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Comparison of Urinary o-Cresol and Hippuric Acid in Drivers, Gasoline Station Workers and Painters Exposed to Toluene in West of Iran

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Abstract: The aim of this study was evaluation of exposed to toluene and compare levels of hippuric acid and o-Cresol in taxi drivers, petrol station workers, car painters with a control group in West of Iran. The urinary o-creasol and toluene in air were analyzed by a gas chromatography and hippuric acid was extracted from urine and analyzed by high performance liquid chromatography. The significant differences in the levels of urinary o-Cresol were found in painters and petrol station workers compared to the control group ($p<0.005$). There was no significant correlation between toluene in air and biomarkers for taxi drivers. The lowest toluene concentration at which urinary hippuric acid increased to a measurable level was approximately 25 to 35 ppm and for o-Cresol was 2 ppm. In conclusion our results was showed that o-Cresol and hippuric acid could separate the exposed to toluene from the non-exposed when toluene in breathing zone of subjects was greater than 3 and 35 ppm, respectively. Hippuric acid and o-Cresol are not suitable biomarkers for occupations such as drivers that exposure to toluene in low concentration.

Key words: Hippuric acid, o-Cresol, toluene, occupations

INTRODUCTION

Toluene is one of the most popular solvents used in industry. Gasoline paints, strippers, glues, some household cleaners and tobacco smoke may be sources of toluene in air[1,2]. Toluene is released to the atmosphere during the production, transport and combustion of gasoline. Occupational overexposure is associated with mucous membrane irritation, decrements in central nervous system function and endocrine distribution[3].

Hippuric acid is most frequently used as a biomarker in the biological monitoring of occupational exposure to toluene. Human metabolism is dominated by the conversion of toluene to benzylalcohol, benzaldehyde and benzoic acid, which is finally conjugated to glycine and excreted as hippuric acid in urine[4]. The determination of urinary cresols (particularly o-Cresol) has been also proposed as a biomarker for exposure to toluene[5,6].

Several studies have been undertaken to test the suitability of these parameters for monitoring purposes[6-8].

Kawai *et al.*[9] reported that hippuric acid concentration did not differ significantly among the exposed groups to toluene in air when the concentration of toluene is less than 30 ppm. Amorim *et al.*[8] reported that o-Cresol values in the urine did not differ significantly among the exposed groups analyzed at the 5% level but some studies concluded that determination of the urinary o-Cresol excretion represents a diagnostically specific and sensitive parameter for the estimation of an individual toluene uptake[7,10]

Most of the studies have been done as experimental or occupational in subjects who exposed to toluene in high concentrations and few data is concerned to exposure to toluene in low concentrations[11]. There is little data on toluene biomarkers for comparison in different occupations[8]. Furthermore, a few data is available on health status of the workers exposed to toluene in the Iran.

The major environmental problem in Iran is air pollution. There are about 6 millions vehicles in Iran and 40% of them are at least two decades old[12]. Low fuel prices and lack of catalytic converters are led to unburned hydrocarbons emits in ambient air. The gasoline stations in Iran are by the streets and workers in these locations refueling gasoline for customers.

The aim of this study was evaluation of exposed to toluene and compare levels of hippuric acid and o-Cresol in taxi drivers, petrol station workers, car painters with a control group in West of Iran (Hamadan state).

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MATERIALS AND METHODS

This study was carried out on samples of the exposed men to toluene in 3 occupational groups in Hamadan city (Centre of Hamadan state at west of Islamic Republic of Iran) from March 2003 to March 2004. Subjects include 60 taxi drivers, 25 of petrol station workers and 42 car painters. The study group was selected from 54 workers at gasoline stations, 60 car painters and 300 drivers by simple random sampling. A control group of 60 non-exposed men living in rural area were selected from the same state. The control group was matched with the study group based on age and smoking status. A detailed questionnaire was completed for study and control group, providing information about personal characteristics, smocking history and eating of some fruits. (Using of alcoholic drinks is banned in Islamic Republic of Iran).

