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Short Communication

Biological monitoring among benzene-exposed workers in Bangalore city, India

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Abstract

Environmental and biological monitoring was carried out in the winter season of 2004 for 30 gasoline station workers (study subjects) and 30 office workers (controls) of Bangalore city, India. Personal air sampling was carried out in the breathing zone of workers using an Anasorb CSC sorbent tube (SKC 226-01) fitted to the low-flow personal samplers (PCXR4 and pocket pump Model No. 210-1002) at a flow rate of 200 ml min^{-1} during the shift work of 8 h. The benzene content adsorbed in the sorbent tube (SKC 226-01) was desorbed with 1 ml of benzene-free carbon disulfide on a developing vibrator and later analysed by Trace GC fitted with MXT-624 column and flame ionization detector. The mean time weighted average benzene concentration found among study and controls was 1.10 ± 1.08 and 0.070 ± 0.035 mg m^{-3}, respectively. Biological monitoring for benzene exposure was performed by measuring *trans*,*trans* muconic acid (*t*,*t*-MA) in the end shift urine samples using HPLC-UV technique. End-shift urine samples (1 ml) were adjusted to pH 7–9 with phosphate buffer pH 7.4 passed through the preconditioned Q-SAX anion-exchange cartridge and the (*t*,*t*-MA) is extracted with 10% acetic acid and later analysed by HPLC-UV detection The mean *t*,*t*-MA found among study and controls were 563.16 ± 281.81 and 266.88 ± 110.65 μg g^{-1} creatinine. About 50% of the study subjects (15) have higher *t*,*t*-MA values than the biological exposure index of the American Conference of Government Industrial Hygienist (ACGIH). Correlation is significant at 5% level ($p < 0.05$) between personal air benzene concentration and urinary *t*,*t*-MA in the study group. Based on these findings, the *t*,*t*-MA can be used as a biomarker for benzene exposure.

Keywords: *Benzene, urine,* trans,trans *muconic acid, biomarker, high-performance liquid chromatography (HPLC), gasoline handlers.*

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Introduction

Benzene, which is a constituent of motor fuel, a by-product of combustion, a constituent of automobile exhaust and present in cigarette smoke, is a widespread environmental pollutant (ATSDR 1997). Since it is classified as a human carcinogen, monitoring of benzene in the environment and its biomarkers is of importance. The concentration of benzene in gasoline varies from 1 to 5% in India. There are several biomarkers for benzene exposure, namely blood and breath benzene, phenol, catechol, hydroquinone, S-phenyl mercapturic acid (S-PMA) and *trans*,*trans*-muconic acid (*t*,*t*-MA). A large number of studies have been performed with respect to phenol as a biomarker for benzene exposure. Hotz et al. (1997) did a comparative study of different biomarkers, namely blood and breath benzene, phenol, catechol, hydroquinone, S-phenyl mercapturic acid and *t*,*t*-MA, in a population of 410 male workers working in garages. The final result showed that *t*,*t*-MA and S-PMA concentrations were good indicators of benzene exposure in the range 0.1–1.0 ppm, even in a population comprising of both smokers and non-smokers. Phenol, hydroquinone, catechol and blood/breath benzene were less suitable biomarkers in the same exposure range. Lee et al. (1998) conducted a similar comparative study of different biomarkers. The end results showed that end-shift urinary *t*,*t*-MA concentrations correlated well with the time weighted average (TWA) benzene concentration in the breathing zone air of workers, whereas no significant correlation could be established for urinary phenol, catechol and hydroquinone with benzene exposure. The results confirmed that urinary phenol appears to be a poor biological monitor for low-level benzene exposure. Very few studies (Verma & Rana 2001) with respect to phenol as a biomarker for benzene exposure among petrol filling workers have been reported in India. However, the ACGIH adopted S-PMA in 1997 and added *t*,*t*-MA in 2000 to the Biological Exposure Index (BEI). Other than the exposure, certain compounding factors such as smoking and dietary habits are the possible interferences in the determination of biological markers, especially *t*,*t*-MA in the population exposed to benzene. Ruppert et al. (1997) reported that a typical dietary intake of 6–30 mg day^{-1} sorbate accounted for 10–50% of the background *t*,*t*-MA excretion in non-smokers and for 5–25% in smokers. In general, a smoker consuming one pack a day inhales a benzene concentration of approximately 1800 μg day^{-1} in mainstream smoke (Wallace 1989). The consumption of packed food, especially among gasoline handlers, is rare in developing country such as India. As the petrol fillers were from a lower socio-economic group, they consume only home-made food. However, in the present study the subjects were informed not to smoke and consume packed food during monitoring period. Biological monitoring with respect to *t*,*t*-MA as a biomarker for benzene exposure has been less studied in India. It is in this context in which the study has been performed.

