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Original Article

Relationship Between the Concentration of Airborne Benzene Pollutant and the Amount of Urinary Metabolites of Trans, Trans-muconic Acid, and Hippuric Acid in Employees Working in Different Plants of Bou Ali Sina Petrochemical Company

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Abstract

Background & Aims: The petrochemical industry as a modern industry, despite the positive outcomes it has brought to mankind, is a source of gaseous and aerosol pollution and industrial effluents on a large scale, which can have direct and indirect destructive effects on the environment and human life. This study investigated the relationship between the amount of airborne benzene with the amount of trans,trans-muconic acid (ttMA) and hippuric acid metabolites in the urine of workers working in petrochemical complexes with different exposure times and methods.

Materials and Methods: For this purpose, breathing the air of different petrochemical plants of Bou Ali Sina was sampled by the National Institute for Occupational Safety and Health 1501 method, and the urine of workers (n = 24) was also sampled in these units. In addition, the amount of benzene in the air samples and the amount of urinary metabolites of ttMA and hippuric acid were analyzed in urine samples sent to the laboratory using a high-performance gas chromatography-mass spectrometry device and gas-liquid chromatography. Finally, urinary creatinine was measured by an ultraviolet-visible spectrophotometer.

Results: The results showed that the concentration of benzene in the aromatic unit had the highest value, which had a higher level of pollution than both standards. The xylene mixing unit with a concentration of 3.6 μg/m^3, the loading unit with a benzene content of 3.4 μg/m^3, and a tank unit with 2.8 μg/m^3 had a lower amount of benzene pollution compared to the Occupational Safety and Health Administration permissible exposure limit-short-term exposure limit (OSHA PEL-STEL) standard but had higher pollution levels in comparison to the OSHA PEL-TWA (time-weighted average) standard. In the sampling unit of the laboratory with a benzene amount of 0.94 μg/m^3 and in the technician unit of the laboratory, the amount of pollution was lower than both OSHA PEL-TWA and OSHA PEL-STEL standards. The aromatic unit demonstrated the highest amount of benzene, while the lowest amount was related to the laboratory section.

Conclusion: The results of the measurement of urinary benzene metabolites revealed that the concentration of urinary phenol and inhaled benzene in evening shift workers was higher than the corresponding amount in the morning shift workers, which may be due to the high level of pollution evenings compared to the morning. On the other hand, the results represented that the average hippuric acid in the exposed people (n = 24) was higher than the control (n = 20) so that it was 0.35 in the exposed and 0.26 in the control subjects. In addition, the average muconic acid in the exposed and control subjects decreased to 1.57 and 0.89, respectively. The minimum and maximum amounts of muconic and hippuric acids in the exposed subjects were 0.97 and 2.62, as well as 0.14 and 0.83, respectively. The maximum and minimum concentrations of muconic and hippuric acids were 2.62 and 0.97, as well as 0.83 and 0.14 in exposed subjects, respectively, which was less than muconic acid.

Keywords: Benzene, Muconic acid, Hippuric acid, Respiration Air F-main urinary metabolite

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1. Introduction

Benzene is one of the pollutants that is dispersed in the air due to the activity of the petrochemical industry. According to Occupational Safety and Health Organization (OSHA), National Institute for Occupational Safety and Health (NIOSH), International Labor Organization (ILO), the World Health Organization, American Conference of Governmental Industrial Hygienists (ACGIH), International Agency for Research on Cancer (IARC), Environmental Protection Agency (EPA), and American Industrial Health Association, it is classified as definite human carcinogens which can lead to many complications such as aplastic anemia, leukemia, acute myeloid, lymphoma, and leukemia in exposed workers [1,2]. Breathing is the main way of contact with benzene in the industry. As a chemical mediator, Benzene is used

