EXPOSURE CHARACTERIZATION AND BIOMARKER EVALUATION OF 1,3-BUTADIENE RESULTING FROM MOBILE SOURCE EMISSIONS

by

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Abstract

Exposure to automobile exhaust is a significant public health concern because it is ubiquitous in communities and it contains numerous hazardous air pollutants (HAPs) including 1,3-butadiene, a known human carcinogen. In addition to being ubiquitous, concentration of automobile exhaust is elevated in densely populated urban areas due to high vehicle density and increasing congestion, further exacerbating risks. This thesis work was designed to advance our understanding of mobile source-related exposure to 1,3-butadiene including the development of methods for measuring internal dose. Together, these advances address critical uncertainties in risk assessment and epidemiological studies linking automobile exhaust and cancer.

To investigate the relationship between vehicle counts and ambient 1,3-butadiene levels, concurrent measurements of both variables were made at the Baltimore Harbor Tunnel (BHT) tollbooth facility in Baltimore, MD, over consecutive 3-hour intervals on 7 weekdays (n=56). The 3-hour outdoor 1,3-butadiene concentrations varied according to time of day and traffic volume. The minimum levels occurred at night (12 a.m.– 3 a.m.) with a mean of 2.0 $\mu g/m^3$ (SD=1.3, n=7) while the maximum levels occurred during the morning rush hour (6 a.m. – 9 a.m.) with a mean of 11.9 $\mu g/m^3$ (SD=4.6, n=7). The corresponding 3-hour traffic counts were 1,413 (SD=144) and 16,893 (SD=692), respectively. Using multivariate regression, a significant association ($p<0.001$) between traffic and curbside pollutant concentrations was observed. As much as 62% of the variability in ambient 1,3-butadiene levels was explained by traffic volume, class, and

meteorology. Results suggest that >2-axle vehicles emit 32 times more 1,3-butadiene than do 2-axle vehicles. This study provides an empirical model for estimating curbside air pollution levels associated with traffic that may be relevant to exposures in the urban environment.

The study was further extended to assess worker exposures. The potential workday exposure of tollbooth workers to 1,3-butadiene (and other HAPs) and the protection offered by the tollbooths were evaluated using simultaneous indoor and outdoor measurements at the BHT tollbooth. Mean outdoor 1,3-butadiene concentrations varied by shift with the morning (10.7 $\mu g/m^3$) exceeding afternoon (7.2 $\mu g/m^3$) and the lowest levels observed during the night (3.7 $\mu g/m^3$) when traffic volume was the lowest. In comparison, considerable protection was provided to workers by the indoor environment where lower concentrations of 1,3-butadiene were observed for all three shifts (2.9, 0.9 and 0.9 $\mu g/m^3$ respectively). Greatest protection offered by the tollbooth was observed during the afternoon shift (8 fold reduction in 1,3 butadiene concentrations) whereas the morning and night shift experienced similar protection (~ 4 fold reduction). Chlorinated hydrocarbons were observed at higher concentrations within the tollbooth indicating the presence of indoor sources and the opportunity for exposure mitigation. Levels of PAHs were similarly reduced from outdoors (50 ng/m^3) to indoors (15.4 ng/m^3). The protective nature of the tollbooth highlighted in this study is likely due to the positive pressure control ventilation system that was present at this specific facility, which represents 55% of tollbooths in Maryland. This study provides an estimate of tollbooth workers potential exposures to various mobile source related pollutants and highlights the protective nature of tollbooths equipped with efficient control ventilation systems.

The final dissertation component considered personal exposures to 1,3-butadiene and evaluated urinary biomarkers. Personal exposure was characterized using personal air samples in three exposure scenarios: weekend suburban exposure, weekday urban exposure and tollbooth worker's workday exposure. Furthermore, the urinary biomarkers of 1,3-butadiene: dihydroxybutyl mercapturic acid (DHBMA) and monohydroxybutyl mercapturic acid (MHBMA), which have been used extensively in high occupational exposure settings, were evaluated to discern subtle differences in environmental exposures occurring between the three exposure scenarios. The mercapturic acids (DHBMA and MHBMA) were analyzed using liquid chromatography with tandem mass spectrometry (LC-MS/MS). Quantitation was achieved using isotopically labeled internal standards. The limit of detection determined using spiked synthetic urine was 0.4 ng/mL for the MHBMA and 3 ng/mL for the DHBMA. Results showed that the three exposure groups differed by personal exposure to 1,3-butdiene with median exposures measuring 0.79, 1.62 and 2.38 ug/m^3 for weekend suburban, weekday urban and tollbooth worker exposure groups respectively. The DHBMA levels (mean, SD) observed for the tollbooth workers (362.3, 192.1 ng/mL) were higher than that observed for the weekend suburban exposure (257.7, 227.7 ng/mL) and the weekday urban exposure (241.0, 161.4 ng/mL). Similarly, MHBMA levels (mean, SD) observed for the tollbooth workers (7.8, 8.2 ng/mL) were higher than that observed for the weekend suburban exposure (6.3, 5.9 ng/mL) and the weekday urban exposure (5.9, 4.9 ng/mL) groups. Although the trends in mean DHBMA and MHBMA were consistent with the group exposure levels, (i.e. tollbooth workers > weekday urban > weekend suburban) no statistically significant differences were found between groups. Further, the consistency in trend with exposure

tollbooth workers > weekday urban > weekend suburban) no statistically significant differences were found between groups. Further, the consistency in trend with exposure was not observed when DHBMA and MHBMA were adjusted for creatinine. There was no significant association between personal exposure and either urinary biomarker (r-square< 0.1 and p>0.05).

The current study has provided significant advancement in the development of precise, sensitive, and accurate method for the measurement of 1,3-butadiene metabolites in environmental settings. However, an absence of association between personal exposure and biomarker level was observed in this study. It is likely that the lack of association is attributable to limitation of study design (small sample size and unexpectedly small exposure differential between groups) and lack of specificity (in the case of DHBMA).

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Coming into the program, I was aware that undertaking doctoral dissertation would be a difficult task. I just had no point of reference, for the "difficult task". Having gone through it, now I have come to a realization that the "difficult task" would have turned into an "impossible task" if it were not for the expert help and guidance from many people.

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Chapter 1: Introduction

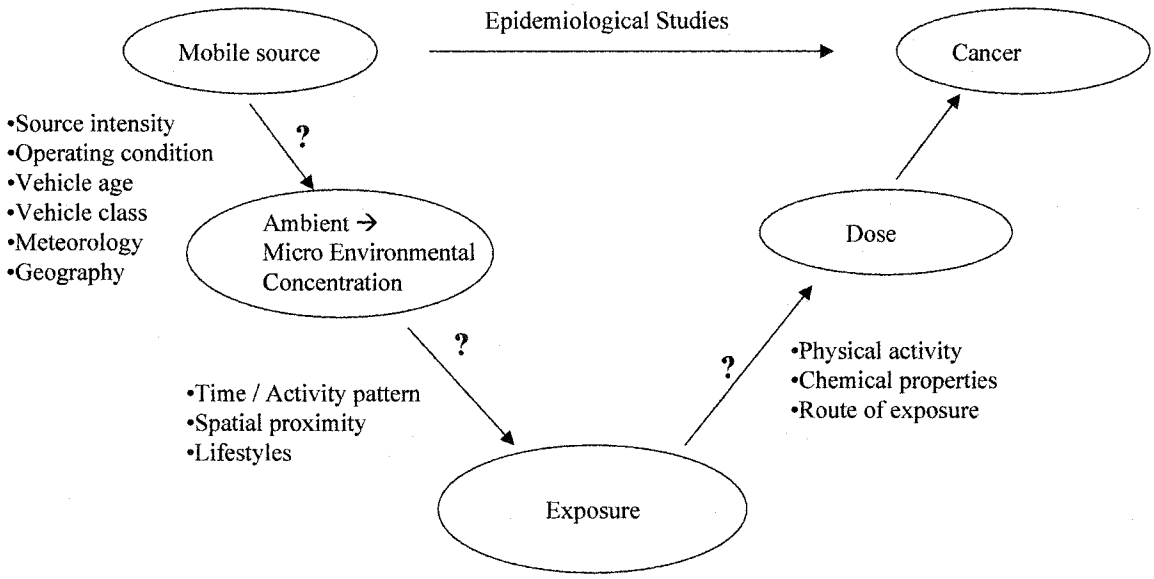
Automobile exhaust has become pervasive in industrialized societies and its presence is intensifying in the less developed parts of the world due to rapid urbanization and subsequent industrialization (Mage 1992). Epidemiological studies have linked exposure to automobile exhaust with elevated risks of lung cancer (Gustavsson *et al.* 1990; Gustavsson *et al.* 2000; Hemminki and Pershagen 1994; Jakobsson *et al.* 1997; Steenland *et al.* 1990), male breast cancer (Hansen 2000), childhood cancers (Feychting *et al.* 1998; Pearson *et al.* 2000; Savitz and Feingold 1989) and leukemia (Crosignani *et al.* 2004; Pearson *et al.* 2000). Despite this evidence, little is known regarding the specific etiologic agents within the complex mixture of automobile exhaust that are responsible for the elevated cancer risks. Identifying these agents, their spatial distribution in ambient air and in micro-environments, and characterizing personal exposures to such etiologic agents will further clarify the relationship between mobile sources and cancer risks (Figure 1.1). Hazardous Air Pollutants (HAPs) such as benzene, polycyclic aromatic hydrocarbons and formaldehyde have been identified as the chemicals of main concern in automobile exhaust. More recently, 1,3-butadiene has emerged as one of the prime candidates for the elevated cancer incidence, accounting for more cancer risks than benzene, formaldehyde and PAH combined (U.S.Environmental Protection Agency Office of Air Quality 2000).

Populations at Risk

The potential for exposure to automobile exhaust is most pronounced in urban locations where heavily commuted roadways transect densely populated communities. Human

exposure to these mobile source emissions can be substantial due to increasing: 1) traffic volume and congestion, 2) vehicle-miles driven, and 3) numbers of heavier, less efficient sport utility vehicles (McAuley 2003).

Figure 1.1: Exposure assessment to reduce uncertainty in cancer epidemiology



In addition to urban populations, mobile source emissions also pose risks to some occupational populations such as tollbooth attendants due to both the proximity and the intensity of the emission sources at their workplace. Tollbooth workers routinely spend a large fraction of their workday within a few feet of vehicles emitting a wide range of toxic pollutants. Traffic volumes at tollbooth facilities can number in the tens of thousands of vehicles per hour. Furthermore, tollbooth related-vehicle operation including acceleration and deceleration are associated with high engine and brake wear

emissions (Cadle *et al.* 2001; Schauer *et al.* 2002a). As a result of these factors, tollbooth facilities potentially represent a likely worst-case scenario for exposure to mobile source-related air pollution. Yet, despite the potential hazards, little has been done to characterize the personal exposures experienced by these at-risk individuals.

Primary Objectives

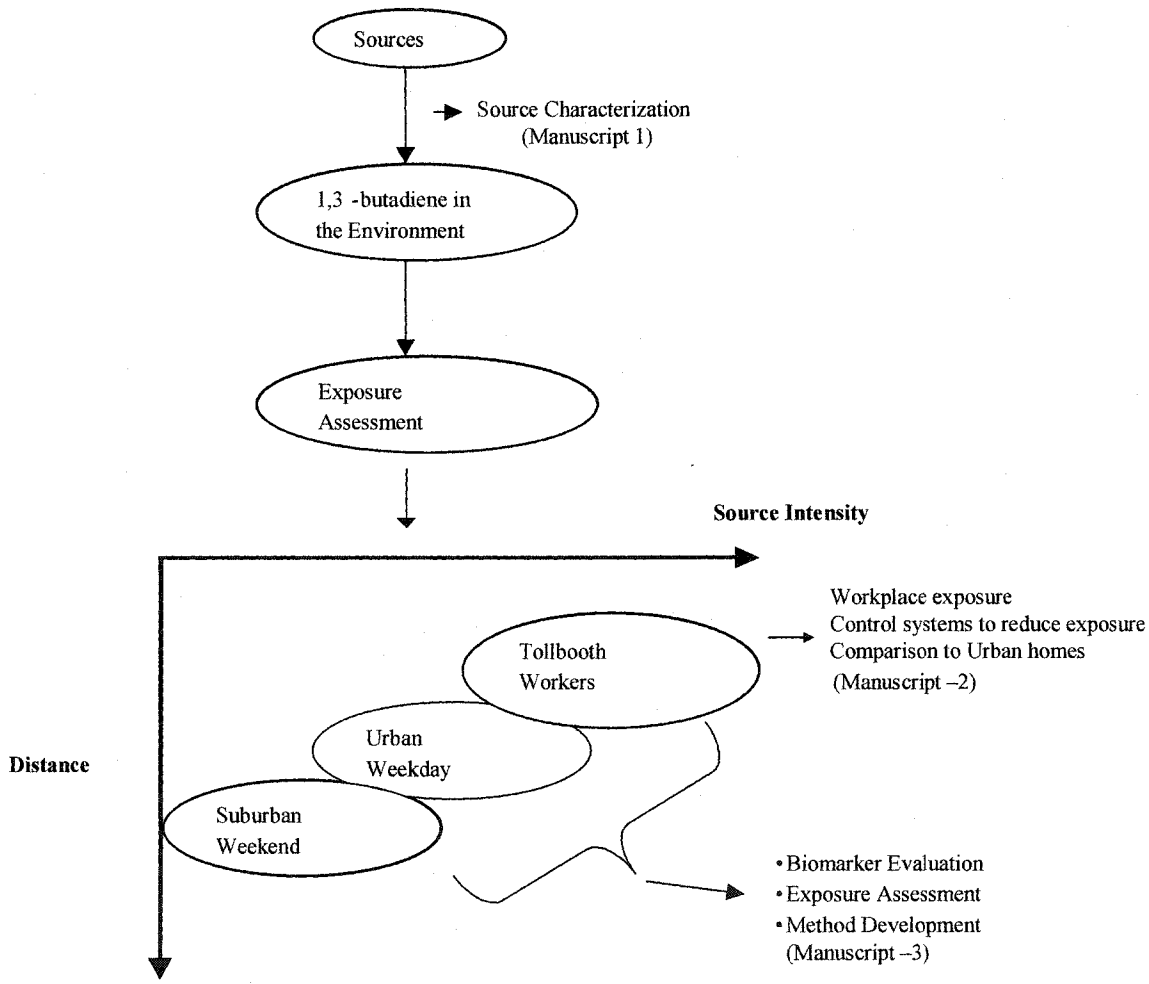
As mentioned above, exposure to automobile exhaust is of great concern to public health because it is not only ubiquitous in the environment but also contains several HAPs including 1,3-butadiene, a known human carcinogen (National Toxicology Program 2000; U.S.Environmental Protection Agency 2002). This thesis work was designed to characterize exposure to 1,3-butadiene across different exposure scenarios and evaluate two urinary biomarkers for environmental exposures to 1,3-butadiene. Specifically, there are three primary objectives of this thesis:

1) Assess curbside levels of mobile source related carcinogens and the differential rates of 1,3-butadiene emissions among various vehicle types in a real world environment,
2) Characterize environmental exposures to 1,3-butadiene related to automobile exhaust, using personal exposure measurements and
3) Evaluate urinary metabolites of 1,3-butadiene as biomarkers to discern subtle differences in environmental exposures.

The study design used to achieve these objectives included mobile source hot spots (an urban location and commuting highways) with three exposure scenarios: weekend

suburban exposure, weekday urban exposure and tollbooth worker exposure. These exposure scenarios differed by virtue of spatial proximity to and intensity of mobile sources (Figure 1.2). The two urinary metabolites examined in this study: monohydroxybutyl mercapturic acid (MHBMA) and dihydroxybutyl mercapturic acid (DHBMA), have been extensively used in occupational settings where exposure levels are in excess of 100 fold higher than those encountered in environmental settings. The goal of this evaluation was to determine whether the urinary biomarkers could be used to discern differences in environmental exposures. The sensitivity required for the analysis was achieved with a newly enhanced method that utilizes liquid chromatography tandem mass spectrometry (LC-MS/MS).

Figure 1.2: Schematic of study design



Thesis Outline

This thesis is organized into 6 different chapters. Details pertaining to 1,3-butadiene, including previous work focusing on personal exposures and biomarkers is presented in the background section (Chapter 2). Chapter 3 is a peer-reviewed published manuscript that describes the measured association between vehicle volume and the resulting curbside ambient pollutant concentrations of 1,3-butadiene, benzene and PAH at a tollbooth facility, and further provides a direct comparison of emission rates between different vehicle types (Sapkota and Buckley 2003).

Chapter 4 is a second peer-reviewed manuscript that examines indoor and outdoor VOC (volatile organic compound) levels at a workplace for one of the populations at-risk, i.e. tollbooth workers (Sapkota *et al.* 2004). For this portion of the research, concurrent measurements of 1,3-butadiene and other VOCs were made outside and inside the tollbooth. By examining the relationship between the indoor and outdoor concentrations, the protection offered by the control ventilation system was evaluated. VOCs that are known to be absent from automobile exhaust were used as negative controls for this evaluation. Finally, the concentrations inside the tollbooths were compared to literature reports of concentrations in typical urban homes from three cities (New York, Los Angeles and Baltimore).

Chapter 5 is a draft of a third manuscript that describes the methods and findings related to the measurements of the mercapturic acids (biomarkers) of 1,3-butadiene and their association with measured personal exposure. Here the biomarkers were evaluated by

examining differences in metabolite levels among three differentially exposed groups: tollbooth workers (high), volunteers located near a busy urban arterial on a weekday (medium) and the same volunteers located at their suburban home on weekend (low). The detection of the urinary metabolites associated with low-level environmental exposure required substantial improvements over previously published analytical methods. Therefore, a major scientific contribution from this effort was the development of a more sensitive analytical method for measuring the urinary metabolites of 1,3-butadiene.

Finally, Chapter 6 is an overall synthesis of the thesis. It discusses the limitations of the research, specific recommendations on addressing these limitations, lessons learned and future research directions. Significant time was invested in developing the analytical method for the measurements of the 1,3-butadiene urinary biomarkers, initially using GC/MS and then using LC-MS/MS. Details of the method development is documented in this chapter so that future students/readers working in similar areas can benefit from the method development aspect. In addition, this chapter presents the mass balance of butadiene exposure and the amount of biomarker excreted using a Monte-Carlo simulation that incorporates the data from this study, as well as other parameters that have been reported in the literature.

Chapter 2: Background

Butadiene (BD) is a colorless gas that is flammable at room temperature (Table 2.1) (US EPA Office of Air and Radiation 1993). It is highly reactive and polymerizes very easily. Since it is a gaseous pollutant, inhalation is the primary route of exposure.

Table 2.1: Physical and chemical properties of 1,3-butadiene

Properties	**Values**
Molecular Wt. (g/mole)	54.1
Melting Point (°C)	-108.9
Boiling Point (°C)	-4.4
Density (g/cm^3)	0.621
Vapor Pressure (atm)	1.2
Flash Point (°C)	-105
Solubility in Water (g/L)	0.74
Conversion Factor	1 ppb= 2.21 ug/m^3
(@ 25°C)	1 ug/m^3=0.45 ppb

Butadiene is primarily used in the manufacturing of resins, plastics and synthetic rubber. The worldwide production of BD is 12 billion pounds per year, of which the U.S. accounts for approximately 25% (Morrow 1990). Of the approximately 4 billion pounds produced in the U.S., about 2.8 billion pounds is used in styrene-butadiene rubber (SBR) and polybutadiene rubber (BR) industries. Neoprene rubber industry is the third largest user of BD, accounting for 360 million pounds annually (US Department of Health and Human Services 1984). Major anthropogenic sources of environmental BD include

automobile exhaust, prescribed burns, manufacturing & processing facilities, plastic and rubber factories, and oil refineries. Of all the anthropogenic sources, automobile exhaust (on highway and off highway) is by far the most dominant source accounting for as much as 60% (33,000 tons) of annual national BD emissions (National Toxicology Program 2000) followed by wildfires and prescribed burns accounting for 30% (20,000 tons). Industrial processes, including organic chemical manufacturing, polymer and resin production, secondary lead smelting and petroleum refining account for an additional 6% (4,000 tons). These national emission statistics clearly indicate that any effort in reducing levels of BD in the environment (and personal exposure) needs to focus on mobile sources.

Personal exposure to 1,3-butadiene has not been well characterized primarily because the method commonly used for sampling VOCs using 3M badges (passive sampling) or the charcoal tubes (active sampling) does not adequately sample 1,3-butadiene (Chung *et al.* 1999; Kim *et al.* 1999). Lately, the increasing use of thermal desorption techniques that utilize multi-bed sorbent tubes consisting of Carbopak B and Carbosieve SIII has enabled more quantitative assessments of 1,3-butadiene exposure. Utilizing this approach, Kim et al. have quantified 1,3-butadiene levels in different microenvironments including homes (1.1 ug/m^3), restaurants (1.5 ug/m^3), pubs (3.0 ug/m^3) and cars (7.9 ug/m^3) respectively (Kim *et al.* 2001). Similarly, Kinney et al have reported mean 1,3-butadiene concentrations of 0.13 ug/m^3 and 1.18 ug/m^3 for outside and inside homes and mean personal exposure of 0.87 ug/m^3 (Kinney *et al.* 2002). More recently, a study conducted in Mexico City reported median 1,3-butadiene concentrations of 0.8, 2.0 ug/m^3 for

outdoor and indoor environment and 2.1 ug/m^3 for personal exposure (Serrano-Trespalacios *et al.* 2004).

Based on several epidemiological studies, 1,3-butadiene (BD) has been classified as a known human carcinogen (National Toxicology Program 2000; U.S.Environmental Protection Agency 2002). The US Environmental Protection Agency (EPA) estimates that the human lifetime excess cancer risk from chronic exposure to BD is $8x10^{-2}$ per ppm ($3.5x10^{-5}$ per ug/m^3) based on a linear extrapolation of increased leukemia risks observed in occupationally exposed workers (U.S.Environmental Protection Agency 2002). Based on these estimates, an acceptable level of lifetime risk (1 cancer case in a million/ 70 years) would result if individuals were exposed to 0.01 ppb BD over their lifetime. There are some limited data available from monitoring sites across the country, which indicate that ambient levels of BD exceed the 0.01 ppb level in most urban areas, including Baltimore City (Maryland Department of the Environment 2000). Therefore, populations living in urban environments are potentially exposed to BD levels that result in cancer rates, which exceed the "acceptable" risk of $1x10^{-6}$. However, such ambient concentrations are not a true reflection of personal exposures experienced by residents living in urban areas. Several other factors, in addition to ambient concentrations, contribute to personal exposures, including occupation, proximity of ones residence to high-traffic roadways, time spent in automobiles, other personal habits and meteorological conditions. Thus, an overall estimate of personal exposures to 1,3-butadiene across different population groups and covering various geographical areas is warranted to identify populations at risk, as well as the magnitude of such risks.

The first known controlled exposure to BD in humans dates back to 1944, when Carpenter et al exposed two humans to 8,000 ppm BD for an 8-hour period (Carpenter *et al.* 1944). BD was considered to be relatively nontoxic until the mid 1980s, as apparent in the high occupational permissible exposure limit (PEL) of 1,000 ppm in air set forth by the Occupational Safety and Health Administration (OSHA). But several studies in the 80s revealed that BD is a potent multi-organ carcinogen in mice when administered via inhalation (Huff *et al.* 1985b; Melnick *et al.* 1988). BD also has been shown to be carcinogenic in rats, but not as potent as in the case of mice. Mice exposed to BD showed increased tumor incidence in the hematopoietic system, heart, lung, forestomach, harderin gland, perpetual gland, liver, mammary gland, ovaries and kidneys (Huff *et al.* 1985a; Melnick *et al.* 1990; National Toxicology Program 1984). Similarly, rats exposed to BD showed increased tumor incidence in the brain, pancreas, testes, thyroid gland, mammary gland, uterus and zymbal gland (Owen *et al.* 1987). These data, along with the epidemiological studies, led both the OSHA and the American Convention of Governmental Industrial Hygienists (ACGIH) to lower their respective PEL and Threshold Limit Value (TLV) to 1 and 2 ppm, respectively.

Acute exposure to BD is associated with irritation of the eyes, nasal passages, throat, and lungs, and neurological effects such as blurred vision, fatigue, headaches, and vertigo (Agency for Toxic Substances and Disease Registry (ATSDR) U.S.Department of Health and Human Services 1992). Using traditional epidemiological approaches, Divine *et al.* (Divine *et al.* 1993) showed that BD exposure is associated with lymphosarcoma and leukemia in routine and non-routine workers who were employed in BD monomer

industries in Port Neches, Texas for less than 10 years. However, the association was not significant for those who worked for more than 10 years. A retrospective cohort study of SBR workers showed that BD exposure was significantly associated with leukemia in hourly workers as compared to the general population (Delzell *et al.* 2001; Macaluso *et al.* 1996). Similarly Matanoski et al studied 12,110 workers from eight SBR industries in the US and Canada and found that BD exposure was associated with leukemia only in certain subgroups (Matanoski *et al.* 1990). A nested case control study of the same population by Santos-Burgoa and Matanoski revealed a stronger association between BD exposure and leukemia (Matanoski *et al.* 1993a; Matanoski and Santos-Burgoa 1994; Santos-Burgoa *et al.* 1992).

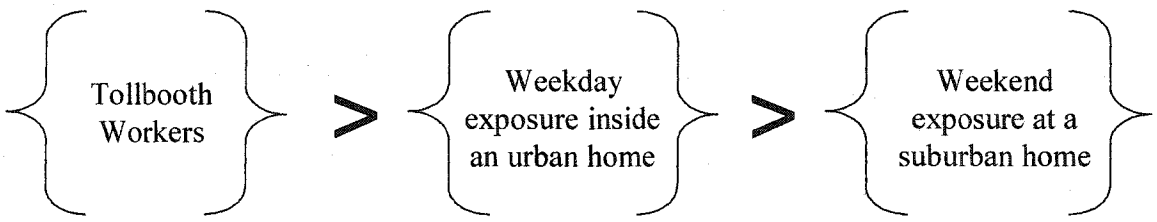
Several investigators have used molecular epidemiological approach to establish a relationship between exposure to 1,3-butadiene and intermediate markers of disease. Such studies have reported conflicting findings. Hays *et al.* reported that the BD polymer production workers in China had higher levels of *N*-(2,3,4-trihydroxybutyl) valine (THBVal) adducts, compared to their unexposed counterparts (Hayes *et al.* 2000). However, the two groups did not differ with regard to the frequency of sister chromatid exchanges, aneuploidy and hypoxanthine–guanine phosphoribosyltransferase (HPRT) somatic mutations, indicating a lack of association between exposure and the markers studied. Albertini *et al.* and Swenberg *et al.* also found no association between BD exposure and HPRT mutations or sister chromatid exchanges (SCE) among exposed workers and non exposed controls working in the same factory (Albertini *et al.* 2001; Swenberg *et al.* 2001). However, other researchers have shown an increase in HPRT

mutant lymphocytes in workers exposed to BD (Ward, Jr. *et al.* 1994; Ward, Jr. *et al.* 1996). In a more recent study of Czech butadiene workers, Albertini et al have reported a significant association between butadiene exposure levels and both HBVal and THBVal hemoglobin adducts, but no association between exposure and HPRT mutations.

The Czech study also utilized the two urinary biomarkers of 1,3-butadiene, dihydroxybutyl meracpturic acid (DHBMA or M1) and monohydroxybutyl meracpturic acid (MHBMA or M2). A significant association was reported between exposure and the urinary biomarkers (Albertini *et al.* 2001; Albertini *et al.* 2003; van Sittert *et al.* 2000). The mean concentration of DHBMA in the urine of workers collected after their shifts were 355, 508 and 1479 ng/mL for the control, monomer and SBR worker respectively. The corresponding MHBMA values were 1.6, 3.6 and 20 ng/mL respectively for the control, monomer and SBR workers. Similarly Ubran et al. studied these two biomarkers in smokers and non-smokers and reported that smokers excreted significantly higher amounts of DHBMA and MHMBA compared to non-smokers (Urban *et al.* 2003). The total amount of DHBMA and MHBMA excreted by smokers in 24 hours was 644 and 86.4 ug, respectively, compared to nonsmokers who excreted 459 and 12.5 ug of the two biomarkers respectively, within the same period of time. In contrast, in a recent study of petrochemical workers in Italy, Fustiononi et al reported no significant difference in the median DHBMA and MHBMA between exposed workers and their non-exposed counterparts (Fustinoni *et al.* 2004). The median DHBMA and MHBMA levels for the control group were 547 and 5.6 ng/mL respectively and those for the exposed workers were 507 and 5.0 ng/mL respectively. Their findings are of particular importance to this

study because the median exposures of the exposed workers 1.5 ug/m^3 (mean 11.5 ug/m^3), and the control workers, 0.4 ug/m^3 (mean 0.9 ug/m^3), are more relevant to this study, compared to the Czech worker's exposures.

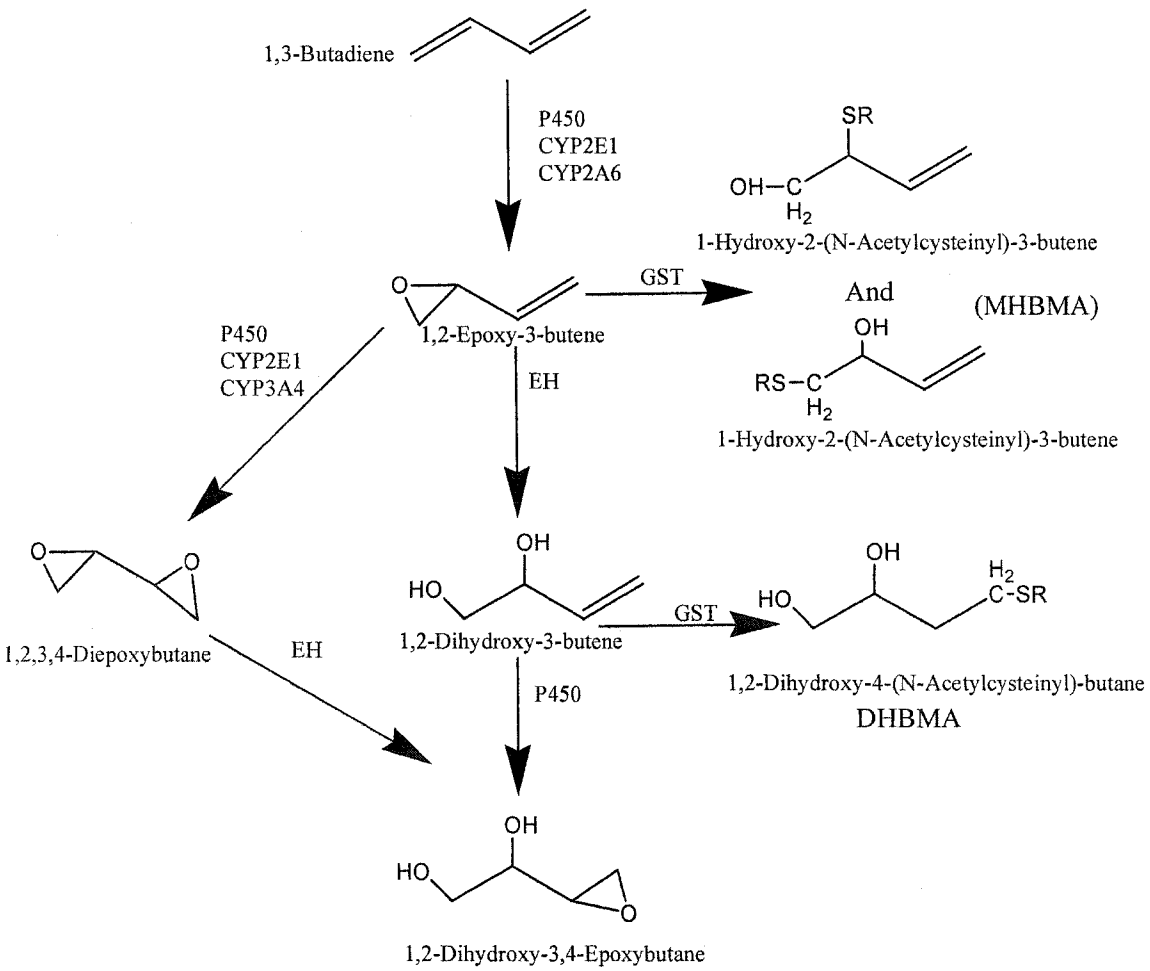
Characterization of exposure using exposure dosimeters such as personal air samples and biomarkers provides epidemiological studies a more direct and comprehensive measure of exposure compared to questionnaires. This in turn enhances the ability of epidemiological studies to identify associations between exposures and disease by minimizing exposure misclassification, enabling a better characterization of risk. Chemical specific biomarkers not only take into account multiple routes of exposure, heterogeneity of the chemical in the environment, duration of exposure and interpersonal variability but also help to examine mechanisms in various biological targets (Groopman and Kensler 1999). Although such markers are useful only in identifying recent exposures (generally less than a few days), they provide a framework for estimating total exposures in a given population and predicting the risk of adverse health effects in such exposed populations, given that the presence of such a marker is associated with disease outcome. This type of information can be very helpful in identifying the most affected populations and subsequently applying risk mitigation strategies for reducing the associated morbidity and mortality. This approach was utilized to characterize 1,3-butadiene exposure across a range of environmental exposures:



Metabolism of 1,3-butadiene

The susceptibility differences between various mammalian species with regard to BD exposures led to a belief that different species metabolize BD differently. However, both in vitro and in vivo studies indicate that BD metabolism is qualitatively similar among different species (Figure 2.1), but quantitatively different (Bechtold *et al.* 1994; Henderson *et al.* 1996).

