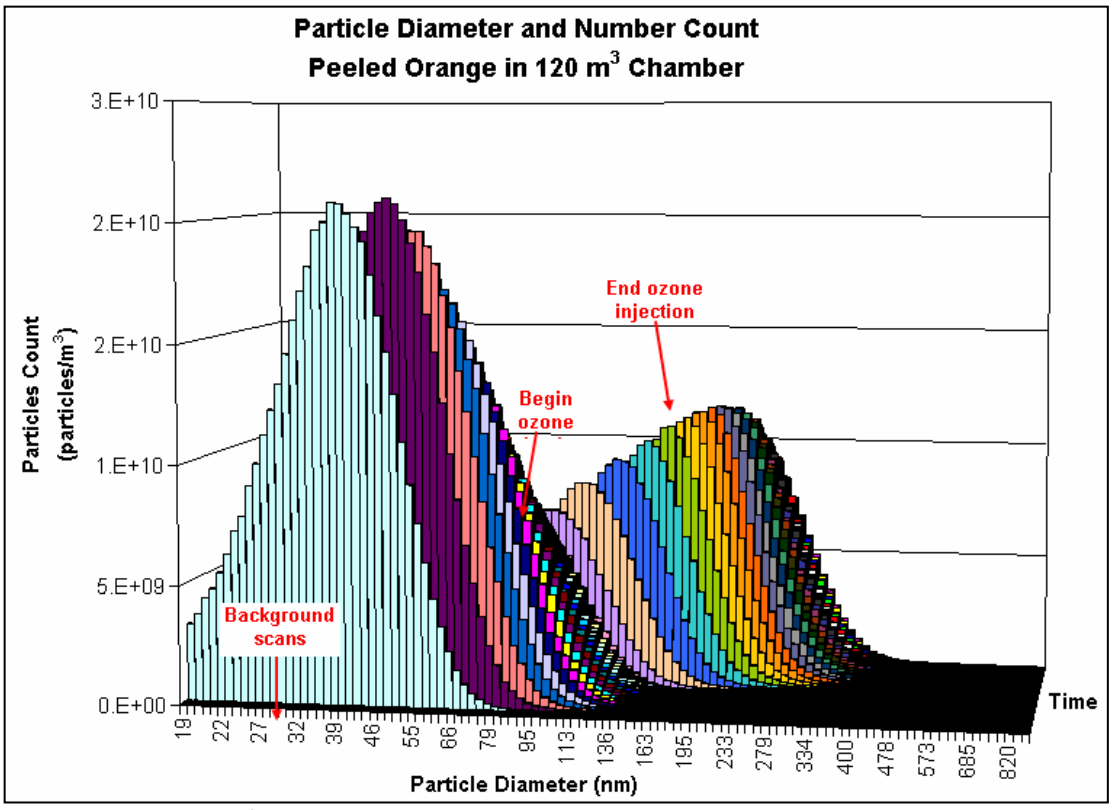


Figure 6.5 – 120 m^3 Chamber Particle Diameter and Number Count

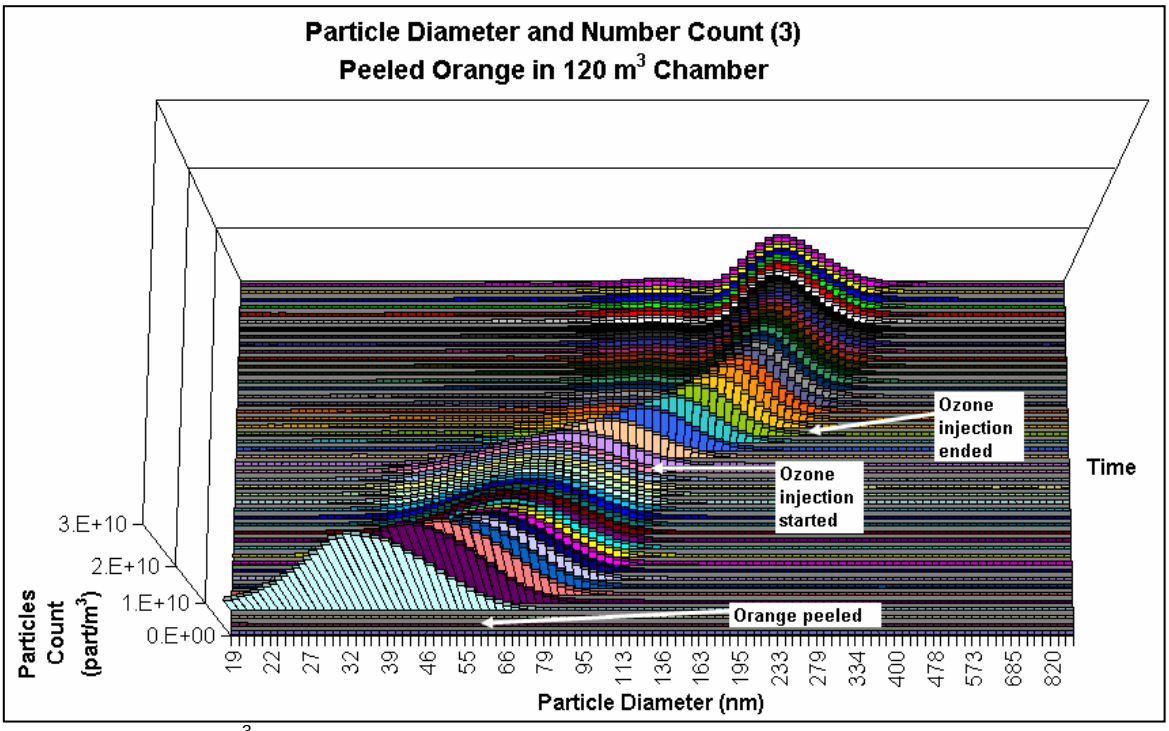


Figure 6.6 – 120 m^3 Chamber Particle Diameter and Number Count – Top View

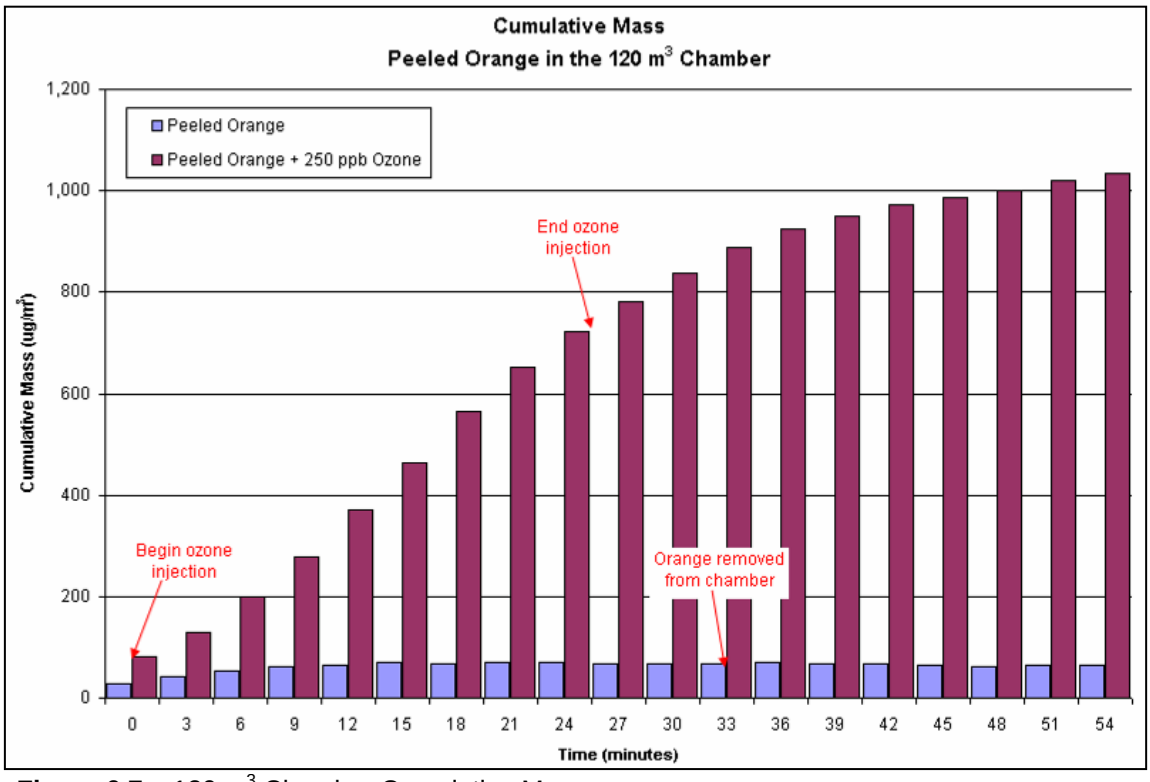


Figure 6.7 – 120 m^3 Chamber Cumulative Mass

Approximately one hour after the orange was peeled (twenty minutes after the orange and its peels were removed from the chamber), 250 ppb ozone was injected into the chamber at a rate of 10 ppb/minute. The addition of ozone to this simulated indoor environment was immediately evident by new particle generation (see Figures 6.5 and 6.6). In this second phase of particle production, the median diameter quickly increased to 188 nm during the 25 minutes of ozone injection, and then slowly continued to increase to 225 nm over the next 50 minutes. This growth was also apparent in the analysis of the cumulative mass concentration (see Figure 6.7). The mass increased rapidly from 60 $\mu g/m^3$ to 650 $\mu g/m^3$ during the ozone injection, and then continued to grow at a slower rate over the next 50 minutes, until it reached a concentration of roughly 1 mg/m^3. These data show how the particles present at the time of ozone injection grew greatly in mass, but decreased in number when ozone was injected into the system.

6.4.4. Gas–phase results

Figure 6.8 shows the gas-phase concentrations of selected components of the chamber mixture. At time 21.00 the orange was peeled in the chamber and it was observed that 28 ppb of ozone was also present in the chamber. NO was immediately added to the chamber to titrate out the ozone, as evidenced by the decrease in ozone and minor increase in formaldehyde concentration in Figure 6.8 (formaldehyde formation was due to ozone oxidation of the VOCs emitted from the orange peel prior to its removal from the system). With no ozone in the chamber, the d-limonene emitted from the orange increased to 370 ppbV in approximately 30 minutes (time 21.50). At this time, the orange and its peels were removed from the chamber, stabilizing the d-limonene concentration in the chamber. At time 22.00 (one hour later), ozone was injected into the chamber at 10 ppb/minute for 25 minutes.

When ozone was re-introduced into the system, it began immediately reacting with d-limonene, decreasing the concentration of each in the chamber. The green line on the graph indicates the expected growth in ozone concentration (had there been no reactions occurring), and the red line shows the actual ozone concentration measured in the chamber. Due to d-limonene's rapid interaction with the ozone, the measured ozone in the chamber did not increase at the rate at which it was being injected, and therefore never reached a measured concentration higher than 86 ppb. Within one hour, the d-limonene concentration decreased from 370 ppbV to 80 ppbV.

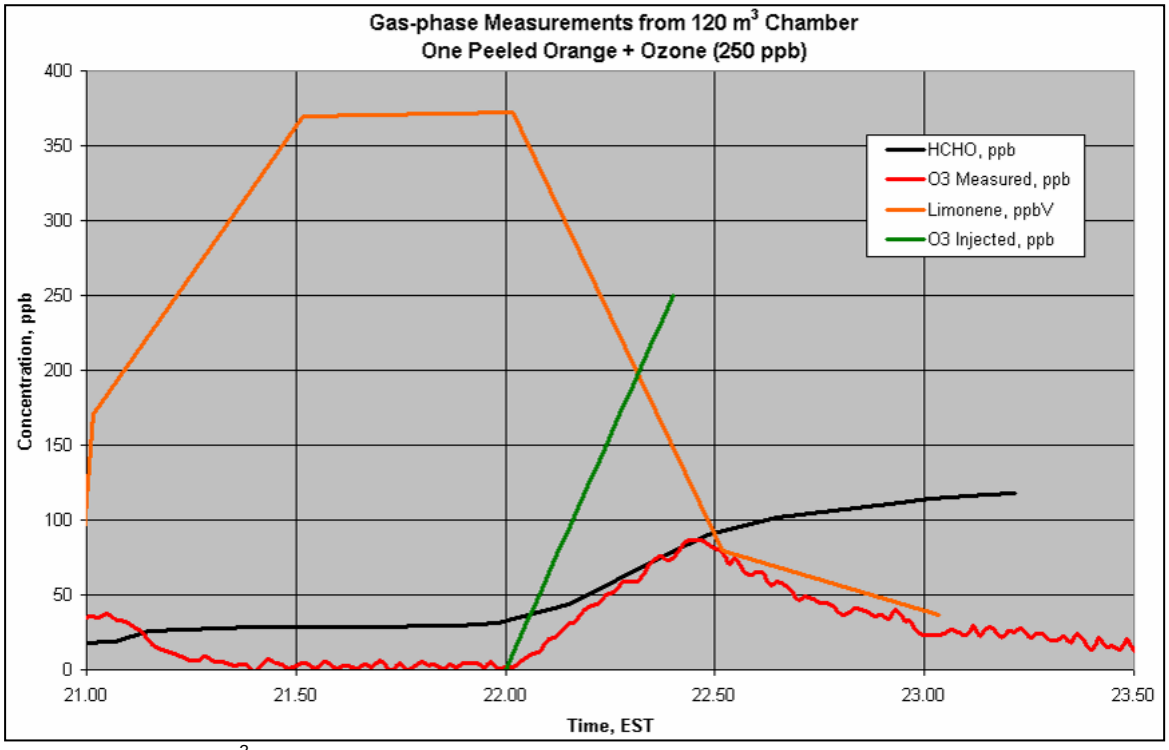


Figure 6.8 – 120 m^3 Chamber Gas-phase Results

d-Limonene's reaction with ozone produces a number of secondary products mentioned earlier, including formaldehyde, limonaldeyde and AMCH. The concentration of formaldehyde in the gas-phase more than doubled in the hour after ozone was introduced to

the system. The dramatic increase in concentration of another secondary product is immediately apparent by inspection of GCMS data (Figure 6.9). The top chromatogram in Figure 6.9 shows the chamber contents approximately 20 minutes after the orange was peeled. The bottom chromatogram shows the same chamber mixture, but 30 minutes after the start of the ozone injection. The first peak, which dwarfs all other peaks, is d-limonene. The second major peak is that of AMCH, a major gas-phase oxidation product of limonene's reaction with hydroxyl radicals and, to a far lesser degree, ozone. These plots show a 64% decrease in d-limonene, which resulted in a 900% increase in AMCH. A second common oxidation product of d-limonene, limonaldehyde, was expected at approximately 52 minutes (Leungsakul, 2004), but it was not detected in the gas phase.

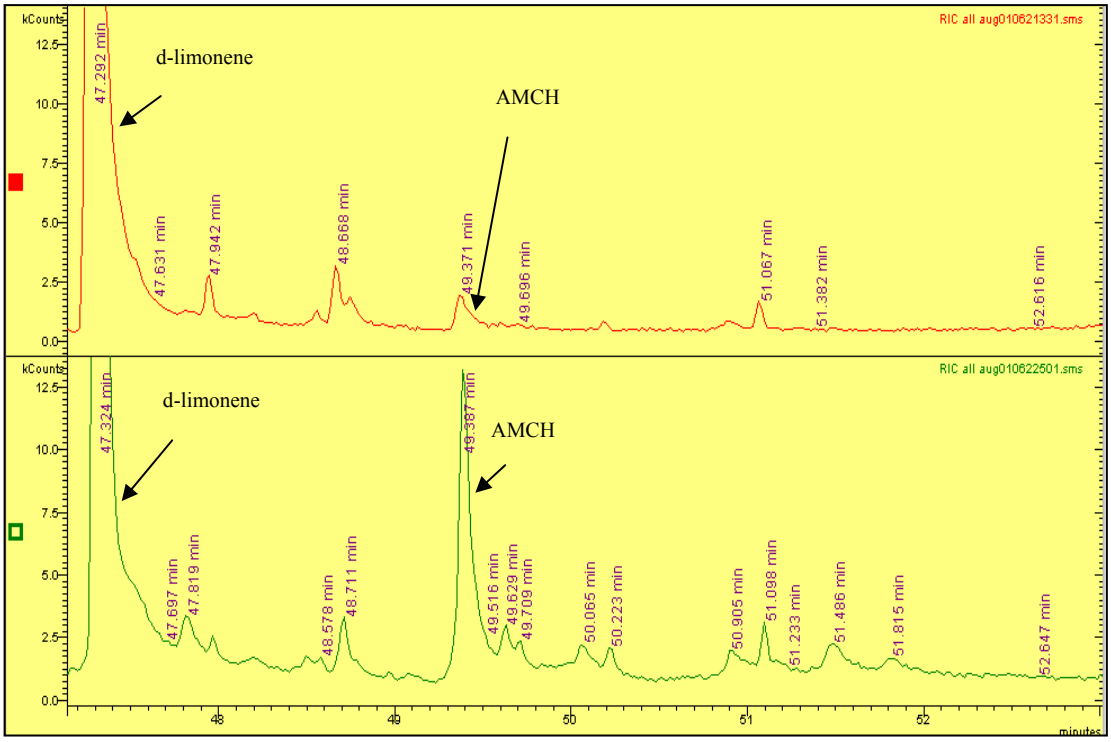


Figure 6.9 – Gas-phase GCMS Peaks

6.5. Conclusions

This research reveals the importance of ozone to the generation of formaldehyde and AMCH, as well as to increasing the mass of SOA in an indoor setting. Although oranges alone can produce a significant number of small particles, the addition of ozone to the system not only results in particle growth, but also produces potentially harmful oxidation products, such as formaldehyde. This research is intended to call attention to the importance of everyday sources of VOCs found indoors. Oranges are just one of many examples of everyday objects (food, furniture, paint, plants, room deodorizers, etc.) that may emit chemicals that have the potential to form particles as well as other products, with and without ozone present.

