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1           **TRAFFIC-RELATED AIR POLLUTION. A PILOT EXPOSURE**

2                           **ASSESSMENT IN BEIRUT, LEBANON**

3  
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22

## 23 ABSTRACT

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

47 benzene and BD at the roadside in central Beirut, are potentially at a higher risk for  
48 development of diseases such as cancer than office policemen.

49

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

52

## 53 1. INTRODUCTION

54 Traffic-related volatile organic compounds (VOCs) pollution has frequently been  
55 demonstrated to be a more serious problem in the developing countries than in the United  
56 States and Europe, as indicated by the VOC data obtained in Thailand, India, Pakistan and  
57 Egypt (Arayasiri et al., 2010; Rekhadevi et al., 2010; Kamal et al., 2012; Ibrahim et al.,  
58 2012). In Beirut, capital of Lebanon, air pollutant concentrations currently exceed air quality  
59 standards and guidelines (Waked and Afif, 2012). About 67% of non methanic VOC  
60 emissions are calculated to originate from the on-road transport sector and the majority of  
61 vehicles operate on gasoline (Waked and Afif, 2012).

62 Since concentrations of VOCs are elevated, albeit to different extents, on and near  
63 roadways, the individuals whose job requires that they spend long periods of time near  
64 vehicles may incur substantial occupational exposures to traffic-related air pollution (Knibbs  
65 and Morawska et al., 2012). It is well known that exposure data from stationary monitoring  
66 sites cannot give the real exposure profile in urban areas, since the level of traffic VOCs  
67 decreases drastically as the distance from the main traffic roads increases (Han and Naehner,  
68 2006). More and until now, no studies have been conducted to assess of the human health  
69 risks from urban air pollution exposure in Beirut. Taken together, we carried out a personal  
70 exposure measurement campaign among traffic policemen to benzene and 1,3-butadiene  
71 (BD), since both compounds are generated from the incomplete combustion of gasoline.

72 Benzene and BD have been classified as Group 1 carcinogens (IARC; 2008, 2009)  
73 based on evidence for an increased genotoxic and epigenotoxic effects in both occupational  
74 exposure assessment (Ruchirawat et al., 2010; Carugno et al., 2012; Peluso et al., 2012; Seow  
75 et al., 2012; Xiang et al., 2012) and in *in vivo* and *in vitro* studies (Dagher et al., 2006; Billet  
76 et al., 2010; Koturbash et al., 2011; Sangaraju et al., 2012; Tabish et al., 2012; Abbas et al.,  
77 2013).

78 Biomarkers of benzene, as urinary trans, trans-muconic acid (*t,t*-MA) and urinary S-  
79 phenylmercapturic acid (S-PMA), have been measured mostly in fuel-related exposure such  
80 as in station attendants, in public transportation or in traffic policemen (Fustinoni et al., 2005;  
81 Barbieri et al., 2008; Manini et al., 2010), while only a few studies of traffic-related  
82 exposures to BD have been performed (Sapkota et al., 2006; Arayasiri et al., 2010).

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

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

98

99 **2. MATERIALS AND METHODS**

100

101 **2.1. Measurements campaign design**

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

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

111

112 **2.2. Sample collection**

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

122

123 **2.3. Analysis of BTEX**

124 BTEX compounds were analyzed as described previously by Avogbe and colleagues  
125 (2011). Exposure to airborne BTEX was assessed by using GABIE (Gas Adsorbent Badge for  
126 Individual Exposure) diffusive samplers (ARELCO, ARC20001UP, France) containing  
127 activated charcoal cartridge. After sampling, the badges were sealed, preserved at -80 °C and  
128 sent for analysis to the “Centre Commun de Mesure”, ULCO, Dunkerque (France). Briefly,  
129 BTEX were desorbed from the activated charcoal by using 2 mL of benzene-free carbon  
130 disulfide (Sigma, France) under agitation for 15 min. The mixture was filtered and 1 µL of  
131 the filtrate was analysed on a Gas Chromatograph (GC) (CP-3800, Varian USA) coupled to a  
132 Mass Spectrometer (1200 TQ, Varian USA) using Factor four VF-5 ms column (0.25 mm  
133 internal diameter, 30 m, film thickness 0.25 µm). The carrier gas was helium, and the flow  
134 rate was set at 1 mL/min. The GC oven was held at 40 °C for 5 min and then increased to 310  
135 °C at the rate of 5 °C/min. The recovery rate of extraction of benzene was 99%. Data were  
136 averaged over each sampling period.

