TRAFFIC-RELATED AIR POLLUTION. A PILOT EXPOSURE ASSESSMENT IN BEIRUT, LEBANON

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ABSTRACT

Traffic-related volatile organic compounds (VOCs) pollution has frequently been demonstrated to be a serious problem in the developing countries. Benzene and 1,3-butadiene (BD) have been classified as a human carcinogen based on evidence for an increased genotoxic and epigenotoxic effects in both occupational exposure assessment and in vivo/in vitro studies. We have undertaken a biomonitoring of 25 traffic policemen and 23 office policemen in Beirut, through personal air monitoring, assessed by diffusive samplers, as well as through the use of biomarkers of exposure to benzene and BD. Personal benzene, toluene, ethylbenzene, and xylene (BTEX) exposure were quantified by GC-MS/MS, urinary trans, trans-muconic acid (t,t-MA) by HPLC/UV, S-phenyl mercapturic acid (S-PMA), monohydroxy-butenyl mercapturic acid (MHBMA) and dihydroxybutyl mercapturic acid (DHBMA) by ultra-performance liquid chromatography-electrospray tandem mass spectrometry (UPLC/ESI(-)-MS/MS) in MRM (Multiple Reaction Monitoring) mode. We found that individual exposure to benzene in the traffic policemen was higher than that measured in traffic policemen in Prague, in Bologna, in Ioannina and in Bangkok. t,t-MA levels could distinguish between office and traffic policemen and showed a better correlation with personal BTEX compounds exposure. However, median MHBMA levels in traffic policemen were slightly elevated, though not significantly higher than in office policemen. Alternatively, DHBMA concentrations could significantly distinguish between office and traffic policemen and showed a better correlation with personal total BTEX exposure. DHMBA, measured in the post-shift urine samples, correlated with both pre-shift MHMBA and pre-shift DHMBA. Moreover, there was not a marked effect of smoking habits on DHBMA. Taken together, these findings suggested that DHBMA is more suitable than MHBMA as biomarker of exposure to BD in humans. Traffic policemen, who are exposed to
benzene and BD at the roadside in central Beirut, are potentially at a higher risk for
development of diseases such as cancer than office policemen.

**Keywords:** Biomarkers; benzene; 1,3-butadiene; occupational exposure; traffic-related air
pollution
1. INTRODUCTION

Traffic-related volatile organic compounds (VOCs) pollution has frequently been demonstrated to be a more serious problem in the developing countries than in the United States and Europe, as indicated by the VOC data obtained in Thailand, India, Pakistan and Egypt (Arayasiri et al., 2010; Rekhadevi et al., 2010; Kamal et al., 2012; Ibrahim et al., 2012). In Beirut, capital of Lebanon, air pollutant concentrations currently exceed air quality standards and guidelines (Waked and Afif, 2012). About 67% of non methanic VOC emissions are calculated to originate from the on-road transport sector and the majority of vehicles operate on gasoline (Waked and Afif, 2012).

Since concentrations of VOCs are elevated, albeit to different extents, on and near roadways, the individuals whose job requires that they spend long periods of time near vehicles may incur substantial occupational exposures to traffic-related air pollution (Knibbs and Morawska et al., 2012). It is well known that exposure data from stationary monitoring sites cannot give the real exposure profile in urban areas, since the level of traffic VOCs decreases drastically as the distance from the main traffic roads increases (Han and Naeher, 2006). More and until now, no studies have been conducted to assess of the human health risks from urban air pollution exposure in Beirut. Taken together, we carried out a personal exposure measurement campaign among traffic policemen to benzene and 1,3-butadiene (BD), since both compounds are generated from the incomplete combustion of gasoline.

Benzene and BD have been classified as Group 1 carcinogens (IARC; 2008, 2009) based on evidence for an increased genotoxic and epigenotoxic effects in both occupational exposure assessment (Ruchirawat et al., 2010; Carugno et al., 2012; Peluso et al., 2012; Seow et al., 2012; Xiang et al., 2012) and in in vivo and in vitro studies (Dagher et al., 2006; Billet et al., 2010; Koturbash et al., 2011; Sangaraju et al., 2012; Tabish et al., 2012; Abbas et al., 2013).
Biomarkers of benzene, as urinary trans, trans-muconic acid (t,t-MA) and urinary S-phenylmercapturic acid (S-PMA), have been measured mostly in fuel-related exposure such as in station attendants, in public transportation or in traffic policemen (Fustinoni et al., 2005; Barbieri et al., 2008; Manini et al., 2010), while only a few studies of traffic-related exposures to BD have been performed (Sapkota et al., 2006; Arayasiri et al., 2010).