Figure 2.1: Schematic for 1,3-butadiene metabolism (Albertani et al. 2001)



The activation of BD to its toxic metabolite is mediated by a cytochrome P450 (CYP 450). As shown in Figure 2.1, BD is oxidized by CYP2E1 and CYP2A6 to the electrophilic 1,2-epoxy-3-butene (also called butadiene mono-epoxide (BDO) or epoxy butane). This intermediate compound can undergo oxidation to form 1,2,3,4-diepoxybutane (BDO_2), also called butadiene diepoxide). BDO also can be hydrolyzed by epoxide hydrolase (EH) forming 1,2-dihydroxy-3-butene (BD-diol). Both BDO_2 and BD-diol can undergo further metabolism to form 1,2-dihydroxy-3,4-epoxybutene (butadiene diol-epoxide, EBD). These three epoxides (BDO BDO2 and EBD) can react with DNA and proteins such as hemoglobin (Boogaard *et al.* 2001), where they form DNA or protein adducts that elicit toxicity. They can be detoxified when they undergo conjugation with glutathione (GSH) or when they are hydrolyzed by epoxide hydrolase (Himmelstein *et al.* 1997; van Sittert *et al.* 2000).

Two out of the three epoxides mentioned earlier (BDO and BDO_2) are believed to be the major contributors for the mutagenicity and carcinogenicity of BD. Both of these epoxides are mutagenic without further metabolic activation in bacterial mutation assays (Adler *et al.* 1997). The mutagenicity of BDO_2 is much higher than that of BDO, in both in vitro and in vivo studies (Adler *et al.* 1997; Cochrane and Skopek 1994; Tates *et al.* 1998), but their exact role in BD induced carcinogenesis is not clear in either BD-exposed humans or experimental animals. Using in vitro studies, Schmidt *et al.* (Schmidt and Loeser 1986) and Csanady *et al.* (Csanady *et al.* 1992) have shown that the rate of BDO_2 formation is highest in mice followed by rats and primates. Similar results also

were obtained by Bond *et al.* (Bond *et al.* 1986b) and Dahl *et al.*(Dahl *et al.* 1991) using in vivo studies. This potentially could explain why BD is highly toxic to mice and not as toxic to rats (Himmelstein *et al.* 1997).

Measuring the detoxified GSH conjugate, which is excreted in urine, is a good way of determining aggregate exposures to BD. Richardson *et al.* (Richardson *et al.* 1999) showed that the major metabolites derived from BDO in both rats and mice are *N*-acetyl-*S*-(3,4-hydroxybutyl)-*L*-cysteine (also called dihydroxybutyl mercapturic acid (DHBMA) or M-1 metabolite) and regio isomers *N*-acetyl-*S*-(1-(hydroxymethyl)-2-propenyl)-*L*-cysteine and *N*-acetyl-*S*-(2-hydroxy-3-butenyl)-*L*-cysteine (also called monohydroxybutyl mercapturic acid (MHBMA) or M-2 metabolite). As shown in Figure 1, the M-1 metabolite is formed when BDO is hydrolyzed to 1,2-dihydroxy-3-butene (DHB, BD-diol) and this product is subsequently conjugated with GSH. Similarly, M-2 metabolite is formed when BDO directly conjugates with GSH. Metabolites formed from BDO_2 and EDB are primarily trihydroxybutyl mercapturic acid (THBMA). Both the M-1 and the M-2 metabolite have been used successfully as a biomarker of exposure to 1,3-butadiene in rubber manufacturing and oil refinery workers, with M-2 more closely predicting a recent exposure (Albertini *et al.* 2001; Albertini *et al.* 2003; van Sittert *et al.* 2000).

Chapter 3: The Mobile Source Effect on Curbside 1,3-Butadiene, Benzene and Particle-Bound PAH Assessed at a Tollbooth

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Abstract

On-road mobile sources contribute substantially to ambient air concentrations of the carcinogens 1,3-butadiene, benzene, and polycyclic aromatic hydrocarbons (PAH). The current study was conducted to evaluate the association between traffic level and type and curbside concentrations of these pollutants. 1,3-butadiene and benzene were measured at the Baltimore City Harbor Tunnel tollbooth over 3-hour consecutive intervals on 7 weekdays (n=56) during July 2001. Particle-bound PAH was measured on a subset of 3 days using a photoionization direct-reading instrument. Traffic was counted and classified by number of axles. The 3-hour outdoor 1,3-butadiene levels varied according to time of day and traffic volume. The minimum occurred at night (12 a.m.– 3 a.m.) with a mean of 2.0 $\mu g/m^3$ (SD=1.3, n=7) while the maximum occurred during the morning rush hour (6 a.m. – 9 a.m.) with a mean of 11.9 $\mu g/m^3$ (SD=4.6, n=7). The corresponding 3-hour traffic counts were 1,413 (SD=144) and 16,893 (SD=692), respectively. During the same intervals, mean benzene concentration varied from 3.0 $\mu g/m^3$ (SD=3.1, n=7) to 22.3 $\mu g/m^3$ (SD=7.6, n=7). Median PAH concentrations ranged from 9 to 199 ng/m^3. Using multivariate regression, a significant association ($p<0.001$) between traffic and curbside pollutant concentration was observed. Much of the pollutant variability (1,3-butadiene-62%, benzene-77%, and PAH-85%) was explained by traffic volume, class, and meteorology. Results suggest >2-axle vehicles emit 60, 32, and 9 times more PAH, 1,3-butadiene, and benzene, respectively, than do 2-axle vehicles. This study provides an empirical model for estimating curbside air pollution levels associated with traffic that may be relevant to exposures in the urban environment.

Implication

Mobile source emissions present a unique public health threat because of toxic emissions and exposure potential resulting from their proximity and integration into U.S. communities. Urban communities are especially susceptible due to population density and dense commuting traffic. The current study provides a quantitative assessment of the relationship between traffic volume and class and the curbside concentration of key environmental carcinogens. This assessment defines an experimental approach and estimate of the mobile source effect on the curbside pollutant concentration under real world meteorological conditions. The resulting models may be useful for evaluating ambient exposure, risk, and control strategies.

Introduction

1,3-butadiene, benzene and PAHs are listed by the U.S. Environmental Protection Agency (EPA) among 31 priority mobile source air toxins (U.S.Environmental Protection Agency Office fo Air Quality 2000a). Recently, 1,3-butadiene was reclassified as a "known human carcinogen" (National Toxicology Program 2000) based on the epidemiological and mechanistic information. Exposure to 1,3-butadiene is associated with lymphosarcoma (Divine *et al.* 1993; Divine and Hartman 2001) and leukemia (Matanoski *et al.* 1993b; Matanoski *et al.* 1997; Santos-Burgoa *et al.* 1992; Santos-Burgoa *et al.* 1997) in occupationally exposed workers. Benzene also has been long established as a 'known human carcinogen' (Agency for Toxic Substances and Disease Registry 1997; IARC 1982; U.S.Environmental Protection Agency 1979). Exposure to benzene is associated with acute nonlymphocytic leukemia (ANLL) and chronic lymphocytic leukemia (CLL) (Aksoy *et al.* 1976; Aksoy 1978; Bond *et al.* 1986a; Rinsky *et al.* 1981; Rinsky *et al.* 1987; Rinsky 1989; Wong 1987a; Wong 1987b).

Emissions of chemicals such as 1,3-butadiene, benzene, and PAH into the environment by mobile sources are of great public health concern because of their carcinogenicity and heightened exposure potential that results from their proximity and integration into U.S. society at all levels (urban, suburban, and rural). Several epidemiological studies have observed higher cancer rates among urban compared to suburban populations (Doll 1991; Greenberg *et al.* 1985; Howe *et al.* 1993; Phillimore and Reading 1992). Air pollution including benzene, 1,3-butadiene, and PAHs is believed to be a contributing risk factor (Doll 1991).

The potential for exposure to automobile exhaust containing these carcinogenic chemicals is most pronounced in urban locations where heavily commuted roadways transect densely populated communities. Human exposure to these mobile source emissions can be substantial due to increasing: 1) traffic volume and congestion, 2) vehicle-miles driven, and 3) numbers of heavier, less efficient sport utility vehicles (SUVs). Increased emissions may be only partially offset by technological gains in emissions control. Based on modeling results from the Assessment System for Population Exposure Nationwide (ASPEN) for 1990, Rosenbaum et al. (Rosenbaum *et al.* 1999) estimates mobile sources contribute 63%, 59%, and 63% to total ambient benzene, 1,3-butadiene, and polycyclic organic matter (POM[1]), respectively. Taking into account point and area sources in addition to mobile sources, for the 60,803 census blocks in the contiguous U.S., Rosenbaum et al. estimates median ambient levels of 1.6, 0.099, and 0.18 $\mu g/m^3$ for the three pollutants, respectively. These modeling results are further substantiated by studies indicating large pollution differences between weekends and weekdays attributable to varying traffic levels. In a series of studies, Vukovich et al. (Vukovich 2000) identified 27% to 42% higher volatile organic compound (VOC) levels in the Northeast and Texas on weekdays relative to weekends. Ilgen et al. (Ilgen *et al.* 2001) reported geometric mean benzene levels of 3.1 and 1.8 $\mu g/m^3$ in Germany homes located on high and low traffic streets, respectively.

[1] Note, POM is the more comprehensive family of organics that subsumes the carbon and hydrogen only containing polycyclic aromatic hydrocarbons (PAHs).

Both indoor and outdoor sources factor into human exposure and risk. There are several known indoor sources of benzene and PAHs such as cleaning products, paints, glues and tobacco smoke, for benzene, and wood burning, cooking and tobacco smoke for PAHs (Wallace 2000; Wallace *et al.* 1987; Wallace 1989a; Wallace 1989b). For 1,3-butadiene, the only known indoor source is tobacco smoke which can elevate indoor 1,3-butadiene concentrations significantly (Kim *et al.* 2001).

Although there is a growing body of literature identifying a substantial mobile source contribution to ambient pollution, these estimates largely rely on dynamometer emissions testing coupled with estimates of vehicle miles driven. The current study is unique in examining the actual measured association between vehicle volume and class and the resulting curbside ambient pollutant concentration providing a real world basis by which to validate models and estimate exposure. The study was conducted at a tollbooth facility where traffic count and type were carefully quantified providing a basis for real world estimates of the mobile source effect on curbside concentration for 1,3-butadiene, benzene, and PAH.

Methods

Study Site and Sampling

This study was conducted at the Maryland Transportation Authority (MTA) operated Baltimore City Harbor Tunnel tollbooth facility. This facility has 14 tollbooths evenly divided between northbound and southbound traffic. Sampling was conducted at a single

northbound tollbooth (number 3). It was selected because it is open and operator-occupied 24 hours/day. Samplers were placed immediately outside the tollbooth on the south side (vehicles approaching) approximately 3 feet above ground. Sampling was conducted over seven weekdays during the period from June 18 to 28, 2001.

Three-hour integrated 1,3-butadiene and benzene samples were collected using a Perkin-Elmer STS-25 Sequential Sampler™ (Perkin-Elmer, Shelton, CT). Samples were collected sequentially onto stainless steel Perkin-Elmer Air Toxic Tubes™ packed with a solid sorbent (Supelco cat # 25051, Bellefonte, PA), using a SKC 210 pocket pump (SKC Inc., Eighty Four, PA) set at a nominal flow rate of 25 mL/min. Pumps were calibrated upon initiation of sampling using a DryCal DC-2 primary standard (BIOS International Corp, Butler, NJ). Sample flows were checked after sampling to account for any drift during sampling.

Every 24 hours, the sampled air toxic tubes were removed from the sequential sampler and returned to the lab for analysis. Samples were thermally desorbed (Perkin-Elmer™ ATD-400; Perkin-Elmer, Shelton, CT), separated by gas chromatography, and detected by mass spectrometry using a Shimadzu GC-17A gas chromatograph and QP-5000 mass spectrometer (Shimadzu Biotech, Columbia, MD). The conditions used for both ATD-400 and GC/MS were adapted from Kim et al. (Kim *et al.* 1999). Chromatographic separation was obtained using Restek Rtx–624 column, 60 m x 0.25 mm ID with 1.4 μm thickness (catalog # 10969; Restek Corp., Bellefonte, PA).

Calibration standards were prepared at 6 levels by diluting 2 mg/ml 1,3-butadiene stock solution (cat #S-406A-10x, Accustandard, New Haven, CT) and 2 mg/mL custom VOC mix (cat #S-2081-R10-10x, Accustandard, New Haven, CT) in methanol. One-microliter injections were made into clean sampling tubes using a modified GC injector port (50^0C, helium flow of 80 ml/min for 10 min). The final amount on sampling tubes ranged from 1 to 25 ng and 1 to 50 ng for benzene and 1,3-butadiene, respectively.

Particle bound PAH was measured using an Ecochem PAS 2000 PAH Ambient Analyzer™ (Ecochem Technologies, West Hills, CA). This is a direct-reading instrument that measures PAH on particles by photoionization. Particles entering the instrument are irradiated with a UV light at 222 nm (6.7 eV). Particles containing PAHs with a photoelectric threshold of less than 6.7 eV will loose an outer shell electron and become positively charged. The charge particles are collected onto a filter resulting in an electrical current proportional to the ions collected. Therefore, all particles with a photoelectric threshold less than 6.7 eV will be ionized and measured as PAH(Niessner *et al.* 1990). Air is sampled at a flow rate of 2 L/min. The inlet is not configured to provide a size specific classification, however, electrons emitted from larger particles are more likely to be recaptured, therefore, ionization and instrument response is most effective for particles containing PAH in the size range <1-2 μm in diameter (Wilson *et al.* 1994). The Echochem PAS 2000 was placed side-by-side with the STS-25 sequential samplers and samples were collected continuously for 2 days during the study period. Measurements were logged in one-minute intervals. These data were combined to give 3-h average concentrations corresponding to the traffic count intervals.

Hourly traffic count data for both northbound and southbound traffic was obtained from the Maryland Transportation Authority (Tollbooth Administration at Baltimore Harbor Tunnel) which maintains an hourly record of total vehicle counts passing through each tollbooth, classified by the number of axles on each vehicle. The axle-based classification was compared to the Federal Highway Authority (FHWA) classification system (Table 3.1) (US Department of Transportation 2002). This table indicates that 2-axle vehicles primarily represent passenger cars, minivans, pickups and single unit trucks, whereas >2-axle vehicles are primarily buses, large trucks and trailers.

Insert [Table 3.1]

Meteorological measurements including temperature, relative humidity, rain, wind speed, and direction were made using a Davis meteorological station (Davis Instrument Corp, Hayward, CA). The meteorological station was located in East Baltimore approximately four miles due north of the toll plaza near the Johns Hopkins Bloomberg School of Public Health.

Field (n=7) and laboratory (n=10) blanks were included in all sampling and analytical runs. Reported concentrations have been corrected for mean field blank levels. All samples were analyzed on the same day that they were returned from the field. Measurement precision was determined from a single measurement made in triplicate using side-by-side sampling. Recovery was determined by spiking air toxic tubes (n=7)

with 15 ng of 1,3-butadiene and 5 ng of benzene. Tubes were cleaned for reuse by conditioning in the ATD 400 at 350°C for 15 min. Conditioned tubes were randomly selected and analyzed to verify that there was no carry-over of residual analytes from one sample to the other.

Data Analysis

The hourly traffic data was summed in a 3-hour interval corresponding to the 3-hour integrated sampling period. A composite traffic volume for a given day was calculated by adding up the vehicle counts for all 14 tollbooths per 3-hour interval. The traffic volume data was grouped into two classes for analysis: 2-axle and >2-axle vehicles. Meteorological data and PAH data were similarly averaged over the same 3-hour sampling interval. All measured concentrations were corrected for recovery and blanks. Multivariate regression model (Equation 1) was used to investigate the relationship between curbside pollution levels, traffic volume, and meteorological conditions using Intercooled Stata, version 7.0 for Windows (Stata Corporation, TX).

Equation 1. $C_i = \beta_{0i} + \beta_{1i} * \textit{2-axel} + \beta_{2i} * \textit{>2-axel} + \beta_{3i} * \textit{Temp} + \beta_{4i} * \textit{Wind Speed} + \varepsilon$

In this model, C_i is the curbside concentration of pollutant i for the three hour sampling interval. The regression coefficient β_{1i} represents an average increase in the curbside concentration of pollutant i (ng/m^3) for a unit increase in 2-axle vehicle number, adjusted for the number of >2-axle vehicles, temperature, and wind speed. Similarly β_{2i} represent an average increase in the curbside concentration of pollutant i (ng/m^3) for a unit increase

in >2-axle vehicle number, adjusted for the number of 2-axle vehicles, temperature, and wind speed. These coefficients have units of ng/m^3/vehicle and provide an indication of the mobile source effect on the curbside pollutant concentration. Hereafter, this effect will be referred to as "the mobile source effect".

The method detection limit was calculated following the Code of Federal Regulations (40CFR136 Appendix B) as discussed in EPA Compendium Method TO-17 (U.S.Environmental Protection Agency 1997). The limit of detection was obtained by multiplying the standard deviation of the 7 spiked samples by the Student's t-value associated with the 99% confidence interval and 6 degrees of freedom.

Results

The recovery for 1,3-butadiene and benzene averaged (± standard deviation) 85±12% and 97±8% respectively for the 7 recovery spike samples analyzed. The analysis of a single sample collected in triplicate yielded a coefficient of variation of 2 and 6% for 1,3-butadiene and benzene respectively. The limit of detection was determined as 0.46 and 0.58 $\mu g/m^3$ for the two respective analytes.

Results from this study relate to traffic levels, vehicle class, and operating conditions at a specific tollbooth facility. The vehicle operating conditions associated with the tollbooth are varied with vehicles decelerating and braking upon approach, idling and traveling slowly to the tollbooth attendant and then accelerating onto the highway. Total vehicle counts (north and south bound) per 3-hour interval over the 7-weekday sampling period

are shown in Figure 3.1. The diurnal distribution is bimodal with modes associated with the morning and evening rush hours, as expected. There is an approximate 8-fold difference between minimum counts occurring during the nighttime hours to the maximum recorded during the rush hour. The total morning rush hour traffic exceeds the mid-afternoon traffic by a factor of about 1.5. However, there is a distinct difference in traffic volume patterns between vehicles with 2-axles relative to vehicles with >2 axles. The morning rush hour is due to increase in both 2-axle and >2-axle vehicles, whereas >2-axle vehicle counts remain elevated into the early afternoon hours while the commuting 2-axle vehicles drop precipitously between the morning and afternoon rush hours. Although the number of >2-axle vehicles continue to increase even after rush hour, the overall traffic count goes down after rush hour because >2-axle vehicles account for only 2-9% of the total vehicle counts. Therefore, the overall decrease in total traffic counts during the afternoon is due to drastic decreases in commuters on the highways during afternoon hours.

[Figure 3.1]

The distributions of 1,3-butadiene and benzene 3-hour integrated outdoor measurements made over the 7-day period are shown in Figure 2 & 3. The concentration profiles of 1,3-butadiene and benzene tracked one another and followed a similar bimodal pattern to the traffic counts with the lowest levels occurring in the early morning hours and peak levels occurring during morning and afternoon rush hours. The lowest 1,3-butadiene levels (median: 2.0 $\mu g/m^3$; range 0.8–4.5 $\mu g/m^3$) were recorded during the interval 12–3 a.m.

whereas maximum levels (median: 13.5 $\mu g/m^3$; range: 6.0–19.0 $\mu g/m^3$) were recorded during the interval 6-9 AM. The corresponding benzene concentrations were 2.7 ug/m^3 (0.7–9.6 $\mu g/m^3$) and 22.3 $\mu g/m^3$ (12.5–32.5 $\mu g/m^3$), respectively. PAH levels followed a slightly different pattern with minimum values observed during the evening interval 9 pm – 12 M (median: 9.3 ng/m^3; interquartile range (IQR) of 10) and maximum levels observed during the 6–9 am interval (median: 199 ng/m^3; IQR of 241). Interquartile ranges are reported for the PAH measurements due to the large variability in the one-minute measurements.

[Insert Figure 3.2 and 3.3]

Meteorological measurements over the 56 three-hour intervals are presented in Table 3.2. Temperature and humidity ranged from 21.8 to 31.6 °C and 38.5 to 76.0%, respectively. Wind speeds ranged from 0.3 to 2.1 m/s with no predominant direction.

[Insert Table 3.2]

The association between traffic volume and curbside concentrations of 1,3-butadiene, benzene, and PAH is illustrated by a scatter plot (Figure 3.4). Simple linear regression of ambient 1,3-butadiene, benzene, and PAH on total traffic volume indicates that 40, 69 and 49% of the pollutant variability is explained by traffic volume.

[Insert Figure 3.4]

Table 3.3 presents a matrix of correlation coefficients for the various pollutant measurements and traffic count and meteorological explanatory variables. As suggested in the concentration profile plots (Figure 3.2 & 3.3), 1,3-butadiene and benzene are significantly correlated ($p \leq 0.05$). The meteorological variables humidity and temperature are similarly significantly correlated ($p \leq 0.05$). PAH and 1,3-butadiene showed a stronger correlation with >2-axle vehicles than with 2-axle vehicles whereas benzene was more strongly correlated with 2-axle vehicles.

[Insert Table 3.3]

The simple linear models were further refined using multivariate analysis that simultaneously took into account vehicle class and meteorological conditions including temperature, wind speed and direction. The results of the multivariate analysis are presented in Table 3.4. In this more complete analysis, traffic volume classified as 2 and >2-axle vehicles was significant ($p \leq 0.05$) for both 1,3-butadiene and benzene. In contrast, for PAH, only >2-axle traffic volume was significant. The multivariate models are a significant improvement over the univariate models as indicated by the increased explained variability in pollutant concentrations: 62%, 77% and 85% for 1,3-butadiene, benzene, and PAH, respectively. It is likely that some of the unexplained variability is attributable to spatial differences in wind speed and direction between the toll plaza and the location of the measurements in East Baltimore.

The traffic volume regression coefficient is indicative of mobile source effect (ng/m^3/vehicle) based on 3-h integrated measurements. Accordingly, a unit increase in 2-axle vehicle increases the curbside concentration of 1,3-butadiene, benzene and PAH by 0.32, 1.0, and 4.5 ng/m^3. The >2-axle vehicle mobile source effect of 10.4, 9.5, and 271 ng/m^3/vehicle for 1,3-butadiene, benzene and PAH, exceeds that of 2-axle vehicles by a factor of 32, 9, and 60, respectively. However, the difference in mobile source effect is partially offset by the traffic volume in each class with 2-axle vehicles out numbering >2-axle vehicles by a factor of 29. Therefore, taking both the mobile source effect and vehicle counts into account, the >2-axle vehicle contribution exceeds that of 2-axle vehicles by a factor of 1.1 and 2.1 for 1,3-butadiene and PAH respectively. For benzene, the inverse is true with the 2-axle vehicle contribution exceeding the >2-axle vehicle contribution by a factor of 3.2.

[Insert Table 3.4]

Discussion

The current study was designed to inform the source to effect continuum for mobile sources and cancer risk by elucidating the association between traffic volume and curbside levels of mobile source-related air pollution. Benzene, 1,3-butadiene, and PAH are of particular concern as environmental carcinogens. Although emission data are available from dynamometer testing (Schauer *et al.* 2002b) and tunnel tests (De Fre *et al.* 1994; Kean *et al.* 2000) and annual ambient estimates have been modeled (Rosenbaum *et al.* 1999; The Office of Transportation and Air Quality (OTAQ) 2000), the current study

represents some of the first time-resolved measurements quantifying the association between outdoor curbside pollutant levels and traffic volume and class. An advantage of the current study approach is that it provides actual *in situ* measurements that take into account a host of meteorological factors including wind speed, temperature, and humidity. Furthermore, because the traffic patterns of 2-axle and >2-axle vehicles differed substantively, it has been possible to resolve their relative contribution to ambient levels.

Although the observed tollbooth 1,3-butadiene and benzene concentrations (means ranging from 2.0 to 11.9 and 3.0 to 22.3 $\mu g/m^3$, respectively) are considerably higher than what has been observed even for urban environments, findings from this study may have particular relevance for urban communities built in close proximity to high traffic arterials as exists in Baltimore City. In comparison, the most recent data from the California Air Resource Board (Californi Air Resource Board (CARB) 2002) indicates annual median 1,3-butadiene levels of 0.60 ug/m^3 (0.18-2.06) and 0.13 ug/m^3 (0.04-0.84) for urban and suburban locations, respectively. The corresponding annual median benzene levels are 3.5 $\mu g/m^3$ (1.27-9.54) and 0.95 $\mu g/m^3$ (0.32-4.13). The annual average (range) urban and suburban 1,3-butadiene levels reported for Maryland in 1999 were 0.35 $\mu g/m^3$ (0.07-1.23) and 0.04 $\mu g/m^3$ (0.0-0.15), respectively. The corresponding values for benzene were 2.2 $\mu g/m^3$ (0.8-5.8) and 0.7 $\mu g/m^3$ (0.3-1.5), respectively (Maryland Department of the Environment 2000). Similar model-based estimates are given by Rosenbaum et al. for all U.S. census tracts showing median annual average benzene and 1,3-butadiene levels of 1.6 and 0.18 $\mu g/m^3$, respectively. Therefore, the high-end

tollbooth 1,3-butadiene and benzene levels typically exceed average urban ambient levels by about an order of magnitude and 20 to 30 fold for the two pollutants, respectively. The observed higher tollbooth levels are due to the proximity and intensity of the source, i.e., ~70,000 vehicles/day, and provide a valuable laboratory for examining the real-world impact of mobile sources on air quality.

The differences between particle-bound PAH levels previously measured in the urban environment relative to the current tollbooth study are less dramatic than for 1,3-butadiene and benzene. The lowest and highest median concentrations of 9.3 ng/m^3 (IQR=10.7) and 199.3 ng/m^3 (IQR=241.3) were observed during 9pm-12am and 6am-9am intervals, respectively. Indoor median concentrations measured in homes without smokers in the Boston region using a PAS monitor ranged from 8 to 19 to 31 ng/m^3 at suburban, semi-urban, and urban locations, respectively (Dubowsky *et al.* 1999). In the same city during the summer of 1998, Dunbar et al. (Dunbar *et al.* 2001) reports median curbside concentrations over five days that ranged from 10 to 20 ng/m^3 (assuming 1 fA/ng/m^3). Based on integrated sampling and laboratory analysis methods, Naumova et al. 2002 (Naumova *et al.* 2002) reports outdoor median particle-phase ΣPAH levels ranging from 1 to 4 ng/m^3 for homes in Los Angeles, Houston, and Elizabeth.

Traffic volume was found to be a strong determinant for curbside concentrations of 1,3-butadiene, benzene, and PAH explaining 62%, 77%, and 85%, respectively, of the air pollution levels, indicating that of the three pollutants, PAH is most strongly associated with traffic. The observed R^2 for 1,3-butadiene and benzene are consistent with US-EPA estimates of 56% and 60% of total 1,3-butadiene and benzene emissions attributable to

on-road mobile sources (US EPA Office of Air and Radiation 1993). In a recent study where the same model EcoChem instrument was used at a busy Boston intersection, Dunbar et al. 2001 attributed 46% of the total particle-bound PAH mass to primary motor vehicle emissions. Of this, 65% of the PAH mass was attributable to buses and trucks and 35% was attributable to cars. Although the basis for vehicle classification differs, these results are comparable to our simple linear regression results that showed 2-axle and >2-axle vehicles explaining 25% and 64% of the particle-bound PAH concentration variability.

Based on 3-h integrated measurements, the coefficient given by the regression of traffic count on curbside ambient concentration provides an estimate of the mobile source effect on curbside concentration relevant to the location and meteorology of sampling. The magnitude of the source effect varied by pollutant and vehicle class. The highest mobile source effect was 0.2711 ng/m^3/vehicle for particle-bound PAH from >2-axle vehicles. This exceeded the 2-axle mobile source effect of 0.0045 ng/m^3/vehicle by a factor of 60. These results compare with Dunbar et al. who attributed 65% of the total PAH mass to buses and trucks that comprised 6% of the total traffic volume suggesting a 29 fold difference in mobile source effect between passenger vehicles and trucks and buses. Additional corroboration for high PM emission of diesel vehicles relative to gasoline vehicles is given by Durbin et al. 1999 (Durbin *et al.* 1999) indicating diesel light-duty vehicles emit 1-2 orders of magnitude more particulate matter relative to gasoline vehicles.

For 1,3-butadiene and benzene, the current study suggests that >2-axle vehicles have a mobile source effect that is 32 and 9 times greater than 2-axle vehicles, respectively. This difference is consistent in direction but higher than the 3 to 4 fold difference in hydrocarbon emissions suggested by EPA (The Office of Transportation and Air Quality (OTAQ) 2000) for light duty gasoline powered vehicles (approximately 0.6 g/mi for 1991-7 vehicles with 50,000 miles) relative to heavy duty diesel powered vehicles (2.1 g/mi for 1991-1997 vehicles with 50,000 miles).

The observed difference in mobile source effect by number of vehicle axles is likely attributable to a combination of fuel type and consumption, i.e., larger vehicles with >2-axles are likely to burn more fuel per mile and are more likely to have diesel engines. From the current study design, it is not possible to disentangle these two possible contributing effects. However, based on the Federal Highway Administration's (FHA) database of vehicle miles traveled (Federal Highway Administration, 1999) it is possible to estimate fuel consumption by vehicle type. In order to compare the vehicle classification from the current study (i.e., by number of axles) to FHA's classification, it is necessary to assign the FHA classification to categories by axle: 2-axle vehicles = motorcycle, light duty gas, and light duty diesel; >2-axle vehicles = heavy-duty gas and heavy duty diesel. Assuming this classification and based on the FHA database for the surrounding vicinity (Baltimore City, Baltimore County and Anne Arundel County)(US EPA Office of Air Quality Planning and Standards 2002), 99.7% of the gasoline is consumed by 2-axle vehicles whereas 72.2% of diesel fuel is consumed by >2-axle vehicles. These data indicate that although the 2-axle vehicle class is nearly all gasoline

powered, the >2-axle vehicle class is comprised of a mixture of diesel and gas, although predominantly diesel. Therefore, these data provide some substantiation that a difference between the vehicle axle categories considered in this study is due to type of fuel.

The regression coefficients for wind speed and temperature were significant in explaining pollutant variability, however, a different effect was observed for the gas phase VOCs relative to particle-bound PAHs. For 1,3-butadiene and benzene, an inverse association was observed such that increasing wind speed was associated with decreased pollutant levels. This effect is likely due to horizontal mixing with relatively less polluted regional air. In contrast, for particle-bound PAH, a direct association was observed with wind speed. The reason for this direct association is unclear. However, it may be due to the instrument's inlet configuration and collection bias due to size. Since PAH adsorption and the instrument's response are both particle size dependent (Offenberg and Baker 1999; Wilson *et al.* 1994), it follows that alteration of the particle collection efficiency by size will alter the PAH concentration measurement. The observed effect is consistent with a bias of greater efficiency in sampling small particles with increased wind speed. Alternatively, this effect could result from re-suspension of surface deposited particle bound PAH. It is unlikely that the observed effect was due to some regional industrial source since no association was observed with wind direction.

