Application of biological monitoring for exposure assessment of 1,3-Butadiene

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Abstract

Background 1, 3-Butadiene is an important industrial compound. Based on sufficient evidence of carcinogenicity in laboratory animals and humans, the International Agency for Research on Cancer, in 1999, classified 1, 3-Butadiene as a probable carcinogen to humans (group 2A). The potential for exposure to vehicle exhaust containing these chemical compounds is very noticeable in urban locations. Exposure to incomplete combustion of gasoline has been a long time concern in many occupational fields, including policemen, service stations, and the petroleum industry but in Iran has rarely been studied.

Methods This study designed to determine the exposure levels for traffic policemen and gas station workers during routine work shift, by biological monitoring. With this aim, 25 policemen engaged in traffic control, 25 gas station workers and 25 occupationally non-exposed persons were investigated. Spot urine samples were obtained prior to and at the end of the work shift from each subject. The urinary levels of selected urinary metabolites (MHBMA and DHBMA) were determined by using LC–MS/MS.

Results There were significant differences among the mean urinary concentrations of DHBMA in pre-shift samples of policemen, gas station workers and occupationally non-exposed persons. The mean urinary concentrations of DHBMA differed significantly among post-shift samples of policemen and gas station (ANOVA: $p < 0.05$ and Kruskal-Wallis test: $p < 0.05$).

Conclusions There was a significant difference in DHBMA concentrations between job categories ($p < 0.05$ by ANOVA and Kruskal-Wallis test), and policemen and gas station workers were found to be probably the most exposed groups in this study.

Keywords 1, 3-Butadiene · Biological monitoring · Urinary metabolites · Exposure assessment

Background

1, 3-Butadiene is an important industrial compound. Extensive research of its potential health impacts have indicated a complex pattern of carcinogenicity in rats and mice, where it is a multi-site cancer-causing agent in both species but with vastly various potencies [1, 2]. Based on sufficient evidence of

carcinogenicity in laboratory animals and partial evidence of carcinogenicity in epidemiological studies in humans, the International Agency for Research on Cancer, in 1999, classified 1, 3-Butadiene as a probable carcinogen to humans (group 2A) (IARC, 1999) [3]. In 2002, the US Environmental Protection Agency classified 1, 3-Butadiene as a human carcinogen by inhalation [4]. Emissions of chemical compounds such as benzene, 1,3-butadiene and PAH into the air and environment by mobile sources are of great public health concern because of their serious side effects such as carcinogenicity and heightened exposure capacity that results from their proximity and integration into society at all levels (rural, suburban and urban). Many epidemiologic studies have showed higher cancer rates among urban compared with suburban populations [5, 6]. Air pollution, including benzene, 1,3-butadiene, and poly aromatic hydrocarbons, is believed to be a contributing risk factor [7]. The potential for exposure to vehicle exhaust containing these chemical compounds is very noticeable in urban locations. Exposure to incomplete combustion of gasoline has been a long time concern in many occupational fields, including

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policemen, service stations, and the petroleum industry. Gas station workers and traffic policemen are two of the groups which are most heavily exposed to vehicle exhausts during their work shifts [8, 9]. According to studies, in estimation of workers' exposure with biological samples, analysis of the concentration of two major urinary metabolites of 1, 3-butadiene, monohydroxybutenyl-mercapturic acids (MHBMA) and dihydroxy-butyl-mercapturic acid (DHBMA), in urine after the work shift seems to give the most valid estimation of exposure [10, 11], so in this study these two metabolites were selected as suitable biomarkers of 1, 3-Butadiene exposure. The aim of this study was to determine the exposure levels for traffic policemen and gas station workers during routine work shift, by biological monitoring.

Materials and methods

Study population

Seventy-five healthy men from Tehran, Iran, were enrolled in the research. The study population consisted of 25 gas station workers and 25 traffic policemen engaged in traffic control in five districts with medium to high traffic level, chose based on traffic stream information recorded by the Regional Agency for Environmental Protection. All of the participants were men between 25 and 55 (mean 40) years of age and none of them were smokers. For the estimation of any background, levels originating from other activities such as vehicle refueling, urine samples were collected from 25 occupationally non-exposed persons in the similar regions acted as referents.

