

## **Exposure of Sweepers to Volatile Organic Compounds Using Urinary Biological Exposure Index**

*\*Bahrami AR. PhD, \*\*Ansari M. MD*

*\*Dept. of Occupational Health, Faculty of Health, Hamadan University of Medical Science, Hamadan, Iran*

*\*\*Dept. of Internal Medicine, Faculty of Medicine, Hamadan University of Medical Science, Hamadan, Iran*

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### **Abstract**

**Background:** Evaluation of exposition to Volatile Organic Compounds (VOC) and compare urinary level of urinary biological exposure index in sweepers in West of Iran.

**Methods:** This study was carried out on 40 street sweepers and 40 non-exposed as control group living in Hamadan City, west of Iran. VOC was analyzed using gas chromatography equipped with a Flame Ionization Detector (FID). The urinary biological exposure index (BEI) was analyzed with High performance Liquid Chromatography (HPLC) equipped with an ultraviolet (UV) detector. The concentration of benzene, toluene, m & p-xylene o-xylene and ethyl benzene was 39.01, 121.60, 27.12, 12.30, and 10.21 ( $\mu\text{g}/\text{m}^3$ ), respectively.

**Results:** No-difference in the levels of hippuric acid in urine was found in sweepers compared with the control group. Significance differences in the level of o-cresol in urine were found in sweepers compared to control group ( $P < 0.005$ ). The poor correlation coefficient was seen between xylenes concentration and urinary MHA for drivers.

**Conclusion:** High VOC are emitted inside of cities at Iran. O-cresol could separate the exposed to toluene from the non-exposed in sweepers. Hippuric acid, methyl hippuric acid and muconic acid are not suitable biomarkers for occupations such as sweepers exposure to VOC in low concentration.

**Keywords:** *Xylene, Methylhippuric acid, Petroleum, Urine, Air*

### **Introduction**

The contamination of urban air by hydrocarbons and inorganic pollutants at high concentration causes serious adverse effects on human health. At typical ambient concentrations, the focus of interest is on benzene because it has been recognized as a class I carcinogenic agent from the International Agency for research on cancer (IARC), as long term exposure to high benzene concentrations, is known to cause bone marrow damage, leukemia and aplastic anemia. Outdoors exposure

to benzene is primarily connected with vehicle emissions, including both exhaust and evaporative losses of benzene, toluene and xylene are known arise from the same sources (1).

Among urban citizens, the highest exposure to air borne pollutants is experienced by outdoor workers, such as policemen, street sweepers, postal workers and newspaper vendors. There are not any studies regarding evaluation the sweepers exposed to these contaminants, although several studies have been undertaken to estimate the relationship between these contaminants and their related metabolites in other occupations (1-4). It is

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**\*Corresponding author:** Dr AR Bahrami, Fax: +98 811 825 5301, E-mail: Bahrami@Umsha.ac.ir,

showed that concentration of VOC is between 89.9 to 178.8  $\mu\text{g}/\text{m}^3$  in Hamadan City, west of Iran (5).

In this study the contribution of the exposure to traffic emissions in the exposure to benzene was assessed in street sweepers. These workers are professionally exposed to vehicle exhaust while sweeping the street. The sweepers exposed directly to volatile organic compounds more than general population of citizens.

## **Materials and Methods**

This study was carried out on a 40 street sweepers that exposed to hydrocarbons in Hamadan City, west of Iran. A control group of 40 non-exposed 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, provided 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 (6). The charcoal tube was attached to the worker's overalls as closely as possible to the face in order to determine the monocyclic aromatic hydrocarbons concentrations in the breathing zone. The pump was operated at 200 ml/min and the duration of sampling was 2-4 h. Benzene was extracted with carbon disulphide ( $\text{CS}_2$ ) from the charcoal. A gas chromatography machine (Model 4600-Unicom Company, England) equipped with Flame Ionization Detector (FID) was used for quantitative measurement. Separation of the compounds was achieved with glass column 1.5m $\times$ 4mm i.d packed with 10% SE 30 on Chromosorb W-AW-DMCS 100-120.

This column temperature was programmed at 50° C for 2 min then increased to 180° C at

a rate of 4° C/min, and finally kept at constant temperature of 180° C for 2 min. The results were calculated in ppm unit over 8 h average.

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

The determination of t,t-MA was carried as earlier stated (7). 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 (2000 rpm for ten min) to separate eventual suspended materials. One ml was subsequently passed through a SAX column. The column 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 chromatograph equipped with a UV detector (Model K-2600 Knauer) was used for analysis. The UV detector was set at 259 nm. The HPLC was an APEX ODS II 3 $\mu\text{m}$  (25cm  $\times$  4.6mm) 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/min. All chemicals and water used were HPLC grade. In these conditions, the retention time for trans, trans muconic acid was about 14-15 min. The determination of MHA and HA was carried out according to NIOSH (8). Initially, 40  $\mu\text{l}$  of HCl and 0.3 gram 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 °C before reconstitution. The residue of samples redissolved in 200 µl of distilled water and 20 µl was injected to 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-acetonitrile-acetic acid (89:10:1) at a flow rate of 1 mL min<sup>-1</sup>.

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

Data analysis was performed using SPSS statistical software for windows. Comparison be-

tween the t, t-MA mean values (creatinine adjusted) was carried out with Mann-whitney test and for benzene concentration was obtained by the student's *t*-test.

