Evaluating the Amount of Exposure to Benzene by Urinary Trans-Trans Muconic Acid Levels Assessment for Workers in Contact with Volatile Organic Compounds (VOCs)

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ABSTRACT: *Benzene is one of the major constituents of the volatile organic pollutants family and one of the major pollutants in air pollution, which contains the most extensive chemical compound used in both natural processes and synthetic processes. The main way to contact benzene in the industry is through the respiratory tract. Exposure to benzene can cause many of the most dangerous adverse health effects, especially leukemia. This study used high-performance liquid chromatography analysis techniques to determine the exposure level of benzene dyeing plant workers and the bioassay of trans-trans muconic acid in urine. This is a case-control study. 65 painting factory workers (in two groups exposed to benzene and not exposed to masks and without respiratory masks) participated in this study. Samples were collected at the beginning and end of the shift. High-performance liquid chromatography (HPLC) was used to determine the concentration of trans-trans muconic acid in urine samples. The results showed that the mean concentration of trans-trans muconic acid as a metabolite of benzene in the urine of exposed workers (mean ± standard deviation) was 2.7025± 3.18126 (ppm) and the mean concentration of trans-trans muconic acid in workers with mask (mean ± standard deviation) is 1.4079±2.73664 (ppm). Statistical analysis of the results between the two groups showed that the exposure to benzene concentration between workers with and without respiratory masks was (p=0.246) and was P> 0.05. Consequently, there was no significant difference between the two groups. The present study shows that the average concentration of transmuconic acid in workers' urine indicates exposure to higher concentrations than standard benzene, so it can be said that these people are at risk for occupational diseases, occupational cancers, and other side effects. are. From exposure to these pollutants.*

Keywords*: Benzene; Liquid Chromatography with Liquid Performance; Trans-trans Muconic Acid; Respiratory Mask.*

INTRODUCTION

People in their environment are exposed to a variety of environmental pollutants, including halogenated hydrocarbons such as chloroform and chloromethane, and aromatic hydrocarbons such as benzene, ethylbenzene, and xylene. Workplace chemical pollutants include gases, vapors, solids and liquids. Benzene is a colorless and fragrant liquid used in the industrial production of materials such as polystyrene, synthetic rubber, and nylon. This liquid is also used in the preparation of detergents and paints [1,2]. The number of chemical manufacturing plants is increasing daily, with the number of workers at increased risk [3,4]. Benzene is one of the major pollutants due to the high concentration of solvents in some US industrial sites, cancer, birth defects, and other diseases [5,6]. Benzene, as an acute toxin, produces a hypnotic and opiate effect, which can have tangible effects on the bone marrow that in some cases cause Bone marrow cancer (brain and bone cancer) [2,7]. Benzene, toluene, xylene, and ethylbenzene are among the most widely used aromatic solvents. These solvents have different health effects depending on the type of composition and concentration, some of which have a bad smell, reduced capacity of respiratory and cancer. Benzene has been declared a definitive human carcinogen by the US Environmental Protection Agency, the American Society of Industrial Health Professionals, and the International Agency for Research on Cancer [2,8,9]. The Center for Environmental and Labor-Health of the country introduces ACGIH Trans-trans Muconic Acid, Hypuric Acid, Hypuric Acid Methyl Isomers, Phenol, and MEK, as the main determinant of urinary metabolism in relation to exposure to benzene, toluene, xylene isomers, phenol, and MEK [10]. Benzene and toluene undergo biotransformation after being absorbed into the body, including the liver, and eventually transform into (Trans-Trans-Muconic Acid) trans-trans muconic acid and orto crezol, respectively. The main purpose of this process is to increase the polarity of the adsorbed compounds and consequently to increase the renal excretion caused by them [4,11-13]. This chemical enters the human body through the respiratory, gastrointestinal, and dermatological systems and, after entering the bloodstream, due to its being lipophilic, its distribution in the body depends on the amount of fat in the organs and ultimately all metabolites derived from the pollutants. It is excreted in the urine. Trans-trans muconic acid is a metabolite of benzene in the urine [14]. However, while the permissible concentration of benzene in respiratory air is 0.5 ppm, toluene is set at 20 ppm [15]. Basically sampling and analyzing pollutants in the air is done solely for the purpose of measuring and controlling chemical airborne in the workplace. However, other modes of exposure such as skin absorption, swallowing, and non-occupational exposure is not included and do not include control measures. In fact, biological monitoring is a way of filling this gap and is a complement to assessing exposure through air sampling and recognizing the timely reversible effects plays an important role in reducing the risks to workers' health. Therefore, the biological contact index is used to determine contact and complement for air samples. In the biological monitoring of volatile compounds due to the polarity of metabolites, andnon-invasive and simple sampling processes, the use of urinary media is the most appropriate [13,16]. Respiratory protective equipment is used to control respiratory exposure to organic solvents such as benzene. Respiratory protection devices are tools that prevent contaminants from entering the respiratory tract. In addition to the use of protective equipment, environmental engineering measures must be taken in contact with contaminants. Environmental control measures include engineering measures such as proper design and installation of equipment, removal or reduction of contaminants in the source of production (stopping the process, replacement of materials, etc.), use of closed systems, enclosure, ventilation (local and incremental), workshop cleaning, stockpiling materials and labeling, and so on. Our goal as a biotechnology chemical engineer is to test the efficacy of respiratory masks in reducing trans-trans muconic concentrations as a marker of urinary metabolites in workers. This metabolite is measured to prevent poisoning and dangerous diseases, including leukemia in workers.

