Original Article

Biological monitoring of petrochemical industry workers exposed to benzene, toluene, xylenes, methyl ethyl ketone, and phenol in Southern Iran

Rahimpoor R, MSc[1], Bahrami AR, PhD[2*], Assari MJ, PhD[3], Ghorbani F, PhD[4], Negahban AR, MSc[1], Rahimnejad S, MSc[1], Mehdizadegan B, BSc[5]

1- MSc in Occupational Health, Dept. of Occupational Health, Centre of Health Researches, School of public Health, Hamadan University of Medical Science, Hamadan, Iran. 2- Professor, Dept. of Occupational Health, Centre of Health Researches, School of public Health, Hamadan University of Medical Science, Hamadan, Iran. 3- Assistant Prof., Dept. of Occupational Health, Centre of Health Researches, School of public Health, Hamadan University of Medical Science, Hamadan, Iran. 4- Associate Prof., Dept. of Occupational Health, Centre of Health Researches, School of public Health, Hamadan University of Medical Science, Hamadan, Iran.5- BSc in Occupational Health, Dept. of Occupational Health, Centre of Health Researches, School of public Health, Hamadan University of Medical Science, Hamadan, Iran.

Abstract

Received: July 2015, **Accepted**: August 2015

Background: Volatile organic compounds (VOCs), including carcinogens and toxic compounds, are produced in petrochemical industries. We undertook this study in order to study workers' respiratory exposure to different VOC concentrations and compare the results with the urinary levels of its metabolites and study the correlation between them in petrochemical industries.

Materials and Methods: Exposure to benzene, toluene, xylene isomers, phenol, and methyl ethyl ketone (MEK) was evaluated in 104 male subjects using personal sampling pump and charcoal sorbent tube at the breathing zone and analyzed using gas chromatography–mass spectrometry (GC-MS). The urine samples were analyzed using high-performance liquid chromatography (HPLC-UV) and gas chromatography-flame ionization detector (GC-FID).

Results: The mean concentration of urinary trans,trans-muconic acid (t,t-MA), was 1431 µg/g creatinine and hippuric acid, methyl hippuric acid, phenol, and MEK were, 0.394, 0.444, 0.098 g/g creatinine, and 0.15 mg/l. The mean concentration of benzene in the breathing zone was greater than the threshold limit value (TLV) recommended by the American Conference of Governmental Industrial Hygienists (ACGIH).

Conclusions: In this study, a more significant relationship was found between benzene in breathing zone and urinary t,t-MA in those exposed to benzene in concentrations higher than 1 ppm ($r^2 = 0.89$) than lower than 1 ppm. Moreover, the same results were observed for other hydrocarbons and their level of urinary biological index. A more significant relationship was observed between phenol in breathing zone and urine through exposure to airborne phenol in concentrations of higher than 0.049 ppm than lower than 0.049 ppm ($r^2 = 0.75$). The relationship between MEK in breathing zone and urinary MEK was more significant in concentrations of higher than 0.1 ppm ($r^2 = 0.79$) than lower than 0.1 ppm.

Keywords: Volatile organic compounds, Biological monitoring, Occupational exposure, benzene, toluene, xylenes, methyl ethyl ketone, phenol.

Introduction

Volatile organic compounds (VOCs) including airborne carbon compounds evaporate under atmospheric pressure and temperature (1). They are commonly found in the environment, work-place, and consumer products (2), and are absorbed into the human body through different routes. However, inhalation is the main route of exposure to these compounds, due to the high vapor pressure of these

* **Corresponding author:** Abdul Rahman Bahrami, Dept. of Occupational Health, Centre of Health Researches, School of public Health, Hamadan University of Medical Science, Hamadan, Iran
Email: A167R@yahoo.com

compounds (3, 4). VOCs can have adverse effects on the human health and environment. Benzene, toluene, xylene isomers, phenol, and methyl ethyl ketone (MEK) are the most consumed VOCs and they are used in various industries (5-7). Benzene is a known human group 1 carcinogen [International Agency for Research on Cancer (IARC)] and skin A1 carcinogen [American Conference of Governmental Industrial Hygienists (ACGIH)], and causes hematotoxicity, aplastic anemia, acute myelogenous leukemia, and lymphoma (8-10). Toluene is not currently classified as a carcinogen, but exposure to toluene can increase the risk of rectal and colon cancer, and effects the kidneys and nervous system (11). Several studies have demonstrated that acute and chronic exposure to xylene is associated with rectal and colon cancer and anemia, and has adverse effects on the lungs, liver, and kidneys (12). Inhalation exposure to phenol may cause adverse health effects such as lung and gastric cancer and leukemia, and cardio-vascular, kidney, and endocrine systems disorder. Acute exposure to MEK can cause neurological and digestive disorders and chronic exposure to MEK can cause skin and behavioral disorders (13).

