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Mireille Borgie, Anne Garat, Fabrice Cazier, Agnes Delbende, Delphine Allorge, Frederic Ledoux, Dominique Courcot, Pirouz Shirali, Zeina Dagher

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

2 **ASSESSMENT IN BEIRUT, LEBANON**

3
4 **Mireille BORGIE^{a,d}, Anne GARAT^{c,d}, Fabrice CAZIER^{b,d}, Agnes DELBENDE^{b,d},**
5 **Camille RICHEVAL^{c,d}, Delphine ALLORGE^{c,d}, Frederic LEDOUX^{a,d}, Dominique**
6 **COURCOT^{a,d}, Pirouz SHIRALI^{a,d}, Zeina DAGHER^{e,f}**

7
8 ^a Unit of Environmental Chemistry and Interactions on Living, EA 4492, University of
9 Littoral-Côte d'Opale (ULCO), Dunkerque, France

10 ^b Common Center of Measurements, ULCO, Dunkerque, France

11 ^c EA 4483, Faculty of Medicine of Lille, University of Lille North of France, Lille, France

12 ^d University of Lille North of France, Lille, France

13 ^e Bioactive Molecules Research Group, Doctoral School of Sciences and Technologies,
14 Lebanese University, Lebanon

15 ^f Department of Biology, Faculty of Sciences, Lebanese University, Lebanon

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18 **Corresponding Author:** Dr Zeina DAGHER, Bioactive Molecules Research Group,
19 Doctoral School of Sciences and Technologies; Department of Biology, Faculty of Sciences,
20 Lebanese University, P.O. Box 90656, Lebanon. Tel +961 (1) 686 981, fax +961 (1) 680 250,
21 email: dagher_z@ul.edu.lb

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 Naeher,
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⁻¹. The limit of quantification (LOQ) was 10 µg L⁻¹. 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 μL 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 μm , 2.1 x 100 mm) at
162 50 °C. Samples (15 μL) 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 μL) 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 ± 2.91 years (mean \pm SD). Urinary
211 cotinine levels were 890 ± 999 and $786 \pm 770 \mu\text{g g}^{-1}$ creatinine for smokers and 36 ± 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 mg m^{-3} for benzene (OSHA, 1999), of 375
296 mg m^{-3} for toluene (OSHA, 1999), of 435 mg m^{-3} for ethylbenzene (OSHA, 1999) and of 435
297 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 = $48.8 \text{ } \mu\text{g m}^{-3}$) 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 *GSTAI1*) 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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392

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398

399 References

- 400 Abbas, I., Garçon, G., Saint-Georges, F., Andre, V., Gosset, P., Billet, S., Goff, J.L., Verdin,
401 A., Mulliez, P., Sichel, F., Shirali, P., 2013. Polycyclic aromatic hydrocarbons within
402 airborne particulate matter (PM_{2.5}) produced DNA bulky stable adducts in a human lung
403 cell coculture model. *J Appl Toxicol.* 33:109-19.
- 404 Albertini, R.J., Sram, R.J., Vacek, P.M., Lynch, J., Wright, M., Nicklas, J.A., Boogaard, P.J.,
405 Henderson, R.F., Swenberg, J.A., Tates, A.D., Ward, J.B., Jr., 2001. Biomarkers for
406 assessing occupational exposures to 1,3-butadiene. *Chem Biol Interact.* 135:429–453.
- 407 Arayasiri, M., Mahidol, C., Navasumrit, P., Autrup, H., Ruchirawat, M., 2010. Biomonitoring
408 of benzene and 1,3-butadiene exposure and early biological effects in traffic policemen.
409 *Sci Total Environ.* 408:4855-62.
- 410 Avogbe, P., Ayi-Fanou, L., Cachon, B., Chabi, N., Delbende, A., Dewaele, D., Aissi, F.,
411 Cazier, F., and Sanni A., 2011. Hematological changes among Beninese motor-bike taxi
412 drivers exposed to benzene by urban air pollution. *Afr. J. Environ. Sc. Technol.* 5: 464-
413 472
- 414 Barbieri, A., Violante, F.S., Sabatini, L., Graziosi, F., Mattioli, S., 2008. Urinary biomarkers
415 and low-level environmental benzene concentration: assessing occupational and general
416 exposure. *Chemosphere.* 74:64-9.
- 417 Billet, S., Paget, V., Garçon, G., Heutte, N., André, V., Shirali, P., Sichel, F., 2010. Benzene-
418 induced mutational pattern in the tumour suppressor gene TP53 analysed by use of a
419 functional assay, the functional analysis of separated alleles in yeast, in human lung
420 cells. *Arch Toxicol.* 84:99-107.
- 421 Carugno, M., Pesatori, A.C., Dioni, L., Hoxha, M., Bollati, V., Albetti, B., Byun, H.M.,
422 Bonzini, M., Fustinoni, S., Cocco, P., Satta, G., Zucca, M., Merlo, D.F., Cipolla, M.,
423 Bertazzi, P.A., Baccarelli, A., 2012. Increased mitochondrial DNA copy number in

