Comparison of Benzene Exposure in Drivers and Petrol Stations Workers by Urinary trans, trans-Muconic Acid in West of Iran

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Abstract: Motor vehicle traffic is the main emission source of benzene. We undertook this study in order to compare benzene exposure and urinary levels of trans,trans-muconic acid (t,t-MA) in taxi drivers and petrol station workers. Air benzene levels were analyzed with gas chromatography using a Flame Ionization Detector. t,t-MA was extracted from urine and analyzed using high performance liquid chromatography. Significant differences in levels of urinary t,t-MA were found in drivers and petrol station workers when compared to a control group (p<0.05). Correlation coefficients between benzene in air and t,t-MA for petrol station workers and drivers were 0.65 and 0.30, respectively. The concentration of benzene in the breathing zone of petrol station workers was 2–3 times higher than drivers, and also 3 times greater than a threshold level (0.5 ppm) recommended by the American Conference of Governmental Industrial Hygienists (ACGIH). The lowest benzene concentration at which urinary t,t-MA increased to a measurable level was approximately 0.17 ppm. In conclusion our results suggested that high benzene levels are emitted in petrol stations in west Iran. t,t-MA analysis was able to separate those exposed from the non-exposed benzene group when benzene in the breathing zone of subjects was greater than 0.17 ppm.

Key words: Benzene, Muconic acid, Drivers, Petrol stations workers

Introduction

Environmental concentrations of benzene have increased significantly in the recent years due to increased road traffic. The major sources of benzene in ambient air of urban areas are car exhaust and evaporation loss during the handling, distribution and storage of petrol1–2).

Urinary metabolites that might serve as indices of occupational or environmental exposure to benzene include phenol, hydroquinone, trans, trans-muconic acid (t,t-MA), and S-phenyl mercapturic acid3–5). Of these, urinary t,t-MA concentration is recognized as a reliable biomarker which is relatively convenient to measure6). t,t-MA is a minor non-phenolic metabolite of benzene that is excreted in urine. The American Conference of Governmental Industrial Hygienists (ACGIH) introduced t,t-MA as biological exposure index for benzene exposure7). Several investigations have reported the urinary concentration of t,t-MA in subjects who were exposed to benzene in manufactures of industrialized country8), nevertheless, few data exist which compare exposure to benzene among different occupations in developing countries9).

The major environmental problem in Iran is air pollution. There are currently around 6 million vehicles in Iran, of
which 40% are at least two decades old). The government phased out leaded gasoline and had replaced it with unleaded gasoline by January 2000, which caused the addition of more aromatic hydrocarbons to gasoline. Petrol stations in Iran located on streets and workers at the stations undertake pumping and services of fuel. During their daily work, workers have direct contact with petroleum products, such that occupational exposure to benzene cannot be avoided. The aims of this study were to evaluate exposure to benzene and compare levels of t,t-MA in taxi drivers and petrol station workers in a region of Western Iran.

Materials and Methods

Subjects

This study was carried out on a sample of men exposed to benzene in two occupational groups in Hamadan state: taxi drivers and petrol station workers. The study groups consisted of 25 workers at petrol stations and 60 taxi drivers. The study group was selected from 54 workers at petrol stations, and 300 drivers by simple random sampling. A control group of 60 non-expose men living in a rural area were selected from the same state. The control group was matched with the study group based on age, smoking status and had not exposure to benzene. A detailed questionnaire was completed for this study and control participants, providing information about personal characteristics, smoking and drinking habits.

Personal monitoring of exposure

A charcoal adsorption tube from (SKC, USA) connected to a small pump (Negretti Automation, Model NR645, England) was used to obtain personal samples. The charcoal tube was attached to the worker’s overalls as closely as possible to the face in order to determine the benzene concentrations in the breathing zone. The pump was operated at 200 ml/minute and the duration of sampling was 2–4 h. Benzene was extracted with carbon disulphide (CS₂) from the charcoal. A gas chromatography machine (Model 4600-Unicam Company, England) equipped with Flame Ionization Detector (FID) was used for quantitative measurement. Separation of the compounds was achieved with glass column 1.5 m x 4 mm i.d packed with 10% SE 30 on Chromosorb W-AW-DMCS 100-120.