Personal monitoring of exposure: A charcoal adsorption tube from (SKC) connected to a small pump (Negretti Automation, Model NR645) was used for sampling air. The charcoal tube was attached to the worker's overalls as closely as possible to the face in order to determine the toluene concentrations in the breathing zone. The pump was operated at 200 mL/min and the duration of sampling time was 2-4 h. Toluene was extracted with carbon disulphide (CS_2) from the charcoal. A gas chromatography (Model 4600-Unicam Company) equipped with a Flame Ionization Detector (FID) was used for quantitative measurement. Separation of the compounds was achieved with glass packing column 1.5 m×4 mm i.d packed with 10% SE 30 on Chromosorb W-AW-DMCS 100-120.

This column had a programmed temperature of 50°C for 2 min followed by an increase to 180°C at a rate of 4°C min^{-1} and finally during analysis a constant temperature of 180°C for 2 min.

Analysis of urine: Exposed subjects and non-exposed control group were asked to pass urine in the end of the shift. Samples were refrigerated immediately, transferred to the analytical laboratory and kept frozen until analyzed.

Hippuric acid in urine: The determination of hippuric acid was carried out according to National Institute for Occupational Safety and Health (NIOSH) method[13]. Initially, 40 μL of HCl and 0.3 g sodium chloride were added to 1 mL of urine into a graduated centrifuge tube. 4 mL of ethyl acetate added to tube and the samples were mixed centrifuged at 1200 rpm for 5 min, then the ethyl acetate layered transferred to tapered test tube by pasture pipette. Samples were evaporated to dryness using a gentle steam of nitrogen in a heating block at 45°C before reconstitution. The residue of samples redissolved in 200 μL of distilled water and 20 μL was injected to High Performance Liquid Chromatography (HPLC) system.

A HPLC chromatograph (Knauer) equipped with an ultraviolet (UV) detector (Model K-2600 Knauer) was used for analysis. The UV detector was set at 254 nm. The HPLC column was an APEX ODS II 3 μm, 25×4.6 mm. Chromatography was isocratic in a mobile phase consisting of water-acetonnitrile-acetic acid (89:10:1) at a flow rate of 1 mL/min .

o-Cresol in urine: The determination of o-Cresol was carried out according to a procedure essentially described by Amorium and Alverz-Leitet[8] 5 mL urine and 1 mL concentrated sulfuric acid were added to a centrifuge tube and placed in a water bath for 1.5 h. After a few minutes of heating, gently release excess pressure in the tubes by uncapping the tubes momentarily. Allow the tubes to cool and then saturated with ammonium sulfate. Add 1 mL of ethyl acetate and mix on a rotary mixer for 10 min. Centrifuge and transfer the organic layer to a glass culture tube containing a small amount of anhydrous sodium sulfate. Five microliter of samples was injected to gas chromatograph.

A gas chromatograph (Model 4600-Unicam Company) equipped with Flame Ionization Detector (FID) was used for analyses of o-Cresol. A glass column (1.5 m×4 mm i.d) packed with 10% SE 30 on Chromosorb W-AW-DMCS 100-120 mesh was used to separation of compounds. The initial column temperature was 120°C for 2 min followed by a temperature gradient of 10°C/min to 180°C for 2 min.

The urinary creatinine was measured by Jaffe kinetic method using a Boehringer Mannheim Hitachi 917 automatic analyzer and were reported following adjustment for creatinine concentration.

Data analysis was performed using SPSS statistical software for windows. Correlation were assessed with regression analysis and the F test was used to test significance between the slopes of different regression lines. Student's t test were used when necessary.

RESULTS

There was significant difference between the level of hippuric acid in urine of painters and the control subjects ($p<0.005$). No-difference in the levels of hippuric acid in urine was found in drivers and gasoline stations compared with the control group (Table 1). Significant differences in the level of o-Cresol in urine were found in painters and petrol station workers compared to control

group ($p<0.005$) but no-significant difference was seen for urinary o-Cresol in taxi drivers compared to control group. The mean concentrations of toluene in ambient air of painters, drivers and gasoline station workers were 45.41, 3.97 and 1.70 ppm, respectively (Table 1)

The lowest toluene concentration at which urinary hippuric acid in subjects has significant difference with control group was 25 to 35 ppm and for o-Cresol was more than 2 ppm (Table 2).