137

## 138 **2.4. Determination of urinary metabolites**

### 139 2.4.1. Urinary *t,t*-muconic acid (*t,t*-MA)

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

148



149 2.4.2. Urinary S-phenylmercapturic acid (S-PMA)

150 Four hundred microlitres of urine containing 10  $\mu\text{L}$  of deuterated internal standards at  
151  $10 \mu\text{g mL}^{-1}$  were added to 0.400 mL of sodium acetate buffer at 10 mM and 1 mL of distilled  
152 water. Sample mixture was homogenized for 0.5 min and centrifuged at 5000 rpm for 5 min.  
153 The supernatant was loaded onto Oasis® MAX 3 cc/60 mg cartridge (Waters, Milford, USA)  
154 pre-conditioned with 2 mL of methanol (Chromanorm HPLC grade) followed by 2 mL of  
155 deionized water (Versol®, Aguetant, Lyon, France). The extraction cartridge was washed  
156 successively with 2 mL of distilled water and 2 mL of methanol. Following the final wash,  
157 the cartridges were dried for 1 min. Analytes elution was effected using 2 mL of a 1% formic  
158 acid in methanol (v/v). The extracted fraction was evaporated under a stream of nitrogen gas,  
159 then dissolved in 0.1 mL 0.1% formic acid/acetonitril (95:5 v/v) (Sigma-Aldrich, France).

160 Urinary S-PMA was analyzed using a Waters Acquity ultra-performance liquid  
161 chromatography (UPLC) performed on a Acquity UPLC BEH C18 (1.7  $\mu\text{m}$ , 2.1 x 100 mm) at  
162 50 °C. Samples (15  $\mu\text{L}$ ) were injected onto the column using a gradient elution of acetonitril  
163 and 0.01% aqueous acetic acid at a flow rate of 0.4 mL/min. Mass spectrometric selective  
164 detection was provided by a Waters Acquity Xevo TQD tandem mass spectrometer running  
165 in negative mode. Negative ions were acquired using electrospray ionization operated in the  
166 MRM (Multiple Reaction Monitoring) mode. The concentration of S-PMA was expressed as  
167  $\mu\text{g g}^{-1}$  creatinine. The LOQ was  $1 \mu\text{g L}^{-1}$ .

168

169 2.4.3. Urinary monohydroxy-butenyl mercapturic acid (MHBMA) and dihydroxybutyl  
170 mercapturic acid (DHBMA)

171 The preparation of urine samples for analysis of MHBMA and DHBMA was carried  
172 out as previously described for the determination of urinary S-PMA. After the clean-up  
173 procedure, samples (15  $\mu\text{L}$ ) were injected onto the column using a gradient elution of

174 acetonitril and 0.1% aqueous formic acid at a flow rate of 0.4 mL/min and were subjected to  
175 analysis by a Waters Acquity TQD tandem mass spectrometer operating in ESI-MRM mode.  
176 Concentrations of urinary MHBMA and DHBMA were expressed as  $\mu\text{g g}^{-1}$  creatinine and the  
177 LOQ was  $5 \mu\text{g L}^{-1}$  and  $50 \mu\text{g L}^{-1}$ , respectively.

178

#### 179 2.4.4. Urinary creatinine

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

182

#### 183 2.4.5. Cotinine assay

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

189

### 190 2.5. Statistical Analysis

191 The Chi2 test was used for comparing categorical variables between groups; when  
192 expected values within cells are  $< 5$ , Fisher exact test was used. For quantitative variables  
193 with normal distribution, Student test or ANOVA were used to compare between two groups.  
194 In case of non normal distribution, the Mann-Whitney or Kruskal-Wallis test were used to  
195 compare between mean ranks for two or different groups. Correlation analysis was performed  
196 with Spearman rank order correlation. Multivariate analysis was also performed to estimate  
197 the influence of BTEX levels on biomarkers. We adjusted our model on several possible  
198 potential confounders including, age, BMI, estimated vehicle counts for the exposure period,

