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Urinary biomarkers of 1,3-butadiene in environmental settings using liquid chromatography isotope dilution tandem mass spectrometry

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Abstract

Although, 1,3-butadiene is a known human carcinogen emitted from mobile sources, little is known about traffic-related human exposure to this toxicant. This pilot study was designed to characterize traffic-related environmental exposure to 1,3-butadiene and evaluate its urinary mercapturic acids as biomarkers of exposure in these settings. Personal air samples and multiple urine samples were collected on two separate occasions from three groups of individuals that differed by spatial proximity as well as intensity of traffic: (i) toll collectors, (ii) urban-weekday and (iii) suburban-weekend group. Air samples were analyzed using thermal desorption followed by GC/MS and urine samples were analyzed using isotope dilution liquid chromatography tandem mass spectrometry (ID-LC-MS/MS) for two mercapturic acids of 1,3-butadiene: monohydroxy-3-butenyl mercapturic acid (MHBMA) and 1,2-dihydroxybutyl mercapturic acid (DHBMA). Exposure differed between groups ($p < 0.05$) with median values of 2.38, 1.62 and 0.88 $\mu g/m^3$ for toll collectors, the urban-weekday group and the suburban-weekend group, respectively. A refined ID-LC-MS/MS method enabled detection of MHBMA, previously detected only in occupational settings, with high frequency. MHBMA and DHBMA were detected in 95 and 100% of urine samples at levels (mean ± S.D.) of 9.7 ± 9.5, 6.0 ± 4.3 and 6.8 ± 2.6 ng/mL for MHBMA and 378 ± 196, 258 ± 133 and 306 ± 242 ng/mL for DHBMA for the three different groups, respectively. Mean biomarker levels were higher among the toll collectors compared to the other two groups, however, the differences were not statistically significant ($p > 0.05$). This study is the first to evaluate 1,3-butadiene biomarkers for subtle differences in environmental exposures. However, additional research will be required to ascertain whether the lack of statistical association observed here is real or attributable to unexpectedly small differences in exposure between groups (<1 $\mu g/m^3$), non-specificity of the biomarker at low exposure, and/or small sample size.
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Keywords: 1,3-Butadiene; VOC; Exposure; Biomarker; Automobile exhaust; Traffic; LC-MS/MS; Tandem mass spectrometry

Abbreviations: DHBMA, 1,2-dihydroxybutyl mercapturic acid; GC/MS, gas chromatography/mass spectrometry; ID-LC-MS/MS, isotope dilution liquid chromatography tandem mass spectrometry; MHBMA, 1- and 2-monohydroxy-3-butenyl mercapturic acid; U.S. EPA, United States Environmental Protection Agency; VOC, volatile organic compounds

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1. Introduction

Environmental exposure to 1,3-butadiene is a significant public health concern because it is a known human carcinogen [1,2], and traffic, which is a dominant source of ambient butadiene [3], is ubiquitous and in close proximity to residential communities. Several epidemiological studies have linked exposure to 1,3-butadiene with lymphohaematopoietic cancers [4,5] and leukemia [6–9] in occupationally exposed individuals. The U.S. Environmental Protection Agency (EPA) estimates that the human lifetime excess cancer risk from chronic exposure to butadiene is 3.5×10^{-5} per $\mu g/m^3$ based on a linear extrapolation of increased leukemia risks observed in occupationally exposed workers [2]. These estimates imply that an acceptable level of lifetime risk (one cancer case in a million) would result if individuals were chronically exposed to 22 ng/m^3 level of butadiene over their lifetimes. The limited data available from monitoring sites across the country indicate that ambient air concentrations of butadiene routinely exceed this level in most urban areas, including Baltimore City [10], with mobile sources accounting for as much as 60% of ambient emissions [3]. Ambient levels contribute to exposure but are not the sole determinant. Additional factors contributing to personal exposures to VOC include occupation, residence proximity to traffic [11], time spent in automobiles [12], and other personal habits including smoking [13].

Characterization of human exposure to a carcinogen such as 1,3-butadiene using personal air samples and/or biomarkers provides epidemiological studies with a more direct and individualized comprehensive measure of exposure. This in turn enhances one's ability to detect associations between exposures and disease by minimizing exposure misclassification. Exposure characterization using personal air monitoring takes into account the heterogeneity of contaminants in different microenvironments and duration of exposure, but such measurements are generally limited to a single route of exposure, i.e. primarily inhalation. Chemical-specific urinary biomarkers, on the other hand, not only integrate multiple routes of exposure, but also address intrinsic individual variability conferred by genetic polymorphisms, and help to examine mechanisms of actions at various biological targets [14]. Biomarkers also offer a clearer demonstration that the toxicant has been absorbed [15] and provide an estimate of total exposure. Furthermore, biomarkers can be invaluable for mitigating risk, because it is useful in identifying specific at-risk subpopulations that can be targeted for interventions.