THEORETICAL SECTION

Method of Sampling

Samples were collected from workers at one of Mazandaran's paint factories. In this case-control study, 65 subjects in both case and control groups who were employed in the paint production unit selected for this study. Products of this factory include the production of industrial paint and construction.

Workers' shifts at the factory include a shift from 8 am to 5:30 pm. Their selection was based on exposure to benzene and no drug use at the time of the study and the number of smokers in the study. This study was performed in two groups. The first group consisted of 35 painters and the second group consisted of 30 office personnel (control group). Factory workers were sampled in two stages during a shift. The first stage was sampling workers at the beginning of the shift and the second stage was at the end of the exposure shift. The exposed individuals had some respiratory masks and some had no respiratory masks. The mask used in this study was a single filter. Urine samples were collected in 50 mL volumes of polyethylene and kept in an ice-cold container until delivery to the laboratory and stored at 4 to 8° C until analysis.

Materials used in the analysis

Trans-trans muconic acid for Sigma - Aldrich Inc., HPLC-grad distilled water from Merck Inc. in Germany, ethyl acetate obtained from Merck Inc., methanol, sodium acetate purchased from Merck inc., tetrahydrofuran prepared from Merck, hydrochloric acid from Merck Inc. with 36.46 g/mol molar mass.

Method of preparation of samples for trans-trans Muconic acid assay

To prepare the sample, take 1 mL of the urine sample and transfer it to the test tube, add 200 µL of 2 mM hydrochloric acid solution and mix the contents of the test tube well, then add 8 mL of ethyl acetate solvent. Add and mix, then vortex the entire contents of the test tube for 2 minutes, then place the samples in a centrifuge (B 3/11 Model and Mark Jouan) and centrifuge for 15 min at 2500rpm. The solution is divided into two phases, the upper phase which is the organic phase with the sampler, and pour it into the rotary balloon and its rotary device. Purify the solvent, then add 1 mL of the mobile phase of the HPLC apparatus consisting of (acetic acid: tetrahydrofuran: methanol: water) in a ratio (8/10/2/1) with a shaker. We shake the balloon and then inject the resulting solution into the HPLC machine, which has the HPLC machine used in this experiment with UV and C18 column (250 cm in diameter x 4.6 mm). For data analysis, the data will first be entered into Excel software and then read through SPSS software.

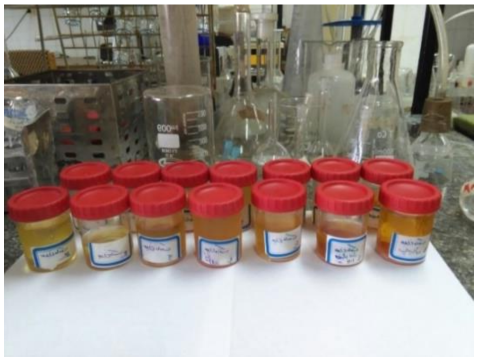

Fig. 1: Urine samples.

Fig. 2: Sample preparation method.