Conclusion

The current study provides unique time resolved measurements of traffic counts and vehicle class combined with the curbside concentrations of three key mobile source-

related environmental carcinogens, i.e., benzene, 1,3-butadiene and particle-bound PAH. An examination of the variability in the source term relative to the resulting pollutant levels using multivariate regression analysis yielded a statistically significant association ($p<0.001$) providing an empirical model for estimating pollutant levels from traffic volume and class taking into account wind speed and temperature. Because the traffic volume profile between 2-axle and >2-axle vehicles differed, it was possible to tease out a mobile source effect term (ng/m^3/vehicle) for these two classes. For all three pollutants, the mobile source effect of >2-axle vehicles exceeded that of 2-axle vehicles by as much as a factor of 60 for particle-bound PAH, to factors of 9 and 32 for benzene and 1,3-butadiene, respectively. However, because the number of 2-axle vehicles outnumber >2-axle vehicles by 29 fold, the overall contribution of 2-axle and >2-axle vehicles to total pollutant levels are within a factor of 1 to 3.

The current study's findings are based on measurements from a single location and season, therefore generalization is limited. However, the high pollutant concentrations measured in close proximity to and during times of high traffic may be relevant to the exposure potential along commuting arterials that transect some urban communities. In cities such as Baltimore, the housing stock has been constructed in very close proximity (i.e., 6 to 10 feet) to these same heavily trafficked roadways. Exposures can be further exacerbated by a custom of stoop sitting and socializing typical in urban communities. Depending on time activity patterns of urban residents (e.g., frequency, duration, and time of day at home), their exposure may be under estimated relative to estimates given by ambient central site monitoring (Californi Air Resource Board (CARB) 2002;

Maryland Department of the Environment 2000) or modeling (Rosenbaum *et al.* 1999). Building on this study and in order to examine the relevance of extrapolating from the current study to the urban environment, additional studies are being conducted to assess traffic volume/class and indoor and outdoor pollution data on a busy urban arterial.

Acknowledgement

This study was supported through the Johns Hopkins Education and Research Center Pilot Project Research Training Fund (T42 / CCT 310419) with additional support provided by the National Institutes of Environmental Health Sciences (ES 03819) and US EPA (R 827436). We are grateful to Dr. Lance Wallace (U.S. EPA) for the use of the EcoChem PAH monitor and Mr. Timothy Reilly of the Baltimore Harbor Tunnel for giving us access to the tollbooth facility and for providing the traffic count data. We acknowledge D'ann Williams for her assistance in conducting the sampling.

Table 3.1: Comparison of FHWA vehicle class and the number of axles, with examples.

FHWA Vehicle Class	Average # axles per Vehicle	Vehicle Types
1	2	motorcycles
2	2	passenger cars
3	2	pickups, vans, campers, minibus
4	2.2	buses
5	2	six-tire, single-unit trucks, including motor homes
6	3	three-axle single unit trucks.
7	4	four axle single unit trucks on single frame
8	4	four or fewer axles consisting of two units
9	5	all five-axles consisting of two units
10	6	vehicles with six or more axle with two units
11	5	five or fewer axle consisting of three units
12	6	all six-axle vehicles with three or more units
13	7	all vehicles with seven or more axles

Table 3.2: Meteorological Results (median and range) by sampling interval

Interval	Temperature (°C)	Relative Humidity (%)	Wind Speed (m/sec)	Dominant Wind Dir
12-3am	23.4 (21.7-26.8)	72.0 (64.2-82.2)	0.3 (0.2-1.2)	NE
3-6am	21.8 (20.2-25.5)	76.0 (70.7-84.7)	0.5 (0.3-1.0)	NNW
6-9am	24.4 (22.7-26.9)	65.5 (60.7-77.0)	0.9 (0.7-1.3)	SE
9-12pm	29.3 (26.1-31.3)	45.7 (39.8-56.7)	1.7 (1.1-1.9)	SSE
12-3pm	31.6 (27.8-33.5)	38.5 (33.8-53.5)	1.4 (1.2-2.7)	N
3-6pm	30.5 (28.3-33.7)	46.3 (41.2-56.8)	2.1 (1.1-3.2)	N
6-9pm	27.8 (27.1-31.5)	53.5 (44.2-61.8)	1.9 (0.7-2.8)	E
9-12am	25.5 (22.6-29.1)	58.5 (53.7-80.0)	0.9 (0.1-1.8)	ENE

Table 3.3: Coefficient of determination (R^2)

2-axle	1.0							
>2-axle	0.5	1.0						
Temp	0.3	0.2	1.0					
Wind_sp	0.3	0.1	0.3	1.0				
Humidity	0.2	0.2	0.7	0.3	1.0			
1,3-butadiene	0.4	0.5	0.2	0.1	0.2	1.0		
Benzene	0.6	0.5	0.4	0.1	0.3	0.7	1.0	
PAH	0.5	0.8	0.0	0.3	0.0	0.6	0.4	1.0
	2-axle	>2-axle	Temp	Wind_sp	Humidity	1,3-butadiene	Benzene	PAH

Table 3.4: Multivariate analysis incorporating different VOCs and vehicle types

Response Variable	Covariates	Reg. Coeff.	R^2	p-value
Outdoor 1,3-butadiene (n=56)	Model		0.62	<0.001
	2-axle	0.00032		0.02
	>2-axle	0.01039		<.01
	Wind speed	-1.25576		0.08
	Temp	0.24694		0.1
	Intercept	-3.97692		0.27
Outdoor Benzene (n=56)	Model		0.77	<0.001
	2-axle	0.00103		<.01
	>2-axle	0.0095		0.01
	Wind speed	-3.43332		<.01
	Temp	0.88081		<.01
	Intercept	-18.8696		<.01
Outdoor PAH (n=14)	Model		0.85	<0.001
	2-axle	0.00451		0.21
	>2-axle	0.27109		0.02
	Wind speed	40.2166		0.04
	Temp	-14.0235		0.48
	Intercept	370.826		0.04

Figure 3.1: Distribution of 3-hr traffic counts for different vehicle types as a function of time. The mean is plotted with error bars representing the standard deviation.

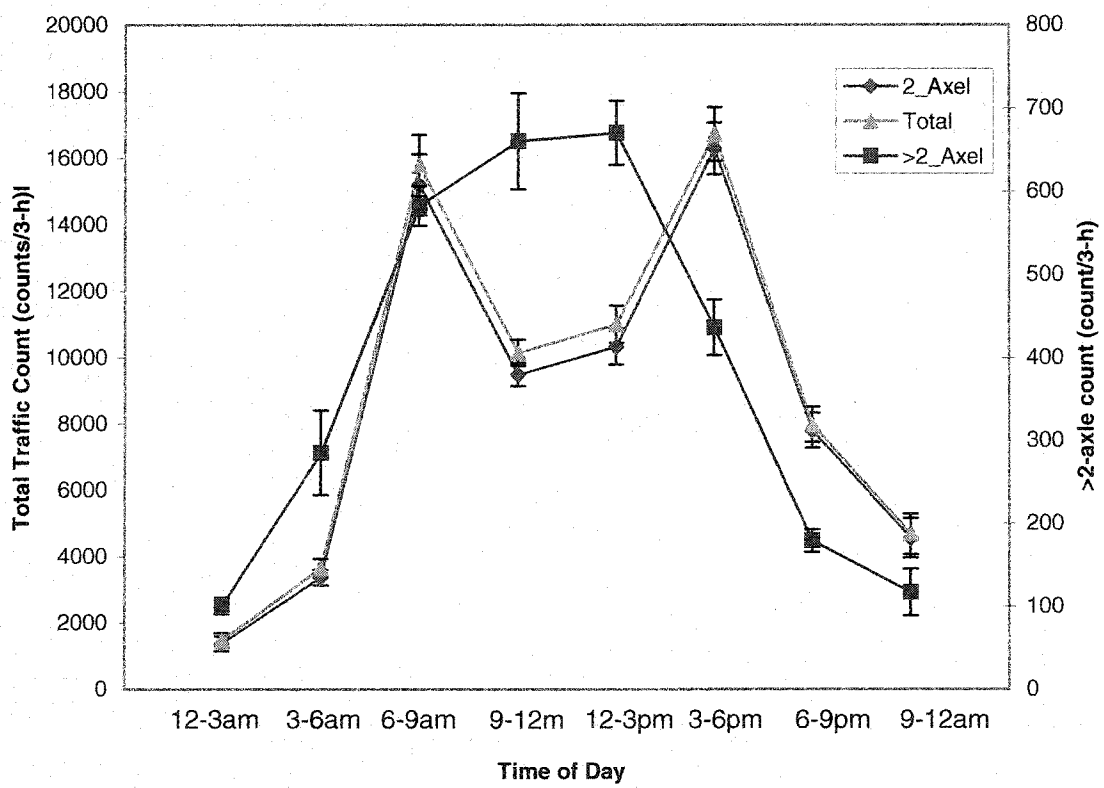


Figure 3.2: Distribution (n=7) of outdoor benzene by time of day. The boxes represent 25th and 75th percentiles, whiskers represent 5th and 95th percentiles and the horizontal bars represent the median value. Individual outliers are represented by the dots.

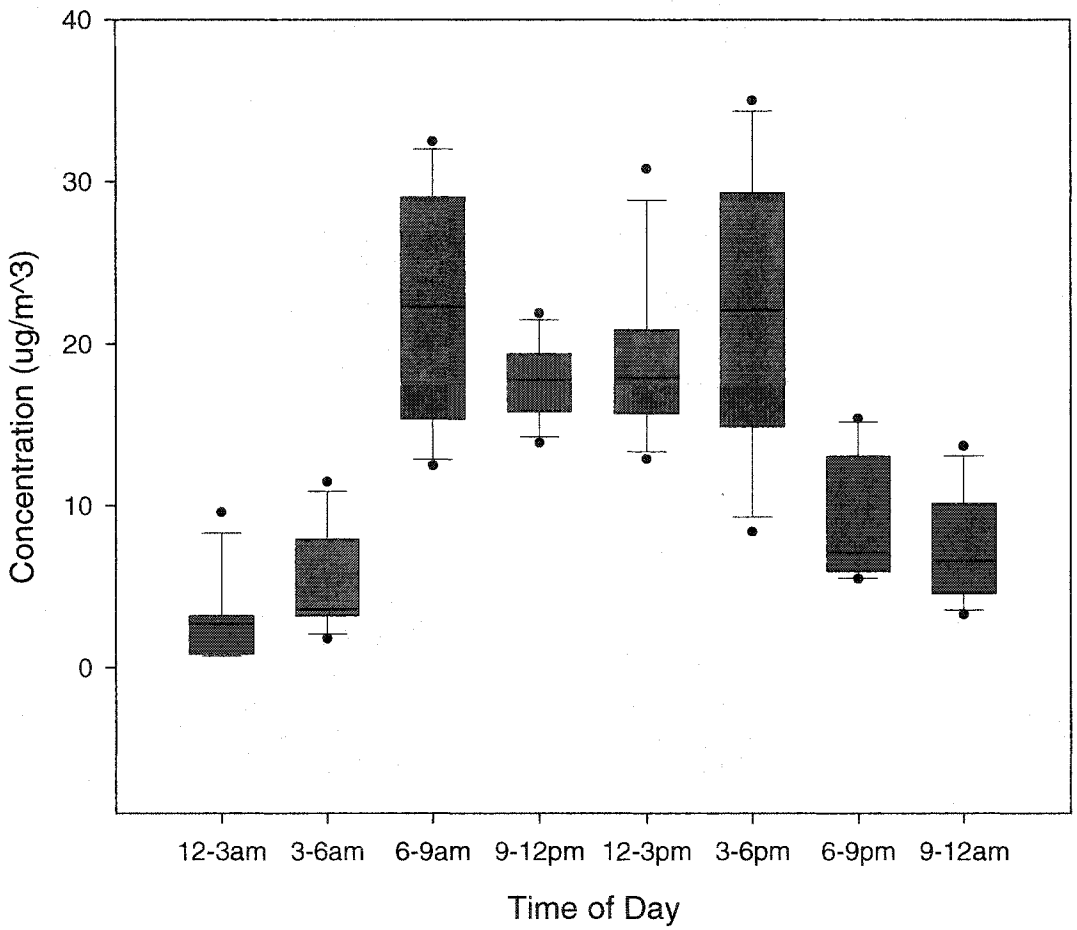


Figure 3.3: Distribution (n=7) of outdoor 1,3-butadiene by time of day. The boxes represent 25th and 75th percentiles, whiskers represent 5th and 95th percentiles and the horizontal bars represent the median value. Individual outliers are represented by the dots

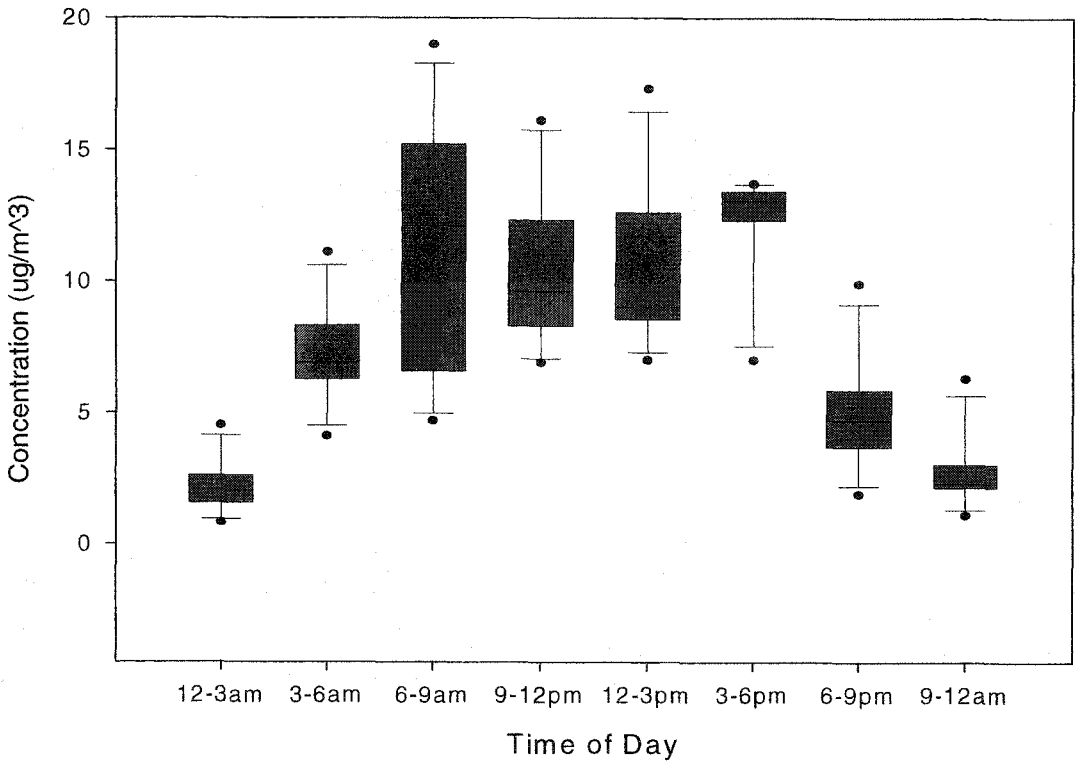
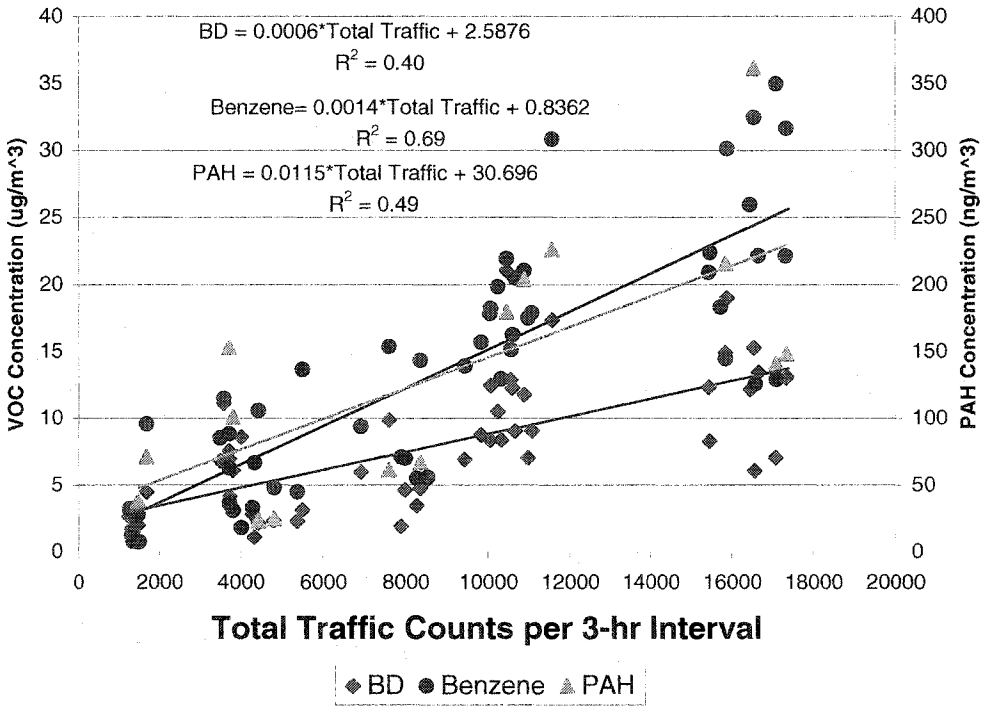


Figure 3.4: Scatter plot with simple linear regression of 1,3-butadiene, benzene and PAH vs. total traffic counts per 3-hour interval. PAH concentration in ng/m^3.



Chapter 4: Tollbooth Workers and Mobile Source Related Hazardous Air Pollutants: How Protective is the Indoor Environment?

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Note: The text and figures presented in this chapter has been submitted for publication

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Abstract

Tollbooth workers are potentially exposed to high levels of mobile source related air pollutants due to the proximity and intensity of the source. To evaluate this worker hazard, we measured the concentration of air toxins including volatile organic compounds (VOCs) and particle bound polycyclic aromatic hydrocarbons (PAHs) inside and outside a Baltimore Harbor Tunnel tollbooth during the summer of 2001. Mean outdoor benzene and 1,3-butadiene concentrations varied by shift with the morning (10.7 and 19.8 $\mu g/m^3$ respectively) exceeding afternoon (7.2 and 14.9 $\mu g/m^3$ respectively) and the lowest levels observed during the night (3.7 and 4.9$\mu g/m^3$ respectively) when traffic volume was the lowest. In comparison, considerable protection was provided to workers by the indoor environment where lower concentrations of 1,3-butadiene and benzene were observed for all three shifts (2.9 and 6.7 $\mu g/m^3$, 0.9 and 3.2 $\mu g/m^3$, and 0.9 and 2.4 $\mu g/m^3$ respectively). Greatest protection offered by the tollbooth was observed during the afternoon shift (5 to 8 fold reduction in indoor concentration) whereas the morning and night shift experienced similar protection (2 to 4 fold reduction). Chlorinated hydrocarbons were observed at higher concentrations within the tollbooth indicating the presence of indoor sources and the opportunity for exposure mitigation. Levels of PAHs were similarly reduced from outdoors (50 ng/m^3) to indoors (15.4 ng/m^3). The protective nature of the tollbooth highlighted in this study is likely due to the positive pressure control ventilation system that was present at this specific facility, which represents 55% of tollbooths in Maryland. This study provides an estimate of tollbooth workers potential

exposures to various mobile source related pollutants and highlights the protective nature of tollbooths equipped with efficient control ventilation systems.

Introduction

The potential for tollbooth worker exposure to high levels of mobile-source related air pollution is of concern due to both the proximity and the intensity of the emission source. Tollbooth workers routinely spend a large fraction of their workday within arms-length of vehicles emitting a wide range of toxic pollutants. Traffic volumes at tollbooth facilities can number in the thousands of vehicles per hour. The exposure potential is further heightened, despite advances in emission control technology due to rising vehicle miles traveled and a growing proportion of heavier, less fuel efficient sport utility vehicles (U.S.Department of Transportation 2000). Furthermore, tollbooth-related vehicle operation including acceleration and deceleration is associated with high engine and brake wear emissions (Cadle *et al.* 2001; Schauer *et al.* 2002a). As a result of these exposures, tollbooth facilities potentially represent a likely worst-case scenario for occupational exposure to mobile-source related air pollution. Yet, despite the potential hazards, little has been done to evaluate exposures associated with working in the indoor tollbooth environment.

Vehicle exhaust is known to contain a wide range of toxic pollutants including particulate matter (PM) (Cadle *et al.* 2001; Chase *et al.* 2000), volatile organic compounds (VOCs), particle-phase organic compounds, carbonyls (Kean *et al.* 2001; Schauer *et al.* 2002a), carbon monoxide (CO), nitrogen oxides (NOX) (Westerholm and Egeback 1994), as well as dioxins and furans (Gullett and Ryan 2002). Exposure to cancer causing pollutants

that are present in vehicle exhaust is of particular interest for assessing risk. Among the vehicle exhaust carcinogens, most of the cancer risk is attributed to benzene, 1,3-butadiene, and particle-bound polycyclic aromatic hydrocarbons (PAH) due to their high concentrations in exhaust and cancer potency (Rosenbaum *et al.* 1999; U.S.Environmental Protection Agency Office fo Air Quality 2000b). A recent study conducted at the same tollbooth facility that is the subject of this investigation, reported that the curbside concentration of these carcinogens is associated with traffic volume and vehicle type(Sapkota and Buckley 2003). A number of studies have linked traffic levels to increased cancer risk (Pearson *et al.* 2000; Savitz and Feingold 1989) including childhood leukemia. While others looking at short term effects have reported statistically significant positive associations for acute irritant effects of the nose and throat along with nausea and headaches (Yang *et al.* 2002), central nervous systems complaints (Strauss *et al.* 1992), and decreased pulmonary function (Evans *et al.* 1988).

Despite the potential for high-level exposures to a variety of hazardous air pollutants among tollbooth workers, little is known of toll collectors exposures in the US. Recent studies conducted in Taiwan have characterized worker exposures to PM, and PAH using a combination of air and biological monitoring (Lai *et al.* 2004a; Lai *et al.* 2004b; Lai *et al.* 2004c; Tsai *et al.* 2002b). In comparison, the studies conducted in the United States are more historical and focus on CO, lead, NO_2, SO_2, asbestos, and particulate matter (PM) (Boeniger 1995; Burgess *et al.* 1977). Recent studies have reported ambient tollbooth pollution levels of carbonyls (Destaillats *et al.* 2002), VOCs and PAHs (Sapkota and Buckley 2003); however, the relevance of such ambient measurements to

worker exposure is unknown. Recognizing the exposure potential and related health risks, the current study was conducted specifically to assess the effectiveness of the indoor tollbooth environment in protecting workers from exposure to mobile source related carcinogens.

Methods

Worker exposure and the protection provided by the indoor environment were evaluated from the source (traffic volume), to resulting curbside pollutant concentrations, and ultimately the concentrations of these pollutants inside the tollbooth. Air pollution measurements were concurrently made inside and outside a single tollbooth at the Baltimore Harbor Tunnel (BHT) facility during seven weekdays from June 18, 2001 to June 28, 2001. The BHT Plaza is located south of Baltimore City on highway 895. At this facility there are 14 tollbooths, 7 each on the Northbound and Southbound lanes. The BHT facility operates 3 shifts per day: morning shift from 6:00 am to 2:00 pm, afternoon shift from 2:00 pm to 10:00 pm and night shift from 10:00 pm to 6:00am.

A single control ventilation system is used for supplying air to the 14 tollbooths as well as the administrative building that are present at the Baltimore Harbor Tunnel Plaza. The air intake for the control ventilation system is located at the back of the administrative building, which faces away from the highway. The distance from the intake of the control ventilation system to the nearest and farthest tollbooth is approximately 55 feet and 300 feet respectively as shown in Figure 4.1. Three air ducts enter each booth from an access tunnel below the plaza. One small vertical supply grille with adjustable vanes (2‘ by

2.95") is in the front "service" door and a large duct is in the middle of the tollbooth. The larger middle ductwork has 2 discharge louvers (18" by 14"), one near the top of the booth and one near the bottom of the booth. Two smaller linear diffuser ducts (3' by 3") in the ceiling introduce air into the booth from the front and the back. Together these ducts are capable of delivering 500 cubic feet of conditioned air, designed to keep the tollbooth under positive pressure (Robeson 2004). A heating unit and a small individual air conditioning unit are in the ceiling of each tollbooth. The heating and individual AC units re-circulate the air from inside the booth conditioning the air for comfort. Air flow to the booth is controlled by a temperature sensor inside the booth. Prior to delivery, the intake air is filtered through a set of prefilters (Heavy Duty Precisionaire™) and then a box filter (Multiflow, Type S, Koch Filter Corp). The box filter is rated to provide 85% efficiency in removing 1 μm particles. The prefilters are replaced every 3 months and the box filters are replaced once a year.

[Insert Figure 4.1]

Hourly traffic count data for both northbound and southbound traffic during the study period were obtained from the Maryland Tollbooth Authority (MDTA). The MDTA maintains hourly records of all vehicles passing through each tollbooth. The hourly traffic data were summed into 3-hour intervals corresponding to the VOC integrated sampling period.

VOC samples were collected inside and outside the tollbooth using a PerkinElmer STS-25 Sequential Sampler™ (PerkinElmer, Shelton, CT). The sampler was set to collect samples every three hours sequentially for 24 hours. Samples were collected onto stainless steel PerkinElmer Air Toxic Tubes™ (3.5 inch long, 0.25 inch outer diameter) packed with a mixed sorbent comprised of carbopack B and corboxen 1000 (Supelco cat # 25051, Bellefonte, PA). A SKC 210 pocket pump (SKC Inc., Eighty Four, PA) set at a nominal flow rate of 25 ml/min was used to draw air through the sampling tubes. Pumps were calibrated upon initiation of sampling using a DryCal DC-2 primary standard (BIOS International Corp, Butler, NJ). Sample flows were checked after sampling to account for any drift during sampling.

Every morning, VOC samples from the previous 24-hour were retrieved from the field and analyzed the same day using a method previously described (Kim *et al.* 1999; Sapkota and Buckley 2003). In short, the samples were thermally desorbed using PerkinElmer™ ATD-400 (PerkinElmer, Shelton, CT), separated and analyzed with a Shimadzu GC-17A/QP-5000 GC/MS (Shimadzu Biotech, Columbia, MD) in selective ion monitoring (SIM) mode. Chromatographic separation was achieved using a Restek Rtx-624 column, 60 m x 0.25 mm ID with 1.4 um film thickness (Restek Corp., catalog no. 10969). A working stock solution consisting of 500 μg/mL 1,3-butadiene and 200 μg/mL VOCs was prepared using a 2 mg/mL 1,3-butadiene stock solution (Accustandard, catalog no. S-406A-10x) and 2 mg/mL custom VOC mix (Accustandard, catalog no. S-2081-R10-10x). Six point calibration standards were prepared in methanol using the working stock. One μl injections of these standards were made onto clean sampling tubes

using a modified GC injector port (50°C, Helium flow of 80 mL/min for 10 min). The final amount on sampling tubes ranged from 0 to 50 ng for 1,3-butadiene and 0 to 20 ng for the rest of the VOCs. To account for any sample contamination during the sampling phase, field (n=7) and laboratory (n=10) blanks were deployed and analyzed. Reported concentrations have been blank adjusted.

Particle bound PAH was measured using an Ecochem PAS 2000 PAH Ambient Analyzer™ (Ecochem Technologies, West Hills, CA). This is a direct-reading instrument that measures PAH on particles by photoionization. Particles entering the instrument are irradiated with a UV light at 222 nm (6.7 eV). Particles containing PAH with photoelectric threshold less than 6.7 eV will lose an outer shell electron and become positively charged. The charged particles are collected onto a filter resulting in an electrical current proportional to the ions collected. Therefore, all particles with a photoelectric threshold less than 6.7 eV will be ionized and measured as PAH (Niessner *et al.* 1990). Air is sampled at a flow rate of 2 L/min and the inlet is not configured to provide a specific size classification. However, electrons emitted from larger particles are more likely to be recaptured, therefore, ionization and instrument response is most effective for particles containing PAH in the size range of <1-2 μm in diameter (Wilson *et al.* 1994). The Echochem PAS 2000 was placed side-by-side with the STS-25 sequential samplers, indoors and outdoors, and samples were collected continuously for 2 days during the study period. Measurements were logged in one-minute intervals. These data were combined to give 3-h average concentrations corresponding to the traffic count intervals.

All statistical analyses were performed using Intercooled Stata, version 7.0 for Windows (Stata Corporation, TX). The normality of VOC and PAH concentration distributions were assessed using the Shapiro-Wilk test. Differences in pollutant levels indoors and outdoors were tested using the non-parametric Mann-Whitney test.

Results

The combined northbound and southbound traffic profile at the BHT ranged from 400 to 5900 vehicles per hour (72,000 per day) with a distinctive bimodal distribution corresponding to the morning and afternoon rush hour (Figure 4.2). These high traffic levels and their large variability highlight the significant exposure potential due to source activity.

[Insert Figure 4.2]

The concentration profile of 1,3-butadiene and benzene outside the tollbooth is in accord with the bimodal traffic pattern observed in Figure 4.2, with one peak associated with the morning rush-hour and a second peak corresponding with the afternoon rush-hour. In contrast to the outdoor concentrations, indoor levels are much lower and lack a distinct bimodal pattern (Figure 4.3A & 4.3B).

[Insert Figure 4.3]

The concentrations of VOCs at the tollbooth varied by location (indoor vs outdoor) and type (Table 1). Inside the tollbooth, chloroform (0.1 $\mu g/m^3$), followed by styrene, (0.4 $\mu g/m^3$) was present at the lowest concentration while MTBE (17.2 ug/ m^3) and dichlorobenzene (95.7 $\mu g/m^3$) were found to be at the highest concentration. Likewise, for measurements made outside of the tollbooth, chloroform (ND, <0.04 $\mu g/m^3$) and trichloroethylene (0.1 $\mu g/m^3$) were the 2 VOCs with the lowest concentrations, while MTBE (35 $\mu g/m^3$) and PAHs (50ng/ m^3) had the highest concentrations.

[Insert Table 4.1]

A statistically significant ($p<0.05$) positive association was observed between indoor and outdoor concentrations of both 1,3-butadiene and benzene based on simple linear regression (Figure 4). These results suggest that outdoor concentrations explain as much as 33% and 27% of the variability in indoor concentrations of 1,3-butadiene and benzene, respectively. Moreover, similar observed slopes (0.20) for these two VOCs provides an indication of the protection offered by the tollbooth indoor environment.

[Insert Figure 4.4]

The protection provided by the indoor environment was further evaluated by examining the indoor/outdoor concentration ratios for each of the measured pollutants (Figure 4.5). The pollutants fall into two distinct groups: those with median ratios less than and greater than one. The latter group is comprised of chlorinated hydrocarbons and is

suggestive of an indoor source. The former group is largely comprised of aliphatic and aromatic mobile source-related hydrocarbons and indicates substantial mitigation from outdoors to indoors.

[Insert Figure 4.5]

The 8-hr time-weighted average (TWA) concentration of selected VOCs for the 3 different shifts were derived from the 3-hour incremental measurements (Table 4.2). For benzene and 1,3-butadiene, the morning shift (6:00am-2:00pm) was characterized by the highest concentration whereas the night shift (10:00pm–6:00am) was characterized by the lowest concentrations both inside and outside the tollbooth. For the indoor environment, the concentration during the morning shift was significantly higher than the afternoon and night shifts ($p<0.05$), but there was no difference between the afternoon and night shift concentrations ($p>0.05$). For the outdoor concentrations, the differences between all shifts were statistically significant ($p<0.05$). Furthermore, the indoor concentrations at any given shift was significantly lower than the concurrent outdoor concentrations ($p<0.05$).

