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SHORT COMMUNICATION
A note on urinary *trans,trans*-muconic acid level among Thai press workers

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Benzene is a common toxic volatile substance associated with many industrial processes. Benzene exposure is of particular concern because recent research indicates that it can result in chronic toxicity and thousands of workers in industrial plants experience ongoing exposure. Therefore, the determination and control of benzene exposure among at-risk workers is very important. Urinary *trans*,*trans*-muconic acid (ttMA) determination is a helpful test for monitoring groups of at-risk workers for exposure to benzene. In this study, 103 urine samples were obtained from 60 controls and 43 occupational exposed press workers in a press factory in Bangkok. All samples were analysed for ttMA using a previously reported method. The average urinary ttMA levels for the control and exposed groups were 0.08 ± 0.03 mg g^{-1} creatinine and 0.56 ± 0.65 mg g^{-1} creatinine, respectively. Significantly higher urinary ttMA levels were observed among the press workers ($p = 0.03$). The introduction of public health policies concerning the prevention of exposure to benzene among at-risk workers is recommended, and more widespread use of biological monitoring for the assessment and control of occupational exposure to industrial chemicals is encouraged.

Keywords: *trans*,*trans*-muconic acid, press worker, benzene.

Introduction

Volatile solvents, particularly toxic agents such as benzene and toluene, constitute significant potential threats to human health in both occupational and environmental settings. Benzene is a common toxic volatile substance associated with many industrial processes. It is considered to be a hazardous chemical agent and human pollutant.

Chronic exposure to benzene is of particular concern: thousands of workers in industrial plants are at risk of chronic intoxication, producing central nervous system depression and memory impairment (Irons and Stillman 1996, Snyder and Hedli 1996). Industrial epidemiological studies have shown that at occupational exposure levels above 16 mg m^{-3} (5 p.p.m.) benzene may induce leukaemia (Irons and Stillman 1996, Snyder and Hedli 1996). Therefore, monitoring of exposure is very important.

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Benzene and its metabolites are potential biomarkers of benzene exposure. Previous studies have indicated that these biomarkers can be used to distinguish populations with different levels of benzene exposure and to determine differences in metabolism (Weisel *et al*. 1996, Medeiros *et al*. 1997). Compared with other routine biomarkers for benzene exposure, urinary *trans*,*trans*-muconic acid (ttMA) determination has been shown to be a reliable marker (Boogaard and van Sittert 1995, Ong *et al*. 1996).

In developing countries, awareness of the public health impact of exposure to volatile solvents is growing, although few of these countries have introduced policies and regulations that combat the problem effectively. Owing to the recent industrialization of Thailand, a developing country in Southeast Asia, many occupations are now at risk of exposure to benzene.

Surprisingly, there have been only a few reports (Suwansaksri and Wiwanitkit 2000, Wiwanitkit *et al*. 2001a) about monitoring benzene exposure among at-risk workers in Thailand. A number of occupation groups, such as press workers, are at risk; these workers have direct contact with benzene in their daily work and specific control measures have yet to be established. This pilot study aimed to determine the difference between the urinary ttMA level in non-exposed subjects and that in press workers.

Materials and methods

Subjects

A total of 103 healthy volunteers were included in this study. The study group comprised 43 subjects who were all press workers working every day in the same press factory in Bangkok. The control group comprised 60 subjects with a low risk for benzene exposure; they were all residents in an area free of factories. All the subjects in this study had similar eating and drinking habits.

Before the study, all subjects were interviewed to determine possible exposure to benzene, especially from smoking or volatile substance abuse, and those with a history of possible exposure were excluded. All subjects gave informed consent. The study was approved by the Faculty of Allied Health Science, Chulalongkorn University. Each subject provided a urine sample for laboratory analysis.

Laboratory analysis

All the urine samples were sent to the laboratory for analysis. Determination of the ttMA level was performed using a high performance liquid chromatography (HPLC) method as described elsewhere (Wiwanitkit *et al*. 2001b).