In general, petrochemical industries including large-industrial are the high important of viewpoint occupational health and industrial safety. They are one of the sources of releasing VOCs in the atmosphere, due to production process, storage tanks, and wastewater of these industries (14-16). Biological monitoring of workers exposed to solvents are supplement environmental monitoring and could be used to evaluate the effects of workload, work habits, exposure to various routes, and in health risk assessment (17, 18). The ACGIH has introduced trans,trans-mocunic acid (t,t-MA), hippuric acid (HA), methyl hippuric acid isomers (2-MHA, 3-MHA, and 4-MHA), phenol, and MEK as urinary metabolites following exposure to benzene, toluene, xylene isomers (O, M and P-xylene), phenol, and MEK, respectively (8).

The aims of this study were to evaluate occupational exposure to VOCs in the breathing zone of workers, compare their urinary biological index, and find correlation between them at the Mahshahr Petrochemical Industries, Khuzestan Province, Iran.

Material and methods

This study was carried out on 104 workers exposed to VOCs in the petrochemical industries in the South of Iran.

A charcoal adsorption tube (SKC, Inc., USA) connected to a small pump (Model 222, SKC, Inc., USA) was used to obtain personal samples (19). The charcoal tube was attached to the workers' overalls as close to the face as possible in order to obtain the samples in the breathing zone. The pump was operated at 200 ml/minute and the duration of sampling was 3.5-4 hours. From each worker, 2 samples were taken to cover all exposure hours. Benzene, toluene, xylene isomers, phenol, and MEK were extracted with carbon disulfide (CS_2) from the charcoal. A gas chromatography (GC) machine (Model CP 3800, Varian, USA) equipped with mass spectrometry was used for qualitative and quantitative measurement. Separation of the compounds was achieved with capillary column 25 m × 0.22 mm × 2.5 µm. The operation conditions were 2.5 ml/minute hydrogen, 25 ml/minute air flow, and detector temperature of 280 °C. Column temperature was programmed at 30 °C for 12 minutes, and then, increased to 180 °C at a rate of 20 °C/minute, and finally, kept at constant temperature of 180 °C for 0.5 minute. The results were calculated in mgm^3 unit over an average of 8 hours (20, 21).

Urine samples of subjects were collected at the end of the shift in a 250 ml polyethylene bottle containing a few crystals of thymol. Samples were refrigerated immediately, transferred to the analytical laboratory at Department of Occupational Health, Hamedan University of Medical Sciences, Iran, and kept frozen until analyzed.

t,t-MA in urine:

The determination of t,t-MA was carried out as stated above (22-24). To improve the recovery, urine samples were brought to a pH of 7-10 by the addition of 1 molar 75% sodium hydroxide aqueous solution before the sample was cleaned using solid phase extraction. Urinary samples were centrifuged (1500 rpm for 5 minutes) to separate suspended materials. Subsequently, 1 ml of samples was passed through a SAX column. The column had been previously conditioned with 3 ml of methanol and 1 ml water. After washing with 2 ml of 1% acetic acid, t,t-MA was eluted from the cartridge with 3 ml of 10% acetic acid. Then, 50 µl of this solution was analyzed by a high-performance liquid chromatography (HPLC) machine. A HPLC chromatograph equipped with an ultraviolet (UV) detector (Model K-2600 Knauer) was used for analysis. For the analysis of t,t-MA, the UV detector was set at 259 nm. The HPLC was an NUCLEOSIL100-5-C18- 5 µm (250 ×4 mm) 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 chemical and water used were HPLC grade. Under these conditions, the retention time for t,t-MA was about 13-15 minutes.

HA and MHA isomers in urine:

The determination of HA and MHA isomers was carried out according to the National Institute for Occupational Safety and Health (NIOSH 8301) (25). Initially, 80 µl of 6 N of HCL and 0.3 g sodium chloride were added to 1 ml of urine in a graduated centrifuge tube. Subsequently, 4 ml ethyl-acetate was added to the tube and samples were centrifuged at 1200 rpm for 5 minutes, then, the ethyl-acetate layer was transferred to a tapered test tube using a pasture pipette. Samples were evaporated to dryness under a gentle stream of nitrogen in a heating block at 45 °C before reconstitution. The residue of samples was redissolved in 200 µl of distilled water, and 5 µl of the product was injected into the HPLC system. A HPLC chromatograph equipped with an ultraviolet (UV) detector (Model K-2600 Knauer) was used for analysis. In the HA analysis, the UV detector was set at 254 nm. The HPLC was an NUCLEOSIL100-5-C18- 5 µm (250 × 4 mm) analytical column. Chromatography was isocratic in a mobile phase consisting of water-acetonitrile-acetic acid (89:10:1). The flow rate was set at 1.5 ml/minute. All chemical and water used were HPLC grade. Under such conditions, the retention time for HA, 2-MHA, 3-MHA, and 4-MHA was about 3, 6, and 6 minutes, respectively.

