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Determination of urinary hippuric acid and *o*-cresol levels as biological indicators of toluene exposure in shoe-workers and glue sniffers

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In this study, groups exposed to toluene either intentionally (glue sniffers) or unintentionally (shoe-workers) were compared. The groups were evaluated in terms of urinary levels of the toluene metabolites hippuric acid and *o*-cresol. Results were also compared with control values. Hippuric acid levels were determined by high performance liquid chromatography and *o*-cresol levels by gas liquid chromatography. The levels of hippuric acid and *o*-cresol were found to be statistically significantly higher in glue sniffers than in shoe-workers ($p < 0.001$) or controls ($p < 0.001$). In addition, the differences between the levels of urinary hippuric acid and *o*-cresol in the shoe-workers and in the controls were statistically significant ($p < 0.05$ and $p < 0.001$, respectively). These results suggest that extremely high levels of urinary hippuric acid and *o*-cresol indicate massive exposure to toluene.

Keywords: toluene, hippuric acid, *o*-cresol, glue sniffing, urine.

Introduction

Most of the data on the human health effects associated with exposure to toluene are derived from case studies of solvent abusers or studies of occupationally exposed workers. Organic solvent abuse is a significant social problem, especially among adolescents (Watson 1984). These solvents are found in glue, adhesives, paint thinners and cleaning fluids. Among these solvents, toluene is reported to be frequently abused (Watson 1982). Many of the commercial glue products used by glue sniffers contain toluene (Jansen *et al*. 1992). Occupational exposure to toluene is also frequent among workers in shoe factories, since toluene is one of the most popular solvents in adhesives used for shoes, sandals or bags (Inoue *et al*. 1986, Murata *et al*. 1994).

Cytochrome P450 enzymes are involved in several steps of toluene biotransformation (Löf *et al*. 1990). By P450-mediated side-chain oxidation, toluene is metabolized into benzoic acid, which is further conjugated with glycine to hippuric acid. Hippuric acid is the main metabolite of toluene (about 80%). Small amounts of absorbed toluene (about 1%) are oxidized at the aromatic ring and excreted into the urine as *o*- and *p*-cresol, and about 20% of the absorbed toluene is excreted unchanged into the expired air. The first study of toluene exposure was reported in 1962 (Ogata *et al*. 1962). Since then, workers exposed to toluene have been studied by several researchers. Among the metabolites excreted in the urine, hippuric acid

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has attracted considerable attention as an indicator of exposure to toluene. However, urinary hippuric acid not only reflects toluene exposure, but is also affected by food habits such as the consumption of benzoic acid-containing soft drinks, alcohol consumption, cigarette smoking, some environmental contaminants and drugs (including paracetamol and acetylsalicylic acid) (Nakajima *et al*. 1993). As the level of hippuric acid excreted in the urine is affected by these compounds, *o*-cresol has been suggested used as a biomarker, especially in the determination of a low level of exposure to toluene. However, urinary *o*-cresol level is reduced by smoking and drinking (Inoue *et al*. 1994), particularly at low levels of toluene.

Various studies have attempted to whether *o*-cresol or hippuric acid is a better indicator of exposure to toluene in the urine. Pierce *et al*. (2002) suggested that the urinary hippuric acid/*o*-cresol ratio is a useful indicator of the time since the cessation of exposure when background metabolite levels are known. The purpose of the present study was to determine and compare the levels of urinary hippuric acid and *o*-cresol in intentionally toluene-exposed glue sniffers and unintentionally toluene-exposed workers in shoe manufacturing, in order to assess their role as biomarkers of exposure to toluene.

Materials and methods

Subjects studied

A group of 20 male glue sniffers (aged 9–20 years) participated in the study voluntarily; 11 of them were smokers. Urine samples were taken a few hours after an episode of glue sniffing. The second group consisted of 54 male workers (aged 13–62 years) from two shoe manufacturing areas who were exposed to toluene at work; 41 of them were also smokers. They worked an 8 h double shift, and used glue throughout their work period. The workers did not wear protective gloves for the prevention of possible skin penetration of toluene and did not wear masks. Spot urine samples were collected before the shift, at midday, and 2 h later at the end of the shift (7 p.m.) on a Monday. The control group (non-exposed subjects) comprised 54 men with a mean ($\pm$SD) age of 27.6 ± 10.4 years (range 14–62 years); 25 of them were smokers. Each person completed a questionnaire to provide personal information such as age, dietary habits, duration of glue sniffing, the number of cigarettes smoked per day, and the number of alcohol units consumed per week. Urine samples from controls were collected in the morning before a meal. All urine samples were collected in small glass bottles, cooled to 4°C and stored at −20°C until analysis.

