Original Article

J Res Health Sci, Vol. 8, No.2, 2008, pp. 61-68

Environmental Exposure to Xylenes in Drivers and Petrol Station Workers by Urinary Methylhippuric Acid

**Bahrami A. Ph.D, *Jonidi-Jafari A. Ph.D, **Mahjub H. Ph.D*

**Dept. of Occupational Health, Faculty of Health, Hamadan University of Medical Science, Iran*
***Dept. of Biostatistics & Epidemiology, Faculty of Health, Hamadan University of Medical Science, Iran*

(Received 1 Apr 2008; Accepted 30 Sep 2008)

Abstract

Background: The aims of this study were evaluation of exposed to xylenes in low concentration and compare urinary level of methyl hippuric acid in taxi drivers and petrol stations workers in West of Iran.

Methods: This observation study was carried out on samples of the exposed men to xylenes in two occupational groups in Hamadan City (west of Iran) from March 2003 to March 2004. Subjects included 45 taxi drivers and 25 petrol station workers. The study group was selected from 54 workers at petrol stations and 300 drivers by simple random sampling. Xylenes was analyzed using gas chromatography equipped with a Flame Ionization Detector (FID). The urinary methyl hippuric acid (MHA) was analyzed with High performance Liquid Chromatography (HPLC) equipped with an ultraviolet (UV) detector.

Results: Total xylene exposure was 1.05±0.55 ppm (mean±SD) with a range of 0.20-2.55 ppm that was about 4 times more than taxi drivers' exposure. The poor correlation coefficient was seen between xylenes concentration and urinary MHA for drivers (r^2= 0.09 to 0.42) but significant associations were noted between urinary MHA and xylene in the breathing zone of petrol station workers (r^2= 0.69 to 0.77; P< 0.05).

Conclusion: High xylenes levels are emitted in petrol stations at Iran. Urinary MHA level has a poor correlation with exposure to xylenes in drivers but has good correlation in petrol station workers.

Keywords: *Xylene, Methylhippuric acid, Drivers, Petrol station workers, Urine, Air, Iran*

Introduction

Xylene can exist in three isomeric forms. These three isomers possess similar properties. Commercial xylene is a mixture of these three isomers: ortho, meta and para, with the meta form usually the principal component (45-70%). As no explosive aromatic hydrocarbons, mixtures of the three (technical xylene) isomers are heavily used in the chemical industry and in the petroleum industry as a solvent and gasoline "antiknock" additives. Xylene will cause depression of the central nervous system. This narcotic effect will impair performance and affect cerebral function (1-3). Xylenes are ubiquitous in the environment, xylenes released to the atmosphere as fugative emissions from industrial sources, in automobile exhaust through volatilization of xylenes used as solvents. Most of the released to the environment partition into air (4). The American Conference of Governmental Industrial Hygienists (ACGIH) recommends a threshold limit value (TLV) of 100 ppm for xylenes and also recommended a biological exposur index for MHA 1.5 g/creatinine measured in urine from end of the shift (5).

Corresponding author: Dr AR Bahrami, Fax: +98 811 8255301, E-mail: A167R@yahoo.com

Xylene is metabolized at a half-life rate of 20 to 30 h. Only about 5% of xylene is exhaled unchanged. The balance of xylene is metabolized by the oxidation of a methyl group to toluic acid. The toluic acid is converted to MHA and excreted in the urine (6-7). There are some investigations about the urinary level of MHA in subjects who were exposed to xylenes in manufactures of industrialized country (8-10). There are not any researches about compare exposure to xylene among different occupations in developing countries.

The major environmental problem in Iran is air pollution, there are about of 6 million vehicles in Iran and 40% of them are at least two decades old (11). The government of Iran phases out leaded gasoline and replace it with unleaded gasoline by January 2000 it cause added more aromatic hydrocarbons to gasoline. The petrol pump stations in Iran are besides of street and workers in this location did the pumping and services of fuel. During their daily work, workers have direct contact with petroleum products, so occupational exposure to xylene cannot be avoided.