Phenol in urine:

The determination of phenol was carried out based on NIOSH 8305 (26). Initially, 1 ml of HCL was added to 5 ml of urine in a 15 ml graduated centrifuge tube. The centrifuge tube stopper was loosely closed and the water bath was heated 95 °C for 1.5 hours. The centrifuge tube was removed from the water bath and 10 µl internal standard (0.6 mg/ml nitrobenzene in methanol) was added to the tube and volume in the centrifuge tube was adjusted to 10 ml with distilled water. Subsequently, 2 ml diethyl ether was added to the tube and it was shaken vigorously for 1 min, then, cooled to 0 °C. The tube was allowed to separate, 0.5 ml of clear diethyl ether and 0.5 mg sodium sulfate were transferred to a 2 ml vial, and 5 µl of the resulting mixture was injected into the GC system. A GC (Model GC 2010-SHIMADZU, RX MAX) equipped with a flame ionization detector (FID) and a 30 m × 25 mm × 25 µm Gas Kuro pack 54 fused-silica capillary column was used. The operation condition was 25 ml/minute nitrogen and temperature injection, column, and detector were 180, 120, and 200 °C, respectively. This column temperature was programmed at 120 °C for 4 minutes, then, was increased to 190 °C at a rate of 16 °C/minute, and finally, kept at a constant temperature of 190 °C for 4 minutes.

MEK in urine:

The determination of MEK was carried out as mentioned above (27). Initially, 1 ml of urine was added to a 2 ml vial and vial stopper was fastened. Finally, the water bath was heated 75 °C for 35 minutes, and using the headspace

technique, 5 µl of the mixture was injected into the GC system. A GC (Model GC 2010-SHIMADZU, RX MAX) equipped with FID and 30 m × 25 mm × 25 µm Gas Kuro pack 54 fused-silica capillary column was used. The operation conditions consisted of 40 ml/minute of nitrogen and temperature injection, column, and detector at 100, 35, and 200 °C, respectively. This column temperature was programmed at 35 °C for 1.5 minutes, then, increased to 80 °C at a rate of 25 °C/minute and kept at a constant temperature of 80 °C for 0.5 minute, then, increased to 120 °C at a rate of 70 °C/minute, and finally, kept at a constant temperature of 120 °C for 2 minutes.

Urinary creatinine was measured through the kinetic Jaffe method using a spectrophotometer (Model Lambda 950 UV/VIS, USA). The spectrophotometer was set at 520 nm and reported any concentration adjustment.

Data analysis was performed using SPSS statistical software for windows (version 16.0, SPSS Inc., Chicago, IL, USA). Least squares linear regression was used to investigate the relationship between the level of benzene, toluene, O-xylene, M-xylene, P-xylene, phenol, and MEK in breathing zone and the level of urinary t,t-MA, HA, 2-MHA, 3-MHA, 4-MHA, phenol, and MEK. The Pearson correlation coefficient was used to evaluate the relationship between the level of benzene, toluene, O-xylene, M-xylene, P-xylene, phenol, and MEK in breathing zone and the level of urinary t,t-MA, HA, 2-MHA, 3-MHA, 4-MHA, phenol, and MEK in smoker and nonsmoker subjects. Student's t-test was used to study the difference in levels of urinary metabolites between the smoker and nonsmoker subjects.

Results

The mean of age, work history, and work hours per week of subjects were 35.73, 11.60, and 54.44, respectively. The concentrations of VOCs in breathing zone and urinary metabolites of subjects are shown in table 1. The results showed that the concentrations of toluene, xylene, phenol, and MEK and their urinary metabolites were lower than standard limits recommended by ACGIH and occupational exposure limits (OELs) by the Occupational and Environmental Centre of the Ministry of Health and Medical Education, Iran. The concentration of benzene in most petrochemical industries is higher than the national standard limits.

Table 1: Environmental and biological monitoring data for all workers

	TLV or BEI	Mean ± SD	Geom. Mean± SD	Variance
Benzene in air (mg/m^3)	1.60	3.81 ± 2.04	0.000 ± 8.13	4.160
t,t-MA in urine (µg/g creatinine)	500.00	1431 ± 472	0.021 ± 1532	12278
Toluene in air (mg/m^3)	75.37	4.96 ± 4.98	1.740 ± 10.23	24.800
HA in urine (g/g creatinine)	1.60	0.39 ± 0.11	0.113 ± 0.84	0.012
O-xylene and M-xylene in air (mg/m^3)	434.23	5.17 ± 5.56	0.560 ± 12.94	30.910
2-MHA and 3-MHA in urine (g/g creatinine)	1.50	0.44 ± 0.11	0.134 ± 0.68	0.012
P-xylene in air (mg/m^3)	434.23	9.86 ± 16.89	0.420 ± 41.38	285.270
4-MHA in urine (g/g creatinine)	1.50	0.10 ± 0.02	0.040 ± 0.11	0.004
Phenol in air (mg/m^3)	19.25	0.34 ± 0.10	0.030 ± 0.63	0.010
Phenol in urine (mg/g creatinine)	250.00	0.58 ± 0.23	0.000 ± 1.13	0.053
MEK in air (mg/m^3)	589.86	6.62 ± 7.21	0.620 ± 25.16	51.980
MEK in urine (mg/l)	2.00	0.15 ± 0.05	0.001 ± 0.12	0.002

