Available online at www.sciencedirect.com
SCIENCE DIRECT®
Chemico-Biological Interactions 153–154 (2005) 75–83
Chemico-Biological Interactions
www.elsevier.com/locate/chembioint

Environmental and occupational exposure to benzene in Thailand

Panida Navasumrit [a], Sirirat Chanvaivit [a], Pornpat Intarasunanont [a], Manaswee Arayasiri [a], Narumon Lauhareungpanya [a], Varaporn Parnlob [a], Daam Settachan [a], Mathuros Ruchirawat [a,b,*]

[a] *Laboratory of Environmental Toxicology, Chulabhorn Research Institute, Vipavadee Rangsit Highway, Lak Si, Donmuang, Bangkok 10210, Thailand*
[b] *Department of Pharmacology, Faculty of Science, Mahidol University, Bangkok 10400, Thailand*

Available online 1 April 2005

Abstract

Exposure to benzene in air is a concern in Thailand, particularly since it was observed that the incidence of blood-related cancers, such as leukemia and lymphoma, has increased in the past few decades. In Bangkok, the mean atmospheric levels of benzene on main roads and in schools were 33.71 and 8.25 ppb, respectively, while in gasoline service stations and petrochemical factories the mean ambient levels were 64.78 and 66.24 ppb, respectively. Cloth vendors (22.61 ppb) and grilled-meat vendors (28.19 ppb) working on the roadsides were exposed to significantly higher levels of benzene than the control group (12.95 ppb; $p < 0.05$). Bangkok school children (5.50 ppb) were exposed to significantly higher levels of benzene than provincial school children (2.54 ppb; $p < 0.01$). Factory workers (73.55 ppb) and gasoline service attendants (121.67 ppb) were exposed to significantly higher levels of benzene than control workers (4.77 ppb; $p < 0.001$). In accordance with the increased benzene exposures, levels of urinary *trans,trans*-muconic acid (MA) were significantly increased in all benzene-exposed groups. In school children, the levels of MA were relatively high, taking into account the much lower level of exposure. Blood benzene levels were also significantly increased in Bangkok school children (77.97 ppt; $p < 0.01$), gasoline service attendants (641.84 ppt; $p < 0.05$) and factory workers (572.61 ppt; $p < 0.001$), when compared with the respective controls. DNA damage, determined as DNA strand breaks, was found to be elevated in gasoline service attendants, petrochemical factory workers, and Bangkok school children ($p < 0.001$). The cytogenetic challenge assay, which measures DNA repair capacity, showed varying levels of significant increases in the numbers of dicentrics and deletions in gasoline service attendants, petrochemical factory workers and Bangkok school children, indicating a decrease in DNA repair capacity in these subjects.

Keywords: Bangkok; Benzene; Biomarkers; Children; DNA repair capacity; DNA strand breaks; *trans,trans*-Muconic acid

1. Introduction

Benzene is classified as a human carcinogen, and an association between exposure to benzene and the development of leukemia is well established [1]. Ex-

* Corresponding author. Tel.: +66 2 574 0615; fax: +66 2 574 0616.
E-mail address: mathuros@tubtim.cri.or.th (M. Ruchirawat).

0009-2797/$ – see front matter © 2005 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.cbi.2005.03.010

posure to benzene in the environment and in certain occupational settings has been a subject of concern in Thailand, particularly since it was observed that the incidence of leukemia has increased in the past few decades [2].

Benzene is an important component of gasoline. In Thailand, the limit for benzene content in gasoline is set at 3.5% [3], while in some industrialized countries, such as the USA, the content is only 1% [4]. Benzene is also used in the petrochemical as well as many other chemical industries. Sources of benzene in the environment, to which the general public may be exposed, are cigarette smoke, burning of coal, and traffic emissions. In many occupational settings, exposure may be from benzene used as a solvent, as a raw material, or in gasoline itself.

