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Medicolegal Aspects of Blood-Urine Toluene and Urinary Ortho-Cresol Concentrations in Toluene Exposure

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Abstract: Toluene is a widely used solvent in different industrial areas and has a depressant effect on the central nervous system. In medicolegal cases, for the possible influence of toluene on actions or conditions prior to death, a reliable indicator of exposure must be precisely investigated. We developed an appropriate method for toluene analysis and investigate the blood-urine toluene levels and urinary ortho-cresol levels in toluene-exposed workers. Blood and urine toluene levels from 50 male subjects were detected by Gas Chromatography-FID (GC-FID) using the head-space method, and urinary o-cresol levels, a metabolite of toluene, were analyzed by GC. The significance of difference of o-cresol/creatinine levels was evaluated by the nonparametric statistics Kruskal-Wallis One

Way Anova, and correlation and regression statistics were determined by Pearson Correlation of bivariate analysis. The toluene concentrations in blood ranged from 0.07 to 2.12 mg/g. Urinary o-cresol levels were not correlated significantly with blood or urine toluene levels. There was no significant difference for ortho-cresol/creatinine levels between the control and worker groups. It is concluded that for medicolegal purposes, urine toluene and urinary o-cresol levels are not reliable markers, and direct toluene analysis in blood must be preferred in toluene exposure.

Key Words: forensic toxicology, toluene, toluene exposure, toluene abuse, ortho-cresol

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Introduction

Toluene (methyl benzene) is a widely used organic solvent in the printing, painting, automotive, shoemaking and pharmaceutical industries. It is also the common substance in glue and thinner sniffed by drug abusers (1-3).

Toluene is a central nervous system (CNS) depressant. The acute effects of toluene are similar to those of alcohol intoxication (2,4,5). Acute poisoning includes initial excitement or euphoria followed by CNS depression with headaches, dizziness, blurred vision, and ataxia. In severe cases, coma, convulsion and death from respiratory failure occur. Chronic poisoning has resulted mainly in nervous system disorders, and renal and hepatic damage (6-8). Toluene can be absorbed into the blood flow from the lungs and the gastrointestinal tract and through the skin and mucosa. Fat-rich tissues such as brain and liver serve as the most extensive reservoir for toluene (2). Occupational exposure to toluene mainly occurs as fume inhalation when the compound is used as a solvent (9).

More than 80% of absorbed toluene is metabolized by mix-oxidase enzyme systems to benzoic and hippuric

acid before excretion into the urine. Some absorbed toluene (0.4 - 1.1%) is hydroxylated and excreted as a mixture of ortho-, para- and meta-cresol (9,10).

Among the metabolites, hippuric acid is a traditional biomarker in the biological monitoring of occupational exposure to toluene (11,12) but it is known that p-cresol and hippuric acid rates are obscured by endogenous and dietary sources (10). Nise also found an association between alcohol consumption and hippuric acid excretion in toluene-exposed printers. Recently it is supposed that o-cresol is the least influenced by background contribution (10,13,14).

Cases in which toluene has forensic relevance can be divided into three groups: traffic violations or accidents where the driver's impairment was caused by toluene inhalation, crimes (usually violent) where toluene may be related to the behaviour of the individual or individuals involved, and cases in which the presence of toluene in blood or tissue has some relationship to the cause of death or the general health of the person (15).

Driving and working under the influence of toluene must be evaluated regarding the possibility of driving

impairment/workplace accidents in legal cases. A case of accidental death due to falling from a height with acute sub-lethal toluene poisoning during painting was reported (4,16).

Recently toluene abuse has become a social problem in Turkey and analysis of this substance is not performed effectively yet. This study was undertaken to develop an appropriate method for toluene analysis and to determine a reliable indicator(s) in toluene exposure for medicolegal purposes. Blood-urine toluene levels were analyzed by a Gas Chromatography-Flame Ionization Detector (GC-FID) with head-space sampling, and urinary ortho-cresol levels were analyzed by GC in toluene-exposed subjects.