424 occupations associated with low-dose benzene exposure. *Environ Health Perspect.*
425 120:210-5.

426 Cheng, X., Zhang, T., Zhao, J., Zhou, J., Shao, H., Zhou, Z., Kong, F., Feng, N., Sun, Y.,
427 Shan, B., Xia, Z., 2012. The association between genetic damage in peripheral blood
428 lymphocytes and polymorphisms of three glutathione S-transferases in Chinese workers
429 exposed to 1,3-butadiene. *Mutat Res.* 750:139-46.

430 Clifford P. Weisel, 2010. Benzene exposure: An overview of monitoring methods and their
431 findings. *Chem-Biol Interact.* 184:58–66

432 Dagher, Z., Garçon, G., Billet, S., Gosset, P., Ledoux, F., Courcot, D., Aboukais, A., Shirali,
433 P., 2006. Activation of different pathways of apoptosis by air pollution particulate matter
434 (PM2.5) in human epithelial lung cells (L132) in culture. *Toxicol.* 225:12-24.

435 EU-RAR, 2002. European Union Risk Assessment Report 1,3-Butadiene.

436 Fustinoni, S., Campo, L., Satta, G., Campagna, M., Ibba, A., Tocco, M., Atzeri, S., Avataneo,
437 G., Flore, C., Meloni, M., Bertazzi, P., Cocco, P., 2012. Environmental and lifestyle
438 factors affect benzene uptake biomonitoring of residents near a petrochemical plant.
439 *Environ Int.* 39: 2-7.

440 Fustinoni, S., Consonni, D., Campo, L., Buratti, M., Colombi, A., Pesatori, A.C., Bonzini,
441 M., Bertazzi, P.A., Foà, V., Garte, S., Farmer, P.B., Levy, L.S., Pala, M., Valerio, F.,
442 Fontana, V., Desideri, A., Merlo, D., 2005. Monitoring low benzene exposure:
443 comparative evaluation of urinary biomarkers, influence of cigarette smoking, and genetic
444 polymorphisms. *Cancer Epidemiol. Biomarkers Prev.* 14, 2237–2244.

445 Han, X., Naeher, L., 2006. A review of traffic-related air pollution exposure assessment
446 studies in the developing world. *Environ Int.* 32:106-20

447 IARC, 2008. Monographs On The Evaluation Of The Carcinogenic Risk Of Chemicals To
448 Humans. Volume 97. 1,3-Butadiene, Ethylene Oxide and Vinyl Halides
449 (<http://monographs.iarc.fr>).

450 IARC, 2009. A Review Of Human Carcinogens, Part F: Chemical Agents And Related
451 Occupations. *Lancet Oncol* 10:1143-1144.

452 Ibrahim, K.S., Amer, N.M., El-Dossuky, E.A., Emara, A.M., El-Fattah, A.E., Shahy, E.M.,
453 2012. Hematological effects of benzene exposure with emphasis on muconic acid as
454 biomarker in exposed workers. *Toxicol Ind Health*. 0748233712458141.

455 Johnson, E.S., Langård, S., Lin, Y.S., 2007. A critique of benzene exposure in the general
456 population. *Sci Total Environ*. 374:183-98.

457 Kamal, A., Malik, R.N., Fatima, N., Rashid, A., 2012. Chemical exposure in occupational
458 settings and related health risks: a neglected area of research in Pakistan. *Environ*
459 *Toxicol Pharmacol*. 34:46-58.