The focus in these experiments was to examine the characteristics of d-limonene emitted from its source. The next set of experiments will look at d-limonene found in other common household products, consumer-cleaning products.

7. Respiratory Health Effects Observed from Exposure to Household Cleaning Products

Manuscript will be submitted to Environmental Health Perspectives with Seth M. Ebersviller[3], Melanie L. Doyle[4], Kim M. deBruijne[5], Kenneth G. Sexton[6] and Harvey E. Jeffries

Title: Toxicity Induced in Cultured Human Epithelial Lung Cells from Exposure to Biogenic and Petrochemical Solvents, Alone and in Household Cleaning Products

[3] Assisted with gas-phase analysis and experiments

[4] Performed toxicological processing

[5] Assisted with toxicological exposures during the experiments

[6] Assisted with all chemical analysis and experiments

7.1. Abstract

There is growing interest in switching from petroleum to biogenic-based cleaning products. States are bringing forth regulation to control product compositions based on reactivity (ozone forming potential), yet there are other health impacts from exposure to these cleaning products that cannot be described merely by assessing their reactivity. Several issues should be considered when evaluating the impacts of consumer products. While determination of the ozone forming potential is important, the possible toxic effects from the components or their reaction products should not be ignored. To look more closely at the chemistry and relative health risks of consumer products, two cleaning products, one containing d-limonene and the other 2-butoxy ethanol (2BE), a glycol ether, were oxidized by mixing with ozone in clean air. To assess the contribution of the individual solvents to the observed chemistry and toxicity of the cleaning products, oxidation experiments were repeated with pure, reagent grade solvents. Cultured human lung cells were exposed to both the gas and particle phases of the mixtures. Detailed chemical and particle composition analyses were performed both before and after oxidative aging, using a standard GC/MS-FID to determine chemical composition and a TSI SMPS instrument to evaluate the size distributions and mass concentrations of the particulate matter. The results from these experiments show that a household spray cleaner containing 2BE induces more respiratory toxicity than a household spray cleaner containing d-limonene. Neither d-limonene nor 2BE exhibited chemical or toxicological behavior that corresponded to the mixtures containing these solvents. Assessing the indoor chemistry and the potential health effects of both primary and secondary toxics presents a more balanced approach for evaluating the environmental and health impacts of consumer products and their formulations.

7.2. Introduction

As interest in air quality increases, the concerned consumer is turning to more "green" purchases and biogenic products are becoming more popular. In fact, in 2002 the federal government implemented the Farm Bill that "requires Federal agencies to establish procurement preference programs for biobased products and to purchase these products" (OFEE, 2006). At the same time, states are bringing forth regulations to control product compositions based on reactivity (ozone forming potential) which may deter some manufacturers from using biobased organic solvents.

For example, California is moving towards regulating volatile organic compounds (VOCs) in consumer products based solely on their ozone forming reactivity, using the maximum incremental reactivity (MIR) scale (CARB, 2007; Carter, 1998). The California Air Resources Board (CARB) initiated this shift after determining that VOC emissions from consumer products accounted for 10% of the total VOC emissions statewide in 2005 (CARB, 2006). Therefore, the state feels that regulating the composition of consumer products is a good way to reduce their outdoor VOC emissions, thereby decreasing the formation of ozone in their urban air and helping the state to meet the ozone National Ambient Air Quality Standard (NAAQS). There is potential danger in only addressing this problem from an outdoor air quality perspective – it does not consider the impacts to the indoor air quality and potential health risks.

Rules limiting the ozone forming reactivity of consumer products based on their outdoor ozone impacts could potentially regulate some solvents out of use. For example, d-limonene, a biogenic solvent derived from orange peels, has an estimated MIR value of 3.99. This higher MIR value could likely exclude it from many product formulations. This chemical is

naturally emitted from oranges and is naturally present in the environment regardless of its use as a solvent in consumer products. It is approved as a food additive and has been used in many confections, such as chewing gum, for decades. If d-limonene is removed from consumer products due to its high MIR value, it may be replaced by materials such as glycol ethers, complex and chlorinated phosphates, petroleum-based surfactants, petroleum distillates and solvents, formaldehyde, amyl acetate, methyl ethyl ketone (MEK), benzene, perchloroethylene, hydrofluoric acid, or chlorinated hydrocarbons (Henneberry, 2005). Several of these chemicals, including glycol ethers, are on a list of "chemicals to avoid, or chemicals considered severe pollutants" by the National Parks Service (NPS, 2003). This assessment was based on the Janitorial Products Pollution Prevention Project, which was funded by several sponsors including the USEPA and CalEPA (WRPPN, 1999). By removing solvents, such as d-limonene, from consumer products used indoors and replacing them with chemicals from the list above, consumer products could pose a greater risk to human health, especially when considering the substantial fraction of time people spend indoors. Therefore, regulations to improve the outdoor environment may inadvertently increase health risks for the consumers that use those products. While the determination of the ozone forming potential of consumer products is very important, the possible toxic effects from the product components or their reaction products cannot be ignored.

In this study, we examined the potential impact of cleaning products on the indoor environment using an example of common household cleaning products containing either a petroleum or biogenic-based solvent. To follow the recent trend in biobased solvents, we chose to study d-limonene, for which no federal inhalation exposure limit has been set. Sweden has an eight-hour time-weighted average (TWA-8hr) exposure limit of 27 ppm

(FDACS, 2004) and the American Industrial Hygiene Association has set a TWA-8hr guideline exposure level of 30 ppm (AIHA, 2005). Indoor concentrations have been measured from 2 to 480 g/m^3 (WHO, 1998). d-Limonene's indoor chemistry has been studied at concentrations ranging from 40-115 ppb (Tamas, 2006), and potential health effects using mouse models have utilized d-limonene concentration ranging from 48-1,599 ppm (Clausen 2001, Larsen 2000, Rohr 2002, Wolkoff 2000). Larsen, using a human-mouse model predicted that the human sensory threshold should be approximately 50 ppm, but 80 ppm has not induced sensory irritation in humans (Larsen, 2000). This suggests that some human-mouse models may not be perfect. The goal of this study is to assess the human health risks from realistic exposure scenarios.

Much of the previous work has examined individual compounds, their chemistry and their health effects, but not as they appear in everyday life: in a mixture. While recent work has been published which examined cleaning products containing glycol ethers and terpenes (Singer 2006), it is hard to extrapolate those results into realistic exposures and effects. Therefore, we propose a two-tiered experimental design; Part 1 utilizes cleaning products to assess their potential health effects. Most of the previous work has looked at d-limonene alone, thus in Part II we want to determine whether it is appropriate to use individual compounds as proxies for their behavior in mixtures. The experiments below examine these compounds, as well as how they behave in a mixture. As reactivity continues to be a major focus in ozone control measures, we also want to examine compounds with different MIR values to determine whether reactivity is a good predictor for indoor chemistry and health risks. Finally, with the current trend towards "greener" products, we want to examine solvents from both biogenic and petrochemical sources.

With all this in mind, we decided to study two solvents: d-limonene and 2-butoxy ethanol (2BE), a glycol ether which is petroleum based and has a lower estimated MIR value (2.88) (Carter, 2003). The experiments described below are designed to investigate the potential respiratory toxicity and indoor chemistry of these two chemicals, individually and in a mixture, before and after oxidation with ozone.

7.3. Methods

7.3.1. Product Choice

To determine which products would be most appropriate for these experiments (e.g. widely available, comparable formulations, similar indoor use), a large variety of general-purpose spray cleaners were obtained. Two household cleaners were chosen, one with d-limonene as an active solvent (HC_{Lim}) and the other with 2BE as the active solvent (HC_{2BE}). The products were chosen because they are from the same manufacturer and intended for the same purpose, suggesting that the basic cleaning product mixtures would be comparable. In addition, they are widely available in supermarkets and home improvement stores. For the individual compounds, both the d-limonene and the 2BE were obtained from Sigma-Aldrich (St. Louis, MO): (R)-(+)-Limonene, 97%, and Ethylene glycol butyl ether ≤ 99%, liquid.

7.3.2. Experimental Design

Nine experiments were conducted to measure the inflammation and cytotoxicity resulting from an acute inhalation exposure to cleaning products and their solvents. A range of concentrations was considered for simulating different levels of use; these amounts were based on previous real-use experiments (Nazaroff, 2006). Two concentrations were chosen for the cleaning solvents, “low” and “high” concentrations, allowing for two different use scenarios. Two concentrations also provided more informative indoor chemistry and health

toxicological data. Four experiments were conducted with d-limonene, two with a d-limonene containing household cleaner (HC_{Lim}) and two with d-limonene alone. Four experiments were conducted with glycol ether, two with a household cleaner (HC_{2BE}) containing 2BE and two with 2BE alone. The household cleaner, or mixture, experiments were conducted first; during which the amount of solvent in the mixture was measured. This measured concentration of the solvent was used for the individual solvent experiments. This method allowed equal amounts of the solvents to be compared for their effects alone and in a mixture. The exact concentrations used in these experiments can be found in Table 1. The amount of 2BE in the low concentration mixture experiment (HC_{2BE} – low) was miscalculated, which resulted in 265 μL of 2BE to be injected for the low individual glycol ether experiment. This error was corrected for the high concentration experiment. The results published below focus on the high concentration experiments.

Table 7.1: Concentrations used in experiments

Experiment	Amount	Concentration of solvent
HC_{Lim} – low	8 mL	5.9 ppmC of d-limonene
d-Limonene – low	480 μL	5.9 ppmC
HC_{Lim} – high	22 mL	16.2 ppmC of d-limonene
d-Limonene – high	1,300 μL	16.0 ppmC
HC_{2BE} – low	11 mL	6.7 ppmC of 2BE
2BE – low	265 μL	2.4 ppmC 2BE
HC_{2BE} – high	22.5 mL	13.7 ppmC of 2BE
2BE – high	1,460 μL	13.8 ppmC 2BE

In all eight of these experiments, measurements and exposures occurred both with and without the presence of ozone. We used a concentration of 100 ppb ozone to simulate

ambient indoor levels. Room-scale experiments with cleaners containing d-limonene used ozone concentrations ranging from 30-250 ppb (Nazaroff 2006). While 40 ppb ozone is generally considered a more realistic urban background level, 100 ppb, a concentration lower than most previous d-limonene experiments, was chosen to examine a "worse-case scenario" and to ensure that we would see the full extent of the chemistry occurring in the indoor simulations. The ninth experiment was conducted with 100 ppb ozone alone, to observe any toxicity induced by the ozone exposure (see Figure 7.1). No significant inflammation or cell death resulted from the ozone only exposure. Therefore, the toxicological results presented in this paper are due solely to the individual solvents, the cleaning product mixtures and their oxidation products.

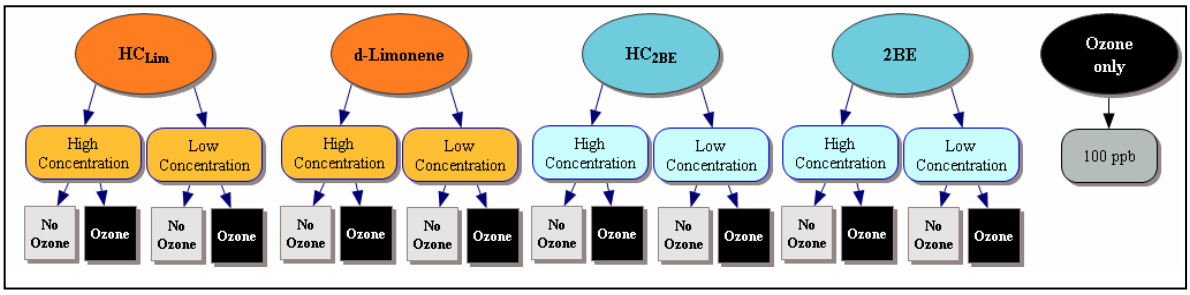


Figure 7.1: Experimental Layout, Two experiments were conducted with each household cleaner and individual solvent, at high and low concentrations. Samples and exposures occurred both before and after oxidation with ozone. An additional experiment exposed the cells to ozone alone.

7.3.3. Smog-Chamber/In-Vitro Exposure System

The experiments in this study were conducted in an outdoor smog chamber coupled with a gaseous exposure system (modular incubator chamber) (Sexton, 2004) and an electrostatic aerosol *in vitro* exposure system (EAVES) (de Bruijne, 2007), see Figure 7.2. This system allows human epithelial lung cells to be exposed to particles and gases from the chamber, while continuous samples are taken for chemical analysis. The smog chamber consists of a 120 m^3 outdoor chamber that is lined with Fluorinated Ethylene Propylene (FEP) Teflon film

and located in Chapel Hill, North Carolina (http://www.oneatmosphere.unc.edu/facilities.html). This chamber, which has a leak rate of 1-2 % per hour, is evaluated at the start of each experiment season and periodically throughout the summer.

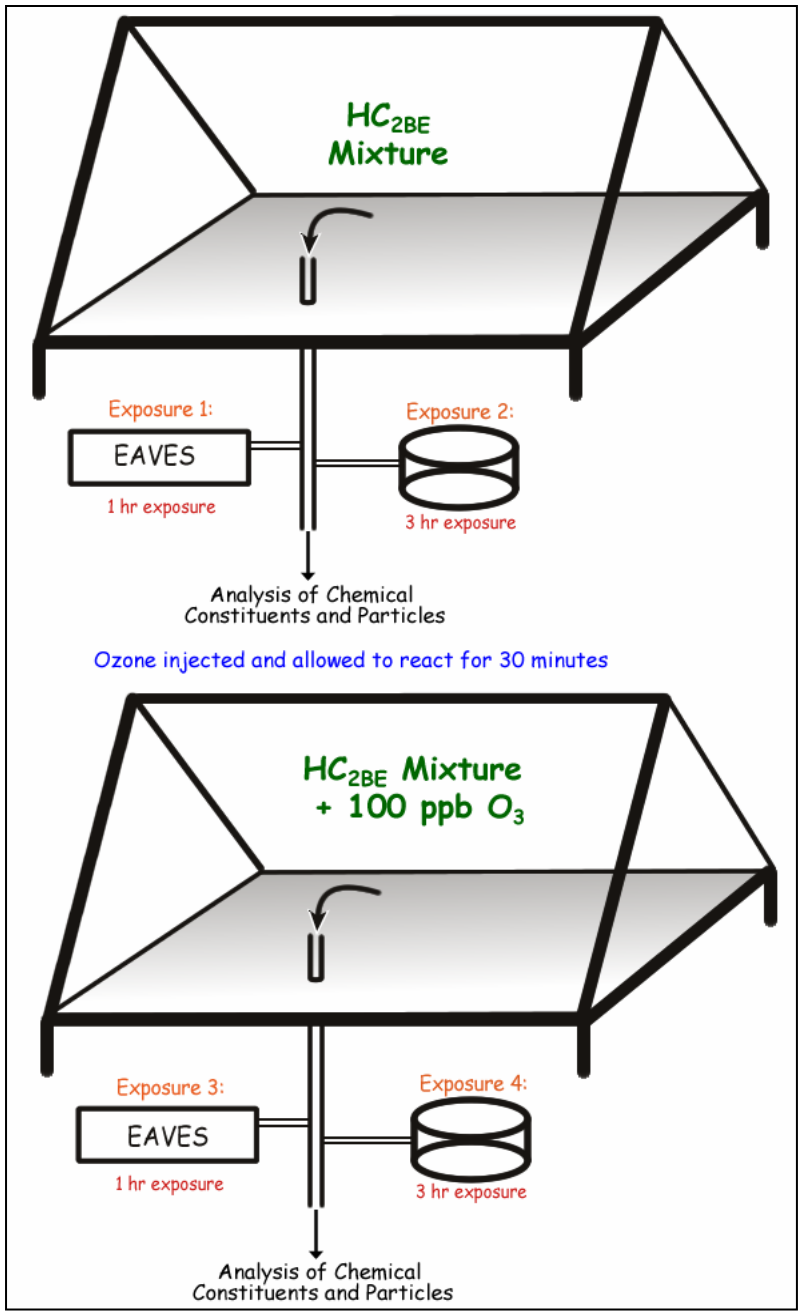


Figure 7.2: Experimental Design, The mixture was placed in the outdoor smog chamber at night to simulate indoor conditions. The cells were exposed to the particles (Exposure 1: EAVES) and the gases (Exposure 2: modular incubator chamber) as samples were taken for chemical analysis. 100 ppb ozone was injected into the chamber and allowed to react for 30 minutes. The cells were then exposed to the particles (Exposure 3: EAVES) and the gases (Exposure 4: modular incubator chamber) as samples were taken for chemical analysis.