Urine analysis

Urinary hippuric acid was analysed using the method of Löf *et al*. (1990), with 2-methylhippuric acid as the internal standard (Ogata *et al*. 1978). High performance liquid chromatography using a Hewlett-Packard Model 1050 equipped with a Li Chrospher 100 RP-100 (5 μm) prepacked column with an ultraviolet detector (240 nm) was used for the analysis.

Urinary *o*-cresol was analysed using the method of Nise (1992), with 3,5-dimethylphenol as the internal standard. Gas–liquid chromatography using a Hewlett-Packard Model 5890 II equipped with a Hewlett-Packard Ultra 1 capillary column and a flame ionization detector was used for the analysis.

All results were corrected for the creatinine content of the samples.

Statistical analysis

A p value < 0.05 was considered to be statistically significant. Statistical computations were performed using SPSS version 10.0 (SPSS Inc., Chicago, Illinois, USA). Firstly a two-way multivariate analysis of variance was performed. The Scheffe multiple comparison test was used to assess statistically significant differences between group means. The effect of age, smoking and alcohol consumption status on *o*-cresol and hippuric acid levels were analysed by multiple regression analysis. As smoking status and alcohol consumption status are classified variables, these two variables were coded as dummy variables.

Results

The general characteristics of the study population are summarized in Table 1. Table 2 presents the levels of urinary hippuric acid and *o*-cresol in glue sniffers, shoe-workers and control subjects.

A significant difference was found between urinary hippuric acid and *o*-cresol levels in the control group and in glue sniffers ($p < 0.001$). A statistically significant difference was also found between shoe-workers and glue sniffers ($p < 0.001$). In addition, the differences between the levels of urinary hippuric acid and *o*-cresol in the shoe-workers and in the controls were statistically significant ($p < 0.05$ and $p < 0.001$, respectively).

In order to determine toluene exposure throughout the day, urine samples were taken from the shoe-workers three times on a Monday: in the morning, at midday, and 2 h after their shift. The mean values of hippuric acid and *o*-cresol in the urine samples collected at various times are shown in Table 3. The average concentration of urinary hippuric acid and *o*-cresol increased markedly and significantly at the end of the work-shift ($p < 0.001$).

There were no statistically significant differences in urinary hippuric acid or *o*-cresol levels between toluene-exposed subjects classified according to their smoking

Table 1. General characteristics of study population

	Glue sniffers ($n = 20$)	Shoe-workers ($n = 54$)	Controls ($n = 54$)
Age (years)			
Mean ± SD	16.20 ± 2.75	31.22 ± 10.75	27.6 ± 10.4
Range	9–20	13–62	14–62
Duration of exposure (years)			
Range	0.5–3	0.5–31	–
Smoking habits (%)			
Non-smoker	45	24.1	53.7
Smoker	55	75.9	46.3
Alcohol intake (%)			
Yes	55	25.9	66.67
No	45	74.1	33.33

(values are mean ± SD).

Table 2. Mean (±SD) urinary hippuric acid and *o*-cresol levels in controls, shoe-workers and glue sniffers

	n	Hippuric acid (g g^{-1} creatinine)	*o*-Cresol (mg g^{-1} creatinine)
Controls	54	$0.16 \pm 0.15^{a,b}$	$0.012 \pm 0.01^{b,c}$
Shoe-workers	54	0.39 ± 0.30^{b}	0.084 ± 0.045^{b}
Glue sniffers	20	11.66 ± 9.5	18.99 ± 20.77

[a] $p < 0.05$ compared with corresponding shoe-workers.
[b] $p < 0.001$ compared with corresponding glue sniffers.
[c] $p < 0.001$ compared with corresponding shoe-workers.

Table 3. Mean (±SD) urinary hippuric acid and *o*-cresol levels at different times of collection from 54 shoe-workers

	Hippuric acid (g/g creatinine)	*o*-Cresol (mg/g creatinine)
Preshift	0.13±0.14[a]	0.012±0.023[a]
Midday	0.17±0.17	0.024±0.029
Postshift	0.39±0.28	0.084±0.045

[a] $p < 0.001$ compared with corresponding postshift values.

and alcohol consumption status (Tables 4 and 5). Glue sniffers were classified according to the duration of toluene abuse into four groups: <1 year ($n=4$), $2-<2$ years ($n=6$), $3-<3$ years ($n=6$), and >5 years ($n=4$). No significant correlation was found between the duration of abuse and hippuric acid and *o*-cresol levels ($p > 0.05$).

