

ORIGINAL ARTICLE

# Efficacy of Urinary Hippuric Acid as a Biomarker of Low Level Exposure to Toluene in Petroleum Depot Workers

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## ABSTRACT

Exposure to toluene can be associate with genotoxicity, neurological dysfunction, reproductive toxicity, and etc. Biomonitoring of hippuric acid (HA) is used for a long time as an occupational exposure index to toluene. The aim of this study was to explore efficiency of HA as a biomarker of exposure to low level of toluene for Iranian Petroleum Depot workers in 2011. Personal monitoring to toluene exposure for 45 workers (exposed group) and 32 staff (control group) were done according to the NIOSH Method No.1501, and simultaneous biological monitoring were conducted as urinary HA for all subjects. Pre and post shift urine samples were collected for analysis of HA by the NIOSH method No.8300 and urinary HA concentrations were corrected with creatinine content. Personal exposure of petroleum loading operators to toluene was  $0.78 \pm 0.37$  ppm (Mean  $\pm$  SE). There was no statistical significant difference between urinary HA of exposed and control groups (staff). There was no statistical correlation between occupational exposure to toluene and internal exposure in term of HA. Occupational exposures to toluene were less than TLV in petroleum products loading operators. Due to exposure with low levels of toluene concentrations, however the content of urinary HA in gasoline operators were higher than BEI (Biological Exposure Index), but of no significant relationship between airborne concentrations of toluene and levels of HA in urine in all exposed groups. In conclusion, urinary HA is not appropriate biomarker of low level exposure to toluene.

**Keywords:** Toluene, biomarker, biological monitoring, Hippuric Acid

## INTRODUCTION

Workers in petroleum products depot and distribution chain are exposed to volatile organic compounds (VOCs). The main VOCs emissions from

installation storage tanks by using US EPA standard emission model has been established to be toluene, hexane and isooctane in compared with other VOCs compounds, by having high vapor pressure and content in the liquid composition in storage tanks [1]. Recently, supposed possible genotoxic effects to toluene exposure in below level of occupational exposure limits, as well as changes in hepatic enzymes and albumin levels [2].

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Exposure to toluene also can cause central nervous system depression and impair to memory. It has been reported symptoms such as headache, drowsiness, fatigue, muscular weakness, poor balance and difficulty in walking [3]. Besides change of renal function in chronic exposure with high concentrations, change of hormone level, increasing in systole blood pressure are counted among complications of toluene exposure [4]. ACGIH has decreased TLV-TWA to toluene exposure from 50 ppm to 20 ppm based on evidences to reproductive toxicity and ocular effects [5].

Occupational exposure to toluene and benzene was reported to be 69-259 and 60-255 ppb respectively at petroleum liquid storage and distribution facilities in India [6]. Top loading operators were reported to have higher exposure than automatic bottom loading operators [7].

Biological monitoring is applied for better assessing of internal absorbed dose related with high workload and work practice, evaluation of protective measures efficiency, recognizing routes of exposure other than inhalation [8]. In general, blood and urine are used for biological monitoring. Urine is applied widely for biomonitoring, especially for unstable chemicals such as chemicals that have short half-life [9]. Several biomarkers are available for assessing internal exposure to toluene, including toluene in blood, urine and exhaled air, and its metabolites in urine as HA, Ortho-cresol, S-p-toluymercapturic acid +S-benzylmercapturic acid [10].

In a study related with toluene markers as biological exposure indices, exertion of HA was estimated 75% of absorbed dose [11]. In comparing between five metabolites for toluene exposure monitoring, HA has been evaluated to be have a better correlation than others metabolite with toluene exposure in air, but for low level of exposure, less than 2 ppm toluene, urinary Benzylmercapturic acid was known as good markers for biomonitoring [12]. The excretion of HA in urine following exposure to toluene vapors was shown to increase two hours after exposure and return to normal after 18 hours [13]. Linear correlation between urinary HA and toluene concentration has been reported in high level of toluene exposure [14]. Another author has expressed that urinary benzylmercapturic acid was superior biomarker to HA and o-Cresol for low level exposure toluene [15].