Environmental and occupational monitoring of benzene exposure and the use of various biomarkers to study benzene exposure and potential toxicity can help to identify the high-risk groups and to determine whether this risk is due to the relative high exposure levels, as in occupational exposures, or the inherently greater susceptibility of the individual, such as in children.

This study has been undertaken to investigate the exposure to benzene in the environment, i.e. from the traffic-related air pollution in Bangkok, and in various occupations, such as in gasoline service attendants and petrochemical factory workers. Environmental and personal air sampling have been used to assess the exposure. Measurement of blood benzene levels and/or urinary *trans,trans*-muconic acid (MA) provides information on the internal doses as well as individual variation in metabolism. DNA damage as measured by the Comet assay [5], and DNA repair capacity as measured by the cytogenetic challenge assay [6], provide information on possible early biological effects of benzene exposure and may be indicative of health risks.

2. Materials and methods

2.1. Study design

2.1.1. Environmental exposure to benzene

In the study involving traffic-related air pollution, seven heavily congested areas of Bangkok were chosen as study locations for roadside and school levels of benzene, namely the Pratunam, Banglampu, Chakrawad, Pratumwan, Hualampong, Surawongse and Victory Monument areas. Street vendors who set up their stalls directly at the roadside and whose work period was approximately 8 h were recruited as subjects. Temple areas located within 500 m of the selected main roads were chosen as control sites, and monks and nuns who spent most of their time in the temples were selected as the controls. Through information provided in the questionnaires, factors such as age and lifestyle (e.g. non-smoking status, medication, type of diet, etc.) were taken into consideration to ascertain that they were well matched between controls and study groups. Schools in Bangkok located within 500 m of the main roads were selected as study sites, while others situated in Chonburi (a provincial area located approximately 110 km from Bangkok) were used as control sites. Students were age-, gender-, and education level-matched between the control and study groups. An explanation of the study was given to all subjects and an informed consent was obtained from all the participants (or parents in the case of the school children).

2.1.2. Occupational exposure to benzene

Attendants from gasoline service stations in Bangkok and workers in the quality control unit of petrochemical factories involved with the production of aromatic compounds, such as benzene and toluene, were recruited in this study. Gasoline service station attendants worked 8-h shifts and only refuelled gasoline. They were not involved in any other responsibilities during their workday. The control subjects were age-, gender-, and lifestyle (non-smoking status, medication, type of diet, etc.)-matched workers in an occupational setting unrelated to the use of benzene.

2.1.3. Selection of study subjects

All adult subjects were non-smoking, healthy volunteers between the ages of 18–40. Mean levels of urinary creatinine lower than 28 μg/mmol creatinine were used to affirm the non-smoking status [7]. Subjects of both genders were involved in the study of benzene exposures in street vendors, monks and nuns, while subjects selected for the study on occupational exposures were all males. School children were healthy, 10–12 years old boys.

2.2. Sample collection

Individual benzene exposure was monitored by attaching diffusive badges (3 M organic vapor monitor 3500) near the breathing zone of study subjects. For ambient air sampling, badges were hung on poles or walls at a height of approximately 150 cm off the ground. Sampling time was 8 h, after which samples were capped, transported to the laboratory, and stored at −20 °C until analysis. Urine samples were collected in the morning (prior to the start of the work or school day) and afternoon (at the end of the work or school day), and stored frozen until the analysis. Blood samples were collected after work shift or school day except in street vendors, who would not consent. Whole blood samples were processed for DNA strand breaks immediately upon arrival in the laboratory.

2.3. Determination of benzene in air samples

Benzene was desorbed from the diffusive badges with carbon disulfide (CS_2). The extracts were then transferred to 2 ml glass vials, and analyzed by gas chromatography equipped with a flame ionization detector (GC-FID, Hewlett Packard 6890). GC conditions were as follows: 99.99% He as the carrier gas, pressure of 15 psi, a 2:1 split ratio injection at 220 °C, and an oven ramp rate of 30–180 °C/min.