Sampling

The exposure assessments were done in August 2019. Radiello passive samplers were used for measuring personal exposure to 1, 3-Butadiene [12, 13]. After exposure, the chemical substances collected on sorbing cartridges were desorbed by thermal desorption procedure and analyzed by GC/MS. Air concentration (expressed in $\mu\text{g m}^{-3}$) was calculated using the equation:

$$\text{Conc.}(\mu\text{g}/\text{m}^3) = \frac{m(\mu\text{g}) \times 10^3}{Q(L/\text{min}) \times t(\text{min})}$$

where m = mass of analytes determined in desorbing solvent; Q = uptake rate of substances (30.5 mL min⁻¹ for 1, 3-Butadiene); t = exposure time (www.sigma-aldrich.com/radiello, 2010) [13]. Urine samples were gathered at the beginning and at the end of the work shift. Urine samples were stored under 4 °C in a cool box until they were gotten by the laboratory, where they were divided into several portions and kept at -20 °C until analysis.

Analysis of urine samples

The urinary samples of 75 volunteers were collected and analyzed for their MHBMA and DHBMA concentrations according to the method of Li et al. [14]. At first, frozen urine samples were allowed to thaw and reach to room temperature. By adding hydrochloric acid, the samples pH were adjusted to 2.0, and then centrifuged at 10 000 rpm for 6 min at 10 °C. Then 100 μL of the working solution of the internal standard was added to 1 mL of each sample. After vortex mixing, solid phase extraction (SPE) procedure was applied for sample preparation. The Oasis HLB phase cartridges were conditioned with 3 mL methanol and 3 mL 0.1% formic acid. Then, 2 mL 0.1% formic acid and 2 mL methanol were eluted through each cartridge under vacuum, respectively. After evaporation of solvent under a nitrogen stream, the residue was dissolved in 1 mL 0.1% formic-methanol (v/v 1/4 9/1), and were analyzed by LC-MS/MS. The concentration of MHBMA and DHBMA were expressed as ng/mg creatinine.

Statistics

Mean urinary concentration of MHBMA and DHBMA among three groups (policemen, gas station workers and occupationally non-exposed persons) were analyzed and because the distribution of data was not normal, the analysis was done by means of two statistical strategies: analysis of variance (one-way ANOVA) followed by Scheffé's post hoc test and Kruskal-Wallis test. Results were showed as mean $\pm$ S.D. and 95% confidence intervals. The level of significance was set to 0.05 and p values > 0.05 were assumed to be nonsignificant.

Results

Table 1 shows the results of environmental measurements ($\mu\text{g m}^{-3}$) in the three groups of traffic policemen, gas station workers and occupationally nonexposed persons during a day work shift. The mean value for exposure of 1, 3-Butadiene in breathing zone of the two groups of traffic policemen, gas station workers were 23.56 and 11.52 $\mu\text{g m}^{-3}$, respectively, which were significantly greater than the occupationally non-exposed groups but below the 2000 ACGIH TLV(TWA) (2.21 mg m⁻³) (OSHA, 2000) [15].

Table 2 shows the results of the mean urinary concentration of MHBMA and DHBMA determinations (ng/mg) in the three groups of workers in two different samples collected: before starting the shift and at the end of the work. The urinary MHBMA and DHBMA concentration of subjects with jobs associated with exposure to gasoline (policemen and gas station workers) before starting the work were compared with the no or low exposure group. The mean urinary concentration of

Table 1 The mean value (SD) for exposure of 1, 3-Butadiene in breathing zone

Groups	Sample size (n)	1, 3-Butadiene (μgm^{-3})	P value*
Traffic policemen,	25	23.56 (35.82)	0.015
Gas station workers	25	11.52 (23.61)	
occupationally nonexposed persons	25	0.92 (1.11)	

*Significance level: ≤ 0.05

MHBMA and DHBMA in policemen were significantly greater than that the other groups. In addition, the mean levels of MHBMA and DHBMA before the work shift in policemen and gas station workers were higher than the occupationally non-exposed group and a statistically significant difference could be observed ($p \leq 0.05$). The concentration of DHBMA increased during the day for all three groups. The increase is more marked among policemen and gas station workers. As is shown in Table 1, excretion of DHBMA in policemen after the work shift were significantly higher than gas station workers and occupationally non-exposed group ($p < 0.001$). The concentration of DHBMA in policemen increased significantly during the day and reached 335.35 (ng/mg). Significant correlations between 1, 3-Butadiene in breathing zone and postshift urine DHBMA concentration was found in this study ($r = 0.514$, $p \leq 0.01$). However, no significant association was found between the level of individual 1,3-butadiene exposure and post-shift MHBMA.