TLV: Threshold limit value; t,t-MA: Trans,trans-muconic acid; HA: Hippuric acid; MHA: Methyl hippuric acid; MEK: Methyl ethyl ketone; BEI: Biological exposure indices

The highest mean concentration of benzene, toluene, O-xylene, M-xylene, P-xylene, phenol, and MEK in breathing zone was related to occupational groups of operator (1.694 ppm), lab sample man (3.428 ppm), repairman (7.327 ppm), lab sample man (6.412 ppm), site man (0.637 ppm), and exploiter (3.439 ppm), respectively. However, the highest mean concentration of urinary t,t-MA, HA, 2-MHA, 3-MHA, 4-MHA, Phenol, and MEK was related to the occupational groups of operator (2441.765 µg/g creatinine), lab staff (0.817 g/g creatinine), repairman (0.742 g/g creatinine), supervisor (0.142 g/g creatinine), site man (1.188 mg/g creatinine), and exploiter (0.371 mg/l), respectively. The concentration of urinary metabolites in different occupational groups is shown in table 2.

Table 2: Concentration of urinary metabolites among various occupational groups in the studied petrochemical industries

Occupational group	N	t,t-MA (µg/g creatinine)	HA (g/g creatinine)	2-MHA and 3-MHA (g/g creatinine)	4-MHA (g/g creatinine)	Phenol (mg/g creatinine)	MEK (mg/l)
Site man	30	1257.980	0.732	0.489	0.132	1.188	0.101
Exploiter	18	552.661	0.545	0.395	0.112	0.348	0.371
Tipper	3	493.065	0.812	0.041	0.094	0.169	0.087
Security office	3	182.061	0.013	0.017	0.056	0.333	0.049
Lab sample man	10	1413.254	0.152	0.140	0.087	0.146	0.158
Operator	13	2441.765	0.782	0.438	0.095	0.423	0.136
Repairman	21	2317.808	0.601	0.742	0.119	0.404	0.198
Lab staff	1	78.455	0.817	0.076	0.052	0.215	0.022
Supervisor	5	1077.137	0.170	0.647	0.142	0.307	0.310
Total	104	1431.150	0.394	0.444	0.098	0.585	0.154

t,t-MA: Trans,trans-muconic acid; HA: Hippuric acid; MHA: Methyl hippuric acid; MEK: Methyl ethyl ketone

The urinary t,t-MA, HA, 2-MHA ,3-MHA, 4-MHA, Phenol, and MEK levels for smokers were 1440 µg/g creatinine, 0.206 g/g creatinine, 0.651 g/g creatinine, 0.329 g/g creatinine, 0.641 mg/g creatinine, and 0.304 mg/l, respectively. For nonsmokers, these levels were 550 µg/g creatinine, 0.426 g/g creatinine, 0.572 g/g creatinine, 0.179 g/g creatinine, 0.103 mg/g creatinine, and 0.013 mg/l, respectively. There was a significant difference between urinary metabolites in smokers and nonsmokers ($P < 0.05$). The Pearson correlation coefficient (R^2) between VOCs exposure and urinary metabolites for smokers and nonsmokers is shown in table 3. A statistically significant correlation was found in all subjects between VOCs in breathing zone and urinary metabolite level (Figures 1 and 2).

Table 3: Pearson correlation coefficient (R2) between VOCs exposure and urinary metabolites for smokers and nonsmokers

Smoking	**Benzene and t,t-MA**	**Toluene and HA**	**O-xylene, M-xylene, 2-MHA, 3-MHA**	**P-xylene and 4-MHA**	**Phenol in air and urine**
Smokers	0.79	0.56	0.81	0.92	0.75
Nonsmokers	0.68	0.78	0.69	0.73	0.57
P-value	0.030	0.075	0.023	0.036	0.024

