OPEN ACCESS
Freely Available Online

JOURNAL OF CANCER GENETICS AND BIOMARKERS
ISSN NO: 2572-3030

Research

DOI: 10.14302/issn.2572-3030.jcgb-20-3407

Assessment of Traffic Policemen Exposure To Benzene by Using Trans, Trans-Muconic Acid Biomarker in Ahvaz City, Iran

Neisi Abdolkazem[1], Mahmoudi Parviz[1,*], Kayedi Neda[1], Behroozi Hamid[2]

[1]Department of Environmental Health Engineering, Air Pollution and Respiratory Diseases Research Center, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran.

[2]Department of Radiologic Technology, Faculty of Paramedicine, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran.

Abstract

Benzene is known as an environmental and industrial pollutant. One way to check the amount of exposure to benzene is to measure its metabolites in the urine. Trans, Trans-Muconic Acid which is one of its metabolites is also as a biological indicator of exposure to benzene. In this study, the relationship between the amount of this biomarker and the environmental benzene concentration was investigated in two groups of policemen working outdoors in the traffic-congested areas and indoor in the same area.

The results showed that the highest amount of benzene was in Salman Farsi Station at 4.44 ppm. At the same time, the biomarker measured in the urine showed 127.20 µg / L, which was the highest amount. In addition, the amount of benzene in the outdoor was approximately 3 times more than indoor and the amount of biomarker measured in outdoor employees was two times more than indoors ones. In general, wherever there was more benzene, the biomarker would also show a higher amount.

Corresponding author: Mahmoudi Parviz, Department of Environmental Health Engineering, Air Pollution and Respiratory Diseases Research Center, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran.

Keywords: Benzene, Trans, Trans-Muconic Acid, Traffic policemen, Ahvaz

Received: May 24, 2020 **Accepted:** Jun 08, 2020 **Published:** Jun 13, 2020

Editor: Jiaqing Hao, University of Louisville School of Medicine.

Introduction

Benzene is a volatile aromatic hydrocarbon that is widely distributed as a natural compound in petroleum, and it also spreads through incomplete combustion in motor vehicles. A greater amount of this pollutant is produced by vehicles due to sudden running of the engine in places where there is more traffic [1].

Benzene is converted into benzene oxide by cytochrome enzyme in the liver. This compound is also converted into several compounds by enzymes and non-enzymes pathways, which can be converted to phenol (74-87%) or intermixed with glutathione (1%) and then converted to the form of s-phenyl mercapturic acid (SPMA). It can also be converted to Trans, trans-muconaldyde, and then to Trans muconic acid through the opening of the ring (2%).

The American Conference of Governmental Industrial Hygienists (ACGIH) has suggested the use of SPMA and t, t-MA as a biomarker for exposure to benzene [1].

In Iran, the standard of Euro 4 is considered as the ideal standard for gasoline. In most refineries of the country, fuel is produced according to the Euro 4 standard. According to the standard of Euro 4, the number of octane gasoline should be between 91 and 95. The octane number indicates slow-burning and dry-burning of fuel consumed [2].

Euro 4 fuel standard administered in 2005. The fuel for this standard should contain at most 35% aromatic materials, 18% olefin compounds, 1% benzene, 2.7% oxygen and 50 ppm sulfur. This fuel is completely unleaded. The Euro 5 standard of Europe was introduced in 2009. Based on this standard, the Euro 5 fuel contain at most 35% aromatic materials, 18% olefin compounds, 1% benzene, 2.7% oxygen and 10 ppm sulfur. The fuel is also unleaded and its octane number is above 95. The amount of benzene in the fuel is a feature which is noticeable in standard actions for fuel consumed in vehicles. According to the standard of Euro 4, the amount of benzene allowed in the gasoline fuel is at most 1% (Iranian National Standards Organization).

It should be noted that only attention to the standardization of fuel is not enough, but it should also be considered in the process of manufacturing new cars. A part of the pollutants is caused by incomplete burning of fuel in the engine of the vehicle. In other words, the engine must match the fuel consumed, otherwise the engine will not work properly and we will see excess emissions of pollutants in the environment.

Traffic police are known as a job in which people should stand and work for a few hours in a day at the crossroads with high passing of vehicles [3].

Materials and Methods

In this study, the work environment of the traffic police (mostly in the crowded crossroads of the city) has been monitored for the concentration of benzene. At the same time, as the urine sample is taken from these individuals, the presence of the biomarker of Trans, Trans muconic acid has been investigated. Also samples were taken from police officers who were not present in the crowded places in order to compare the results. Finally, the amount of exposure to benzene was investigated in the police officers of Ahvaz city.

Study Population and Sampling

In order to perform this study, 40 policemen were placed in two groups of traffic police engaged in traffic control and policemen only involved in office work in the same districts. All of the policemen were male and their age, and duration of employment is shown in Table 1.