This column temperature was programmed at 50°C for 2 minutes then increased to 180°C at a rate of 4°C/minute, and finally kept at constant temperature of 180°C for 2 min. The results were calculated in ppm unit over 8 h average.

Results

The characteristics of subjects and control groups were shown in Table 1. Statistical tested showed that there were no differences in age as well as smoking status between the subjects and control group (p>0.05).

Exposed subjects and non-exposed control were asked to pass urine at the end of the shift. Samples were refrigerated immediately, transferred to the analytical laboratory, and kept frozen until analysis.

The determination of t,t-MA was carried out according to the method of Boogaard & Van Sittert. To improve the recovery, urinary samples were brought to PH 7–10 by the addition of 35% (w/v) sodium hydroxide aqueous solution before the sample was cleaned using solid phase extraction. Urinary samples were centrifuged (2,000 rpm for ten minutes) to separate suspended materials and 1 ml was subsequently passed through a SAX column which had been previously conditioned with 3 ml of acetonitrile and 3 ml of water. After washing with 3 ml of 1% percent acetic acid, t,t-MA was eluted from the cartridge with 4 ml of 10% acetic acid. Twenty micro liters of this solution were analyzed by high performance liquid chromatography (HPLC).

A HPLC system equipped with a UV detector (Model K-2600 Knauer, Germany) was used for analysis. The UV detector was set at 259 nm. The HPLC column was an APEX ODS II 3 µm (250 x 4.6 mm) (Beckman, USA) analytical column. Chromatography was isocratic in a mobile phase consisting of water-methanol-acetic acid (89:10:1). The flow rate was set at 1 ml/minute. All chemicals and water used were HPLC grade. In these conditions, the retention time for t,t-MA is about 14–15 min.

Urinary creatinine was measured by the Jaffé’s kinetic method using a Boehringer Mannheim Hitachi 917 automatic analyzer, and were used to adjust the concentration of t,t-MA.

Data analysis was performed with SPSS statistical software for windows. Comparison between the mean t,t-MA values (creatinine adjusted) was carried out with Mann-whitney test and between benzene concentration was tested by the student’s t-test. A logistic regression was used to define the border level of t,t-MA between the exposed group and control group.
subjects \((p<0.05)\). The mean concentrations of benzene in the ambient air of drivers, and petrol station workers were 0.30 and 1.41 ppm respectively. There was a significance difference between the levels of t,t-MA in the urine of smokers and non-smokers \((p<0.05)\). The mean value of urinary t,t-MA in the group of non-smokers and smokers were 0.80 ± 0.92 and 1.25 ± 1.40 mg/g creatinine respectively.

To determine contribution of smoking to urinary t,t-MA levels, the relation between benzene exposure and urinary t,t-MA in all of subjects (drivers and petrol station workers) for non-smokers, and both groups (smokers and non-smokers) were shown in Figs. 3 and 4 respectively. In non-smokers, the mean and standard deviation value of urinary t,t-MA in drivers, petrol station workers and control group were (0.27 ± 0.21), (2.11 ± 1.14) and (0.14 ± 0.03) mg/g creatinine, respectively. The results were shown there are significant difference for urinary t,t-MA between the exposure groups comparing to the control group \((p<0.05)\).

To determine the cut point for exposure to benzene, we used the logistic regression analysis. The logistic regression was used to define the border level of t,t-MA between the exposed group and control group wherever the smoking status was controlled. Based on the results of the logistic regression, the border level for t,t-MA was 0.32mg/g creatinine. The critical value of benzene concentration from the line constructed by regressing t,t-MA on the benzene concentration was obtained 0.17ppm wherever smoking status was controlled \((y=0.5 + 1.57x, \text{Fig. 3})\).

The critical urinary of t,t-MA for benzene concentration at 0.17 ppm based on Figs. 2–4 was 0.38, 0.35 and 0.34 mg/g creatinine respectively. The critical concentration of MA at Fig. 1 is different to Figs. 2–4 because the wide range is between 0.2 to 3.1 ppm.