**199** smoking and alcohol consumption. A level of  $p < 0.05$  was considered statistically significant

**200** (SPSS version 16.0 software).

**201**

## 202 3. RESULTS

203

### 204 3.1. Demographic characteristics

205 Table 1 represents the distribution of subjects with respect to age, smoking, duration  
206 of exposure and body mass index (BMI). Statistical results for alcohol current use are not  
207 shown due to poor positive responses to the related question. The two groups studied had  
208 similar demographic characteristics. On an average, the work shift was 7 h/d and 5 h/d for  
209 traffic and office policemen, respectively. All participants were of the same social class.  
210 Within both groups, the length of employment was  $4.47 \pm 2.91$  years (mean  $\pm$  SD). Urinary  
211 cotinine levels were  $890 \pm 999$  and  $786 \pm 770 \mu\text{g g}^{-1}$  creatinine for smokers and  $36 \pm 15.5$  and  
212  $27 \pm 6 \mu\text{g g}^{-1}$  creatinine for nonsmokers sampled from traffic and office policemen,  
213 respectively. In line with WHO (WHO, 1996) recommendations, only urine samples with  
214 creatinine concentration in the range  $0.3\text{--}3.0 \text{ g L}^{-1}$  were considered.

215

### 216 3.2. BTEX in breathing zone air

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

223 A close examination at the results reveals that individual exposure to benzene values  
224 are high to previously campaigns conducted in Bologna/Italy (Maffei et al., 2005), in  
225 Ioannina/Greece (Pilidis et al., 2008), in Prague/Czech Republic (Rossnerova et al., 2009)  
226 and in Bangkok/Thailand (Arayasiri et al., 2010). It is necessary to clarify that policemen

227 working outdoor were performing exclusively traffic regulation throughout the entire  
228 exposure period, during which the policemen stay in the middle of the intersections and they  
229 are exposed to the direct emissions of vehicles, where the concentrations are elevated. In  
230 addition, linear regression analysis estimated that each exposure to one vehicle increases the  
231 level of individual exposure to benzene, to o-xylene and to p-xylene of  $3.23 \mu\text{g m}^{-3}$  ( $p =$   
232  $0.017$ ),  $2.6 \mu\text{g m}^{-3}$  ( $p = 0.05$ ) and  $3.16 \mu\text{g m}^{-3}$  ( $p = 0.05$ ), respectively. No significant  
233 relationships were observed between individual toluene or ethylbenzene exposure levels and  
234 estimated vehicle counts for the exposure period.

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

240

### 241 **3.3. Biomarkers of exposure**

#### 242 **3.3.1. Benzene**

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

250

#### 251 **3.3.2. 1,3-Butadiene**

252 The median level of pre-shift MHBMA in traffic policemen was 6.5-fold higher than  
253 that of office policemen ( $p < 0.001$ ). The concentration of post-shift MHBMA in traffic  
254 policemen was not shown to be significantly different from that of the office policemen  
255 (Table 4). The median level of pre-shift DHBMA in traffic policemen was 2.7-fold higher  
256 than that of office policemen ( $p < 0.001$ ) and 2.8-fold higher at post-shift ( $p < 0.01$ )  
257 compared between traffic and office policemen. A strong significant correlation between  
258 personal toluene, ethylbenzene, m-xylene, o-xylene, p-xylene or total BTEX exposure and  
259 urinary post-shift DHBMA (Table 3). The association between personal total BTEX exposure  
260 and post-shift DHBMA may indicate the same source of emissions, *i.e.* automobile  
261 exhaust. Significant difference was found between sub-groups of non-smokers, light to  
262 moderate smokers and heavy smokers only at pre-shift *t,t*-MA levels ( $p = 0.01$ ) (Kruskal-  
263 Wallis test). Median levels of urinary post-shift MHBMA in the urine of heavy smokers was  
264 5.6-fold higher than in non-smokers ( $p = 0.025$ ) (Mann-Whitney test). Urinary S-PMA and  
265 DHBMA values did not discriminate exposure resulting from smoking habits and no  
266 significant difference was found between smokers and non-smokers (data not shown).  
267 Nevertheless, significant difference was found between sub-groups of non-smokers, light to  
268 moderate smokers and heavy smokers at pre-shift and post-shift MHBMA results ( $p = 0.007$   
269 and  $p = 0.003$ ) (Kruskal-Wallis test). Median levels of urinary post-shift MHBMA in the  
270 urine of light to moderate smokers and heavy smokers were, respectively, 4.9-fold and 4.1-  
271 fold higher than in non-smokers.