Table 1. Study population consist outdoor and indoor policemen

Group	n	Age (y) mean (SD)	Years of employment
Traffic police	20	29.85 (3.32)	3.6 (1.53)
Office police	20	29.25 (3.19)	4 (1.56)
Total	40	29.55 (3.23)	3.8 (1.54)

Simultaneously with sampling of these subjects, the concentration of benzene was measured in five points of Daneshgah square, Naderi (Salman Farsi) crossroad, Kianpars crossroad, Chahar-Shir square and Sa'at square, which are the most traffic congestion parts of Ahvaz. Policemen were selected from police stations operating in these areas.

For sampling, 20 traffic policemen and 20 office policemen (who work only in the indoor environment) were selected with restriction on no-smokers [4]. Sampling was done at 8:00 am while the subjects were fasting. 100 mL of urine was sampled from each person and transferred to the laboratory for analysis.

Samples Preparation and Measurement of Trans-Trans Muconic Acid Biomarker

Urine samples are collected in polypropylene containers without any preservative. Samples could be kept at a temperature of -10 °C to -18 °C for a maximum of 30 days [1]. In the first stage, we brought 0.5 ml of urine sample to pH = 7.5 by using 5 ml phosphate buffer. Then, the sample was extracted and enriched by using solid-phase extraction with an anion-exchange cartridge (SPE SAX), 500 mg stationary phase, 3 mL volume which were previously activated using 3 mL methanol and 3 mL distilled water. Then, the absorbent was washed with 3 ml of 1% acetic acid and finally, the target Analyte was washed with 4 mL of 10% acetic acid and was collected in a 5 ml flask; then, it was reached to the volume of 5 ml with distilled water. After that, 100 µL of the sample was ready to be injected into the HPLC according to the instructions below [1, 5, 6].

In the second stage, chemical analysis was carried out by using an HPLC apparatus equipped with a UV detector with a wavelength of 259 nm and also by using a moving phase (water, methanol and acetic acid at a ratio of 69, 30 and 1 respectively) at a flow rate of 1 mL / min in C-18 analytical column. The detection limit is 0.01 µg / mL with this method [1, 5, 6].

Statistical Methods of Results Analysis

T-test was used to compare the mean values of biomarker concentration in two groups. Pearson correlation analysis was used to determine the relationship between the concentrations of biomarkers and the concentration of benzene in the air. Regression analysis was used to investigate the relationship between air pollutants and trans, trans- muconic acid biomarker.

Results and Discussion

Concentration of Trans, Trans Muconic Acid Biomarker in Policemen Urine Samples in Indoor and Outdoor Environment

Based on the results of this study, 70% of policemen had over 4 years of work experience and 30% of them had 4 to 7 years of work experience. The results of the measurement of the trans, trans- muconic acid biomarker in policemen urine samples in Ahvaz are shown in Table 2.

As it is shown, at all stations, the mean concentration of this biomarker in policemen who work outdoor is more than those who work in the office. This indicates that the exposure to benzene has been higher in the outdoor environment. In a similar study, Lovreglio et al. concluded that benzene and toluene were much more in workers who were exposed to benzene than in the control group. In that study, the validity of using trans, trans muconic acid was confirmed for the biological monitoring of exposure to low-benzene concentrations [7]. Also, Protano et al. in a study in 2012 showed that by increasing urbanism and living in more crowded places, the amount of t , t-muconic acid biomarker has increased in the urine samples of the subjects [8].

Among policemen working outdoor at 5 different stations, those working at Salman Farsi Street showed the highest mean biomarker concentration at a rate of 127.20 µg / L, while the lowest rate for policemen working in the Saat square was 33.75 µg / L. In addition, among policemen working in administrative offices, the workers in these two locations also had the highest and lowest mean biomarker concentrations at the rate of 69.00 µg / L and 17.5 µg / L, respectively. These results could be due to the influence of air polluted from the environment into the workplace of those working in the office environment.

The Concentration of Benzene Indoor and Outdoor

As shown in Table 2, the highest mean concentration of benzene measured outdoor was at the station of Salman Farsi Street with a mean concentration of 4.44 ppm while the lowest mean concentration has

Table 2. Trans, trans- muconic acid biomarker in the urine samples of policemen and concentration of benzene

location	workplace	Trans, trans muconic acid		Benzene Con.	
		Mean (µg/L)	SD	Mean (ppm)	SD
Salman Farsi St	indoor	69.00	0.07	1.51	0.12
	outdoor	127.25	0.01	4.44	0.07
	Total	98.12	0.03	2.97	1.57
Shahid Bandar Sq	indoor	47.00	0.03	1.30	0.03
	outdoor	99.00	0.08	4.05	0.15
	Total	73.00	0.03	2.67	1.47
Kianpars St	indoor	39.25	0.02	1.29	0.03
	outdoor	75.00	0.01	3.76	0.12
	Total	57.13	0.02	2.52	1.32
Sa`at Sq	indoor	17.50	0.02	0.83	0.02
	outdoor	33.75	0.02	3.35	0.07
	Total	25.63	0.08	2.09	1.34
University Sq	indoor	23.50	0.01	0.77	0.02
	outdoor	42.75	0.08	2.82	0.07
	Total	33.13	0.01	1.80	1.09
Total	indoor	39.25	0.02	1.14	0.29
	outdoor	75.55	0.03	3.68	0.58
	Total	57.40	0.03	2.41	1.36