Although, BD is a known human carcinogen emitted from mobile sources, little is known about traffic-related human exposure to this toxicant. BD is metabolized in vivo to reactive epoxides which are supposedly responsible for the observed carcinogenic effects (category 1A; EU-RAR 2002). A main metabolic pathway for these epoxides is the reaction with glutathione, leading to a urinary excretion of 3,4-dihydroxybutyl mercapturic acid and 3-monohydroxybutenyl mercapturic acids (DHBMA and MHBMA). Up to now, DHBMA and MHBMA have already been used in population surveys as a biomarker of exposure to BD (Arayasiri et al., 2010; Ruchirawat et al., 2010; Cheng et al., 2012).

Hence, it will be of great interest to conduct a biomonitoring pilot study in order to assess traffic-related VOCs in central Beirut, and to evaluate the use of biomarkers of benzene and BD exposure of urban traffic policemen. This work was, therefore, undertaken to determine t,t-MA, SPMA, MHBMA and DHBMA in urine spot samples before and at the end of a working shift, and personal air monitoring to airborne benzene, toluene, ethylbenzene, and xylene (BTEX) during the work shift. The influence of personal exposure, job activity and personal characteristics on biomarkers excretion was evaluated.
2. MATERIALS AND METHODS

2.1. Measurements campaign design

The campaign took place during May-June 2011 and included measurements of BTEX personal exposure to 47 healthy volunteers. All participants were males. The volunteers group includes 24 traffic policemen and 23 office policemen which constitute the control group. The traffic policemen group consisted of officers whose activity consists exclusively of traffic regulation at intersections of central roads in the city. All participants were carrying passive samplers for BTEX during the working hours.

All participants kept a questionnaire requesting information about lifestyle and health status and a personal daily questionnaire, where they referred to the duration of the performed activities during sampling time.

2.2. Sample collection

The sampling was conducted on Monday, which is the first day of the work week after a two days holiday over the weekend to minimize residual exposure from the previous week. The work shift was 7 h/d and 5 h/d for traffic and office policemen, respectively. Individual air samples were attached to the clothing in the breathing zone of study subjects and throughout the entire work shift. After air sampling was completed, samples were capped, transported to the laboratory, and stored at 4 °C until analysis within 8 days by the end of the sampling campaign. Urine samples were collected at both pre-shift and post-shift and stored at -80 °C until analysis. All participants gave their informed consent and the study was approved by the Ethics Committee of the Lebanese University.

2.3. Analysis of BTEX
BTEX compounds were analyzed as described previously by Avogbe and colleagues (2011). Exposure to airborne BTEX was assessed by using GABIE (Gas Adsorbent Badge for Individual Exposure) diffusive samplers (ARELCO, ARC20001UP, France) containing activated charcoal cartridge. After sampling, the badges were sealed, preserved at -80 °C and sent for analysis to the “Centre Commun de Mesure”, ULCO, Dunkerque (France). Briefly, BTEX were desorbed from the activated charcoal by using 2 mL of benzene-free carbon disulfide (Sigma, France) under agitation for 15 min. The mixture was filtered and 1 μL of the filtrate was analysed on a Gas Chromatograph (GC) (CP-3800, Varian USA) coupled to a Mass Spectrometer (1200 TQ, Varian USA) using Factor four VF-5 ms column (0.25 mm internal diameter, 30 m, film thickness 0.25 μm). The carrier gas was helium, and the flow rate was set at 1 mL/min. The GC oven was held at 40 °C for 5 min and then increased to 310 °C at the rate of 5 °C/min. The recovery rate of extraction of benzene was 99%. Data were averaged over each sampling period.