2.4. Determination of blood benzene

Benzene from 1 ml of blood was absorbed onto a solid phase micro extraction (SPME) fiber (Carboxene^{TM}/polydimethylsiloxane) placed in the headspace above the sample for 30 min, and then desorbed by heat onto a GC column (HP-5MS). GC conditions were as follows: 99% He as the carrier gas, pressure of 15 psi, a 2:1 split ratio injection at 220 °C, and an oven ramp rate of 30–180 °C. MS conditions were as follows: EI mode ion source, MS quad temperature of 150 °C, MS source temperature of 230 °C, a solvent delay of 1.3 min, a resulting EM voltage of 1200 V and a run time of 12.83 min.

2.5. Determination of trans,trans-muconic acid

An aliquot of 1 ml alkalinized urine (pH 7–10) was applied to a strong anion exchange (SAX) column and subsequently washed with 2 ml H_2O and 1 ml 1% (v/v) acetic acid. The muconic acid was eluted with 1 ml 10% (v/v) aqueous acetic acid and analyzed by HPLC equipped with a UV detector operated at 262 nm with the following conditions: mobile phase of aqueous acetic acid:MeOH (90:10 (v/v)); reverse phased C18 column (Phenomenex Luna) at 20 °C; flow rate of 1.2 ml/min; and a 20 μl injection volume. MA levels were expressed as mg/g creatinine, as well as ng/ml urine. Urinary creatinine was analyzed using a Sigma Diagnostic creatinine kit.

2.6. Determination of DNA strand breaks

DNA strand breaks were determined by way of the alkaline Comet assay, as described in detail elsewhere [8,9]. Briefly, 20 μl of whole blood was mixed with LMP agarose, and embedded in an agarose precoated slide. Slides were submerged in cold lysis solution for at least 1 h at 4 °C. Subsequently, slides were transferred to a electrophoresis chamber and covered with alkaline solution (pH 13) for 20 min before electrophoresis at 300 mA, 24 V for 20 min. After electrophoresis, slides were neutralized with 1 M ammonium acetate and stained with 50 μl Sybr® solution (1:5000). A total of 50 cells from each of the duplicated slides were examined randomly under an epi-fluorescence microscope (Axioplan 2, Zeiss, Germany). The extent of DNA damage was measured quantitatively using the CometScan image analysis software (MetaSystems), and expressed as Olive tail moment.

2.7. Determination of DNA repair capacity by the cytogenetic challenge assay

The challenge assay used in this study was carried out according to the methods that have been previously described [10,11]. At 24 h after blood culture, the cells were irradiated with 100 cGy using a ^{137}Cs-source at a dose rate of 5 Gy/min. Fifty hours after culture initiation, cells were blocked with Colcemid (final concentration of 0.1 μg/ml) for 1.5 h and harvested using the standard procedure. Cytological preparations were made, coded and stained with a 10% Giemsa solution for 15 min. Fifty metaphase cells were analyzed from each of the duplicated slides under the microscope. The presence of dicentric chromosomes

and chromosome deletions per metaphase were determined.

2.8. Statistical analysis

Mean exposures and various biomarker levels were compared between the control and exposed groups by way of the Mann–Whitney *U*-test (SPSS v10). A *p*-value ≤ 0.05 was considered statistically significant.

3. Results

3.1. Atmospheric levels of benzene at various sites

The atmospheric levels of benzene at various study sites are summarized in Table 1. Benzene levels on the major roadsides measured at seven locations in Bangkok ranged from 15.49–65.70 ppb, with a mean of 33.71 ppb. These roadside levels were significantly higher ($p < 0.05$) than the mean ambient level of three temple areas, which was 12.39 ppb. Bangkok schools had a mean benzene level of 8.25 ppb, which was significantly higher than that of the provincial schools (2.71 ppb, $p < 0.01$). In workplace settings, such as in gasoline service stations and in factories, the ambient levels of benzene were 64.78 and 66.24 ppb, respectively. These levels were considerably higher than that in the control workplace, which was only 1.57 ppb ($p < 0.001$).