Data were categorized according to mean concentrations of benzene exposure, in three groups: 1) less than 0.17 ppm (all of them were drivers) 2) data between 0.17 ppm to 0.5 ppm (23 drivers and 1 petrol station worker) 3) data more than 0.5 ppm (4 drivers and 24 petrol station workers). The statistical showed mean urinary t,t-MA of that drivers

Table 1. Characteristics of subjects and control groups

<table>
<thead>
<tr>
<th></th>
<th>Smokers (%)</th>
<th>Non-smokers (%)</th>
<th>Total</th>
<th>Age (x ± SD)</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Taxi Drivers</td>
<td>20 (34%)</td>
<td>40 (66%)</td>
<td>60</td>
<td>31.76 ± 9.17</td>
<td>24–53</td>
</tr>
<tr>
<td>Petrol station workers</td>
<td>9 (36%)</td>
<td>16 (64%)</td>
<td>25</td>
<td>34.13 ± 7.92</td>
<td>20–57</td>
</tr>
<tr>
<td>Control group</td>
<td>18 (30%)</td>
<td>42 (70%)</td>
<td>60</td>
<td>32.18 ± 6.22</td>
<td>22–59</td>
</tr>
</tbody>
</table>

Table 2. Results of benzene in ambient air and biological monitoring of trans,trans-muconic acid at different occupations and control group

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Taxi Drivers</th>
<th>Petrol station workers</th>
<th>Control group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benzene in air (ppm)</td>
<td>n=60</td>
<td>n=25</td>
<td>N=60</td>
</tr>
<tr>
<td>x ± SD</td>
<td>0.31 ± 0.22**</td>
<td>1.40 ± 0.80**</td>
<td>–</td>
</tr>
<tr>
<td>Range</td>
<td>0.07–0.95</td>
<td>0.2–3.1</td>
<td></td>
</tr>
<tr>
<td>t,t-MA (mg/litr)</td>
<td>x ± SD</td>
<td>0.42 ± 0.51*</td>
<td>3.10 ± 0.64*</td>
</tr>
<tr>
<td></td>
<td>Range</td>
<td>0.11–1.51</td>
<td>1.70–3.5</td>
</tr>
<tr>
<td>t,t-MA (mg/g creatinine)</td>
<td>x ± SD</td>
<td>0.31 ± 0.44*</td>
<td>2.64 ± 0.47*</td>
</tr>
<tr>
<td></td>
<td>Range</td>
<td>0.09–1.27</td>
<td>1.20–3.28</td>
</tr>
</tbody>
</table>

*Significant difference comparing with control group.
** Significant difference between concentration of benzene in the breathing zone of drivers and petrol station workers \((p<0.05)\).
exposed to benzene at concentration less than 0.17 ppm, did not differ significantly from the control group. Whereas, mean urinary t,t-MA of drivers and also workers of petrol station workers who exposed to benzene concentration more than 0.17 ppm, (group 2 and 3) differ significantly from control group (Table 3).

Figs. 1 and 2 show the scatter diagrams between benzene concentration in the breathing zone, and urinary t,t-MA of petrol station workers and drivers respectively. Significant associations were noted between urinary t,t-MA and benzene in the breathing zone for petrol station workers (r=0.65; p<0.05).

**Discussion**

The present study was undertaken to evaluate exposure to benzene and the relationship between t,t-MA and atmospheric benzene among taxi drivers and petrol station workers. The mean concentration of benzene in breathing zones of petrol station workers was 2–3 times higher than that of the drivers, and also 3 times greater than the threshold level (0.5 ppm) recommended by the American Conference of Governmental Industrial Hygienists (ACGIH). The mean concentration of benzene in the breathing zone of drivers was less than the threshold level, but greater than reported...
studies from Asia, Australia, and America\textsuperscript{13–17}. The mean urinary \textit{t,t}-MA in workers of petrol stations and drivers was slightly less than gas station attendants in Bangkok (4 mg/g creatinine)\textsuperscript{19} but greater than research results carried out in Tunis (0.11 mg/g creatinine)\textsuperscript{19}.

Low fuel prices, old motor technology and a lack of catalytic converters has led to unburned hydrocarbons being emitted into the ambient air of gasoline stations and also to the inside of vehicles in Iran. Other ancillary factors are cited as well, evaporation of volatile organics from car carburetors and petrol tanks, the relatively old age of cars and the consequent inefficiencies fuel burning of their motors.