[Insert Table 4.2]

Discussion

As evidenced by this study, as well as other peer-reviewed reports (Tsai *et al.* 2002b; Tsai *et al.* 2002a), tollbooth workers are potentially exposed to elevated levels of toxic

mobile source emissions. Worker exposures are influenced both by source terms (e.g., traffic volume, operating conditions, and proximity to passing vehicles) as well as mitigation through the control ventilation system and/or respiratory protection. This study is unique in characterizing indoor and outdoor mobile source related pollutant levels and assessing the effectiveness of the tollbooth control ventilation system in protecting workers.

The Maryland Transportation Authority reports that there are 7 toll facilities with 78 toll booths and 292 toll booth workers in the State of Maryland. Of the 78 tollbooths, 55% are equipped with control ventilation systems comparable to the BHT. The remaining facilities are ventilated using single unit fans and/or air conditioners. National data are not available by which to assess the representativeness of these Maryland data. Results of the current study are likely to be relevant to the 55% of tollbooth facilities equipped with comparable control ventilation systems.

The concentration profile of 1,3-butadiene and benzene measured outside the tollbooth clearly shows a traffic related increase during the morning and afternoon rush hours. This increase, however, is not manifested indoors, demonstrating the protection offered by the tollbooth. We observed that the outdoor 1,3-butadiene and benzene concentrations varied by shift both indoors and outdoors. Inside the tollbooth, the morning shift concentrations were significantly higher ($p<0.05$) than the afternoon and the night shifts; however there was no difference between the afternoon and night shift ($p>0.05$). For the outdoor measurements, both 1,3-butadiene and benzene concentrations were higher than the night

shift concentration ($p<0.05$). These results are in agreement with a recent tollbooth study by Tsai et al (Tsai *et al.* 2002a) conducted in Taiwan that measured indoor concentrations of BTEX and MTBE. The authors reported indoor benzene levels of 19.9, 21.75 and 11.75 $\mu g/m^3$ (geometric mean) for afternoon (8:00am-4:00pm), night (4:00pm-12:00am) and late night shifts (12:00am-8:00am) respectively. However, the magnitude of these concentrations are far greater than what we observed at the BHT likely due to the absence of any control ventilation at the Taiwanese facility. Another potential explanation for the differences is that the Taiwanese study was conducted during the winter months in contrast to our summer-time study.

The observed 8-hr TWA concentrations of 1,3-butadiene and benzene (indoors or outdoors) were far below occupation guidelines of ACGIH's (American Convention of Governmental Industrial Hygiene Association) threshold limit value of 4.4 and 1.6 mg/ m^3 or that of OSHA's (Occupational Safety and Health Administration) permissible exposure level: 2.2 and 3.2 mg/ m^3.

A recent study conducted at the same tollbooth facility (Sapkota and Buckley 2003) has identified the traffic (volume and class) and meteorological determinants for select gas and particle-phase curbside pollutant concentrations. Due to a combination of traffic and meteorological conditions, vehicle emission hazards are greatest for the morning shift when outdoor concentrations are at their highest. Protection afforded by the indoor environment varied with shift. The greatest protection was observed during the afternoon shift where 5 to 8 fold reductions in VOC concentrations were observed. For the

morning and night shifts, reductions were comparable and ranged from 2 to 4 fold. The differences in protection by shifts are potentially due to differential rates in ventilation operation. Because the control ventilation operates through a temperature dependent variable demand system, it is likely that flow increased during the afternoon shift when temperatures were higher resulting in higher positive pressure within the booth thereby decreasing the infiltration of outdoor air. These findings suggest that additional protection can be provided to workers by increasing flow to the tollbooth.

Some of the VOCs measured are not emitted from mobile sources and therefore serve as a negative control for the impact of mobile source emissions on the indoor tollbooth environment. All of the mobile source pollutants (e.g. 1,3-butadiene, benzene, MTBE, ethylbenzene, PAHs) were characterized by indoor/outdoor ratios that were on average less than one. In contrast, the chlorinated hydrocarbons including chloroform, tetrachloroethylene, methylene chloride, dichlorobenzene and trichloroethylene were all characterized by ratios greater than one suggesting an indoor source contribution. Methylene chloride is used as a solvent in consumer products and dichlorobenzene is used in air fresheners. The higher indoor concentration is likely associated with cleaning activities and the use of air fresheners within the tollbooths. Likewise, trichloroethylene and tetrachloroethylene are commonly used in dry-cleaning operations, therefore, it is likely that the higher indoor concentration is due to workers wearing dry cleaned uniforms. The indoor/outdoor ratio for chloroform and trichloroethylene may be biased because compared to indoor samples; more outdoor samples were below the limit of detection.

[Insert Table 4.3]

The observation of indoor/outdoor ratios >1 for the chlorinated hydrocarbons is indicative of indoor sources and is consistent with reports for the residential environment (Gordon *et al.* 1999; Payne-Sturges *et al.* 2004; Sax *et al.* 2004). Sax et al (Sax *et al.* 2004) reported indoor/outdoor ratios >1 for chloroform and dichlorobenzene for homes in New York City and Los Angeles. Ratios were mixed for styrene, methylene chloride, toluene, and benzene i.e.greater than one among New York homes but less than one among homes in Los Angeles. The mean ratios for the remaining VOCs (i.e., ethylbenzene o-xylene, m,p-xylene MTBE tetrachloroethylene, trichloroethylene and carbon tetrachloride) were all close to one for both New York and Los Angeles homes. Similarly, ratios of 1.3 and 4.6 were reported for benzene and toluene across homes in EPA Region 5 (Gordon *et al.* 1999). Of particular relevance, for a South Baltimore community within 5 km of the tollbooth facility, Payne-Sturges et al (Payne-Sturges *et al.* 2004), reported mean VOC ratios close to one for MTBE, carbon tetrachloride, and trichloroethylene in contrast to mean ratios >1 for tetrachloroethylene, methylene chloride, chloroform, ethylbenzene, xylene, toluene, and benzene. In contrast to Sax et al and Payne-Sturges et al, numerous VOCs measured at the tollbooth (butadiene, benzene, MTBE, toluene, carbon tetrachloride, xylene, trimethylbenzene, and ethylbenzene) had indoor/outdoor ratios less than one. The likely difference in the observed indoor/outdoor ratios between this study and the residence-based reports is attributable to different factors that are specific to each environment. At the tollbooth, high outdoor

concentrations result from the high activity and proximity of the source (vehicles) while effective control ventilation helps to maintain relatively low indoor concentrations, which result in indoor/outdoor ratios less than one. In contrast, residential indoor/outdoor ratios tend to be greater than or equal to one due to lower activity and proximity of the source outdoors combined with the presence of other VOC sources inside (e.g. gasoline storage, deodorants, air fresheners, household cleaners, various solvents, dry cleaned cloths and paints).

[Insert Table 4.4]

The outdoor VOC concentrations observed at the tollbooth tended to be higher than what has been observed for the urban residential environment. Outdoor concentrations of mobile source related VOCs (MTBE, benzene and ethylbenzene) at the tollbooth were 1.3 (ethylbenzene) to 8 (MTBE) fold higher than outdoor concentrations at urban locations (Table 4.4) (Payne-Sturges *et al.* 2004; Sax *et al.* 2004). In the urban environment 1,3-butadiene was reported to be below the limit of detection (0.06 $\mu g/m^3$), whereas we observed a mean concentration of 6.5 $\mu g/m^3$ outside the tollbooth. The chlorinated hydrocarbons including tetrachloroethylene, chloroform and trichloroethylene were the exceptions where concentrations were observed to be 1.1 to 5 fold lower outside the tollbooth compared to the reported outdoor urban residential environment.

The indoor VOC comparisons between tollbooth and urban homes were very different. Here, mobile source related VOC levels were comparable or less than those observed in the indoor urban environment. In contrast, the concentration of the chlorinated hydrocarbons including carbon tetrachloride, methylene chloride, trichloroethylene and dichlorobenzene, tended to range from 2 (carbon tetrachloride) to 24 (1,4-dichlorobenzene) fold higher inside the tollbooth. For the mobile source-related VOCs, the fact that the outdoor concentrations tended to be much higher while indoor levels tended to be comparable to the urban residential environment highlights the protection offered by the tollbooth with the control ventilation system.

Similar to the mobile-source related VOCs measured outdoors, the concentration of particle bound PAH inside the tollbooth (15.4 ng/m^3) was comparable to indoor non-smoking semi-urban homes, reported by Dubowsky et al (Dubowsky *et al.* 1999), who observed PAH concentrations of 8, 19 and 31 ng/m^3 for suburban, semi-urban and urban locations, respectively. In contrast, median outdoor PAH concentrations (50 ng/m^3) at the tollbooth far exceeded the indoor urban location (outdoor levels were not reported in this study).

Despite the high outdoor levels, the fact that workers are experiencing indoor exposures similar to the urban indoor residential environment indicates the effectiveness of the ventilation control at the tollbooth facility. Yet some mobile source-related VOCs (1,3-butadiene, MTBE) were still higher inside the tollbooth compared to the indoor environment at city homes. The higher concentration of chlorinated VOCs (methylene

chloride, TCE and dichlorobenzene) inside the tollbooth compared to the city homes are likely to be related to cleaning solvents and/or off-gassing from dry cleaned uniforms. Indoor source contributions for these chlorinated VOCs presents an opportunity for intervention to reduce exposure and risk.

The data presented in this study indicate that high concentrations of mobile source-related VOCs are present outside of tollbooth facilities due to the intensity and proximity of mobile sources. At the particular site investigated in the current study, we observed that the control ventilation system was effective in reducing worker potential exposures comparable to what is experienced in the urban indoor residential environment. The low indoor/outdoor ratios, ranging from 0.7 (styrene) to 0.2 (1,3-butadiene) demonstrate the protection offered by the control ventilation equipped tollbooth to the harmful pollutants that are associated with mobile sources. However, it is likely that the indoor measurements represent the best scenario for worker exposure since their job task involves leaning out of the booth for toll collection. Additional research is needed to assess exposure using personal and/or biological monitoring techniques. The observed higher concentration of chlorinated hydrocarbons within the tollbooth indicates an indoor source contribution suitable for remediation.

Acknowledgements

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Table 4.1: Distribution of VOCs ($\mu g/m^3$) and PAH (ng/m^3) measured indoors and outdoors

	Indoor						Outdoor					
Analyte	Min	25th percentile	Median	Mean	75th percentile	Max	Min	25th percentile	Median	Mean	75th percentile	Max
1,3-Butadiene	0.23	0.23	1.31	1.60	2.12	8.09	0.23	3.13	6.46	7.24	11.41	20.49
Benzene	0.29	1.21	3.55	4.12	6.33	14.91	0.73	5.53	12.91	13.29	20.16	34.99
PAH	1.87	6.30	15.40	34.75	37.60	284.85	2.92	18.20	50.00	134.96	148.40	1130.25
MTBE	0.50	13.00	17.20	18.80	25.50	43.80	0.17	22.30	35.00	39.60	51.20	121.40
Toluene	0.23	3.81	9.00	9.75	13.86	40.41	0.23	8.49	17.61	17.87	25.98	45.18
Carbon tetrachloride	0.13	1.44	1.90	1.98	2.36	3.90	0.13	1.96	3.44	3.06	4.17	6.03
mp_Xylene	0.08	3.15	5.13	6.64	7.79	31.83	0.08	5.50	9.13	10.59	15.40	27.56
O_Xylene	0.05	1.22	1.83	2.04	2.51	7.64	0.37	1.97	3.25	3.65	5.45	8.53
Trimethylbenzene	0.08	2.01	2.67	2.83	3.69	6.62	0.08	2.58	4.47	5.35	7.90	13.82
Ethylbenzene	0.06	1.30	2.01	2.81	2.94	11.80	0.06	1.69	3.18	3.67	4.76	21.66
Styrene	0.05	0.26	0.40	0.45	0.53	1.19	0.05	0.20	0.53	0.61	1.00	1.68
Chloroform	0.04	0.04	0.10	0.63	0.75	5.39	0.04	0.04	0.04	0.05	0.04	0.49
Methylene chloride	0.07	5.80	8.41	18.06	11.13	364.55	0.07	0.85	1.33	2.17	2.16	25.28
Tetrachloroethylene	0.10	1.33	2.10	1.95	2.71	4.23	0.10	0.10	0.25	0.39	0.59	2.11
Dichlorobenzene	0.19	82.46	95.73	97.43	112.64	153.52	0.19	2.75	4.36	4.50	5.42	13.65
Trichloroethylene	0.06	1.92	3.11	3.19	4.49	6.89	0.06	0.06	0.06	0.08	0.06	0.56

Table 4.2: Time weighted average concentrations of benzene and 1,3-butadiene inside and outside the tollbooth by workshift. [a]Significantly higher than afternoon shift ($p<0.05$). [b]Significantly higher than night shift. [c]Significantly lower than outdoor concentration during same shift.

Location	Workshift	Butadiene ($\mu g/m^3$)	Standard Deviation	Benzene ($\mu g/m^3$)	SD
Inside Tollbooth	Morning Shift	$2.9^{a,b,c}$	0.9	$6.7^{a,b,c}$	1.3
	Afternoon	0.9^{c}	0.4	3.2^{c}	1.2
	Night	0.9^{c}	0.5	2.4^{c}	1.4
Outside Tollbooth	Morning Shift	$10.7^{a,b}$	3.2	$19.8^{a,b}$	2.8
	Afternoon	7.2^{b}	1.7	14.9^{b}	4.7
	Night	3.7	0.9	4.9	2.2

Table 4.3: Median indoor/outdoor ratios observed at the BHT tollbooth facility compared with published reports for urban residential locations

VOC	Tollbooth	NY Homes (Sax et al)	LA Homes (Sax et al)	Baltimore (Payne-Sturges et al)
1,3-Butadiene	0.2	-	-	-
Benzene	0.3	1.4	1.1	1.4
PAH	0.3	-	-	-
MTBE	0.5	1.1	1.0	1.0
Toluene	0.5	2.0	1.3	3.1
Carbon tetrachloride	0.6	0.9	1.0	0.9
m,p-xylene	0.6	1.3	1.1	1.9
o-xylene	0.6	1.3	1.0	-
1,2,4-Trimethylbenzene	0.6	-	-	-
Ethylbenzene	0.6	1.3	1.1	2.0
Styrene	0.8	2.3	1.5	1.7
Chloroform	2.7	10.2	4.3	10.5
Methylene chloride	6.3	2.1	1.6	2.7
Tetrachloroethylene	8.3	1.3	1.1	1.8
1,4-Dichlorobenzene	21.9	4.2	2.7	-
Trichloroethylene	50.9	1.5	1.1	1.1

Table 4.4: VOCs ($\mu g/m^3$) and PAH (ng/m^3) concentrations measured at BHT tollbooth relative to homes located in urban centers

	Indoor Concentration				Outdoor Concentration			
	New York Homes	Los Angeles Homes	Baltimore Homes	Inside Tollbooth	New York Homes	Los Angeles Homes	Baltimore Homes	Outside Tollbooth
Chloroform	2.15	0.40	2.30	0.10	0.20	0.10	0.22	0.04
1,4-Dichlorobenzene	7.50	3.95		95.73	1.85	1.55		4.36
Styrene	0.75	0.90	0.43	0.40	0.30	0.60	0.25	0.53
1,3-Butadiene	0.70	0.50		1.31	ND	ND		6.46
Methylene chloride	1.80	1.50	0.95	8.41	0.75	0.80	0.35	1.33
Toluene	11.00	15.00	12.12	9.00	5.85	11.95	3.88	17.61
Trichloroethylene	0.25	0.15	0.18	3.11	0.30	0.10	0.17	0.06
Benzene	2.55	3.30	2.45	3.55	1.60	3.25	1.79	12.91
Ethylbenzene	1.60	2.30	1.95	2.01	1.15	2.40	1.00	3.18
o-Xylene	1.70	3.00		1.83	1.30	3.35		3.25
m,p-Xylene	5.00	8.45	7.60	5.13	3.65	8.95	3.97	9.13
MTBE	12.50	12.50	4.25	17.20	10.45	14.50	4.30	35.00
Tetrachloroethylene	2.75	1.60	0.50	2.10	1.40	1.40	0.28	0.25
Carbon tetrachloride	0.55	0.55	0.85	1.90	0.55	0.55	0.90	3.44
Trimethylbenzene				2.70				4.47
PAH				15.40				50.00

Figure 4.1: Schematic of Baltimore Harbor Toll Plaza illustrating the location of air intake for the control ventilation system that supplies the administration building and the individual tollbooths.

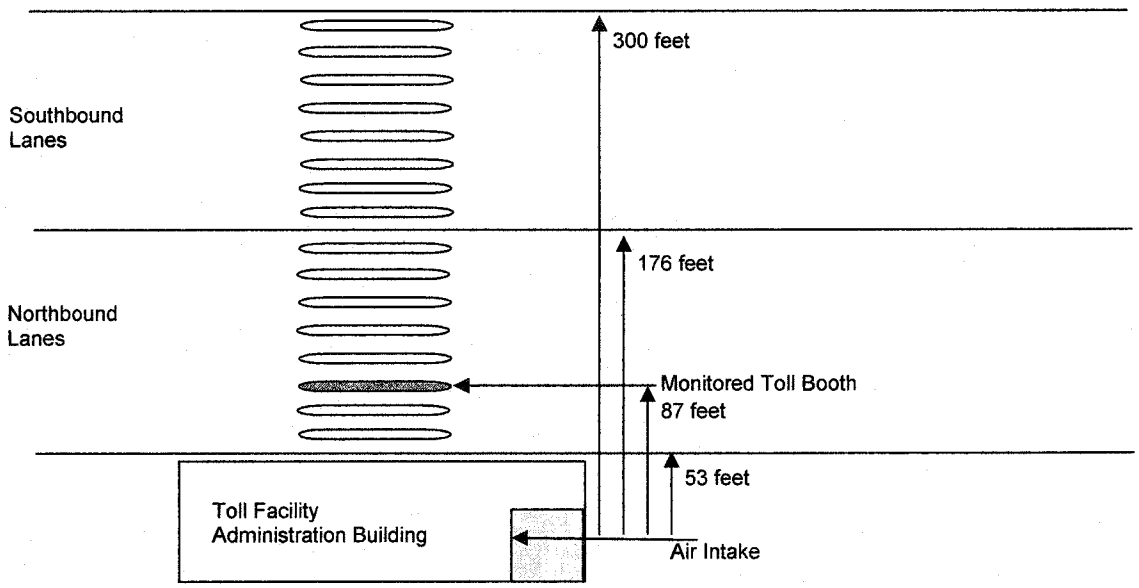


Figure 4.2: Hourly traffic volume (weekday) at the Baltimore Harbor Tollbooth facility by time of day. The horizontal line in the box represents the median; the box represents the 25th and the 75th percentile, the whiskers represent the 10th and the 90th percentile and the dots represent the 5th and the 95th percentile.

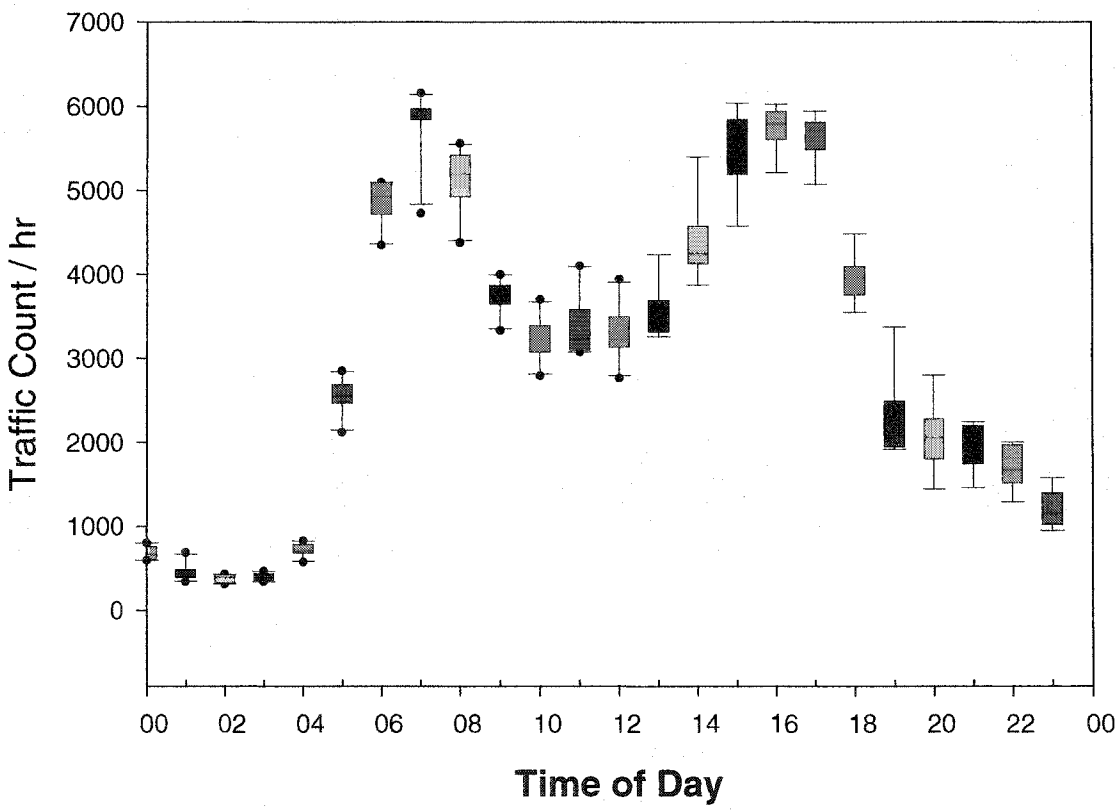


Figure 4.3: Indoor and outdoor concentration of butadiene and benzene at the BHT tollbooth facility by time of day. The actual points on the figure represent the median concentration and the whiskers represent the 5th and 95th percentile.

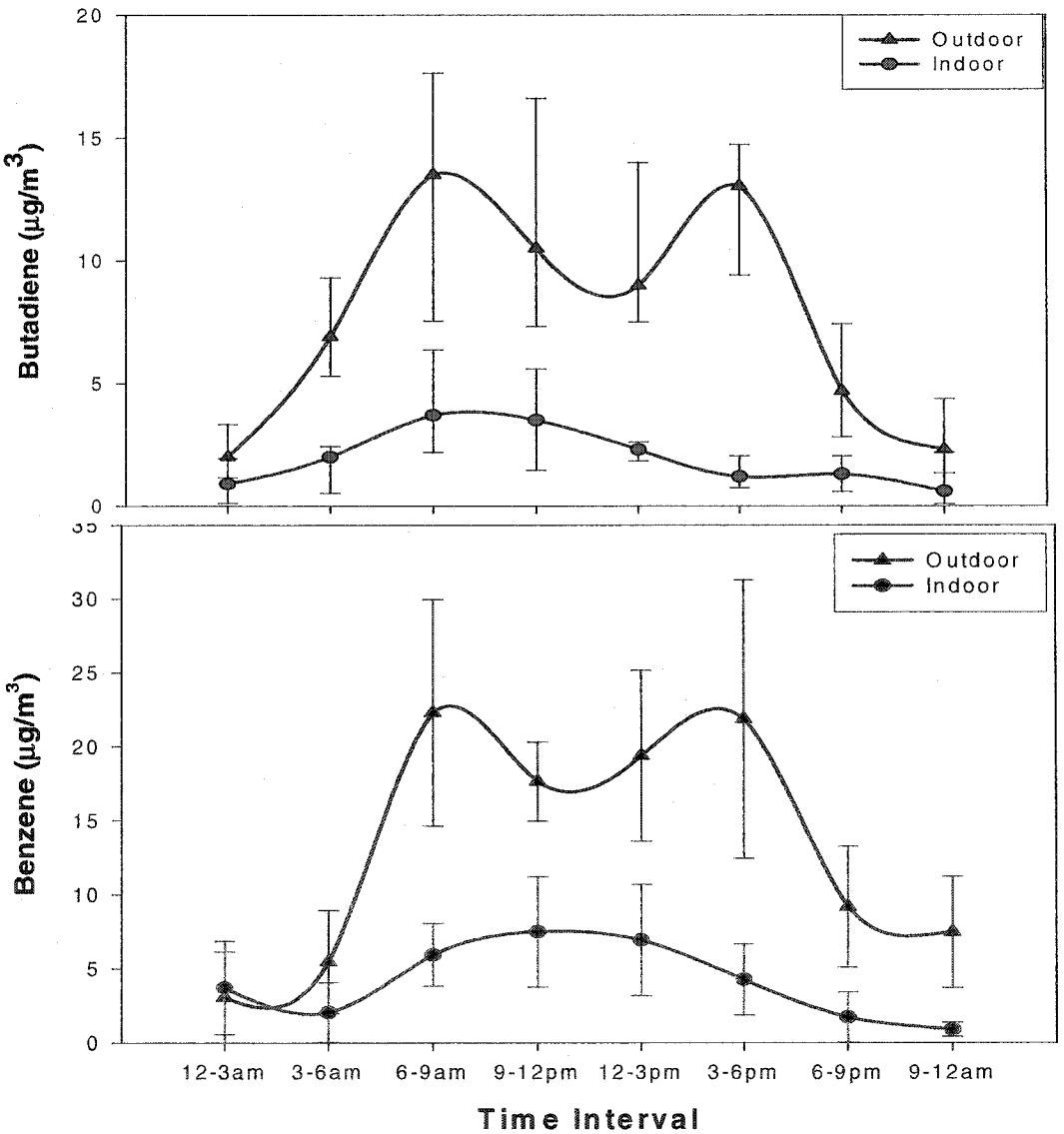


Figure 4.4: Scatter plot and linear regression showing the association between 1,3-butadiene and benzene measured inside relative to outside the tollbooth (n=56).

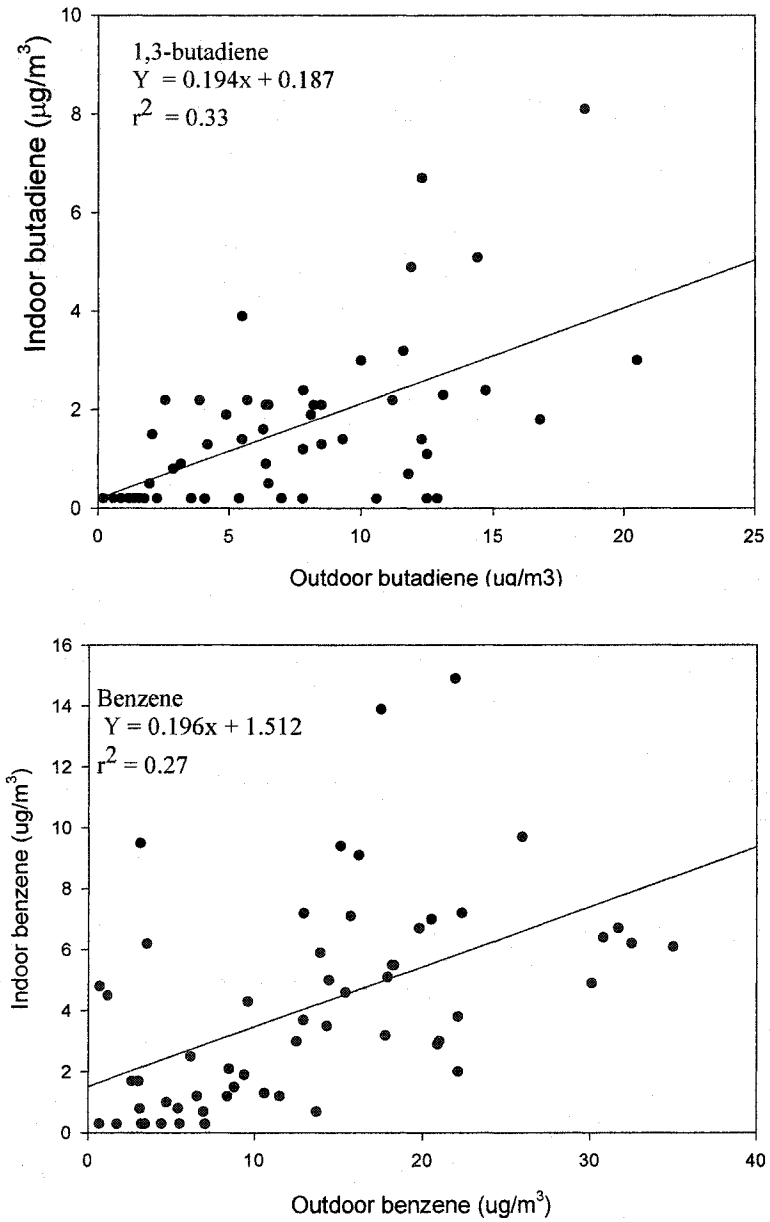
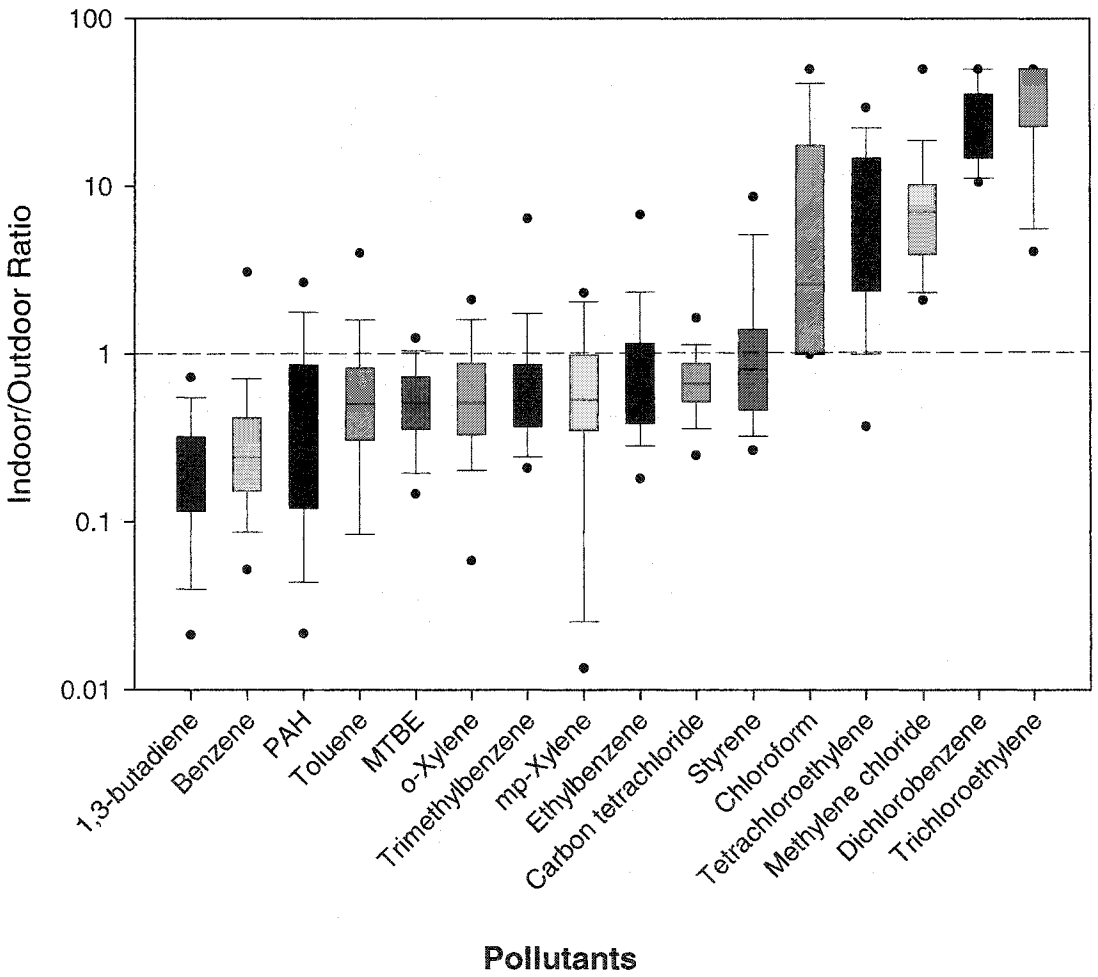


Figure 4.5: Indoor/Outdoor VOC and PAH ratios measured at the Baltimore Harbor Tunnel tollbooth. The horizontal line in each box represents the median, the box ends indicate the 25th and the 75th percentile, the whiskers represent the 10th and the 90th percentile, and the dots represent the 5th and the 95th percentile. The dotted line across the graph corresponds to a ratio of 1, i.e. same indoor/outdoor concentration.