460 Kandyala, R., Raghavendra, S.P., Rajasekharan, S.T., 2010. Xylene: An overview of its
461 health hazards and preventive measures. *J Oral Maxillofac Pathol*. 14:1–5.

462 Knibbs, L.D., Morawska, L., 2012. Traffic-related fine and ultrafine particle exposures of
463 professional drivers and illness: An opportunity to better link exposure science and
464 epidemiology to address an occupational hazard? *Environ Int*. 49:110-4.

465 Koturbash, I., Scherhag, A., Sorrentino, J., Sexton, K., Bodnar, W., Tryndyak, V.,
466 Latendresse, J.R., Swenberg, J.A., Beland, F.A., Pogribny, I.P., Rusyn, I., 2011.
467 Epigenetic alterations in liver of C57BL/6J mice after short-term inhalational exposure
468 to 1,3-butadiene. *Environ Health Perspect*. 119:635-40.

469 Maffei, F., Hrelia, P., Angelini, S., Carbone, F., Forti, G. V., Barbieri, A., 2005. Effects of
470 environmental benzene: Micronucleus frequencies and haematological values in traffic
471 police working in an urban area. *Mutat. Res*. 583 :1–11.

472 Manini, P., De Palma, G., Andreoli, R., Mozzoni, P., Poli, D., Goldoni, M., Petyx, M.,
473 Apostoli, P., Mutti, A., 2010. Occupational exposure to low levels of benzene:
474 Biomarkers of exposure and nucleic acid oxidation and their modulation by polymorphic
475 xenobiotic metabolizing enzymes. *Toxicol Lett.* 193:229-35.

476 Occupational Safety and Health Administration (OSHA), US. Department of Labor. OSHA
477 standard -29 CFR 1910.1028 (7/1/1999), from National Library of Medicine, HSDB:
478 Benzene, CASRN: 71-43-2; 1999 ([http://toxnet.nlm.nih.gov/cgi-](http://toxnet.nlm.nih.gov/cgi-bin/sis/search/f?./temp/~F3eDcl:1)
479 [bin/sis/search/f?./temp/~F3eDcl:1](http://toxnet.nlm.nih.gov/cgi-bin/sis/search/f?./temp/~F3eDcl:1) Last accessed 07/12/2012).

480 OSHA, OSHA standard -29 CFR 1910.1000 (2/10/2008), from U.S. National Archives and
481 Records Administration's Electronic Code of Federal Regulations, HSDB: Xylene,
482 CASRN: 1330-20-7; 2008 (Last accessed 07/12/2012).

483 OSHA, OSHA standard -29 CFR 1910.1000 (7/1/1999), from National Library of Medicine,
484 HSDB: Toluene, CASRN: 108-88-3; 1999 (Last accessed 07/12/2012).

485 OSHA, OSHA standard -29 CFR 1910.1000 (7/1/1999), from National Library of Medicine,
486 HSDB: Ethylbenzene, CASRN: 100-41-4; 1999 (Last accessed 07/12/2012).

487 Pamela M. Vacek, Richard J. Albertini, Radim J. Sram, Patricia Upton, James A. Swenberg,
488 Hemoglobin adducts in 1,3-butadiene exposed Czech workers: Female–male
489 comparisons, *Chemico-Biological Interactions*, Volume 188, Issue 3, 5 December 2010,
490 Pages 668-676.

491 Peluso, M., Bollati, V., Munnia, A., Srivatanakul, P., Jedpiyawongse, A., Sangrajrang, S.,
492 Piro, S., Ceppi, M., Bertazzi, P.A., Boffetta, P., Baccarelli, A.A., 2012. DNA
493 methylation differences in exposed workers and nearby residents of the Ma Ta Phut
494 industrial estate, Rayong, Thailand. *Int J Epidemiol.* 41:1753-60.

495 Pilidis, G.A., Karakitsios, S.P., Kassomenos, P.A., Kazos, E.A., Stalikas, C.D., 2009.
496 Measurements of benzene and formaldehyde in a medium sized urban environment.

497 Indoor/outdoor health risk implications on special population groups. *Environ Monit*
498 *Assess.* 150:285-94.

499 Qu, Q., Melikian, A.A., Li, G., Shore, R., Chen, L., Cohen, B., Yin, S., Kagan, M.R., Li, H.,
500 Meng, M., Jin, X., Winnik, W., Li, Y., Mu, R., Li, K., 2000. Validation of biomarkers in
501 humans exposed to benzene: urine metabolites. *Am J Ind Med.* 37:522-31.