272

### 273 3.4. Multiple regression analysis

274

275 Based on multiple regression analyses, smoking consumption was not found to have a  
276 significant influence on DHBMA which was strongly related to logBTEX ( $\beta = 0.535$ ;  $p <$

277 0.001), showing that there is a difference in sensitivity between the two urinary metabolites  
278 of BD exposure measured in this study. Nevertheless, age was negatively related to  
279 logDHBMA ( $\beta = -0.286$ ;  $p = 0.045$ ).

280

## 281 4. DISCUSSION

282

283 Environmental exposure to VOCs among workers has been already described  
284 elsewhere (Barbieri et al., 2008; Pilidis et al., 2008; Arayasiri et al., 2010; Manini et al.,  
285 2010), whereas it has not been reported yet for Beirut traffic policemen. For these reasons, we  
286 have undertaken an extensive investigation of the occupational exposure to VOCs, notably,  
287 benzene and BD, which are carcinogenic substances in urban air from incomplete combustion  
288 of fossil fuels, through personal air monitoring, as well as through the use of biomarkers of  
289 exposure. In a previous studies conducted in Beirut, it has been reported that the spatial  
290 distribution of VOCs emissions are mostly over Beirut and its suburbs, where there are dense  
291 populations and heavy traffic (Waked et al., 2012). While ambient air monitoring of VOCs-  
292 compounds has not been reported at roadsides in Beirut area, individual exposure levels to  
293 BTEX compounds in traffic policemen were significantly higher than in office policemen.  
294 These levels were far below the Occupational Safety and Health Administration (OSHA)  
295 exposure limits, 8-h time weight average of  $3.2 \text{ mg m}^{-3}$  for benzene (OSHA, 1999), of 375  
296  $\text{mg m}^{-3}$  for toluene (OSHA, 1999), of  $435 \text{ mg m}^{-3}$  for ethylbenzene (OSHA, 1999) and of 435  
297  $\text{mg m}^{-3}$  for xylene (OSHA, 2008). Moreover, our results have shown that each exposure to  
298 one vehicle increases the level of individual exposure to benzene, o-xylene and to p-xylene  
299 due to the fact that xylene is primarily released from motor vehicle exhaust where it is used  
300 as a solvent (Kandyala et al., 2010). Evidence presented herein extended these observations  
301 by showing that these personal exposure levels indicated higher emissions related to vehicles  
302 in Beirut area that are highly traffic congested and to the fact that in Lebanon personal  
303 vehicles are the prevailing mode of transport.

304 In addition, individual exposure to benzene (mean =  $48.8 \mu\text{g m}^{-3}$ ) in the traffic policemen in  
305 this study was higher than that measured in traffic policemen in Bologna/Italy (mean =  $17.3$



306  $\mu\text{g m}^{-3}$ ) (Barbieri et al., 2008), in Ioannina/Greece (mean =  $30 \mu\text{g m}^{-3}$ ) (Pilidis et al., 2008), in  
307 Prague/Czech Republic (mean concentration in February = 6.99 and in May =  $4.53 \mu\text{g m}^{-3}$ )  
308 (Rossnerova et al., 2009) and in Bangkok/Thailand (mean= $38.24 \mu\text{g m}^{-3}$ ) (Arayasiri et al.,  
309 2010). The same for other BTEX compounds, individual exposure to toluene, ethylbenzene,  
310 m-xylene and o-xylene in the traffic policemen in this study were strongly higher than that  
311 measured in traffic policemen in Prague/Czech Republic (mean concentration in May =  
312 13.99, 3.25, 10.95 and  $3.75 \mu\text{g m}^{-3}$ , respectively) (Rossnerova et al., 2009). Difference in  
313 many of environment factors, such as traffic characteristics, quality of fuel, meteorological  
314 conditions, building characteristics of the area, and difference in physical activity in the  
315 workplace may contribute to the difference in the levels of individual VOCs exposure.  
316 Although excellent spearman correlations ( $p < 0.001$ ) were found between toluene,  
317 ethylbenzene, m-xylene, o-xylene, p-xylene and total BTEX exposure levels, we did not find  
318 significant correlation between benzene and none of BTEX compounds. Even if these  
319 compounds were emitted from the same source (Barbieri et al., 2008), we suggest that lack of  
320 any such correlation may be due to the fact that some individual values reported for benzene  
321 exposure were below the LOD ( $< 0.3 \mu\text{g m}^{-3}$ ).