## Results

The results of concentration of volatile organic compounds in breathing zone of sweepers and control group are shown in Table 1. No-difference in the levels of non-smoker workers were found compared with the smoker group. The results of the levels of urinary biological index in sweepers and control group are shown in Table 2. No-difference in the levels of hippuric acid in urine was found in sweepers compared with the control group. Significance differences in the level of o-cresol in urine were found in sweepers compared to control group (*P* < 0.005).

**Table 1:** The mean concentration of volatile organic compounds exposure (µg/m<sup>3</sup>) in sweeper

		Whole group	Smoker	Non-smoker
Benzene	x±SD	39.01±10.93	40.45±13.29	37.34±10.11
	Range	53.27-21.48	53.27-34.45	42.21-21.48
Toluene	x±SD	121.60±33.72	115.45±37.87	130.56±30.98
	Range	160.37-73.34	145.21-73.34	160.37-110.60
M & p-xylene	x±SD	27.12±8.83	24.34±9.42	30.20±7.80
	Range	35.25-13.36	30.21-13.36	35.25-22.38
O-xylene	x±SD	12.30±4.30	11.24±4.87	13.67±5.76
	Range	18.47-7.45	15.21-7.45	18.47-10.34
Ethyl benzene	x±SD	10.21±5.45	12.35±5.75	8.45±3.90
	Range	15.51-7.34	15.51-9.87	10.90-7.34

**Table 2:** The results of levels of urinary biological index in sweepers and control group

		study group	control group	<i>P</i>
t,t-MA (µg/g creatinine)	x ± SD	103.45 ± 110.35	125.09 ± 134.61	0.35
	Range	10.25-245.21	2.75-261.12	
MH (µg/g creatinine)	x ± SD	270.45 ± 135.23	255.71 ± 140.87	0.43
	Range	101.34-351.27	121.65-374.38	
o-cresol (µg/l)	x ± SD	85.12 ± 32.23	15.98 ± 12.45	0.005
	Range	49.56-121.19	0.00- 23.98	
3MHA (mg/g creatinine)	x ± SD	3.34 ± 4.37	1.21 ± 1.19	0.05
	Range	0.00-8.56	0.00-2.89	
4MHA (mg/g creatinine)	x ± SD	2.41 ± 3.56	1.18 ± 2.21	0.05
	Range	0.00-6.01	0.00-3.65	
2MHA (mg/g creatinine)	x ± SD	1.85 ± 2.75	0.67 ± 1.61	0.05
	Range	0.00-4.15	0.00-2.34	
MHA (mg/g creatinine)	x ± SD	7.67 ± 8.35	3.04 ± 5.91	0.05
	Range	0.00-16.34	0.00-6.90	

## Discussion

The mean concentration of VOC in the breathing zone of sweepers was less than the threshold level (9), but greater than reported studies in other occupations (10, 11). Low fuel prices, old motor technology and a lack of catalytic converters have led to VOC being emitted into the ambient air from car exhaust. The exposure to VOC in some other environmental sources such as exposure to aromatic hydrocarbons at home, cigarette or sources from street other than inside of vehicle; effects to urinary biological index. The other factors that made variation in urinary biological index include anatomical and physio-logical difference between people, individual work practice, and difference between inhaled VOC concentrations. Data from this study showed that MHA had a poor correlation coefficient with low concentration of xylenes in sweepers. There was also a weak correlation between urinary t,t-MA and personal exposure level to benzene in sweeper workers when benzene in air was less than 0.17 ppm. In concentrations less than 0.17, the use of t,t-MA as a benzene biomarker is complicated because t,t-MA is also a metabolite of sorbic acid (12). Flavored drinks and sweet snack foods result in the excretion of large amounts of t,t-MA in adults and children (13). There are some differing research results considering the association between urinary t,t-MA and the low level benzene exposure. Some authors have found excellent correlations. For example, Bergamachi et al. (14) examined exposure in 24 nonsmoking bi-cyclists during 2-h rides on urban and rural routes. They measured benzene in personal sampled air, blood, and urine. Urinary 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 t,t-MA between pre- to post-ride.

There was a good correlation between o-cresol and toluene in sweeper worker but a weak correlation found for methyl hippuric acid with toluene. The urinary excretion of o-cresol represents a specific and sensitive indicator of an individual toluene uptake. Angerer found a correlation coefficient of 0.65 for subjects that exposed to toluene at 64.8 ppm (15). No-difference in the levels of hippuric acid in urine was found in sweepers compared with the control group.

We have not seen the effect on smoking habits on the level of VOC in urinary biological index but Huang et al. reported that metabolism of xylenes was significantly reduced among smokers or drinkers compared with non-smokers and non-drinkers (16). Some other studies reported significant difference between smoker and non-smokers that exposed to benzene in ambient air (17, 18). In conclusion our results suggest that high VOC are emitted inside of cities at Iran. We suggest that extensive attention to benzene exposure is needed for maintaining the health of sweepers in Iran. Our result showed that o-cresol could separate the exposed to toluene from the non-exposed in sweepers. Hippuric acid, methyl hippuric acid and muconic acid is not suitable biomarkers for occupations such as sweepers exposure to VOC is in low concentration.

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