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in the production of chemicals such as styrene, cumene, cyclohexane, synthetic rubber and adhesives and oils, varnishes, polishing oil, paints, pharmaceuticals, and agricultural chemicals. It is also employed as a solvent in paints, thinners, inks, and adhesives. Occupational exposure to benzene occurs in rubber industries, oil refining, chemical factories, shoe manufacturing, gasoline storage equipment, and gasoline stations. Exposure to benzene can also occur by eating contaminated food products, but the absorption of food is less than 1% of the average daily benzene absorbed in the general population [1]. The measurement of phenol in the urine is the usual biological sign of exposure to benzene [3]. However, studies have shown that when exposed to low levels of benzene, which is less than 5 ppm, urine phenol may not be a reliable biological sign. Therefore, trans,trans-muconic acid (ttMA) and hippuric acid seem to be the most reliable benzene metabolites for monitoring benzene exposure with low levels between 0.25 and 3.5 ppm. These acids are used as useful biological parameters for exposure to benzene between 1 and 68 ppm in many studies. Studies conducted in the petrochemical industry of Iran indicate that workers in the petrochemical industry are exposed to volatile organic compounds, including benzene, through breathing [4]. In several studies, the concentration of the metabolites of these compounds in workers' urine samples was investigated to evaluate the level of workers' exposure to benzene compounds and the like [5,6]. In the petrochemical industry, workers of various plants are exposed to benzene substances, and there are many concerns about the adverse effects of constant exposure to this substance; unfortunately, a limited number of studies have so far focused on the cancer risk of exposure to benzene in workers working in petrochemical plants, refineries, and industrial factories such as cement, steel, and iron smelting factories. Thus, this study aimed to investigate the amount of airborne benzene and its relationship with the amount of urinary benzene metabolites, including phenol, ttMA, and hippuric acid, in the urine sample of workers working in petrochemical complexes with different exposure times and methods.

2. Materials and Methods

This cross-sectional descriptive study was conducted to evaluate the relationship between the amount of benzene in breathing air and the amount of urinary benzene metabolites in workers working in six petrochemical plants of Bou Ali, Bandar Imam in the spring of 2017. Data were collected using a questionnaire, as well as analysis of the urine samples obtained from 24 people working in the industry and 20 people as the control group. To eliminate possible errors and contaminations during sampling or transfer, a number of samples were selected as witnesses from the employees of the study department and prepared similarly.

2.1. Sampling and analysis of benzene in air samples

The sampling of air and workers was performed in aromatic, mixing xylene, loading, tanks, sampler, and laboratory units. After analyzing the relevant samples, each worker completed a questionnaire in which his demographic data were provided, including work experience, age, current job details, drug use, smoking, and the like.

The sampling and measurement of benzene compounds were conducted using the American NIOSH method No. 1501. A gas chromatography method equipped with a mass detector and a 20-microliter loop at a wavelength of 254 nm was employed to qualitatively and quantitatively identify benzene. Isolation and identification of benzene under the study was done using a siloxane column with performance specification RR-c18 e 100*4.6 mm, introduced by the US EPA [7]. Benzene, obtained from Sigma-Aldrich Company at concentrations of 10, 30, 50, 100, 200, 500, and 1000 ppm, was applied to draw standard curves to determine the type (using retention time) and amount (using the area under the curve). After drawing the standard curve of benzene, the original samples were injected into the gas chromatography-mass spectrometry device to determine the concentration. After determining the concentration by Eq. (1), the concentration of benzene in each of the air samples was determined.

$$C = \frac{(W + W_f + W_b - B - B_f - B_b)}{V} \qquad (1)$$

where W and W_f represent the mass of the analyte found on the filter and the amount of analyte in the front part of the absorber, respectively. Moreover, W_f and B denote the amount of analyte in the rear part of the absorber and the mean value of the analyte on the blank filter bed, respectively. Further, B_f, B_b, V, and C are the amount of analyte in the front part of the blank absorber, the amount of analyte in the back part of the blank absorber, the sampled air volume in liters, and the concentration of the analyte in mg/m^3, respectively [8,9].

2.2. Measurement and determination of benzene metabolites in employees' urine samples

After informing the workers and obtaining consent to receive urine samples from them, the information related to the investigation of the influencing factors in the concentration of urinary volatile organic compound metabolites, including age, work experience, weekly working hours, and smoking, was collected through a questionnaire. According to ACGIH instructions, urine samples were collected in polyethylene containers at the end of the work shift on the same day. To determine the concentration of ttMA in the urine sample, the solid phase extraction method was employed by a strong exchange cartridge. After calculating the area under the peak of the unknown sample, the concentration of ttMA and hippuric acid compounds in the original sample were calculated using the calibration curve and considering the correction factor related to the ratio of the volume of the urine sample to the volume of the extractant solution [10].

2.3. Data analysis

SPSS 16, Student's t-test, and linear regression were used to statistically analyze the collected data and to examine the correlation of quantitative variables with each other and the status of each of the studied variables based on qualitative variables.

3. Results

The results of the amount of environmental contact of employees in different workplaces are displayed in Figure 1. As shown, the amount of benzene in the ambient air of the aromatic and loading units was higher than that of the other units.