322 In our study, *t,t*-MA concentration was found to be a better indicator of the levels of  
323 benzene exposure than S-PMA. Urinary post-shift *t,t*-MA levels were significantly higher in  
324 traffic policemen compared with office policemen (Table 4). In a previous studies with Italian  
325 policemen who had levels of individual benzene exposure of  $17.3 \mu\text{g m}^{-3}$  (Barbieri et al.,  
326 2008), *i.e.* lower than in this study, post-shift *t,t*-MA levels were comparable ( $44 \mu\text{g m}^{-3}$  for  
327 non-smokers and  $120 \mu\text{g m}^{-3}$  for smokers) (Manini et al., 2010), while the post-shift S-PMA  
328 levels were much lower in both studies (approximately 7-fold). However, the levels of S-  
329 PMA and *t,t*-MA were not significantly different between pre- and post-shift samples,  
330 excepted for *t,t*-MA results within office policemen group ( $p = 0.03$ ). A close examination at

331 individual results reveals a decrease of urinary S-PMA by the end of the work shift. A  
332 previous study, conducted by Qu and colleagues (2000), estimated the urinary half life of  
333 elimination ( $t_{1/2}$ ) of S-PMA to be 12.8 h. Thus, higher levels in pre-shift S-PMA could be the  
334 result of cumulative exposure to benzene during the previous day. Moreover, ingestion of  
335 unknown amounts of dietary sorbic acid and glutathione-S-transferase (*GSTM1*, *GSTT1* and  
336 *GSTA1*) polymorphism may influence variability in metabolites levels (Manini et al., 2010).  
337 Moreover, we denote some urinary S-PMA and *t,t*-MA values below the LOQ ( $< 1 \mu\text{g L}^{-1}$   
338 and  $< 10 \mu\text{g L}^{-1}$ , respectively). It seems that biotransformation of benzene to its metabolites is  
339 reduced by co-exposure to other chemicals and additives emitted from gasoline vehicles  
340 (Barbieri et al., 2008) such as toluene (a competitive inhibitor of benzene for CYP  
341 metabolism) which would affect the pharmacokinetics and metabolism of benzene, including  
342 conjugation with glutathione and subsequent mercapturic acid excretion (Johnson et al.,  
343 2007). Accordingly to previous studies, no significant associations were found between  
344 personal benzene or personal BTEX compounds exposure and S-PMA or *t,t*-MA levels  
345 (Table 3) (Barbieri et al., 2008; Arayasiri et al., 2010). The lack of any such correlation may  
346 be due to the confounding effect of smoking on metabolite excretion (Carrieri et al., 2010;  
347 2012) or to the fact that some values reported for benzene exposure, urinary PMA and *t,t*-MA  
348 were below the LOQ. Moreover, urinary *t,t*-MA level may be affected by the diet of the  
349 subjects examined (Carrieri et al., 2010; Weisel, 2010). For BD exposure in ambient air, a  
350 limited number of biomonitoring studies have been conducted. Pre- and post-shift DHBMA  
351 levels in traffic policemen were significantly higher than in office policemen (Table 4). We  
352 found that urinary pre-shift MHBMA and DHBMA concentrations in traffic policemen were  
353 significantly higher than office policemen, which indicates that elimination of those  
354 metabolites from previous BD exposure is not yet completed. This suggests that the average  
355 urinary  $t_{1/2}$  of MHBMA and DHBMA is somewhat protracted compared to the  $t_{1/2}$  of other

356 mercapturic acids, such as the benzene metabolite S-PMA and *t,t*-MA, which have an average  
357  $t_{1/2}$  of 12.8 h and 13.7 h, respectively (Qu et al., 2000; van Sittert et al., 2000; Albertini et al.,  
358 2003). A close examination at individual results reveals a decrease of urinary MHBMA level  
359 by the end of the work shift. In view of the complexity of the metabolic pathways involved in  
360 the biotransformation of butadiene, we expected an effect of the genetic polymorphisms in  
361 xenobiotic metabolizing enzymes which affects the excretion of urinary metabolites. We also  
362 suggest that co-exposure to aromatic hydrocarbons (*e.g.* toluene) may cause a decrease in  
363 epoxide metabolites levels, namely formation via cytochrome P-450 2E1, and a decreased  
364 excretion urinary metabolites of BD (Johnson et al., 2007; Vacek et al., 2010; Fustinoni et al.,  
365 2012).