The aim of this study was to evaluate of the occupational exposure to toluene in Iranian Petroleum Depot workers and investigate of urinary HA as biomarker of occupational exposure to toluene.

## MATERIALS AND METHODS

### Subjects

The main routes of exposure in occupational groups have been observed inhalation and skin contact. Because of loading method (top loading), operators are

exposed to condensed vapors during loading operation, especially at the moment of opening the door of tanker and measuring of fuel level by dipstick, and also in while testing of some fuel properties such as flash point (in quality control group) and as well as by possible and accidental spillage in during of loading operation. SEGs guideline was used for selecting correct number of samples in each group for air and biological monitoring [16]. This study was conducted in Ray City Petroleum Depot Workers in 2011. The company is based in Tehran, Iran. Tehran Oil Refining Co. operates as a subsidiary of National Iranian Oil Refining And Distribution Company. All participants were male, nonsmoker and their consent for participating in this study was received. They also completed a questionnaire about their age, education, experience at work, passive and active smoking, having special dietary, using any prescription drugs and secondary job for possible exposure to toluene. Nonsmoking subjects were chosen based on including criteria that they had no exposure to toluene from other sources. Personal air and biological monitoring were conducted on for 77 subjects consisted of 45 exposed (operational workers) and 32 controls (staff). Exposed groups were divided in nine SEGs by activity and exposure with special petroleum product [16]. In general each operator loads 12 tankers per day which the loading time varies by tank capacity, number of tankers that are loaded simultaneously and average loading time was estimated to take 20 min for each tanker. All workers were shift work.

### Air sampling and analysis

Air samplings and analysis were conducted according to NIOSH method No. 1501. Nine SEGs were exposed to gasoline and other petroleum products including: unleaded gasoline, jet fuel and Compressed Natural Gas (CNG), gas oil and heavy oil, rail road fuel (consisted of two products; heavy oil and gas oil). Loading operations were implemented as top loading by standing in loading rack and filling products with nozzle arms through an opening compartment in the top of tanker. Quality control operators conducted some tests for determining type of fuel, quality and properties such as distillation, flash point of fuels and so on. Other operators had supervision duties. Air temperature and pressure were measured in the days of air sampling by thermometer and digital monometer.

Personal monitoring of exposed and control groups were conducted for 4-5 hours using charcoal tube (SKC No. 226-02) by standard personal sampler pump (SKC Co.) and flow rate of 100 L/min. Extraction of toluene from samples and blanks were done by pure CS<sub>2</sub> (99.99% purity from Merck Co). Prepared samples were analyzed with gas chromatography equipped with flame ionization detector (model GC-17A, Shimadzu) equipped with 30 m BP1 capillary column (100% dimethyl polysiloxene purchased from SGE Company). Thermal setting of GC was programmed to start from

**Table 1.** Personal monitoring results for exposure to toluene

Group	No. of samples	Min (ppm)	Max (ppm)	Median (ppm)	Mean (ppm)	Std. Error (ppm)	Geom. Mean (ppm)	Geom. Std. Dev (ppm)
Gasoline	5	0.223	15.315	1.36	4.44	2.79	1.870	4.80
Petroleum Products	5	0.023	0.200	0.043	0.095	.037	0.068	2.58
Quality Control	5	0.042	4.453	0.205	1.000	0.86	0.243	5.82
Gas Oil	7	0.012	0.382	0.043	0.099	0.05	0.059	2.91
Heavy Oil	5	0.001	0.292	0.045	0.089	0.05	0.029	9.65
Rail Road	5	0.034	5.359	0.043	1.11	1.06	0.114	8.69
Seal	6	0.039	0.116	0.043	0.060	0.13	0.055	1.57
Motor	3	0.007	0.043	0.043	0.031	0.01	0.024	2.77
Safety Supervisors	4	0.028	0.043	0.043	0.039	0.00	0.038	1.23
Staff (control group)	32	0.019	0.294	0.043	0.049	0.01	0.044	1.48

45 °C for 4 min and increasing to 105 °C with gradient temperature of 50 °C/min and fixed temperature of 105 °C for 4 min. The flow of column was set to 1.5 ml/min and the temperature of FID was set at 200 °C.