According to Figure 1, the concentration of benzene in the aromatic unit had the highest value, which has a higher level of pollution than both standards. The mixed xylene unit, loading unit, and tank unit with a concentration of 3.6, 3.4, and 2.8, respectively, had lower benzene pollution compared to the Occupational Safety and Health Administration permissible exposure limit-short-term exposure limit (OSHA PEL-STEL) standard, but the amount of pollution was higher in comparison to the OSHA PEL-time-weighted average (TWA) standard. The laboratory sampling unit with 0.94 benzene and the laboratory technician unit had less pollution than both OSHA PEL-TWA and OSHA PEL-STEL standards.

4. Discussion

4.1. Complications and Effects of Benzene

The evaluation of the symptoms and side effects of benzene demonstrated that among the acute side effects, headache and burning eyes are more common among 24 exposed people than 20 controls, but the prevalence of other acute and chronic side effects and symptoms such as bad smell in the nose, abnormal taste in the mouth, imbalance, and dizziness were more frequent in the exposed subjects than the control ones. There was no significant relationship between the two groups at the 0.05 level. According to the research, benzene vapors at medium and high concentrations (acute poisoning) affect the central nervous system and cause symptoms such as dizziness, weakness, headache, nausea, vomiting, incoordination of body parts, blurred vision, loss of consciousness, and confusion. Additionally, high concentrations of benzene vapors have an irritating effect on the mucous membranes of the eyes, nose, throat, and respiratory system [8,9]. However, due to the correct use of appropriate personal protective equipment such as gloves, masks, and filters suitable for absorbing organic vapors and working against the wind direction, most of the symptoms and complications mentioned in the two groups represented no significant differences.

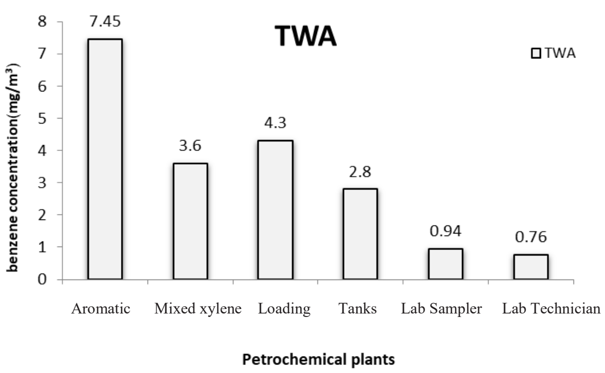

Figure 1. Concentration of benzene in the air of the breathing zone of different petrochemical units in Bou Ali Sina Petrochemical Company. *Note*. TWA: Time-weighted average.

The effect of benzene and its side effects on the employees of a petrochemical complex were measured, and it was revealed that the highest number and percentage of the exposed people had headache symptoms, and then the number of people exposed to eye-burning was the highest. According to Table 1, the number of exposed people was higher than that of the control, and the effects of benzene were mostly related to headache and burning eyes. The least number of burning in the nose and the imbalance were observed in the exposed subjects and the controls, respectively.

Based on the obtained results, it was observed that short-term concentration (19.5 ppm), mixed xylene occupational group (18.76), short-term concentration loading (17.43 ppm), and the laboratory sampler (14.7 ppm) were more than the maximum standard in the aromatic occupational group, and they were less than the maximum standard in the breathing zone of the laboratory technicians and tanks. Among the reasons for the excessive concentration of the loading unit standard is the mismatch of the tanker valve with the arm funnel; due to the carelessness of the loading staff in discharging the remaining liquid benzene in the arm and its trap or the technical defect of the loading system after filling the tanker and removing the arm from it, liquid benzene is occasionally poured on the ground in a small amount and sometimes drop by drop, causing environmental pollution.

In the tank unit, to comply with safety and environmental principles, the tanks in this unit are covered with nitrone. In addition, the benzene tank, which has a high vapor pressure, is built with an internal floating roof. Therefore, due to the closed system, there is no pollution of benzene vapors in this unit. There is no air pollution in the laboratory area due to the presence of a local (range hood) and general ventilation system and the performance of laboratory work under the hood. However, the users of laboratory samplers are exposed to benzene from different parts of the unit during sampling and are exposed to the same concentration (14.7 ppm) in the short term.