Discussion

In this research, we have investigated exposure to 1,3-butadiene, which is carcinogenic substance in urban air from incomplete combustion of fossil fuels, by using personal air monitoring, as well as through the use of biomarkers of exposure. The levels of individual exposure in traffic policemen and gas station workers (mean concentration were 23.56.62 and 11.52 μgm^{-3}) were far below the OSHA exposure limits, 8-hours time weight average (TWA) of 2.2 mgm^{-3} for 1,3-butadiene (OSHA, 2000) [15]. However, since 1,3-butadiene is carcinogen and can cause DNA damage, NIOSH suggests that exposure should be reduced to protect people's health

[16]. One study of 1,3-butadiene exposure in ambient air was conducted in toll booth workers and faculty and university staff in an urban area in Baltimore, Maryland, US (mean concentration = 2.88 and 1.47 μgm^{-3} , respectively) [17]. These levels of 1,3-butadiene in the toll booth workers were lower than in Tehran traffic policemen and gas station workers (mean concentration = 3.15 μgm^{-3}), which indicated higher emissions of 1,3-butadiene from vehicles in areas in the center of Tehran that are highly traffic congested. The levels of individual exposure of 1,3-butadiene in this study were comparable to the Italy study [18]. Difference in many of environment factors, such as quality of fuel, traffic characteristics, building characteristics of the area, and meteorological conditions and difference in physical activity in the workplace may cause the differences in the levels of individual 1,3-butadiene exposure. Different biomarkers of exposure to 1,3-butadiene were examined to assess suitability for use as indicators of exposure to this compound in urban air. For 1,3-butadiene exposure in ambient air, a few number of biomonitoring researches has been conducted. Levels of urinary MHBMA reported by Sapkota et al. [17], were lower than those in this work, despite the level of individual exposure being quite similar. Reasons behind these differences may include the effects of genetic polymorphisms or different analytical procedures or differing lifestyles between people in these countries. The results of this study showed that MHBMA levels in traffic policemen and gas station workers were slightly elevated, and significantly higher than in occupationally nonexposed persons. The correlation between MHBMA and individual exposure levels of 1,3 butadiene was not statistically significant, indicating a complex metabolism of 1,3-butadiene at low levels, or that MHBMA is not a major metabolite of 1,3-butadiene in humans [19]. This was also reported in a previous study

Table 2 Mean urinary concentration (S.D.) MHBMA and DHBMA in three groups (n = 25) before and after starting work

Time of sampling	Compounds (ng/mg)	Groups			P value*
		Policemen	Gas station workers	Occupationally non-exposed persons	
Before shift	MHBMA	35.26 (13.73)	28.39 (15.39)	21.71 (11.14)	0.095
	DHBMA	305.12 (123.25)	174.18 (85.62)	146.01 (96.36)	0.034
After shift	MHBMA	37.09 (23.51)	30.12 (17.41)	22.11 (9.68)	0.11
	DHBMA	335.35 (154.12)	199.32 (92.85)	151.23 (88.36)	0.025

*Significance level: ≤ 0.05

[17]. Another interesting result was that the levels of MHBMA in the urine samples before starting work were similar to that of the post-shift urinary concentrations and there are no significant differences between them. This may be because of the longer elimination half-life of MHBMA [19], further researches are needed to assess the effects of elimination half-life, genetic polymorphisms and lifestyles on the levels of MHBMA excretion following exposure to 1,3-butadiene in urban air. Alternatively, urinary concentrations of DHBMA were found to be a better biomarker of the levels of 1,3-butadiene exposure in urban air than MHBMA, as showed previously [19, 20]. Pre and post-shift DHMBA urinary concentrations could significantly distinguish between traffic and occupationally nonexposed persons and showed a better correlation with personal total 1,3-butadiene exposure.

Conclusions

In conclusion, these results indicated that traffic policemen, who are exposed to 1,3-butadiene at the roadside in central of Tehran, are potentially at a higher risk for development of diseases such as cancer than other groups. Traffic jam conditions, high rate of passenger cars, the quality of fuel, and increased rate of exposure would result a higher risk for subjects such as policemen. Further studies need to focus on the genetic factors and lifestyle that may affect the background levels of DHBMA and MHBMA.

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Authors' contributions RA and NR participated in the design of the study. RA did the analyses and MGH interpreted the analyzed results. NR was the main investigator, supervised the work, drafted and revised the paper critically for important intellectual content and compiled the work in accordance to journal format. All authors have read and approved the final manuscript.

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Data availability The data will not be shared with a reason.

Compliance with ethical standards

Declaration Not applicable.

Ethics approval and consent to participate The research protocol was approved by Ethics Committee of Tehran University of Medical Sciences (IR.TUMS.SPH.REC.1396.2335).

Consent for publication Not applicable.

Conflict of interest The authors declare that they have no competing interests.

Abbreviations IARC, International Agency for Research on Cancer; MHBMA, Monohydroxybutenyl-mercapturic acid; DHBMA, Dihydroxy-butyl-mercapturic acid; SPE, Solid phase extraction; TWA, Time weight average

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