Chapter 5: Exposure Characterization and Biomarker Evaluation of 1,3-butadiene Associated with Mobile Sources

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Abstract

Exposure to automobile exhaust is of significant concern to public health because it contains hazardous chemicals such as 1,3-butadiene, a known human carcinogen. It is ubiquitous in the environment with higher concentrations in the urban areas due to high vehicle density and increasing congestion, further exacerbating cancer risks in these densely populated areas. The goal of this study was to characterize mobile source related environmental exposure to 1,3-butadiene and to evaluate mercapturic acids of butadiene as a biomarker of exposure to discern subtle differences in environmental exposures.

The mercapturic acids (urinary biomarker) that have been used in occupational studies were evaluated using three differentially exposed groups: tollbooth workers (high), volunteers located near a busy urban arterial on a weekday (medium) and the same volunteers located at their suburban home on weekend (low). Personal air samples were analyzed with gas chromatography mass spectrometry (GC/MS). Two mercapturic acids of butadiene: dihydroxybutyl mercapturic acid (DHBMA) and monohydroxybutyl mercapturic acid (MHBMA) were analyzed using liquid chromatography with tandem mass spectrometry (LC-MS/MS). Quantitation was achieved using isotopically labeled internal standards. The limit of detection determined using spiked synthetic urine was 0.4 ng/mL for MHBMA and 3 ng/mL for DHBMA. Results showed that the three exposure groups differed by personal exposure to 1,3-butdiene with median exposures measuring 0.79, 1.62 and 2.38 ug/m^3 for weekend suburban, weekday urban and tollbooth worker exposure groups respectively. The DHBMA levels (mean, SD) observed for the tollbooth workers (362.3, 192.1 ng/mL) were higher than that observed for the weekend suburban

exposure (257.7, 227.7 ng/mL) and the weekday urban exposure (241.0, 161.4 ng/mL). Similarly, MHBMA levels (mean, SD) observed for the tollbooth workers (7.8, 8.2 ng/mL) were higher than that observed for the weekend suburban exposure (6.3, 5.9 ng/mL) and the weekday urban exposure (5.9, 4.9 ng/mL) groups. Although the trends in mean DHBMA and MHBMA were consistent with the group exposure levels, (i.e. tollbooth workers > weekday urban > weekend suburban) no statistically significant differences were found between groups. Further, the consistency in trend with exposure was not observed when DHBMA and MHBMA were adjusted for creatinine. There was no significant association between personal exposure and either urinary biomarker (r-square< 0.1 and p>0.05).

The current study has provided significant advancement in the development of precise, sensitive, and accurate method for the measurement of 1,3-butadiene metabolites in environmental settings. However, an absence of association between personal exposure and biomarker level was observed in this study. It is likely that the lack of association is attributable to limitation of study design (small sample size and unexpectedly small exposure differential between groups) and lack of specificity (in the case of DHBMA).

Introduction

Characterization of human exposure to toxic agents using personal air samples and/or biomarkers provide epidemiological studies with a more direct and comprehensive measure of exposures. This in turn enhances ones ability to detect associations between exposures and disease by minimizing exposure misclassification. Characterization of exposure using personal air monitoring takes into account the heterogeneity of contaminants in different microenvironments and duration of exposure, but such measurement are generally limited to a single route of exposure, i.e. primarily inhalation. Chemical specific urinary biomarkers, on the other hand, not only take into account multiple routes of exposure, but also address intrinsic individual variability such as genetic polymorphisms, and help to examine mechanisms of actions at various biological targets (Groopman and Kensler 1999). Biomarkers also offer a clearer demonstration that the toxicant has in fact entered into the human body (Groopman and Kensler 1993) and provide a cross-sectional estimate of total exposure in a population. Furthermore biomarkers are also helpful in predicting the risk of adverse health outcomes in exposed populations, given that the presence of a certain marker is associated with a specific disease outcome. Such information can be invaluable for mitigating risk, because it can identify specific at-risk subpopulations to which proper interventions can be directed.

Before using biomarkers in epidemiological studies, they must undergo an extensive validation process to ascertain the sensitivity, specificity, accuracy and reliability (Groopman and Kensler 1993). In the past decade, two novel urinary biomarkers 1,2-dihydroxybutyl mercapturic acid (DHBMA) and monohydroxy-3-butenyl mercapturic

acid (MHBMA) have shown promise as biomarkers for occupational exposure to 1,3-butadiene (Bechtold *et al.* 1994; Sabourin *et al.* 1992). More recently, these urinary biomarkers have been validated as exposure biomarker in a large occupational study of styrene butadiene rubber (SBR) workers in the Czech Republic (Albertini *et al.* 2001; Albertini *et al.* 2003; Boogaard *et al.* 2001; van Sittert *et al.* 2000). These studies have shown that MHBMA is more accurate in predicting recent exposure to 1,3-butadiene. However, to date no one has evaluated these biomarkers for environmental exposure to 1,3-butadiene, which are several orders of magnitude lower than occupational exposures.

Based on several epidemiological studies, 1,3-butadiene (BD) is classified as a known human carcinogen (National Toxicology Program 2000; U.S.Environmental Protection Agency 2002). The US Environmental Protection Agency (EPA) estimates that the human lifetime excess cancer risk from chronic exposure to BD is $8x10^{-2}$ per ppm ($3.5x10^{-5}$ per ug/m^3) based on a linear extrapolation of increased leukemia risks observed in occupationally exposed workers (U.S.Environmental Protection Agency 2002). These estimates imply that an acceptable level of lifetime risk (1 cancer case in a million) would result if individuals were chronically exposed to 0.01 ppb (0.022 ug/m3) level of BD over their lifetimes. There are some limited data available from monitoring sites across the country that indicate ambient levels of BD exceed the 0.01 ppb level in most urban areas, including Baltimore City (Maryland Department of the Environment 2000). However, such ambient concentrations are not a true reflection of personal exposures experienced by residents living in urban areas. In addition to ambient concentration, several other factors contribute to personal exposures, including occupation, proximity of the residence

to high-traffic roadways, time spent in automobiles, other personal habits including smoking, and meteorological conditions. So an overall estimate of personal exposures to 1,3-butadiene across different population groups and covering various geographical area is warranted to identify mobile source hot spots, populations at risk, and the magnitude of the risk.

Major anthropogenic sources of environmental BD include automobile exhaust, prescribed burns, manufacturing & processing facilities, plastic and rubber factories, and oil refineries. Of all the anthropogenic sources, automobile exhaust (on-highway and off-highway) is the dominant source accounting for as much as 60% (33,000 tons) of the annual national BD emissions (National Toxicology Program 2000) followed by wildfires and prescribed burns accounting for 30% (20,000 tons) of the total emissions. Industrial processes, including organic chemical manufacturing, polymer and resin production, secondary lead smelting and petroleum refining account for an additional 6% (4,000 tons). These national emission statistics clearly indicate that any effort in reducing ambient BD levels (and resulting personal exposures) needs to focus on mobile sources.

Butadiene is one of the most potent carcinogens emitted in automobile exhaust. Besides BD, automobile exhaust contains several other hazardous air pollutants (HAPs) such as benzene, formaldehyde, polycyclic aromatic hydrocarbons and others. The US EPA estimates that the cancer risk attributable to 1,3-butadien is three times that of benzene, formaldehyde and acetaldehyde (other major mobile source pollutants of concern) combined (U.S.Environmental Protection Agency Office fo Air Quality 2000c). Despite

this, exposure to BD has not been well characterized primarily because the traditional methods used for VOC sampling using 3M badges or charcoal tubes do not adequately sample 1,3-butadiene that are present in typical ambient environment (Chung *et al.* 1999; Kim *et al.* 1999). Lately, the increasing use of thermal desorption techniques that utilize multi-bed sorbent tubes has enabled more quantitative assessments of 1,3-butadiene exposure (Kim *et al.* 1999; Kim *et al.* 2001; Kinney *et al.* 2002; Sax *et al.* 2004). This paper is focused on characterizing low-level environmental personal exposure to BD resulting from mobile sources, using personal air sampling. It further evaluates the suitability of two urinary biomarkers that have been validated for high occupational exposures, for predicting low-level environmental exposure.

Metabolism of 1,3-Butadiene

The activation of BD to its toxic metabolite is mediated by a cytochrome P450 (CYP 450). As shown in Figure 1, BD is oxidized by CYP2E1 and CYP2A6 to the electrophilic 1,2-epoxy-3-butene (also called butadiene monoepoxide (BDO) or epoxy butane). This can undergo oxidation to form 1,2,3,4-diepoxybutane (BDO_2, also called butadiene diepoxide). BDO also can be hydrolyzed by epoxide hydrolase (EH) forming 1,2-dihydroxy-3-butene (BD-diol). Both BDO_2 and BD-diol can undergo further metabolism to form 1,2-dihydroxy-3,4-epoxybutene (butadiene diol-epoxide, EBD). These three epoxides (BDO BDO_2 and EBD) can react with DNA and proteins such as hemoglobin (Boogaard *et al.* 2001), where they form DNA or protein adducts that elicit toxicity. They can be detoxified when they undergo conjugation with glutathione (GSH) or when hydrolyzed by epoxide hydrolase (Himmelstein *et al.* 1997; van Sittert *et al.* 2000).

The hydrolysis of BDO to 1,2-dihydroxy-3-butene (DHB, BD-diol) and subsequent conjugation of this product with GSH leads to formation of *N*-acetyl-*S*-(3,4-hydroxybutyl)-*L*-cysteine (also called dihydroxybutyl mercapturic acid (DHBMA) or M-1 metabolite). When BDO directly undergoes conjugation with GSH, the resulting product is *N*-acetyl-*S*-(1-(hydroxymethyl)-2-propenyl)-*L*-cysteine & *N*-acetyl-*S*-(2-hydroxy-3-butenyl)-*L*-cysteine (also called monohydroxybutyl mercapturic acid (MHBMA) or M-2 metabolite). In vitro and in vivo studies indicate that BD metabolism is qualitatively similar among different species (Figure 5.1), but quantitatively different (Bechtold *et al.* 1994; Henderson *et al.* 1996). Richardson *et al.* (Richardson *et al.* 1999) showed that the major metabolites derived from BDO in both rats and mice were DHBMA and MHBMA. Sabourin et al showed that the relative amount of these two metabolites excreted varied between different species, and that this variation was proportional to the level of epoxide hydrolase activity found in that species. Both DHBMA and MHBMA have successfully been used as a biomarker of exposure to 1,3-butadiene in rubber manufacturing and oil refinery workers, with MHBMA more closely predicting a recent exposure (Albertini *et al.* 2001; Albertini *et al.* 2003; van Sittert *et al.* 2000).

[Insert Figure 5.1]

Methods

Study Population

Individuals representing different exposure scenarios were recruited, that differed by spatial proximity and intensity of the mobile sources: weekend suburban exposure (far from source and low source intensity), weekday urban exposure (close proximity to source and medium source intensity) and tollbooth workers workday exposure (very close proximity and high source intensity). For the first and second scenario, 7 faculty and staff from the School of Public Health were recruited as a convenient sample. For the third scenario, 9 tollbooth workers from Baltimore Harbor Tunnel were recruited. All individuals were non-smokers living in non-smoking household. The last two scenarios were chosen because they represent mobile source "hot spots". The first scenario was chosen because it further increases the range of exposure for evaluating the urinary biomarkers. The study was approved by Committee on Human Research (CHR) at The Johns Hopkins University Bloomberg School of Public Health. Prior to collecting any samples, a written consent was obtained from all study participants.

Personal air sampling

Personal exposure for the weekend suburban scenario was monitored on Sunday from 12pm to 8pm. For the weekday urban scenario, personal exposures were monitored of the same individuals while they spent 4 hours inside an urban home by a busy street during morning rush-hour (6am-10am). For the high exposure group, tollbooth workers were monitored from 6am to 2pm. In all cases, personal air samples were collected within the breathing zone of the participants using stainless steel Perkin-Elmer Air Toxic Tubes™

(3.5 inch long, 0.25 inch outer diameter) packed with a mixed sorbent comprised of carbopack B and corboxen 1000 (Supelco cat # 25051, Bellefonte, PA) and SKC 210 pocket pump (SKC Inc., Eighty Four, PA). The Air Toxic tube was clamped directly onto the lapel of the participant. The SKC pocket pump was operated at a nominal flow rate of 100 ml/min. To avoid sample breakthrough, the flow through the pump was split using an adjustable low flow tube holder (SKC Inc, Cat # 224-26-01) and a constant pressure regulator (SKC Inc. cat # 224-26-CPC) such that the flow through the sampling tube was only 12mL/min. The actual flow through the sampling tube was calibrated before initiation of sampling using a DryCal DC-2 primary standard (BIOS International Corp, Butler, NJ). Sample flows were checked again at the end of the monitoring period.

The personal air samples were analyzed within 24 hour of collection using method previously described (Kim *et al.* 1999; Sapkota and Buckley 2003) In short, the VOCs were thermally desorbed with Perkin-Elmer™ ATD-400 (Perkin-Elmer, Shelton, CT), separated by gas chromatography, and detected with mass spectrometry (GC/MS) in selective ion monitoring (SIM) mode using a Shimadzu GC-17A gas chromatograph and QP-5000 mass spectrometer (Shimadzu, Columbia, MD). Chromatographic separation was achieved using Restek Rtx-624 column, 60 m x 0.25 mm ID with 1.4 um film thickness (Restek Corp., catalog no. 10969). Lab blanks and field blanks were analyzed along with the personal samples. Six level calibration standards were prepared in methanol using a 2mg/mL 1,3-butadiene stock solution (Accustandard, catalog no. S-406A-10x). One microliter injection of these standards were made onto a clean sampling

tubes using a modified GC injector port (50°C, Helium flow of 80 ml/min for 10min). The final amount of BD on sampling tubes ranged from 1 to 50 ng.

Urine Collection

Once the personal air sampling was initiated on Sunday (low exposure group), the volunteers were instructed to collect all the urine samples that day. When the volunteers were at the urban home, urine samples were collected upon their arrival, and instructed to collect all the urine voids for that day. For the tollbooth workers, urine sample were collected prior to their workshift, at lunch break, after shift, before bed and the first urine void the following day (Figure 5.2). All urine samples were stored at –70°C until analysis.

[Insert Figure 5.2]

Chemicals

Isomeric mixture of R,S-1-hydroxy-2-(N-acetylcysteinyl)-3-butane and R,S-2-hydroxy-1-(N-acetylcysteinyl)-3-butane (MHBMA), their duterated analogue R,S-1-hydroxy-2-(N-acetylcysteinyl)-3-butane-[d_6] and R,S-2-hydroxy-1-(N-acetylcysteinyl)-3-butane-[d_6] along with R,S-1,2-dihydroxy-4-(N-acetylcysteinyl)-butane (DHBMA) and it's duterated analogue R,S-1,2-dihydroxy-4-(N-acetylcysteinyl)-butane-[d_7] were purchased from Toronto Research Chemicals, Ontario, Canada (Cat# A179005, A179007, A173710 and A173712 respectively). HPLC grade water (Burdick & Jackson Cat# 365-4) and MS grade methanol (Burdick & Jackson Cat # GC230-4) were obtained from VWR

International, West Chester, PA. Synthetic urine was obtained from Spectrum Labs, Cincinnati, OH.

Urine Sample Preparation

Urine samples were thawed at room temperature until the frozen samples were completely dissolved. Aliquots of 10 ul of 100 ng/mL MHMBA-[d_6] and DHBMA-[d_7] in methanol were added to 1mL urine samples and pH was adjusted to 2.5 by addition of 1 and 0.1 N HCl. Samples were then extracted with Waters Oasis HLB cartridges (Waters Corp, Milford MA. Cat # WAT094226) that were pre-equilibrated with 1mL methanol followed by 1 mL water. After loading, the cartridges were washed with 1 mL water, and elution was performed with 1 mL of 50:50 methanol/water at <0.5 ml/min.

Sample Analysis

The analytical instrumentation consisted of Thermo Electron Surveyor HPLC and autosampler system coupled with Thermo Quantum Ultra triple quadruple mass spectrometer. Using an autoinjector, 10 ul of the sample was injected. Analytical separation was achieved using a mixed-mode (C8/anion exchange) column that was custom packed (Alltech Associates, Deerfield IL. Part# C-6008, 7 □m particle size, 100 mm x 2.1 mm id) and pre-column filter (Phenomenex, Torrance CA. Part# AJ0-4286, 4mm Lx 2 mm id), maintained at 35°C. The mobile phase was isocratic, consisting of 30:70 water/methanol with 5 mM ammonium formate at a flow rate of 300 ul/min with a total run time of 6 min. Under these condition, both the analytes and the respective internal standards eluted between 2.5-3.5 min. Using divert valve, the LC eluent coming

before 2 min and after 4 min was directed to waste, to avoid excess ammonium formate entering into the spray chamber. The MS/MS was operated in negative ionization selective reaction monitoring (SRM) mode using electrospray ionization (ESI) probe. The instrument conditions are described in detail in Table 5.1. DHBMA was detected using m/z 250.06→ 121.06 and quantified using the DHBMA- [d_7] internal standard, m/z 257.11 → 128.11. Similarly MHBMA was detected using m/z 232.05 → 103.08 and quantified using the MHBMA-[d_6], m/z 238.09→109.11. Synthetic urine was used to prepare a 9-point calibration curve for M-1 (0-1000 ng/mL) and M-2 (0-200 ng/mL). The instrument response increased linearly over the entire range for both analytes, with r-square value greater than 0.996.

[Insert Table 5.1]

Sample recovery: Since all urine samples had some detectable amount of DHMBA and MHMBA, synthetic urine was used for the purpose of determining sample recovery and limit of detection. Two sets of 3x1 ml aliquots synthetic urine were spiked with 10 ul of 100 ng/mL MHMBA-[d_6] and DHBMA-[d_7] internal standards. The first set of synthetic urine samples was further spiked with 10 ul of low stock (1000 ng/ml M-1 and 200 ng/mL of M-2), and the second set with 10 ul of high stock (2000 ng/ml M-1 and 400 ng/mL of M-2). All spiked synthetic urine were pH adjusted to 2.5 and the analytes were extracted as discussed earlier. Sample recovery at each spike (low and high) was determined as the % of analyte recovered relative to the spiked amount. To determine the limit of detection, a set of 7x1 ml aliquots of synthetic urine was spiked with 10 ul of

100 ng/mL MHMBA-[d_6] and DHBMA-[d_7] internal standards followed by 10 ul of low stock containing 1000 ng/ml M-1 and 200 ng/mL of M-2. These samples were pH adjusted to 2.5 and extracted as discussed earlier. Limit of detection was determined by multiplying the standard deviation of the 7 spiked samples by the Student's t-value associated with the 99% confidence interval and 6 degrees of freedom.

Precision: Method precision was determined using two different approaches. In the first approach, a pooled urine sample was spiked withe DHBMA and MHMBA, divided into 1mL aliquots, and stored in the freezer. Whenever urine samples were extracted, 1-2 aliquots of the spiked urine samples were also included. At the end, precision was determined using all the spiked urine samples that were analyzed over the entire sample analysis period. In the second approach, 2 urine samples were randomly selected in each batch of extraction. These randomly selected samples were then extracted in duplicates. Method precision was determined based on the agreement between duplicate extracts analyzed over the entire study period.

Statistical Analysis

All statistical analysis on exposure data was performed after log transformation using Intercooled Stata, version 7.0 for Windows (Stata Corporation, TX). For the urinary biomarker data group differences were ascertained using a non parametric Mann-Whitney test, without any transformation. The association between personal exposure and urinary biomarkers were evaluated by grouping all the study participants into a single category. The association between the exposure and biomarker was further explored using a

multiple linear regression model with a random intercept, to allow each individual to have their own intercept.

Results

The characteristics of the study participants are listed in Table 1. The two groups are similar with regards to age, but differed with respect to race and sex. Most of the tollbooth workers were African Americans (67%), whereas the suburban weekend and urban weekday group were primarily Caucasians (50%) and Asians (36%). The tollbooth workers were mostly females (67%) whereas the comparison groups were primarily male (64%). The control group had a considerably lower BMI (22.0±2.3) compared to tollbooth workers (28.5±6.0).

Personal Exposure

The distribution of 1,3-butadiene exposure varied by groups with median levels of 0.88, 1.62 and 2.38 $\mu g/m^3$ observed for the weekend suburban, weekday urban and tollbooth workers respectively. Although the trend in median levels was consistent with the study design, differences of <1 $\mu g/m^3$ between groups were less than expected (Figure 5.3). The tollbooth workers' exposure along and the urban weekday exposure both were higher than the suburban weekend exposure ($p<0.05$).

[Insert Figure 5.3]

Urinary Biomarker

At the instrumental conditions specified in Table 1, m/z 103.08 and 121.06 were the dominant daughter ions of the molecular base ion of MHBMA$^-$ (233-H^+ = 232) and DHBMA$^-$ (251 – H^+ = 250) respectively (Figure 5.4). Under the specified chromatographic conditions (Table 2), DHBMA and DHBMA-d_7 eluted prior to MHBMA and MHMBA-d_6 (Figure 5.5).

[Insert Figure 5.4]

[Insert Figure 5.5]

Recoveries based on the spiked synthetic urine were (mean±SD) 86±11% and 92±12% for the high-level and low-level spikes of DHBMA. For MHBMA, recoveries were 85±12% and 85±8% for the high and the low-level spikes. The LOD was estimated to be 0.4 ng/mL and 3.7 ng/mL for MHBMA and DHBMA, respectively. Using this LC-MS/MS method, DHMBA was detected in 100% of the samples collected, while MHBMA was detected in 95% of the samples. Those samples that were below the LOD were assigned a value of ½ LOD, an approach that is suitable for skewed data, as previously described by Hornung and Reed (Hornung and Reed 1990). For samples extracted and analyzed in duplicates throughout the study period, there was good agreement between the duplicates for both DHBMA and MHBMA, as indicated by slope close 1 and r-square greater than 0.9 (Figure 5.6).

[Insert Figure 5.6]

Linear regression (Figure 5.7) between DHBMA and MHBMA showed that there was a significant association between the two urinary biomarkers ($p<0.05$, $R^2=0.48$). The linear In all cases, DHBMA was the dominant marker representing greater than 90% of the total biomarker excreted. The mean (SD) metabolic ratio determined as the ratio of DHBMA/(DHBMA+MHBMA) was 0.974 (0.021) with min and max of 0.897 and 0.999 respectively.

[Insert Figure 5.7]

The distribution of DHBMA and MHMBA levels (Figure 5.8) across the three exposure scenarios shows some trend for increasing biomarker levels. However, this trend is very weak compared to the one observed for personal exposures in Figure 5.3. As shown in Table 3, the DHBMA levels (mean, SD) observed for the tollbooth workers (362.3, 192.1 ng/ml) was higher than that observed for the weekend suburban exposure (257.7, 227.7 ng/mL) and the weekday urban exposure (241.0, 161.4 ng/mL), but not statistically significant ($p>0.05$). Similarly, the MHBMA levels (mean, SD) observed for the tollbooth workers (7.8, 8.2 ng/ml) was higher than that observed for the weekend suburban exposure (6.3, 5.9 ng/mL) and the weekday urban exposure (5.9, 4.9 ng/mL), but not statistically significant ($p>0.05$). However, the consistent increasing trend with exposure group disappeared when the biomarker levels were adjusted for creatinine. In addition to the findings of this study, Table 5.3 also provides the comparison of personal

exposures and the urinary DHBMA and MHMBA levels to some recently published studies.

[Insert Figure 5.8]

[Insert Table 5.3]

The association between the personal exposure to 1,3-butadiene and the urinary biomarker was investigated by combining all the exposure level and biomarker level into a single group. An exploratory analysis using scatter plot (Figure 5.9) showed a lack of distinct pattern between exposure and biomarker level.

[Insert Figure 5.9]

The relationship between personal exposure, urinary biomarker and the exposure group was examined using multiple regression with random intercept and an interaction term for levels of personal exposure and exposure group:

$\log Y_{it} = \beta_0 + \beta_1 \log X_i + \beta_2 \log X_i * I + \varepsilon$

Y_{ij} = Urine Concentration of biomarker for individual i at time t (ng/mL);

X_i = Personal exposure of individual i ($\mu g/m^3$),

I = Exposure group (1 if tollbooth workers, 0 otherwise)

The results showed that there was no significant association between personal exposure and either biomarker level (negative β_1). This relationship between personal exposure and

biomarker was modified by the exposure group (positive β_2) but was not statistically significant.

Discussion

In this study, personal exposure to 1,3-butadiene was characterized for three scenarios: suburban weekend, urban weekday (rush-hour) and tollbooth workers. The urban and the tollbooth environments were characterized by close proximity and high traffic intensity, often referred to as mobile source "hot spots". Additionally, we used this hot spot exposures, included in the study design, to evaluate urinary metabolite of 1,3-butadiene (MHBMA and DHBMA) as biomarkers of exposure.

This study provides, for the first time, tollbooth workers personal exposure to 1,3-butadiene. The tollbooth workers (morning shift) median personal exposure (2.38 $\mu g/m^3$) was similar to the indoor morning shift concentration (2.9 $\mu g/m^3$) previously reported for the same facility (Sapkota *et al.* 2004). These relatively low indoor concentration (morning shift: 2.9 $\mu g/m^3$) occurred despite 3 fold higher outdoor concentration (morning shift: 10.7 $\mu g/m^3$). Sapkota et al reported this reduction to the effective ventilation system which maintained the tollbooth under positive pressure.

The tollbooth workers' median exposure (2.38 $\mu g/m^3$) was higher than the median urban weekday exposure (1.62 $\mu g/m^3$) and the median suburban weekend exposure (0.88 $\mu g/m^3$). However, the absolute differences between these groups are relatively small

(0.76 and 0.74 $\mu g/m^3$ respectively). The overall concentration of 1,3-butadiene inside the tollbooth (1.31 $\mu g/m^3$) reported previously by Sapkota et al. was only slightly higher than what has been observed inside urban homes (0.7 $\mu g/m^3$) as reported by Sax et al (Sax *et al.* 2004). Given this background, the findings that the personal exposure of tollbooth workers at the BHT tollbooth was only slightly higher compared to the personal exposure of individuals spending time inside an urban home located by a busy street during morning rush-hour is not surprising. It is possible that the personal exposure of tollbooth workers working at different facility that lacks efficient control ventilation system (as present in this facility) is likely to be similar to the outdoor concentration at the tollbooth, and significantly higher than the exposure at urban homes. A study investigating the differences in exposure at the two different type of tollbooth facility is warranted.

Careful examination of the time activity questionnaire revealed that the amount of time spent driving during the off-day was higher amongst tollbooth workers compared to the faculty and staff. In extreme cases, a worker reported driving as much as 6 hours during his off-day, while other reported mowing the lawn for 3 hours. The personal exposure to 1,3-butadiene in such cases was found to be very high (results not shown). This could result in high exposure variability during the off day, in contrast to workday exposure, which tend to be similar due to the identical work environment. The small sample size limited the further data analysis evaluating the effect of driving and mowing the lawn on personal exposure and compare to that of working in tollbooth.

The method utilizing SPE extraction followed by LC-MS/MS analysis showed excellent sensitivity, recovery and precision for both urinary metabolite of 1,3-butadiene resulting from environmental exposure. The close agreement between the duplicate extracts for both DHMBA and MHMBA highlight the precision of the current method. The close to 1 slope factor for the duplicate extracts shows that the results were reproducible and the high r-square (> 0.9) indicate that the results are precise.

Although the two urinary biomarkers of butadiene are present at very different levels, they are closely related to each other (r-square 0.48). The ratio of the two biomarkers DHBMA/(DHBMA+MHBMA) is an indication of the metabolic pathway. This ratio, initially reported by Sabourin et al (Sabourin *et al.* 1992) has been shown to increase from mice (0.2) to rats (0.52) to humans (>0.97), paralleling the increase in epoxide hydrolase activity amongst these species (Bechtold *et al.* 1994). Based on this data, the authors hypothesized that in humans, the majority BDO is metabolized by hydrolysis to butenediol instead of direct conjugation with GSH, as in the case with mice. The mean metabolic ratio of 0.974 observed in this study is consistent with these previously reported human studies. A recent occupational study of Czech butadiene worker reported a median metabolic ratio of 0.995, 0.987 and 0.981 for control, monomer and polymer worker respectively (Albertini *et al.* 2001; Albertini *et al.* 2003; van Sittert *et al.* 2000), with the control workers having significantly higher metabolic ratios. In a study comparing smokers and non-smokers, Urban et al (Urban *et al.* 2003) reported a metabolic ratio of 0.970 for non-smokers and 0.859 for smokers. In the current study, we did not observe any difference in the metabolic ratios amongst different groups.

The higher (but not significant) biomarker levels observed for the weekend suburban exposure settings compared to the weekday urban exposure settings is unexpected. One explanation is that the clearance rate for these biomarkers are longer than that for typical mercapturic acids, such that the exposures from the previous day was influencing the biomarker levels collected during the weekend. The tollbooth workers had higher level of DHBMA and MHMBA compared to the weekday urban exposure settings, but this relationship disappeared when the biomarker levels were adjusted for creatinine. For the weekday exposure settings, 36% of the population were females as opposed to the tollbooth workers which had 67% females. Thus, different creatinine levels between the sexes, which in turn differed between the study groups, is likely to have reversed the association between the creatinine adjusted and unadjusted values.

There was no direct association between exposure and either biomarkers. This lack of association is potentially attributable to very narrow range of exposure observed for this study and small sample size. The overall 1,3-butadiene exposure observed in this study was significantly lower than what has been reported in the literature, evaluating the biomarker. In the Czech workers (Albertini *et al.* 2001; Albertini *et al.* 2003; van Sittert *et al.* 2000), the median exposure for the highest exposure group (Polymer workers: 293 ug/m3) was 123 fold (compared to tollbooth worker) to 431 fold (compared to suburban weekend) higher. Similarly the median exposure of the lowest exposure group (13 $\mu g/m^3$) was 5.5 fold (tollbooth workers) to 19 fold (suburban weekend) higher. Although the method described in this study was highly sensitive to detect even MHMBA in 95% of

the samples collected, the small variability in exposure and the limited sample size might have contributed to the non-significant association for the exposure and biomarker. The 100 fold higher exposure levels in the Czech workers, and larger sample size might have enabled the authors to discern the relationship between the urinary biomarker and the 1,3-butadiene exposure. On the contrary, in a study of petrochemical workers in Italy, Fustiononi et al (Fustinoni *et al.* 2004) reported no significant difference in DHMBA and MHMBA between exposed workers and their non-exposed counterparts. Their findings is of particular importance to this study because the median exposure of the exposed worker 1.5 ug/m3 (mean 11.5 ug/m3) and the control workers 0.4 ug/m3 (mean 0.9 ug/m3) are more relevant to this study, compared to the Czech worker's exposure.

The presence of DHBMA (median 160.8 ng/mL) even in the lowest exposure group is consistent with what has been reported in the literature. These levels are lower than the DHBMA levels reported by Fustioni et al for their unexposed control population. The high concentration of DHBMA relative to the low levels of exposure raises the concern of specificity for this biomarker. In comparison, the level of MHBMA levels in the low exposure group is low and very similar, both in this study and Fustioni et al. The presence of MHMBA reported here, even in the lowest exposure group who did not smoke, serves to document the presence of this biomarker in general population. This is expected because 1,3-butadiene is ubiquitous in the environment, so everybody is exposed to some level of it.

In conclusion, this study characterizes subtle differences on the personal exposure to 1,3-butadiene in different environmental settings: tollbooth workers > urban weekday > suburban weekend. However, the absolute difference between the exposure is very small. This small difference in exposure is likely attributable to the efficient control ventilation system present at this specific tollbooth facility, as previously reported (Sapkota and Buckley 2003). This study further provides a LC-MS/MS method for detection of urinary biomarkers of 1,3-butadiene resulting from environmental exposure. The two biomarkers (DHBMA and MHMBA) were present at an elevated level in the urines of tollbooth workers, compared to that of the suburban weekend exposure group and the urban weekday exposure group, but the differences were not statistically significant.