The results of the t-test showed that the average amount of phenol in the urine after the working shift of the exposed

Table 1. Mean values measured in the studied groups

Acute symptoms	Study group				*P* value
	Exposure		Control		
	No.	%	No.	%	
Burning eyes	12	50	10	41.66	0.604
Burning nose	3	12.5	2	8.33	0.397
Abnormal sense of smell	9	37.5	3	12.5	0.013
Sore throat	10	41.66	2	8.33	0.0362
Imbalance	5	20.83	1	4.16	0.091
Burning	6	25	2	8.33	0.282
Dizziness	8	33.33	5	20.83	0.397
Abnormal taste in the mouth	5	20.83	3	12.5	0.469
Blurred vision	10	41.66	9	37.5	0.822
heavy-headedness	4	16.66	10	41.66	0.159
Headache	19	79.16	18	75	0.715

subjects was higher than that of the control subjects, but it was still lower than the allowed amount (10 mg of phenol per gram of creatine). Based on the test results, the average amount of urine phenol after the work shift of the exposed group was more than its value before the working shift of the same group, but the average amount of urine phenol before and after the work shift of the control group had no difference, indicating that although there is contact with benzene in the exposed group, due to the use of suitable personal respiratory protective equipment and gloves, as well as the presence of an engineering control system (local ventilation) in the required places and compliance with the principles of professional hygiene, its metabolite in the urine is less than the allowed amount (Figure 2).

The average concentration of phenol, which is determined as a biological indicator of contact with benzene, is lower in the morning shift, while it is higher in the evening shift than the standard recommended by Iran's Technical Committee of Occupational Health. The standard limit of phenol in the urine is 25 mg/g of creatine. Moreover, the concentration of benzene is lower than the 8-hour standard (0.5 ppm), which is recommended by Iran's Technical Committee of Occupational Health and the American Industrial Hygiene Association [10]. In this research, the concentration of phenol in the workers' urine and the inhaled benzene in the evening shift workers is more than in the morning shift workers, which may be related to the higher pollution in the evenings. The pollutant concentration in the air in the evening is higher than in the morning air due to the concentration of the pollutant during the daytime hours. Therefore, evening shift workers inhale pollutants more than their morning shift counterparts, and the concentration of phenol excreted in this group is usually higher. Phenol is expected to further increase when the amount of benzene pollutants is higher in the work environment. Research has confirmed the relationship between phenol excreted from workers' urine and benzene in the air (Table 2).

The low amount of urine phenol in the morning shift workers can be related to the concentration of benzene in the air of different units because, in the morning, the air of benzene production units has a lower concentration of pollutants such as benzene. This concentration increased with the increase of the production time. The absence of significant changes in the amount of phenol in the urine during the work shift may be associated with the half-life of phenol, which is one of the indicators of benzene and is 28 hours. Considering that the length of each work shift is 8 hours, which is far less than the half-life of phenol, phenol does not change much during the work shift. In their study, Rastkari et al found that if benzene in the work environment is less than 1 ppm, the excretion of phenol from the urine is the same, while ttMA, which is also produced from the metabolism of benzene, represents an increase [1]. Fang et al reported that if the concentration of benzene is lower than 1 ppm in the air, the concentration of phenol after starting work and phenol before starting work does not differ significantly [3]. The

Figure 2. Comparison of the average amount of phenol before and after the working shift in the exposed and control subjects using Duncan's mean comparison test (α = 1 %).

findings of another study by Maghsoodi Moghadam et al demonstrated that at the concentrations of 0.7-13.6 μg/m^3 of benzene in the air, phenol in the urine of workers was constant during work [4], but the concentration of ttMA and phenylmercapturic acid increased during the work shift in this study (Table 3).

Based on the results (Figure 3), the maximum and minimum concentrations of muconic and hippuric acids were 2.62 and 0.97, as well as 0.83 and 0.14 in the exposed people, which is less than muconic acid. Urine phenol levels in workers with high experience (15-23 years) were lower than phenol in workers with low experience (less than 15 years). This can be related to the kinetics of benzene in the body. Long-term contact with organic solvents increases the possibility of creating other metabolic forms in the body so that in addition to phenol, catechol, hydroquinone, muconic acid, and phenylmercapturic acid are excreted from the urine. Therefore, by increasing the kinetic pathways in the body of workers with high experience, the phenol excreted in their urine will be significantly reduced compared to workers with low experience. Considering the constancy of phenol in the urine during shift work and the studies conducted on ttMA and phenylmercapturic acid at concentrations less than 1 ppm, the measurement of the above metabolites in the urine in conditions where the concentration of benzene in the air is less than 1 ppm, benzene is preferable in measuring urine phenol.