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

370 Multiple regression analysis applied to evaluate the contribution of each predictor to the  
371 variability of urinary biomarkers of benzene and BD. Traffic and office policemen stated to  
372 have smoked during working. Although levels of BD in the breathing zone of study subjects  
373 have not been reported, it seems that smoking habits influence the total intake of BD (WHO,  
374 2000). Unexpectedly, there was not a marked effect of smoking habits on DHBMA. Multiple  
375 regression analysis showed that the levels of individual BTEX exposure significantly  
376 contributed to increase urinary DHBMA ( $\beta=0.535$ ,  $p < 0.001$ ). Accordingly, DHBMA values  
377 did not discriminate exposure resulting from smoking habits and no significant difference  
378 was found between smokers and non-smokers.

379 In conclusion, these results indicated that traffic policemen, who are exposed to benzene  
380 and BD at the roadside in central Beirut, are potentially at a higher risk for development of

381 diseases such as cancer than office policemen. Further studies need to focus on the lifestyle  
382 and genetic factors that may affect the background levels of S-PMA, *t,t*-MA, MHBMA and  
383 DHBMA. Given chaotic traffic conditions, quality of fuel, high rate of passenger cars  
384 ownership, meteorological conditions, building characteristics of the area, and high pollution  
385 rates observed in Beirut, increased urinary exposure biomarker would result in a higher risk  
386 in the exposed subjects. Accordingly, public authorities should particularly set policies aimed  
387 to reduce traffic-related air pollution.  
388

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**398**

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534

**535 TABLES**

**536**

**537** **Table 1.** Summary of information collected by questionnaire.

	Traffic policemen (n=24)	Office policemen (n=23)	<i>p</i> value
Age (years, mean $\pm$ SD)	27.4 $\pm$ 4.6	29.1 $\pm$ 3.6	0.06
Percentage of never/current-smokers	37	56	0.19
Cigarettes/d (mean $\pm$ SD)	21 $\pm$ 18	26.2 $\pm$ 23	0.633
Sampling period (h, mean $\pm$ SD)	33.25 $\pm$ 3.1	45.2 $\pm$ 3.4	<0.001
BMI (kg m <sup>-2</sup> , mean $\pm$ SD)	25.6 $\pm$ 4.6	27.9 $\pm$ 4.3	0.125

**538** Significant at  $p < 0.05$ . Student's *t* test.

**539 Table 2.** Distribution of individual VOCs exposure in subgroups of policemen. Values are expressed as geometric medians, means and  
**540** geometric standard deviations. Concentrations are expressed as  $\mu\text{g}/\text{m}^3$ .

Parameter	Traffic policemen (n=24)			Parameter	Office policemen (n=23)			<i>p value</i>
	Median	Min-Max	Mean $\pm$ SD		Median	Min-Max	Mean $\pm$ SD	
Benzene (n=21)	0.3	0.3-867.8	48.8 $\pm$ 189.3	Benzene (n=22)	0.3	0.3-10.8	1.2 $\pm$ 2.3	n.s.
Toluene (n=20)	101.8	29.8-9788.4	11520. $\pm$ 2393.0	Toluene (n=22)	9.3	0.44-46.3	12.1 $\pm$ 11.3	<0.001
Ethylbenzene (n=21)	58.1	6.7-5845.2	662.3 $\pm$ 1420.3	Ethylbenzene (n=22)	4.5	0.88-19.1	5.7 $\pm$ 4.4	<0.001
mXylene (n=21)	102.8	29.1-1209.6	196.7 $\pm$ 287.6	mXylene (n=22)	2.0	0.65-10.6	2.5 $\pm$ 2.2	<0.001
oXylene (n=21)	21.3	1.0-1839.1	177.7 $\pm$ 432.3	oXylene (n=22)	1.7	0.58-5.64	2.0 $\pm$ 1.3	<0.001
pXylene (n=15)	3.9	1.2-30.1	9.6 $\pm$ 10.0	pXylene (n=20)	0.4	0.18-2.1	0.6 $\pm$ 0.4	<0.001
BTEX (n=21)	295.1	85.8-18709.1	2189.0 $\pm$ 4450.8	BTEX (n=22)	21.4	3.6-84.1	24.3 $\pm$ 19.6	<0.001