2.4. Determination of urinary metabolites

2.4.1. Urinary t,t-muconic acid (t,t-MA)

Urine samples were thawed at room temperature for 15 min with frequent stirring, and then centrifuged at 3000 g for 10 min. Aliquots of alkalized urine were applied to a strong anion exchange (SAX 500 mg 3cc) column (Varian) and subsequently washed with 3 mL 1% (v/v) acetic acid. The t,t-muconic acid was eluted with 3 mL 10% (v/v) aqueous acetic acid and then analyzed by HPLC equipped with a UV detector (Waters, Milford, USA). The concentration of t,t-MA was expressed as μg/g creatinine. The limit of detection (LOD) was 3 μg L⁻¹. The limit of quantification (LOQ) was 10 μg L⁻¹. The coefficient of variation of the method was within 12% for inter-day determination.
2.4.2. Urinary S-phenylmercapturic acid (S-PMA)

Four hundred microlitres of urine containing 10 μL of deuterated internal standards at 10 μg mL\(^{-1}\) were added to 0.400 mL of sodium acetate buffer at 10 mM and 1 mL of distilled water. Sample mixture was homogenized for 0.5 min and centrifuged at 5000 rpm for 5 min. The supernatant was loaded onto Oasis® MAX 3 cc/60 mg cartridge (Waters, Milford, USA) pre-conditioned with 2 mL of methanol (Chromanorm HPLC grade) followed by 2 mL of deionized water (Versol®, Aguettant, Lyon, France). The extraction cartridge was washed successively with 2 mL of distilled water and 2 mL of methanol. Following the final wash, the cartridges were dried for 1 min. Analytes elution was effected using 2 mL of a 1% formic acid in methanol (v/v). The extracted fraction was evaporated under a stream of nitrogen gas, then dissolved in 0.1 mL 0.1% formic acid/acetonitril (95:5 v/v) (Sigma-Aldrich, France).

Urinary S-PMA was analyzed using a Waters Acquity ultra-performance liquid chromatography (UPLC) performed on a Acquity UPLC BEH C18 (1.7 μm, 2.1 x 100 mm) at 50 °C. Samples (15 μL) were injected onto the column using a gradient elution of acetonitril and 0.01% aqueous acetic acid at a flow rate of 0.4 mL/min. Mass spectrometric selective detection was provided by a Waters Acquity Xevo TQD tandem mass spectrometer running in negative mode. Negative ions were acquired using electrospray ionization operated in the MRM (Multiple Reaction Monitoring) mode. The concentration of S-PMA was expressed as μg g\(^{-1}\) creatinine. The LOQ was 1 μg L\(^{-1}\).

2.4.3. Urinary monohydroxy-butyl mercapturic acid (MHBMA) and dihydroxybutyl mercapturic acid (DHBMA)

The preparation of urine samples for analysis of MHBMA and DHBMA was carried out as previously described for the determination of urinary S-PMA. After the clean-up procedure, samples (15 μL) were injected onto the column using a gradient elution of
acetonitril and 0.1% aqueous formic acid at a flow rate of 0.4 mL/min and were subjected to
analysis by a Waters Acquity TQD tandem mass spectrometer operating in ESI-MRM mode.
Concentrations of urinary MHBMA and DHBMA were expressed as \( \mu g \, g^{-1} \) creatinine and the
LOQ was 5 \( \mu g \, L^{-1} \) and 50 \( \mu g \, L^{-1} \), respectively.

2.4.4. Urinary creatinine

The concentration of creatinine in the urine samples was performed by the Jaffé
method using a Roche Diagnostics kit (Roche Diagnostics, France). The LOD was 34 \( \mu g \, L^{-1} \).

2.4.5. Cotinine assay

Urinary cotinine levels, as the major nicotine metabolite, were analyzed using DRI
cotinine enzyme immunoassay (Microgenics GmbH, Passau, Germany) to check the tobacco
smoke exposure reported in the lifestyle questionnaires. Subjects with cotinine levels greater
than 500 \( \mu g \, g^{-1} \) of creatinine were considered active smokers. The LOD was 34 \( \mu g \, L^{-1} \). All
the tests were performed according to the manufacturer's instructions.

