

## RESEARCH COMMUNICATION

# Genotoxic Monitoring and Benzene Exposure Assessment of Gasoline Station Workers in Metropolitan Bangkok: Sister Chromatid Exchange (SCE) and Urinary Trans, Trans-Muconic Acid (t,t-MA)

Tanasorn Tunsaringkarn<sup>1\*</sup>, Jamsai Suwansaksri<sup>2</sup>, Suphan Soogarun<sup>3</sup>, Wattasit Siriwong<sup>1</sup>, Anusorn Rungsiyothin<sup>1</sup>, Kalaya Zupuang<sup>1</sup>, Mark Robson<sup>4,5,6</sup>

### Abstract

Early warning of the potential of mutagens or carcinogens caused by benzene exposure that might occur in gasoline station workers can be achieved by examining 2 major biomarkers: sister chromatid exchange (SCE) and trans, trans-muconic acid (t,t-MA), a urinary metabolite of benzene. The main objective of this study was to assess benzene exposure and monitor the genotoxic effect of gasoline station workers in Bangkok, Thailand. Blood and urine samples were collected from 33 gasoline station workers, working in Pathumwan district area, central Bangkok, Thailand, for SCE and t,t-MA analysis, from April to June 2009. Control samples were collected from 30 office workers and students in the same area at the same period. Our results indicated significantly higher frequencies of SCE in gasoline exposed workers were than in controls ( $p < 0.01$ ), independent of gender. Urinary t,t-MA and t,t-MA/creatinine levels of gasoline exposed workers were also significantly higher than the control groups ( $p < 0.05$ ) were significantly higher in women than men workers ( $p < 0.01$ ). Calculated chromosomal damage relative risk (RR) of gasoline station workers was 3.00 (95% CI = 1.81 - 4.98,  $p < 0.001$ ) compared to controls. The gasoline exposed workers had potentially higher risk of chromosomal damage and cancer development because of direct contact to benzene.

**Keywords:** Gasoline exposure - genotoxicity - sister chromatid exchange - trans, trans-muconic acid

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### Introduction

Air pollution caused by traffic transportation, fuel combustions and volatile organic compounds (VOCs) composition of gasoline, has progressed to be a serious health problem in Bangkok, Thailand, especially among occupational workers as gasoline station workers (Land transportation department, 2000; Muttamara and Leong, 2000). Exposure to gasoline vapors is classified by the International Agency for Research on Cancer as possibly carcinogenic to humans, mainly on the basis of the established carcinogenicity of some component chemicals such as benzene (IARC, 1989).

Benzene is one of primary interest of petroleum constituents which effects to human health. Human exposure to benzene is a global health problem. The major effects of benzene are manifested via chronic (long-term)

exposure through the blood. Benzene damages the bone marrow and can cause a decrease in red blood cells, leading to anemia. It can also cause excessive bleeding and depress the immune system, increasing the chance of infection. Benzene causes leukemia and is associated with other blood cancers and pre-cancers of the blood (Rana and Verma, 2005; Huff, 2007). While, t,t-MA is a minor metabolite of benzene and serves for the assessment of an occupational benzene exposure (Panev et al., 2002). The mechanism of benzene toxicity, particularly its leukemogenic effects, is far from being fully understood (Snyder et al., 1993, Fabiani et al., 2001). Contamination of the environment with VOCs has become an important issue, since many of these compounds are toxic and may pose health risks of various concerns.

In order to assess any biological risks caused by gasoline vapor, the biological monitoring using biological

<sup>1</sup>College of Public Health Sciences, Chulalongkorn University, Bangkok, Thailand, <sup>2</sup>Department of Clinical Chemistry, Faculty of Allied Health Sciences, Chulalongkorn University, Bangkok, Thailand, <sup>3</sup>Department of Clinical Microscopy, Faculty of Allied Health Sciences, Chulalongkorn University, Bangkok, Thailand, <sup>4</sup>School of Environmental and Biological Sciences, Rutgers University, New Brunswick, NJ, USA, <sup>5</sup>UMDNJ-School of Public Health, Piscataway, NJ, USA, <sup>6</sup>Thai Fogarty ITREOH Center, Chulalongkorn University, Bangkok, Thailand. \*For correspondence : tkalayan@chula.ac.th