For an exposure biomarker to be effectively deployed in studies of exposure, risk or epidemiology, its sensitivity, specificity, accuracy and reliability need to be characterized [15]. In the past decade, two novel urinary biomarkers 1- and 2-monohydroxy-3-butenyl mercapturic acid (MHBMA) and 1,2-dihydroxybutyl mercapturic acid (DHBMA) have shown promise as biomarkers for occupational exposure to 1,3-butadiene [16,17]. MHMBA is formed when butadiene monoepoxide (BDO) undergoes direct conjugation with glutathione, while DHBMA is formed when BDO is hydrolyzed to the diol that subsequently undergoes conjugation with glutathione [18,19]. Previous studies have shown that variation in the relative amount of MHBMA and DHBMA excreted is proportional to the level of epoxide hydrolase activity in different species [16,17,20], with mice excreting the lowest amount of DHBMA (20% of the total biomarker) and humans excreting the highest amount of DHBMA (>97% of the total biomarker). Accordingly, prior studies that have used solid phase extraction followed by derivatization of the biomarker and subsequent analysis using gas chromatography/mass spectrometry (GC/MS) have been unsuccessful in detecting MHBMA even for highly exposed workers, owing to the poor sensitivity of the GC/MS method [17].

More recently, both MHBMA and DHBMA have been used as biomarkers of exposure in a large occupational study of Czech workers in the styrene butadiene rubber industry [18,19,21,22]. Measurements performed by GC–MS/MS analysis of chemically derivatized organic extracts of urine found that MHBMA was more accurate in predicting recent exposure to butadiene than DHBMA. Of late, new methods have been developed for analyzing MHBMA and DHBMA that use liquid chromatography tandem mass spectrometry (LC-MS/MS). These methods provide significant improvement in sensitivity compared to traditional GC/MS method, simultaneously reducing the time required to prepare samples [23,24]. However, neither DHBMA nor MHBMA has been evaluated in environmental settings, where exposures are several orders of magnitude lower than those experienced in occupational settings. To address these research gaps, the current pilot study was designed to (i) assess environmental exposure to butadiene using personal exposure monitors and (ii) apply/modify the existing LC-MS/MS method [23,24] to evaluate MHBMA and DHBMA as biomarkers of butadiene exposure in environmental setting using three exposure scenarios: suburban-weekend exposure, urban-weekday exposure and toll collectors workday exposure.

2. Methods

2.1. Study population

Individuals representing three exposure scenarios differing by spatial proximity as well as intensity of mobile sources were recruited. These were: (1) toll collectors' workday exposure (very close proximity and high source intensity); (2) urban-weekday exposure (close proximity to source and medium source intensity) and (3) suburban-weekend exposure (far from source and low source intensity). For the first group, nine toll collectors working the morning shift (6 a.m.–2 p.m.) at the Baltimore Harbor Tunnel were recruited. For the suburban-weekend and the urban-weekday exposure groups, seven faculty and staff from the School of Public Health were recruited as a convenient sample. All individuals were non-smokers living in non-smoking households. The study was approved by the Johns Hopkins Bloomberg School of Public Health Committee on Human Research (CHR). Prior to collecting any samples, a written consent was obtained from all study participants.

2.2. Personal air sampling

Exposure for each of the three groups was assessed using personal air monitors. Each group of participants was monitored on two separate occasions. The duration of personal air monitoring was 8, 4 and 8 h for the suburban-weekend, urban-weekday and the toll collectors, respectively. For the suburban-weekend exposure group, monitoring was conducted on Sundays from 12 to 8 p.m. For the urban-weekday exposure group, monitoring was conducted on the same individuals while they spent 4 h inside an urban row home located on a busy street during morning rush-hour (6–10 a.m.). Toll collectors were monitored during their 8-h shift (6 a.m.–2 p.m.). The altered sampling duration for the urban-weekday group was necessary for logistical reasons (e.g. need to return to work). 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 in. long, 0.25 in. o.d.) packed with a mixed sorbent comprised of carbopack B and corboxen 1000 (Supelco cat. # 25051; Bellefonte, PA). The air toxic tube was clamped directly onto the lapel of the participant. A small battery operated pump (SKC 210 pocket pump, SKC Inc.; Eighty Four, PA) was used to sample air at a constant rate of 100 mL/min. To avoid sample breakthrough, a portion of the pump flow was diverted 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 12 mL/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.