This research was carried out in Hamadan city, west of Iran. Hamadan is the centre of Hamadan Province with high vehicular density including 1000-2500 per hour in the centre of city and 20-250 per hour in the gasoline stations.

The aims of this study were evaluation of exposed to xylenes in low concentration in taxi drivers and petrol stations workers and compare level of methyl hippuric acid in West of Iran.

Materials and Methods

This observational study was carried out on samples of the exposed men to xylenes in two occupational groups in Hamadan City (west of Iran) from March 2003 to March 2004. Subjects included 45 taxi drivers and 25 petrol station workers. The study group was selected from 54 workers at petrol stations and 300 drivers by simple random sampling. A detailed questionnaire was completed for subjects, providing information about personal characteristics and smoking history. We remove the subjects that used food interference but affect of smoking and age were reported in paper.

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 (11). The charcoal tube was attached to the worker's overalls as closely as possible to the face in order to determine the xylenes concentrations in the breathing zone. The pump was operated at 200 ml/min and the duration of sampling was 2-4 h. Xylenes were extracted with carbon disulphide (CS_2) from the charcoal. A gas chromatography (Model 4600-Unicam Company, England) equipped with a Flame Ionization Detector (FID) was used for quantitative measurement. Separation of the compounds was achieved with glass packing column 1.5m×4 mm i.d packed with 10% SE 30 on Chromosorb W –AW-DMCS 100-120. This column had a programmed temperature of 50° C for 2 min followed by an increase to 180° C at a rate of 4° C min^{-1} and finally during analysis a constant temperature of 180°C for 2 min.

Analysis of urine

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

MHA in Urine

The determination of MHA was carried out according to National Institute for Occupational Safety and Health (NIOSH) method (12). Initially, 40 µl of HCl and 0.3 gr sodium chloride were added to 1 mL of urine into a graduated centrifuge tube. Four mL of ethyl acetate added to tube and the samples were mixed centrifuged at 1200 r.p.m for 5 min, then the ethyl acetate layered transferred to tapered test tube by pasture pipette. Samples were evaporated to dryness using a gentle steam of nitrogen in a heating block at 45 ºC before reconstitution. The residue of samples redissolved in 200 µl of distilled water and 20 µl was injected to High Performance Liquid Chromatography (HPLC) system. A HPLC chromatograph (Knauer) equipped with an ultraviolet (UV) detector (Model K-2600 Knauer) was used for analysis. The UV detector was set at 254 nm. The HPLC column was an APEX ODS II 3µm, 25×4.6 mm. Chromatography was isocratic in a mobile phase consisting of water-acetonnitrile-acetic acid (89:10:1) at a flow rate of 1 mL min^{-1}.

The urinary creatinine was measured by Jaffe kinetic method using a Boehringer Mannheim Hitachi 917 automatic analyzer and was reported following adjustment for creatinine concentration.

Data analysis was performed using SPSS statistical software for windows (version 13.0). Kolomogorov-Smirnov test was used for normality distribution of variables. If any variable was not normal, Log_{10} trasformation was used to get a normal distribution. For association between two non-normal variables, Spearman's correlation coefficient was used. For fitting first-order or second-order factors, log transformation of variables was used. In addition, for non-normal data, median and geometric mean was calculated. For comparing between smokers and non-smokers Mann-Whitney U test was used.

Results

The levels of MHA in urine and xylenes concentration in the breathing zone of drivers and petrol station workers are shown in Table 1. There was a significant difference between the levels of MHA in urine of drivers and petrol station workers ($P< 0.05$). The mean concentrations of xylenes in the ambient air of drivers, and petrol station workers were 0.24 and 1.05 ppm respectively. The urinary level of m-MHA is more than o- and p-MHA in both occupations. The urinary MHA in three samples of drivers were not observed in subjects exposed to xylene less than 0.02 ppm.