Materials and Methods

Subjects

In this study, 50 male subjects, 41 workers exposed to toluene from three different industrial areas and 9 healthy male subjects for control, were selected. Group 1: 15 male workers from the painting sector of the automotive industry (age 20-60 years, weight 56-115 kg and working period 5-35 years), Group 2: 10 male workers who repair car hoods (age 22-49 years, weight 64-85 kg and working period 3-27 years), Group 3: 16 male workers from the shoemaking industry (age 12-48 years, weight 45-70 kg and working period 2-39 years). Blood and urine sampling time of workers was at the end of the eight hour workshift on the sixth day of a working week.

Urine samples could not be obtained from 2 and 1 workers in groups 1 and 3 respectively. A blood sample could not be obtained from 1 subject in group 2.

The control group consisted of 9 healthy nonexposed male subjects (age 23-27 years, weight 70-85 kg).

Biological specimens

Blood samples were extracted from the cubital vein of the workers into 9 mL vacutainer tubes containing sodium citrate (Merck) as the anticoagulant. After pouring 5 mL of blood into a 10 mL glass head space vial (with Teflon septa and aluminum cap-HP), the vial was capped immediately and kept frozen at -20°C until analysis (15).

Urine samples from each worker were collected in 20 mL screw-capped bottles containing 60 mg sodium

fluoride (Riedel) as the preservative. After pouring 5 mL of urine into a 10 mL glass head space vial, the vial was capped immediately and kept frozen at -20°C until analysis. Urine samples left in the bottle were kept for urinary o-cresol analysis under the same storage conditions (10,17).

Toluene analysis

Toluene standards were prepared in 25 mL screw-capped glass tubes using human blood obtained from a blood bank. Calculated volumes of toluene (Merck, HPLC Grade) were added to known volumes of ice-cold blood using a Hamilton microsyringe (HP). We started with the highest concentration quantity of toluene (50 $\mu\text{g/g}$) and, by dilution with blood each time, the lowest concentration was reached (0.780 $\mu\text{g/g}$). Only a small volume of air (< 5%) was allowed above the blood. The tubes were sonicated (Ultrasonic LC 30) at 4°C for 1 h. Blood was poured into 10 mL vials and capped immediately. Two vials for each concentration were prepared and stored at -20°C . Seven standard concentrations were used: 0.780, 1.560, 3.125, 6.250, 12.5, 25 and 50 $\mu\text{g/g}$. For each concentration, five injections were performed.

Instruments: Hewlett Packard HP 6890 GC system equipped with a flame ionization detector (FID). The column used was Supelcowax 10, 2-5301 (30.0 m x 530.00 μm i.d., film thickness 1.00 μm). The carrier gas was helium, 5.4 mL/min, column temperature was 80°C , detector temperature was 250°C , inlet temperature was 80°C and split ratio was 3:1.

Biological samples kept at -20°C were left to thaw at room temperature over 20 min. Then the samples were heated at 50°C for 20 min and 0.5 mL of head space air was injected into a gas chromatograph with a warmed 5 mL glass gas syringe (Supelco SGE Syringe, 5 MAX-HSV, 2-3984) (17). For each individual, duplicate analysis was performed.

O-cresol analysis

O-cresol (Sigma) standards, 0.625, 1.250, 2.5 and 5 $\mu\text{g/mL}$ prepared in carbon disulfide (Sigma, HPLC Grade), were spiked with 25 mg/L 3,5-xyleneol (Merck) as an internal standard. For each concentration, triplicate injections were performed.

Instruments: GC-FID, column used was Supelcowax 10. The carrier gas was helium, 6.2 mL/min, column temperature was programmed as 2 min hold at 100°C ,

8°C/ min from 100-200°C, 5 min hold at 200°C. Detector temperature was 250°C, inlet temperature was 210°C and the split ratio was 50:1.

After warming the urine samples to room temperature, 0.5 mL 15% HCl (Merck) was added to 1 mL of urine and the mixture was heated at 100°C in a water bath (Kottermann) for 1 h. After cooling the urine samples to room temperature under tap water, 0.1 mL of 3,5-xylenol (25 mg/L water) was added as an internal standard and the resulting solution was mixed by shaking for 10 min with 2 mL of carbon disulfide, and centrifuged at 2000 rpm for 3 min. The organic phase of the extract was treated with 0.5 g of anhydrous sodium sulfate (Merck) and centrifuged at 2000 rpm for 10 min again. The carbon disulfide solution was evaporated to 100 µL at 30°C under a stream of nitrogen gas in an evaporator and 1 µL was injected into GC (10). Ortho-cresol concentrations were corrected for urinary flow by dividing by the urinary creatinine concentrations.