Personal air samples were analyzed within 24 h of collection using a method previously described [25,26]. Briefly, the VOCs were thermally desorbed with Perkin-Elmer™ ATD-400 (Perkin-Elmer; Shelton, CT), resolved by gas chromatography (GC), and detected with mass spectrometry (MS) in selected ion monitoring (SIM) mode using a Shimadzu GC-17A gas chromatograph and QP-5000 mass spectrometer (Shimadzu; Columbia, MD). Chromatographic separation was achieved using a Restek Rtx-624 column, measuring 60 m × 0.25 mm i.d. with 1.4 μm film thickness (Restek Corp.; cat. # 10969). To account for any sample contamination during the sampling phase, field ($n = 18$) and laboratory ($n = 20$) blanks were deployed and analyzed. Reported concentrations have been blank adjusted.

2.3. Urine collection

Urine sample collection was tailored to each of the respective exposure scenarios. For each groups, urine samples were collected before, during and after exposure. The before exposure collection was comprised of a single void. The during exposure collection varied by scenario due to differing durations and work-related logistics. Whereas all voids were collected during exposure for the suburban and urban scenarios, for the tollbooth workers, samples were collected during their lunch break and at the end of shift. Urine samples collected post exposure included the last void of the day (before bed) and the first void of the following morning. Consequently, the post exposure collection period varied from 10 (suburban-weekend group) to 18 h (urban-weekday group). All urine samples were stored at −70 °C until analysis.

2.4. Chemicals

Isomeric mixture of *R*,*S*-1-hydroxy-2-(*N*-acetylcysteinyl)-3-butene and *R*,*S*-2-hydroxy-1-(*N*-acetylcysteinyl)-3-butene (MHBMA), their deuterated analogs *R*,*S*-1-hydroxy-2-(*N*-acetylcysteinyl)-3-butene-[d_6] and *R*,*S*-2-hydroxy-1-(*N*-acetylcysteinyl)-3-butene-[d_6] along with *R*,*S*-1,2-dihydroxy-4-(*N*-acetylcysteinyl)-butane (DHBMA) and its deuterated analogue *R*,*S*-1,2-dihydroxy-4-(*N*-acetylcysteinyl)-butene-[d_7] were purchased from Toronto Research

Chemicals, Ont., 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.

2.5. Urine sample preparation

Urine samples were thawed at room temperature. Aliquots of 10 μL of 100 ng/mL MHBMA-[d_6] and DHBMA-[d_7] in methanol were added to 1 mL urine samples and the pH was adjusted to 2.5 by the addition of HCl. Samples were then extracted using Waters Oasis™ HLB cartridges (Waters Corp.; Milford, MA; cat. # WAT094226) pre-equilibrated with 1 mL methanol followed by 1 mL water. Following loading of the sample, cartridges were washed with 1 mL water, and analytes were eluted with 1 mL of 50:50 methanol/water at <0.5 mL/min.

2.6. Sample analysis

Purified organic extracts were placed in a Surveyor HPLC and autosampler system (Thermo Electron, San Jose, CA) coupled to a Quantum Ultra triple quadruple tandem mass spectrometer (Thermo Electron) and analyzed using a modification of a method previously described by McDonald et al. [23]. In brief, a 10-μL sample aliquots were injected onto the HPLC system and analytes were separated on a mixed-mode (C8/anion exchange) custom packed column (Alltech Associates; Deerfield IL; Part# C-6008, 7 μm particle size, 100 mm × 2.1 mm i.d.) with a pre-column filter (Phenomenex, Torrance CA; Part# AJ0-4286; 4 mm × 2 mm i.d.). The isocratic mobile phase maintained at 35 °C consisted of a 30:70 mixture of water and methanol, containing 5 mM ammonium formate flowing at a rate of 300 μL/min with a total runtime of 6 min. Analytes were introduced into the mass spectrometer using an electrospray ionization (ESI) probe operated in negative mode. The MS/MS was operated in selected reaction monitoring (SRM) mode using the instrument conditions provided in detail in Table 1. MHBMA was detected using the transition of *m/z* 232.0 → 103.1 and quantified using the internal standard MHBMA-[d_6], *m/z* 238.1 → 109.1. Similarly DHBMA was detected using *m/z* 250.1 → 121.1 and quantified using DHBMA-[d_7] *m/z* 257.1 → 128.1. A nine-point calibration curves for MHBMA (0–200 ng/mL) and DHBMA (0–1000 ng/mL) was prepared in methanol/water for each batch of analysis. The instrument response increased linearly over the entire range for both analytes, with R^2-values greater of than 0.996.