Briefly, 0.5 ml of urine was mixed with 2 ml of Tris buffer containing vanillic acid as the internal standard. This mixture was percolated through a preconditioned ion-exchange column. The column was rinsed with phosphoric acid solution, acetate buffer and deionized water, the analytes were eluated with 2 ml of an equivolume solution of 1.5 mol l^{-1} sodium chloride and methanol, and 100 μl was injected into the HPLC column. The mobile phase consisted of 10 ml l^{-1} acetic acid, 100 ml l^{-1} methanol and 5 mmol l^{-1} sodium acetate. The initial flow rate was 1.7 ml min^{-1}. The ttMA and internal standard levels were detected at 5.5–6.1 and 15.1–16.7 min, respectively. The lowest detection limit was 0.05 mg l^{-1}.

Statistical analysis

Statistical analysis of the results was carried using the SPSS 7.0 for Windows program. The results for both exposed and control subjects were shown to have a normal distribution using the Kolmogorov–Smirnov test. The unpaired *t*-test was used to compare values for ttMA in the control and exposed groups. Differences were considered to be statistically significant at $p < 0.05$.

Results

A total of 103 subjects were included in the study. There were 96 men (93.2%) and seven women (6.8%), with a mean ± SD age of 32.84 ± 8.46 years. The mean ttMA level in the control group (all males) was 0.08 ± 0.03 mg g^{-1} creatinine. The mean urinary ttMA level in the press workers (36 males, seven females) was 0.56 ± 0.65 mg g^{-1} creatinine. The urinary ttMA levels among the press workers were significantly higher ($p < 0.01$) (Table 1).

Discussion

Benzene is an aromatic hydrocarbon solvent that is widely used in industry. Prolonged exposure can cause chronic toxicity, especially to the haematological systems (Irons and Stillman 1996, Snyder and Hedli 1996). International organizations such as the Agency for Toxic Substances and Disease Registry (ATSDR) (1997) have documented benzene toxicity and recommend the monitoring of benzene exposure in at-risk groups. In many countries the work environment is strictly regulated with regard to the air concentration of benzene. However, in the evaluation of exposure and risk, biological monitoring of benzene has several advantages over technical assessment of exposure. Urinary ttMA, a peripheral biomarker of benzene exposure, is a useful monitoring tool for the early detection of dangerous exposure and can be used to promote improvements in the work environment (Angerer 1979, Lauwerys and Buchet 1988).

In many newly industrial countries, the rapid growth of industrialization without adequate policies and regulations for the management of pollutants has led to an increase in occupational health problems. Hence, monitoring toxic substances in the workers is necessary. Despite the promotion of pollution control in Thailand, high levels of volatile solvent metabolites of benzene, indicating high exposure, have been reported in recent studies (Suwansaksri and Wiwanitkit 2000, Wiwanitkit *et al*. 2001a).

In this pilot study, the use of urinary ttMA level determination as a biomarker for benzene exposure is reported. Press workers were selected as representative of occupationally exposed workers as they are constantly in contact with benzene while working. Furthermore, most of them live in rooms near the workplace. However, these workers are largely forgotten and are not protected by environmental control strategies; none recalled a past health check-up for benzene exposure.

Theoretically, we expected to find a difference between the urinary ttMA level in the occupational exposed group and that in the non-exposed controls. When the

Table 1. Urinary ttMA levels in control and press worker groups.

		Urinary ttMA level (mg g^{-1} creatinine)	
Group	*n*	Mean ± SD	Range
Controls	60	0.08 ± 0.03	0–0.60
Press workers	43	0.56 ± 0.65	0–3.12

mean urinary ttMA levels in the press workers were compared with those in the control group, significantly higher levels were detected. This result is consistent with those in previous studies (Suwansaksri and Wiwanitkit 2000, Wiwanitkit *et al*. 2001a), which indicated high urinary ttMA levels among high-risk workers. In our study all the subjects were from the same area with a reported benzene level of 0.76–4.14 p.p.m. (Muttamara *et al*. 1999); therefore the difference in urinary ttMA levels can be attributed to the occupational exposure. Of interest, we detected a lower ttMA level in our control population compared with that reported in Western populations (Weisel *et al*. 1996). This finding might relate to increased pollutants in developed and industrialized Western countries.

A number of forgotten high-risk workers can be found in Thailand. Monitoring benzene exposure in these workers is important. The introduction of public health policies concerning the prevention of exposure to benzene among at-risk workers is recommended, and more widespread use of biological monitoring for the assessment and control of occupational exposure to industrial chemicals is encouraged. A regular check-up including determination of the urinary ttMA level as a marker for benzene exposure in such workers is recommended.

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