502 Rekhadevi, P.V., Rahman, M.F., Mahboob, M., Grover, P., 2010. Genotoxicity in filling
503 station attendants exposed to petroleum hydrocarbons. *Ann Occup Hyg.* 54:944-54.

504 Rossnerova, A., Spatova, M., Rossner, P., Solansky, I., Sram, R.J., 2009. The impact of air
505 pollution on the levels of micronuclei measured by automated image analysis. *Mutat*
506 *Res.* 669:42-7.

507 Ruchirawat, M., Navasumrit, P., Settachan, D., 2010. Exposure to benzene in various
508 susceptible populations: co-exposures to 1,3-butadiene and PAHs and implications for
509 carcinogenic risk. *Chem Biol Interact.* 184:67-76.

510 Sangaraju, D., Goggin, M., Walker, V., Swenberg, J., Tretyakova, N., 2012. NanoHPLC-
511 nanoESI(+)-MS/MS quantitation of bis-N7-guanine DNA-DNA cross-links in tissues of
512 B6C3F1 mice exposed to subppm levels of 1,3-butadiene. *Anal Chem.* 84:1732-9.

513 Sapkota, A., Halden, R.U., Dominici, F., Groopman, J.D., Buckley, T.J., 2006. Urinary
514 biomarkers of 1,3-butadiene in environmental settings using liquid chromatography
515 isotope dilution tandem mass spectrometry. *Chem Biol Interact.* 160:70-9.

516 Seow, W.J., Pesatori, A.C., Dimont, E., Farmer, P.B., Albetti, B., 2012. Urinary Benzene
517 Biomarkers and DNA Methylation in Bulgarian Petrochemical Workers: Study Findings
518 and Comparison of Linear and Beta Regression Models. *PLoS One* 7:e50471.

519

520 van Sittert, N., Megens, H., Watson, W., Boogaard, P., 2000. Biomarkers of exposure to 1,3-
521 butadiene as a basis for cancer risk assessment. *Toxicol Sci.* 56:189-202. Tabish, A.M.,

522 Poels, K., Hoet, P., Godderis, L., 2012. Epigenetic factors in cancer risk: effect of
523 chemical carcinogens on global DNA methylation pattern in human TK6 cells. PLoS
524 One. 7:e34674.

525 Waked, A., Afif, C., Seigneur, C., 2012. An atmospheric emission inventory of
526 anthropogenic and biogenic sources for Lebanon. Atmos. Environ. 50,88-96

527 WHO, 1996. Biological Monitoring Of Chemical Exposure In The Workplace, vol. 1,
528 Geneva: World Health Organization.

529 WHO, 2000. Butadiene Air Quality Guidelines. Chapter 5.3 - Second Edition. Regional
530 Office for Europe, Copenhagen, Denmark.

531 Xiang, M., Ao, L., Yang, H., Liu, W., Sun, L., Han, X., Li, D., Cui, Z., Zhou, N., Liu, J., Cao,
532 J., 2012. Chromosomal damage and polymorphisms of metabolic genes among 1, 3-
533 butadiene-exposed workers in a matched study in China. Mutagenesis. 27:415-21.

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 ⁻² , 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 ¹	S-PMA ²	<i>t,t</i> -MA ¹	<i>t,t</i> -MA ²	MHBMA ¹	MHBMA ²	DHBMA ¹	DHBMA ²
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 ¹								0.467**	0.464**	-	0.389**	-	0.384**	0.356*
S-PMA ²									-	-	-	0.374*	-	0.320*
<i>t,t</i> -MA ¹										-	0.385**	-	-	-
<i>t,t</i> -MA ²											-	-	-	-
MHBMA ¹												0.352*	0.538**	0.540**
MHBMA ²													-	-
DHBMA ¹														0.716**

544 Missing values correspond to lack of correlation between parameters. Only significant data are represented. ¹ Pre-shift; ² 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 ^a	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 ^a	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 ^a	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 ^a	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 ^b	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 ^a	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 ^a $p < 0.001$ or ^b $p < 0.01$.

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