Table 1
Analytical parameters for LC-ESI-MS/MS analysis of monohydroxy-3-butenyl mercapturic acid (MHBMA) and dihydroxybutyl mercapturic acid (DHBMA)

HPLC parameters			
Injection volume			10 μL
Column temperature			35 °C
Runtime			6 min
MS/MS parameters			
Ion source			ESI negative
Spray voltage			−2800 V
Sheath gas pressure			35 arbitrary unit
Aux gas pressure			14 arbitrary unit
Capillary temperature			375 °C
Source CID			10 V
Collision pressure			0.9 mTorr
Peak widths			$Q1 = 0.5$; $Q3 = 0.7$
Analyte	Parent ion *m/z*	Daughter ion *m/z*	Collision energy
SRM table			
MHBMA	232.0	103.1	17
MHBMA-[d_6]	238.1	109.1	15
DHBMA	250.1	121.1	16
DHBMA-[d_7]	257.1	128.1	19

2.7. Sample recovery

Since all urine samples had some detectable level of MHBMA and DHBMA, synthetic urine was used for the purpose of determining sample recovery and limits of detection. A 3 × 1 mL aliquots of synthetic urine were spiked with 10 μL of 100 ng/mL MHBMA-[d_6] and DHBMA-[d_7] internal standards followed by 10 μL of standard mix (1000 ng/mL DHBMA and 200 ng/mL of MHBMA). These samples were prepared and analyzed as described above. Sample recovery was determined as the percentage of analyte recovered relative to the spiked amount. To determine the limit of detection, a set of 7 × 1 mL aliquots of synthetic urine was spiked with 10 μL of 100 ng/mL internal standards (MHBMA-[d_6] and DHBMA-[d_7]) followed by 10 μL standard mix containing 500 ng/mL DHBMA and 100 ng/mL of MHBMA, and extracted as described above. The limit of detection was determined by multiplying the standard deviation of seven spiked samples by the Student's *t*-value associated with the 99% confidence interval and 6 ($n - 1$) degrees of freedom [27].

2.8. Precision

Method precision was determined using two different approaches. In the first approach, a pooled urine sample was spiked with MHBMA and DHBMA, divided into 1 mL aliquots, and stored in the freezer. Whenever urine samples were extracted, one or two aliquots of the spiked urine samples were also included. Overall analytical precision was determined as the coefficient of variation (CV) for all the spiked urine samples analyzed over the entire analysis period. In the second approach, two urine samples were randomly selected in each batch of extraction. These randomly selected samples were extracted in duplicates and analyzed separately. Method precision was determined based on the agreement between duplicate extracts analyzed over the entire study period.

2.9. Statistical analysis

All statistical analyses were performed using Intercooled Stata, Version 7.0 for Windows (Stata Corporation, College Station, TX). The normality of the personal exposure and biomarker data were assessed using Shapiro–Wilk test. Group differences for personal exposure as well as urinary biomarker levels were evaluated by the non-parametric Mann–Whitney test. The association between personal exposure and urinary biomarker levels was evaluated across all subjects by simple linear regression, after log transformation. The association between the exposure and biomarker was further explored using a multiple linear regression model with a random intercept (random effect model), to allow each individual to have their own intercept. A criterion of $p < 0.05$ was set for determining statistical significance.

3. Results

The personal air sampling method had a recovery rate (mean ± S.D.) of 85 ± 12% and a coefficient of variation of 2% for 1,3-butadiene, as described in detail previously [26]. The corresponding limit of detection (LOD) was 0.46 μg/m^3. For the ID-LC-MS/MS method, recoveries based on the spiked synthetic urine were (mean ± S.D.) 86 ± 11% and 85 ± 12% for DHBMA and MHBMA, respectively. The LOD for the ID-LC-MS/MS method was estimated to be 0.4 and 3.7 ng/mL for MHBMA and DHBMA, respectively. Those samples that were below the LOD were assigned a value of 1/2 LOD, an approach that is suitable for skewed data [28]. Duplicate analyses conducted throughout the study period showed good reproducibility for both DHBMA and MHBMA, as indicated by a slope of close to unity (0.98 and 0.95 for DHBMA and MHBMA, respectively) and a R^2-value greater than 0.9 The pool spiked urine samples analyzed over the entire sample analysis period had a coefficient of variation of less than 12% for both DHBMA and MHBMA. For the samples analyzed in duplicates over a range of concentration, the coefficient of variation was 9.2% for DHBMA and 13.1% for MHBMA.