2.5. Statistical Analysis

The Chi2 test was used for comparing categorical variables between groups; when
expected values within cells are < 5, Fisher exact test was used. For quantitative variables
with normal distribution, Student test or ANOVA were used to compare between two groups.
In case of non normal distribution, the Mann-Whitney or Kruskal-Wallis test were used to
compare between mean ranks for two or different groups. Correlation analysis was performed
with Spearman rank order correlation. Multivariate analysis was also performed to estimate
the influence of BTEX levels on biomarkers. We adjusted our model on several possible
potential confounders including, age, BMI, estimated vehicle counts for the exposure period,
smoking and alcohol consumption. A level of \( p < 0.05 \) was considered statistically significant (SPSS version 16.0 software).
3. RESULTS

3.1. Demographic characteristics

Table 1 represents the distribution of subjects with respect to age, smoking, duration of exposure and body mass index (BMI). Statistical results for alcohol current use are not shown due to poor positive responses to the related question. The two groups studied had similar demographic characteristics. On average, the work shift was 7 h/d and 5 h/d for traffic and office policemen, respectively. All participants were of the same social class. Within both groups, the length of employment was 4.47 ± 2.91 years (mean ± SD). Urinary cotinine levels were 890 ± 999 and 786 ± 770 µg g\(^{-1}\) creatinine for smokers and 36 ± 15.5 and 27 ± 6 µg g\(^{-1}\) creatinine for nonsmokers sampled from traffic and office policemen, respectively. In line with WHO (WHO, 1996) recommendations, only urine samples with creatinine concentration in the range 0.3–3.0 g L\(^{-1}\) were considered.

3.2. BTEX in breathing zone air

The results of personal exposure monitoring are presented in Table 2. The levels of BTEX compounds exposure were much higher in traffic policemen than in the office population. Traffic policemen were exposed to significantly higher (14-fold) BTEX compounds concentrations than office policemen (\(p < 0.001\)). Traffic policemen were currently exposed to a wide range of benzene, toluene, ethylbenzene, m-xylene, o-xylene and p-xylene levels.

A close examination at the results reveals that individual exposure to benzene values are high to previously campaigns conducted in Bologna/Italy (Maffei et al., 2005), in Ioannina/Greece (Pilidis et al., 2008), in Prague/Czech Republic (Rossnerova et al., 2009) and in Bangkok/Thailand (Arayasiri et al., 2010). It is necessary to clarify that policemen
working outdoor were performing exclusively traffic regulation throughout the entire exposure period, during which the policemen stay in the middle of the intersections and they are exposed to the direct emissions of vehicles, where the concentrations are elevated. In addition, linear regression analysis estimated that each exposure to one vehicle increases the level of individual exposure to benzene, to o-xylene and to p-xylene of 3.23 µg m\(^{-3}\) (p = 0.017), 2.6 µg m\(^{-3}\) (p = 0.05) and 3.16 µg m\(^{-3}\) (p = 0.05), respectively. No significant relationships were observed between individual toluene or ethylbenzene exposure levels and estimated vehicle counts for the exposure period.

Moreover, excellent spearman correlation (p < 0.001) were found between toluene, m-xylene, ethylbenzene, o-xylene, p-xylene and total BTEX exposure levels in the breathing zone (Table 3): in fact, emissions and combustion products from gasoline vehicles include a large variety of chemicals and additives, e.g. toluene; when those compounds are emitted from the same source, they have a nearly constant emission ratio (Barbieri et al., 2008).

### 3.3. Biomarkers of exposure

#### 3.3.1. Benzene

The median level of post-shift t,t-MA in traffic policemen was 13-fold higher than that of office policemen (p < 0.001)p = p = (Table 4). The concentrations of pre- and post-shift t,t-MA were significantly different in office policemen (p = 0.03). The concentration of post-shift S-PMA in traffic policemen was not shown to be significantly different from that of the office policemen. No significant associations were found between personal benzene, toluene, ethylbenzene, m-xylene, o-xylene, p-xylene or total BTEX exposure and S-PMA or t,t-MA levels (Table 3).