3.2. Environmental exposure to benzene from traffic-related air pollution

Two groups of street vendors, namely cloth vendors and grilled-meat vendors, participated in the study. Cloth vendors are exposed to benzene mainly from traffic emissions, while grilled-meat vendors may also be exposed to benzene through the burning of charcoal. Benzene exposure and urinary MA levels in street vendors are summarized in Table 2. Exposure levels were highest in the grilled-meat vendors (28.19 ppb) followed by the cloth vendors (22.61 ppb). These levels were significantly higher than that in monks and nuns (12.95 ppb, $p < 0.05$) residing in nearby temples. MA levels in the morning were low in all the groups with no significant difference among groups. However, the levels increased in the afternoon, indicating signifi-

Table 1
Atmospheric levels of benzene at various sites

Study locations	Number of sites	Number of sampling areas	Temperature (°C)	Relative humidity (%)	Wind speed (km/h)	Level of benzene (ppb)
Main road (Bangkok)						
Temple	3	6	29.2 ± 0.2	66.7 ± 2.0	5.3 ± 0.5	12.39 ± 1.83
			29.4 (28.4–29.8)	66.8 (60.5–75.0)	4.9 (3.7–6.6)	11.21 (9.09–21.32)
Roadside	7	10	31.6 ± 0.4	61.4 ± 3.4	5.4 ± 0.3	33.71 ± 6.90*
			31.9 (29.5–32.6)	63.5 (49.0–74.5)	4.9 (4.7–6.6)	28.32 (15.49–65.70)
School area						
Provincial (Chonburi)	2	9	28.7 ± 0.2	74.0 ± 0.6	4.7 ± 0.7	2.71 ± 0.38
			28.6 (28.3–29.1)	74.0 (73.0–75.0)	4.3 (3.7–6.1)	2.51 (1.20–5.40)
Bangkok	4	12	27.7 ± 0.3	69.3 ± 4.4	3.9 ± 0.3	8.25 ± 0.78**
			27.9 (26.8–28.4)	68.0 (61.0–80.0)	3.8 (3.3–4.6)	8.60 (6.93–8.87)
Workplace						
Control workplace	2	14	28.5 ± 0.3	66.4 ± 3.5	NA	1.57 ± 0.62
			28.6 (27.1–30.8)	60.8 (52.5–80.8)		1.14 (0.30–22.03)
Gasoline service stations	20	20	29.4 ± 0.6	65.3 ± 2.7	NA	64.78 ± 17.59***
			30.0 (27.5–31.0)	66.8 (51.8–72.2)		35.29 (1.74–238.76)
Factory	4	18	26.7 ± 0.3	64.8 ± 1.7	NA	66.24 ± 24.33***
			27.1 (24.6–27.5)	64.8 (55.8–74.0)		10.69 (1.64–545.13)

Values are expressed as mean ± S.E. on the first line and median (min–max) on the second line of each parameter.
(*,**,***) Statistically significant difference from the corresponding control at $p < 0.05$, 0.01, 0.001, respectively.
NA, not applicable.

Table 2
Benzene exposure and urinary *t,t*-muconic acid (MA) levels in street vendors

Parameters	Study groups		
	Monks and nuns ($n = 18$)	Cloth vendors ($n = 22$)	Grilled-meat vendors ($n = 21$)
Individual exposures (ppb)	12.95 ± 0.61	22.61 ± 1.32*	28.19 ± 2.23*
	12.47 (8.63–18.77)	21.08 (13.94–40.66)	24.61 (16.80–52.04)
Urinary MA (mg/g creatinine)			
Morning	0.05 ± 0.01	0.07 ± 0.01	0.05 ± 0.01
	0.04 (0.01–0.1)	0.05 (0.01–0.16)	0.05 (0.01–0.10)
Afternoon	0.06 ± 0.01	0.12 ± 0.02 *,§	0.11 ± 0.02 *,§
	0.06 (0.01–0.19)	0.11 (0.03–0.30)	0.08 (0.02–0.34)
Urinary MA (ng/ml urine)			
Morning	49.01 ± 5.51	67.00 ± 11.66	70.28 ± 12.08
	45.35 (11.48–96.64)	48.42 (13.95–250.49)	45.86 (9.23–251.98)
Afternoon	75.74 ± 7.95§	118.85 ± 14.51*,§	159.40 ± 16.66 ***,§
	83.23 (30.63–148.16)	103.76 (37.14–275.31)	161.62 (29.64–340.91)