t,t-MA: Trans,trans-muconic acid; HA: Hippuric acid; MHA: Methyl hippuric acid

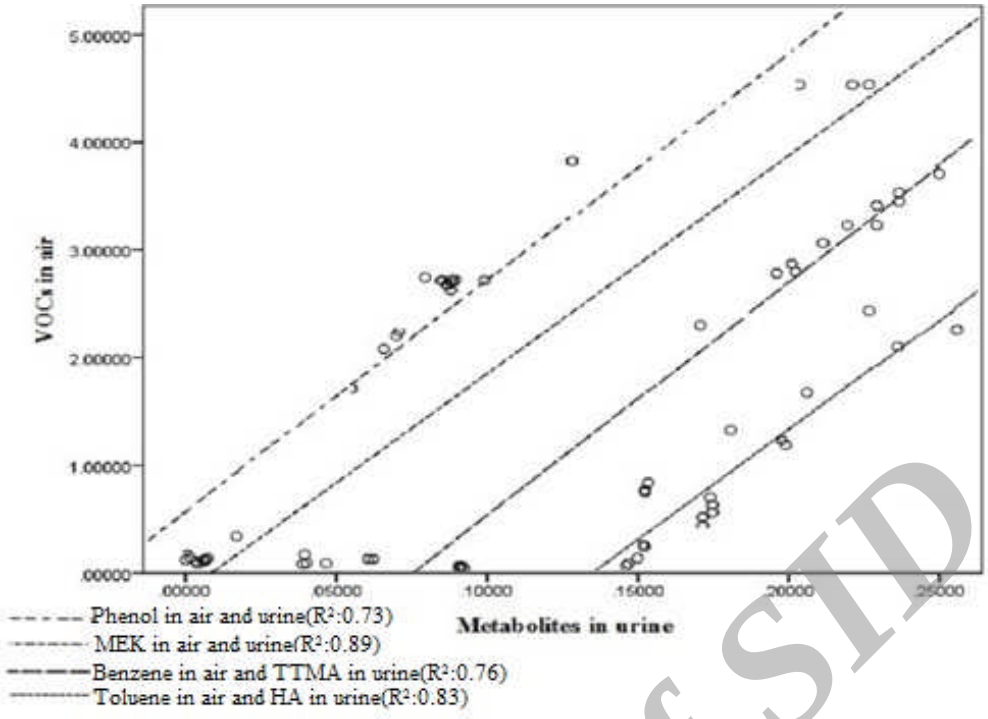

Figure 1: Correlation between VOCs exposure levels and urinary metabolites for all samples

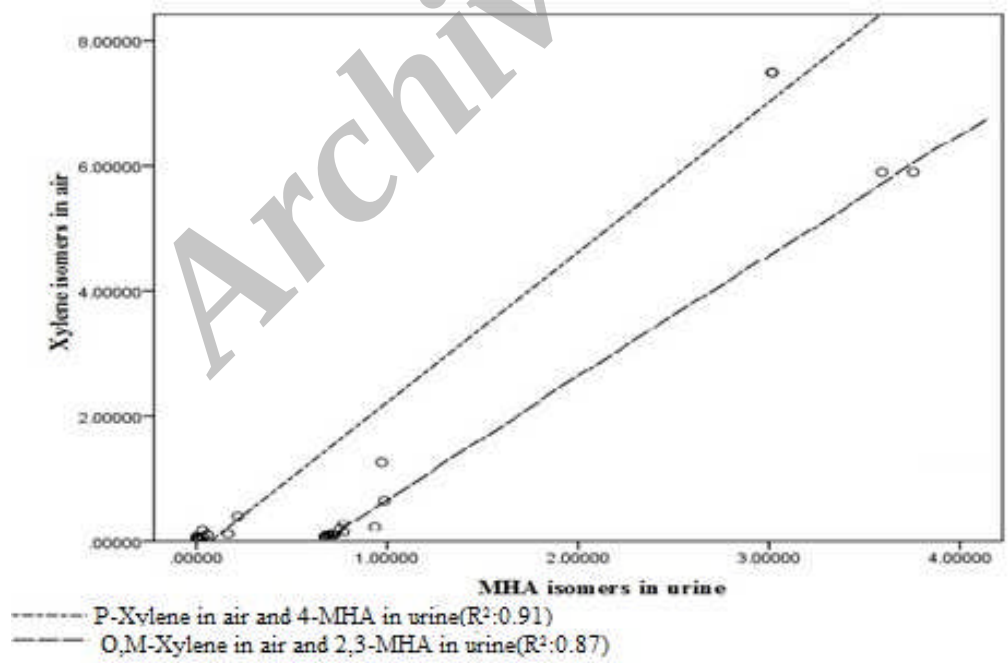

Figure 2: Correlation between Xylene isomers exposure levels and urinary MHA isomers for all subjects

Discussion

The present study was undertaken to investigation exposure to VOCs and urinary metabolites in petrochemical industry workers. The mean concentration of benzene in breathing zone was higher than the TLV recommended by ACGIH, but the concentration of other compounds was lower than the standard limits (8). Although benzene is not a raw material of most industries and is only used in two industries, it was found in the ambient air of all industries. It seems the wind transfers benzene to all industrial sites in the petrochemical area. Bahrami et al. reported that those exposed to benzene could be separated from non-exposed groups through urinary t,t-MA level when benzene level in the breathing zone of subjects was higher than 0.17 ppm. In concentrations lower than 0.17 ppm, t,t-MA is a metabolite of sorbic acid. Thus the use of t,t-MA as a biomarker of benzene is complicated. The use of flavored drinks and sweet snacks can cause the urinary excretion of t,t-MA (28).