#### 3.3.2. 1,3-Butadiene
The median level of pre-shift MHBMA in traffic policemen was 6.5-fold higher than that of office policemen \( (p < 0.001) \). The concentration of post-shift MHBMA in traffic policemen was not shown to be significantly different from that of the office policemen (Table 4). The median level of pre-shift DHBMA in traffic policemen was 2.7-fold higher than that of office policemen \( (p < 0.001) \) and 2.8-fold higher at post-shift \( (p < 0.01) \) compared between traffic and office policemen. A strong significant correlation between personal toluene, ethylbenzene, m-xylene, o-xylene, p-xylene or total BTEX exposure and urinary post-shift DHBMA (Table 3). The association between personal total BTEX exposure and post-shift DHBMA may indicate the same source of emissions, \textit{i.e.} automobile exhaust. Significant difference was found between sub-groups of non-smokers, light to moderate smokers and heavy smokers only at pre-shift \( t,t\)-MA levels \( (p = 0.01) \) (Kruskal-Wallis test). Median levels of urinary post-shift MHBMA in the urine of heavy smokers was 5.6-fold higher than in non-smokers \( (p = 0.025) \) (Mann-Whitney test). Urinary S-PMA and DHBMA values did not discriminate exposure resulting from smoking habits and no significant difference was found between smokers and non-smokers (data not shown). Nevertheless, significant difference was found between sub-groups of non-smokers, light to moderate smokers and heavy smokers at pre-shift and post-shift MHBMA results \( (p = 0.007 \) and \( p = 0.003) \) (Kruskal-Wallis test). Median levels of urinary post-shift MHBMA in the urine of light to moderate smokers and heavy smokers were, respectively, 4.9-fold and 4.1-fold higher than in non-smokers.

### 3.4. Multiple regression analysis

Based on multiple regression analyses, smoking consumption was not found to have a significant influence on DHBMA which was strongly related to \( \text{logBTEX} \) \( (\beta = 0.535; p < \)
0.001), showing that there is a difference in sensitivity between the two urinary metabolites of BD exposure measured in this study. Nevertheless, age was negatively related to logDHBMA ($\beta = -0.286; p = 0.045$).
4. DISCUSSION

Environmental exposure to VOCs among workers has been already described elsewhere (Barbieri et al., 2008; Pilidis et al., 2008; Arayasiri et al., 2010; Manini et al., 2010), whereas it has not been reported yet for Beirut traffic policemen. For these reasons, we have undertaken an extensive investigation of the occupational exposure to VOCs, notably, benzene and BD, which are carcinogenic substances in urban air from incomplete combustion of fossil fuels, through personal air monitoring, as well as through the use of biomarkers of exposure. In a previous studies conducted in Beirut, it has been reported that the spatial distribution of VOCs emissions are mostly over Beirut and its suburbs, where there are dense populations and heavy traffic (Waked et al., 2012). While ambient air monitoring of VOCs-compounds has not been reported at roadsides in Beirut area, individual exposure levels to BTEX compounds in traffic policemen were significantly higher than in office policemen. These levels were far below the Occupational Safety and Health Administration (OSHA) exposure limits, 8-h time weight average of 3.2 mg m$^{-3}$ for benzene (OSHA, 1999), of 375 mg m$^{-3}$ for toluene (OSHA, 1999), of 435 mg m$^{-3}$ for ethylbenzene (OSHA, 1999) and of 435 mg m$^{-3}$ for xylene (OSHA, 2008). Moreover, our results have shown that each exposure to one vehicle increases the level of individual exposure to benzene, o-xylene and to p-xylene due to the fact that xylene is primarily released from motor vehicle exhaust where it is used as a solvent (Kandyala et al., 2010). Evidence presented herein extended these observations by showing that these personal exposure levels indicated higher emissions related to vehicles in Beirut area that are highly traffic congested and to the fact that in Lebanon personal vehicles are the prevailing mode of transport.