All of the experiments occurred during the summer months, between late May and early August on evenings with approximately the same weather conditions, thus minimizing fluctuating exposure and chamber conditions that may modify the experimental outcome. The chamber is located on the roof of a building on the University of North Carolina at Chapel Hill campus, directly above the lab where chemical sampling and cell exposure were conducted. This allowed short sample lines (approximate length 14 feet or 4.3 meters) to connect directly from the chamber to the sampling instruments. The experiments were conducted at night to simulate indoor conditions by not allowing any photochemical reactions. During the afternoon prior to an experiment, the chamber was vented with a high efficiency particulate air (HEPA) filter, closed and dehumidified to prevent condensation on the chamber walls after sundown. The dew point and temperature were monitored throughout the night to ensure that no condensation occurred. Any ozone remaining in the chamber was titrated out with the addition of NO once the sun dropped below the horizon. Total solar and ultra violet (UV) radiation were continuously monitored in the chamber to ensure an experiment did not start until 0 UV was measured in the chamber, simulating an indoor environment.

Once the sun dropped below the horizon, the household cleaning product was injected into the chamber using a nebulizer system. This system flushes air through the mixture to create a fine mist that was injected directly into the chamber – which attempts to simulate the mist expelled from a spray cleaner's bottle during actual use. For the individual solvent experiments, the chemicals, precisely measured with a syringe (Hamilton GASTIGHT ®, Reno Nevada), were evaporated into the chamber using a u-tube and a heat gun. All of these injections occurred near a mixing fan to ensure uniform distribution of the injected material.

Once the injection was complete, the cells were exposed to air in the chamber. The actual cell exposure procedure is described in the next section. After the first cell exposures were complete, 100 ppb of ozone was injected into the chamber with an ozone generator for 10 minutes at a 10 ppb/min flow rate, and mixing fans were turned on for approximately ten minutes. Once the ozone was allowed to react for 30 minutes, the second set of cells were exposed to the air in the chamber. Samples for the chemical analysis were taken continuously prior to and throughout the experiment and are described in further detail below.

7.3.4. Toxicological Exposure and analysis

A549 cells, a cultured human lung epithelial cell line, were cultured in F12K medium plus 10% fetal bovine serum and 1% penicillin and streptomycin (all from Invitrogen, Carsbad, CA) (Doyle, 2004; Sexton, 2004; Jaspers, 1997). Upon confluency, the culture medium was replaced with serum-free media (F12K, 1.5 µg/ml bovine serum albumin, and antiobiotics). Just before each exposure, media located in the apical chamber was aspirated, while media in the basolateral compartment remained. This facilitates direct exposure of lung epithelial cells to pollutants without significant interference of media, yet the cells are maintained with nutrients throughout the exposure.

The cells were exposed to the gas-phase portion of the chamber contents for three hours. This system consists of a modular incubator chamber through which chamber air was pulled at a rate of 1 lpm. The cells were exposed to the particle-phase portion of the chamber contents for one hour using EAVES. EAVES is an electrostatic precipitator that pulls air from the chamber through the instrument between two charged plates. The cells are located between these two plates in a dish that is even with the bottom plate, thus not affecting

airflow. The particles are charged on entry and deposited on the bottom plate; the cells are located within this deposition area. This system has been tested and does not expose the cells to the gas being pulled through the system; the corona wire, the electric charge in the plate, and the airflow do not affect the lung cells. Please see de Bruijne, 2007 for a further explanation of this instrument. Both of these exposure systems were kept in an incubator, maintaining a constant temperature of 37C.

The cell exposures occurred both before and after the ozone injection into the chamber. For each exposure a set of control cells, prepared the same as the exposure cells, were kept in the incubator throughout each experiment. The gas exposures and controls were done in triplicate and the particle exposures and controls were done in quadruplicate. Clear Teflon sample lines connect the gas exposure system to the chamber, while carbon impregnated Silicone tubing connect EAVES to the chamber to reduce particle loss. For a detailed explanation of the smog-chamber/in-vitro system, please see (Sexton, 2004; Doyle, 2004; Doyle, 2006).

Once the exposure was complete and the cells were taken out of the exposure system, the media was removed from the cells and replaced with fresh serum-free media. Approximately 9 hours post-exposure the basolateral supernatants were stored at -80°C until analysis for cytotoxicity and inflammatory gene expression were conducted. For the analysis of cytotoxicity, the cells were analyzed for the release of cell lactate dehydrogenase (LDH) using a coupled enzymatic assay (Promega, Madison, WI or Takara, Japan), as per the suppliers instructions. To determine inflammatory response, the cells were analyzed for IL-8 protein levels by ELISA (R&D Systems, Minneapolis, MN), as per the supplier's instructions. Cytotoxicity and inflammation were expressed as fold increase over the

individual clean air control. To determine whether the ozone exposure affected the cells, an ozone-only experiment was conducted. For this experiment, cells were exposed to 100 ppb of ozone only for three hours in the modular incubator and for one hour in EAVES. The analysis confirmed that the ozone exposure induced no significant inflammation or cell death over the control. Statistical analysis was performed on this data using a one-way ANOVA followed by Dunnett's multiple comparison test. When comparing only two sets of data, a two-tailed t-test was used. Results were considered significant if their p-value < 0.05.

7.3.5. Instrumentation and measurements

TwoVarian Star 3800 gas chromatographs, one with a Saturn Ion Trap mass spectrometry (GCMS) instrument equipped with flame ionization detectors (FID) and a liquid nitrogen cryotrap injection system, and another with FID, was used to measure the VOC chemical composition in the chamber. Samples were drawn continuously and injected every 30 or 70 minutes from the chamber and analyzed throughout the experiment. A TSI SMPS - Scanning Mobility Particle Sizer™ Spectrometer was used to measure the size distributions and mass yields of the particulate matter. The parameters on the SMPS are optimized for particles 20-890 nm. The SMPS connected to the chamber with twelve feet of grounded stainless steel tubing and carbon impregnated Silicone tubing to reduce particle loss to the sides of the tubing. It was sampled continuously throughout the experiment. A Teflon filter-filter sampling cartridge measured total suspended particulate through a glass sampling manifold connected directly under the chamber for one hour intervals throughout each experiment. An impinger using PFBHA derivitization solution was sampled directly under the chamber through Teflon tubing for a three hour period during both of the exposure periods, which measured carbonyls and formaldehyde (Yu, 1995; Liu, 1999). For both the

2BE experiments, the formaldehyde was also measured using the automated Dasgupta-diffusion-tube sampler (Dasgupta, 1988). Ozone, temperature and dew point were measured and monitored continuously throughout the experiments.

7.4. Results

In this section, we present the particle and gas-phase results and the toxicological effects from these exposures. All of the results presented here are from the high concentration experiments (see Table 7.1). The toxicological, particle-phase and initial gas-phase results from all of the experiments can be found in Appendices B, C and D. A detailed carbonyl-specific chemical analysis of the gases and the particles collected on the filters will be presented in a future publication (Ebersviller et.al.).

7.4.1. Particle-Phase Results

Figurer 7.3 shows the cumulative mass concentrations and Figure 7.4 shows the number count for each of the four high concentration experiments. Note that the concentrations of d-limonene in the individual and HC_{Lim} experiments were equal and the concentrations of 2BE in the individual and HC_{2BE} experiments were equal. Before oxidized with ozone, both d-limonene and 2BE have low mass concentrations, less than 9 $\mu g/m^3$, and particle counts, less than 2.5 x 10^9 particles/cm^3. After 100 ppb ozone was injected into the chamber, there was no significant growth in the glycol ether particle-phase. Conversely, the oxidation of d-limonene resulted in a 400-fold increase of mass in the chamber. Figure 7.4 illustrates a rapid increase in particle formation after d-limonene oxidation. The size distribution of these particles is very small, see Figure 7.5a. With minimal background mass in the chamber, these particles are the result of self-nucleation of d-limonene or products of secondary reactions. There appears to be no secondary organic aerosol formation from the oxidation of

the 2BE with ozone (Figure 7.5b).

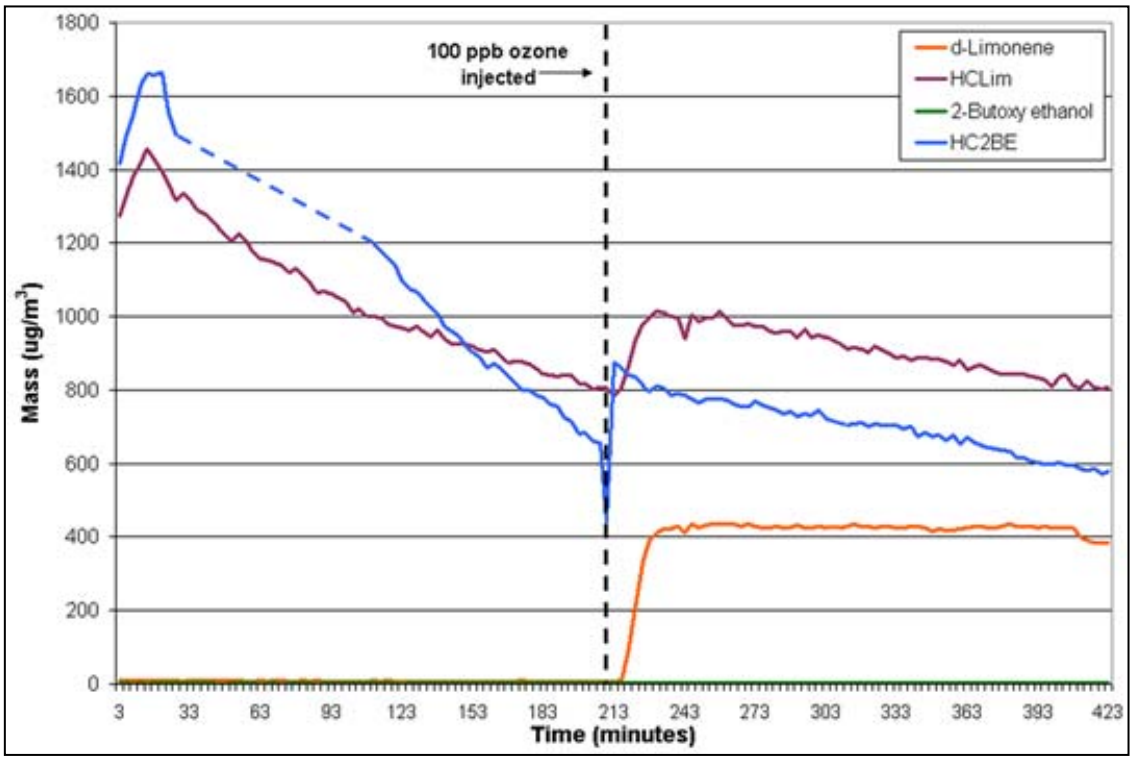


Figure 7.3: Cumulative Particle Mass Concentration

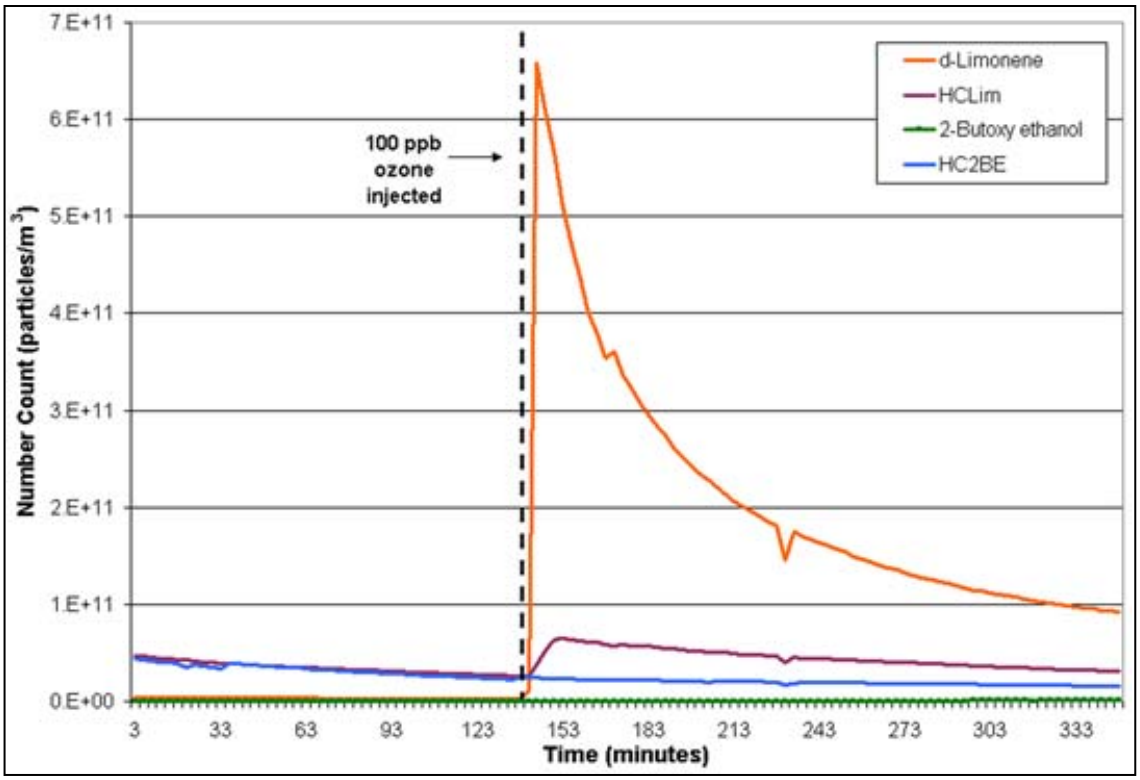


Figure 7.4: Particle Number Count

The injection method used for the HC_{Lim} and HC_{2BE} produced the particles present in the chamber at the start of these experiments (see Figure 7.3). As described above, the household cleaners were injected into the chamber using a nebulizer to blow air through the cleaning product liquid, producing a fine mist. This mist simulated the indoor use of the spray cleaners, although the size distributions of these particles are slightly smaller than the normal spray nozzle produces. The peak in particles in the initial minutes identifies when the injection of the cleaning products ended. Figure 7.3 shows a general decline in mass over time for these experiments. When the 100 ppb of ozone was injected into the chamber, both the HC_{Lim} and HC_{2BE} mass concentrations increased approximately 200 $\mu g/m^3$, which was a 20% increase for HC_{Lim} and a 30% increase for HC_{2BE}, approximately. Figure 7.4 shows that while the number of particles increased for HC_{Lim}, the count remained fairly constant for HC_{2BE}. The number distribution for HC_{2BE} shows that the particles simply grew in size once the ozone was injected (Figure 7.5b). Something quite different occurred when the HC_{Lim} was oxidized. As the oxidation with ozone evolved, a bi-modal distribution of particle size developed. One peak of larger particles grew as the HC_{Lim} particles already present in the chamber grew, similar to

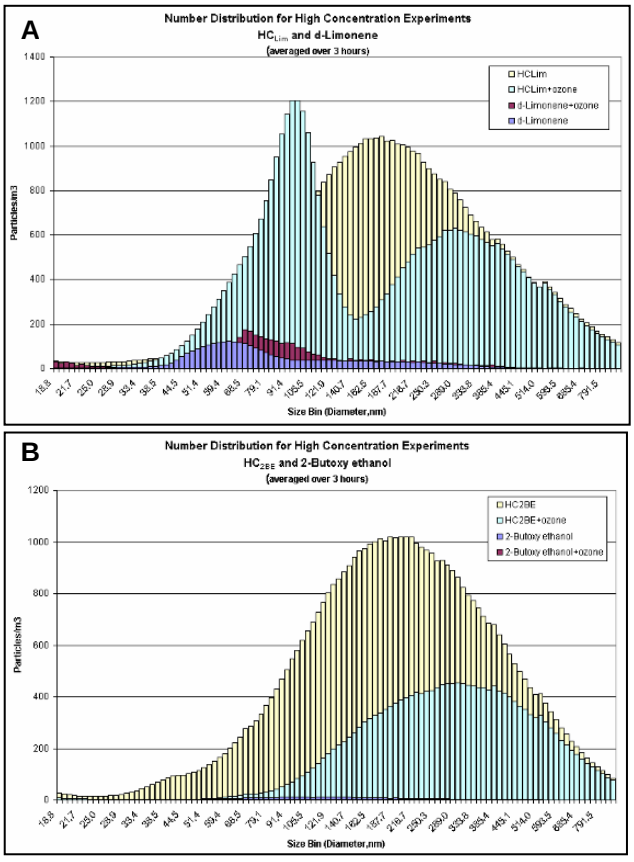


Figure 7.5: Particle Size Distributions

what happened with HC_{2BE}. A second peak of smaller particles also grew in the chamber; this peak was a result of self-nucleation of d-limonene present in the HC_{Lim} formulation. The lack of this bimodal peak supports the evidence that no secondary organic aerosol is forming because of the 2BE oxidation with ozone.