Table 3. Correlation between trans, trans-muconic acid and benzene concentration

			Concentration of Trans, trans muconic acid	Concentration of Benzene
4 years' work experience and less	Concentration of Trans, trans muconic acid	Pearson Correlation	1	.721**
Between 4 and 7 years' work experience	Concentration of Trans, trans muconic acid	Pearson Correlation	1	.891**
Total	Concentration of Trans, trans muconic acid	Pearson Correlation	1	0.75

**. Correlation is significant at the P value: 0.01

been reported at the station of Daneshgah Square with a concentration of 2.82 ppm. Also, the highest mean concentration indoor was at Salman Farsi Station with a mean concentration of 1.51 ppm and the lowest mean concentration was at the station of Daneshgah square with a concentration of 0.77 ppm.

Considering permissible concentration of exposure to benzene, which is 9.39 ppb [9], even the exposure is more than permissible limit indoor. Also, the results showed that there is a significant difference between the concentration of benzene at different stations with each other and indoor and outdoor with 95% confidence level. The results of this study are consistent with the study results of Dehdari Rad et al. in Ahvaz in 2014. That study concluded that the concentration of benzene in traffic congested environments is 3.5 times more than its concentration indoor. [10].

Relationship Between Benzene Concentration and Trans, Trans-Muconic Acid Concentration

As shown in Table 3, there is 75% significant relationship between the concentration of benzene and the concentration of trans, trans-muconic acid biomarker, which is 72% in the group with less than 4 years of work experience and 89% in the group of between 4 and 7 years of work experience.

Conclusion

In general, this study shows that policemen in Ahvaz are exposed to benzene more than the permissible limit; this amount in those who work outdoor in the traffic congested crossroads is three times higher than those who work indoor. Also, in areas where benzene was more observed, the biomarker measured in the urine sample showed a higher value, which could be due to the penetration of benzene through the respiration of individuals into the body and its deformation in the form of a trans, trans muconic acid biomarker in the body. Due to chronic effects of benzene on policemen, environment factors, such as traffic characteristics, quality of fuel, meteorological conditions, building characteristics of the area, and difference in physical activity in the workplace may contribute to the difference in the levels of every individual benzene exposure. It is suggested to change their workplace alternatively and prevent a certain number of individuals from permanent exposure.

Acknowledgements

This work was financially supported by Department of Environmental Health of Ahvaz Jundishapur University of Medical Sciences (grant number: U-96155).

References

1. Aprea, C., et al., Reference Values of Urinary Trans,trans-muconic Acid: Italian Multicentric Study. Archives of Environmental Contamination and Toxicology, 2008. 55(2): p. 329-340.
2. de Sá Borba, P.F., et al., BTEX Emissions from the Largest Landfill in Operation in Rio de Janeiro, Brazil. Bulletin of Environmental Contamination and Toxicology, 2017. 98(5): p. 624-631.
3. Kinjal, C. and P. Minarva, Air Pollution Exposures And Their Effect on Traffic Police. International Journal of Innovative Reserch in Technology, 2014. 1(9): p. 12-15.
4. Wiwanitkit, V., J. Suwansaksri, and S. Soogarun, A note on urine trans, trans muconic acid level among a sample of Thai police: Implication for an occupational health issue. Vol. 76. 2003. 103-8.
5. Ducos, P., et al., Improvement in HPLC analysis of urinary trans, trans-muconic acid, a promising substitute for phenol in the assessment of benzene exposure. International Archives of Occupational and Environmental Health, 1990. 62(7): p. 529-534.
6. Shahtaheri, S.J., F. Ghamari, and F. Golbabaei, Sample preparation followed by high performance liquid chromatographic (HPLC) analysis for monitoring muconic acid as a biomarker of occupational exposure to benzene. Int J Occup Saf Ergon, 2005. 11(4): p. 377-88.
7. Lovreglio, P., et al., Validity of new biomarkers of internal dose for use in the biological monitoring of occupational and environmental exposure to low concentrations of benzene and toluene. International Archives of Occupational and Environmental Health, 2010. 83(3): p. 341-356.
8. Protano, C., et al., Urinary trans, trans-muconic acid and S-phenylmercapturic acid are indicative of exposure to urban benzene pollution during

childhood. Science of The Total Environment, 2012. 435-436: p. 115-123.

9. Agency, U.S.E.P., Integrated Risk Information System (IRIS) on Benzene. National Center for Environmental Assessment. Office of Research and Development, Washington, DC., 2009.
10. Rad, H.D., et al., Levels and sources of BTEX in ambient air of Ahvaz metropolitan city. Air Quality, Atmosphere & Health, 2014. 7(4): p. 515-524.