In addition, individual exposure to benzene (mean = 48.8 μg m$^{-3}$) in the traffic policemen in this study was higher than that measured in traffic policemen in Bologna/Italy (mean = 17.3
μg m$^{-3}$) (Barbieri et al., 2008), in Ioannina/Greece (mean = 30 μg m$^{-3}$) (Pilidis et al., 2008), in Prague/Czech Republic (mean concentration in February = 6.99 and in May = 4.53 μg m$^{-3}$) (Rossnerova et al., 2009) and in Bangkok/Thailand (mean=38.24 μg m$^{-3}$) (Arayasiri et al., 2010). The same for other BTEX compounds, individual exposure to toluene, ethylbenzene, m-xylene and o-xylene in the traffic policemen in this study were strongly higher than that measured in traffic policemen in Prague/Czech Republic (mean concentration in May = 13.99, 3.25, 10.95 and 3.75 μg m$^{-3}$, respectively) (Rossnerova et al., 2009). Difference in many of environment factors, such as traffic characteristics, quality of fuel, meteorological conditions, building characteristics of the area, and difference in physical activity in the workplace may contribute to the difference in the levels of individual VOCs exposure. Although excellent spearman correlations ($p < 0.001$) were found between toluene, ethylbenzene, m-xylene, o-xylene, p-xylene and total BTEX exposure levels, we did not find significant correlation between benzene and none of BTEX compounds. Even if these compounds were emitted from the same source (Barbieri et al., 2008), we suggest that lack of any such correlation may be due to the fact that some individual values reported for benzene exposure were below the LOD (< 0.3 μg m$^{-3}$).

In our study, $t,t$-MA concentration was found to be a better indicator of the levels of benzene exposure than S-PMA. Urinary post-shift $t,t$-MA levels were significantly higher in traffic policemen compared with office policemen (Table 4). In a previous studies with Italian policemen who had levels of individual benzene exposure of 17.3 μg m$^{-3}$ (Barbieri et al., 2008), i.e. lower than in this study, post-shift $t,t$-MA levels were comparable (44 μg m$^{-3}$ for non-smokers and 120 μg m$^{-3}$ for smokers) (Manini et al., 2010), while the post-shift S-PMA levels were much lower in both studies (approximately 7-fold). However, the levels of S-PMA and $t,t$-MA were not significantly different between pre- and post-shift samples, excepted for $t,t$-MA results within office policemen group ($p = 0.03$). A close examination at
individual results reveals a decrease of urinary S-PMA by the end of the work shift. A previous study, conducted by Qu and colleagues (2000), estimated the urinary half life of elimination ($t_{1/2}$) of S-PMA to be 12.8 h. Thus, higher levels in pre-shift S-PMA could be the result of cumulative exposure to benzene during the previous day. Moreover, ingestion of unknown amounts of dietary sorbic acid and glutathione-S-transferase ($GSTM1$, $GSTT1$ and $GSTA1$) polymorphism may influence variability in metabolites levels (Manini at el., 2010). Moreover, we denote some urinary S-PMA and $t,t$-MA values below the LOQ ($< 1 \mu g \ L^{-1}$ and $< 10 \mu g \ L^{-1}$, respectively). It seems that biotransformation of benzene to its metabolites is reduced by co-exposure to other chemicals and additives emitted from gasoline vehicles (Barbieri et al., 2008) such as toluene (a competitive inhibitor of benzene for CYP metabolism) which would affect the pharmacokinetics and metabolism of benzene, including conjugation with glutathione and subsequent mercapturic acid excretion (Johnson et al., 2007). Accordingly to previous studies, no significant associations were found between personal benzene or personal BTEX compounds exposure and S-PMA or $t,t$-MA levels (Table 3) (Barbieri et al., 2008; Arayasiri et al., 2010). The lack of any such correlation may be due to the confounding effect of smoking on metabolite excretion (Carrieri et al., 2010; 2012) or to the fact that some values reported for benzene exposure, urinary PMA and $t,t$-MA were below the LOQ. Moreover, urinary $t,t$-MA level may be affected by the diet of the subjects examined (Carrieri et al., 2010; Weisel, 2010). For BD exposure in ambient air, a limited number of biomonitoring studies have been conducted. Pre- and post-shift DHBMA levels in traffic policemen were significantly higher than in office policemen (Table 4). We found that urinary pre-shift MHBMA and DHBMA concentrations in traffic policemen were significantly higher than office policemen, which indicates that elimination of those metabolites from previous BD exposure is not yet completed. This suggests that the average urinary $t_{1/2}$ of MHBMA and DHBMA is somewhat protracted compared to the $t_{1/2}$ of other
mercapturic acids, such as the benzene metabolite S-PMA and \( t,t \)-MA, which have an average
\( \text{t} \frac{1}{2} \) of 12.8 h and 13.7 h, respectively (Qu et al., 2000; van Sittert et al., 2000; Albertini et al.,
2003). A close examination at individual results reveals a decrease of urinary MHBMA level
by the end of the work shift. In view of the complexity of the metabolic pathways involved in
the biotransformation of butadiene, we expected an effect of the genetic polymorphisms in
xenobiotic metabolizing enzymes which affects the excretion of urinary metabolites. We also
suggest that co-exposure to aromatic hydrocarbons (e.g. toluene) may cause a decrease in
epoxide metabolites levels, namely formation via cytochrome P-450 2E1, and a decreased
excretion urinary metabolites of BD (Johnson et al., 2007; Vacek et al., 2010; Fustinoni et al.,
2012).