7.4.2. Particle-Phase Toxicology Results

The toxicological effects induced from exposure to the particles are presented in this section. The LDH release, cytotoxicity or cell death, induced from each exposure is presented in Figure 7.6. In all of the toxicological results figures, the control - the measured response from exposure to clean air - is depicted by a horizontal dashed line. Also note that the cells showed no increased response from exposure to the ozone alone. An asterisk designates the results that are statistically significant compared to the control. In each of the figures, the empty bars represent the individual solvents and the hash-marked bars represent the household cleaner mixtures. The non-colored bars are unoxidized, while the colored bars represent oxidation with ozone, denoted as O_3 in the graphs. Figure 7.6a shows that there is no statistically significant cell death caused from the 2BE particle phase, which is not surprising due to the low mass

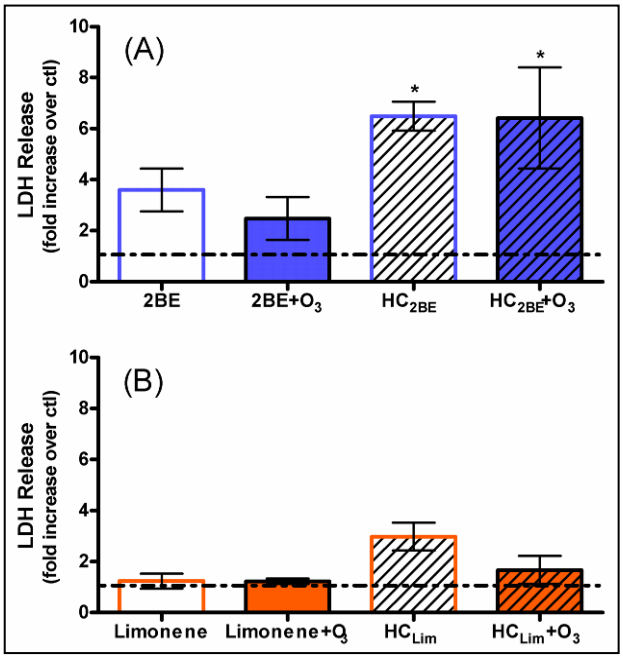


Figure 7.6: These results show the LDH release (cell death) from exposure to the particles; compounds and mixtures both before and after oxidation. These results demonstrate that the potential respiratory effects from individual components do not mirror the health effects seen from exposure to the mixtures. * Significant ($p \leq 0.05$) to the control (dotted line)

concentration of the particles, both before and after oxidation. On the other hand, the household cleaning product containing 2BE induced significant cell death, both before and after oxidation with ozone. d-Limonene and HC_{Lim} exposure did not result in significant cell death, even though their particle mass and number count were greater than HC_{2BE}. Although 2BE caused no significant cell death, HC_{2BE} did cause increased cytotoxicity. One potential explanation may be that the 2BE is cytotoxic to respiratory epithelial cells, but does not partition to the particles easily when alone. Alternatively, the spray application of the HC_{2BE} allows a high concentration of 2BE to be present in the particle phase, which may increase the overall toxicity. An alternative explanation may involve an ingredient in the mixture other than the glycol ether that is causing the adverse effects. This second option may be unlikely, since HC_{2BE} and HC_{Lim} are from the same manufacturer and the particles from HC_{Lim} do not induce cytotoxicity significant from the control. A complete list of the HC_{2BE} and HC_{Lim} formulation ingredients is required to determine the similarity of the two cleaning products.

Figure 7.7 presents the IL-8 protein, an indicator of inflammation, induced from exposure to the particle phase. Figure 7.7a shows that d-limonene alone caused no significant increase in inflammation compared to the control,

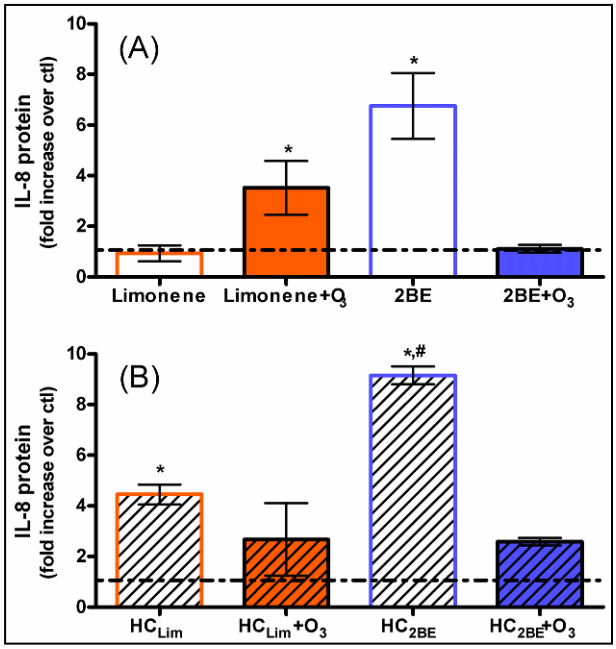


Figure 7.7: These results show the IL-8 protein (inflammation) from exposure to the particles from the individual products and the two mixtures both before and after oxidation. Overall, there is a greater increase in IL-8 release from exposure to the cleaning product containing glycol ether than to the one containing d-limonene.
*Significant ($p \leq 0.05$) to the control (dotted line)
HCG is significantly different from HCL+ozone

until it was oxidized with ozone. Exposure to 2BE alone resulted in significant inflammation, while no increased inflammation was induced from exposure to the particles formed from the oxidized 2BE. Therefore, the oxidation products of the d-limonene induce inflammation, while the ozone oxidation process reduces the significant inflammation potential of 2BE to non-significant levels. The dramatic increase in particle mass and number from the oxidation of d-limonene explains the increase in inflammation. With no observed change in the particle phase of 2BE before or after oxidation with ozone, the explanation for the change in inflammation is less clear. Additional analysis of the filter extractions is needed to determine changes in the particle compositions. For the household cleaning products, HC_{Lim} alone caused significant IL-8 induction over the control, though not when oxidized. The same held true for HC_{2BE}, though the HC_{2BE} alone caused significantly more inflammation than HC_{Lim}, designated by the #. None of the particles resulting from oxidation with ozone induced significantly more inflammation.

7.4.3. Gas-Phase Toxicology Results

The toxicological effects from the gas exposures were much less than the exposures to the particles. Note the change in the scale of the Y-axis in Figures 7.8 and 7.9, the previous particle-toxicology results ranged from 0-10 fold increase over the control and these results only range from 0-5 fold increase over the control.

Figure 7.8 shows the LDH release from exposure to the gases. There was no observed cell death from exposure to the gases from either the d-limonene or the household cleaning product containing d-limonene, as seen in Figure 7.8a. The oxidation of both the d-limonene and the HC_{Lim} did not increase the amount of LDH released from exposure to the reaction products. Figure 7.8b shows that 2BE alone and HC_{2BE} did not show an increase in LDH

over the control. Once both the 2BE and HC_{2BE} were oxidized with ozone and the cells were exposed to the reaction products, a significant level of cell death did occur. These results show that the interaction of 2BE with the ozone may be the source of the toxic secondary products. Therefore, the presence of ozone may be an important factor when working with 2BE, a petroleum-based solvent with low ozone forming reactivity. Alternately, the gas-phase reaction products from the oxidized biogenic solvent, both alone and in a mixture, did not cause significant cell death, even with a much higher estimated MIR value.

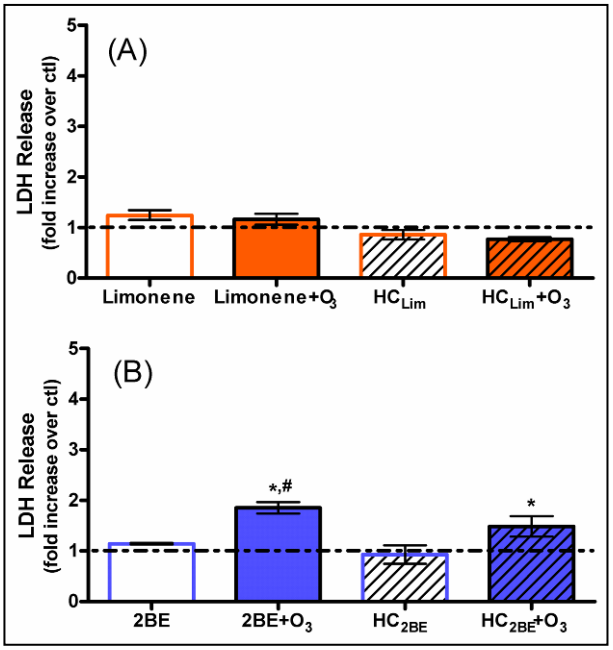


Figure 7.8: These results show the LDH release (cell death) from exposure to the gas-phase; compounds and mixtures both before and after oxidation. These results indicate that the presence of ozone may be an important factor when working with glycol ether, a petroleum-based solvent with low reactivity. The biogenic solvent, both alone and in a mixture did not cause any significant cell death. *Significant ($p \leq 0.05$) to the control (dotted line)

The inflammation data illustrates a different scenario from the LDH data. None of the exposures to the high concentrations resulted in significant levels of the IL-8 protein, compared to the control (see Figure 7.9). The results indicate that none of the reaction products caused a significant increase in inflammation. Note how both 2BE and the HC_{2BE} result in significant cell death, but not increased inflammation (see Figures 7.8b and 7.9). One would expect a gaseous mixture that induced cell death would similarly result in some level of inflammation. One potential explanation for these results may be that the cells are dying before they have a chance to respond to the environmental stimulant and release the IL-8 protein.

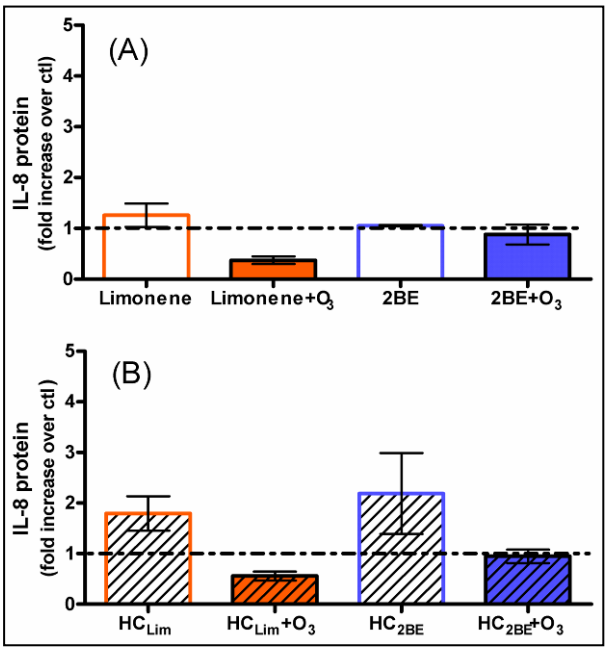


Figure 7.9: These results show the IL-8 protein (inflammation) from exposure to the gas-phase from the individual products and the two mixtures, both before and after oxidation. Note how both the glycol ether and the HCG result in significant cell death (in the previous graphs), but not increased inflammation. One potential explanation may be that the cells are dying before they have a chance to respond to the environmental stimulus and release the IL-8 protein. *Significant ($p \leq 0.05$) to the control (dotted line)

7.5. Discussion

This study set out to examine the health effects and indoor chemistry of two individual products and mixtures containing those compounds. The purpose was three-fold: (1) to determine whether a biogenic solvent, d-limonene, is associated with different potential health effects than a petroleum-based product, glycol ether; (2) to examine whether the reactivity, estimated MIR value, of a solvent correlates to its potential toxicity when oxidized with ozone; and (3) to determine whether these individual compounds could be used as proxies for mixtures when assessing chemistry and health effects.

7.5.1. Biogenic vs. petroleum-based solvents and reactivity

The particles from d-limonene and from HC_{Lim} did not induce significant cell death, nor did the particles from 2BE. The particle-phase of HC_{2BE} did result in increased LDH release, significant to the control (Figure 7.6). Both 2BE and HC_{2BE} showed significantly higher levels of IL-8 protein compared to d-limonene and HC_{Lim} (Figure 7.7). These results show

that given this exposure model both 2BE and HC_{2BE} particles induce greater respiratory toxicity than the particle-phases of d-limonene and HC_{Lim}.

The gas-phase results also support this theory. d-Limonene and HC_{Lim} did not cause LDH or IL-8 release either before or after oxidation with ozone (see Figures 7.6b, 7.7). Although 2BE and HC_{2BE} did not induce significantly more inflammation than the control, they both caused significant cell death when oxidized. Thus the petroleum-based solvent, after oxidation, both alone and in a mixture, showed increased toxicity in the gas-phase, while the biogenic solvent had no significant toxicity.

These results show that d-limonene, alone or in a mixture, has less potential to cause respiratory cytotoxicity or inflammation than 2BE, alone or in a mixture. In this study, the biogenic solvent induced fewer toxic effects than the petroleum-based solvent. Furthermore, although d-limonene is the more reactive solvent, it induced fewer levels of toxic endpoints than 2BE. These results demonstrate why ozone-forming reactivity cannot be the only factor when developing consumer product regulations. The toxicity of these solvents needs to be evaluated and incorporated into the policy decision-making process.

7.5.2. Individual products as proxies for mixtures

The particle data clearly demonstrates that the individual components of the mixtures cannot be used as proxies for those mixtures. First examining d-limonene and HC_{Lim}, their initial particle masses were very different from one another. Once ozone was injected into the system, the mass of the d-limonene particles increased by 400%, while the HC_{Lim} particle mass only increased by 20%. If d-limonene experimental results were used to predict HC_{Lim} behavior, it would greatly over estimate the increase in mass and number of the particles as

well as misrepresent the number distribution of the particles. Thus, d-limonene cannot be used as a surrogate to predict the particle-phase behavior of a mixture containing d-limonene.

On closer inspection of the glycol ether experiments, the particle-phase 2BE results (see Figures 7.6a, 7.7a), 2BE only induced significant effects for inflammation when ozone was not present. In the gas-phase (see Figures 7.8b, 7.9a), the only significant effects observed were LDH release after 2BE was oxidized with ozone. There results show that a reaction is occurring during oxidation that affects the toxicity of 2BE. This pattern is not seen with HC_{2BE}, the oxidation of this mixture does not mimic the behavior of 2BE alone, reaffirming the inability for individual products to act as proxies for mixtures.

The only data that supports the use of individual components as a proxy is the gas-phase toxicological data (Figures 7.8, 7.9). The cytotoxicity and inflammation induced from exposure to the mixtures is similar to the individual products. Yet, because the majority of the toxicity was due to the particle-phase, this does not give much strength to the proxy argument.