Various studies have been conducted by several authors at different industrial cities in order to understand airborne VOCs distributions and their sources (29-31). VOCs emitted from industrial sources into the atmosphere may cause pollution on a local scale. In an industrial city, VOC emissions could not only be the result of industrial sources, but also could be affected by surrounding traffic sources (32).

We found that concentrations of urinary t,t-MA, 2-MHA, 3-MHA, phenol, and MEK in outdoor workers (operator, repairman, site man, exploiter, lab sample man, and tipper) were significantly higher than that in indoor workers (security officer, lab staff, and supervisor). However, the concentration of urinary HA and 4-MHA of the two groups were not significantly different. This observation could be explained by the fact that concentration of VOCs in the outdoor environment is significantly higher than the indoor environment. In addition, outdoor workers in workstations depending on the type of job were chronically exposed to VOCs. Therefore, concentrations of urinary metabolites of these compounds in outdoor workers were higher than that in indoor workers.

Data from this study showed that t,t-MA, HA, and MHA have a good correlation with concentrations of the related hydrocarbons. Many studies have confirmed these results (33-35), but some studies reported a poor correlation between t,t-MA and benzene (36). Angerer et al. reported a poor correlation between HA and toluene (37). A more significant relationship was observed between respiratory benzene and urinary t,t-MA, xylene isomers, and MHA isomers concentrations (r^2 = 0.89, r^2 = 0.86, and r^2 = 0.95, respectively) in those exposed to benzene and xylene isomers in concentrations lower than 1 ppm compared to higher than 1 ppm (r^2 = 0.16, r^2 = 0.024, and r^2 = 0.042, respectively). However, a more significant relationship was observed between respiratory toluene and urinary HA concentrations (r^2 = 0.879) in concentrations of higher than 1 ppm than lower than 1 ppm (r^2 = 0.18). The results showed that the relationship between respiratory phenol and urinary phenol concentrations (r^2 = 0.75) was more significant in concentration higher than 0.05 ppm than lower than 0.05 ppm (r^2 = 0.4). Furthermore, the relationship between respiratory MEK and urinary MEK concentrations (r^2 = 0.79) was more significant in concentrations lower than 0.1 ppm than higher than 0.1 ppm (r^2 = 0.25).

The results showed urinary t,t-MA, 2-MHA, 3-MHA, 4-MHA, phenol, and MEK levels in smokers were higher than that in nonsmokers. This confirmed that cigarette smoking is the most important confounding factor when biomonitoring occupational VOCs exposure. Our results showed a more significant correlation between benzene, xylene isomers, phenol, and MEK in breathing zone and their urinary metabolites in smokers than nonsmokers (P = 0.03, P = 0.041, P = 0.02, P = 0.039, and P = 0.042, respectively). Some

other studies reported significant differences between smokers and nonsmokers exposed to benzene in ambient air (15, 38).

The distribution of pollutants from petrochemical factories into the ambient air mainly depends on the process activities. The petrochemical industries in the studied location include industries such as petrochemical refining and storage, polypropylene, paints, and solvents. In some factories, leakage takes place from the reservoirs, transferring pipes, and installations. The concentration of pollutants ultimately goes beyond the recommended standard level.

Benzene is a carcinogenic compound causing leukemia. The World Health Organization (WHO) has estimated that a lifetime exposure of 1 $\mu g/m^3$ of benzene through inhalation leads to about 6 additional cases of leukemia per million inhabitants (39). This might be due to evaporations and petrochemical industrial activities. Data from this study showed that MHA had a poor correlation coefficient with low concentration of xylenes in workers. It seems that the moving of air pollution plays a major role in benzene distribution in the whole petrochemical region. The most important pollutant emitted in the petrochemical complex is benzene. The concentration of benzene and its urinary metabolites was higher than standard limits. As benzene was distributed in most industries, it seems the wind was the cause of its spreading.

Finally, we suggest that more consideration of benzene and MEK exposure is needed for maintaining the health of petrochemical industry workers.

Conclusion

The mean concentration of benzene in breathing zone and urinary t,t-MA was higher than the TLV and biological exposure indices (BEI) recommended by ACGIH, but the concentrations of other compounds were lower than the standard limits. The results showed that urinary t,t-MA, 2-MHA, 3-MHA, 4-MHA, phenol, and MEK levels in smokers were higher than nonsmokers. Data from this study showed that t,t-MA, HA, MHA, and MEK have a significant correlation with concentrations of the related hydrocarbons. In the petrochemical industry, urinary VOC metabolites excretion rates in the workers with chronic exposure to VOCs were higher than other workers.

Acknowledgements

The authors would like to thank the Hamedan University of Medical Science for providing financial support and laboratory facilities.

