Section A-Research paper

Environmental and biological monitoring of benzene and ethylbenzene at a petroleum refining company in Alexandria, Egypt

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Abstract

Introduction: Occupational exposure to benzene and ethylbenzene has a serious impact on human health as they cause a wide range of health hazards ranging from irritation to carcinogenicity. In the literature, there is no enough data regarding the relationship between environmental levels of benzene and ethylbenzene and the biological monitoring data. This study aimed to measure the environmental levels of benzene and ethylbenzene and their metabolites in urine; and examine the possible relationship in between.

Methods: A cross-sectional comparative study was carried out at a petroleum refining company in Egypt where 37 workers exposed to benzene and ethylbenzene were compared with 37 unexposed workers. Environmental levels of benzene and ethylbenzene were measured; workers were interviewed, and urine samples were collected to measure S-Phenyl mercapturic acid (SPMA) as benzene metabolite and mandelic acid (MA) and phenylglyoxylic acid (PGA) as ethylbenzene metabolites.

Results: Environmental levels of benzene and ethylbenzene were significantly higher at the exposed worksites ($p<0.001$). Exposed workers had significantly higher levels of SPMA, MA, PGA metabolites in urine than the unexposed workers. Environmental levels of benzene and ethylbenzene were positively correlated with SPMA, MA, and PGA levels.

Conclusion: Occupational exposure to benzene and ethylbenzene at exposed worksites was found to be associated with the biological monitoring data. There was a significant positive correlation between environmental levels of benzene and ethylbenzene and levels of their metabolites in urine among the studied workers. These results reveal the importance of implementation of effective control measures through environmental and biological monitoring, and health surveillance at highly exposed areas to reduce the level of these hazardous pollutants at the workplace and consequently prevent their adverse health effects.

Keywords: environmental levels; biological monitoring; petroleum workers; benzene; ethylbenzene

1. Introduction

Occupational exposure to benzene and ethylbenzene has a serious impact on human health as they cause a wide range of health hazards ranging from irritation to carcinogenicity [1-3]. They are among the top chemicals used in petroleum industries and are considered the building blocks for producing a wide range of chemical intermediates and final products [4,5]. These compounds are considered a common occupational hazard for many workers, including those working in petroleum refineries [6,7].

Benzene and ethylbenzene are well absorbed as they are lipophilic in nature. Distribution occurs in adipose-rich tissues such as brain, bone marrow, and body fat and then metabolized and excreted [8]. Metabolism occurs primarily in the liver through cytochrome P450 [9]. The main urinary metabolite for benzene is S-phenylmercapturic acid (SPMA) which is considered as a specific biomarker of benzene exposure [10,11]. While the main urinary metabolites of ethylbenzene used as biomarkers in the biological monitoring are mandelic acid (MA) and phenylglyoxylic acid (PGA) [12-14].

Exposure to benzene is associated with serious detrimental impacts on human health as it is responsible for a wide range of cancerous and non-cancerous health effects [15,16]. The main target systems in the human body affected by benzene exposure are the hematopoietic system, nervous system, respiratory and immune system [16,17]. The International Agency for Research on Cancer (IARC) has classified benzene as a group 1 human carcinogen [18,19]; being responsible for acute myeloid leukemia, acute lymphocytic leukemia, multiple myeloma, non-Hodgkin lymphoma and lung cancer [20,21]. Short-term exposure to high levels of ethylbenzene can cause eye and throat irritation, vertigo, and dizziness. As regards prolonged period of exposure, ethylbenzene is associated with damage to the inner ear, hearing loss and kidney damage [12]. According to the IARC, ethylbenzene is classified as a possible human carcinogen (group 2B) [12,22].

Prevention and control of adverse health effects of these compounds through environmental and biological monitoring, and health surveillance is essential. At workplace, the American Conference of Governmental Industrial Hygienists (ACGIH) recommends an 8-hour Threshold Limit Value (TLV) of 0.5 ppm for benzene and 20 ppm for ethylbenzene [23]. In addition, biological monitoring through analyzing benzene and ethylbenzene metabolites in urine are valuable exposure biomarkers [24].

In Egypt, the rapid development in the petroleum industry is becoming a major source of occupational exposure to benzene and ethylbenzene. In the literature, there is no enough data regarding the relationship between environmental levels of benzene and ethylbenzene and the biological monitoring data. This study was conducted among petroleum workers to 1) measure the environmental levels of benzene and ethylbenzene at the studied petroleum company; 2) measure the metabolites of benzene and ethylbenzene in urine among the studied workers; and 3) examine a possible relationship between the environmental levels of benzene and ethylbenzene and their metabolites.

2. Methods

2.1. Research design and setting

The present study adopted a cross sectional comparative approach that was conducted from April to August 2022 in a petroleum refining company in Egypt.