3.1. Personal exposure

The distribution of butadiene exposure varied by groups with median levels of 0.88, 1.62 and 2.38 μg/m^3 observed for the suburban-weekend, urban-weekday and toll collectors, respectively (Fig. 1). There was a clear trend in median exposure levels, i.e. suburban-weekend < urban-weekday < toll collectors, but the measured differences in median exposure between groups were rather small (<1 μg/m^3). The toll collectors' exposure and the urban-weekday exposure were both higher than the suburban-weekend exposure ($p < 0.05$).

3.2. Urinary biomarker

Compliance with the urine collection protocol was excellent. Toll collectors provided all six of the required urine samples with the exception of one individual who provided five. Furthermore, the time of collection was very consistent across toll collectors owing to their well defined work schedule. Sample collections associated with the urban-weekday and suburban-weekend exposure groups were similarly compliant but more variable in time of collection due to differing individual activities. At the instrumental conditions specified in Table 1, *m/z*

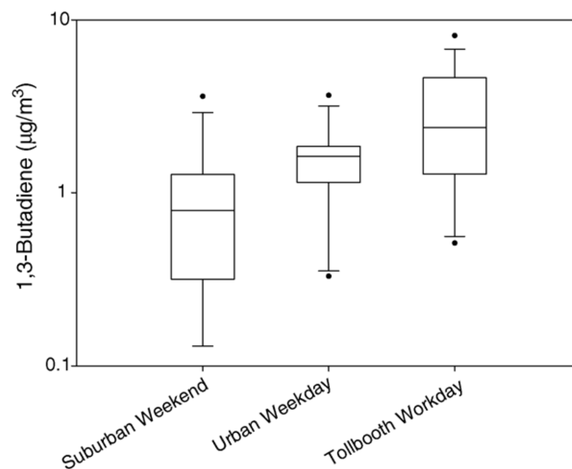

Fig. 1. Personal exposure to 1,3-butadiene among the three study groups.

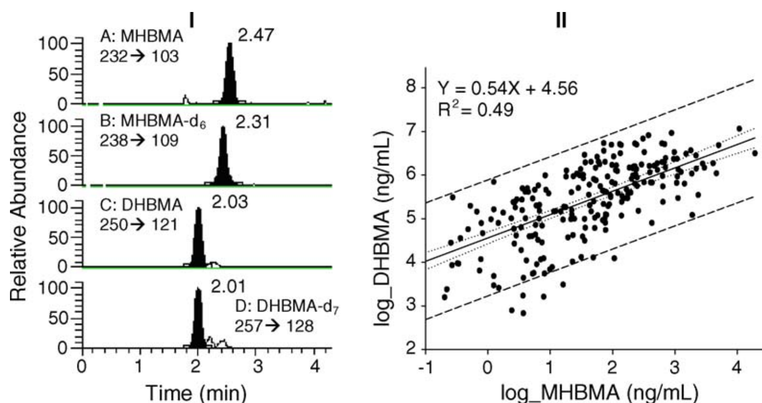

Fig. 2. Panel I—a representative chromatogram of urine samples showing (A) MHBMA, (B) MHBMA-d_6, (C) DHBMA and (D) DHBMA-d_7; Panel II—relationship between MHBMA and DHBMA.

103.1 and 121.1 were the dominant daughter ions of the molecular base ions $[M-H]^-$ of MHBMA (m/z 232.0) and DHBMA (m/z 250.1), respectively. Under the specified chromatographic conditions (Table 1), DHBMA and DHBMA-d_7 eluted prior to MHBMA and MHBMA-d_6 (Fig. 2, Panel I). Using this ID-LC-MS/MS method, DHBMA was detected in 100% of the samples collected, whereas MHBMA was detected in 95% of the samples.

Linear regression (Fig. 2, Panel II) between DHBMA and MHBMA showed a significant association between the two urinary biomarkers ($p<0.05$, $R^2=0.49$). In all cases, DHBMA was the dominant marker, representing greater than 90% of the total biomarkers excreted. The mean (S.D.) metabolic ratio determined as the ratio of DHBMA/(DHBMA + MHBMA) was 0.974 (0.021) with a minimum and a maximum of 0.897 and 0.999, respectively.