RESULTS AND DISCUSSION

The study included 65 samples of urine from workers in the paint factory, all male workers, and 3 smokers. The factory lounge includes ceiling ventilation and a number of windows around the lounge. The temperature at the time of sampling was 26 °C. The purpose of the standard curve is to obtain the concentration and the equation of the unknown line. Fig. 3. is the standard curve for trans-trans muconic acid. After diluting and making the standard sample at various concentrations and injecting it into the HPLC machine, sub-peak 6 and concentration numbers in Excel are inserted and the standard curve is obtained.

Table 1: Sub-peak area values for drawing a standard curve.

Peak level	Concentration (ppm)
6313767	1000
2182475	500
14364921	250
1082914	125

Fig. 3: Trans-Trans Muconic Acid Standard Curve.

Sampling was done from paint factory workers for two consecutive days (first day without a respirator mask and the second day with a respirator mask). Samples were prepared according to the procedure described earlier. Then, the standard sample was injected into the device to calibrate the device, and then the urine sample was injected into the HPLC. Considering the outflow time of trans-trans muconic acid in the injected standard sample, now with injecting the urine sample and the desired peak outflow at the same time, the value of the peak area concentration has been put in the slope equation that is obtained by injecting the standard sample into the device and drawing a standard curve based on its numbers. The obtained numbers are as follows.

The data in this table show that due to the maximum and minimum amounts of urinary trans-trans muconic acid concentration, using individual respiratory masks to prevent the entry of benzene contaminants into the respiratory system of wasted workers has no effect and had no role in reducing the concentration of urinary metabolites of benzene in workers exposed to benzene in the workplace.

Considering the significant difference between the case and control study regarding benzene from the respiratory system and trans-trans muconic acid excretion through urine, the table above also shows a significant difference between respiratory mask users and non-masked users. Therefore, the use of the mask examined during the study had no effect on reducing the exposure of benzene contaminants to the respiratory system of exposed workers.

CONCLUSIONS

Benzene is an important compound of pollutants that is commonly found in indoor and outdoor air and has attracted much attention from researchers. In recent years, many human resources have been contaminated in human societies. Benzene comes into the air from controlled and uncontrolled sources and is exposed to these compounds due to its widespread use in various industries and businesses [1,19]. The main purpose of occupational health is to prevent or appropriately control the contact of people with harmful factors in the workplace and to evaluate the correct contact in designing valuable and useful control strategies. Occupational hazard assessment is done by contact evaluation and comparison with standard values of permitted concentrations of air pollution. Biological monitoring methods have been of great interest in recent years. It is now recognized that evaluation of the biological dose absorbed in the body, such as occupational monitoring, is important to provide information to control the effects of hazardous chemicals. Since benzene is known to be one of the most dangerous chemical agents in the workplace, it is imperative to assess exposure to workers in order to prevent dangerous poisoning and diseases such as leukemia in workers[2, 20,21]. The results of studies carried out by *Rahimnejad et al.* In 2005 individual exposure to benzene and determination of trans-trans muconic acid concentration showed that the average concentration of this substance was 6 to 7 times higher than the standard level, increasing the risk of leukemia. Compared to the results of our research we can say that one of the reasons for the high concentration is the lack of proper protective equipment. Another reason is the lack of proper ventilation for the work environment[22]. In 2014 a study on the urinary metabolites of volatile organic compounds and its related factors in workers in the petrochemical industry was conducted by *Rahimpour et al.*, Urinary muconic acid exceeded the standard set by the National Center for

Table 2: The concentration of urinary Trans-Trans Muconic acid in workers of paint factory with benzene at the beginning of the shift.

Number	Peak Level	Urinary Trans Muconic Acid Concentration in Exposed People (PPM)	Peak Level	Urinary Trans Muconic Concentration in Non-Contact People (PPM)
1	4014	0.66	6125	0.75
2	4014	0.66	3989	0.16
3	8574	0.88	2760	0.12
4	6125	0.75	2760	0.12
5	2760	0.12	3417	0.56
6	6125	0.75	2760	0.12
7	2777	0.45	2760	0.12
8	3155	0.26	2777	0.45
9	2760	0.12	3155	0.26
10	3417	0.56	3155	0.26
11	8574	0.88	2760	0.12
12	6125	0.75	9172	0.91
13	7990	1.31	3979	0.16
14	3155	0.26	2777	0.45
15	6125	0.75	8574	0.88
16	3989	0.16	6125	0.75
17	4014	0.66	3989	0.16
18	2760	0.12	3417	0.56
19	7990	1.31	8574	0.88
20	3417	0.56	6125	0.75
21	8574	0.88	2777	0.45
22	2777	0.45	2760	0.12
23	2760	0.12	2777	0.45
24	7990	1.31	2760	0.12
25	3417	0.56	3155	0.26
26	3155	0.26	2777	0.45
27	7990	1.31	3417	0.56
28	6125	0.75	8574	0.88
29	3989	0.16	2777	0.45
30	2777	0.45	2760	0.12
31	7990	1.31	13692	1.85
32	2760	0.12	-	-
33	3417	0.56	-	-

Table 3. The concentration of urinary Trans-Trans Muconic acid in workers of paint factory with benzene and without exposure at the end of a shift and with or without using a breathing mask.