The correlation coefficient between hippuric acid and toluene for subjects that exposed to toluene is greater than 35 ppm is 0.55 and for subjects exposed to less than 35 ppm is 0.01. The correlation coefficient between o-Cresol and toluene for subjects that exposes to toluene greater than 2 ppm is 0.60 and for subjects exposed to less than 2 ppm is 0.09 (Table 2).

There was not significant correlation between toluene in air and biological index for taxi drivers (Table 3).

No impact of smocking habits on the levels of toluene in o-Cresol and hippuric acid confirmed within the present study.

Table 1: Results of toluene concentrations in ambient air and biological monitoring of hippuric acid and o-Cresol at different occupations and control group

Parameters		Car painters (n=42)	Taxi drivers (n=60)	Petrol station workers (n=25)	Control group (n=60)
Toluene in air (ppm)	x±SD	45.41±17.34	1.70±1.49	3.97±2.84	0.01±0.09
	Range	17.14-69.95	0.01-4.50	0.90-8.74	0.00-0.2
	p-value*	0.00	0.00	0.00	1
Hippuric acid (g L^{-1})	x±SD	01.64±0.49	0.37±0.21	0.37±0.34	0.29±0.14
	Range	00.58-2.07	0.04-0.91	0.03-1.47	0.12-0.65
	p-value*	0.00	0.79	0.84	1
Hippuric acid (g g^{-1} cr)	x±SD	1.31±0.59	0.34±0.30	0.34±0.38	0.27±0.21
	Range	0.41-1.98	0.02-1.83	0.05-1.83	0.10-0.61
	p-value*	0.00	0.99	0.99	1
o-Cresol (mg L^{-1})	x±SD	0.44±0.55	0.09±0.21	0.18±0.31	0.07±0.13
	Range	0.12-1.39	0.09-0.37	0.09-0.80	0.00-0.27
	p-value*	0.00	0.85	0.03	1
o-Cresol (mg g^{-1} ct)	x±SD	0.38±0.50	0.08±0.17	0.16±0.32	0.06±0.10
	Range	0.10-1.29	0.08-0.34	0.07-0.78	0.00-0.24
	p-value*	0.00	0.18	0.03	1

*compare with control group

Table 2: The mean levels of urinary hippuric and o-Cresol at different concentrations of toluene exposure

Concentration of toluene	n (n=127)	Hippuric acid		o-Cresol	
		X±SD	p-value*	x±SD	p-value*
<2	57	0.33±0.22	0.70	0.08±0.20	0.56
2-5	23	0.27±0.14	0.93	0.17±0.37	0.04
5-25	18	0.28±0.20	0.60	0.20±0.33	0.00
25-35	8	0.59±0.22	0.05	0.32±0.32	0.00
35-45	9	0.90±0.48	0.00	0.50±0.41	0.00
45<	14	1.51±0.55	0.00	0.80±0.44	0.00

*compare with control group

Table 3: Calculated regression lines for toluene (tol) in air, urinary o-Cresol (Oc) and Hippuric acid (Ha) at different occupations

Occupations	Y=A+Bx X-Y	Correlation coefficient	A	B	Level of significance
Car painters	tol (ppm)-Oc (mg L^{-1})	0.80	0.52	0.69	0.000
	tol (ppm)-Oc (mg g^{-1} cr)	0.75	1.53	0.05	0.004
	tol (ppm)-Ha (g L^{-1})	0.28	0.19	0.001	0.230
	tol (ppm)-Ha (g g^{-1}cr)	0.25	0.16	0.11	0.343
	Oc (mg/l)-Ha (g g^{-1}cr)	0.61	0.52	0.46	0.005
Taxi drivers	tol (ppm)-Oc (mg L^{-1})	0.08	0.09	0.58	0.204
	tol (ppm)-Oc (mg g^{-1}cr)	0.08	0.29	0.04	0.525
	tol (ppm)-Ha (g L^{-1})	0.07	0.04	-0.01	0.610
	tol (ppm)-Ha (g g^{-1}cr)	0.01	0.04	-0.01	0.602
	Oc mg L^{-1})-Ha (g g^{-1}cr)	0.15	-0.75	0.25	0.221
Petrol station workers	tol (ppm)-Oc (mg L^{-1})	0.60	0.53	0.47	0.004
	tol (ppm)-Oc (mg g^{-1}cr)	0.57	0.66	0.05	0.003
	tol (ppm)-Ha (g L^{-1})	0.25	0.34	-0.01	0.115
	tol (ppm)-Ha (g g^{-1}cr)	0.19	-0.09	0.01	0.671
	Oc (mg L^{-1})-Ha (g g^{-1}cr)	0.59	0.62	0.56	0.002