Alternatively, DHBMA concentrations were found to be a better indicator of the levels of
BD exposure in urban air than MHBMA, as reported previously (Sapkota et al., 2006). Pre-
and post-shift DHMBA levels could significantly distinguish between office and traffic
policemen and showed a better correlation with personal total BTEX exposure.

Multiple regression analysis applied to evaluate the contribution of each predictor to the
variability of urinary biomarkers of benzene and BD. Traffic and office policemen stated to
have smoked during working. Although levels of BD in the breathing zone of study subjects
have not been reported, it seems that smoking habits influence the total intake of BD (WHO,
2000). Unexpectedly, there was not a marked effect of smoking habits on DHBMA. Multiple
regression analysis showed that the levels of individual BTEX exposure significantly
contributed to increase urinary DHBMA (\( \beta=0.535 \), \( p < 0.001 \)). Accordingly, DHBMA values
did not discriminate exposure resulting from smoking habits and no significant difference
was found between smokers and non-smokers.

In conclusion, these results indicated that traffic policemen, who are exposed to benzene
and BD at the roadside in central Beirut, are potentially at a higher risk for development of
diseases such as cancer than office policemen. Further studies need to focus on the lifestyle and genetic factors that may affect the background levels of S-PMA, t,t-MA, MHBMA and DHBMA. Given chaotic traffic conditions, quality of fuel, high rate of passenger cars ownership, meteorological conditions, building characteristics of the area, and high pollution rates observed in Beirut, increased urinary exposure biomarker would result in a higher risk in the exposed subjects. Accordingly, public authorities should particularly set policies aimed to reduce traffic-related air pollution.
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TABLES

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Table 1. Summary of information collected by questionnaire.

<table>
<thead>
<tr>
<th></th>
<th>Traffic policemen (n=24)</th>
<th>Office policemen (n=23)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years, mean ± SD)</td>
<td>27.4±4.6</td>
<td>29.1±3.6</td>
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<tr>
<td>Percentage of never/current-smokers</td>
<td>37</td>
<td>56</td>
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<tr>
<td>Cigarettes/d (mean ± SD)</td>
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<td>26.2±23</td>
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<tr>
<td>Sampling period (h, mean ± SD)</td>
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<td>45.2±3.4</td>
<td>&lt;0.001</td>
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<tr>
<td>BMI (kg m², mean ± SD)</td>
<td>25.6±4.6</td>
<td>27.9±4.3</td>
<td>0.125</td>
</tr>
</tbody>
</table>

Significant at p < 0.05. Student’s t test.
**Table 2.** Distribution of individual VOCs exposure in subgroups of policemen. Values are expressed as geometric medians, means and geometric standard deviations. Concentrations are expressed as µg/m\(^3\).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Traffic policemen (n=24)</th>
<th>Office policemen (n=23)</th>
</tr>
</thead>
</table>
|           | Median | Min-Max | Mean ± SD | Median | Min-Max | Mean ± SD | p value  
| Benzene (n=21) | 0.3    | 0.3-867.8 | 48.8±189.3 | 0.3    | 0.3-10.8 | 1.2±2.3 | n.s.  
| Toluene (n=20) | 101.8  | 29.8-9788.4 | 11520.±2393.0 | 9.3    | 0.44-46.3 | 12.1±11.3 | <0.001  
| Ethylbenzene (n=21) | 58.1   | 6.7-5845.2 | 662.3±1420.3 | 4.5    | 0.88-19.1 | 5.7±4.4 | <0.001  
| mXylene (n=21) | 102.8  | 29.1-1209.6 | 196.7±287.6 | 2.0    | 0.65-10.6 | 2.5±2.2 | <0.001  
| oXylene (n=21) | 21.3   | 1.0-1839.1 | 177.7±432.3 | 1.7    | 0.58-5.64 | 2.0±1.3 | <0.001  
| pXylene (n=15) | 3.9    | 1.2-30.1 | 9.6±10.0 | 0.4    | 0.18-2.1 | 0.6±0.4 | <0.001  
| BTEX (n=21) | 295.1  | 85.8-18709.1 | 2189.0±4450.8 | 21.4   | 3.6-84.1 | 24.3±19.6 | <0.001  