Conflict of interest: None declared

References

1. United States Enviromental Protection Angency. Volatile Organic Compounds (VOCs) [Internet]. 2012 [updated 2012 May 21; cited 2012 Aug 12]; Available from: http://www.epa.gov/iaq/voc2.html.
2. Zabiegała B, Gorecki T, Przyk E, Namiesnik J. Permeation passive sampling as a tool for the evaluation of indoor air quality. Atmos Environ 2002: 36(17):2907-16.
3. Al Zabadi H, Ferrari L, Sari-Minodier I, Kerautret MA, Tiberguent A, Paris C, et al. Integrated exposure assessment of sewage workers to genotoxicants: an urinary biomarker approach and oxidative stress evaluation. Environ Health 2011; 10:23.
4. Bahrami A, Jonidi-Jafari A, Folladi B, Mahjub H, Sadri Q, Motamedzadeh M. Comparison of urinary O-cresol and hippuric acid in drivers, gasoline station workers and painters exposed to toluene in west of Iran. Pak J Biol Sci 2005; 8(7):1001-5.
5. Casas L, Fernández MF, Llop S, Guxens M, Ballester F, Olea N, et al. Urinary concentrations of phthalates and phenols in a population of Spanish pregnant women and children. Environ Int 2011; 37(5):858-66.
6. Chang TY, Huang KH, Liu CS, Shie RH, Chao KP, Hsu WH, et al. Exposure to volatile organic compounds and kidney dysfunction in thin film transistor liquid crystal display (TFT-LCD) workers. J Hazard Mater 2010; 178(1-3):934-40.
7. Lovreglio P, D'Errico MN, Fustinoni S, Drago I, Barbieri A, Sabatini L, et al. Air quality-models and applications. In: Popovic D, editors. Croatia: InTech; 2011. Chapter 16, Assessment of environmental exposure to benzene: traditional and new biomarkers of internal dose; p.321-40.
8. American Conference of Industrial Hygienists. TLVs and BEIs: Based on the Documentation of the Threshold Limit Values for Chemical