markers of oxidative chromosomal damage (Lambert et al., 1982; Carrano and Natarajan, 1988) such as frequency of sister chromatid exchange (SCE) and the novel biomarker for accumulated uptake of benzene of the trans, trans-muconic acid (t,t-MA), an urinary metabolite of benzene, (Scherer et al., 1998) on workers in gasoline station had been conducted. Information obtained from the analysis could provide useful knowledge about the genotoxic risk associated with exposure to this carcinogenic agent like benzene.

## Materials and Methods

### Population study

A cross sectional survey was conducted by collecting blood samples from 33 gasoline station workers from 11 gasoline stations in Phatumwan district, Bangkok, Thailand, from April to June 2009. Control samples were collected from 30 office workers and students in the same area and at the same period. All subjects were healthy and had worked more than six months. All subjects were given informed consents before the study. The Ethical Review Committee for Research Involving Human Research Subjects, Health Science Group, Chulalongkorn University, which approved by Forum for Ethical Review Committees in the Asian and Western Pacific Region (FERCAP)/Strategic Initiative for Developing Capacity in Ethical Review (SIDCER) allowed for this study.

### Sample collection

The 2 ml of venous blood and 20 ml of urine samples were collected at 8 hours after the start of work shift. Blood samples were collected in glass heparinized vacuum tube, stored at room temperature before SCE analysis within 6 hours. Urine samples were collected in glass bottle with sodium azide and frozen at -20°C before t,t-MA and creatinine analysis.

### SCE analysis

Examination for SCE frequency was performed as described by Tucker and Preston (1996). 0.5 ml of heparinized blood sample was added to 5.0 ml of the culture medium containing RPMI 1640 media (Hyclone, Utah, USA), supplemented with 15% fetal bovine serum, 2.5% PHA (Sigma, Germany) and 1% penicillin-streptomycin. 100  $\mu$ l of 1.3 mg/ml 5'-bromodeoxyuridine (BrdU, Sigma Chemical Co.) was added to the medium and incubated in the dark room for 96 hours at room temperature. Immediately adding 0.2  $\mu$ g/ml colchicine (Sigma Chemical Co) and then collected cultured cells and treated with 0.075 M KCl at 37°C for 10 minutes, and fixed with methanol-acetic acid (3:1). Standard harvest procedure was performed by spreading a drop of harvested cell pellets on clean glass slide and stained by Hoechst No.22358 plus Giemsa technique (Koto et al., 1975). The stained slides were examined by a light microscope (Nikon, E200) in regard to SCE frequency per metaphase cell. Counting SCE frequency was done using oil immersion. The total of 15 well-spread metaphases was evaluated in worker and control groups.

### Urinary t,t-MA analysis

Analysis of the urinary t,t-MA was conducted following the modified method described by Lee et al., (1993), using solvent extraction and changing the ingredients of mobile phase for isocratic elution instead of gradient elution which prolong the analysis time. Urine samples were thawed at room temperature for 15 min with frequent stirring and then centrifuged at 3000 x g for 10 min. Aliquots of 10 ml urine samples were mixed with 200  $\mu$ l 0.1% vanillic acid solution as internal standard and extracted with 10 ml ethyl acetate. The evaporated residues were re-dissolved in 500  $\mu$ l HPLC mobile phase (consisting of 5% acetate, 5% THF, and 30% methanol) and 5  $\mu$ l mobile phase portions were injected in Shimadzu HPLC Model class 10A VP series using 150 mm x 4.6 mm Spherisorb 5 ODS-2 type column as immobile phase and UV detector setting at 259 nm and column oven temperature at 40°C. Calibration curve was constructed using 0.5, 2 and 5 mg/L t,t-MA against peak area ratio.