Fig. 1 and 2 show the scatter diagrams between xylene concentration in the breathing zone, and urinary MHA of drivers and petrol station workers, respectively.

There was not any significant difference for the levels of urinary o-MHA with o-xylenes and poor correlations were observed between m & p-xylenes with urinary m & p-MHA in drivers (Table 2 and Fig. 1). Significant associations were noted between urinary MHA and xylene in the breathing zone for petrol station workers (Table 2 and Fig. 2). No differences were detected between smokers and non-smokers and also age groups either in drivers or in petrol station workers. None of subjects in both groups used drinks because using of alcohol banned in Iran.

Table 1: Results of xylenes in ambient air and biological monitoring of MHA at different occupations

Parameter		Taxi Drivers n=45	Petrol station workers n=25
M&p-xylene	x ± SD[1]	0.15±0.16	0.65±0.36
(ppm)	Range	0.00-0.70	0.12-1.65
	Median	0.09	0.58
	GM[2]	0.06	0.56
o-xylene	AM ± SD	0.09±0.10	0.39±0.20
(ppm)	Range	0.00-0.39	0.08-0.90
	Median	0.06	0.38
	GM	0.03	0.34
Xylenes	AM ± SD	0.24±0.26	1.05±0.55
(ppm)	Range	0.00-1.09	0.20-2.55
	Median	0.14	0.96
	GM	0.10	0.92

Table 1: Continued…

m-MHA (mg/g creatinine)	AM ± SD	8.88±12.77	31.53±14.46
	Range	0.00-80.34	18.95-110.78
	Median	4.32	25.66
	GM	2.34	29.21
p-MHA (mg/g creatinine)	AM ± SD	4.82±8.92	18.44±16.69
	Range	0.00-59.31	8.42-70.32
	Median	2.21	11.40
	GM	1.21	14.68
o-MHA (mg/g creatinine)	AM ± SD	3.59±4.97	15.61±13.30
	Range	0.00-23.12	7.02-67.77
	Median	1.20	9.75
	GM	0.51	12.76
MHA (mg/g creatinine)	AM ± SD	17.30±24.86	65.59±42.76
	Range	0.00-151.86	34.39-203.00
	Median	8.12	46.57
	GM	5.55	57.27

[1].SD; Standard division
[2]. GM; Geometric means

Table 2: The regression line between xylenes in ambient air exposure and urinary methyl hippuric acid at different occupations

Occupations		**Regression line Formula**	**Determination Coefficient (R^2)**
Petrol station Workers	o-xylene and o-MHA	Log o-MHA=1.75 +2.34 Log o-xylene + 1.50 Log (o-xylene)^2	0.77
	m & p-xylene and m&p-MHA	Log m&p-MHA=1.77 +1.08 Log m&p-xylene + 1.93 (Log m&p-xylene)^2	0.69
Drivers	o-xylene and o-MHA	Log o-MHA=0.78 +0.43 Log o-xylene	0.09
	m & p-xylene and m&p-MHA	Log m&p-MHA=1.62 +0.71 Log m&p-xylene	0.38