Table 5.1: Analytical parameters for LC-MS/MS

LC Parameters			
Mobile Phase	70:30 H20/MeOH; 5mM Ammonium Formate		
Flow rate	300 ul/min		
Injection volume	10 µl		
Column Temperature	35°C		
Runtime	6 min		
MS-MS Parameters			
Ion Source	ESI Negative		
Spray Voltage	2800		
Seath Gas Pressure	35		
Aux Gas Pressure	14		
Capillary Temp	375 °C		
Source CID	10		
Collison Pressure	0.9 mTorr		
Peak Widths	Q1=0.5; Q3=0.7		
SRM Table			
Analyte	Parent Ion	Daughter Ion	Collison Energy
MHBMA	232.05	103.08	17
MHBMA-[D_6]	238.09	109.11	15
DHBMA	250.06	121.06	16
DHBMA-[D_7]	257.11	128.11	19

Table 5.2: Characteristics of Study Population

		Traffic Site	Tollbooth Workers
Age		41.3±8.1	47.2±8.4
Race	White (%)	50	33
	Black (%)	14	67
	Asian (%)	36	0
Sex	Male (%)	64	33
	Female (%)	36	67
BMI		22.0±2.3	28.5 ± 6.0
Driving (min)		34.2 ± 9.9	20.1 ± 7.8

Table 5.3: Comparison of the urinary biomarkers and personal exposure with literature values

Investigator	Exposure	BD Exposure (ug/m3)		DHBMA (ng/mL)		MHBMA (ng/mL)	
		Mean (SD)	Median (Min-Max)	Mean (SD)	Median (Min-Max)	Mean (SD)	Median (Min-Max)
Current Study	Weekend Suburban	1.22 (1.09)	0.88 (0.23-4.36)	257.7 (227.7)	160.8 (45.9-766.4)	6.3 (3.0)	5.6 (2.3-11.3)
	Urban Weekday	1.47[a] (0.73)	1.62 (0.33-3.66)	241.0 (161.4)	202.4 (34.2-496.2)	5.9 (4.9)	3.4 (1.9-15.8)
	Tollbooth Workers	2.88[a] (2.10)	2.38 (0.51-8.12)	362.3 (192.1)	357.7 (62.6-808.9)	7.8 (8.2)	5.2 (0.6-26.4)
Albertini et al	Control	26 (30)	13 (2-125)	353 (157)	355 (197-747)	1.7 (1.54)	1.6 (0.05-7.3)
	Monomer	643 (2056)	74 (2-19909)	764 (728)	508 (52-3522)	9.44 (12.97)	3.6 (0.05-44.0)
	Polymer	1760 (4692)	293 (2-39030)	4647 (6630)	1479 (190-26207)	120.17 (228.17)	20 (1.7-962.0)
Fustioni et al	Control	0.9 (1.0)	0.4 (<.1-3.8)	602 (207)	547 (232-1009)	7.5 (7.0)	5.6 (<1.0-21.8)
	Petrochemical Workers	11.5 (35.8)	1.5 (<0.1-220.6)	605 (409)	507 (62-1643)	10.5 (13.7)	5.0 (<1.0-50.6)
Urban et al	Non Smoker	NR	NR	459 (72)*	NR (209-898)*	12.5 (1.0)*	NR (7-18.1)*
	Smoker	NR	NR	644 (90)*	NR (116-1084)*	86.4 (14.0)*	NR (15.2-145.1)*

NR: Not Reported. * concentration expressed as ug/24 hour. [a] statistically higher than the suburban weekend (student t-test with unequal variance, unpaired)

Figure 5.1: Schematic for 1,3-butadiene metabolism. Source: Albertani et al 2001

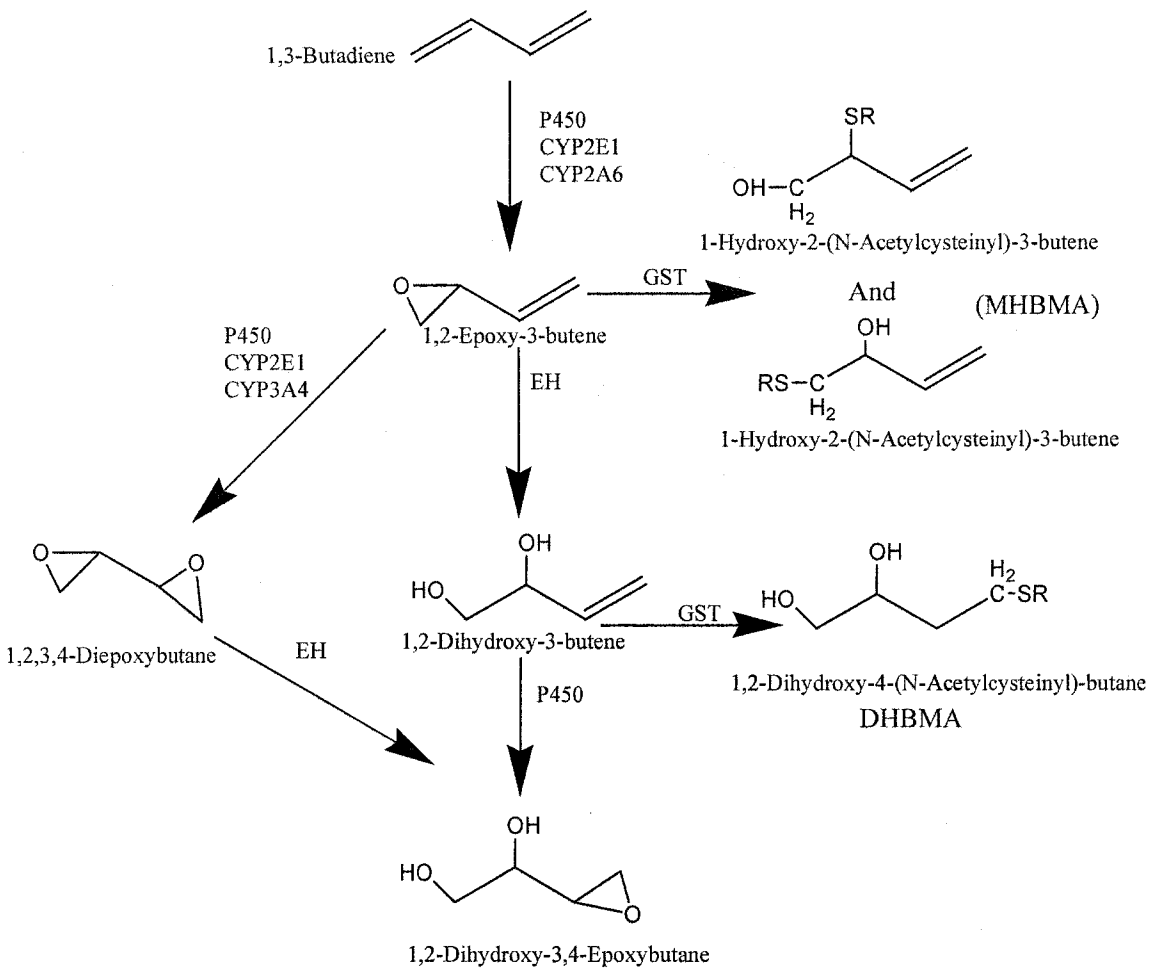


Figure 5.2: Schematic of sample collection for tollbooth workers

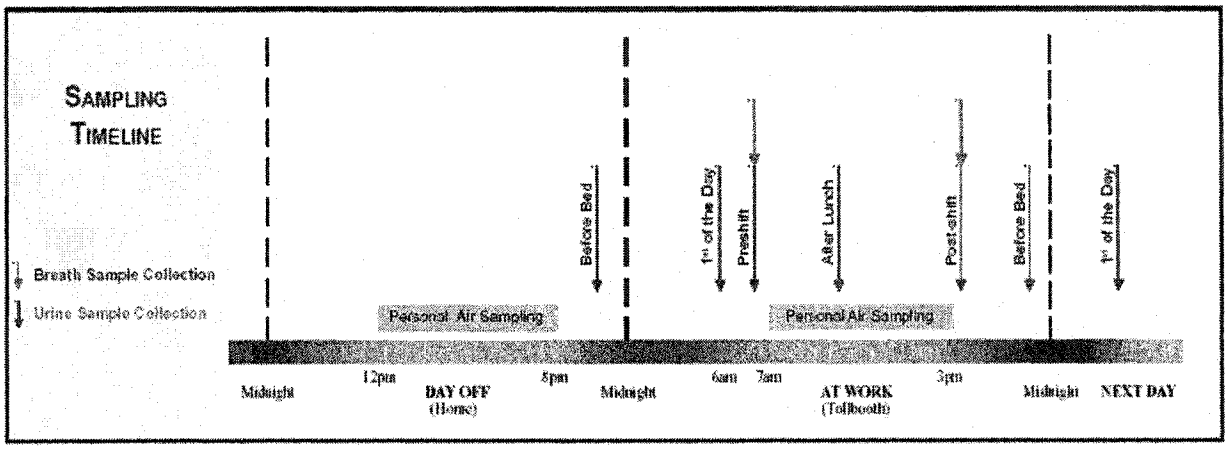


Figure 5.3: Personal exposure to 1,3-butadiene amongst the three groups

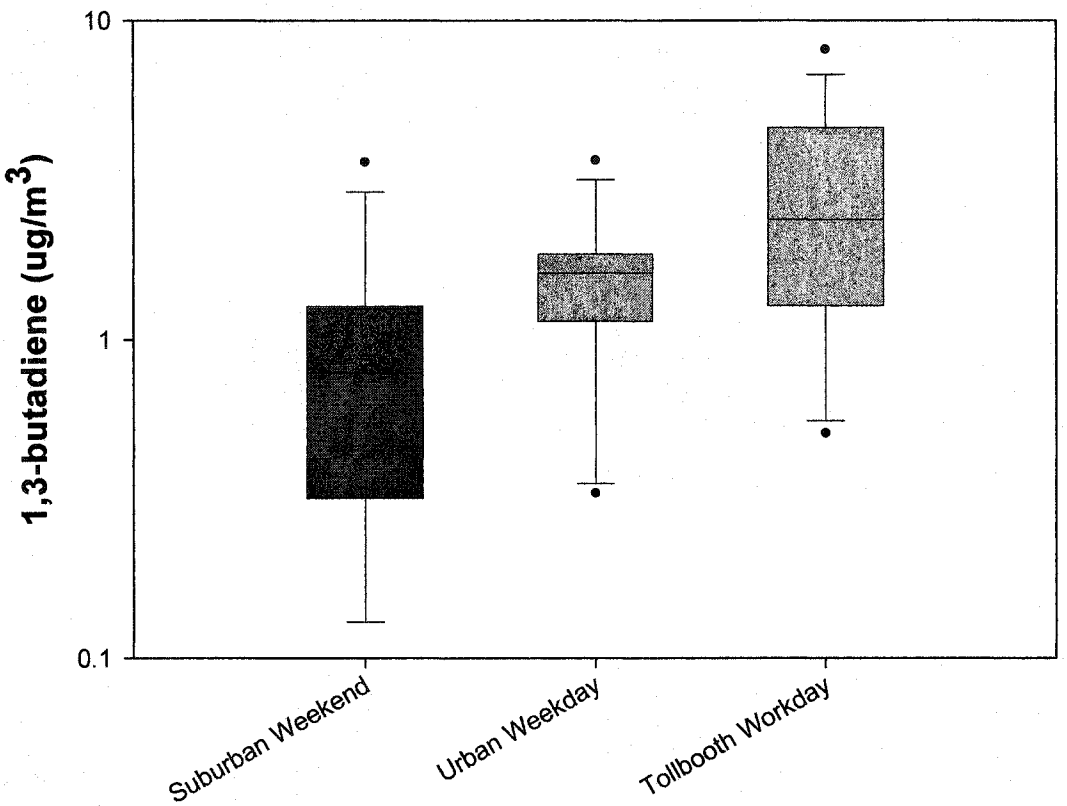


Figure 5.4: Breakdown of MHMBA and DHBMA at collision energy of 17eV and 21eV

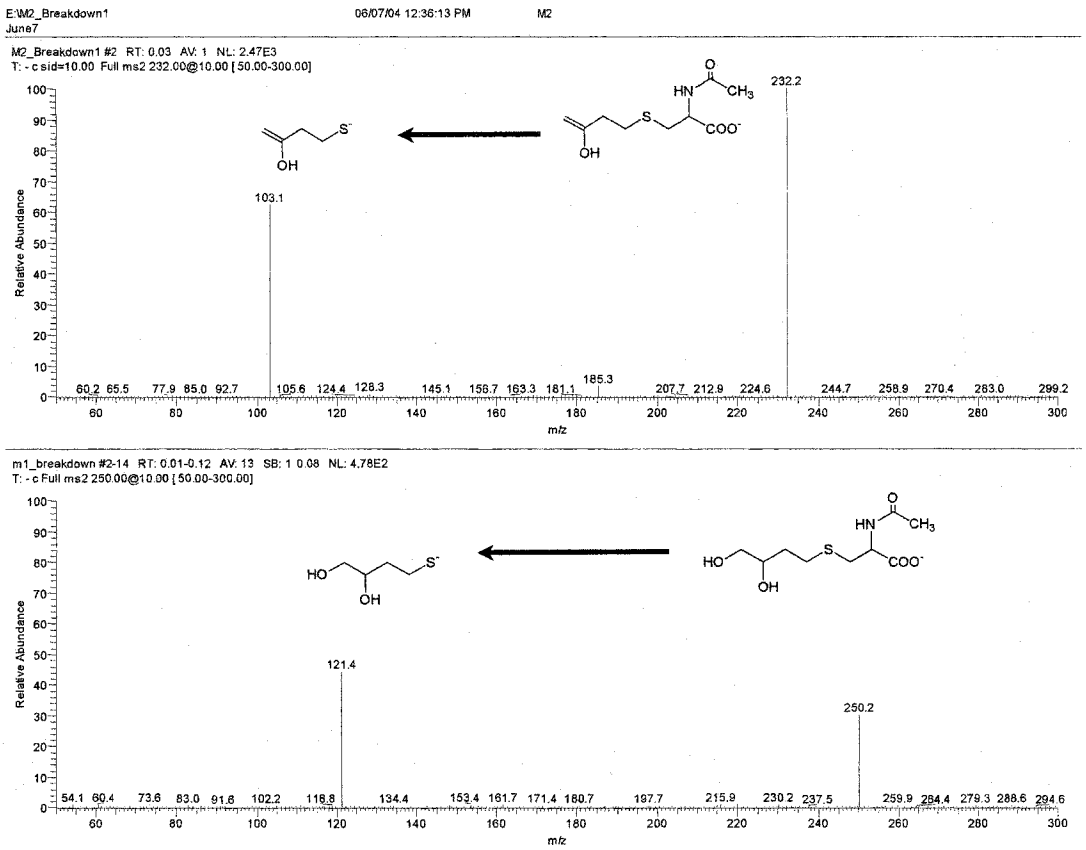


Figure 5.5: A representative chromatogram of urine samples showing both analytes and the internal standards.

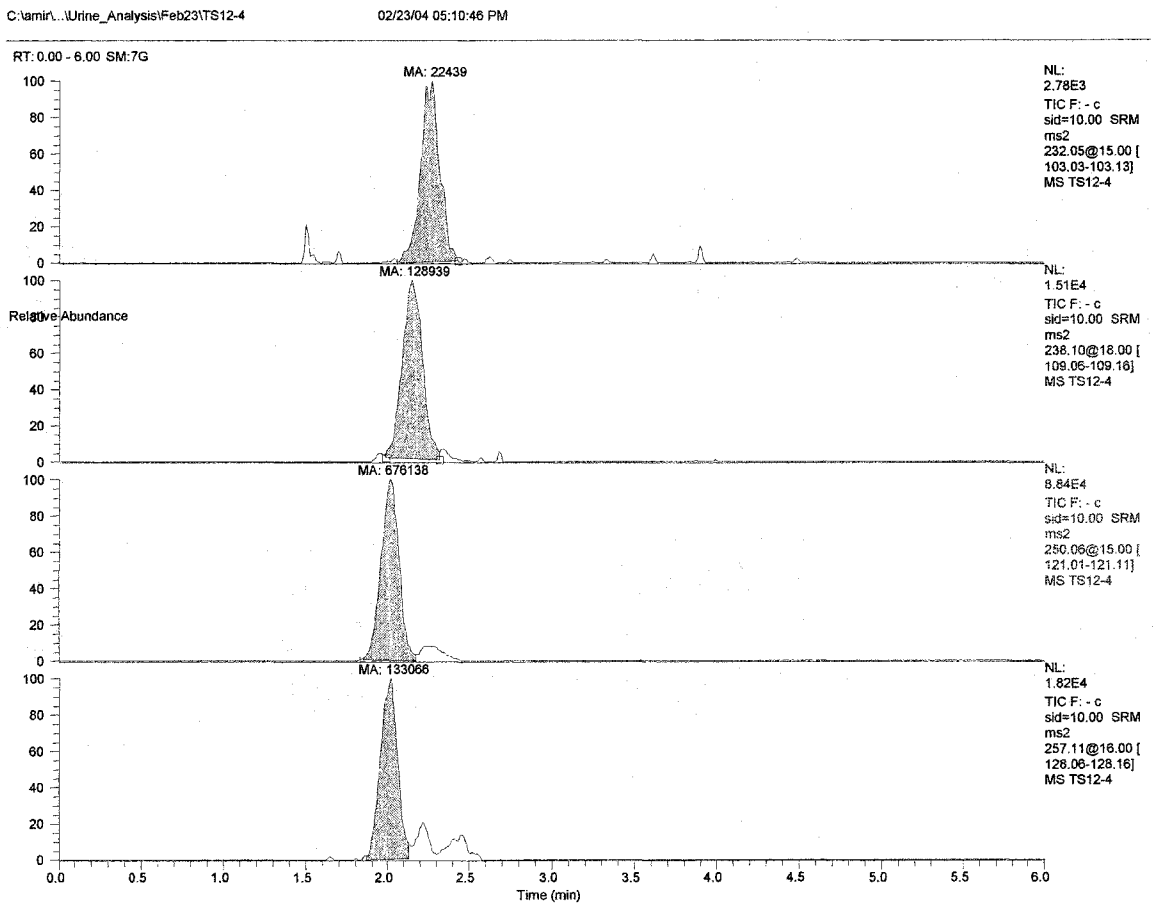
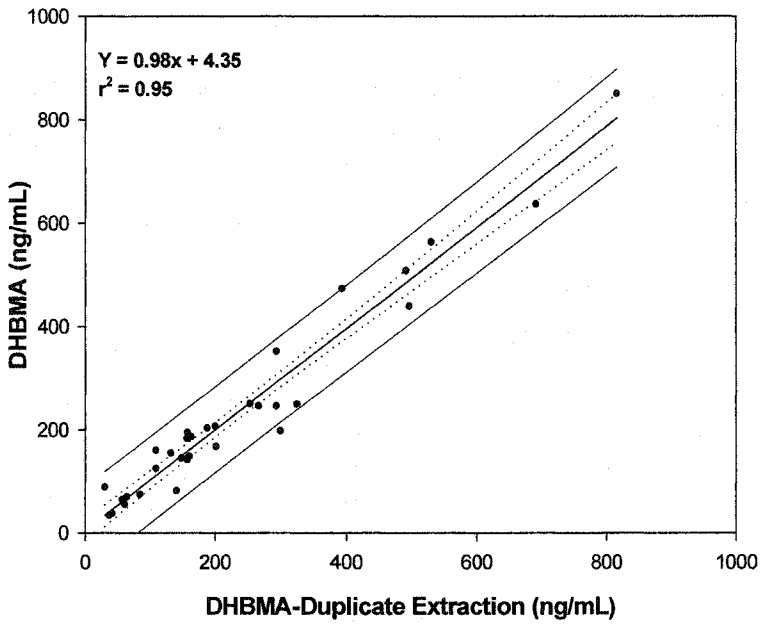


Figure 5.6 Precision based on duplicate extracts of real samples for DHBMA and MHMBA



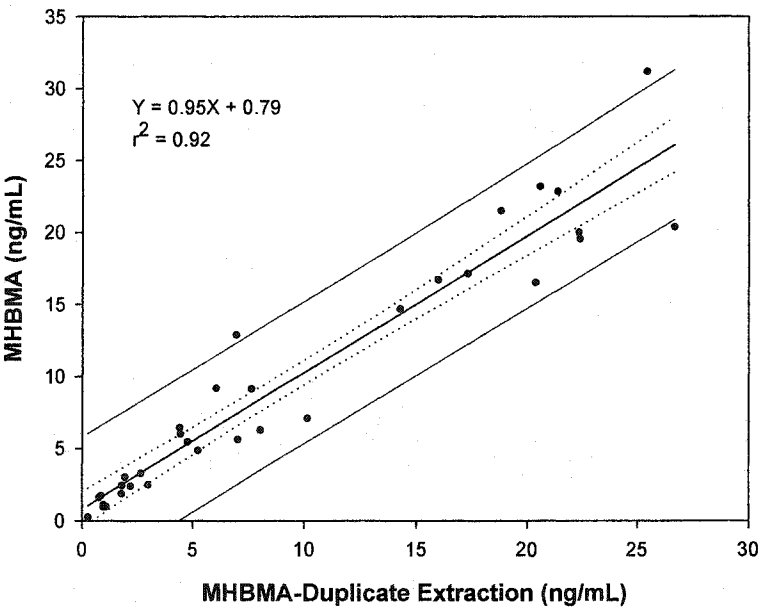


Figure 5.7 Relationship between the urinary biomarker DHBMA and MHBMA

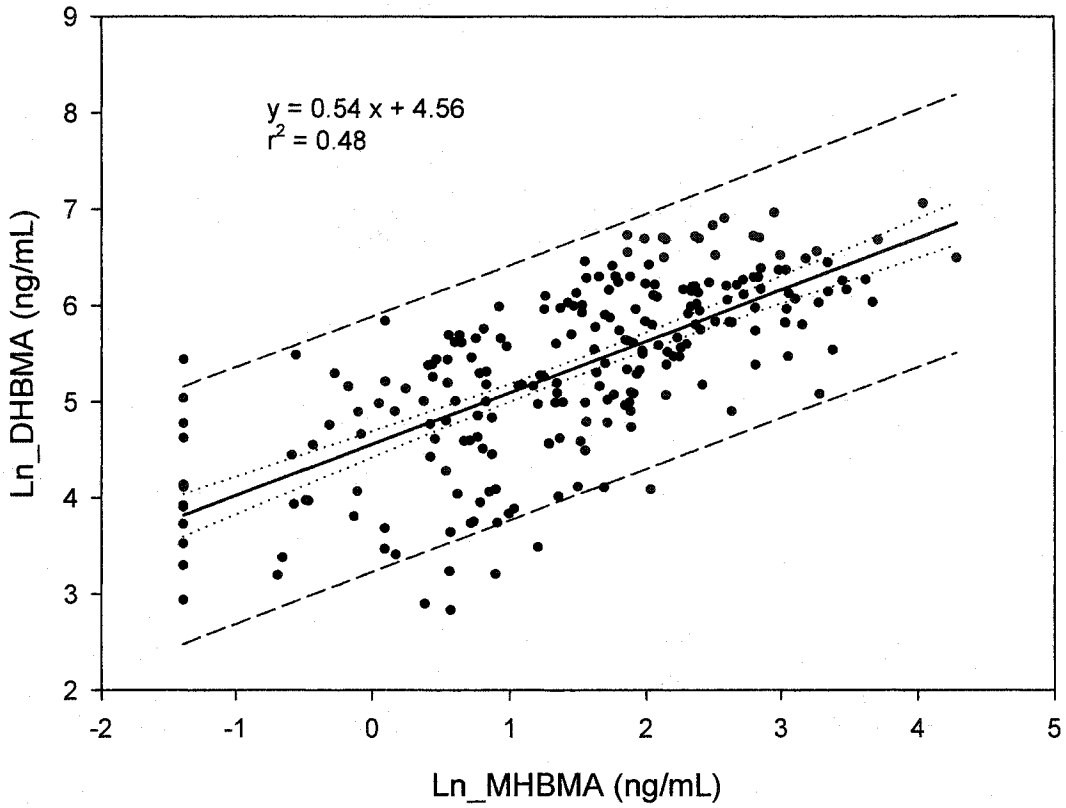


Figure 5.8: Distribution of MHBMA and DHBMA by Exposure Group

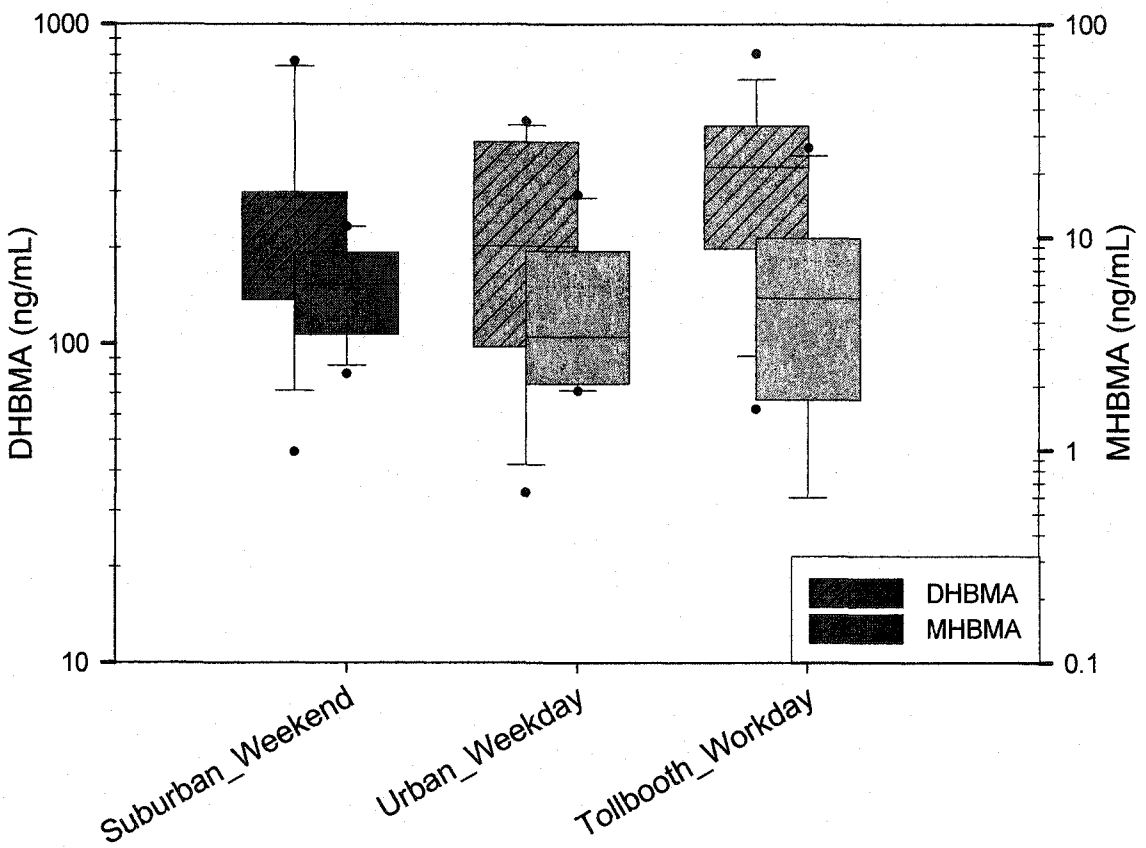
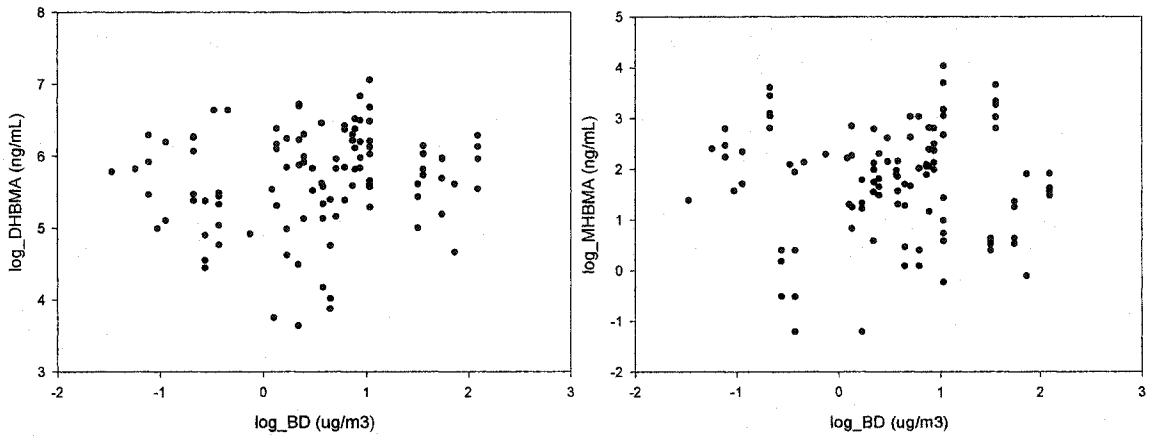


Figure 5.9: Scatter plot of urinary biomarker (A: DHBMA, B: MHBMA) vs personal exposure



Chapter 6. Synthesis: Lessons Learned and Future Directions

This chapter is dedicated to documenting the lessons learned, future directions and the limitations for each of the three main chapters that constituted individual manuscripts. Significant time and resources were spent in developing a suitable assay for the urinary biomarkers using GC/MS and LC/MS/MS, and the discussion presented herein is reflective of this. Finally, the issue of mass balance, i.e. the amount of 1,3-butadiene inhaled vs the amount of biomarker excreted during the same time interval is explored using both deterministic and probabilistic approach. This helps to shed some light onthe appropriateness of the two urinary biomarkers.

1. On the Mobile Source Emission Front

This study was unique in that it estimated the source strength (emission rate) of different vehicle types (based on the number of axles) in a real world setting. The study showed that vehicles with more than 2-axles emit far greater amounts of 1,3-butadiene, benzene and particle bound PAHs than vehicles with 2 axles. But characterization of traffic based on the number of axles is not sufficient. For example, 2-axle vehicles consist of motorcycles, sedans, SUVs, pickups and single unit trucks. Since the market share of less fuel efficient SUVs have been on the rise lately, it would be highly beneficial to quantify the emission rate for different vehicle types that fall within the 2-axle category (sedans vs SUVs vs trucks). Studies that quantify the differences in emission rates between these vehicle types will be assets in terms of educating the public and formulating policies that focus on limiting the number of less fuel-efficient vehicles on the road. Furthermore,

such studies conducted in different seasons will help shed some light on seasonal variability. Future studies should also consider multiple locations for sampling, i.e. both upwind and downwind, and utilize detail meteorological characterization. This will enable the investigators to better characterize the effect of mobile sources on ambient concentrations, adjusting for the background level and the meterological effects.

2. On the Tollbooth Work-Environment Front

This portion of the thesis highlighted the protection offered by tollbooth to the workers using concurrent indoor and outdoor measurements. Although the outdoor concentrations at the tollbooths were much higher than the outdoor concentrations at urban locations, the indoor concentrations within the tollbooths were comparable to the indoor concentrations at urban homes, highlighting the protection provided by the tollbooth. However, the control ventilation system discussed in chapter 4, responsible for this protection, is installed in only 55% of the tollbooths in Maryland. Thus, the current study does not provide an indication of potential exposures that exist within tollbooths lacking such a control ventilation system. It would be beneficial to do the same type of evaluation at tollbooths without control ventilation systems in order to see how they compare with the types of tollbooths examined in this study. Furthermore, such future studies should include multiple seasons, multiple tollbooths and also include a measure of air exchange rate between the indoor and outdoor environments.

3. On the Biomarker Front

Although the exact mechanism of butadiene-induced carcinogenesis is unclear, there is strong scientific evidence indicating that it is mediated by the three genotoxic epoxides resulting from 1,3-butadiene metabolism (Figure 1): 1,2-epoxy-3-butene (butadiene monoepoxide), 1,2-dihydroxy-3,4-epoxybutane (butadiene diol-epoxide) and 1,2,3,4-diepoxybutane (butadiene diepoxide). In light of this, the ideal biomarker for characterizing cancer risk resulting from 1,3-butadiene exposure would be to measure these genotoxic epoxides themselves within the biological sample. Recent studies have reported the excretion of butadiene diepoxides in the urine of rodents that were exposed to 1,3-butadiene using GC/MS (Henderson *et al.* 2001). The metabolite was stable for 42 days when stored at –80°C (total duration investigated). So the suitability of this biomarker for use in human populations should be investigated along with the potential use of butadiene monoepoxide and butadiene diol-epoxide. Using sensitive methods such as LC-MS/MS can help to accurately quantify low levels of epoxides in urine, that are otherwise undetectable with single quad GC/MS systems.