2.2. Participants

Petroleum workers exposed to benzene and/or ethylbenzene for at least five years and agreed to participate were eligible for this study. An equal number of workers not exposed to benzene or ethylbenzene from the same petroleum company were enrolled and served as a comparison group. Workers with previous history of exposure to non- occupational sources of benzene or ethylbenzene were excluded from this study; in addition, workers with history of exposure to heavy metals or organic solvents; had chronic organic diseases; or consumed antioxidant rich food and supplements.

2.3. Sampling and Sample size

Sample size was calculated (using Open-Epi online calculator Version 3.3a, OpenEpi, Atlanta, GA, USA) based on the previously reported urinary levels of SPMA (µg/g creatinine) among workers employed at oil refinery and controls. In order to detect the least difference in the mean urinary levels of SPMA of 3.6 µg/g creatinine [25], with a ratio between the exposed group and the controls of 1:1, at a power of 80% and confidence level of 0.95 ($\alpha = 0.05$), the minimum required sample size was 74 workers. In the present study, 37 workers exposed to benzene and ethylbenzene, and 37 unexposed workers were included in the analysis [25].

2.4. Data collection

2.4.1. Environmental monitoring of benzene and ethylbenzene

It included measurement of environmental levels of benzene, and ethylbenzene at exposed and unexposed worksites of the petroleum refining company using the TIGER handheld VOC detector (IONSCIENCE) [26]. Environmental levels of benzene and ethylbenzene were measured in five exposed worksites in the company, from which the exposed workers were selected, and two other worksites from which the comparison group of unexposed workers was selected. Direct reading of benzene and ethylbenzene levels was done during the working hours, where 15-20 readings were taken from each worksite, then, the average was calculated as the measurement value (mg/m3) [26].

2.4.2. Self-structured interviewing questionnaire

A self-structured interviewing questionnaire was designed to interview all studied workers. The questionnaire collected information about the age, level of education, smoking habit, occupational characteristics (job duration, working hours, personal protective equipment), medical history of chronic diseases.

2.4.3. Biological monitoring of benzene and ethylbenzene

End of shift urine samples were collected from all studied workers. Levels of benzene and ethylbenzene metabolites in urine were measured using the high-performance liquid chromatography (HPLC) [27,28]. S-Phenyl mercapturic acid (SPMA) was measured as a metabolite of benzene. While mandelic acid (MA) and phenylglyoxylic acid (PGA) were measured as metabolites of ethylbenzene exposure. All investigations were done at the Alexandria Faculty of Medicine's Central Laboratories.

2.5. Statistical analysis

The SPSS v.22 (IBM Corp. Released 2011. IBM SPSS Statistics for Mac, Armonk, NY, USA) was used for data analysis. For qualitative data, they were represented as frequencies and percentages. In order to investigate the association between the categorical variables, the Chi Square test, the Monte Carlo test, and the Fisher exact test were used to examine possible associations. As for quantitative data, the Kolmogorov–Smirnov test was used to test the normality of the data [29]. For normally distributed data, the student's t test was used to detect any significant differences between the two studied groups, while for not normally distributed data, the Mann-Whitney test was used. In addition, spearman correlation analysis was done to measure the strength of association between environmental levels of benzene and ethylbenzene and levels of their metabolites in urine among the studied workers (n = 74). The level of significance used for all statistical analyses was 5% ($\alpha = 0.05$).

2.6. Ethical considerations

The study was approved by the Alexandria Faculty of Medicine Research Ethics Committee affiliated with the Alexandria University (Serial Number: 034523154). All procedures performed in the study were in accordance with ethical standards. Data confidentiality was ensured by the researchers.

3. Results

3.1. Workers' sociodemographic and occupational characteristics

The majority of exposed and unexposed workers were aged from 40 to less than 50 years (63.5%). In addition, most of the studied workers had job durations from 10 to less than 20 years (62.2%). A significant difference was found between the two groups as regards age ($p = 0.012$) and job duration ($p = 0.029$). Regarding the level of education, there was no significant difference between the studied groups. All exposed workers and most of unexposed workers (91.9%) got university education. The majority of the studied workers were current smokers (55.4%). There was no significant difference regarding smoking habits ($p = 0.483$). (Table 1)

Table 1: Sociodemographic and occupational characteristics among the studied workers (n = 74)