Values are expressed as mean ± S.E. on the first line and median (min–max) on the second line of each parameter.
(*, ***) Statistically significant difference from monks and nuns group (control group) at $p < 0.05$, 0.001, respectively.
§ Statistically significant difference from the corresponding morning at $p < 0.05$.

cant exposure to benzene during the workday ($p < 0.05$) in cloth vendors (0.12 mg/g creatinine) and grilled-meat vendors (0.11 mg/g creatinine). These MA levels were significantly higher than that in monks and nuns (0.06 mg/g creatinine; $p < 0.05$). It appears that exposure to benzene in the two groups of street vendors may be predominantly traffic-related, since street vendors with different activities, of which burning of charcoal may contribute to benzene exposure, had comparable exposure levels. The association between the amount of time spent near the street and MA level has been reported in another study [12].

Benzene exposure and various biomarkers in school children are summarized in Table 3. Individual exposure to benzene in Bangkok school children (5.50 ppb) was significantly higher than in provincial school children (2.54 ppb, $p < 0.01$). This is confirmed by the levels of blood benzene, which were significantly higher in Bangkok school children (77.97 ppt) than in the provincial school children (46.23 ppt, $p < 0.01$). MA levels measured in the afternoon were also significantly higher in Bangkok school children ($p < 0.01$), indicating a greater exposure. In Bangkok school children, afternoon MA levels (0.17 mg/g creatinine) were significantly higher than morning levels (0.07 mg/g creatinine; $p < 0.05$). Olive tail moment was used to determine the level of DNA strand breaks through the Comet assay. The level of DNA strand breaks was significantly higher in samples from Bangkok school children than in samples from provincial school children ($p < 0.001$). The cytogenetic challenge assay showed a statistically significant increase ($p < 0.05$) in the number of dicentrics but not in the number of deletions per metaphase in samples from Bangkok school children.

3.3. Occupational exposure to benzene in gasoline service stations and petrochemical factories

Benzene exposure levels and various biomarkers in occupationally exposed workers are summarized in Table 4. Individual exposure levels in gasoline service attendants were relatively high, at 121.67 ppb, while in factory workers the mean level was 73.55 ppb, and in controls the mean level was 4.77 ppb. The levels in the two exposed groups were significantly different from controls ($p < 0.001$). Blood benzene concentration reflected personal exposure levels. In gasoline service attendants (641.84 ppt) and factory workers (572.61 ppt), mean blood benzene concentrations were significantly higher ($p < 0.001$) than in controls (82.18 ppt). MA levels in the morning prior to the work shift in the three groups were comparable but increased significantly ($p < 0.05$) after the work shift in both gasoline service attendants and factory workers.

DNA strand breaks in both groups of workers were significantly higher than in controls, as can be seen in

