RESEARCH NOTE

HIGH URINE TTMA LEVELS AMONG FISHERMEN FROM A THAI RURAL VILLAGE

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Abstract. The ongoing industrialization of Thailand, a developing country in Southeast Asia, has put many occupations at high risk of benzene exposure. However, there are few reports about monitoring the biomarkers of benzene exposure among Thais. In this study, we report on high urine trans, trans-muconic acid (ttMA) levels among the fishermen of a rural community. Using high-performance liquid chromatography (HPLC) for urine ttMA determination, 49 subjects (30 fishermen and 19 control subjects) were studied. The mean urine ttMA level in fisherman (0.180 ± 0.130 mg/g creatinine) was significantly higher than that of the control group (0.015 ± 0.053 mg/g creatinine) ($p < 0.05$). We recommend the monitoring of urine ttMA in these workers. The monitoring of the possible benzene contamination of the water and fish is recommended for further study.

Benzene is a widely used chemical agent. Studies of the relationship between the metabolism and toxicity of benzene indicate that several metabolites of benzene play significant roles in generating benzene toxicity (Irons and Stillman, 1996; Snyder and Hedli, 1996). The main sources of human exposure to benzene are associated with personal or professional activities (Wallace *et al*, 1982; 1984a). Investigation of the metabolism of benzene and its metabolites will allow us to determine their toxicity and facilitate the monitoring of at-risk workers (Wallace *et al*, 1982; 1984b; Inoue *et al* 1989; Bechtold *et al*, 1991; Lee *et al*, 1993; Weisel *et al*, 1996; Medeiros *et al*, 1997). At present, the benzene metabolite urine trans, trans - muconic acid is acceptable as a reliable marker in tests of benzene exposure (Lee *et al*, 1993).

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In developing countries, awareness of the public health impact of exposure to benzene is growing, but few of these countries have introduced policies and regulations for significantly combating the problem. In Thailand, there is limited data about benzene monitoring in at-risk populations. We performed a previous study to test the feasibility of urine ttMA determination as a biomarker for benzene exposure among mechanics. This study indicated that it was feasible to use this method to differentiate between the occupationally exposed and non-exposed groups (Suwansaksri and Wiwanitkit, 2000).

Due to the ongoing industrialization of Thailand, a developing country in Southeast Asia, many occupations seem to be at high risk of benzene exposure. In our previous study, the first report of monitoring of benzene exposure among high-risk workers in Thailand, high exposure among mechanics was observed (Suwansaksri and Wiwanitkit, 2000). However, there are also other groups of at-risk workers for whom monitoring of benzene exposure may

be necessary. Here, we report high benzene exposure among the fisherman of a rural Thai community that is not threatened by either a nearby industrial plant or a business area.

A total of 49 subjects were included in this study. The first group (the study group) was comprised of 30 fishermen from a rural community in Chantaburi Province, Thailand. The study considered two fishing villages from two districts: Pak Nam Laem Sing (Laem Sing district) and Koh Kwang (Muang district). These villages are about 320 km from Bangkok. The second group, of 19 male subjects, was the control group (low risk of benzene exposure). All 19 were villagers from a rural area of Lam Luk Ka district, Pathum Thani Province; the area was free of nearby factories. The selection of exposure and control subjects was performed as described in our previous study (Suwansaksri and Wiwanitkit, 2000). All the subjects gave their informed consent. Each subject gave a random urine sample for laboratory analysis; samples were provided between 14.00 and 19.00 hours, some 4 - 8 hours after the subjects had started work.

The samples were sent to the clinical chemistry laboratory, Faculty of Allied Health Science, Chulalongkorn University, for analysis of ttMA level. Determination of ttMA level was performed using the high-performance liquid chromatography (HPLC) methodology that was described in a previous study (Wiwanitkit *et al*, 2001). Briefly, 0.5 ml of urine was mixed with 2 ml of Tris buffer (pH 10) containing vanillic acid as internal standard (IS concentration 1,000 mg/l). This mixture was then percolated through a 1 cm (diameter) preconditioned ion-exchange column [Dowex I (chloride form, 100 - 200 mesh); 100 mm high]. After rinsing the column with phosphoric acid solution, acetate buffer, and deionized water, we eluated the products with 2 ml of an equivolume solution of 1.5 mol/l sodium chloride and methanol. Of this, 10 μl were injected into the HPLC column [Lichrocart C18 cartridge (120 x 4mm; Merck)]. The mobile phase consisted of, per liter, 10 ml of acetic acid, 100 ml of methanol, and 5 mmol/l sodium acetate. The flow rate was started at 1.2 ml/minute. The ttMA and IS were detected at 4.2 to 4.4 and 12.6-13.3 minutes respectively. The lowest detection limit was 0.05 mg/l.

Mean and standard deviations of urine ttMA levels in both groups were calculated. The average urine ttMA levels of each group were compared using the unpaired two tailed *t*-test with $p \leq 0.05$ considered statistically significant. The mean urine ttMA level in fisherman (0.180 ± 0.130 mg/g creatinine) was significantly higher than that of the control group (0.015 ± 0.053 mg/g creatinine) ($p < 0.05$; Table 1).

Benzene toxicity involves both bone marrow depression and leukemogenesis caused by damage to multiple classes of hematopoietic cells and a variety of hematopoietic cell functions. Since benzene is hematoxic and has been classified as a human carcinogen, the monitoring and control of benzene exposure is of importance (Irons and Stillman, 1996; Snyder and Hedli, 1996). Exposure is by inhalation of benzene vapors.

Apart from the at-risk workers in industrial plants, who have direct contact with benzene, there are other groups of workers at risk. In our study, comparing the average urine ttMA levels of the fishermen to those of the control group, a significantly higher level was detected in the fishermen (Table 1). These ttMA levels of the fishermen are about half of those of the mechanics, who have to work in the benzene contaminated area (Suwansaksri and Wiwanitkit, 2000). This occurred despite the fact that benzene contamination in the

Table 1
Urine ttMA levels in control and fishermen groups.

Group	Number	Urine ttMA level (mg/g creatinine)	
		Range	Average
Control group	19	0-0.19	0.015±0.053
Fishermen	30	0-0.40	0.180±0.130

atmosphere of the studied setting was not found. A likely explanation is that these subjects had been exposed to fuel used in the ship during their fishing trips. Since they had to live in these ships for a long time during fishing trips, they could not avoid these petroleum products. Another possible explanation, given in other industrialized countries (Phillips *et al*, 2001; Roose *et al*, 2001), is that there may be benzene contamination of sea fish, as important food in the community that we studied. Since there are a number of industrial plants on the Thai seacoast, some may illegally drain their waste into the sea, contaminating the gulf of Thailand.

These fishermen can be classified as a population at high risk of benzene exposure. They are in everyday contact with petroleum products, the fuel used for their fishing trips, and may also be exposed to contaminated food. We conclude that the monitoring of benzene exposure in fishermen is important. The urine ttMA of these workers should be routinely checked. Monitoring of the benzene contamination of the water and fish is recommended as an area for further study.

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