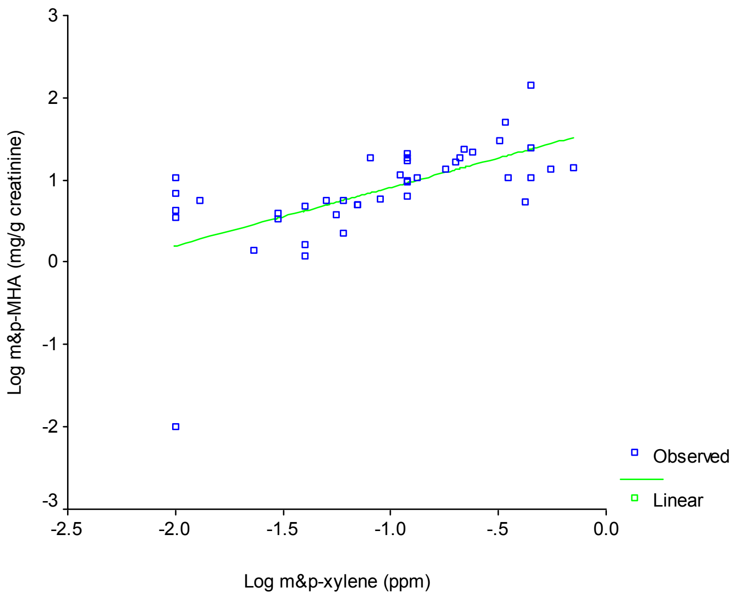

Fig. 1: Correlation between MHA in urine and xylenes in the breathing zone of drivers

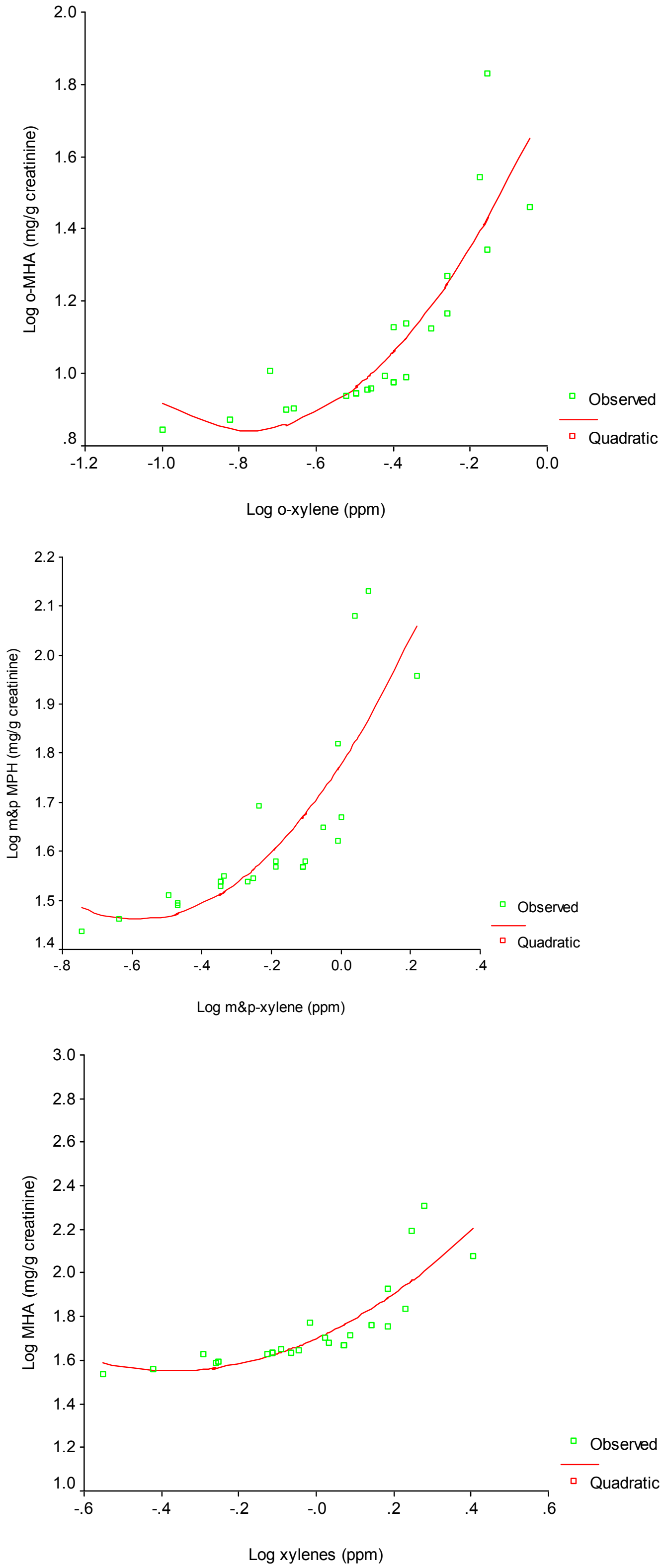

Fig. 2: Correlation between MHA in urine and xylenes in the breathing zone of petrol station workers