Significant at \( p < 0.05 \). Mann-Whitney test, n.s. = not significant.
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<th>Tol</th>
<th>Ethylbz</th>
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<th>oXyl</th>
<th>pXyl</th>
<th>BTEX</th>
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<th>S-PMA₂</th>
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<th>t,t-MA₂</th>
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</table>
Missing values correspond to lack of correlation between parameters. Only significant data are represented. ¹ Pre-shift; ² Post-shift. ** \( p < 0.001; \)

\* \( p < 0.05 \)
Table 4. Biomarkers of exposure in traffic and office policemen.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Study groups</th>
<th>Traffic policemen</th>
<th>Office policemen</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Pre-shift</td>
<td>Post-shift</td>
</tr>
<tr>
<td>Urinary ( t,t )-MA (μg/g creatinine)</td>
<td></td>
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<tr>
<td>Pre-shift</td>
<td>64.7±141.5</td>
<td>14±13.3</td>
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</tr>
<tr>
<td></td>
<td>8.2 (3-586.7)</td>
<td>11.2 (2.5-56.5)</td>
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<tr>
<td></td>
<td>(n=24)</td>
<td>(n=23)</td>
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<tr>
<td>Post-shift</td>
<td>84.4±100.6(^a)</td>
<td>55.5±87(^a)</td>
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<tr>
<td></td>
<td>61 (2.4-444.8)</td>
<td>18.3 (2.5-343.4)</td>
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<td></td>
<td>(n=24)</td>
<td>(n=22)</td>
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<tr>
<td>Urinary S-PMA (μg/g creatinine)</td>
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<tr>
<td>Pre-shift</td>
<td>6.2±6(^a)</td>
<td>2.6±2</td>
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<tr>
<td></td>
<td>4 (1.1-25)</td>
<td>1.7 (0.9-8.1)</td>
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<tr>
<td>Post-shift</td>
<td>5.3±9</td>
<td>2.7±2.4</td>
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<td>2.6 (0.5-45.5)</td>
<td>1.9 (0.4-10.2)</td>
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<tr>
<td>Urinary MHBMA (μg/g creatinine)</td>
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<tr>
<td>Pre-shift</td>
<td>24.7±23.3(^a)</td>
<td>17.7±38.7</td>
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<td></td>
<td>20.1 (2.4-108.3)</td>
<td>3.1 (1.3-147.6)</td>
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<tr>
<td>Post-shift</td>
<td>18.7±20.1</td>
<td>18.8±31</td>
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<td>10.2 (0.2-88.6)</td>
<td>7.5 (1.2-120)</td>
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<tr>
<td></td>
<td>Pre-shift</td>
<td>Post-shift</td>
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<tr>
<td><strong>Urinary DHBMA (μg/g creatinine)</strong></td>
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<tr>
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<td>180.4±73.8&lt;sup&gt;a&lt;/sup&gt;</td>
<td>69.2±43.6</td>
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<td>182.9 (47.6-333.3)</td>
<td>66.5 (13.2-239.3)</td>
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<td><strong>Urinary Cotinine (μg/g creatinine)</strong></td>
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<td>24.6 (8.5-1200.8)</td>
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<td>601.0±780.6</td>
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<tr>
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<td>459.3 (12.1-3583.9)</td>
<td>19.9 (7.9-1362.4)</td>
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<td>(n=24)</td>
<td>(n=22)</td>
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</table>

Values are expressed as mean±SD on the first line and median (min-max) on the second line of each parameter. Statistically significant difference from office policemen at <sup>a</sup><em>p</em> < 0.001 or <sup>b</sup><em>p</em> < 0.01. Statistically significant difference from the corresponding pre-shift at <em>p</em> = 0.03.