7.6. Conclusions

This research examined the indoor impacts of controlling the reactivity of household cleaning products and their role in outdoor VOC emissions. This work has shown that this approach may increase indoor health effects since the data shows that increased reactivity does not result in increased toxicity. People spend a majority of their time indoors; therefore, this approach for regulating consumer products may increase consumers' exposure to toxic secondary reaction products. From a policy standpoint, rule makers must not only consider the outdoor impacts of their regulations when the controlled product is primarily for indoor use. From a research standpoint, more work needs to go towards looking at chemicals in a

realistic environmental setting. These results demonstrate that compounds behave differently in mixtures. How their behavior is altered and how this change affects human health and particle formation, especially in the presence of ozone, needs to be understood further.

8. Management Implications of Consumer Product Regulation

Manuscript will be submitted to Environmental Science and Technology with Douglas J. Crawford-Brown and Harvey E. Jeffries

Introduction

The 1990 Clean Air Act Amendments first required the Environmental Protection Agency (EPA) to look at the impacts of consumer products on VOC emissions. Since the implementation of these amendments, EPA has set limits on the amount of VOCs contained in consumer products (EPA, 2007). In 1988 California had already passed its own state Clean Air Act which required regulations to achieve maximum feasible mass reductions of VOCs in consumer products. As of 2005, there were over 100 categories of consumer products regulated in California (CARB, 2007). The state passed amendments in 2004 to meet the State Implementation Plan (SIP) requirements for VOC levels, which included Antiperspirants and Deodorants Regulation, Consumer Products Regulations, Alternative Control Plan, Aerosol Coating Products Regulation, and Hairspray Credit Program Regulation. These new regulations may require the consumer product industry to decrease or remove chemicals with higher estimated reactivity values from their cleaning product composition to control VOC emissions outdoors. While the majority of California's VOC regulations are mass-based, they are beginning to move towards reactivity-based regulations.

Reactivity is a term that refers to a chemical's estimated potential to form ozone. Reactivity-based regulations base their framework around an evaluation of reactivity, and presently the California Air Resources Board (CARB) uses the maximum incremental reactivity (MIR) scale (Carter, 2000). This scale assigns a MIR value to chemicals, which represents their estimated ozone formation potential. According to this scale, the lower estimated MIR values will have less of an impact on ozone formation. Therefore, regulators would prefer product formulations to contain VOCs with lower MIR values. These regulations were implemented to reduce anthropogenic VOC emissions outdoors to help

control the formation of ozone and other reaction products. These control strategies are focused on outdoor air quality, yet many of these sources are used mainly indoors. These regulations do not take into account potential impacts to the indoor environment. Drawing on the experiments presented in the previous chapter, the results from both the low and high-concentration consumer-product experiments will provide insight into a major component of risk that is overlooked in the rule making process.

8.1. Case study with Household Cleaning Products

8.1.1. Experimental Summary

Two cleaning products, using either d-limonene or 2-buyoxy ethanol (2BE) as solvents, were used to assess the potential health risks from their indoor use. These spray cleaners were chosen to compare a biogenic solvent with a higher estimated reactivity, d-limonene, to a petrochemical solvent with lower estimated reactivity, 2BE. For these experiments, the cleaning products were injected into the UNC outdoor smog chamber (see Figures 6.4, 7.2) at night to simulate indoor conditions. Human epithelial lung cells were exposed to the mixtures in the chamber, both before and after oxidation with 100 ppb ozone. The cells were exposed to the gas and particle phases separately, to distinguish between potential health risks. The cells were analyzed for inflammation and cell death. For each cleaning product, two concentrations were used for the development of dose response curves. For further information on the methods and experimental protocol, see Chapter 7. For the detailed toxicological data, see Appendix B.

8.1.2. Dose-Response Curves

The toxicological results in Chapter 7 only examined the high concentration results. The dose-response curves below employ the results from both the high and low concentration

experiments. The results marked with an asterisk (*) denote statistically significant responses over the control. The purpose of developing these curves is to look at the potential risk people are exposed to when using these two cleaners indoors. Figure 8.1 shows the inflammation induced from exposure to both the gas and particle phases of the two cleaning products, both before and after oxidation with ozone. While none of the responses were significant at the lower concentrations, exposure to the cleaner containing d-limonene (HC_{Lim}) did cause significant inflammation at the higher concentration (see Figure 8.1a). This inflammation was induced from the particles before oxidation with ozone. A similar pattern is seen with the household cleaner containing 2BE (HC_{2BE}). For both of these products, there is no significant increase in inflammation at the higher concentrations, except from exposure to the particles before oxidation. Furthermore, a t-test revealed that the response from HC_{2BE} is significantly greater than the response from HC_{Lim}. Therefore, at higher concentrations, the cleaner with the lower reactivity solvent (HC_{2BE}) induced greater health effects indoors than the higher estimated reactivity solvent (HC_{Lim}).

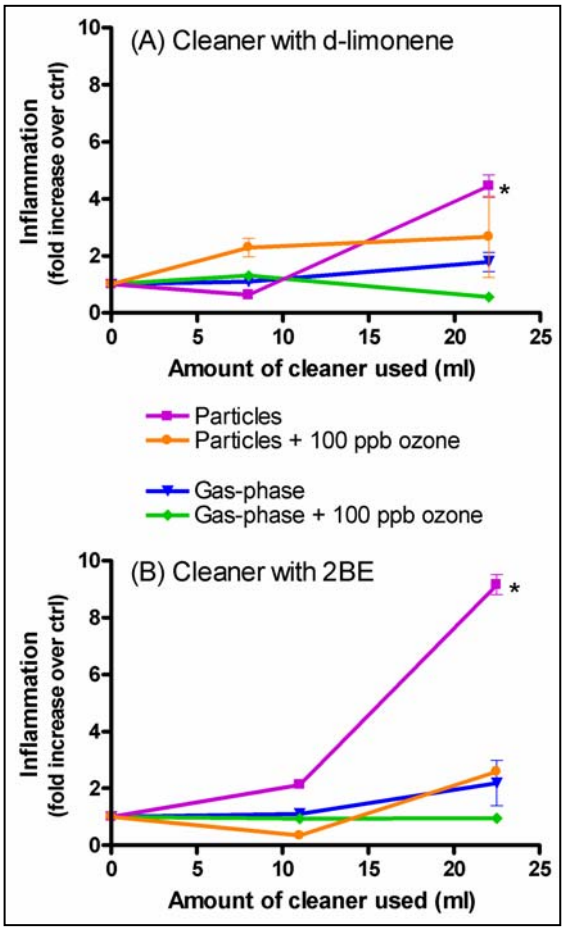


Figure 8.1: Inflammation Dose-Response Curves

Figure 8.2 depicts the dose-response curves for the cell death, or cytotoxicity, induced from exposure to the cleaning products. There was no statistically significant increase in the

cell death caused from higher concentrations of HC_{Lim} (See Figure 8.2a). There is a statistically significant increase in toxicity for HC_{2BE} at the higher concentration (see Figure 8.2b). The particles, both before and after oxidation, and the oxidized gas phase caused significant cell death. There is an obvious increase in risk with a move from HC_{Lim} to HC_{2BE}. The particles present at the higher concentrations of HC_{2BE} can induce a 6-fold increase in risk. Therefore, this data only reinforces the assertion that the lower reactivity solvent product induces significantly greater health effects than the higher reactivity solvent product.

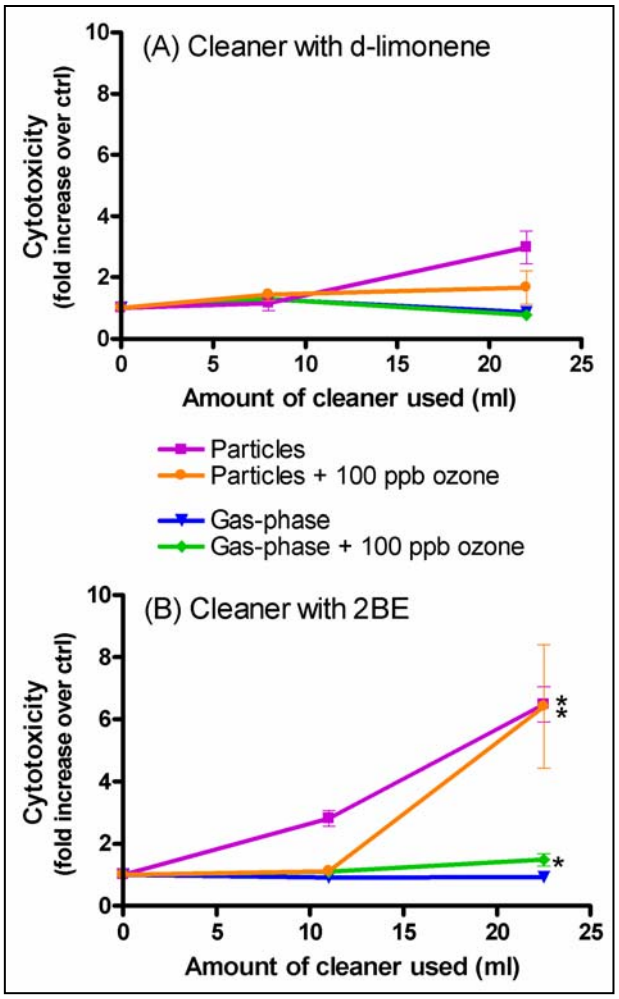


Figure 8.2: Cytotoxicity Dose-Response Curves

These dose-response curves demonstrate that altering the VOC content of consumer product formulations based on reactivity may have a negative effect on indoor air quality at higher concentrations. Why would state and federal governments enact regulations that could increase the risk for consumers using these products? The answer is considered in an assessment of how the EPA is structured.

8.2. Fragmentation and Risk

The EPA was established in 1970 because of a reorganization of the federal government to better assess and control environmental issues. Offices, such as the Federal Water Quality Administration, the National Air Pollution Control Administration, the Bureau of Solid Waste Management, and Council of Environmental Quality were relocated into this new agency. EPA "inherited a cluster of media-specific programs enacted by Congress under the leadership of numerous congressional committees. The programs had been administered by a variety of departments in the federal government, and had been run on a day-to-day basis by diverse groups of professional bureaucrats, each with specific program orientations (air, water, land)" (Krier, 1992). EPA's mandate gave them the authority to identify pollutants and follow their path through the ecological chain, assessing human and environmental exposures and identifying appropriate control measures. By moving and maintaining established offices into EPA, this new agency was able to hit the ground running and not suffer stagnation from a complete reorganization.

The initial media-specific structure of the EPA was reinforced by the environmental regulations enacted over the following decades, such as the Clean Air Act, the Clean Water Act, and the Toxic Substances Control Act. Although this level of specialization facilitated "the digestion of information and the accumulation of skills" (Graham, 1995), it also produced a regulatory process that does not encourage cross-office collaboration. This, in turn, propagates a decision-making process that facilitates risk tradeoffs.

8.2.1. Risk Tradeoffs

Risk tradeoffs occur when a countervailing risk results from an action reducing a targeted risk. Considering the consumer product example, by reducing the risk from outdoor exposure to air pollutants, the risk to indoor pollutants is increased. There are different types of risk tradeoffs, shown in Table 8.1. The type of risk tradeoff is determined by the change in the type of risk and susceptible populations exposed to the countervailing risk. For this case study the type of risk is different. The target risk is outdoor air pollution, which undergoes photochemical reactions and has a large variety of emissions sources affecting the chemical make-up of the atmosphere. The countervailing risk exposes populations to indoor air, in which photochemistry is not important and which has a set of emission sources different from the outdoors. Therefore, the indoor air is comprised of a different mixture of compounds. In addition, the outdoor pollutants reacting with each other would be different from those found indoors. The exposed population is somewhat harder to determine. People who work outdoors or are physically active would be exposed to outdoor air for a longer period than people who work indoors or the elderly and infirmed. Additionally, people who work on janitorial staffs would have an increased exposure to indoor air at potentially higher concentrations. These populations may have different exposures, resulting in a risk transfer. Alternatively, many people do spend a good amount of time both indoors and outdoors, making the risk tradeoff a risk substitution.

Table 8.1: Typology of Risk Tradeoffs

	Compared to the Target Risk, the Countervailing Risk is:		
Compared to the Target Risk, the Countervailing Risk affects:		SAME TYPE	DIFFERENT TYPE
	SAME POPULATION	Risk Offset	Risk Substitution
	DIFFERENT POPULATION	Risk Transfer	Risk Transformation

(Graham, 1995)

8.2.2. Decision-making Process

Regardless of the *type* of risk, an acknowledgment of the risk needs to be incorporated into the decision-making process. This would ensure that the final analysis of the risk is included. This process may alter how policy makers handle the timing and the distribution of the risk to benefit the entire population. There are already some procedures in place to accomplish this. When a regulation is proposed, the Federal Registrar publishes it and the public have an opportunity to comment. It is the responsibility of the EPA to respond to these comments when moving ahead with the final regulation. One potential problem with this approach again relates to the "stovepipe" organization of the EPA. Due to the increased specialization in certain scientific areas, the writers of the regulation incorrectly interpret the significance of a comment, if it is outside their area of expertise. Another procedural step to help control the number of countervailing risks is the involvement of other offices during the process. The writers of the regulation may include enforcement/compliance staff, legal staff, as well as policy/innovations staff in the regulation development process. Yet many people in those offices are also media specific, if not even more specialized. This specialization heightens the level of technical expertise involved in developing and reviewing the

regulation, which is vital, but it also increases the potential to underestimate the significance of an issue outside one's area.

8.3. Conclusions

Potential risk tradeoffs present in the consumer-product regulation scenario bring to light a major component of risk that was overlooked in the decision-making process. The most productive resolution to this problem is unclear. There are steps in the regulatory process that attempt to involve all the potential stakeholders, yet this process is imperfect. In 2004 the National Research Council (NRC 2004) published a report addressing air quality management in the US. It recommended a new approach to the management structure, moving from a single-pollutant approach to a multi-pollutant approach. NRC felt this structure would enable the EPA to better consider interactions between pollutants and control measures. In response, the Office of Air Quality Planning and Standards has recently reorganized to follow this recommendation. This is one-step towards a more holistic approach to air quality. Only after the fragmented arrangement of the EPA offices come together and improve communication and coordination, will the rate of neglected countervailing risks decline.

9. Study Conclusions

These preliminary investigations have explored the potential inhalation risks to consumers from the implementation of some potential consumer product regulation. This work demonstrated that replacing d-limonene with 2-butoxy ethanol to decrease the ozone forming reactivity of consumer product formulations will decrease the quality of indoor air, resulting in increased risk to people indoors.

My hypothesis for this research: Regulating household consumer products based solely on their reactivity (ozone formation potential) and possible detrainment outdoors may replace currently used biogenic solvents with petrochemical solvents that, while feasibly decreasing the localized anthropogenic volatile organic compound emissions, could increase the risk for health effects for consumers indoors. The indoor orange experiments demonstrated the particle forming potential of the natural emission source of d-limonene. These results show the importance of understanding the variety of sources of indoor VOC emissions. These experiments also demonstrate the ability of the UNC smog chamber to analyze indoor environments.

The consumer product research conducted here is the first set of experiments (reported in published literature) to mimic indoor use of consumer products and directly expose human epithelial lung cells to those atmospheres. The UNC smog chamber/*in-vitro* system provides a unique analytic capability for assessing indoor health impacts. These experiments demonstrated that when examining the potential impacts of consumer products indoors, the

actual mixtures must be analyzed. Individual components of these mixtures cannot be used as proxies for the mixtures.

The analysis of the dose-response curves further demonstrates the potential increase in inflammatory and cytotoxic responses from exposure to the cleaning products containing 2BE. The consumer product regulations appear to be missing this major component of the risk. The quality of indoor air must be considered when regulating consumer products, even when those regulations focus on outdoor air quality issues.

9.1. Study Limitations

To understand the findings from this research, it is helpful to discuss the study limitations.

By employing an *in vitro* experimental protocol for this research, the exposures were not conducted in a realistic setting for the cell cultures. A "major limitation of *in vitro* is the cells are removed from their normal environment. There are no neighboring cells or tissues to interact with, and no blood to supply, potentially important factors or nutrients" (Devlin et al. 2005). Additionally, A549 cells, a model of human epithelial lung cells, were used to predict potential health risks. These cells are not representative of sensitive populations or other regions of the respiratory tract, which may be affected differently from the exposure atmospheres.

The consumer product experiments simulated indoor use of the spray cleaners by employing a nebulizer to inject the material into the chamber. This resulted in particles that are slightly smaller than those emitted from an actual spray bottle. The background air in the chamber was "clean air" and not representative of typical indoor atmospheres. Additionally

cleaning surfaces were not present in the chamber, which might play a factor in the chemistry of actual product use.