Refueling at petrol stations and staying inside the vehicles have been shown to contribute to increased exposure to benzene. During refueling, the exposure level of petrol station workers varies according to the benzene content of fuel and the amount of time spent at the station. The proportion of petrol stations to number of vehicles in Iran is low, and for cities with populations of between 0.5 to 1 million there are usually 7 to 10 stations. This cause vehicles stay for a long time (between 5–30 min) waiting for refueling and therefore hydrocarbons such as aromatic compounds may be emitted from car exhausts to these locations.

Benzene in the air inside vehicles is largely derived from engine exhaust emissions or evaporative losses, and varies in concentration according to the vehicle type, age, the type of fuel used, traffic variables such as density and speed, and ventilation characteristics\textsuperscript{19}.

There was a good correlation between urinary \textit{t,t}-MA and personal exposure level to benzene when benzene in air is higher than 0.17 ppm. In concentrations less than 0.17, the use of \textit{t,t}-MA as a benzene biomarker is complicated because \textit{t,t}-MA is also a metabolite of sorbic acid\textsuperscript{20}. Flavored drinks and sweet snack foods result in the excretion of large amounts of \textit{t,t}-MA in adults and children\textsuperscript{21}. The consumption of sorbic acid as preserved food is common in Iran, as this research showed that \textit{t,t}-MA in control group was between 0.01 to 0.35 mg/g creatinine, and critical concentration for 0.17 ppm benzene is around 0.35 mg/g creatinine wherever the smoking status was controlled.

There was no correlation between the ambient level of benzene and urinary \textit{t,t}-MA among drivers, of those the personal exposure level of benzene was low. There are some differing research results considering the association between urinary \textit{t,t}-MA and the low level benzene exposure. Some authors have found excellent correlations. For example, Bergamachi et al.\textsuperscript{22} examined exposure in 24 nonsmoking bicyclists during 2-h rides on urban and rural routes. They measured benzene in personal sampled air, blood, and urine. Urinary \textit{t,t}-MA was also measured. A statistically-significant correlation coefficient of 0.59 was found between air benzene (ranging from 1.2 to 26.1 ppb) and the difference of urinary \textit{t,t}-MA between pre- to post-ride. Others however, have not found significant correlations. Examples include a study of 80 bus drivers, whose benzene exposure based on urinary benzene, were calculated to range from 3 to 313 ppb, during which urinary benzene and urinary \textit{t,t}-MA were not correlated\textsuperscript{20} and another study showing a lack of the correlation at occupational exposure levels <0.25 ppm\textsuperscript{21}. In addition, several authors have reported unexpectedly high urinary \textit{t,t}-MA levels (corresponds exposure of 1 ppm benzene) in non occupationally-exposed control subjects\textsuperscript{23, 25}.

As there was significant difference between urinary \textit{t,t}-MA in smokers and non-smokers during this study, our results are in agreement with other researches\textsuperscript{27–29} where the normal levels of \textit{t,t}-MA in non-smokers at control group was less than 0.06 mg/g creatinine, while higher levels were found in active smokers (more than 0.3 mg/g creatinine).

In conclusion our results suggest that high benzene levels are emitted in petrol stations at Iran. Urinary \textit{t,t}-MA level was able to separate those exposed to benzene from the non-exposed group when benzene in the breathing zone of subjects was greater than 0.17 ppm. We suggest that extensive attention to benzene exposure is needed for maintaining the health of gasoline station workers in Iran, as elsewhere.

**References**


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**Table 3. The mean levels of urinary \textit{t,t}-MA at different concentrations of benzene exposure**

<table>
<thead>
<tr>
<th>Concentration of benzene (ppm)</th>
<th>\textit{t,t}-MA (mg/g creatinine)</th>
<th>\textit{p}-value\textsuperscript{*}</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;0.17</td>
<td>0.24 ± 0.40\textsuperscript{†}</td>
<td>0.24</td>
</tr>
<tr>
<td>0.17–0.5</td>
<td>0.43 ± 0.60\textsuperscript{*}</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>0.5&lt;</td>
<td>2.80 ± 0.75\textsuperscript{*}</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

\textsuperscript{*}Not significant difference compare with control group.

\textsuperscript{†}Significant difference compared with control group.
acid by workers occupationally exposed to benzene. Occup Environ Med 55, 705–11.
7) American Conference of Governmental Industrial Hygienists (ACGIH) (2003) Threshold limit values for chemical substances and physical agents & bibliological exposure indices, ACGIH worldwide, Cincinnati.