There were no statistically significant correlations between age and urinary hippuric acid ($R^2 = 0.013$, $p = 0.435$) or *o*-cresol ($R^2 = 0.108$, $p = 0.240$) levels in glue sniffers. Similar results were also obtained for shoe-workers (hippuric acid: $R^2 = 0.017$, $p = 0.344$; *o*-cresol: $R^2 = 0.001$, $p = 0.864$) and controls (hippuric acid: $R^2 = 0.000$, $p = 0.927$; *o*-cresol: $R^2 = 0.058$, $p = 0.084$).

The effect of alcohol intake on hippuric acid and *o*-cresol levels was also analysed. Hippuric acid excretion did not differ between drinkers and non-drinkers in controls ($R^2 = 0.036$, $p = 0.267$), shoe-workers ($R^2 = 0.272$, $p = 0.056$) or glue sniffers ($R^2 = 0.001$, $p = 0.924$). Similarly, *o*-cresol excretion was not different in drinkers and non-drinkers in controls ($R^2 = 0.000$, $p = 0.960$), workers ($R^2 = 0.004$, $p = 0.873$) or glue sniffers ($R^2 = 0.127$, $p = 0.281$) (Table 5).

Discussion

Volatile solvent abusers prefer glues because they are cheap, easy to obtain and there are no legal difficulties in their marketing. Toluene abuse in glue is a more common phenomenon than abuse of other volatile solvents (Watson 1982). In our study, the group of shoe-workers were unintentionally exposed to toluene in their working area, whereas the glue sniffing group abused toluene intentionally. Workers in shoe manufacturing are usually exposed to a complex mixture of solvents originating from glues and their diluents (Scarpelli *et al*. 1993, Burgaz *et al*. 1997). A combination of hippuric acid and *o*-cresol values could possibly be used to indicate the magnitude of toluene exposure. Therefore both hippuric acid and *o*-cresol levels were determined in this study. There are few reports (Kira *et al*. 1989, Mevlenbelt *et al*. 1990, Yamazaki *et al*. 1992) concerned with monitoring cresols in urine samples collected from glue sniffers.

Although the present study did not attempt to determine the toluene concentrations in the plastic or paper bag from which the glue was sniffed, it is clear that during glue sniffing excessive levels of toluene are inhaled over a short time and that these levels are well above the occupational limits (Meadows and Verghese 1996, Steffee *et al*. 1996). The concentration of toluene inhaled under conditions of abuse can approach 30 000 p.p.m., which is the saturation

Table 4. Effect of smoking status of subjects on mean (±SD) hippuric acid and *o*-cresol levels

	Controls			Shoe-workers			Glue sniffers		
	n	Hippuric acid (g g^{-1} creatinine)	*o*-Cresol (mg g^{-1} creatinine)	*n*	Hippuric acid (g g^{-1} creatinine)	*o*-Cresol (mg g^{-1} creatinine)	*n*	Hippuric acid (g g^{-1} creatinine)	*o*-Cresol (mg g^{-1} creatinine)
Smokers	25	0.20±0.16	0.010±0.009	41	0.39±0.30	0.087±0.085	11	11.09±7.40	19.40±20.54
Non-smokers	29	0.13±0.12	0.013±0.013	13	0.39±0.32	0.078±0.088	9	12.34±12.03	18.49±22.28

Table 5. Effect of drinking status of subjects on mean (±SD) hippuric acid and *o*-cresol levels

	Controls			Shoe-workers			Glue sniffers		
	n	Hippuric acid (g g^{-1} creatinine)	*o*-Cresol (mg g^{-1} creatinine)	*n*	Hippuric acid (g g^{-1} creatinine)	*o*-Cresol (mg g^{-1} creatinine)	*n*	Hippuric acid (g g^{-1} creatinine)	*o*-Cresol (mg g^{-1} creatinine)
Drinkers	36	0.15±0.17	0.012±0.014	14	0.38±0.36	0.082±0.087	11	13.80±5.40	19.52±12.98
Non-drinkers	18	0.13±0.14	0.014±0.015	40	0.39±0.42	0.081±0.086	9	9.00±12.80	18.33±21.41