Number	Peak Level	Urinary Trans Muconic Acid Concentration in Exposed People (PPM)	Peak Level	Urinary Trans Muconic Concentration in Non-Contact People (PPM)
1	6881	1.135	6125	0.75
2	4014	0.66	3989	0.16
3	81148	13.39	72835	10.57
4	7990	1.31	7831	1.22
5	20440	3.37	20123	2.87
6	28606	4.72	26254	3.37
7	2777	0.45	2760	0.12
8	13818	2.28	13692	1.85
9	20310	3.35	20199	2.87
10	3417	0.56	3155	0.26
11	14304	2.36	12715	1.13
12	9295	1.53	9172	0.91
13	11892	1.97	11735	1.20
14	12360	2.03	1196	1.47
15	8716	1.43	8574	0.88
16	6881	1.135	6125	0.75
17	4014	0.66	3989	0.16
18	81148	13.39	72835	10.57
19	7990	1.31	7831	1.22
20	20440	3.37	20123	2.87
21	28606	4.72	26254	3.37
22	2777	0.45	2760	0.12
23	13818	2.28	13692	1.85
24	20310	3.35	20199	2.87
25	3417	0.56	3155	0.26
26	14304	2.36	12715	1.13
27	7990	1.31	7831	1.23
28	20440	3.37	20123	2.87
29	28606	4.72	26254	3.37
30	2777	0.45	2760	0.12
31	13818	2.28	13692	1.85
32	20310	3.35	20199	2.87
33	3417	0.56	-	-

Table 4: Mean and standard deviation of paint factory workers exposed to benzene and urinary trans-trans muconic acid at the end of shift work with and without using a respiratory mask

Workers' Group	Number of workers	Mean	Standard deviation	95% Average Trust Level		Minimum	Maximum
				Lower bound	Upper bound		
Without Mask	20	2.7025	3.18126	9407	4.4642	0.45	13.39
With Mask	15	1.4079	2.73664	9407	4.4642	0.12	10.57

Table 5: Comparison of the difference between exposure of workers to benzene and urinary trans-trans Muconic acid in two groups.

	Sum of Squares	df	Mean Square	F	P-Value
Between groups	12.570	1	12.570	1.428	0.242
Within the group	246.525	28	8.805		

Workplace Health in accordance with the results of the present study. Cigarette smoking also increases urinary excretion of all metabolites except hippuric acid[18]. In a study of the rate of exposure to benzene in gas station workers through environmental assessment and biomarker monitoring conducted by *Rastkaari et al.* In 1994, the results of their study showed a good correlation between trans-urinary acid concentration, trans-muconic acid, and benzene in respiratory air. People working at the gas station are exposed to high concentrations of benzene and the main route of inhalation confirms the findings of the present study[19]. Also, in a study by *Carrie et al.* (2010) on petrochemical industry workers, it was found that the mean concentrations of respiratory air benzene and trans-muconic uric acid were lower than those specified by the American Conference of Governmental Industrial Hygienists. The results of the present study are inconsistent, which may be due to the short half-life of benzene in the body[23]. The purpose of the study was to determine the effect of respiratory masks on the reduction of benzene. The concentration of trans-trans muconic acid in workers without masks (mean ± standard deviation) was 2.7025 ± 3.18126 and the concentration of trans-trans muconic acid in workers with masks (mean ± standard deviation) was 1.4079 ± 2.73664. Also, $p=0.242$ so it is $p>0.05$. Considering the results of the comparison between the two groups in the exposure to benzene indicates that the single filtered mask made in Iran does not have high efficiency in protecting workers against contaminants and there is no significant difference between the workers with masks or without masks. Therefore, we can say that there is a risk of occupational diseases, occupational cancers and other adverse effects of exposure to these pollutants. It is advisable to have regular periodic examinations on a monthly basis.

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