Stubstances and Physical Agents & Biological Exposure Indices. 7th ed. Cincinnati: American Conference of Industrial Hygienists (ACGIH); 2010.
9. International Agency For Research On Cancer (IARC). Carcinogenic to humans [Internet]. 2013 [updated 2012 Nov 7; cited 2013 March 2]. Available from: http://monographs.iarc.fr/ENG/Classification/index.php.
10. Sarma SN, Kim YJ, Ryu JC. Differential gene expression profiles of human leukemia cell lines exposed to benzene and its metabolites. Environ Toxicol Pharmacol 2011; 32(2):285-95.
11. Moro AM, Brucker N, Charão M, Bulcão R, Freitas F, Baierle M. Evaluation of genotoxicity and oxidative damage in painters exposed to low levels of toluene. Mutat Res 2012; 746(1):42-8.
12. Caro J, Gallego M. Environmental and biological monitoring of volatile organic compounds in the workplace. Chemosphere 2009; 77(3):426-33.
13. Pandey SK, Kim KH, Kim KH. Simultaneous determination of odorous volatile organic compounds with gas chromatography and a thermal desorber: A case study on methyl ethyl ketone, methyl isobutyl ketone, butyl acetate, toluene, and xylene. Microchem J 2009; 91(2):245-52.
14. Cetin E, Odabasi M, and Seyfioglu R. Ambient volatile organic compound (VOC) concentrations around a petrochemical complex and a petroleum refinery. Sci Total Environ 2003; 312(1-3):103-12.
15. Chalbot MC, Vei IC, Kavouras IG, Baetzis I. Effects of smoking on the levels of urinary biomarkers of aromatic hydrocarbons in oil refinery workers. Stoch Environ Res Risk Assess 2012; 26(5):731-8.
16. Clayton GD, Clayton FE. Pattys Industrial Hygien and Toxicology. New York: Wiley Interscience; 2001. (Vol 2, Part F edition).
17. Angerer J, Aylward LL, Hays SM, Heinzow B, Wilhelm M. Human biomonitoring assessment values: Approaches and data requirements. Int J Hyg Environ Health 2011; 214(5):348-60.
18. Heinrich-Ramm R, Jakubowski M, Heinzow B, Christensen JM, Olsen E, Hertel O. Biological monitoring for exposure to volatile organic compounds (VOCs) (IUPAC recommendations 2000). Pure Appl Chem 2000; 72(3):385-36.
19. The National Institute for Occupational Safety and Health (NIOSH). NIOSH Manual of Analytical Methods (NMAM). USA: The National Institute for Occupational Safety and Health (NIOSH); 2003 April 23. Report No: 2549. Available from: www.cdc.gov/niosh/docs/2003-154/pdfs/2549.pdf.
20. Bahrami AR. The Comparative Distribution of Volatile Aromatic Hydrocarbons in Ambient Air of Hamadan (West of Iran) with Gasoline Stations. Asian Journal of Water, Environment and Pollution 2005; 2(2):95-8.
21. Maghsoodi- Moghadam R, Bahrami A, Mahjoob H, Ghorbani F. Evaluation of Benzene, ToluneAnd p,m&o-Xylene Contaminants at Mahshahr Petrochemical Complex During 2008-9. Journal of Ilam University of Medical Sciences 2011; 19(2):49-59.
22. Boogaard PJ, Van-Sittert NJ. Biological monitoring of exposure to benzene: a comparison between S-phenylmercapturic acid, trans, trans-muconic acid, and phenol. Occup Environ Med 1995; 52(9):611-20.
23. Bahrami AR, Joneidi-Jafari A, Ahmadi H, Mahjub H. Comparison of benzene exposure in drivers and petrol stations workers by urinary trans, trans-muconic acid in west of Iran. Ind Health 2007; 45(3):396-401.
24. Bahrami A, Edwards J. Determination of trans, trans-muconic acid in Children Living in Adelaide Based on HPLC Developed Method. Pak J Biol Sci 2005; 8(12):1703-6.
25. The National Institute for Occupational Safety and Health (NIOSH). NIOSH Manual of Analytical Methods (NMAM). USA: The National Institute for Occupational Safety and Health (NIOSH); 2003 April 23. Report No: 8301. Available from: www.cdc.gov/niosh/docs/2003-154/pdfs/8301.pdf.
26. The National Institute for Occupational Safety and Health (NIOSH). NIOSH Manual of Analytical Methods (NMAM). USA: The National Institute for Occupational Safety and Health (NIOSH); 2003 April 23. Report No: 8305. Available from: www.cdc.gov/niosh/docs/2003-154/pdfs/8305.pdf.
27. Yoshikawa M, Kawamoto T, Murata K, Arashidani K, Katoh T, Kodama Y. Biological monitoring of occupational exposure to methyl ethyl ketone in Japanese workers. Arch Environ Contam Toxicol 1995; 29(1):135-9.
28. Bahrami A, Ansari M. Exposure of Sweepers to Volatile Organic Compounds Using Urinary Biological Exposure Index. J Res Health Sci 2007; 7(1):1-5.
29. Chang CC, Sree U, Lin YS, Lo JG. An examination of 7: 00–9: 00pm ambient air volatile organics in different seasons of Kaohsiung city, southern Taiwan. Atmos Environ 2005; 39(5):867-84.
30. Hsieh CC, Tsai JH. VOC concentration characteristics in Southern Taiwan. Chemosphere 2003; 50(4):545-56.
31. Ohura T, Amagai T, Fusaya M. Regional assessment of ambient volatile organic compounds in an industrial harbor area, Shizuoka, Japan. Atmos Environ 2006; 40(2):238-48.
32. Liu PW, Yao YC, Tsai JH, Hsu YC, Chang LP, Chang KH. Source impacts by volatile organic compounds in an industrial city of southern Taiwan. Sci Total Environ 2008; 398(1-3):154-63.
33. Kawai, T, Zhang ZW, Takeuchi A, Miyama Y, Sakamoto K, Higashikawa K, et al. Methyl isobutyl ketone and methyl ethyl ketone in

urine as biological markers of occupational exposure to these solvents at low levels. Int Arch Occup Environ Health 2003; 76(1):17-23.
34. Philippat C, Wolff MS, Calafat AM, Ye X, Bausell R, Meadows M, et al. Prenatal Exposure to Environmental Phenols: Concentrations in Amniotic Fluid and Variability in Urinary Concentrations during Pregnancy. Environ Health Perspect 2013; 121(10):1225-31.
35. Miyasaka M, Kumai M, Koizumi A, Watanabe T, Kurasako K, Sato K, et al. Biological monitoring of occupational exposure to methyl ethyl ketone by means of urinalysis for methyl ethyl ketone itself. Int Arch Occup Environ Health 1982; 50(2):131-7.
36. Hopf NB, Kirkeleit J, Bråtveit M, Succop P, Talaska G, Moen BE. Evaluation of exposure biomarkers in offshore workers exposed to low benzene and toluene concentrations. Int Arch Occup Environ Health 2012; 85(3):261-71.
37. Angerer J, Schaller KH. o-, m-, p-Methylhippuric Acids (Toluric Acids) Hippuric Acid [Biomonitoring Methods, 1985]. The MAK Collection for Occupational Health and Safety 2012; DOI: 10.1002/3527600418.bi49569ompe0001
38. Carrieri M, Tranfo G, Pigini D, Paci E, Salamon F, Scapellato ML, et al. Correlation between environmental and biological monitoring of exposure to benzene in petrochemical industry operators. Toxicol Lett 2010; 192(1):17-21.
39. World Health Organization (WHO). International Programme on Chemical Safety (Benzene) [Internet] 1999 [cited 2013 Nov 12]. Available from: http://www.who.int/ipcs/assessment/public_health/benzene/en/.