9.2. Further Research Questions

- How does the smog chamber/*in vitro* exposure system compare to *in vivo* work with animals, such as mice? In much of the literature, the mouse-human models do not correlate well with respect to individual solvents. How would they compare to these *in vitro* results? How would these compare to exposures to humans in actual use scenarios?

- Would different endpoints provide a more detailed picture of how the exposures are affecting the respiratory system? What information would other endpoints provide? Is there a protective response from the cells when particle concentrations reach a particular concentration?

- Further investigations into potential exposures and risk for more realistic scenarios should include longer experiments to monitor the decay of products and possible further interactions with initial SOA, with varying humidity with simulation of natural indoor surfaces and normal household and workplace ventilation rates, and with mixtures of other expected indoor pollutants for potential complex chemical interactions.

- What would the dose-response curves look like with more concentrations? Additional experiments with a larger variety of concentrations of the cleaning products could determine NOELs and LOELs for consumer products.

Appendices
Appendix A - Experiment List

1. 2/1/06, **preliminary d-limonene and consumer product exposure to ozone**.
   Approximately 1.3 μL of pure d-limonene (approx. 164 ppmC) was injected into a 19.41 L glass jar reactor and sampled with GC/MS, FID, Carle I and III and TSI SMPS. Ozone was injected (approx. 100 ppb), allowed to react for 30 minutes and sampled with GC/MS, FID, Carle I and III, TSI SMPS and formaldehyde measurements were taken. For the second half of the experiment, 600 μL of HC_{Lim} was injected into the reactor and after decreasing the concentration to 153.36 ppmC, it was sampled with GC/MS, FID, Carle I and III, TSI SMPS and a formaldehyde measurement was taken. Ozone was injected (approx. 100 ppb), allowed to react for 30 minutes and sampled with GC/MS, FID, Carle I and III, TSI SMPS and a formaldehyde measurement was taken. The average temperature of the lab was 73 degrees and the atmospheric pressure was 27.74.

2. 2/24/06, **peeling an orange with and without ozone present.**
   We used a simple Teflon lined glove-box reactor (300 L Reactor) for the orange peeling experiment. This reactor has lab gloves with Teflon sleeves mounted to the walls of the reactor, allowing us to peel the orange inside the sealed reactor. We injected 81.6 ppb ozone into the 300 L Reactor, peeled the orange, and then sampled with the GC/MS, FID, Carle I and III, TSI SMPS and formaldehyde instruments. We sampled the contents of the reactor with the GC/MS immediately after peeling the orange and again one hour after the first measurement. We then flushed the box with clean air and ensured all the ozone was removed. We peeled an orange in the 300 L Reactor and sampled with the GC/MS, FID, Carle I and III, TSI SMPS, and formaldehyde instruments immediately after peeling the orange and again an hour later.

3. 4/19/06, **preliminary consumer product (with and without limonene) exposure to ozone**
   Approximately 200 μL of HC_{Lim} was injected into the reactor and after achieving a concentration of 164.16 ppmC, it was sampled with GC/MS, FID, Carle I and III, TSI SMPS and a formaldehyde measurement was taken. Ozone was injected (approx. 100 ppb), allowed to react for 33 minutes and sampled with GC/MS, FID, Carle I and III, TSI SMPS and a formaldehyde measurement was taken. For the second half of the experiment, 150 μL of HC_{2BE} was injected into the reactor and after achieving a concentration of 112.48 ppmC, it was sampled with GC/MS, FID, Carle I and III, TSI SMPS and a formaldehyde measurement was taken. Ozone was injected (approx. 100 ppb), allowed to react for 37 minutes and sampled with GC/MS, FID, Carle I and III, TSI SMPS and a formaldehyde measurement was taken.

4. 5/24/06, **HC_{Lim} low concentration in UNC Smog Chamber**
   8 ml of HC_{Lim} was injected into the chamber after sundown with a nebulizer. The injection took 30 minutes. The three GC instruments and the SMPS were sampled continuously throughout the experiment. The cells were exposed to the gases for 3 hours and the particles for 1 hour. The impinger sampled for 3 hours. The first set of filter

samples were not usable, therefore only one 1-hr filter sample was taken. After the exposures were complete, 100 ppb ozone was injected into chamber at 10 ppb/min for exactly 10 minutes and allowed to react for another 20 minutes. The mixing fans were turned on at the start of the ozone injection and turned off after 20 minutes. The second set of cells were exposed to gases for 3 hours and the particles for 1 hour. The impinger was sampled for 3 hours and three 1-hour filter samples were taken. The approximate dew point was 54 ºF.

5. 6/8/06, **d-Limonene low concentration in UNC Smog Chamber**
   480 µl (5.88 ppmC) of d-limonene was injected into the chamber after sundown with a u-tube evaporated with a heat gun. The injection took 5 minutes. The three GC instruments and the SMPS were sampled continuously throughout the experiment. The cells were exposed to the gases for 3 hours and the particles for 1 hour. The impinger sampled for 3 hours and the mister was sampled twice – once for an hour and once for 30 minutes. One 1-hr filter sample was taken. After the exposures were complete, 100 ppb ozone was injected into chamber at 10 ppb/min for exactly 10 minutes and allowed to react for another 20 minutes. The mixing fans were turned on at the start of the ozone injection and turned off after 2 hours. The second set of cells were exposed to gases for 3 hours and the particles for 1 hour. The impinger was sampled for 3 hours, the filter for 1 hour, and the mister was sampled twice for 30 minutes and 75 minutes. The approximate temperature was 64 ºF with a dew point of 59 ºF.

6. 6/13/06 **HC_{Lim} high concentration in UNC Smog Chamber**
   22 ml of HC_{Lim} was injected into the chamber after sundown with a nebulizer. The injection took 1 hour and 13 minutes. The three GC instruments and the SMPS were sampled continuously throughout the experiment. The cells were exposed to the gases for 3 hours and the particles for 1 hour. The impinger sampled for 3 hours and the mister was sampled twice for 30 minutes. One 1-hr filter sample was taken. After the exposures were complete, 100 ppb ozone was injected into chamber at 10 ppb/min for exactly 10 minutes and allowed to react for another 20 minutes. The mixing fans were turned on at the start of the ozone injection and turned off after 20 minutes. The second set of cells were exposed to gases for 3 hours and the particles for 1 hour. One complication occurred during the second half of this experiment. When the cells were removed from EAVES after an hour, we noticed that the injection line for the gas exposures had been disconnected, therefore the cells had not been exposed to anything for the first hour. We hooked up the sample line and started the 3 hour gas-phase exposure at that time. The impinger was sampled for 3 hours, the filter for 1 hour, and the mister was sampled three times for 30 minutes. The approximate dew point was 59 ºF.

7. 6/15/06, **d-Limonene high concentration in UNC Smog Chamber**
   1,300 µl (16.017 ppmC) of d-limonene was injected into the chamber after sundown with a u-tube evaporated with a heat gun. The injection took 25 minutes. The three GC instruments and the SMPS were sampled continuously throughout the experiment. The cells were exposed to the gases for 3 hours and the particles for 1 hour. The impinger sampled for 3 hours and the mister was sampled twice for 30 minutes. One 1-hr filter sample was taken. After the exposures were complete, 100 ppb ozone was injected into

chamber at 10 ppb/min for exactly 10 minutes and allowed to react for another 20 minutes. The second set of cells were exposed to gases for 3 hours and the particles for 1 hour. The impinger was sampled for 3 hours, the filter for 1 hour, and the mister was sampled three times for 30 minutes. The approximate temperature was 77 ºF with a dew point of 58 ºF.

8. 6/21/06, **Ozone-only experiment in UNC Smog Chamber**
   100 ppb of ozone was injected into the chamber, with nothing else in the chamber. The cells were exposed to gases for 3 hours and the particles for 1 hour.

9. 6/28/06 **HC_{2BE} low concentration in UNC Smog Chamber**
   11 ml of HC_{2BE} was injected into the chamber after sundown with a nebulizer. The injection took 31 minutes. Part of the nebulizer is made out of copper and during the injection the copper was oxidized. The three GC instruments and the SMPS were sampled continuously throughout the experiment. The cells were exposed to the gases for 3 hours and the particles for 1 hour. The impinger sampled for 3 hours and the mister was sampled three times for 30 minutes. One 1-hr filter sample was taken. After the exposures were complete, 100 ppb ozone was injected into chamber at 10 ppb/min for exactly 10 minutes and allowed to react for another 20 minutes. The mixing fans were turned on at the start of the ozone injection and turned off after 12 minutes. The second set of cells were exposed to gases for 3 hours and the particles for 1 hour. The impinger was sampled for 3 hours, the filter for 1 hour, and the mister was sampled three times for 30 minutes.

10. 7/11/06, **HC_{2BE} low concentration in UNC Smog Chamber**
    22.5 ml of HC_{2BE} was injected into the chamber after sundown with a nebulizer. The injection took one hour. Part of the nebulizer is made out of copper and during the injection the copper was oxidized. The three GC instruments and the SMPS were sampled continuously throughout the experiment. The cells were exposed to the gases for 3 hours and the particles for 1 hour. After the EAVES exposure was complete, the SMPS intake valve needed to be cleaned. The impinger sampled for 3 hours and one 1-hr filter sample was taken. After the exposures were complete, 100 ppb ozone was injected into chamber at 10 ppb/min for exactly 10 minutes and allowed to react for another 20 minutes. The mixing fans were turned on at the start of the ozone injection and turned off after 20 minutes. The second set of cells were exposed to gases for 3 hours and the particles for 1 hour. The impinger was sampled for 3 hours and the filter for 1 hour. The approximate dew point was 64 ºF.

11. 7/25/06, **2-Butoxy ethanol low concentration in UNC Smog Chamber**
    265 µl of 2BE was injected into the chamber after sundown with a u-tube evaporated with a heat gun. The injection took 8 minutes. The three GC instruments, the formaldehyde instrument and the SMPS were sampled continuously throughout the experiment. The cells were exposed to the gases for 3 hours and the particles for 1 hour. The impinger sampled for 3 hours and one 1-hr filter sample was taken. After the exposures were complete, 100 ppb ozone was injected into chamber at 10 ppb/min for exactly 10 minutes and allowed to react for another 20 minutes. The second set of cells were exposed to

gases for 3 hours and the particles for 1 hour. The impinger was sampled for 3 hours and the filter for 1 hour. The approximate dew point was 66 ºF.

12. 8/1/06, **Peeled orange in UNC Smog Chamber**
    The three GC instruments, the formaldehyde instrument and the SMPS were sampled continuously throughout the experiment. After sundown, an orange was peeled in the chamber. The orange and peels were left in the chamber for 35 minutes and then removed. 250 ppb of ozone was then injected into the chamber and allowed to react. The mixing fans were on the entire time the ozone was injected and turned off when the injection was complete.

13. 8/8/06, **2-Butoxy ethanol low concentration in UNC Smog Chamber**
    1,460 µl of 2BE was injected into the chamber after sundown with a u-tube evaporated with a heat gun. The injection took 28 minutes. The three GC instruments, the formaldehyde instrument and the SMPS were sampled continuously throughout the experiment. The cells were exposed to the gases for 3 hours and the particles for 1 hour. The impinger sampled for 3 hours and one 1-hr filter sample was taken. After the exposures were complete, 100 ppb ozone was injected into chamber at 10 ppb/min for exactly 10 minutes and allowed to react for another 20 minutes. The second set of cells were exposed to gases for 3 hours and the particles for 1 hour. The impinger was sampled for 3 hours and the filter for 1 hour. The approximate temperature was 83 ºF.

Appendix B: Toxicological Results

In this appendix, the complete toxicological results are provided for both the low and high concentrations. The actual measurements are presented in the tables in Section B1. The graphs representing this data are found in Section B2. These graphs represent the fold increase of the response over the control value represented by the dotted line.

B1. IL-8 Protein and LDH Release Measurements

Household cleaner containing d-limonene (HC_{Lim}), low concentration

LDH Release

EAVES control 1		EAVES exposure 1		
1	0.234	5	0.135	**0.624**
2	0.205	6	0.244	**1.128**
3	0.259	7	0.385	**1.780**
4	0.167	8	0.240	**1.110**
	0.216			

CS control 1		CS exposure 1		
9	0.350	12	0.383	**1.133**
10	0.398	13	0.431	**1.275**
11	0.266	14	0.492	**1.456**
	0.338			

EAVES control 2		EAVES exposure 2		
15	0.197	19	0.264	**1.285**
16	0.224	20	0.199	**0.968**
17	0.219	21	0.377	**1.835**
18	0.182	22	0.339	**1.650**
	0.206			

CS control 2		CS exposure 2		
23	0.285	26	0.397	**1.369**
24	0.198	27	0.341	**1.176**
25	0.387	28	0.396	**1.366**
	0.290			

IL-8 Protein

EAVES control 1		EAVES exposure 1		
1	18.549	5	10.013	**0.247**
2	47.762	6	25.799	**0.636**
3	62.011	7	27.704	**0.683**
4	33.889	8	38.029	**0.938**
	40.553			

CS control 1		CS exposure 1		
9	78.765	12	76.041	**0.871**
10	92.014	13	104.453	**1.196**
11	91.219	14	109.640	**1.255**
	87.333			

EAVES control 2		EAVES exposure 2		
15	8.697	19	31.693	**1.565**
16	25.868	20	38.858	**1.918**
17	19.016	21	59.588	**2.942**
18	27.438	22	55.645	**2.747**
	20.255			

CS control 2		CS exposure 2		
23	76.631	26	96.397	**1.201**
24	90.555	27	119.237	**1.485**
25	73.686	28	98.606	**1.228**
	80.291			

Household cleaner containing d-limonene (HC_{Lim}), high concentration

LDH Release

EAVES control 1		EAVES exposure 1		
1	0.079	5	0.274	4.248
2	0.053	6	0.170	2.636
3	0.066	7	0.109	1.690
4	0.060	8	0.216	3.349
	0.065			

CS control 1		CS exposure 1		
9	0.554	12	0.429	0.833
10	0.629	13	0.530	1.029
11	0.362	14	0.370	0.718
	0.515			

EAVES control 2		EAVES exposure 2		
15	0.062	19	0.091	1.510
16	0.063	20	0.036	0.598
17	0.056	21	0.193	3.203
18	0.060	22	0.082	1.361
	0.060			

CS control 2		CS exposure 2		
23	0.160	26	0.104	0.711
24	0.125	27	0.111	0.759
25	0.154	28	0.124	0.847
	0.146			

IL-8 Protein

EAVES control 1		EAVES exposure 1		
1	1.368	5	12.110	4.961
2	1.659	6	8.963	3.671
3	3.412	7	NA	NA
4	3.326	8	11.537	4.726
	2.441			

CS control 1		CS exposure 1		
9	22.169	12	67.932	2.393
10	51.559	13	50.193	1.768
11	11.449	14	34.681	1.221
	28.392			

EAVES control 2		EAVES exposure 2		
15	1.285	19	1.411	0.946
16	1.054	20	2.873	1.926
17	1.425	21	10.293	6.899
18	2.204	22	1.369	0.918
	1.492			

CS control 2		CS exposure 2		
23	5.945	26	2.970	0.437
24	5.840	27	4.993	0.735
25	8.589	28	3.434	0.506
	6.791			

Pure d-limonene, low concentration

LDH Release

EAVES control 1		EAVES exposure 1		
1	0.097	5	0.821	**5.130**
2	0.253	6	0.273	**1.706**
3	0.074	7	0.143	**0.893**
4	0.216	8	0.104	**0.650**
	0.160			

CS control 1		CS exposure 1		
9	0.415	12	0.420	**1.266**
10	0.273	13	0.431	**1.299**
11	0.307	14	0.411	**1.239**
	0.332			

EAVES control 2		EAVES exposure 2		
15	0.047	19	0.132	**2.146**
16	0.059	20	0.373	**6.065**
17	0.065	21	0.142	**2.309**
18	0.075	22	0.128	**2.081**
	0.062			

CS control 2		CS exposure 2		
23	0.408	26	0.449	**1.058**
24	0.403	27	0.407	**0.959**
25	0.462	28	0.277	**0.653**
	0.424			

IL-8 Protein

ESP and CLAM controls very high

EAVES control 1		EAVES exposure 1		
1	11.403	5	2.165	**0.171**
2	20.965	6	1.065	**0.084**
3	13.096	7	5.214	**0.412**
4	5.145	8	1.472	**0.116**
	12.652			

CS control 1		CS exposure 1		
9	9.736	12	10.094	**0.789**
10	16.494	13	16.493	**1.289**
11	12.143	14	6.283	**0.491**
	12.791			