Ethics

The investigation conformed to the principles outlined in the Declaration of Helsinki.

Statistical Analysis

The significance of difference of o-cresol/creatinine levels among the groups was evaluated by the nonparametric statistics Kruskal-Wallis One-way Anova of SPSS (Statistical Packages for Social Sciences). Correlation and regression statistics were determined between blood, urine toluene and o-cresol/creatinine levels by Pearson Correlation of bivariate analysis.

Results

Under the GC conditions described, the retention times (RT) of toluene, o-cresol and 3,5-xylenol were 3.45, 13.59 and 15.78 min respectively. The recovery of o-cresol ranged from 84 to 96%. The toluene calibration curve was linear up to 6.250 µg/g concentrations, with a correlation coefficient of 0.998. The detection limit for toluene was 0.02 µg/g. The coefficient of variation (CV) of the method for the multiple runs on the same day was 3.8%. For o-cresol, a linear calibration curve was obtained with a correlation coefficient of 0.997. The detection limit of o-cresol was 0.01 µg/mL.

The minimum and maximum levels of blood toluene, urine toluene and urinary o-cresol/creatinine levels for three worker groups and the control group are presented in the Table. In all the study groups toluene was detected in the blood. The toluene concentrations in blood ranged from 0.07 to 2.12 µg/g. On the other hand, no toluene was detected in the control blood and urine samples. Toluene was not detected in the fourth and seventh urine samples of groups I and II respectively.

No correlation was found between blood toluene and urine toluene levels in workers exposed to toluene. O-cresol was detected in all urine samples (except one) of the control group and the levels of o-cresol/creatinine were not significantly different from those of the exposed subjects. In addition, no correlation was found between o-cresol/creatinine and blood/urine toluene levels.

Group	Blood toluene* min-max	Urine toluene* min-max	O-cresol/creatinine• min-max
I (n=15)	0.09-2.12 n=15	0.02-0.14 n=13	0.17-0.97 n=13
II (n=10)	0.09-0.31 n=9	0.02-0.09 n=10	0.04-0.89 n=10
III (n=16)	0.07-0.30 n=16	0.03-0.23 n=14	0.01-0.72 n=14
Control (n=9)	ND	ND	0.03-0.76 n=8

Table
Blood toluene, urine toluene and urinary ortho-cresol/creatinine concentrations of three worker groups and the control group.

*: µg/g

•: mg/g

ND: Not Detected

Discussion

In our study, blood toluene concentrations ranged from 0.07 to 2.12 µg/g. This concentration range was consistent with the range given by Miyazaki et al. (17), in which mild intoxication findings begin, and so this range might possess risk. For medicolegal purposes, it is very important to develop a reliable toluene exposure indicator in individuals who are exposed to toluene, regarding the occurrence of traffic violations or accidents, workplace accidents, solvent abuse and death (5,15). The presence of toluene in all workers and the possibility of it becoming part of legal cases at any moment must be taken into consideration. Blood specimen collection for toluene analysis is another important legal fact that must be taken into account, because losses of toluene in blood can be caused by handling and storage conditions, and sample preparation and sampling (15).

In two subjects, urine toluene was not detected and for all the subjects there was no correlation between blood and urine toluene concentrations. Therefore the results of our study showed that urine toluene levels cannot be used as a reliable indicator in toluene exposure. However, Kawai et al. (18) reported that urine toluene is a better marker of exposure than urinary metabolites.

Some studies suggest that the determination of urinary o-cresol excretion represents a diagnostically specific and sensitive parameter for the estimation of an individual toluene uptake (10,19,20). However, in the present study we found no correlation between

blood/urine toluene levels and urinary o-cresol/creatinine levels. Furthermore, the results of our study showed that the o-cresol/creatinine levels of the control group were not significantly different from those of the workers. Inoue et al. (21) have pointed out that urinary o-cresol levels were significantly reduced by smoking, drinking and the two habits combined and they cannot be considered a reliable indicator of exposure to toluene.

We concluded that, in medicolegal cases, toluene analysis should be performed effectively and direct toluene analysis in blood must be preferred to urine toluene and urinary o-cresol analysis.

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