Characteristics	Total (n = 74)	Exposed workers (n =37)		Unexposed workers (n =37)		p-value
	No. (%)	No.	%	No.	%	
Age (years)						
30 - < 40	14 (18.9)	2	5.4	12	32.4	
40 - < 50	47 (63.5)	27	73.0	20	54.1	0.012[*a]
≥ 50	13 (17.6)	8	21.6	5	13.5	
Job duration (years)						
<10	13 (17.6)	2	5.4	11	29.7	0.029[*a]
10 - < 20	46 (62.2)	26	70.3	20	54.1	
≥ 20	15 (20.3)	9	24.3	6	16.2	
Level of education						
Middle or high school education	3 (4.05)	0	0.0	3	8.1	0.077[b]
University education	71 (95.9)	37	100.0	34	91.9	
Smoking						
Smoker	41 (55.4)	22	59.5	19	51.4	0.483[c]
Non-smoker or ex-smoker	33 (44.6)	15	40.5	18	48.6	

[a] Monte Carlo test
[b] Fisher exact test
[c] Chi square test
[*] $p \leq 0.05$

3.2. Environmental monitoring of benzene and ethylbenzene

Environmental monitoring of benzene and ethylbenzene at the studied worksites revealed that the mean environmental level of benzene was significantly higher at the exposed worksites (106.59 ± 2.49 mg/m^3) than the unexposed worksites (6.66 ± 1.79 mg/m^3) (p<0.001). The measured benzene levels exceeded the recommended Threshold Limit Value (TLV) of the ACGIH of 0.5 ppm at all studied worksites. (Table 2)

Similarly, the mean environmental level of ethylbenzene was significantly higher at the exposed worksites (50.47 ± 1.51 mg/m^3) than the unexposed worksites (11.45 ± 3.26 mg/m^3) (p<0.001). The measured ethylbenzene was within the recommended limit of the ACGIH of 20 ppm at all studied worksites. (Table 2)

Table 2: Environmental levels of benzene and ethylbenzene at different worksites at the studied petroleum refining company

Environmental levels	Exposed worksites (n = 5)	Unexposed worksites (n = 2)	p-value
Benzene (mg/m^3)			
Mean ± SD	106.59 ± 2.49	6.66 ± 1.79	<0.001[*a]
Min. – Max.	101.60 – 110.40	4.0 – 10.0	
Ethylbenzene (mg/m^3)			
Mean ± SD.	50.47 ± 1.51	11.45 ± 3.26	<0.001[*a]
Min. – Max.	48.60 – 53.70	7.0 – 16.10	

Abbreviations: SD, standard deviation; Min; minimum; Max., maximum
[a] Student's t test
[*] $p \leq 0.05$

3.3. Biological monitoring of benzene and ethylbenzene

The median level of SPMA was significantly higher among exposed workers [158.00 µg/L (84.97)] compared with unexposed workers [67.50 µg/L (59.73)] (p<0.001). SPMA level exceeded the biological exposure indices (BEIs). In addition, the median levels of PGA and MA were significantly higher among exposed workers [144.54 mg/L (289.47)] and [237.73 mg/L (205.75)] respectively, compared with unexposed workers [25.16 mg/L (44.11)] and [98.40 mg/L (134.01)] respectively (p<0.001). (Table 3)

Table 3: Levels of metabolites of benzene and ethylbenzene in urine among the studied workers (n=74).

Metabolites of benzene and ethylbenzene	Exposed workers (n=37)	Unexposed workers (n=37)	U	p-value
SPMA (µg/L)				
Mean ± SD.	165.05 ± 81.98	78.93 ± 41.71		
Median (IQR)	158.00 (84.97)	67.50 (59.73)	222.0[*]	<0.001[*]
Min. – Max.	49.26 – 386.52	13.74 – 195.18		
PGA (mg/L)				
Mean ± SD.	269.42 ± 250.26	28.02 ± 26.72		
Median (IQR)	144.54 (289.47)	25.16 (44.11)	54.0	<0.001[*]
Min. – Max.	32.63 – 801.17	0.0 – 94.07		
MA (mg/L)				
Mean ± SD.	272.09 ± 151.02	115.11 ± 69.00		
Median (IQR)	237.73 (205.75)	98.40 (134.01)	230.0	<0.001[*]
Min. – Max.	83.0 – 654.55	11.48– 234.71		

Abbreviations: SPMA, S-Phenyl mercapturic acid; PGA, Phenylglyoxylic acid; MA, Mandelic acid; SD: Standard deviation; U: Mann Whitney test
[*] $p \leq 0.05$

3.4. Correlation between environmental levels of benzene and ethylbenzene and levels of their metabolites in urine

A significant positive moderate correlation was found between urinary level of SPMA and environmental level of benzene (r_s =0.493, p<0.001). In addition, a significant positive moderate correlation was found between urinary level of PGA and environmental level of ethylbenzene (r_s =0.688, p<0.001), as well as between urinary level of MA and environmental level of ethylbenzene (r_s =0.522, p<0.001). (Table 4)

Table 4: Correlation between environmental levels of benzene and ethylbenzene and levels of their metabolites in urine among the studied workers (n=74)