Material and methods

The subjects/gasoline handlers were involved in the refilling of gasoline into automobiles. The main route of exposure was only through inhalation. Gasoline exposure through skin contact is not possible as they were handling modern quick-refilling machines. It is observed that the gasoline handlers were not using any type of personal protective equipment including gloves. Twenty-nine gasoline handlers (study

subjects) and 30 office staff (controls) were covered for environmental and biological monitoring in the winter season of 2004 in Bangalore city, India.

The personal air benzene exposure (TWA) was determined by Occupational Safety and Health Administration Method No. 12 (August 1980, revised). Personal air sampling was carried out in the breathing zone of workers through an Anasorb CSC sorbent tube (SKC 226-01; SKC Inc., PA, USA) fitted to personal air samplers (PCXR4 and pocket pump Model No. 210-1002) at a flow rate of 200 ml min^{-1} during the daytime covering the entire shift of 8 h. The benzene content adsorbed in the sorbent tube (SKC 226-01) was desorbed with 1 ml benzene-free carbon disulfide (E. Merck, India) on a developing vibrator (SKC, Inc., USA) and later analysed by Trace GC (Thermo Finnegan, Italy) fitted with an MXT-624 column (Restek, Inc., USA) and a flame ionization detector.

The method followed that of Ducos et al. (1990, 1992) and was followed for the determination of *t*,*t*-MA in the urine samples of study and controls. End-shift urine samples were collected in polythene bottles from both studies, and those from control group workers at the end of the shift were bought to the laboratory in an ice pack and refrigerated. The pH of the urine samples was adjusted to 7–9 using a phosphate buffer of pH 7.4. QSAX anion exchange 100 mg/3 cc cartridges (Analchem India) were fixed to the Visiprep vacuum manifold (Supelco, USA) and conditioned with 3 ml methanol and 3 ml Milli Q ultra-pure water. Before the cartridge becomes dry, 1 ml alkalized urine was loaded on the cartridge and passed through very slowly. The cartridges were washed twice with 3 ml 1% aqueous acetic acid in water. Finally, the *t*,*t*-MA was eluted with 1 ml 10% acetic acid at a flow rate of 1 ml/30 min. An aliquot of 20 µl of acetic acid layer was injected into the HPLC system attached with PDA detector (LC-10 AT VP Shimadzu, Japan). The column used for the analysis was a Shimpack C8 stainless steel column at 30°C with a mobile phase of 10% methanol:90% of 1% (v/v) aqueous acetic acid at a flow rate of 1 ml min^{-1}. The detector wavelength used was 259 nm. The *t*,*t*-MA standard (Catalogue No. m 9000-3) procured from M/s Aldrich Chemical Co., USA and Sigma Aldrich Foreign Holding Co., Bangalore, India was used in the preparation of standard calibration curve in the range 0.2–1.0 µg ml^{-1}. The extraction efficiency of the Q-SAX cartridge was 90%. The concentration of urinary *t*,*t*-MA was calculated with an external standard calibration curve of *t*,*t*-MA in the range 0.2–1.0 µg ml^{-1}.

The urinary creatinine was estimated with Bayer's improved kit (Cat. No. 748) method (Picrate Method) using an RA-50 Chemistry analyser (M/s Ames Technicon-Miles, Inc., USA). Urine (1 ml) was diluted with 24 ml distilled water (Milli Q Ultra pure water) and made up to 25 ml. The working reagents were prepared according to the procedure mentioned therein. Reagent 1 (Picrate), which contains picric acid 34.9 mmol l^{-1} and sodium hydroxide 45 mmol l^{-1}, and reagent 2 (sodium hydroxide 0.26 mol l^{-1}) were mixed in equal volumes in a clean beaker. A total of 100 µl diluted urine sample were mixed with 1.0 ml mixed reagent (1+2) and immediately fed into the RA-50 instrument. The creatinine concentration was measured at 500 nm. The standardization of the procedure was carried out using standard creatinine (2 mg dl^{-1}) supplied by the company. The urine creatinine concentrations were measured as mg dl^{-1} and finally expressed as g l^{-1}. The *t*,*t*-MA concentrations were expressed as µg g^{-1} creatinine and compared with the American Conference of Government Industrial Hygienists (2005) BEI for *t*,*t*-MA.