Previous research focusing on animal model have indicated that females are more sensitive to 1,3-butadiene exposures. However, there is a lack of data to address this question in humans. A molecular epidemiology approach investigating the genotoxic epoxides, as discussed above, will be able to answer questions related to differences in 1,3-butadiene toxicity among the two sexes. The relative amount of the epoxides excreted vs the detoxified mercapturic acids might shed light on the differences between sexes.

The lack of clear relationship between personal exposure and the urinary biomarker can be explained using the data presented in Chapter 4. As discussed in Chapter 4, the indoor concentration of butadiene within the BHT tollbooth was much lower than originally anticipated because of the control ventilation system. As a result, the workers exposure was only slightly higher than the exposure of the suburban weekend and urban weekday exposures of the individuals from university. This lack of variability in exposure (dose) led to statistically insignificant differences in biomarker levels between the workers and the controls. A larger sample size is needed to compensate for this lack in variability between exposure groups. In addition, choosing population that have higher differences in exposure levels will help to illustrate the relationship between exposure and biomarker levels. In this context, it is advisable to recruit workers from tollbooth facilities that do not have the control ventilation systems described in Chapter 4, as a high exposure group.

In addition, the levels of creatinine differed significantly between groups. Since the volume of urine excreted is directly influenced by the amount of liquid an individual consumes, it seems logical to normalize the urine concentration by creatinine level. The association between personal air measurements and biomarker levels seemed to change direction based on whether or not the values were creatinine-adjusted. A more reliable approach would be to collect all void urine volume in 24 hours and look at the total amount of biomarker excreted within 24 hour instead of volume-based concentrations or the creatinine-adjusted concentration. Alternatively, all individuals could be asked to collect all urine voids for per-determined number of hours and express the biomarker as elimination rate i.e ug/hr.

In this study, the metabolic ratio (DHBMA/DHBMA+MHBMA) was somewhat different between the sexes, but definitive conclusions regarding this difference could not be made because of the disproportionate number of females and males between the two study groups. A new study with a larger number of study participants will be necessary to answer this question.

Furthermore, this study would have benefited from having two additional groups of study population, to increase the range of exposure. The two recommended groups are: individuals living in rural areas and tollbooth workers working in tollbooths without the control ventilation system. So, the ideal study population would look like this:

Group A: Rural Residents

Group B: Urban Residents

Group C: Toll Collectors with control ventilation system at workplace

Group D: Toll Collectors without the control ventilation system

Such study will not only allow to correctly evaluate the cancer risk associated to 1,3-butadiene exposure that is attributable to mobile sources, it can also provide a unique opportunity to answer the question whether or not there is sex differences with respect to 1,3-butadiene metabolism in humans. Further, it will provide a true evaluation of the urinary biomarkers ability in predicting low-level recent 1,3-butadiene exposure. In evaluating exposure it will be helpful to measure the exposure and collect urine samples

multiple days, instead of multiple urine samples in single day. This will help to evaluate an individual's variability in BD metabolism given different doses of exposure, instead of looking at time profile of biomarker exposure related to single exposure.

Finally, the urine sample will provide a unique opportunity to evaluate the biomarkers of benzene, butadiene, PAH and MTBE, all mobile source related pollutants, in best describing the traffic exposure. The findings will be very helpful in epidemiological studies aiming to link cancer incidence and exposure to automobile exhaust.

5. On the Mass Balance of Biomarker excreted vs Butadiene inhaled

In this study, the median DHBMA level in the low exposure group (student, faculty and staff) was 178 ng/mL, whereas median MHBMA in the same population was 4.6 ng/mL. Such high levels of DHBMA in the urine of individuals in the low exposure group have been reported by other researchers as well (Fustinoni *et al.* 2004; Urban *et al.* 2003) and is of interest for further investigation. It has been hypothesized that high levels of DHBMA excreted in the urine of individuals within low exposure groups is likely due to some endogenous processes. But the exact mechanism of this process is still unknown.

In this chapter, a mass balance calculation is performed using both probabilistic and deterministic approach. In deterministic approach, the point estimates of parameters are used for calculating the amount of butadiene inhaled by nonsmoker in 24 hour period and the amount of biomarker excreted during the same duration. In probabilistic approach, Monte-Carlo Analysis is utilized which incorporates the entire distribution of the

parameters. The output from this calculation provides an entire distribution of the population, instead of point estimates.

Parameters for calculation:

- Concentration in indoor air (mean $\pm$ SD): 1 $\pm$ 1.5 ug/m3
  Concentration in outdoor air (mean $\pm$ SD): 0.7 $\pm$ 1.1 ug/m3
- Amount of time spent indoors (mean $\pm$ SD): 85 $\pm$ 13 %
- Volume of air inhaled by a healthy individual in 24 hrs: 13 $\pm$ 7m3
- Non-smoking individual, so the only source of butadiene exposure is through inhalation of contaminated air.
- Concentration of DHBMA in urine of the low exposure group (median; 95th percentile) 178; 678 ng/mL
- Concentration of MHBMA in urine of the low exposure group (median; 95th percentile) 4.6; 16.4 ng/mL
- Volume of urine excreted/24 hrs (mean $\pm$ SD): 1.2 $\pm$ 0.8 L

Mass balance based on deterministic calculation:

Amount of butadiene inhaled/24 hr =

(% time spent indoor * concentration indoor + % time spent outdoor *concentration outdoor) * volume of air inhaled/24 hour

= (0.85*1+0.15*0.7) ug/m^3 * 13 m^3

= 12.21 ug.

Amount of MHBMA excreted /24 hr =

Concentration of MHMBA * volume of urine excreted/24 hr

= 4.6 ng/mL * 1200 mL * 1ug/(1000ng)

= 5.52 ug

Amount of DHBMA excreted/24 hr = 178 ng/mL*1200 mL * 1ug/(1000 ng)

= 213.6 ug

As shown by this deterministic calculation, the excretion of MHBMA per 24 hr is within the exposure limit. But as for DHBMA, the amount of biomarker excreted per 24 hour exceeds the amount of 1,3-butadiene inhaled in the same duration by 17 fold. The same

calculation can be performed using a Monte-Carlo simulation (probabilistic approach) that will provide information that the overall distribution of population and the extreme case scenarios. For example, someone who lives on the most polluted areas, performs intense physical labor and drinks very little liquid. Does the DHBMA excreted by such individual fall within the amount of 1,3-butadiene inhaled by this individual?

Using the above parameters a Monte-Carlo simulation was applied with 1000 iterations using Crystal Ball software to obtain the amount of butadiene inhaled per 24 hours and the amount of each metabolite excreted during the same duration. The frequency distribution of the amount of 1,3-butadiene inhaled and the amount of MHBMA excreted in the same duration is similar (Figure 6.1 A& B). On the contrary, the frequency distribution of 1,3-butadiene intake (ug/24hr) and the amount of DHBMA excreted are very different, with the DHBMA range exceeding the BD intake range substantially. This is clearly presented in the cumulative plot (Figure 6.1 A&C). Here, the cumulative plot of MHBMA and BD intake are very similar with the MHBMA lagging the BD intake curve slightly, whereas the DHBMA curve far exceeds the BD intake curve (Figure 6.2).

From this calculation, it is evident that even the lower 5^{th} percentile of the amount of DHBMA excreted exceeds the upper 95^{th} percentile of the amount of 1,3-butadiene inhaled in the same duration. Further, the amount of DHBMA excreted per day exceeds the median 1,3-butadiene inhaled by a factor of 24. On the contrary, the amount of MHBMA excreted per day is less then the amount of 1,3-butadiene inhaled per day, which is more plausible because some fraction of the inhaled butadiene is absorbed, while others are metabolized through different pathways.

This calculation assumes that the only source of 1,3-butadiene exposure in non-smokers (population considered here) is through inhalation of contaminated air. To date, there is no report of 1,3-butadiene exposure through diet or other routes. Given this background, the results from this calculation clearly show that the amount of DHBMA excreted far exceeds the amount of BD inhaled, hence it is likely that a significant portion of DHBMA is the product of some endogenous processes, as has been suggested by several other researchers (Albertini *et al.* 2001; Albertini *et al.* 2003; Bechtold *et al.* 1994; Fustinoni *et al.* 2004; van Sittert *et al.* 2000).

Figure 6.1: Frequency distribution of 1,3-butadiene inhaled (ug) in 24 hours and the biomarkers excreted (ug) during the same period

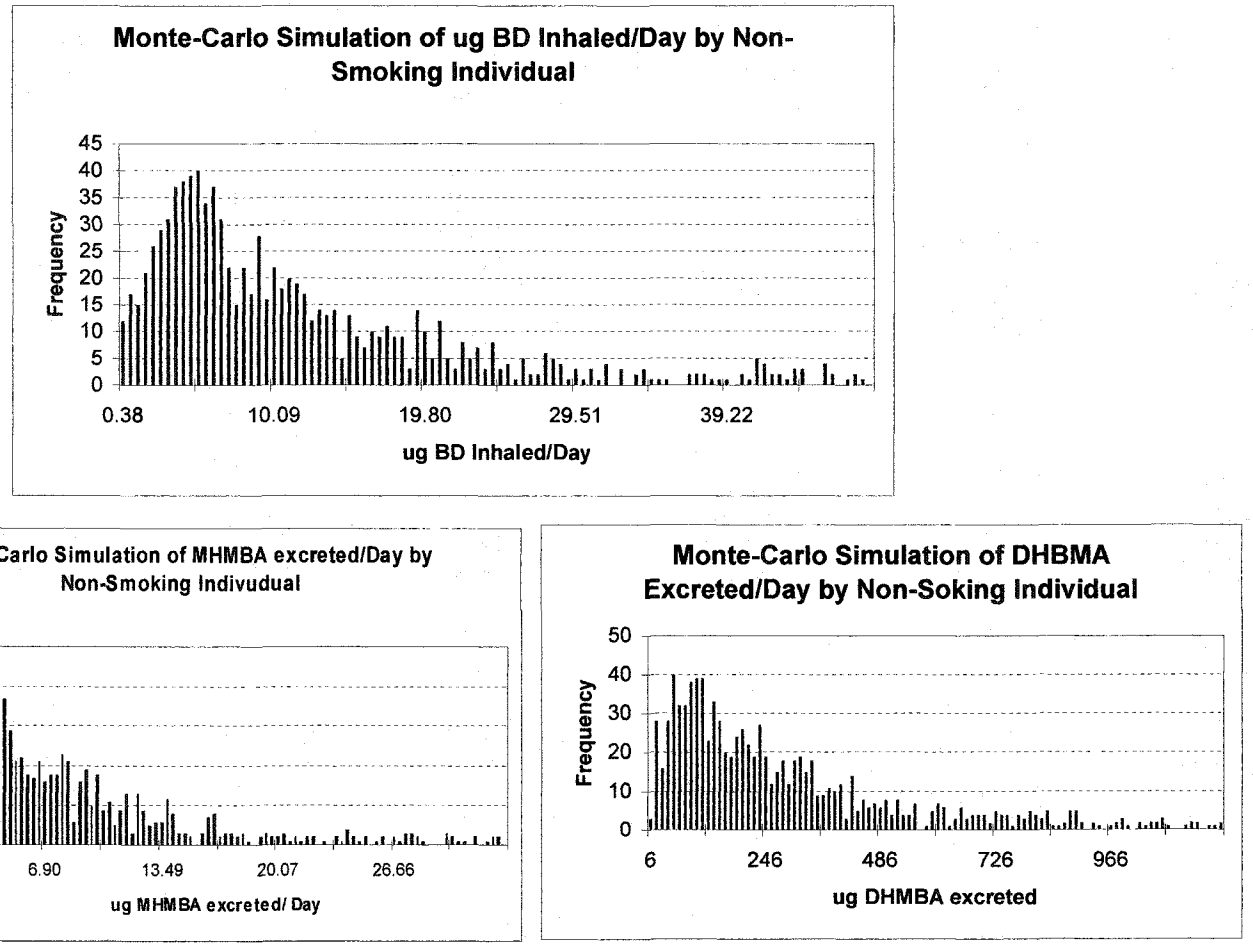
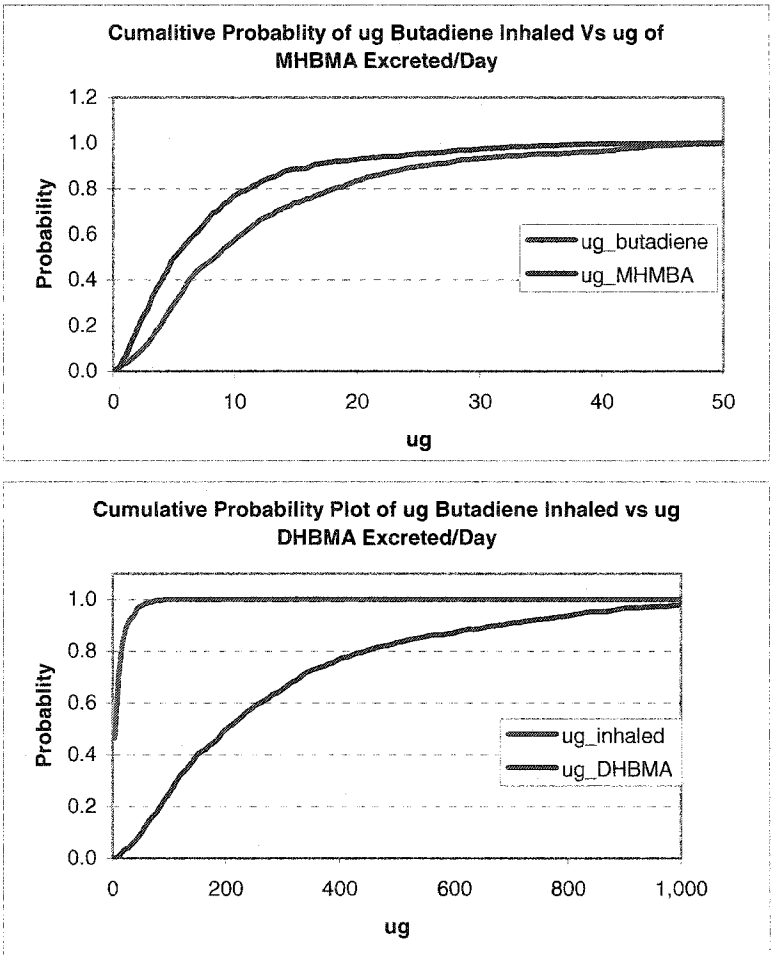


Table 6.1: Percentiles of 1,3-butadiene inhaled (ug) in 24 hours and the biomarkers excreted (ug) during the same period

Percentile	ug of Butadiene Inhaled	ug of DHBMA Excreted	ug of MHBMA Excreted
5	1.6	30.6	1.0
10	2.7	55.3	1.4
25	4.7	105.5	2.8
50	8.9	210.4	5.2
75	16.8	402.6	9.8
90	28.4	747.8	16.9
95	34.5	985.1	26.1

Figure 6.2: Cumulative probability plot of 1,3-butadiene inh aled (ug) in 24 hours and the biomarkers excreted (ug) during the same period



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APPENDIX

(The text and figures included in this section has been accepted for publication in ES&T)

Impact of the 2002 Canadian Forest Fires on PM Air Quality in Baltimore City

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Abstract

With increasing evidence of adverse health effects associated with particulate matter (PM), the exposure impact of natural sources, such as forest fires, has substantial public health relevance. In addition to the threat to nearby communities, pollutants released from forest fires can travel thousands of kilometers to heavily populated urban areas. There was a dramatic increase in forest fire activity in the Province of Quebec, Canada during July 2002. The transport of particulate matter (PM) released from these forest fires was examined using a combination of MODIS satellite image, back trajectories using hybrid single particle Lagrangian integrated trajectory (HYSPLIT), and local light detection and ranging (LIDAR) measurements. Time and size-resolved PM was evaluated at three ambient and four indoor measurement sites using a combination of direct reading instruments (laser, time-of-flight aerosol spectrometer, nephelometer, and an oscillating microbalance). The transport and monitoring results consistently identified a forest fire related PM episode in Baltimore that occurred the first weekend of July 2002 and resulted in a 30-fold increase in ambient fine PM. Based on tapered element oscillating microbalance (TEOM) measurements, the 24-h $PM_{2.5}$ concentration reached 86 $\mu g/m^3$ on July 7th, 2002, exceeding the 24-h national ambient air quality standards (NAAQS). The episode was primarily comprised of particles less than 2.5 μm in aerodynamic diameter, highlighting the preferential transport of the fraction of PM that is of greatest health concern. Penetration of the ambient episode indoor was efficient (median indoor-to-outdoor ratio 0.91) such that the high ambient levels were similarly experienced indoor. These results are significant in demonstrating the impact of a natural source thousands of kilometers away on ambient levels of and potential exposures to air

pollution within an urban center. This research highlights the significance of trans-boundary air pollution and the need for studies that assess the public health impacts associated with such sources and transport processes.

Introduction

Forest fires are a significant source of air pollution. Every year, fires consume millions of hectares of forest in North America generating vast air pollution emissions that pose significant potential for wide-scale human exposure. Biomass emissions from large-scale forest fires include particulate matter (PM), carbon monoxide (CO), polycyclic aromatic hydrocarbons (PAHs), aldehydes, and semi-volatile and volatile organic compounds (VOCs) (1). Plumes of hot gases containing airborne pollutants ascend from forest fires into the mixed layer. These plumes can then detrain from the mixed layer and be advected over long distances in the free troposphere by prevailing winds (2,3). Transport of air pollutants in the free troposphere is more efficient than in the mixed layer because of fewer loss mechanisms and stronger winds (4). Previous research has shown that this long-range transport can affect pollutant concentrations thousands of kilometers from the source (5-10). Wotawa and Trainer (11) reported that extensive forest fires in Canada during 1995 influenced concentrations of CO and ozone (O_3) in the southeastern United States. Building on these findings, McKeen and colleagues(12) showed that transport of nitrogen oxide (NOx) led to elevated O_3 levels over eastern U.S. urban centers. In addition to gaseous pollutants, several investigators (13-15) have reported high concentrations of PM in different areas of Southeast Asia during the Indonesian forest fires of 1997. Though these studies have shown biomass burning to be a major source of

ambient PM, to date, there are no published findings that have evaluated the impact on indoor environment in population centers that are located thousands of kilometers away.

Historic events characterized by unusually high levels of air pollution provide evidence that increased concentrations of airborne pollutants adversely impact human health (16-18). In considering the health impact of air pollutants such as PM, penetration of particles of outdoor origin into the indoor environment is a key consideration because the indoor environment is the dominant venue for exposures to air pollutants due to the large fraction of time spent indoor (19). Penetration is determined by a combination of particle type, size, building, and ventilation characteristics (20).

The current study examines the impact of a significant source event (Canadian forest fires) on the PM levels in an urban center (Baltimore) located thousands of kilometers from the source. The PM exposure continuum is examined including the source, fate and transport, ambient concentrations, size distribution of particles comprising the episode and the indoor concentrations of PM within homes of susceptible individuals, i.e., asthmatic children. This examination illustrates the global significance of biogenic air pollution sources and the meteorological conditions that determine transport and impact on ambient and indoor environments.

Methods

The impact of the July 2002 Canadian forest fires on PM air quality in Baltimore was assessed across the exposure continuum using an array of methods. Characterization of the source term was based on data available from the Canadian Ministry of Forestry including reports tracking the number of hectares of forest burned per month. The transport of the smoke plume from the forest fires to Baltimore was illustrated using satellite and Hybrid Single Particle Lagrangian Integrated Trajectory (HYSPLIT) modeling. The existence of the smoke plume above Baltimore before the peak of the episode and its' re-entrainment into the mixed layer during the episode was determined using Light Detection and Ranging (LIDAR). The effect of this long range-transport on the ambient PM air quality in Baltimore was determined using monitors at three locations within the city. Finally, the overall impact of the episode on the indoor environment was assessed by measurement of PM within children's bedroom.

The fate and transport of the forest fire plume from Quebec to Baltimore was assessed using back-trajectory calculations from HYSPLIT. This model employs archived Eta Data Assimilation System (EDAS) weather model outputs to trace the origins of an air parcel at a specific latitude, longitude and height over the continental United States. This model is well suited and validated for assessing long range air pollution transport (21-24). Sets of back-trajectories with endpoints over Baltimore (39.29° N, 76.55° W) were computed every 6 hours with the end heights of 100, 500 and 1000 meters above ground level (AGL). Each trajectory was computed in 1-hour increments for a 72-hour period. These data were then imported into the software package MATLAB (The MathWorks,

Inc., Natick, Massachusetts) and plotted over the U.S. cartographic boundary files from the 2000 Census. The plume's fate and transport was further assessed qualitatively through publicly available satellite images acquired with Moderate Resolution Imaging Spectroradiometer (MODIS).

The time-resolved vertical aerosol profile was measured at site A1 (Figure 1) using the Johns Hopkins University Miniature Elastic Backscatter LIDAR (JHU-MEBL) as described by Pahlow et al (3). The LIDAR instrument was operated in vertical upward pointing mode on a daily basis during the EPA Baltimore Supersite summer intensive sampling period (see www.jhu.edu/~dogee/mbp/supersite2001/ for details on the measurements and data visualization). A laser emits light pulses at a wavelength of 1.06 micrometers parallel to the axis of a 26 cm, f/10, Cassegrain telescope. In the lower troposphere, laser light is scattered mainly by aerosols, whose size is on the order of the wavelength of the laser light. During an ideal backscatter event, the laser light is reflected 180 degrees downward and is collected by the telescope. Reflected light passes through an interference filter and a lens that focuses the energy on a 3 mm diameter, IR-enhanced silicon avalanche photodiode. Both the travel time of the light emitted from the laser to its collection and the intensity of the backscatter radiation are digitized. In this way a vertical profile of relative aerosol backscatter is obtained at a vertical resolution of 3.6 meters, a range of 6 kilometers, and a time step of 5 seconds. The strong vertical mixing in the daytime mixed layer (ML) and the weak mixing above the temperature inversion on top of the ML lead to an accumulation of aerosol, heat and moisture within the ML and a strong drop in these quantities in the free troposphere. Due to this vertical

gradient in aerosol backscatter across the ML top, the ML height can be determined with LIDAR.

[INSERT FIGURE 1]

Time and size-resolved outdoor PM was measured at two different locations (see Figure 1) using two different methods. In central Baltimore (site A2), 5-minute measurements were made with a laser particle counter (Climet CI-500, Climet Instrument Company, Redlands, CA). The CI-500 was connected to an isokinetic port of a stainless steel sampling manifold. The manifold and Climet were located on the second floor of a three-story row home. The CI-500 registers counts based on light scattered by particles passing through a laser beam. The amount of light scatter is directly proportional to the size of the particle, which is then converted to an electrical pulse by a photo-detector. The amplitude of the pulse is compared to a reference voltage and the particle generating the pulse is assigned to pre-set size bins of 0.3-0.5, 0.5-1.0, 1.0-2.5, 2.5-5, 5-10 μm respectively. The instrument was operated at 2.8 L/min and was factory calibrated within one year of use. The second monitoring site was the Baltimore Supersite (site A1) located approximately 4 miles to the east of the first location on the southeastern edge of the city. Measurements at this location were made using both an Aerodynamic Particle Sizer™ (APS, TSI, Inc., Shoreview, MN) and Tapered Element Oscillating Microbalance™ (TEOM, Rupprecht & Patashnick Co., Inc., Albany, NY). The APS was programmed to provide integrated particle counts every 5 minutes over 52 size bins

between 0.452 and 20 μm. The TEOM was equipped with a 2.5 μm sampling inlet and was operated at 30^0C providing mass concentration results in 30 minute interval.

PM concentrations were measured in the bedrooms of four children participating in a study of childhood asthma in the urban environment and on the rooftop of a three-story row home (site A3) centrally located in the neighborhood where the children lived. Within each home, PM was measured over 72-hours using a portable direct reading nephelometer (MIE pDR1000s, ThermoElectron, Franklin, MA). The four study participants were 6 to 12 years of age and lived in East Baltimore. Three of the four children were asthmatic. Indoor source activity (e.g. smoking, cooking, vacuuming) and ventilation (windows, AC) during the monitoring period was obtained from a daily questionnaire completed by an adult respondent within each home. Details of this study and baseline exposure levels of the entire cohort are described elsewhere (26).

The nephelometer uses a pulsed emitting diode producing near-infrared light at 880 nm that is scattered by particles entering the sensing cell. The intensity of light scattered by the passing particles is proportional to particle concentration. The nephelometer's inlet is designed to passively sample particles ≤ 10 μm. The manufacturer specifies a measurement range from 0.1 to 10 μm, however, it has been shown that particles in the range of 0.3 to 2 μm are preferentially detected (27,28). The nephelometers were factory calibrated using SAE Fine (ISO Fine) test dust characterized as having a mass median diameter of 2 to 3 μm, a bulk density of 2.6 to 2.65 g/cm^3, and a refractive index of 1.54 (Powder Technology, Inc, Schofield WI). Before being deployed to the field,

nephelometers were zeroed against clean air obtained using a HEPA filter according to the manufacturer's operation manual.

Meteorological variables were measured at site A1 using a variety of instruments with sensors positioned on an 11 meter tower. In particular, incoming solar radiation was measured with an Eppley Pyranometer at 11 meters, temperature and relative humidity were logged from a Vaisala HMP45AC probe at 4.9 meter, rain was collected by a Campbell Scientific TE525MM tipping bucket rain gauge, and wind speed and turbulence were measured by a Campbell Scientific three component anemometer-thermometer CSAT3 at 9 meters.

Results

The impact of the Canadian forest fires on Baltimore PM concentrations was assessed by examining the source activity, fate and transport of the plume, resulting ambient PM concentrations, and ultimately, its infiltration into the homes and bedrooms of asthmatic children. The source activity was evaluated based on the Canadian Forest Service estimate of hectares forest combusted in the Canadian province of Quebec during the period of interest. For the forest fire season of 2002, there was more than a 100-fold increase in hectares of forest burned during the month of July compared to other months (Figure 2). Specifically, during the first week of July, there were 85 active wildfires reported in the province of Quebec (29).

[Insert Figure 2]

The transport of the plume across the Eastern seaboard was evaluated both qualitatively and quantitatively. A qualitative evaluation was provided by a satellite image (MODIS) taken on July 7th at 10:35 EDT showing a coherent smoke plume extending from the forest fires in Quebec to the U.S. Northeastern seaboard (Figure 3A).

[Insert Figure 3A and B]

A quantitative assessment of the plume transport was provided using HYSPLIT. Multiple trajectories were run representing a range of heights (100, 500, and 1000 meters) and times (every six hours), however, results were consistent in showing air

parcels contributing to the pollution episode originated in the Quebec region, whereas those before and after the episode originated elsewhere. For clarity of illustration, a single plot per day, capturing the conditions before, during and after the episode is given in Figure 3B. The trajectories shown are for 13:00 EDT and represent a height of 500 m AGL. The selection of the 500 m height is justified because the model is less accurate at lower altitudes due to incomplete resolution of near surface frictional and turbulent effects. In addition, air pollution transport is better represented at heights above the inversion layer, especially during the summer when strong nocturnal inversions occur (25). The selection of 13:00 EDT as an end time for the trajectories is justified because atmospheric mixing is well established. Back trajectories are shown for the five days including and surrounding the PM episode. The back-trajectories clearly show that the air mass arriving in Baltimore on July 6^{th}, 7^{th}, and 8^{th} originated from the area of Quebec where the forest fires were burning. In contrast, on July 5^{th} and 9^{th}, the arriving air mass originated from Western Canada and the U.S. Midwest, respectively.

The local plume was observed aloft using LIDAR measurements. LIDAR provides relative aerosol concentration as a function of time and height z above ground level (Figure 4). Shortly after 9:00 EDT on July 7th, the forest fire smoke plume was situated at a height between 1400 and 1800 meters. The LIDAR verifies that the smoke cloud shown by MODIS images (recorded at 10:35 EDT) existed at heights between 1100 and 1800 meters above ground level before entrainment into the mixed layer. The daytime turbulent mixed layer can be identified due to the homogeneous vertical mixing within it. The mixed layer height increased from 600 meters at 10:00 EDT to approximately 1000

meters at 11:00 EDT that day. Until that time, the aerosol concentration in the mixed layer was lower than in the smoke layer aloft. Shortly after 11:00 EDT, the mixed layer reached the height of the smoke plume. Large eddies infringed the lower interface of the smoke plume and started to mix the smoke-laden air with surface air beginning at 11:30 EDT. This process increased in intensity during the afternoon. Thus, heavily polluted forest fire smoke entered the mixed layer from aloft resulting in elevated PM levels at ground level. After 18:00 EDT the mixing became less intense. The ML height slowly diminished to around 650 meters and the forest fire plume moved upward to >1000 meters at 20:30 EDT. Pahlow et al. (2004) provides further details on the mixed layer dynamics during this event.

[Insert Figure 4]

The entrainment of the smoke plume into the mixed layer evident from the LIDAR measurements coincided with a dramatic increase in ground level PM concentrations at both ambient monitoring sites (sites A1 and A2), clearly defining the forest fire pollution episode. Figure 5 shows the increase in PM concentration (at site A1) across various particle size ranges during the episode compared to background. The background was estimated by the 4-week average (2 weeks before and after the episode) PM concentration matched by day and time. Consistent with LIDAR observations previously described, the peak of the forest fire episode in Baltimore was observed on July 7th between 11:00 and 18:00 EDT. Much of the increase in PM concentration was

attributable to particles less than 2.5um in size. During the peak of the episode, there was as much as 30-fold increase in PM concentration in the 0.8-0.9 μm size range.

[Insert Figure 5]

A similar episode-related particle concentration profile was observed at site A2 based on laser particle count measurements. Figure 6 presents the outdoor particle count concentration profiles for July 6th and 7th relative to the average concentrations for all other weekends in June and July 2002. This figure shows a marked increase in the concentration of smaller particles during the peak of the episode with little or no change in PM concentration for particles ≥2.5 μm, similar to that observed at site A1 measured with the APS. The beginning of the forest fire episode is clearly identified by the elevated levels of PM concentration over background starting at 16:00 EDT on July 6th, reaching maximum levels on July 7th between 11:00 and 18:00 EDT.

[Insert Figure 6]

An episode-related increase in ambient PM (site A3) was observed within the bedrooms of children (n=4, sites I1-I4) participating in a study of asthma and air quality (Figure 7, panel A). Consistent with the ambient measurements, the peak in PM concentrations inside the homes occurred between 11:00 and 18:00 EDT on July 7, 2002. The indoor measurements show a high level of correspondence to the associated outdoor central site, A3 where PM was measured using the same method. The median indoor-to-outdoor ratio during the episode was 0.91 (quartiles of 0.74 and 1.05), indicating efficient

particle penetration. The daily questionnaire revealed some smoking activity in two homes during the measurement period (I-1 and I-4) and incense burning in a third home (I-3). Further, all homes reported at least two windows open during July 7th. No health data was collected during the period of monitoring. The $PM_{2.5}$ concentration profile at site A1 measured with a TEOM (Figure 8 panel B) agrees closely with the bedroom and rooftop measurements, but differs in absolute value. The maximum PM concentrations measured at sites A1 ($PM_{2.5}$ TEOM), A3 (PM_{10} MIE), and I1 through 1-4 (PM_{10} MIE) were 199, 645, and 590 $\mu g/m^3$, respectively. Based on the TEOM measurements, the 24-h integrated $PM_{2.5}$ concentration on July 7th was 86 $\mu g/m^3$, exceeding the 24-hour average National Ambient Air Quality Standard (65 $\mu g/m^3$). The 24-h $PM_{2.5}$ concentration remained elevated on July 8th and 9th (56 and 44 $\mu g/m^3$ respectively), before decreasing to 25 $\mu g/m^3$ on July 10th.