DISCUSSION

The present study was undertaken to evaluate the relationship between two biological exposure indicators (hippuric acid and o-Cresol) to toluene at air in painters, taxi drivers and gasoline station workers. There was a good correlation between urinary o-Cresol and exposed toluene when toluene in air is more than 3 ppm for painters and gasoline stations workers and also between hippuric acid and toluene when concentration of toluene in ambient air is more than 35 ppm (for painters). No correlation coefficient was found between toluene and biological index for taxi drivers at this study.

The use of o-Cresol and hippuric acid as exposure indicators of toluene exposure has already been the subject of several reports which revealed some discrepancies in opinions about their respective usefulness. The results of a field study by Angerer and Krämer[7] showed that the determination of the urinary excretion of o-Cresol represents a specific and sensitive indicator of an individual toluene uptake. They found a correlation coefficient of 0.65 for subjects that exposed to toluene at 64.8 ppm. Nise[6] investigated 58 workers exposed to toluene at 26 ppm and found that o-Cresol correlated with toluene in air with a correlation coefficient of 0.57 while hippuric acid correlated even less closely. In the present study, painters and petrol station workers exposed to toluene at 46 and 4 ppm, respectively and o-Cresol correlated with toluene in these occupations at air with correlation coefficients of 0.75 and 0.57, respectively, so the results concern to painters and workers of gasoline station are similar to Angerer and Nise researches.

In a recent study Inoue *et al.*[14] reported that benzylmercapturic acid is more sensitive than hippuric acid and o-Cresol in low concentration of toluene[14]. The results of hippuric acid and o-Cresol in urine of drivers at present study agreement with Inoue et al finding in that neither hippuric acid nor o-Cresol showed a high correlation when toluene in air is low concentration. Kawai et al reported that determination of unchanged toluene in urine is better marker of exposure to toluene vapor than hippuric and o-Cresol in urine[9].

In the current study hippuric acid in urine of painters has a significant correlation coefficient with toluene while the concentration of toluene is more than 35 ppm, similar results were reported by Kawai *et al.*[9] who found that hippuric acid level in urine resulting from 8 h of occupational exposure could be distinguished from background levels only at exposure levels of ≥30 ppm.

In the present study, the mean of urinary hippuric acid in control group was 0.29 g L^{-1} and results is less than described by Alvarez-Leite[15], this may concern to ban of using alcohol drink in Iran country, because using of alcohol drink decrease in the elimination of hippuric acid into urine[16,17]. Urinary hippuric acid also related to dietary contribution of benzoic acid (in fruits, vegetable and food preservatives] and benzoic acid precursors (in prunes, cranberries and plums)[17].

We have not seen the effect on smocking habits on the levels of toluene in o-Cresol and hippuric acid. Alvarez-Leite *et al.*[15] reports that the individual habits of smoking and drinking either separately or combined, did not significantly alter urinary hippuric acid levels[15].

In present study, the urinary of o-Cresol in control group ranged from 0-0.27 mg g^{-1} creatinine and may related to by smocking and environmental toluene exposure

At the present time, the American Conference of Governmental Industrial Hygienists recommends both urinary hippuric acid and o-Cresol in addition to toluene in blood as marker of exposure to toluene[18], Deutsche Forschungsgemeinschaft recommends o-Cresol in urine and toluene in blood[19] because of diagnostic specificity and also toluene and o-Cresol do not exit in biological samples if the control subjects are not exposed to toluene. In conclusion our results was showed that o-Cresol and hippuric acid could separate the exposed to toluene from the non-exposed when toluene in ambient air was greater than 3 and 35 ppm, respectively. Hippuric acid and o-Cresol are not suitable biomarkers for occupations such as drivers that exposure to toluene is in low concentration, so others biomarkers such as toluene in urine and blood and also benzylmercapturic acid for environmental exposure must be evaluated.

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