Results and discussion

The mean TWA concentrations of benzene found among 29 gasoline handlers and 30 office staff were 1.10 ± 1.08 and 0.070 ± 0.035 mg m^{-3}, respectively. After data analysis, it was found that among six gasoline handlers/study subjects, 20.69% of the TWA concentration of benzene exceeded the ACGIH Threshold Value Limit (TLV) of 1.60 mg m^{-3}. The minimum and maximum TWA concentrations of benzene found among study and controls were 0.19–5.27 and 0.018–0.173 mg m^{-3}, respectively. These large variations of benzene concentrations found were dependent on the amount of gasoline sold and the exposure to the vapours during delivery of the petrol into the vehicle tank. During monitoring, it was noted that some employees lowered their face towards the tank to check the quantity being delivered. Hence, in such situations, the concentration may go up depending upon the duration of exposure.

The mean levels of *t*,*t*-MA among study and controls found in the present study are given in Table I.

ACGIH BEI for t,t-*MA: 500 μg* g^{-1} *creatinine*

The mean *t*,*t*-MA concentration found among gasoline handlers (study subjects) and office staff (controls) were 563.16 ± 281.81 and 266.88 ± 110.65 μg g^{-1} creatinine, respectively. The *t*,*t*-MA concentrations among 15 (50%) gasoline handlers exceeded the ACGIH BEI of 500 μg g^{-1} creatinine. Although the study subjects were informed not to smoke during the monitoring period, only three study subjects were found to be habitual smokers (one to two cigarettes/day) and they smoked during lunch and teatime. The individual concentrations of *t*,*t*-MA found in three gasoline handlers (smokers) were found: 652.14, 312.63 and 190.14 μg g^{-1} creatinine. The individual concentrations of *t*,*t*-MA found in five controls (smokers) were 268.88, 143.0, 292.0, 138.28 and 454.66 μg g^{-1} creatinine. The first four controls (corresponding to 268.88, 143.0, 292.0 and 1138.28 μg g^{-1} creatinine) smoked one to two cigarette(s) during the monitoring period. But one smoker smoked five to six cigarettes during monitoring period (*t*,*t*-MA = 454.66 μg g^{-1} creatinine). However, none of *t*,*t*-MA concentrations among office staff (controls) exceeded the ACGIH BEI. A significant correlation ($p < 0.05$, $r = 0.442$) was found between personal air benzene concentration and urinary *t*,*t*-MA concentration in the present study.

Using HPLC, Wiwanitkit et al. (2001) found for a Bangkok gas station that attendants had a urinary *t*,*t*-MA concentration of 4.00 mg g^{-1} creatinine (4000 μg g^{-1} creatinine), which was three times more than the controls. The values found were more than the ACGIH BEI. Radhouane et al. (2002) monitored benzene

Table I. Mean levels of urinary *t*,*t*-MA among study and controls.

Job category	*t*,*t*-MA (μg g^{-1} creatinine)	Number of values exceeding the Biological Exposure Index (BEI) of 500 μg g^{-1} creatinine
Gasoline handlers (study subjects)	563.16 ± 281.81 (163.43–1196.46) 29	15 (50.0%)
Office staff (controls)	266.88 ± 110.65 (112.44–454.66) 30	nil

exposure and urinary *t*,*t*-MA among 20 tanker fillers and ten filling station attendants (study subjects) and 20 non-occupationally exposed subjects. The average environmental benzene concentration was 0.17 ppm. The benzene air concentrations were well correlated with post-shift *t*,*t*-MA concentration ($r = 0.76$). However, in our present study the mean *t*,*t*-MA found among study subjects was twice more than the controls.

Carrer et al. (2000) studied the daily exposure to volatile organic compounds and performed biological monitoring among office workers in Milan, Italy. The mean benzene and toluene concentrations found were 21.2 and 35.2 μg m^{-3}, respectively. Biological monitoring was performed by measuring blood benzene and toluene concentrations together with urinary *t*,*t*-MA and cotinine at the end of the monitoring period. All the mean levels of biological indicators were significantly higher in active smokers than in non-smoking subjects not exposed to environmental tobacco smoke. But in the present study, the mean benzene concentrations found among office staff were three times higher than the Milan office workers.

From our results, it is concluded that 51.72% of gasoline handlers (15) have higher *t*,*t*-MA values than the ACGIH BEI for *t*,*t*-MA. During the study, the workers did not consume canned food and none smoked during the monitoring period. Hence, it can be assumed that the higher levels of *t*,*t*-MA were mainly due to a higher concentration of benzene in the occupational environment. It is concluded that the gasoline handlers are at risk due to higher levels of benzene in gasoline filling stations.

The benzene content in the occupational environment can be reduced to a greater extent with a reduction of the benzene content in refineries and the introduction of a vapour recovery system at gasoline filling stations. In the near future after the introduction of Bharat III-grade improved fuel, a follow-up study is planned.

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