Table 3
Benzene exposure and various biomarkers in school children

Parameters	School locations	
	Provincial (Chonburi)	Bangkok
Individual exposures (ppb)	2.54 ± 0.23 2.2 (1.2–5.4) *n* = 30	5.50 ± 0.40** 4.6 (2.4–12.3) *n* = 41
Blood benzene (ppt)	46.23 ± 4.32 47.24 (7.03–92.76) *n* = 30	77.97 ± 11.67 ** 65.63 (18.81–470.75) *n* = 41
Urinary MA (mg/g creatinine)		
Morning	0.04 ± 0.04 0.03(0.01–0.11) *n* = 28	0.07 ± 0.01** 0.06 (0.02–0.36) *n* = 35
Afternoon	0.06 ± 0.01 0.05 (0.01–0.18) *n* = 28	0.17 ± 0.03 **,§ 0.13(0.01–0.66) *n* = 35
Urinary MA (ng/ml urine)		
Morning	45.51 ± 5.28 35.81 (9.55–101.67) *n* = 28	45.25 ± 4.67 37.02 (6.28–111.66) *n* = 35
Afternoon	60.45 ± 9.14 59.37 (13.77–122.16) *n* = 28	152.26 ± 35.44*,§ 81.84 (30.76–1025.00) *n* = 35
DNA strand breaks		
Olive tail moment (μm)	0.13 ± 0.01 0.11 (0.00–0.28) *n* = 28	0.22 ± 0.01*** 0.23 (0.03–0.45) *n* = 41
DNA repair capacity		
Dicentric/metaphase	0.19 ± 0.01 0.19 (0.12–0.28) *n* = 18	0.25 ± 0.02* 0.24 (0.14–0.42) *n* = 21
Deletion/metaphase	0.27 ± 0.01 0.27 (0.20–0.34) *n* = 18	0.31 ± 0.02 0.28 (0.18–0.54) *n* = 21

Values are expressed as mean ± S.E. on the first line and median (min–max) on the second line of each parameter.
(*, **, ***) Statistically significant difference from control group at $p < 0.05$, 0.01, 0.001, respectively.
§ Statistically significant difference from the corresponding morning at $p < 0.05$.

the statistically significant differences in Olive tail moment ($p < 0.001$). DNA repair capacity, as measured by the cytogenetic challenge assay, was significantly reduced in both groups of exposed workers compared to controls. The number of dicentric chromosomes per metaphase was significantly higher in gasoline service attendants and factory workers than in the controls ($p < 0.01$). The number of chromosome deletions per metaphase was also statistically significantly higher in gasoline service station attendants than in the controls ($p < 0.001$).

4. Discussion

Atmospheric levels of benzene in Bangkok were almost three- to four-fold higher at the roadside compared to ambient levels in areas approximately 500 m away, such as in nearby temple grounds and schools. People working at the roadsides, such as street vendors, are exposed to benzene at levels of 33.71 ppb. This level is approximately two-fold higher than those reported in a number of major cities in Italy, which ranged between 12.19–18.93 ppb [13].

Table 4
Benzene exposure and various biomarkers in occupationally exposed workers

Parameters	Study groups		
	Controls	Gasoline station attendants	Factory workers
Individual exposures (ppb)	4.77 ± 1.54 1.60 (0.17–63.30) *n* = 45	121.67 ± 14.37 *** 86.40 (2.80–439.90) *n* = 50	73.55 ± 21.37 *** 13.43 (2.11–423.10) *n* = 30
Blood benzene (ppt)	82.18 ± 11.48 50.89 (4.32–267) *n* = 45	641.84 ± 46.87 ***,# 446.73 (50.71–1697.30) *n* = 50	572.61 ± 146.77 *** 82.35 (18.6–1830.54) *n* = 30
Urinary MA (mg/g creatinine)			
Morning	0.08 ± 0.02 0.04 (0.01–0.77) *n* = 45	0.09 ± 0.03 0.04 (0–2.38) *n* = 50	0.06 ± 0.01 0.03 (0–0.48) *n* = 30
Afternoon	0.06 ± 0.01 0.03 (0–0.31) *n* = 45	0.18 ± 0.02 ***,§ 0.12 (0.01–1.23) *n* = 50	0.18 ± 0.04 ***, § 0.09 (0.03–1.18) *n* = 30
Urinary MA (ng/ml urine)			
Morning	47.76 ± 6.86 33.85(2.84–247.82) *n* = 45	72.25 ± 7.65 60.79(2.46–245.96) *n* = 50	55.16 ± 17.45 32.68 (2.50–537.87) *n* = 30
Afternoon	59.48 ± 9.80 39.04(1.75–314.95) *n* = 45	217.30 ± 30.29 ***,#,§ 147.75(7.95–1267.22) *n* = 50	124.27 ± 21.5 ***,§ 84.20(4.46–571.76) *n* = 30
DNA strand breaks			
Olive tail moment (μm)	0.24 ± 0.01 0.25 (0.16–0.31) *n* = 27	0.44 ± 0.06 *** 0.31 (0.23–1.17) *n* = 29	0.38 ± 0.02 *** 0.39 (0.21–0.57) *n* = 23
DNA repair capacity			
Dicentric/metaphase	0.13 ± 0.01 0.14 (0.04–0.22) *n* = 27	0.18 ± 0.02 ** 0.18 (0.06–0.34) *n* = 29	0.17 ± 0.02 ** 0.16 (0.06–0.31) *n* = 23
Deletion/metaphase	0.16 ± 0.01 0.16 (0.06–0.26) *n* = 27	0.38 ± 0.03 *** 0.32 (0.22–0.84) *n* = 29	0.20±0.02 0.18 (0.10–0.34) *n* = 23