EAVES control 2		EAVES exposure 2		
15	1.961	19	1.538	**1.253**
16	1.042	20	10.102	**8.228**
17	0.526	21	0.720	**0.586**
18	1.382	22	NA	
	1.228			

CS control 2		CS exposure 2		
23	3.492	26	19.164	**3.854**
24	2.508	27	10.071	**2.025**
25	8.918	28	23.165	**4.658**
	4.973			

Pure d-limonene, high concentration

LDH Release

EAVES control 1		EAVES exposure 1		
1	0.059	5	0.062	**0.768**
2	0.081	6	0.159	**1.969**
3	0.104	7	0.117	**1.449**
4	0.079	8	0.062	**0.768**
	0.081			

CS control 1		CS exposure 1		
9	0.134	12	0.171	**1.398**
10	0.103	13	0.130	**1.063**
11	0.130	14	0.156	**1.275**
	0.122			

EAVES control 2		EAVES exposure 2		
15	0.060	19	0.098	**1.441**
16	0.045	20	0.087	**1.279**
17	0.045	21	0.083	**1.221**
18	0.122	22	0.065	**0.956**
	0.068			

CS control 2		CS exposure 2		
23	0.084	26	0.106	**0.975**
24	0.120	27	0.127	**1.169**
25	0.122	28	0.147	**1.353**
	0.109			

IL-8 Protein

EAVES control 1		EAVES exposure 1		
1	10.852	5	2.061	**0.175**
2	5.686	6	16.961	**1.438**
3	12.428	7	17.190	**1.457**
4	18.229	8	7.708	**0.653**
	11.799			

CS control 1		CS exposure 1		
9	42.470	12	29.297	**0.827**
10	50.722	13	57.352	**1.619**
11	13.100	14	47.021	**1.327**
	35.431			

EAVES control 2		EAVES exposure 2		
15	5.135	19	12.139	**5.645**
16	1.466	20	1.869	**0.869**
17	1.160	21	10.264	**4.773**
18	0.840	22	5.988	**2.785**
	2.150			

CS control 2		CS exposure 2		
23	12.987	26	3.448	**0.307**
24	15.190	27	3.304	**0.295**
25	5.472	28	5.840	**0.521**
	11.216			

Household cleaner containing 2-butoxy ethanol (HC_{2BE}), low concentration

LDH Release

EAVES control 1		EAVES exposure 1		
1	0.054	5	0.185	3.379
2	0.054	6	0.164	2.995
3	0.054	7	0.146	2.667
4	0.057	8	0.121	2.210
	0.055			

CS control 1		CS exposure 1		
9	0.118	12	0.091	0.848
10	0.093	13	0.090	0.839
11	0.111	14	0.112	1.043
	0.107			

EAVES control 2		EAVES exposure 2		
15	0.043	19	0.075	1.000
16	0.098	20	0.089	1.187
17	0.081	21	0.101	1.347
18	0.078	22	0.068	0.907
	0.075			

CS control 2		CS exposure 2		
23	0.082	26	0.086	1.032
24	0.082	27	0.108	1.296
25	0.086	28	0.079	0.948
	0.083			

IL-8 Protein

EAVES control 1		EAVES exposure 1		
1	17.550	5	37.189	1.902
2	22.350	6	47.905	2.450
3	10.087	7	39.941	2.043
4	28.211	8	41.081	2.101
	19.550			

CS control 1		CS exposure 1		
9	143.096	12	148.365	1.120
10	128.768	13	140.143	1.058
11	125.619	14	146.323	1.104
	132.494			

EAVES control 2		EAVES exposure 2		
15	30.626	19	9.200	0.333
16	31.967	20	13.256	0.480
17	20.618	21	4.504	0.163
18	27.294	22	10.013	0.362
	27.626			

CS control 2		CS exposure 2		
23	96.657	26	85.646	0.976
24	77.156	27	56.861	0.648
25	89.345	28	104.369	1.190
	87.719			

Household cleaner containing 2-butoxy ethanol (HC_{2BE}), high concentration

LDH Release

EAVES control 1		EAVES exposure 1		
1	0.065	5	0.485	**8.186**
2	0.057	6	0.356	**6.008**
3	0.053	7	0.345	**5.823**
4	0.062	8	0.351	**5.924**
	0.059			

CS control 1		CS exposure 1		
9	0.122	12	0.075	**0.560**
10	0.146	13	0.153	**1.142**
11	NA	14	0.144	**1.075**
	0.134			

EAVES control 2		EAVES exposure 2		
15	0.052	19	NA	**NA**
16	0.056	20	0.372	**6.932**
17	0.053	21	0.147	**2.739**
18	NA	22	0.514	**9.578**
	0.054			

CS control 2		CS exposure 2		
23	0.102	26	0.105	**1.189**
24	0.080	27	0.166	**1.879**
25	0.083	28	0.123	**1.392**
	0.088			

IL-8 Protein

EAVES control 1		EAVES exposure 1		
1	2.598	5	21.714	**10.131**
2	1.619	6	19.127	**8.924**
3	1.862	7	18.117	**8.453**
4	2.494	8	19.532	**9.113**
	2.143			

CS control 1		CS exposure 1		
9	4.429	12	7.724	**1.614**
10	5.141	13	18.049	**3.772**
11	NA	14	5.616	**1.174**
	4.785			

EAVES control 2		EAVES exposure 2		
15	3.399	19	NA	**NA**
16	3.744	20	9.673	**2.817**
17	3.160	21	7.935	**2.310**
18	NA	22	9.066	**2.640**
	3.434			

CS control 2		CS exposure 2		
23	32.633	26	17.104	**0.715**
24	NA	27	22.407	**0.936**
25	15.233	28	28.400	**1.187**
	23.933			

Pure 2-butoxy ethanol, low concentration

LDH Release

EAVES control 1		EAVES exposure 1		
1	0.050	5	0.164	3.094
2	0.045	6	0.152	2.868
3	0.052	7	0.224	4.226
4	0.065	8	0.240	4.528
	0.053			

CS control 1		CS exposure 1		
9	0.092	12	0.139	1.376
10	0.094	13	0.090	0.891
11	0.117	14	0.091	0.901
	0.101			

EAVES control 2		EAVES exposure 2		
15	0.054	19	0.250	4.739
16	0.064	20	0.108	2.047
17	0.042	21	0.418	7.924
18	0.051	22	0.309	5.858
	0.053			

CS control 2		CS exposure 2		
23	0.081	26	0.170	1.932
24	0.091	27	0.154	1.750
25	0.092	28	0.169	1.920
	0.088			

IL-8 Protein

EAVES control 1		EAVES exposure 1		
1	1.428	5	1.739	1.146
2	1.334	6	3.345	2.204
3	1.811	7	3.657	2.409
4	1.499	8	3.036	2.000
	1.518			

CS control 1		CS exposure 1		
9	6.445	12	3.061	0.365
10	8.278	13	3.168	0.378
11	10.426	14	6.722	0.802
	8.383			

EAVES control 2		EAVES exposure 2		
15	2.427	19	2.958	1.533
16	1.715	20	1.886	0.978
17	1.884	21	5.751	2.981
18	1.691	22	4.698	2.435
	1.929			

CS control 2		CS exposure 2		
23	5.267	26	9.737	1.851
24	4.912	27	6.661	1.266
25	5.603	28	6.860	1.304
	5.261			

Pure 2-butoxy ethanol, high concentration

LDH Release

EAVES control 1		EAVES exposure 1		
1	0.048	5	0.131	2.817
2	0.043	6	0.283	6.086
3	0.054	7	0.115	2.473
4	0.041	8	0.141	3.032
	0.047			

CS control 1		CS exposure 1		
9	0.057	12	0.068	1.140
10	0.055	13	0.067	1.123
11	0.067	14	0.070	1.173
	0.060			

EAVES control 2		EAVES exposure 2		
15	0.064	19	0.137	2.514
16	0.049	20	0.071	1.303
17	0.054	21	0.264	4.844
18	0.051	22	0.068	1.248
	0.055			

CS control 2		CS exposure 2		
23	0.051	26	0.089	1.658
24	0.052	27	0.110	2.050
25	0.058	28	0.099	1.845
	0.054			

IL-8 Protein

EAVES control 1		EAVES exposure 1		
1	1.110	5	4.387	4.400
2	0.857	6	10.440	10.471
3	1.088	7	5.994	6.012
4	0.933	8	6.118	6.136
	0.997			

CS control 1		CS exposure 1		
9	6.197	12	6.150	1.079
10	5.011	13	6.026	1.058
11	5.884	14	5.838	1.025
	5.697			

EAVES control 2		EAVES exposure 2		
15	0.932	19	1.507	1.128
16	1.012	20	1.305	0.977
17	0.965	21	2.049	1.533
18	2.436	22	1.098	0.822
	1.336			

CS control 2		CS exposure 2		
23	3.013	26	3.997	1.266
24	3.300	27	2.221	0.703
25	3.161	28	2.123	0.672
	3.158			

Ozone only (100 ppb)

B2.
LDH Release

EAVES control 1		EAVES exposure 1		
1	0.057	4	0.067	1.322
2	0.051	5	0.052	1.026
3	0.044	6	0.080	1.579
	0.051			

CS control 1		CS exposure 1		
7	0.074	10	0.072	0.986
8	0.073	11	0.094	1.288
9	0.072	12	0.100	1.370
	0.073			

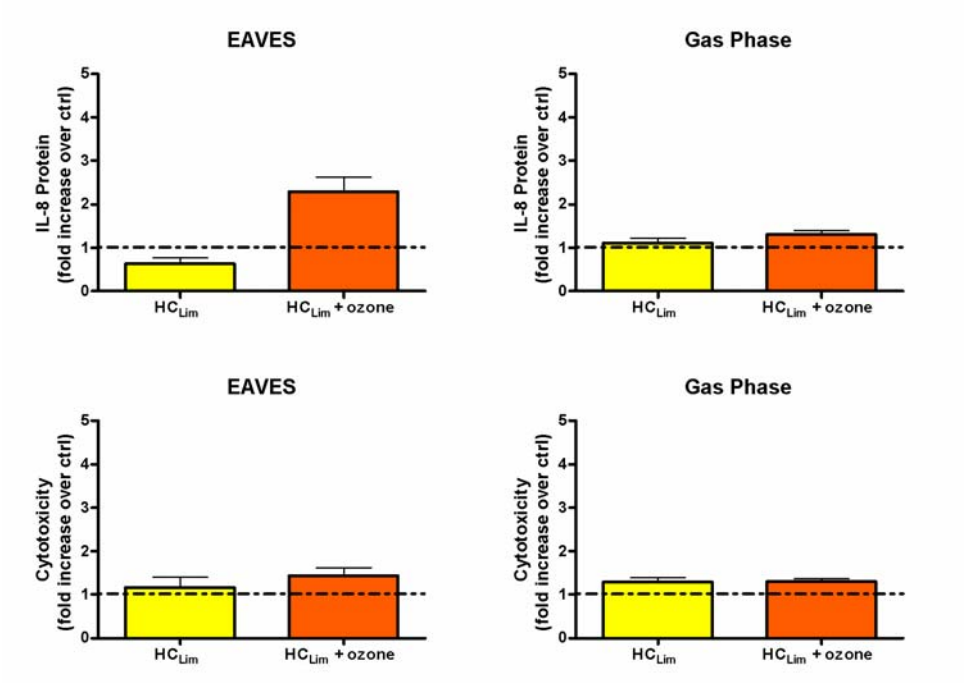
IL-8 Protein

EAVES control 1		EAVES exposure 1		
1	2.483	4	1.545	0.616
2	3.305	5	1.500	0.598
3	1.733	6	1.570	0.626
	2.507			

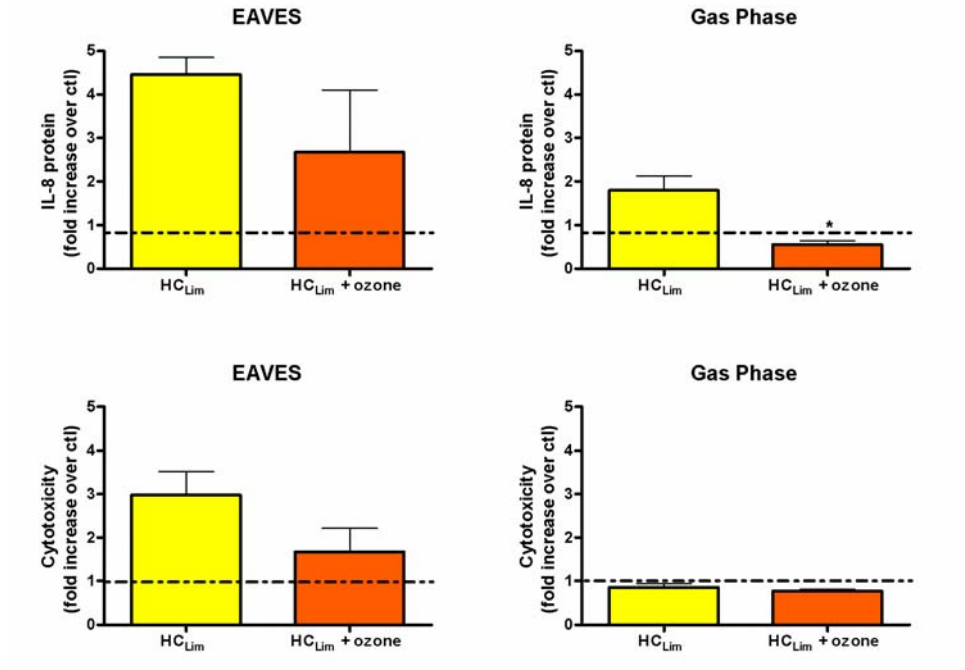
CS control 1		CS exposure 1		
7	4.366	10	9.023	1.214
8	14.331	11	6.124	0.824
9	3.595	12	11.265	1.516
	7.431			

Graphs of Inflammation and Cytotoxicity Data

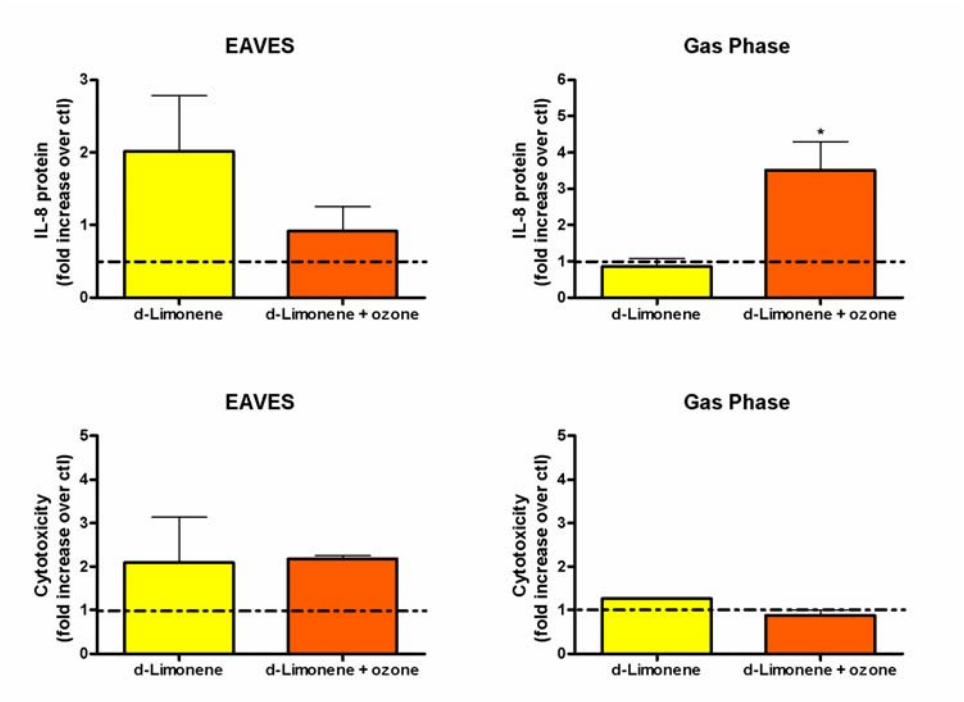
Household cleaner containing d-limonene (HC_{Lim}), low concentration