#### Urine sampling and analysis

Urine samples were collected simultaneous with air sampling for all individuals. All urine samples were collected after passing two days of shift work, and each subject provided two voids urine samples for pre and end of shift while personal monitoring were conducted in breathing zone. Urine samples were analyzed by the NIOSH method No. 8300 by HPLC. Urine samples were collected from subjects in 50ml polyethylene bottles and they were frozen and kept for few days at -20 °C until analysis. Urine samples were divided in two parts for determining HA and creatinine separately. In the initial stage 10ml of urine samples were centrifuged in 5000 RPM for 10 min, then 5ml of its was poured to another bottle and 20 ml ethyl acetate plus 1.5 g sodium chloride and plus 200 µl hydrochloric acid were added, samples were subsequently centrifuged for 10 min at 5000 RPM. Then 0.5 ml of top organic layer samples transferred to in polyethylene bottle, samples were dried by using gentle stream of pure nitrogen gas. Dried samples were mixed with 10 ml of mobile phase (250 µl acetic acid+ 840 ml distilled water+160 ml acetonitrile) and the concentration of HA were determined by HPLC equipped with UV detector at 254 nm.

For analysis of urinary creatinine, 5ml of urine samples were taken in polyethylene bottles and centrifuged two times at 5000 RPM for 10 min in separate bottles at and subsequently 10 µl of cleaned samples were taken and mixed with 990 µl of mobile phase. 20 µl of prepared samples were injected to

HPLC-UV with flow rate 2 L/min and were detected at 240 nm [17].

#### Statistical analysis

Statistical analysis was conducted by AIHA tools (EASC-IHSTAT-V229.xls) and SPSS program (version 16.0). The censored data were adjusted by substitution of  $LOD/\sqrt{2}$  [18]. Limit of detection was 0.06 ppm for toluene. Normal distributions of variables were checked by Kolmogorov-Smirnov test. Whereas the difference between two groups (exposed and control group) in age was significant at Alfa=0.05, for adjusting its effect on urinary HA content, we used analysis of covariance. Pearson's test was used for surveying correlation and urine pre and post shift changing in exposed groups were analyzed by Paired *t*- test. Statistical significant differences were considered on basis of *p*-value<0.05.

#### RESULTS

The mean of ages in the exposed and control group were 31±7.6 and 42.5±7.6 year, respectively that there was significant difference between them in regard of age (*p*<0.001). Work experience was 7±7.6 and 16.5±12 year, for exposed subjects and controls, respectively. Weather conditions include of air temperature and pressure were measured 13.5±7°C and 899±6.8mmHg as Mean±SD, respectively. Body Mass Index (BMI) was estimated 25.5±3.7 and 24.7±3 for exposed subjects and controls, respectively.

The mean of exposure to toluene was determined to be 0.78±0.37 and 0.05±0.01 ppm (Mean±SE) for exposed and control groups, respectively. Air samples results for nine operation groups and also control group has been shown in Table 1. There was significant difference between exposed and control group for toluene exposure (*p*<0.05).

Table 2. Descriptive Statistic for urinary HA samples

Group	No. of samples	Creatinine								Hippuric acid						<i>p</i> -value*
		Pre shift		Post shift		Pre shift <sup>a</sup>		Post shift <sup>a</sup>		Pre shift <sup>b</sup>		Post shift <sup>b</sup>				
		(g/l)	(g/l)	(g/l)	(g/l)	(g/l)	(g/l)	(g/g creatinine)	(g/g creatinine)	(g/g creatinine)	(g/g creatinine)					
		Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD			
Gasoline	5	0.04	0.04	0.04	0.04	0.02	0.01	0.02	0.01	0.63	0.33	2.97	5.84	0.411		
Petroleum Products	5	0.05	0.04	0.05	0.05	0.01	0.00	0.04	0.04	0.50	0.29	1.46	0.87	0.61		
Quality Control	5	0.02	0.01	0.02	0.01	0.01	0.01	0.04	0.04	0.92	0.59	2.06	1.06	0.62		
Gas Oil	7	0.02	0.01	0.05	0.05	0.02	0.00	0.02	0.01	1.08	0.80	1.75	1.02	0.109		
Heavy Oil	5	0.09	0.07	0.03	0.04	0.02	0.01	0.02	0.02	1.39	0.52	1.42	0.99	0.963		
Rail Road	5	0.04	0.02	0.03	0.02	0.01	0.00	0.01	0.00	0.41	0.33	0.59	0.32	0.326		
Seal	6	0.03	0.02	0.02	0.02	0.01	0.00	0.01	0.00	0.61	0.36	0.85	0.43	0.206		
Motor	3	0.04	0.00	0.05	0.04	0.02	0.01	0.01	0.00	0.51	0.19	0.35	0.14	0.481		
Safety Supervisors	4	0.02	0.02	0.02	0.00	0.01	0.00	0.02	0.02	0.89	0.68	1.37	0.84	0.049		
Staff (control group)	32	0.02	0.02	0.01	0.01	0.02	0.01	0.01	0.00	1.20	0.80	1.49	1.22	0.248		

