

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

Rahimpoor R, MSc<sup>1</sup>, Bahrami AR, PhD<sup>2\*</sup>, Assari MJ, PhD<sup>3</sup>, Ghorbani F, PhD<sup>4</sup>, Negahban AR, MSc<sup>1</sup>, Rahimnejad S, MSc<sup>1</sup>, Mehdizadegan B, BSc<sup>5</sup>

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

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**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, Biologic 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<sub>2</sub>) 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<sup>3</sup> 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
<b>Benzene in air (mg/m<sup>3</sup>)</b>	1.60	3.81 ± 2.04	0.000 ± 8.13	4.160
<b>t,t-MA in urine (µg/g creatinine)</b>	500.00	1431 ± 472	0.021 ± 1532	12278
<b>Toluene in air (mg/m<sup>3</sup>)</b>	75.37	4.96 ± 4.98	1.740 ± 10.23	24.800
<b>HA in urine (g/g creatinine)</b>	1.60	0.39 ± 0.11	0.113 ± 0.84	0.012
<b>O-xylene and M-xylene in air (mg/m<sup>3</sup>)</b>	434.23	5.17 ± 5.56	0.560 ± 12.94	30.910
<b>2-MHA and 3-MHA in urine (g/g creatinine)</b>	1.50	0.44 ± 0.11	0.134 ± 0.68	0.012
<b>P-xylene in air (mg/m<sup>3</sup>)</b>	434.23	9.86 ± 16.89	0.420 ± 41.38	285.270
<b>4-MHA in urine (g/g creatinine)</b>	1.50	0.10 ± 0.02	0.040 ± 0.11	0.004
<b>Phenol in air (mg/m<sup>3</sup>)</b>	19.25	0.34 ± 0.10	0.030 ± 0.63	0.010
<b>Phenol in urine (mg/g creatinine)</b>	250.00	0.58 ± 0.23	0.000 ± 1.13	0.053
<b>MEK in air (mg/m<sup>3</sup>)</b>	589.86	6.62 ± 7.21	0.620 ± 25.16	51.980
<b>MEK in urine (mg/l)</b>	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
<b>Total</b>	<b>104</b>	<b>1431.150</b>	<b>0.394</b>	<b>0.444</b>	<b>0.098</b>	<b>0.585</b>	<b>0.154</b>

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 (R<sup>2</sup>) 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
<b>P-value</b>	<b>0.030</b>	<b>0.075</b>	<b>0.023</b>	<b>0.036</b>	<b>0.024</b>

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

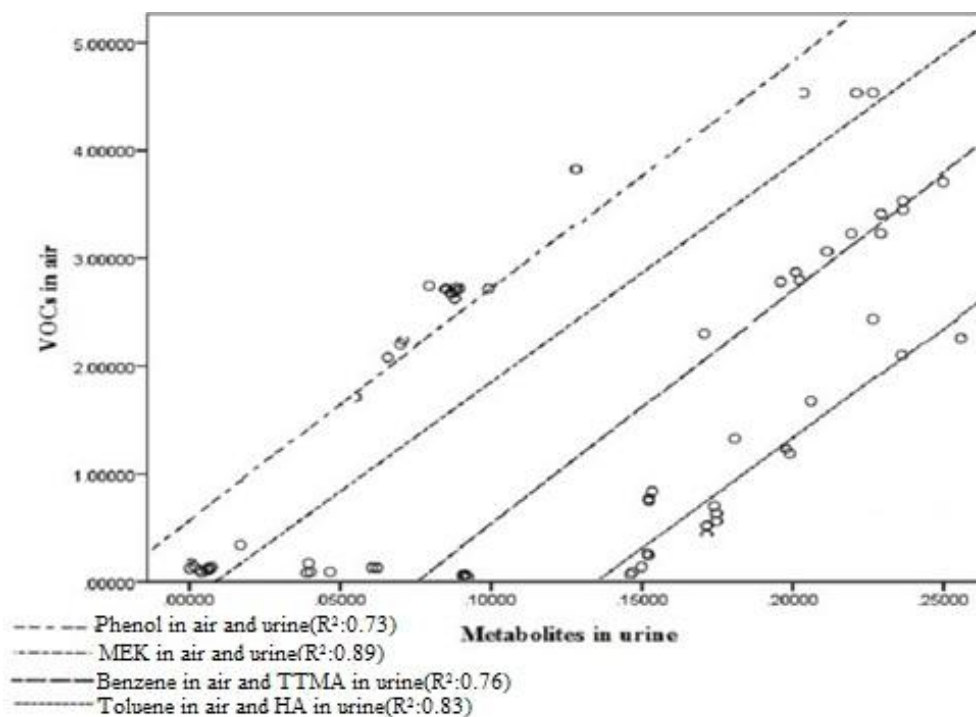


Figure 1: Correlation between VOCs exposure levels and urinary metabolites for all samples

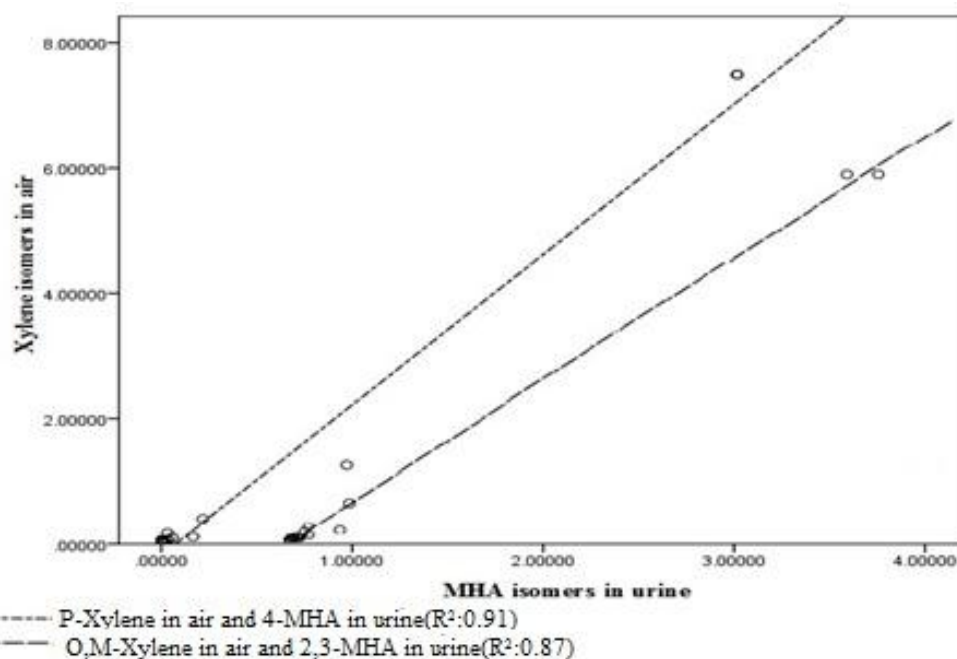


Figure 2: Correlation between Xylene isomers exposure levels and urinary MHA isomers for all subjects

## Discussion

The present study was undertaken to investigate 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  $1 \mu\text{g}/\text{m}^3$  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.

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**Conflict of interest:** None declared

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