Values are expressed as mean ± S.E. on the first line and median (min–max) on the second line of each parameter.
(**, ***) Statistically significant difference from control group at $p < 0.01$, 0.001, respectively.
[#] Statistically significant difference from factory workers at $p < 0.05$.
[§] Statistically significant difference from the corresponding morning at $p < 0.05$.

In our study, personal exposure levels were similar to ambient levels in all the exposed groups, except for the gasoline service attendants. Due to the nature of gasoline refuelling, attendants are exposed to pulses of concentrated vapor from the refilling nozzle, much higher in concentration than diluted ambient levels. It is thought that the measured individual exposure levels for gasoline service attendants may be exaggerated due to these short pulses of concentrated vapor.

Blood benzene levels in school children and workers appear to correlate well with personal exposure levels. However, MA may be a more suitable biomarker of internal dose for the routine monitoring of benzene exposure in humans due to the non-invasive nature of urine sample collection, compared with the collection of blood samples. However, expressed as mg/g creatinine, urinary MA levels do not appear to correlate as well with personal benzene exposures in various occupational groups (Table 4) as non-adjusted MA levels

(ng/ml urine). This is because urinary creatinine may be influenced by factors such as age, diet, and physical activity. Several researchers consider creatinine-adjusted MA levels to be less reliable than unadjusted levels [14].

Our results show no significant differences in MA levels among the three control groups, i.e. monks/nuns, provincial school children and workers in an occupational setting unrelated to the use of benzene. The morning and afternoon levels varied slightly between 0.04 and 0.08 mg/g creatinine.

In the exposed groups, i.e. street vendors, Bangkok school children, gasoline service attendants and petrochemical factory workers, MA levels reflected the extent of exposure. Morning levels were in the same range as controls, but afternoon levels were significantly increased. In this study, and in several other studies [15,16], there were good correlations between MA levels and levels of benzene exposure in adult subjects.

In children, it was observed that the levels of MA excreted were relatively high when compared to those of adults, taking into account the lower exposure levels. Street vendors, monks and nuns were exposed to four to five times higher levels of benzene than the school children, yet urinary MA levels were comparable to those measured in school children. This indicates an age-related variation in benzene metabolism. It has been reported that children can metabolize a higher proportion of benzene to MA than adults [12].

Although benzene is known to cause DNA strand breaks and results from the Comet assay indicate that the Bangkok school children have significantly higher levels of DNA strand breaks than in controls, it is not possible to conclude that such observations are due to benzene exposure alone. Concurrent exposures to other genotoxic compounds cannot be ruled out.