Discussion

The present study was undertaken to evaluate the relationship between MHA and atmospheric xylenes among taxi drivers and petrol station workers. Data from this study showed that MHA had a poor correlation coefficient with low concentration of xylenes in drivers. The exposure to xylenes in some other environmental sources such as exposure to aromatic hydrocarbons at home, cigarette or sources from street other than inside of vehicle, effected on urinary level of MHA. The other factors that made variation in urinary of MHA included anatomical and physiological difference between people, individual work practice, difference between inhaled xylenes concentration. The mean concentration of xylenes in ambient air for drivers in current study was 0.24 differences to other reports. Jacobson and Mclean found that biological monitoring of occupational xylene exposure at level <15 ppm using urinary MHA had a good correlation with atmospheric levels and was a valid complement to ambient monitoring (8). Also Jang et al showed significant correlation between xylene and MHA wherever the mean concentration for xylenes was 12.77 ppm (14). The American Conference of Governmental Industrial Hygienists (ACGIH, 2006) has recommended a biological exposure index for MHA of 1.5 g MHA/g creatinine measured from an end of shift urine sample after a TWA exposure to 100 ppm (15). There are several studies in which correlation between occupational xylene exposure and urinary MHA exist. The results of this study showed that urinary m-MHA was greater than o- and p- MHA, it may concern to metabolism of m-xylene. In a study, Miller and Edwards was found that m-xylene isomers undergo preferential metabolism compared with o-and p-xylene (6), but study by Kawai et al. on individual xylene isomers showed that the slopes of the regression lines for o- and m-isomers were similar, whereas that for p-xylene was larger (10). Studies of rats indicate that m-xylene in some efficiently absorbed through the skin than toluene, benzene or hexane (17). Takeuchi et al. found weakly correlation between the concentration exposure to the xylenes and unmetabolized xylene concentration in the end-of-the shift urine samples (17).

We have not find any significant difference between urinary MHA smokers and nonsmokers but Huang et al. reported that metabolism of xylenes was significantly reduced among smokers or drinkers compared with non-smokers and non-drinkers (18). The mean concentration of xylenes in breathing zones of petrol station workers was 4-5 times higher than that of the drivers. The mean concentration of xylenes in the breathing zone of drivers was greater than reported studies from Asia, Australia, and America (19-20). The Refueling at petrol stations and staying inside the vehicles have been shown to contribute to increased exposure to aromatic hydrocarbons. During refueling, the exposure level of petrol station workers varies according to the xylenes 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.

In conclusion our results suggest that high xylene levels are emitted in petrol stations at Iran. Urinary MHA could not be applying as good biomarkers in drivers wherever subjects exposed to low level of xylenes in ambient air but it is good biomarkers for exposure of xylene in petrol station workers.

Acknowledgements
This research was supported by Hamadan University of Medical Science. The authors declare that they have no conflict of interests.