Environmental levels		Metabolites of benzene and ethylbenzene	
Benzene (mg/m^3)		**SPMA**	
	r_s	0.493*	
	p	<0.001*	
Ethylbenzene (mg/m^3)		**PGA**	**MA**
	r_s	0.688*	0.522*
	p	<0.001*	<0.001*

Abbreviations: SPMA, S-Phenyl mercapturic acid; PGA, phenylglyoxylic acid; MA, Mandelic acid
$_s$ Spearman correlation coefficient
* $p \leq 0.05$

4. Discussion

4.1. Environmental monitoring of benzene and ethylbenzene

In the present study, the environmental levels of benzene exceeded the recommended TLV of the ACGIH at all studied worksites, while ethylbenzene level was within the recommended exposure limits. These results come in agreement with the results of Mihajlovi´et al study (2021) in a petrochemical plant. Air monitoring reveals that benzene concentration was 10.3 mg/m^3 and exceeded the occupational exposure limit. Whereas the mean value for ethylbenzene was below the exposure limit (0.23 mg/m^3) [30]. Similarly, Harati et al. (2018) monitored the concentrations of these chemicals in an automobile manufacturing factory and found that benzene measured (0.775±0.12 ppm) was significantly higher than TLV recommended by ACGIH. On the other hand, the value of ethylbenzene was below the recommended standard level [31]. these results are also in consistent with the findings of Jalilian et al (2022) study conducted in a petroleum refinery; where the average benzene concentration in air sampling was above the permitted level; while ethylbenzene concentration was lower than the TLV-TWA recommended by ACGIH [32].

On the other hand, other studies conducted at gasoline stations found that the level of benzene and ethylbenzene measured exceeded the TLVs of the ACGIH [33-35]. These results could reveal the variability in the control measures implemented and the importance of continuous environmental monitoring at highly exposed areas to lower the level of these hazardous pollutants at the workplace.

Moreover, in the current study, the mean environmental levels of benzene and ethylbenzene were significantly higher at exposed worksites than at unexposed worksites. This would be suggested as an explanation for the significantly higher levels of metabolites of benzene and ethylbenzene among exposed workers than among the unexposed workers.

Similarly, other studies found that the levels of benzene and ethylbenzene in air samples were significantly higher at exposed areas compared with unexposed areas, even at low concentrations below the TLV, and were associated with adverse health effects [36,37]. Those findings would raise the importance for conducting further research on workers exposed to low levels of benzene and ethylbenzene below the permissible exposure limits and to understand the adverse effects of long-term exposure to low levels of these compounds in order to reach a conclusion that support lowering the currently applied TLVs.

4.2. Relationship between environmental levels of benzene and ethylbenzene and levels of their metabolites in urine

In the current study, the median levels of S-PMA, PGA, MA; were significantly higher among exposed workers compared with unexposed workers. A significant positive correlation was found between urinary level of SPMA and environmental level of benzene and between urinary level of PGA, MA and environmental level of ethylbenzene indicating higher occupational exposure to benzene and ethylbenzene at exposed refinery worksites. Moreover, the two studied groups in the current study were similar regarding smoking habit, therefore the significantly higher SPMA level found among exposed workers would probably be due to the occupational exposure.

SPMA is a sensitive and specific biomarker of exposure to benzene even at low exposure [25]. Carrieri et al study (2018) conducted a biological monitoring for benzene in an oil refinery and confirmed the validity of SPMA as a good biomarker of benzene even at low levels of exposure [25]. Biological monitoring was carried out in previous studies by measuring the urinary metabolites of these compounds. Carrieri et al study (2019) measured the urinary concentration of SPMA among gasoline pump attendants to explore the relationship between exposure biomarker and nucleic acid oxidation biomarkers. SPMA values were high in all exposed subjects, being even higher or equal to the ACGIH limit, indicating a high occupational exposure to benzene [38].

MA is a specific biomarker of exposure to ethylbenzene even at low exposure, as found by Chang et al who measured urinary MA as biological index for ethylbenzene exposure among spray painters [39].

5. Conclusion and recommendations

The current study revealed a significant positive moderate correlation between environmental levels of benzene and ethylbenzene and levels of their metabolites among the studied workers. Implementation of effective control measures at the petroleum refining company through regular environmental and biological monitoring of occupational exposure is essential to prevent adverse health effects of benzene and ethylbenzene exposure. Future research exploring the adverse effects of exposure to low levels of these compounds below the permissible levels is recommended.

List of Abbreviations

ACGIH: American Conference of Governmental Industrial Hygienists

MA: Mandelic acid

PGA: Phenylglyoxylic acid

SPMA: S-phenylmercapturic acid

TLV: Threshold Limit Value

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