### Urinary creatinine analysis

Examination of creatinine concentration in urine samples was done using Creatinine Liquicolor Reagent kit (Human, Germany). Urine sample was diluted to 1:10 and analyzed using LIASYS II automated biochemical analyzer (AMS, Italy) in two-point kinetic mode. Alkaline picrate reagent in Jaffe's reaction was mixed at 3:23 ratio by volume before use. All urine samples were performed at a standard Spacial Laboratory, Bangkok, Thailand. Acceptable limits on urinary creatinine concentrations were between 0.3 and 3.0 g/L (ACGIH, 2010) as The World Health Organization (WHO) has adopted guidelines.

### Statistical analyses

Descriptive statistics was used to analyze the characteristic and concentration of blood biological markers of gasoline station worker and control groups. The frequencies of SCE were dichromatomized into high- and low-frequency groups based on their means plus standard error (S.E.) values of control groups. The chromosomal damage relative risk was calculated by comparing the proportion of workers with frequency of SCE higher than the mean plus standard error value to proportion of workers with frequency of SCE lower than the mean plus standard error value. All the statistical analyses were performed using SPSS 17.0 for Windows Program. A statistically significant difference was accepted at a *p*-value of <0.05 similar to the other medical studies.

## Results

Results of our statistical analyses revealed no significant differences of means of ages of worker and control groups in both men and women (32.6 versus 29.1 years) (Table 1). Mean of frequencies of SCE of workers was significantly higher than those of controls (8.52) (Table 2). Both urinary t,t-MA and urinary t,t-MA/creatinine were significantly higher in worker group (Independent t-test, *p*<0.05) when compared to control group. In addition, values in women were higher than in men gasoline workers. The mean urinary creatinine of

**Table 1. Frequency of SCE and Urinary t,t-MA, Creatinine and t,t-MA/ Creatinine of Controls and Gasoline Workers**

Parameters (number)	Age	Frequency of SCE	U_t,t-MA	U_creatinine	U_t,t-MA/creatinine
	(years)	(SCEs/cell)	(mg/L)	(mg/dl)	(mg/g)
	(Mean ± S.E.)	(Mean ± S.E.)	(Mean ± S.E.)	(Mean ± S.E.)	(Mean ± S.E.)
Controls (30)	29.1 ± 2.5	8.52 ± 0.40 <sup>a</sup>	1.05 ± 0.29 <sup>b</sup>	108.8 ± 16.14	1.03 ± 0.25 <sup>b</sup>
Men (11)	38.8 ± 4.4	7.92 ± 0.69	0.66 ± 0.28	126.9 ± 31.25	0.52 ± 0.21
Women (19)	29.0 ± 3.4	8.84 ± 0.48	1.21 ± 0.38	103.6 ± 19.09	1.22 ± 0.32
Gasoline Workers (33)	32.6 ± 1.7	13.47 ± 0.26 <sup>a</sup>	1.74 ± 0.25 <sup>b</sup>	138.4 ± 15.23	2.38 ± 0.63 <sup>b</sup>
Men (18)	33.2 ± 2.0	13.74 ± 0.40	1.20 ± 0.37*	141.7 ± 18.97	0.81 ± 0.15**
Women (15)	32.1 ± 2.9	13.16 ± 0.31	2.39 ± 0.23*	134.4 ± 25.31	4.26 ± 1.23**

<sup>a,b</sup> between controls and workers at  $p < 0.01$  and  $p < 0.05$ ; \*, \*\* between male and female workers at  $p < 0.05$  and  $p < 0.01$

**Table 2. Comparison of Mean SCE Frequencies between Worker and Control Groups With and Without Dichotomization by Mean of Control Group**

Parameters	Control Group (number)	Worker Group (number)	p-value
SCE frequency/cell	8.52 ± 0.40	13.47 ± 0.26	<0.01 <sup>a</sup>
Dichotomized by mean of control group			
SCE frequency < 8.92	20	0	
SCE frequency ≥ 8.92	10	33	<0.001 <sup>b</sup>

<sup>a</sup>Independent Sample Test; <sup>b</sup>  $\chi^2$  - test, RR = 3.00, 95% = 1.81-4.98

worker and control groups were 138.38 and 108.81 mg/dl which were not significant differences. However, these levels were higher than ACGIH BEI.