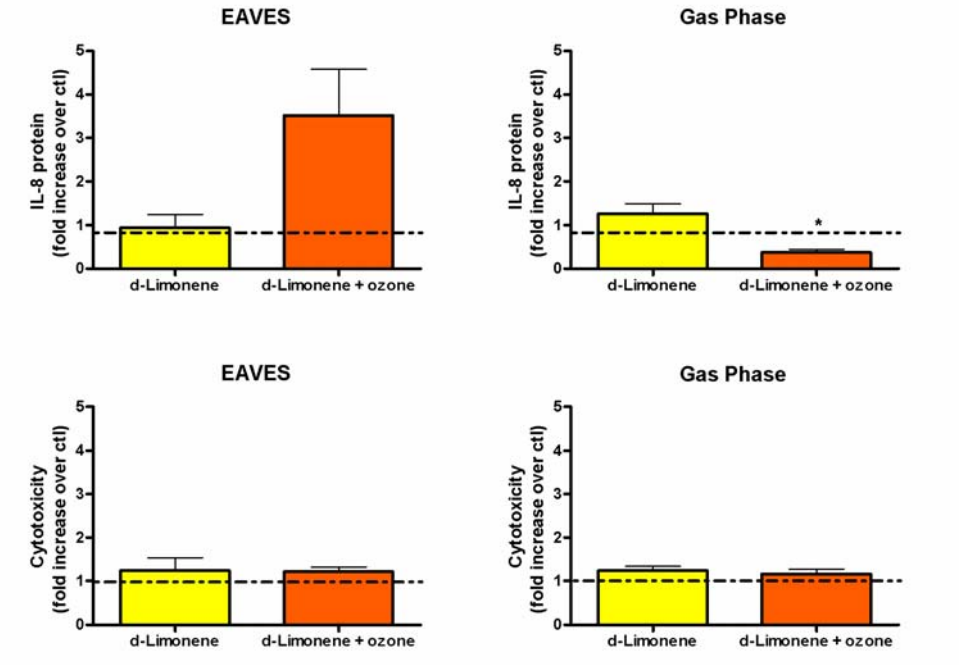
Household cleaner containing d-limonene (HC_{Lim}), high concentration



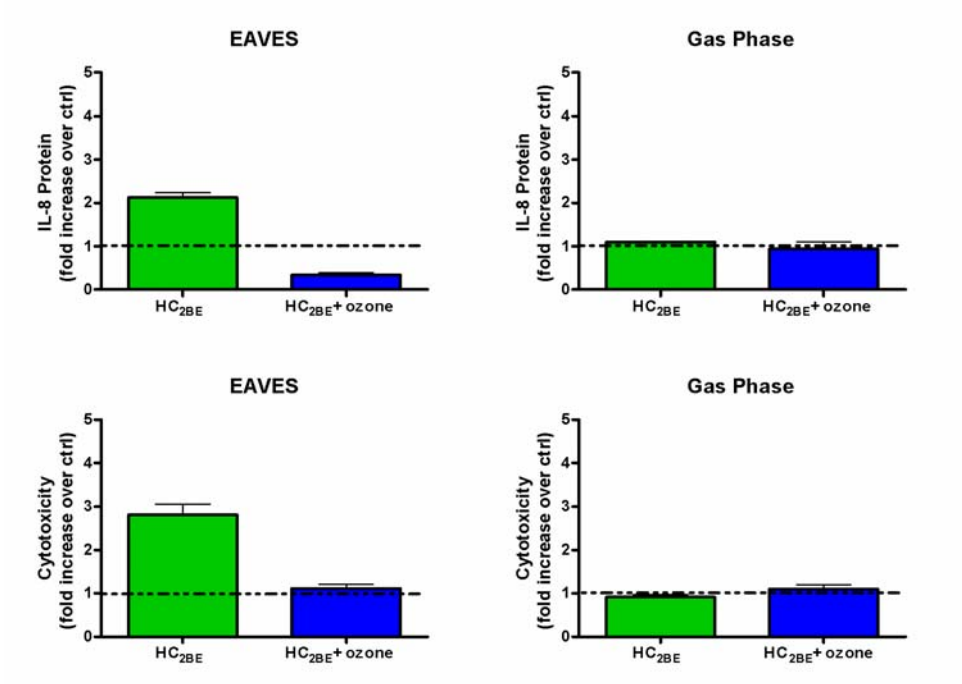
d-Limonene, low concentration



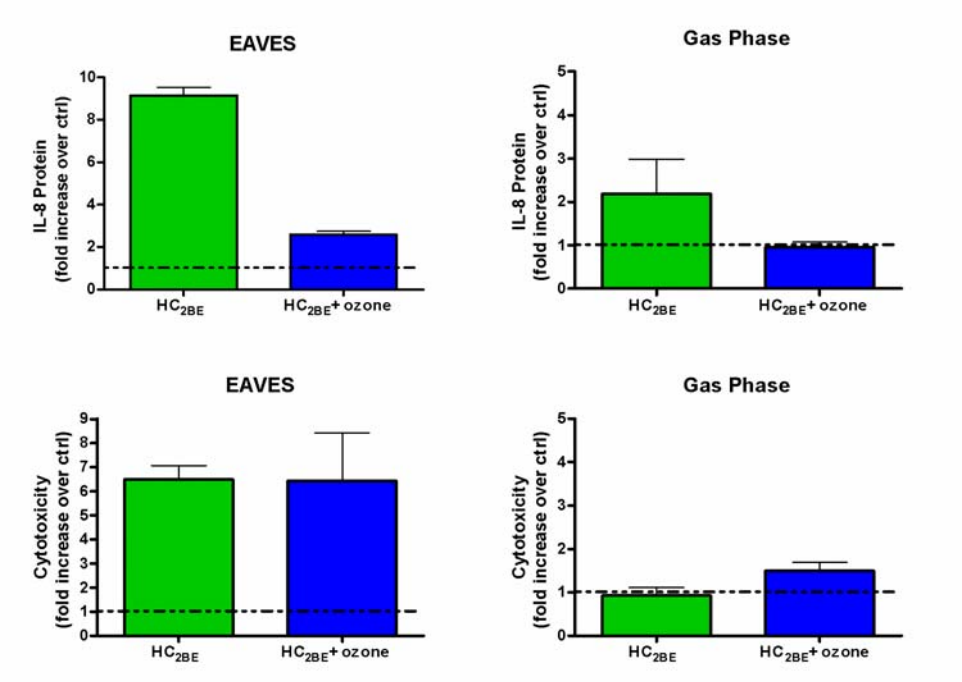
d-Limonene, high concentration



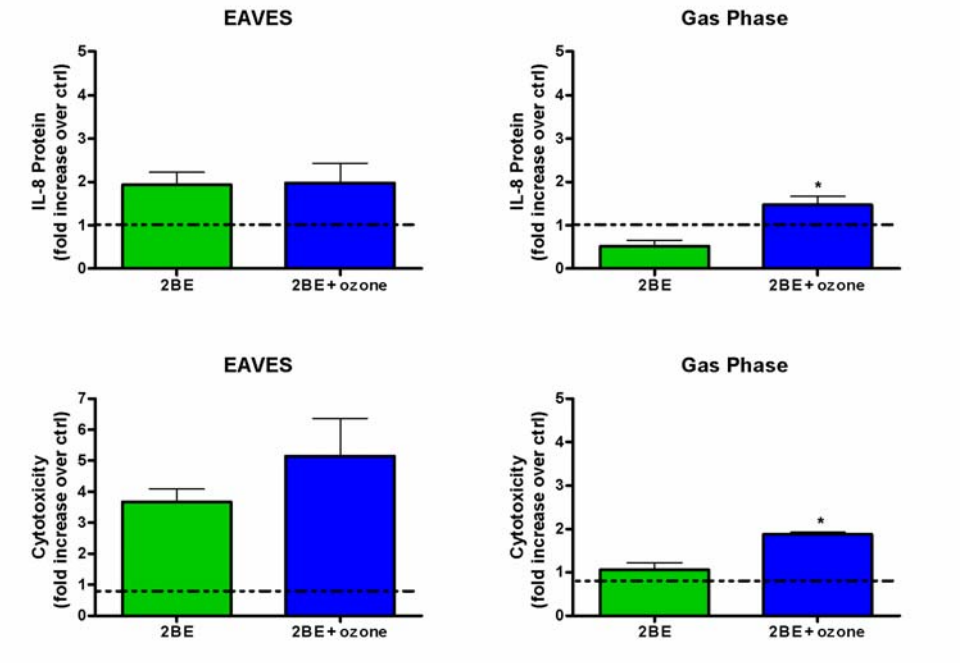
Household cleaner containing 2-butoxy ethanol (HC_{2BE}), low concentration



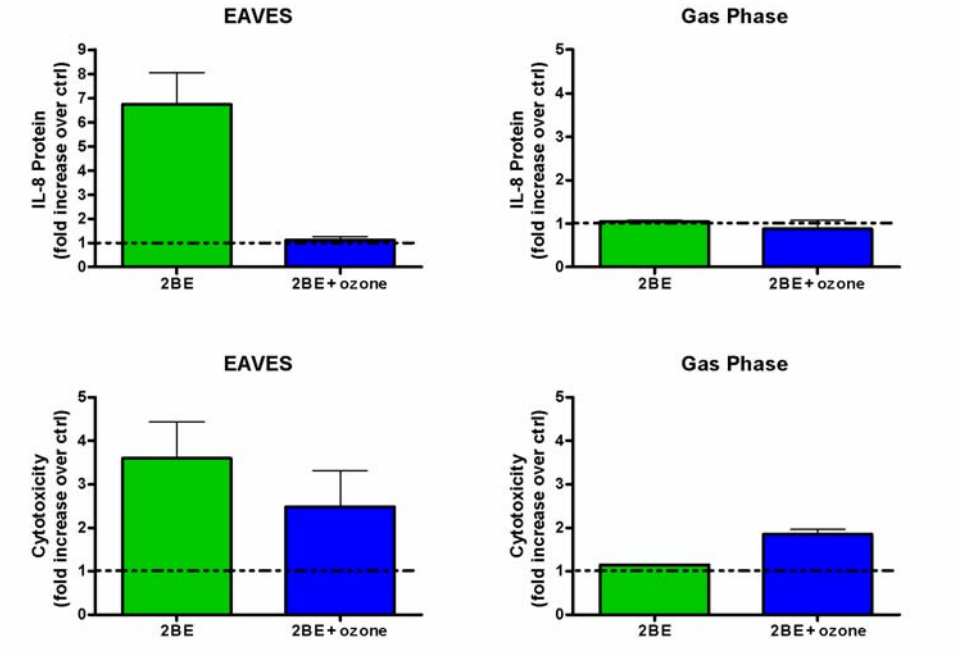
Household cleaner containing 2-butoxy ethanol (HC_{2BE}), high concentration



2-butoxy ethanol, low concentration



2-butoxy ethanol, high concentration



Appendix C – Summary of Particle-Phase Data for Toxicological Exposures

d-Limonene (low concentration)

SMPS	Cumulative Mass	Number Count
d-limonene low		
1hr, EAVES	1.71 μg/m^3 (1.31 – 2.32)	3.04 x 10^8 particles/m^3 (0.74 – 5.81)
3hr, GAS-PHASE	1.69 μg/m^3 (1.22 – 2.33)	3.03 x 10^8 particles/m^3 (0.41 – 9.18)
d-limonene low + ozone		
1hr, EAVES	593 μg/m^3 (570 – 620)	3.34 x 10^{11} particles/m^3 (2.35 – 4.58)
3hr, GAS-PHASE	551 μg/m^3 (467 – 620)	2.35 x 10^{11} particles/m^3 (1.10 – 4.69)

d-Limonene (high concentration)

SMPS	Cumulative Mass	Number Count
d-limonene high		
1hr, EAVES	8.60 μg/m^3 (7.86 – 9.29)	6.59 x 10^{10} particles/m^3 (0.26 – 130)
3hr, GAS-PHASE	8.18 μg/m^3 (7.31 – 9.29)	2.47 x 10^{10} particles/m^3 (0.17 – 130)
d-limonene high + ozone		
1hr, EAVES	430 μg/m^3 (424 – 437)	2.57 x 10^{11} particles/m^3 (1.63 – 3.91)
3hr, GAS-PHASE	422 μg/m^3 (297 – 437)	1.66 x 10^{11} particles/m^3 (0.65 – 4.02)

HC_{Lim} (low concentration)

SMPS	Cumulative Mass	Number Count
HC_{Lim} low		
1hr, EAVES	0.99 mg/m^3 (0.06 – 1.30)	3.46 x 10^{10} particles/m^3 (0.38 – 5.04)
3hr, GAS-PHASE	0.66 mg/m^3 (0.06 – 1.30)	2.26 x 10^{10} particles/m^3 (0.32 – 6.35)
HC_{Lim} low + ozone		
1hr, EAVES	0.85 mg/m^3 (0.74 – 0.89)	3.10 x 10^{10} particles/m^3 (2.60 – 3.56)
3hr, GAS-PHASE	0.77 mg/m^3 (0.03 – 0.89)	2.65 x 10^{10} particles/m^3 (0.29 – 3.63)

HC_{Lim} (high concentration)

SMPS	Cumulative Mass	Number Count
HC_{Lim} high		
1hr, EAVES	1.20 mg/m^3 (1.07 – 1.34)	5.63 x 10^{10} particles/m^3 (4.37 – 7.50)
3hr, GAS-PHASE	1.02 mg/m^3 (0.80 – 1.34)	4.16 x 10^{10} particles/m^3 (2.37 – 7.50)
HC_{Lim} high + ozone		
1hr, EAVES	0.96 mg/m^3 (0.79 – 1.01)	5.18 x 10^{10} particles/m^3 (3.90 – 6.03)
3hr, GAS-PHASE	0.82 mg/m^3 (0.65 – 0.92)	3.37 x 10^{10} particles/m^3 (2.17 – 4.62)

2-butoxy ethanol (low concentration)

SMPS	Cumulative Mass	Number Count
2BE low		
1hr, EAVES	1.64 μg/m^3 (1.32 – 2.19)	2.32 x 10^8 particles/m^3 (0.67 – 4.41)
3hr, GAS-PHASE	1.48 μg/m^3 (1.08 – 2.19)	2.08 x 10^8 particles/m^3 (0.36 – 4.76)
2BE low + ozone		
1hr, EAVES	1.93 μg/m^3 (1.58 – 2.57)	2.49 x 10^8 particles/m^3 (0.70 – 4.97)
3hr, GAS-PHASE	1.99 μg/m^3 (1.50 – 2.63)	3.13 x 10^8 particles/m^3 (0.47 – 7.52)

2-butoxy ethanol (high concentration)

SMPS	Cumulative Mass	Number Count
2BE high		
1hr, EAVES	1.93 μg/m^3 (1.49 – 2.82)	2.22 x 10^8 particles/m^3 (0.50 – 4.34)
3hr, GAS-PHASE	2.03 μg/m^3 (1.49 – 2.86)	2.32 x 10^8 particles/m^3 (0.26 – 5.42)
2BE high + ozone		
1hr, EAVES	3.05 μg/m^3 (2.12 – 4.01)	4.32 x 10^8 particles/m^3 (1.18 – 8.48)
3hr, GAS-PHASE	3.91 μg/m^3 (2.12 – 5.08)	5.57 x 10^8 particles/m^3 (0.89 – 12.0)

HC_{2BE} (low concentration)

SMPS	Cumulative Mass	Number Count
HC_{2BE} low		
1hr, EAVES	1.07 mg/m^3 (0.90 – 1.29)	7.13 x 10^{10} particles/m^3 (5.17 – 9.88)
3hr, GAS-PHASE	0.89 mg/m^3 (0.64 – 1.29)	5.32 x 10^{10} particles/m^3 (2.89 – 10.3)
HC_{2BE} low+ ozone		
1hr, EAVES	0.58 mg/m^3 (0.52 – 0.61)	2.79 x 10^{10} particles/m^3 (2.28 – 3.36)
3hr, GAS-PHASE	0.54 mg/m^3 (0.44 – 0.63)	2.54 x 10^{10} particles/m^3 (1.82 – 3.37)

HC_{2BE} (high concentration)

SMPS	Cumulative Mass	Number Count
HC_{2BE} high		
1hr, EAVES	0.88 mg/m^3 (0.63 – 1.29)	4.74 x 10^{10} particles/m^3 (3.03 – 6.64)
3hr, GAS-PHASE	0.87 mg/m^3 (0.44 – 1.29)	3.72 x 10^{10} particles/m^3 (1.46 – 7.17)
HC_{2BE} high + ozone		
1hr, EAVES	0.73 mg/m^3 (0.42 – 0.78)	2.05 x 10^{10} particles/m^3 (1.35 – 2.36)
3hr, GAS-PHASE	0.68 mg/m^3 (0.42 – 0.78)	1.85 x 10^{10} particles/m^3 (1.22 – 2.36)

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