The distribution of DHBMA and MHBMA levels showed a rather weak trend for increasing biomarker levels across the three exposure groups (Fig. 3). As shown in Table 2, the DHBMA levels (mean ± S.D.) observed for the toll collectors (378 ± 196 ng/mL) were higher than that observed for the suburban-weekend exposure group (306 ± 243 ng/mL) and the urban-weekday exposure (258 ± 133 ng/mL), but the difference was not statistically significant ($p>0.05$). Similarly, the MHBMA levels (mean ± S.D.) observed for the toll collectors (9.7 ± 9.5 ng/mL) were higher than those observed for the suburban-weekend group (6.8 ± 2.6 ng/mL) and the urban-weekday exposure group (6.0 ± 4.3 ng/mL), but these differences were not statistically significant either ($p>0.05$). Table 2 further compares the findings of this study with personal exposures and urinary biomarker levels published in recent studies conducted by other investigators [19,24,29].

The association between individual personal exposure to butadiene and the urinary biomarker level was explored using simple linear regression (Fig. 4). No apparent trend was observed between exposure level and either biomarker ($R^2<0.2$ and $p>0.05$).

$$\log Y_{it} = \beta_0 + \beta_1 \log X_i + \beta_2 \log X_i^* I + \varepsilon \quad (1)$$

Y_{it} = Urine Concentration of biomarker for individual i at time t (ng/mL)
X_i = Personal exposure for individual i (μg/m^3)
I = Exposure group (1 if toll collectors, 0 otherwise)

The relationship between personal exposure, urinary biomarker and the exposure group was further examined

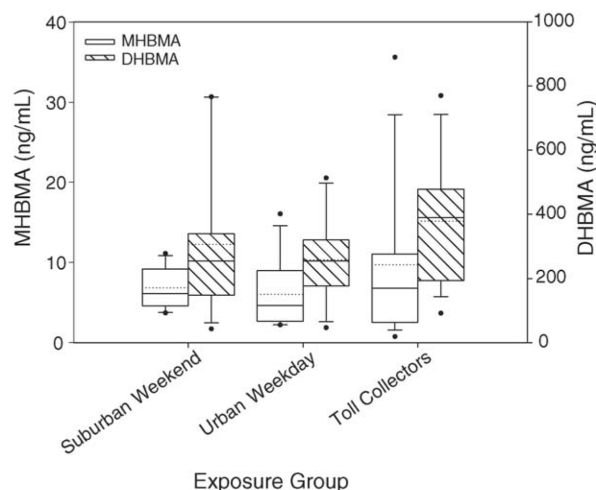

Fig. 3. Distribution of monohydroxy-3-butenyl mercapturic acid (MHBMA) and dihydroxybutyl mercapturic acid (DHBMA) by exposure group. The boxes represent 25th and 75th percentiles, whiskers represent 5th and 95th percentiles, solid horizontal bars represent the median and the dotted line represent the mean.

Table 2
Comparison of personal exposure and urinary biomarkers levels determined in the present study to values reported in the literature

Investigator	Exposure	BD exposure ($\mu g/m^3$)		DHBMA (ng/mL)		MHBMA (ng/mL)	
		Mean (S.D.)	Median (min–max)	Mean (S.D.)	Median (min–max)	Mean (S.D.)	Median (min–max)
Current study	Suburban-weekend	1.22 (1.09)	0.88 (0.23–4.36)	306.5 (242.7)	254.8 (42.8–766.4)	6.8 (2.6)	6.1 (3.7–11.1)
	Urban-weekday	1.47 (0.73)	1.62 (0.23–3.66)	257.8 (133.2)	244.1 (46.3–513.3)	6.0 (4.3)	4.7 (2.2–16.1)
	Tollbooth workers	2.88 (2.10)	2.38 (0.51–8.12)	378.5 (196.0)	390.2 (91.5–770.1)	9.7 (9.5)	6.8 (0.75–35.6)
Van Sittert 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)
Fustinoni et al.	Control	0.9 (1.0)	0.4 (<0.1–3.8)	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)[a]	NR (209–898)[a]	12.5 (1.0)[a]	NR (7–18.1)[a]
	Smoker	NR	NR	644 (90)[a]	NR (116–1084)[a]	86.4 (14.0)[a]	NR (15.2–145.1)[a]

NR, not reported.
[a] Concentration expressed as μg/24 h.

using multiple regression analysis with random intercept and an interaction term for levels of personal exposure as shown in Eq. (1). For this analysis, the urban-weekday and suburban-weekend exposure groups were combined into one group to enhance statistical power. The results of this analysis showed no significant association between personal exposure and either biomarker at the individual level ($\beta_1 = -0.31$, $p = 0.15$; $\beta_2 = 0.35$, $p = 0.25$ for MHBMA and $\beta_1 = -0.21$, $p = 0.24$; $\beta_2 = 0.43$, $p = 0.08$ for DHBMA).