Determining of the chromosomal damage risk of high SCE individuals was done by dichotomizing the distribution of SCE frequencies by adding the mean and standard error (8.52 + 0.40) of control groups (Table 1), which was equal to 8.92 SCEs/cell. The chromosomal damage relative risk of workers compared to controls was 3.00 (95% CI = 1.81 - 4.98,  $p < 0.001$ ) (Table 2).

## Discussion

Gasoline station worker is a representative of an occupational group who permanently exposed to hazardous air pollutants (HAPs) from the fumes of fuels and vehicle emissions. This worker is exposed to various gasoline products via different routes, e.g. ingestion and dermal absorption, but the most likely route of exposure is by inhalation (Flanagan and Meredith, 1996). It is possible long term effects on human health upon entering blood circulation which exerts cytotoxic and genotoxic properties (Crebelli et al., 2001; Sun et al., 2009; Rekhadevi et al., 2010).

Our results are in line with previous studies by Pitarque et al., (1997), Celi and Akba (2005) and Soogarun et al., (2006), but there was no significant difference between men and women. Therefore, the SCE was not related to gender which supported some previous studies that showed no correlation between SCE frequency, duration of exposure, smoking habit and age (Hoet et al., 2008; Ulker et al., 2008). On the other hand, Carere et al., (2002) and Fustinoni et al., (2005) studies showed cigarette smoking give the prevailing contribution to individual genotoxic burden and Bukvic et al., (1998) study presented SCE frequencies were significantly related with age and

smoking habits but there were no relation between SCE and length of employment.

Our findings on the urinary t,t-MA showed significantly higher in gasoline worker than control groups ( $p < 0.05$ ). This could be derived from the fact that the gasoline workers directly exposed to benzene. However the urinary creatinine of worker and control groups was not significantly difference. It meant that the excretion rate of workers and controls were not different and with least variation in the individuals. As the result, the urinary t,t-MA/creatinine was significantly higher in gasoline workers than controls group. Both the urinary t,t-MA and urinary t,t-MA/creatinine were significantly higher in women than men workers ( $p < 0.01$ ) because heart rate dynamics are higher in women than men (Ryan et al., 1994). The higher blood benzene level in women than men could be the causative factor (Unpublish data). Result of blood benzene level and its metabolite were higher in women than men which supported the study done by Melikian (2002). Hoet et al., (2008) found that at low levels of benzene exposure (<0.1 ppm), t,t-MA is definitely not a reliable biomarker of benzene exposure. Benzene concentration in blood and in urine as well as S-phenylmercapturic acid (SPMA), but not t,t-MA, were significantly higher in smokers than in non smokers (Hoet et al., 2008). Our results exhibited that t,t-MA reflected to the internal dose of blood benzene with almost similar accuracies and gender was as a confounding factor. In addition, the urinary t,t-MA/creatinine of control and worker groups were higher than the limited concentration of ACGIH BEI (2007, 0.5 mg/g creatinine). This finding could be caused by high benzene exposure of ambient air in urban area. Several studies (Navasumrit et al., 2005; Manini et al., 2006; Bahrami et al., 2007; Davis et al., 2007; Manini et al., 2008; Weisel et al., 2010) reported of the level of benzene exposure in workers who are part of the transportation industries, such as drivers and service station workers, and individuals in occupation that are near traffic, such as police officers found that the level of exposure was as high as tens to hundreds of ppb, which were 2-33 times higher than levels in controls (Wiwanitkit et al., 2008). The importance of such variations in benzene metabolism on an extrapolation of health risk from occupational exposures is not known. Disagreements still exist in the literature as to what causes and contributes to this metabolite.

In conclusion, the biomonitoring programs of benzene exposure, urinary t,t-MA and urinary t,t-MA/

creatinine were proved to be the good and sensitive biomarkers. While the study of frequency of SCE was a sensitive cytotoxic method for determining genotoxic risk with carcinogenic agent exposure like benzene. The combination of these valid biomarker measurements can be very useful to determine any chemical exposure levels and also lead to define how best to reduce these exposures. Those analyses are recommended for public institutions concerned with primary prevention for health risk reduction and environmental management for air quality of community health.

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