**541** Significant at  $p < 0.05$ . Mann-Whitney test, n.s. = not significant.

**542**

**543 Table 3.** Spearman correlation coefficients between different variables.

	Tol	Ethylbz	mXyl	oXyl	pXyl	BTEX	S-PMA <sup>1</sup>	S-PMA <sup>2</sup>	<i>t,t</i> -MA <sup>1</sup>	<i>t,t</i> -MA <sup>2</sup>	MHBMA <sup>1</sup>	MHBMA <sup>2</sup>	DHBMA <sup>1</sup>	DHBMA <sup>2</sup>
Bz	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Tol		0.962**	0.982**	0.939**	0.933**	0.989**	0.358*	-	-	-	-	-	0.505**	0.610**
Ethylbz			0.965**	0.931**	0.950**	0.975**	0.354*	-	-	-	0.317*	-	0.496**	0.639**
mXyl				0.951**	0.944**	0.988**	0.381*	-	-	-	0.357*	-	0.490**	0.607**
oXyl					0.946**	0.944**	0.394**	-	-	-	-	-	0.411**	0.565**
pXyl						0.943**	0.398*	-	-	-	-	-	0.370*	0.597**
BTEX							0.401**	-	-	-	0.345*	-	0.495**	0.616**
S-PMA <sup>1</sup>								0.467**	0.464**	-	0.389**	-	0.384**	0.356*
S-PMA <sup>2</sup>									-	-	-	0.374*	-	0.320*
<i>t,t</i> -MA <sup>1</sup>										-	0.385**	-	-	-
<i>t,t</i> -MA <sup>2</sup>											-	-	-	-
MHBMA <sup>1</sup>												0.352*	0.538**	0.540**
MHBMA <sup>2</sup>													-	-
DHBMA <sup>1</sup>														0.716**

**544** Missing values correspond to lack of correlation between parameters. Only significant data are represented. <sup>1</sup> Pre-shift; <sup>2</sup> Post-shift. \*\*  $p < 0.001$ ;

**545** \*  $p < 0.05$



546 **Table 4.** Biomarkers of exposure in traffic and office policemen.

547

Parameter	Study groups	
	Traffic policemen	Office policemen
Urinary <i>t,t</i> -MA (µg/g creatinine)		
Pre-shift	64.7±141.5	14±13.3
	8.2 (3-586.7)	11.2 (2.5-56.5)
	(n=24)	(n=23)
Post-shift	84.4±100.6 <sup>a</sup>	55.5±87*
	61 (2.4-444.8)	18.3 (2.5-343.4)
	(n=24)	(n=22)
Urinary S-PMA (µg/g creatinine)		
Pre-shift	6.2±6 <sup>a</sup>	2.6±2
	4 (1.1-25)	1.7 (0.9-8.1)
	(n=24)	(n=23)
Post-shift	5.3±9	2.7±2.4
	2.6 (0.5-45.5)	1.9 (0.4-10.2)
	(n=24)	(n=22)
Urinary MHBMA (µg/g creatinine)		
Pre-shift	24.7±23.3 <sup>a</sup>	17.7±38.7
	20.1 (2.4-108.3)	3.1 (1.3-147.6)
	(n=24)	(n=23)
Post-shift	18.7±20.1	18.8±31
	10.2 (0.2-88.6)	7.5 (1.2-120)
	(n=24)	(n=22)

Urinary DHBMA ( $\mu\text{g/g}$  creatinine)

Pre-shift	180.4 $\pm$ 73.8 <sup>a</sup>	69.2 $\pm$ 43.6
	182.9 (47.6-333.3)	66.5 (13.2-239.3)
	(n=24)	(n=23)
Post-shift	207.5 $\pm$ 112.2 <sup>b</sup>	73.3 $\pm$ 45.3
	188.6 (80-588.6)	67.4 (23.9-186.7)
	(n=23)	(n=22)

Urinary Cotinine ( $\mu\text{g/g}$  creatinine)

Pre-shift	767.8 $\pm$ 1138.5 <sup>a</sup>	188.6 $\pm$ 339.2
	486.9 (16.1-5240)	24.6 (8.5-1200.8)
	(n=24)	(n=23)
Post-shift	601.0 $\pm$ 780.6	205.5 $\pm$ 393.2
	459.3 (12.1-3583.9)	19.9 (7.9-1362.4)
	(n=24)	(n=22)

**548** Values are expressed as mean $\pm$ SD on the first line and median (min-max) on the second line

**549** of each parameter.

**550** Statistically significant difference from office policemen at <sup>a</sup>  $p < 0.001$  or <sup>b</sup>  $p < 0.01$ .

**551** \* Statistically significant difference from the corresponding pre-shift at  $p = 0.03$ .