It was expected that smokers would be more exposed to benzene due to smoking and being in the area of the site, thus an independent *t* test was used to compare the variable of exposure to benzene and the variable of smoking, and the result of the test showed no statistically significant relationship between smoking and non-smoking and exposure to benzene in the air of the workplace. Moreover, the statistical *t* test/limit value of 5 one-sample tests was employed to compare the amount of exposure to benzene with 1 ppm, and considering the 1 ppm exposure to benzene in the air/mean of 52 working environments, it was found that there was a significant difference with the permissible benzene threshold, and it was equal to this permissible threshold. Additionally, the results of personal exposure evaluations conducted among different units, shifts, and work groups to determine the concentration of benzene in the air of the work environment demonstrated that the concentration of benzene in the evening shift, compared to the morning shift, had a higher mean concentration in different work groups. This can be considered due to the heat of the air and environmental conditions in the morning shift when the workers and employees spend less time on the site, as well as in the night shift considering that the operational power of the unit is lower than its normal level and the mean concentration of the exposure of people in this shift will be lower than the other two shifts.

Table 2. Average results of biological monitoring of benzene (urine phenol) before and after the shift in the exposed and control subjects

Measured parameters	Study group	Status: Before work shift	Status: After work shift	*P*-value
		Mean ± SD	Mean ± SD	
Phenol	Exposure	4.76 ± 1.97	5.94 ± 3.46	0.016
	Control	3.24 ± 2.18	3.57 ± 2.54	0.892

Note. SD: Standard deviation.

Table 3. Mean urinary metabolites of hippuric and muconic acids in the exposed and control subjects

Variable	Status	No.	Min.	Max.	Mean	SD
Hippuric acid	Exposure	24	0.14	0.83	0.35	0.16
	Control	20	0.1	0.61	0.26	0.12
Muconic acid	Exposure	24	0.97	2.62	1.57	0.071
	Control	20	0.56	1.53	0.89	0.21

Note. Min. Minimum; Max.: Maximum; SD: Standard deviation.

5. Conclusion

The results revealed that the concentration of benzene in the aromatic unit had the highest value, which had a higher level of pollution than the other two standards. The mixed xylene unit with a concentration of 3.6, loading unit with a benzene amount of 3.4, and tank

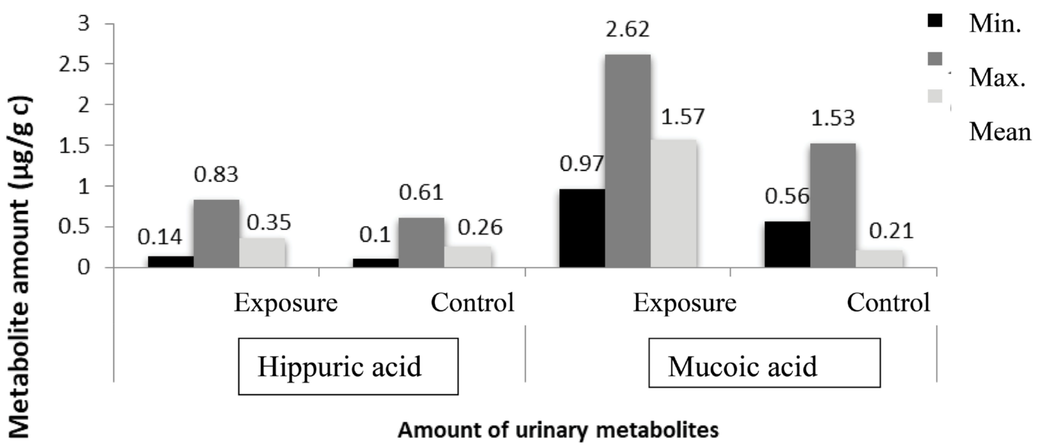

Figure 3. Mean concentration of hippuric and muconic acids in the exposed and control subjects. *Note*. Min.: Minimum; Max.: Maximum.