4. Discussion

Urban and tollbooth environments, characterized by close proximity and high intensity of traffic are often referred to as mobile source "hot spots". Exposures to mobile source-related hazardous air pollutants (HAPs), especially human carcinogens such as 1,3-butadiene, in these hot spots are of significant concern to public health. In the current study we address this concern in two ways. First, exposure was characterized across a range of conditions of varying source intensity and proximity. Second, the observed exposure formed the basis for an evaluation of the predictive validity of 1,3-butadiene urinary biomarkers. Whereas the exposure characterization provides key information to inform risk assessment, the evaluation of the biomarker is necessary to gauge its utility for epidemiological studies, especially the ones dealing with low environmental exposures.

The present study provides, for the first time, toll collectors' personal exposure to butadiene. The morning shift toll collectors' median personal exposure (2.38 $\mu g/m^3$) was similar to the indoor air concentration of butadiene (2.9 $\mu g/m^3$) inside the tollbooth, reported previously for the same facility [30]. In that study, the relatively low indoor concentrations inside the tollbooth compared to the high outdoor concentration (2.9 $\mu g/m^3$ versus 10.7 $\mu g/m^3$) was attributed to the effective ventilation system that maintained the tollbooth under positive pressure. As expected, the toll collectors' median exposure (2.38 $\mu g/m^3$, min/max: 0.51/8.12) was higher than the median urban-weekday exposure (1.62 $\mu g/m^3$, min/max: 0.33/3.66) and the median suburban-weekend exposure (0.88 $\mu g/m^3$, min/max: 0.23/4.36). However, the absolute differences between these groups are relatively small (0.76 and 0.74 $\mu g/m^3$, respectively). The overall concentration of butadiene inside the tollbooth (1.31 $\mu g/m^3$) reported previously [30] was only slightly higher than what has been observed inside urban homes (0.7 $\mu g/m^3$) by Sax et al. [31]. Therefore, it is not surprising that the personal exposure of toll collectors at the Baltimore Harbor Tollbooth were only slightly higher

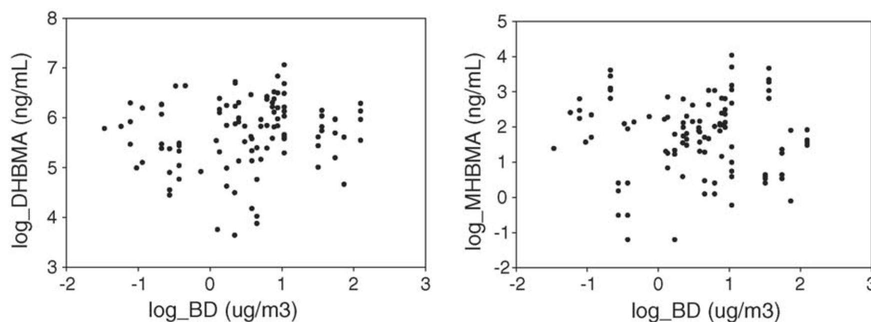

Fig. 4. Scatter plot of monohydroxy-3-butenyl mercapturic acid (MHMBA) and dihydroxybutyl mercapturic acid (DHBMA) vs. personal exposure.

than the personal exposure of individuals spending time inside an urban home. It is likely that the exposure of toll collectors working at facilities lacking control ventilation systems (as present in this facility) will 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 types of tollbooth facilities is warranted.

This study provides a rapid ID-LC-MS/MS method that is sensitive enough for analyzing both DHBMA and MHBMA in human urine resulting from environmental exposures to butadiene. This method requires minimal sample cleanup, low sample volume (1 mL), and does not require a concentration step. Use of this sensitive ID-LC-MS/MS method, with similar SRM transition as described previously [23,24], enabled detection of DHBMA and MHBMA in 100 and 95% of the samples. The method showed excellent sensitivity, recovery and precision for both urinary metabolites of butadiene resulting from environmental exposure. The close agreement between the duplicate extracts for both DHBMA and MHBMA, as indicated by a slope factor approaching unity, and a high R^2 (>0.9) highlight reproducibility and precision of the method. The method precision is further illustrated by the low CV observed. Although neither of the biomarkers was evaluated for storage loss, extensive storage study has been previously reported [24] that observed no losses up to 18 months (entire duration of experiment) when urine samples were stored at −20 °C. In the present study, samples were stored at −70 °C and the longest storage period was 12 months, so storage losses were unlikely to have occurred.