References
1. National Occupational Health and Safety. *NIOSH Pocket Guide to Chemical Hazards*. Cincinnati, NIOSH, 2005. pp12-56
2. Yang PY, Lin JL, Hall AH, Tsao TC, Chern MS. Acute ingestion poisoning with insecticide formulations containing the pyrethroid permethrin, xylene, and surfactant: a review of 48 cases. *J Toxicol Clin Toxicol*. 2002; **40:**107-13.
3. Ukai H, Inui S, Takada S, Dendo J, Ogawa J, Tsobe K, Ashida T, Tamura M Tabuki K, Ikeda M. Types of organic solvents used in small- to medium-scale industries in Japan; a nationwide field survey. *Arch Occup Environ Health*. 1997; **70:** 385-92.
4. Leung PL, Edwards JW. Evaluation of personal exposure to monoaromatic hydrocarbons. *Occup Environ Med*. 1998; **55**:249-57.
5. American Conference of Governmental Industrial Hygienists. Threshold limit values for chemical substances and physical agents & biliological exposure Indices. Cincinnati: *ACGIH worldwide*; 2003.
6. Miller MJ. Possible preferential metabolism of xylene isomers following occupational exposure to mixed xylenes. Int Arch. Occup. *Environ Health Mar*. 1999; **72:** 89-97.
7. Langman JM. Xylene: its toxicity, measurement of exposure levels, absorbtion metabolism and clearance. *Pathology*. 1994; **26**: 301-309.
8. Jacobson GA, Mclean S. Biological monitoring of low level occupational xylene exposure and the role of recent exposure. *Ann Occup Hyg*. 2003; **47**: 331-36.
9. Inoue O, Seiji K, Kawai T, Jin Ch, Cai S-X, Chi L Qu Q-S, Zhang T, Ikeda M. Excretion of methyl hippuric acids in urine of workers exposed to a xylene mixture. Comparison among three xylene isomers and toluene. *Int Arch Occup Environ Health*. 1993; **64**: 533-9.
10. Kawai T, Misname K, Yasugi T, Horiguchi S, Uchida Y, Twami O, Iguchi H, Ikdea M. Urinary methylhippuric acid isomer levels after occupational exposure to a xylene mixture. *Int Arch Occup Environ Health*. 1991; **63**: 69-75.
11. Center of Iran statistic. Tehran statistical handbook, Tehran, Budget and program Organization: 2002.
12. National Institute for Occupational Safety and Health Hydrocarbons. Manual of analytical methods. Aromatic: METHOD 1501, dated 15 March 2003, Cincinnati: NIOSH; 1997.
13. National Institute for Occupational Safety and Health Hydrocarbons. Manual of analytical methods. Aromatic: METHOD 8301, Cincinnati, NIOSH, 1997.
14. Jang Y. Biological monitoring of workers exposed to ethylbenzene and coexposed to xylene. *Int Arch Occup Health*. 2001; **74**: 31-7.
15. Hinwood AL, Rodriguez C, Runnion T, Farrar D, Murray F, Horton A, Glass D, Sheppeard V, Edwards JW, Denison L, Whitworth T, Eiser C, Bulsara M, Gillett RW, Powell J, Lawson S, Weeks I, Galbally I. Risk factors for increased BTEX exposure in four Australian cities. *Chemosphere*. 2006; **3**:533-41.
16. Takeuchi A, Kawai T, Zhang ZW, Miyama Y, Sakamoto K, Higashikawa K, Ikeda I. Toluene, xylenes and xylene isomers in urine as biological indicators of low level exposure to each sol-

vent; a comparative study *Int Arch Occup Environ Health.* 2005; **75**: 387-93.
17. Huang MY. Exposure of workers to a mixture of toluene and xylenes. I metabolism. *Occup and Environ Med*. 1994; **51**: 42-6.
18. Lee SC, Chiu MY, Ho KF, Zou SC, Wang X. Volatile organic compounds in urban atmosphere of Hong Kong. *Chemosphere*. 2002; **48**: 375-82.
19. Gee IL, and Sollars CJ. Ambient air levels of volatile organic compounds in latin American and Asian cities. *Chemosphere.* 1998; **36**: 2497-506.
20. Brocco D, Fratarcangeli R, Lepore L, Petricca M and Venrrone I. Determination of aromatic hydrocarbons in urban air of Rome. *Atmos Environ*. 1997; **31**: 551-66.