unit with an amount of 2.8, compared to the OSHA PEL-STEL standard, had a lower amount of benzene pollution; however, in comparison to the OSHA PEL-TWA standard, the amount of pollution was higher. The laboratory sampling unit with 0.94 benzene and the laboratory technician unit had less pollution than both OSHA PEL-TWA and OSHA PEL-STEL standards. On the other hand, the evaluation of the symptoms and side effects of benzene represented that among the acute side effects, headache and burning eyes were more common among the exposed subjects than the control subjects, but the prevalence of other acute and chronic side effects such as the sense of abnormal smell in the nose, abnormal taste in the mouth, imbalance, dizziness, despite their frequency, was higher in the exposed subjects than in the control subjects. The mean concentration of phenol, determined as a biological indicator of contact with benzene, was lower in the morning shift, while it was higher in the evening shift than the standard recommended by Iran's occupational health technical committee. On the other hand, the results showed that the concentration of benzene in the evening shift, compared to the morning shift, had a higher mean concentration in different work groups. The concentration of phenol in the workers' urine and benzene in inhaled air was higher in evening shift workers than in morning shift workers, which might be related to the higher pollution in the evenings than in the mornings. The pollutant concentration in the air in the evening was higher than the morning air due to the concentration of the pollutant during the daytime hours. Therefore, evening shift workers inhaled pollutants more than morning shift workers, and the concentration of phenol excreted in this group of workers was usually higher. According to the results of measuring the concentration of benzene in the aromatic working group, mixed xylene and the average loading of exposure to this chemical substance were several times higher than the standard, indicating the faster start of control measures. Benzene is the final product of these two units and it is impossible to remove it from the production source, thus management control measures such as reducing the hours of exposure to this substance by increasing the number of workers can be suggested as an effective management control solution. Another control measure regarding this hazardous substance is to check the gaskets of gasoline pumps at specified intervals and replace them if there is any defect. It is also recommended that direct reading devices should be used to monitor the possibility of leakage.

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Author Contributions

All authors contributed to conceptualization: data management: formal analysis: funding acquisition: review: methodology: project management: resources: software: monitoring: validation: visualization: writing - original draft: writing - review and editing .

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this manuscript. Furthermore, the ethical issues have been completely observed by the authors including plagiarism, informed consent, misconduct, data fabrication and/or falsification, double publication and/or submission, and redundancy.

References

1. Rastkari N, Izadpanah F, Yunesian M. Exposure to benzene in gas station workers: environmental and biological monitoring. Iran J Health Environ. 2015;8(2):163-70. [Persian].
2. Lovreglio P, D'Errico MN, Fustinoni S, Drago I, Barbieri A, Sabatini L, et al. Assessment of environmental exposure to benzene: traditional and new biomarkers of internal dose. In: Popovic D, ed. Air Quality-Models and Applications. IntechOpen; 2011. p. 321-40. doi: 10.5772/16915.
3. Fang MZ, Shin MK, Park KW, Kim YS, Lee JW, Cho MH. Analysis of urinary S-phenylmercapturic acid and trans, trans-muconic acid as exposure biomarkers of benzene in petrochemical and industrial areas of Korea. Scand J Work Environ Health. 2000;26(1):62-6. doi: 10.5271/sjweh.511.
4. Maghsoodi Moghadam R, Bahrami A, Mahjoob H, Ghorbani F. Evaluation of benzene, toluene and p-xylene contaminants at Mahshahr petrochemical complex during 2008-9. J Ilam Univ Med Sci. 2011;19(2):49-59. [Persian].
5. Bahrami AR, Ansari M. Exposure of sweepers to volatile organic compounds using urinary biological exposure index. J Res Health Sci. 2007;7(1):1-5.
6. Kim JA, Kim S, Kim HJ, Kim YS. Evaluation of formaldehyde and VOCs emission factors from paints in a small chamber: The effects of preconditioning time and coating weight. J Hazard Mater. 2011;187(1-3):52-7. doi: 10.1016/j.jhazmat.2010.10.094.
7. International Agency for Research on Cancer (IARC). Carcinogenic to Humans. USA: World Health Organization; 2013. Available at: http://monographs.iarc.fr/ENG/Classification/index.php. Accessed March 2, 2013.
8. American Conference of Governmental Industrial Hygienists (ACGIH). TLVs and BEIs: Based on the Documentation of the Threshold Limit Values for Chemical Stubstances and Physical Agents & Biological Exposure Indices. ACGIH; 2010. p. 120-50.
9. EPA. Volatile Organic Compounds (VOCs). United States Environmental Protection Agency; 2012. Available from: http://www.epa.gov/iaq/voc2.html. Updated May 21, 2021. Accessed August 12, 2012.
10. National Institute for Occupational Safety and Health (NIOSH). NIOSH Manual of Analytical Methods (NMAM) 8305. USA: NIOSH; 2003. Available at: www.cdc.gov/niosh/docs/2003-154/pdfs/8305.pdf. Accessed April 23, 2013.