The ratio of the two biomarkers DHBMA/(DHBMA + MHBMA) is an indication of the metabolic pathway. This ratio, initially reported by Sabourin et al. [16] has been shown to increase from mice (0.2) to rats (0.52) to humans (>0.97), paralleling the increase in epoxide hydrolase activity across these species [17,20]. The authors hypothesized that in humans, the majority of BDO is metabolized by hydrolysis to butenediol instead of direct conjugation with GSH. The mean metabolic ratio of 0.974 observed in this study is consistent with these previously reported human studies. A recent occupational study of butadiene workers from the Czech Republic reported a median metabolic ratio of 0.995, 0.987 and 0.981 for control, monomer and polymer worker, respectively [18,19,21], with the control workers having significantly higher metabolic ratios. In a study comparing smokers and non-smokers, Urban et al. [24] 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 by exposure group, sex or age.

The relatively subtle differences in exposure were not reflected by the measured biomarkers when considered at the group or individual level. At the group level, the toll collectors had higher levels of MHBMA and DHBMA compared with both urban-weekday and suburban-weekend exposure groups, however the differences were not statistically significant ($p > 0.05$). At the individual level, no association between exposure and either biomarker was identified. This lack of association may be attributable to a number of study limitations that could lead to obscuration of the association between exposure and biomarker level including unexpectedly small differences in exposure between groups and relatively small sample size. In addition, several other metabolites of 1,3-butadiene have been reported in mammals besides MHMBA and DHBMA [32]. It is possible that for low-level exposures as has been observed in the current study, metabolic differences between individuals may be obscuring the association between exposure and biomarker level. During the study design, 1,3-butadiene was assumed to have a clearance time similar to that of benzene [33,34], but recently Albertini et al. have suggested that the clearance of MHBMA in humans may be longer than initially expected [19]. This can be an additional source of variability given the slightly differ-

ent exposure duration monitored for the urban-weekday group.

The overall butadiene exposure observed in this study was lower than what has been previously reported in the literature. In the Czech study [18,19,21], the median exposure of the highest exposure group (polymer workers: 293 $\mu g/m^3$) was 123- and 431-fold higher than the personal exposure observed for toll collectors and the suburban-weekend exposure group in this study. Additionally, the difference in personal exposure between high and low exposure groups in the Czech worker study was 280 $\mu g/m^3$ compared to <2 $\mu g/m^3$ observed in the current study. Such large exposure differences and sample size might have enabled the authors of the Czech study to discern the relationship between the urinary biomarker and butadiene exposure. However, in a study of petrochemical workers in Italy, Fustinoni et al. [29] reported no significant difference in DHBMA and MHBMA between exposed workers and their non-exposed counterparts. Their findings are of particular relevance to this study because the median exposure levels of the exposed worker 1.5 $\mu g/m^3$ (mean 11.5 $\mu g/m^3$) and the control workers 0.4 $\mu g/m^3$ (mean 0.9 $\mu g/m^3$) are more comparable.

The presence of DHBMA (mean 306 ng/mL) even in the lowest exposure group is consistent with what has been reported in the literature [29]. However, it is unclear whether this metabolite is specific to 1,3-butadiene inhalation exposure, formed from other exogenous chemical in diet, water or air, or formed endogenously. Albertini et al. hypothesized that DHBMA may be formed from endogenous butadiene-diol, but additional work is necessary to substantiate this. In comparison, MHBMA was observed at low levels similar to what was reported by Fustinoni et al. The presence of MHBMA reported here, even in the lowest exposure group, all non-smokers living in non-smoking households, serves to document the presence of this metabolite among individuals exposed to low-level environmental concentrations of traffic origin. In conclusion, this study characterized subtle differences in personal exposure to butadiene in different environmental settings: toll collectors > urban-weekday > suburban-weekend. Despite the different settings, actual exposures differed only modestly (difference < 1 $\mu g/m^3$ between groups). This small difference in exposure is likely attributable to the efficient control ventilation system present at this specific tollbooth facility, as previously reported [26]. This study further provides an ID-LC-MS/MS method for the detection of urinary biomarkers of butadiene resulting from environmental exposure.

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