concentration at 20°C (US Environmental Protection Agency 1983). Yamazaki and coworkers (1992) reported that there is a correlation between urinary hippuric acid ($p < 0.05$) or *o*-cresol ($p < 0.01$) and expired air toluene concentrations in glue sniffers. In another study, Devathasan *et al*. (1984) reported toluene blood concentrations of glue sniffers of up to 2%. Abusers with mild signs of intoxication were reported to have blood levels of 1–2.5 μg g^{-1} (Raikhlin-Eisenkraft *et al*. 2001), but it is difficult to correlate blood toluene levels with signs and symptoms in solvent abusers. Coma or death was associated with a blood toluene level >10 μg g^{-1} (Miyazaki *et al*. 1990), and in fatal cases the blood toluene concentration ranged from 10–119 μg g^{-1} (Carlisle *et al*. 1991).

Some studies have reported the effects of alcohol consumption and smoking on the excretion of hippuric acid and *o*-cresol in urine (Dossing *et al*. 1984, Inoue *et al*. 1993, 1994). Therefore both of these confounding factors were evaluated in the present study.

Of the various studies on the effect of smoking on toluene metabolism, some have reported a higher level of *o*-cresol excretion in smokers compared with non-smokers (Dossing *et al*. 1984), whereas others have reported a higher level of *o*-cresol excretion in non-smokers (Nise 1992). In the present study the *o*-cresol levels in glue sniffers and controls were highly different from each other (18.99 ± 20.77 mg g^{-1} creatinine for glue sniffers; 0.012 ± 0.01 mg g^{-1} creatinine for controls), the role of smoking in these differences were ignored. Nevertheless, no differences were observed between smokers and non-smokers in the glue sniffing group ($p > 0.05$). In addition, no differences were found between smokers and non-smokers in the shoe-workers group in terms of *o*-cresol and hippuric acid excretion ($p > 0.05$). In the light of these results, it appears that smoking has no effect on toluene metabolism in this study.

In addition to smoking, the effect of alcohol consumption on *o*-cresol levels was also investigated in this study. Dossing *et al*. (1984) found a reduction in the excretion of both hippuric acid and *o*-cresol when ethanol was consumed during experimental exposure, suggesting that alcohol inhibits both the side-chain (to hippuric acid) and ring (to *o*-cresol) oxidation of toluene. For this reason our subjects were divided in two groups – alcohol consumers and non-consumers – but no intergroup differences were obtained for hippuric acid or *o*-cresol ($p > 0.05$).

As seen in Table 2, the mean urinary hippuric acid and *o*-cresol concentrations of glue sniffers were 30-fold and 226-fold higher, respectively, than those observed in industrial workers, and 73-fold and 1582-fold higher, respectively, than control values. The differences in urinary hippuric acid and *o*-cresol concentrations between these three groups were statistically significant. Our results are much more higher than the values obtained in the study of Yamazaki *et al*. (1992), in which glue sniffers and industrial workers were compared. They reported hippuric acid and *o*-cresol values of 9.76 ± 6.80 g g^{-1} creatinine and 7.31 ± 4.94 mg g^{-1} creatinine, respectively, for glue sniffers, and 0.050 ± 0.51 g g^{-1} creatinine and 0.095 ± 0.168 mg g^{-1} creatinine, respectively, for industrial workers.

Many authors have discussed the use of urinary metabolites as indicators of toluene exposure in industrial workers. In the present study, urine samples were collected from shoe-workers at different times during the work shift, and it was

found that levels of *o*-cresol and hippuric acid increased with time of exposure. Because end-of-shift urinary hippuric acid and *o*-cresol measurements are used for the biological exposure index (BEI) established for workplace exposure by the American Conference of Governmental Industrial Hygienists (ACGIH 2000), in the present study the end-of-shift values of the toluene metabolites were used for comparison with the values obtained from glue sniffers.

Studies so far have not demonstrated any genotoxic or carcinogenic effects on humans from low levels of toluene exposure (McGregor 1994, Pitarque *et al*. 1999), so the significance of the present study's findings with regard to the risks associated with the levels of toluene found is controversial. Nevertheless, in a study using the Comet assay, we found significant damage in the peripheral blood lymphocytes of glue sniffers compared with controls ($p < 0.001$) (I. Cok, unpublished data).

The most rational way to reduce the risk of toluene exposure is the removal of toluene from products, together with the temporary restrictions on the sale of toluene-based chemicals until it has been substituted with non-solvent-based products. Future research should include evaluation of the genotoxic effects of high levels of toluene exposure and assessment of risk after exposure.

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