HA: Hippuric Acid

<sup>a</sup> Observed values<sup>b</sup> Corrected values*p*-value\*: differences between pre and post shift urinary Hippuric Acid corrected by creatinine (Paired *t*-test)

The Mean of pre shift of urinary HA were measured  $0.79 \pm 0.56$  and  $1.20 \pm 0.8$  g/g creatinine in exposed and control group, also mean content of urinary HA for post shift were measured  $1.47 \pm 2.03$  and  $1.49 \pm 1.22$  g/g creatinine in two groups as Mean $\pm$ SD, respectively. The ANCOVA for adjusting of age of urinary HA in pre shift samples was shown significant difference between exposed and control group ( $P$ -value=0.02), but the result of ANCOVA for adjusting of age and pre shift effect of urinary on content of urinary HA in post shift samples wasn't shown any significant difference between exposed and control group. Mean content level of HA in gasoline operators was 2.97 g/g creatinine and also airborne toluene concentration was assessed higher compared with other operational groups ( $4.44 \pm 2.79$  ppm). Paired *t*-test did not revealed any significantly difference for urinary HA in pre and post shift urine samples in operational groups, exception in safety supervisor but Pearson's test did not show any significant relation between air samples and post shift of HA metabolite in all operational groups (Table 2).

## DISCUSSION

The mean concentrations of toluene in breathing zone were below the Occupational Exposure Limit (TLV=20 ppm) for all of operational groups [19]. Level of toluene exposure in gasoline loading operators was estimated higher than other products loading operators and also higher than exposure of workers in gasoline terminals of India [6]. The low estimated exposure concentration to toluene may be due to decrease of customers demand in Iran and decreasing in the loading numbers.

Among of all studied groups, gasoline operators showed urinary HA higher than BEI (1.6 g/g creatinine) [19] but there was not found any significant relation between airborne of toluene concentration and level of urinary HA in this group, this results might be due to limited numbers samples in this surveyed group or low level of toluene exposure ( $0.78 \pm 0.37$  ppm as Mean $\pm$ SE) may justify non-significant relationship between level of urinary HA and airborne toluene concentration in operational groups. Results of this study can confirm the results of Duydu et al. study that reported no significant correlation in low level of exposure for HA [20] and also some recent articles suggesting benzylmercapturic acid and blood toluene measurements as proper means of biological monitoring of the low levels toluene exposure [12, 15, 21]. Bahrami et al. also stated that significant correlation of toluene exposure and urinary hippuric acid were seen just for exposures higher than 35ppm [22].

The Mean of urinary HA (post shift) in control group was  $1.49 \pm 1.22$  g/g creatinine in this study which was higher than their Japanese counterpart without active exposure to organic solvents (0.23 g/g creatinine) [23] and also higher than Brazilian people which were reported to have level of urinary HA up to  $0.18 \pm 0.01$  g/g creatinine. They also reported that age and sex decrease urinary creatinine due to decreasing the muscles mass and glomerular filtration rate with increasing age [24]. This study also confirmed negative correlation between age and urine creatinine content ( $p$ -value=0.013), and this phenomena could justify higher ratio of urinary HA per creatinine in this study.

## CONCLUSION

Exposure of top loading operators was very low compared to other similar studies and below respective TLV and in such cases biological monitoring should focus on alternative biomarkers rather than urinary HA.

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