[Insert Figure 7]

Meteorology during the episode was characterized by a long period without precipitation (June 28th – July 9th) and strong northerly winds. Local wind directions oscillated around a northern flow before the episode with average wind speeds of 4 m/s noted for July 5th and 6th. A short period of weak winds (approximately 1 m/s during the night of July 6th) coincided with the buildup of PM. Then, wind speeds recovered to previous levels (approximately 3 to 4 m/s) during the daytime hours of July 7th, the day with the strongest impact from the forest fire smoke plume. A strong decrease in solar radiation (a decline of 35% compared to measures from July 5th) could be attributed to the local arrival of the smoke cloud from the fires. Temperatures during the episode were

lower than the thirty-day average. Relative humidity in Baltimore was low during the period from July 5th to July 7th ranging from 30% to 60%. The arrival of a cold front on July 9th brought rains that totaled 2.5 mm from 19:00 EDT to 02:00 EDT on July 10th when $PM_{2.5}$ levels decreased to 25 $\mu g/m^3$.

Discussion:

Using a combination of satellite images, back trajectory models, and LIDAR data, we have linked a PM episode observed in Baltimore to forest fires burning in Quebec, Canada. Although the episode was of limited duration, it dramatically increased local PM concentrations. Based on TEOM measurements, the 24-h $PM_{2.5}$ concentration reached 86 $\mu g/m^3$ during the episode, exceeding the 24-h NAAQS. The peak of the episode occurred at 13:00 EDT on July 7th, 2002, when ambient $PM_{2.5}$ reached a maximum concentration of 199 $\mu g/m^3$ representing an eight-fold increase over background levels. The high concentrations of PM observed in this study are consistent with elevated levels of gaseous mercury and carbon monoxide (CO) produced by the same fires (30) and CO and ozone produced by earlier fires (11). In addition to the Canadian fires, PM generated by forest fires in Central America have been reported to reach the southeastern United States (31). Other researchers have described PM episodes in Southeast Asia resulting from Indonesian forest fires that occurred in 1997. During the peak of these events, PM_{10} concentration in excess of 400 $\mu g/m^3$ was observed in Kuala Lumpur, Malaysia (14), Singapore (13) and Darussalam, Brunei (15). The 1997 Indonesian forest fire destroyed as much as 5.2 million hectares of forest (32), whereas the total amount of forest destroyed in Quebec during the 2002 fire season was

approximately one million hectares (33). http://www.nrcan-rncan.gc.ca/cfs-scf/science/prodserv/firereport/archives/canada_report_2002.pdf. The 2002 Quebec forest fires were the worst in the region during the past ten years and exceeded the mean area burned for the previous nine years (267,212 hectares) by nearly four-fold.

Our results illustrate the local effects on ambient and indoor levels of PM due to long-range transport of PM from Canadian forest fires to the densely populated eastern United States. Much of the increase in PM concentration during this episode was primarily observed in the fine fraction (particles less than 2.5 μm). This is consistent with the results of Gillies et al. (34) who reported transport of particles less than 5 μm in size arriving from a source 500 kilometers away. It has been established that larger particles (those greater than 10 μm) tend to settle closer to their source due to gravitational settling while ultra-fine particles (those less than 100 nm) tend to coagulate, leading to loss of particles in that size fraction (35,36). The fine fraction PM is preferentially transported over long distances because these particles are too small to settle by gravity and too large to coagulate (37). Such transport has particular public health relevance because it is this same fraction that more readily penetrates indoor where people spend most of their time. Furthermore, particles in the fine fraction are capable of penetrating deeper into the lungs and have been associated with increased mortality and morbidity (37-40).

Concurrent MIE measurements made at the rooftop (site A3) and in children's bedroom (sites I-1 through I-4) showed comparable concentrations. The median indoor / outdoor ratio of 0.91 measured across all four homes is comparable to Abt et al (41) who reported

penetration efficiencies as high as 0.94 for particles less than 0.5 μm and 0.53 for particles 0.7-10 μm size, while others (42,43) have reported a PM_{10} penetration factor close to unity. In addition to the fine particle size, opening of windows during the episode also potentially enhanced the particle penetration indoor. This observation agrees with the findings by Riely et al (20) noting that particle penetration indoor is dictated by factors such as particle size, building types and ventilation characteristics.

Although the outdoor $PM_{2.5}$ concentrations measured using the TEOM at site A1 closely tracked the PM_{10} measurements made using MIE inside the bedrooms (I-1 through I-4) and rooftop (site A3), the absolute values differed. Based on the TEOM measurements at site A1 and the MIE measurements at site A3 (both outdoors), we observed a median TEOM to MIE ratio of 0.62. This compares with Wallace et al. who reported a ratio of 0.66 (44). Using the observed ratio, it is estimated that the maximum PM2.5 concentration inside the homes of children reached 366 ug/m^3 during the peak of the episode.

The public health significance of the episode is further established by documenting its occurrence within bedrooms of inner-city children with asthma. The near 1:1 indoor to outdoor concentration ratio during the episode indicates a lack of protection within the indoor environment. Thus, health advisories recommending that susceptible individuals stay indoor would have been relatively ineffective in reducing exposure. These findings point to a need for a more comprehensive health advisory to alleviate exposures during pollution episodes resulting from long-range transport. In addition to staying indoor,

factors such as closing windows, running air conditioners in recirculation mode, using air purifiers, and encouraging use of adequately ventilated community centers needs to be appropriately evaluated for potential to reduce exposures during such episodes.

The phenomenon of long-range transport of pollutants is of significant concern both for regulatory compliance and public health. Using a suite of time-resolved PM measurements made throughout Baltimore, we show uniform evidence of increased air pollution resulting from the long-range transport of smoke plumes released from Canadian forest fires. The significance of such long-range transport has recently been highlighted by Holloway et al. (4) who recommended a hemispheric-scale treaty to manage the intercontinental transport of pollution. Although the source event discussed in this paper is natural, emissions from man-made sources are likely to get transported in a similar manner. Furthermore, the current study highlights the need and provides the exposure assessment underpinnings for the epidemiological study of short term high exposure events.

Acknowledgements

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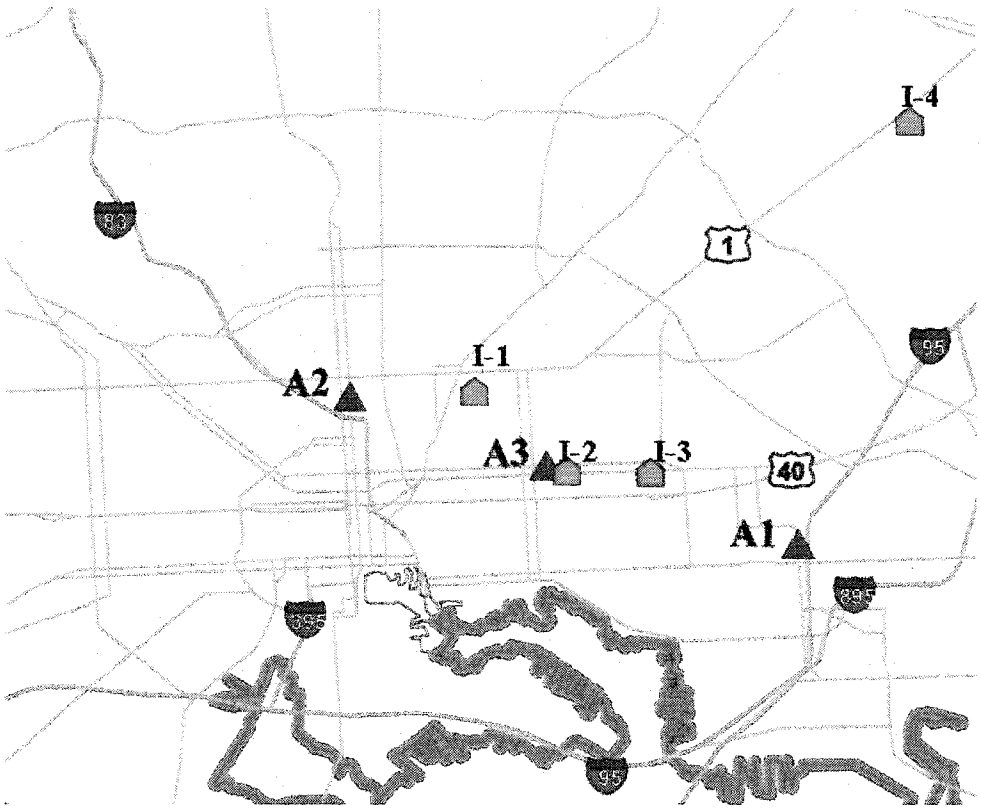
Figure 1: Baltimore City map showing locations of ambient and residential indoor monitoring sites.



A1. Baltimore Supersite Ambient monitoring station. Measurements include the Aerodynamic Particle Sizer™, the Tapered Element Oscillating Microbalance™, Lidar, and meteorological monitoring.

A2. The Baltimore Traffic Study monitoring station. Indoor and outdoor particle counts are made using a switching manifold and a Climet-CI500 laser particle counter.

A3. Ambient monitoring station. PM concentration measured with a MIE DataRam™.

I-1-4. Indoor measurements made within homes (child's bedroom) participating in a study of Childhood Asthma in the Urban Environment. Measurements were made using a MIE DataRam™.

Figure 2: Hectares of forest burned in Quebec during 2002 fire season.

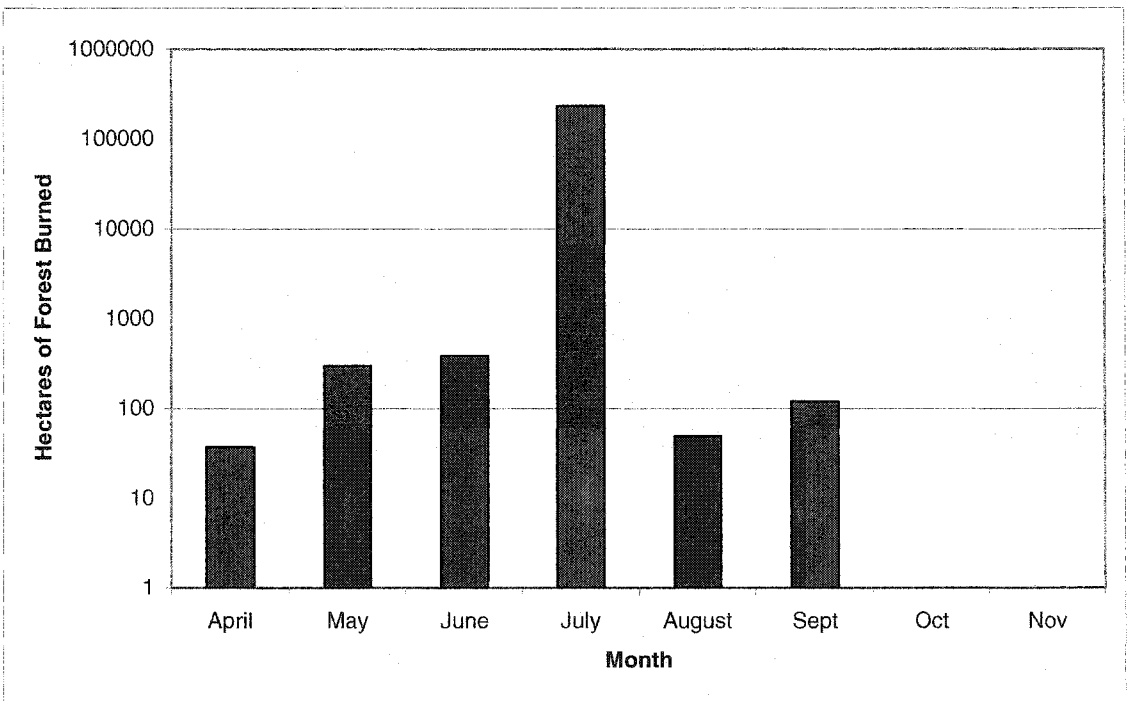


Figure 3: A) MODIS satellite image taken on July 7, 2002, 10:35 EDT. The red circles mark areas of high forest fire activity. MODIS satellite image courtesy of Land Rapid Response Team, NASA/GSFC, Greenbelt, MD.

B) Back trajectories of air parcels ending at Baltimore, MD from the HYSPLIT model. The trajectory end times are shown in the legend and the end height is 500m AGL. Each segment of the trajectory lines indicate a 12-h interval.

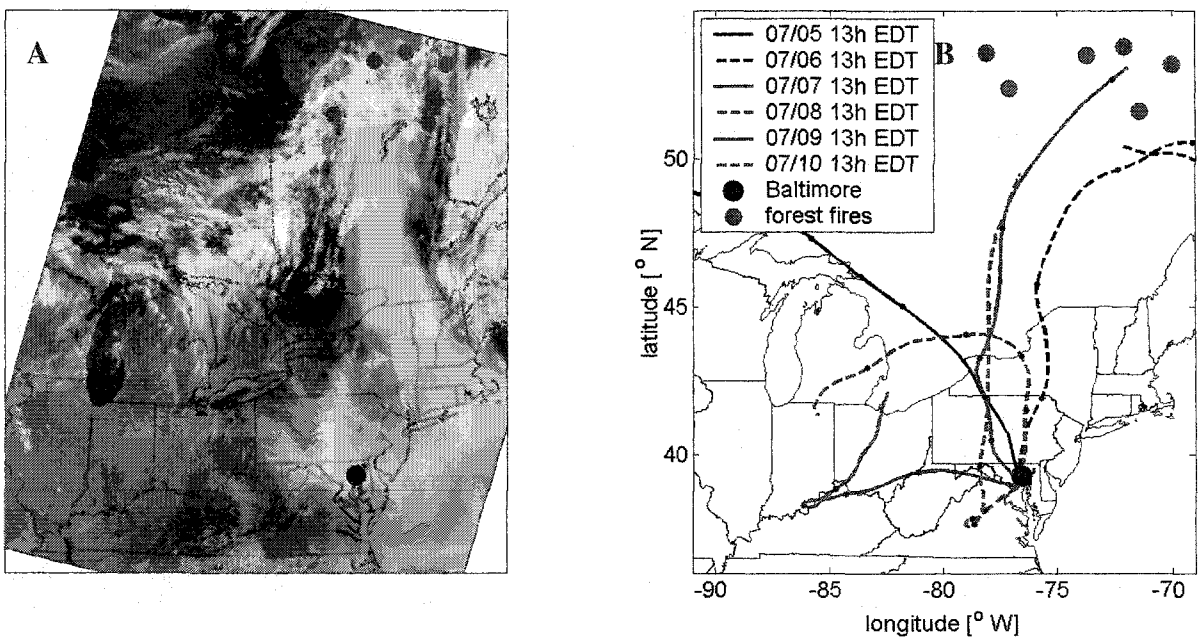


Figure 4: Time profile of range-corrected relative aerosol backscatter (Pr^2) in arbitrary units from ground-based lidar measurements over Baltimore on 7/7/2002 measured at site A1. Clean air scatters less light and is shown in blue, whereas the strongly scattering smoke-laden air is shown in red.

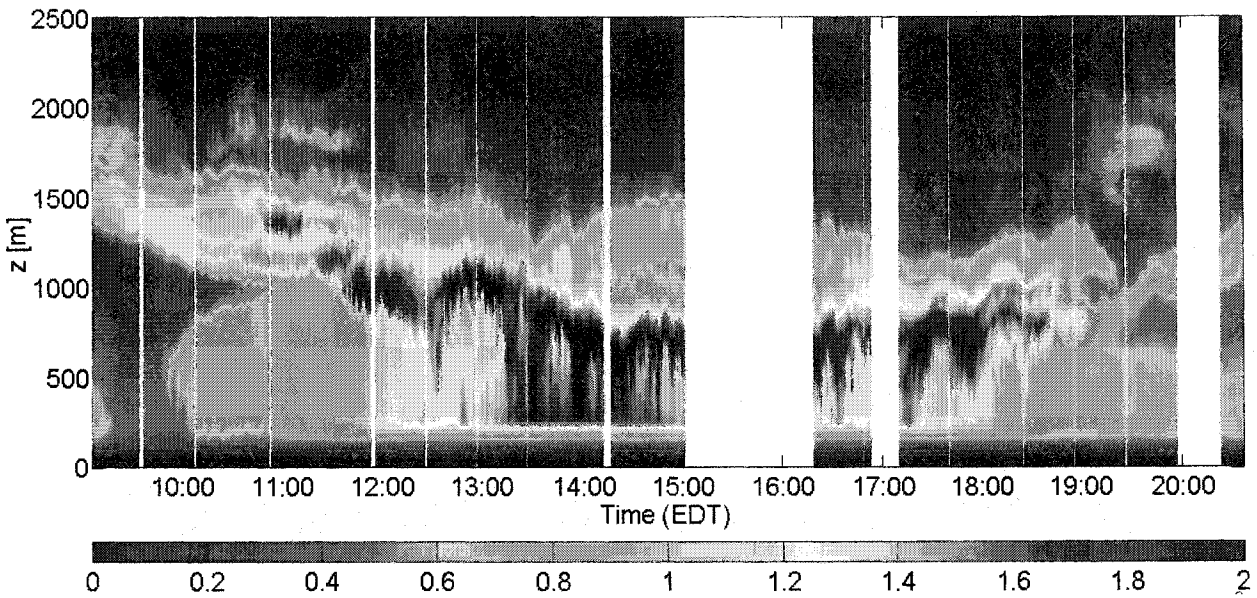


Figure 5: Three dimensional plot showing the time profile of the particle mass concentration ratio (episode:background) by size as measured with the Aerodynamic Particle Sizer™ at site A1. The background level is given by the mean mass concentration on the same weekend days preceding and following the episode.

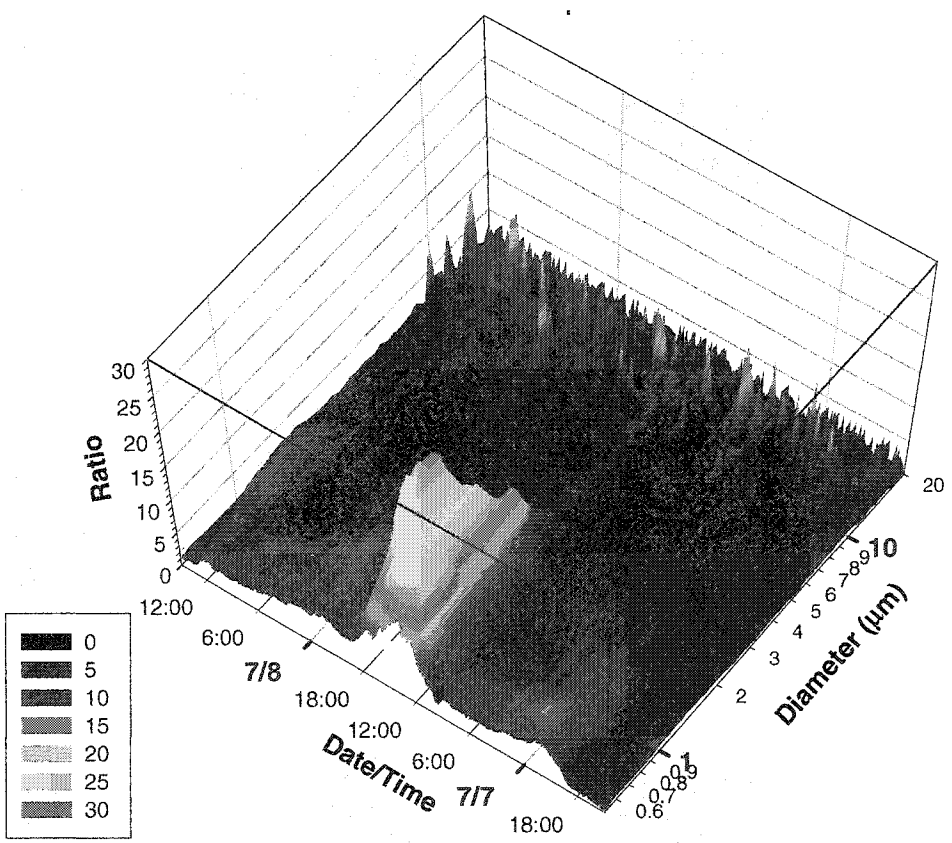
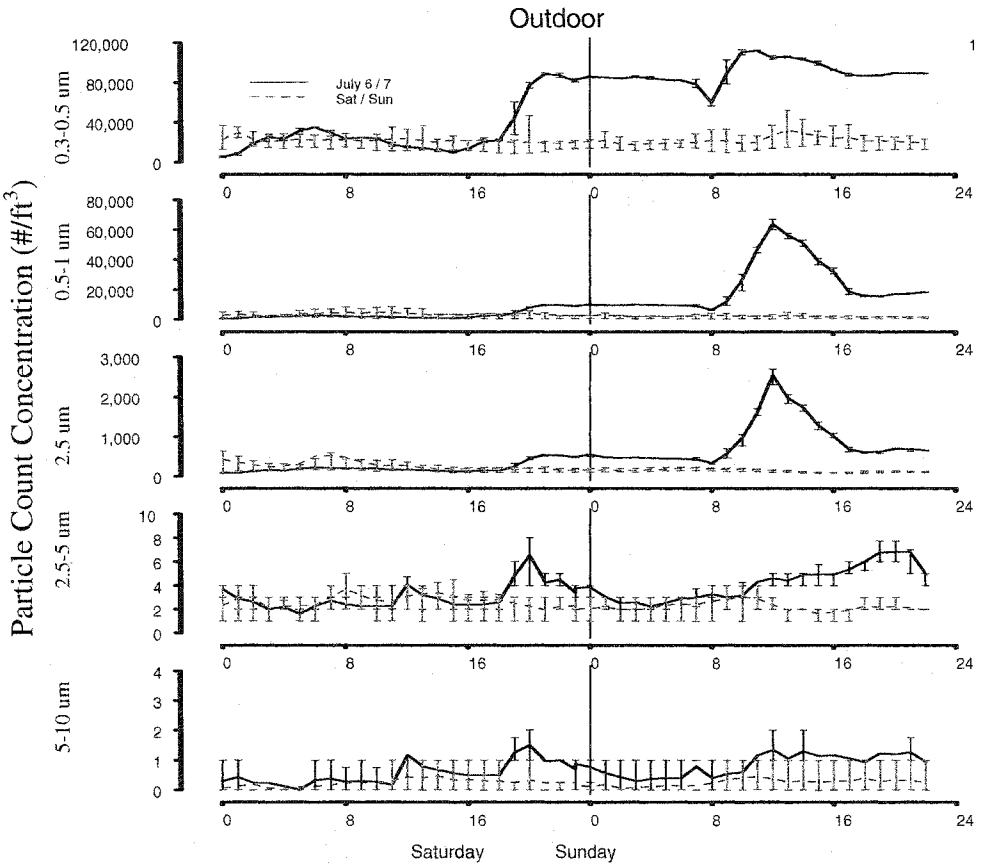


Figure 6. Particle number concentration measured with a Climet CI-500 outdoor at site A2 on July 6 and 7 (Saturday and Sunday) compared to all other Saturdays and Sundays in June and July. Each line indicates the mean particle number concentration; the bars indicate the 25th and 75th percentile.



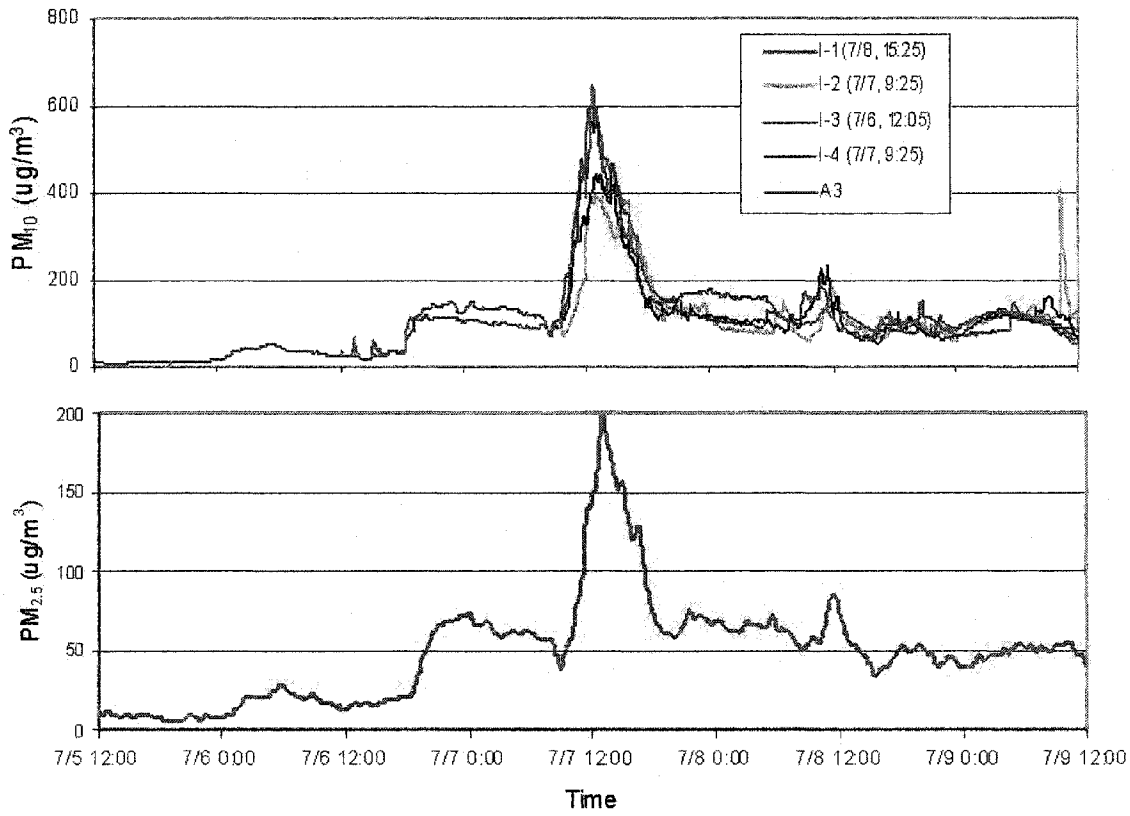


Figure 7. Panel A: Indoor (I-1 through I-4) and outdoor (A3) PM_{10} concentration profiles measured with the MIE DataRam™ . For the indoor sites, the time that monitoring began is indicated in the legend. **Panel B**: Outdoor $PM_{2.5}$ concentration profile measured at site A1 with a TEOM™.

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EDUCATION

Johns Hopkins University Bloomberg School of Public Health, Baltimore, MD
Doctor of Philosophy in Environmental Health Sciences, 2004
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Clark University, Worcester, MA
Bachelor of Science in Chemistry, 1998, magna cum laude
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EXPERIENCE

8/99-Present **Johns Hopkins University Bloomberg School of Public Health, Department of Environmental Health Sciences**, Baltimore, MD

Co-Investigator

Designed and directed a study to assess personal exposures to 1,3-butadiene (a known human carcinogen) resulting from automobile exhaust among tollbooth workers, as well as a suburban and an urban population. Developed a more sensitive analytical method for measuring urinary biomarkers of butadiene. Quantified the differential emissions of 1,3-butadiene between various classes of automobiles.

Research Assistant

Conducted a risk assessment to estimate cancer risks associated with Air Toxics in Maryland, and compared the calculated risk estimates to the observed patterns of cancer mortality in each census tract. Prepared and presented a report of these results, along with a geographical information systems analysis, to the Maryland Department of Health and Mental Hygiene. Conducted a gas chromatography/mass spectrometry analysis of Volatile Organic Compounds (VOCs) in support of an urban community exposure study.

Teaching Assistant

Worked with course instructors to develop curricula. Lectured, advised and evaluated students.

4/04-5/04 **Nepal Nutritional Intervention Project Sarlahi (NNIPS)**, Kathmandu, Nepal
Study Coordinator
Conducted a pilot study in Sarlahi, Nepal to estimate exposures to particulate matter (PM10, PM2.5 and total dust) resulting from indoor cook-stoves. Evaluated reductions in PM exposures after the installation of improved stoves fitted with chimneys.

5/00-7/00 **World Health Organization**, Geneva, Switzerland
Researcher (Intrenship)
Assisted in designing an intervention study to reduce childhood exposures to environmental tobacco smoke in 16 developing countries in Asia and Eastern Europe.

3/98-8/99 **Harvard School of Public Health**, Boston, MA.
Chemist

Conducted a biomarker-based exposure assessment of asphalt workers exposed to polycyclic aromatic hydrocarbons (PAHs). Successfully improved analytical methods for measuring PAHs in personal air samples and 1-hydroxypyrene in the urine samples.

9/96-9/97 **Clark University**, Worcester, MA
Research Assistant, Department of Environmental Sciences
Conducted a meta-analysis of work-space and dietary cadmium exposures and their resulting health effects in Japan. Assessed whether smoking confounded the relationship between the studied exposures and health outcomes in this population.

1/94-8/98 **Clark University**, Worcester, MA
Research Assistant, Department of Chemistry
Conducted laboratory experiments and analyzed data in the following areas: isolation and purification of pig kidney diamine oxidase; design of a coupled assay of mandelonitrile lyase and horse liver alcohol dehydrogenase for the kinetic resolution of racemic cyanohydrins using ß-nicotinamide adenine dinucleotide as a co-factor; scaling-up of the above procedure for commercial purposes with an NADH recycling system.

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Brown S, Burke T, Sapkota A, Kanarek N, Buckley TJ. A Community Linkage Approach in Setting Local Environment Related Cancer Prevention Priorities in Maryland. Environmental Health Perspective (Submitted)

Sapkota A., Williams D, Buckley TJ. Tollbooth Workers and Mobile Source Related Hazardous Air Pollutants: How Protective is the Indoor Environment. Environmental Science and Technology (Submitted)

Sapkota A, Halden R, Groopman JD, Dominici F, Buckley TJ. Use of LC/MS-MS to Determine the Major Mercapturic Acids of 1,3-butadiene Resulting From Exposure to Automobile Exhausts (In prep.)

PRESENTATIONS

Sapkota A, Halden R, Groopman JD, Dominici F, Buckley TJ. Use of LC/MS-MS to Determine the Major Mercapturic Acids of 1,3-butadiene Resulting from Low Level Environmental Exposure. Current Bioanalytical Applications in Mass Spectrometry, The National Institutes of Health, Bethesda, April 2004.

Sapkota A, Symons JM, Kleissl J, Ondov J. Buckley TJ. The Impact of Canadian Forest Fires on the Air Quality in Baltimore City: A Case Study of Long-Range Pollutant Transport. International Society for Exposure Analysis 13th Annual Conference, Stresa Italy, September 2003.

Sapkota A, Buckley TJ. Mobile Source Related Personal Exposure to 1,3-Butadiene. International Society for Exposure Analysis 13th Annual Conference, Stresa Italy, September 2003.

Sapkota A, Buckley TJ. The mobile source effect on curbside 1,3-butadiene, benzene, and particle-bound polycyclic aromatic hydrocarbons assessed at a tollbooth. The 95th Annual Conference of the Air and Waste Management Association, Baltimore, MD 2002.

Sapkota A, Geyh A, Moradian R and Buckley TJ. Traffic Related Indoor and Outdoor VOCs in an Urban Environment. International Society for Exposure Analysis 10th Annual Conference, Monteray California, October 2000

HONORS & AWARDS

- Cornelius W. Kruse Award for Outstanding Graduate Studies, Johns Hopkins University 2004·
- The Johns Hopkins School of Hygiene and Public Health Scholarship, 1999-2004
- National Institutes of Occupational Safety and Health, Education and Research Center, Pilot Project Award 2000-2001
- International Society for Exposure Analysis Travel Scholarship, 2000
- Clark University International Scholarship, 1993-1998
- Clark University, Deans List (First Honors), 1993, 1994, 1997

SKILLS

- Liquid chromatography tandem mass spectrometry (LC/MS-MS)
- Gas chromatography/mass spectrometry (GC/MS)
- High-Pressure Liquid Chromatography
- Nuclear Magnetic Resonance
- Electron Spin Resonance
- Infrared, and Fluorescence Spectroscopy
- Stata statistical package
- Arcview Geographical Information Systems (GIS)
- Epi Info
- Fluent in English, Nepali and Hindi

AFFILIATIONS

- International Society for Exposure Analysis, 2000-Present
- Air and Waste Management Association, 2001-Present