The level of exposure in gasoline service attendants was 121.67 ppb while that in factory workers was 73.55 ppb. These levels are under the ACGIH limit of 0.5 ppm [17], while the levels in gasoline service attendants is right at the NIOSH limit of 0.1 ppm [18]. They are also much lower than those reported in the workers in other countries in which chromosome aberrations and cytogenetic changes in specific chromosomes were detected [19,20]. The Comet assay has also revealed a significant increase in DNA strand breaks in our occupationally exposed groups. This is in agreement with data from other studies in workers exposed to low levels of benzene [21,22]. By using the challenge assay to detect mutagen sensitivity [6] in exposed groups, a significant decrease in DNA repair capacity, measured by an increase in the dicentric chromosomes and chromosome deletions per metaphase, was observed in the gasoline service station attendants and factory workers. The levels of dicentrics per metaphase in both the groups were comparable but significantly higher than in the controls. It is interesting that the workers in this study showed significant responses to the challenge assay, while an earlier study by Hallberg et al. [23] utilizing the host cell reactivation assay had found no abnormal responses to the challenge assay in workers exposed to less than 0.3 ppm of benzene. Also in this study, we observed a significant decrease in DNA repair capacity in Bangkok school children when compared with that of provincial school children. Since school children were exposed to a relatively low level of benzene (2.54–5.50 ppb), such an observation may indicate individual variations in response to the exposure, i.e. a greater sensitivity in children.

Manifestation of the abnormal DNA repair response in the challenge assay used in this study may be due to a combination of two factors, i.e. concentration of genotoxic compounds and individual susceptibility, and may be indicative of an increased health risk for diseases such as cancer.

At high exposure levels, benzene can cause leukemia. Chromosomal aberrations and DNA damage are also evident. However, it is not clear whether or not there is an exposure level below which no significant health effects will occur with prolonged or continuous environmental exposures.

In our study, we have been able to detect an increase in DNA damage and a decrease in DNA repair capacity in Bangkok school children when compared with controls, despite the relatively low environmental exposures. These results provide an indication that children may be more vulnerable to the effects of genotoxic environmental contaminants. However, due to the small size of the study, a further investigation involving a larger number of subjects is necessary.

Acknowledgements

This project was supported in part by research grants from the Chulabhorn Research Institute, the

Bangkok Metropolitan Authority, the Petroleum Authority of Thailand, and the Post-Graduate Education, Training and Research Program in Environmental Science, Technology and Management under the Higher Education Development Project of the Commission on Higher Education, Thailand.

References

[1] International Agency for Research on Cancer, IARC Monographs on the Evaluation of Carcinogenic Risks to Humans, Suppl. 7, Overall Evaluations of Carcinogenicity: An Updating of IARC monographs, vols. 1–42, 1987.
[2] E.J. Mitacek, K.D. Brunnemann, A.P. Polednak, T. Limsila, K. Bhothisuwan, C.F. Hummel, Rising leukemia rates in Thailand: the possible role of benzene and related compounds in cigarette smoke, Oncol. Rep. 96 (2002) 1399–1403.
[3] Notifications of specification and quality of gasoline, Department of Energy Business, Ministry of Energy, Thailand, published in the Royal Government Gazette No. 121, dated January 22, B.E.2547, 2004.
[4] United States Environmental Protection Agency, 'Fuel controls': draft technical support document: control of emissions of hazardous air pollutants from motor vehicles and motor vehicle fuels, Washington DC: Office of Air and Radiation, EPA 420-D-00-003, July 2000.
[5] P. Møller, L.E. Knudsen, S. Loft, H. Wallin, The Comet assay as a rapid test in biomonitoring occupational exposure to DNA-damaging agents and effect of confounding factors, Cancer Epidemiol. Biomarkers Prev. 9 (2000) 1005–1015.
[6] W. Au, Mutagen sensitivity assays in population studies, Mutat. Res. 544 (2003) 273–277.
[7] R. Greaves, L. Trotter, S. Brenneck, E. Janus, A simple high-pressure liquid chromatography cotinine assay: validation of smoking status in pregnant women, Ann. Clin. Biochem. 38 (2001) 333–338.
[8] N.P. Singh, M.T. McCoy, R.R. Tice, E.I. Schneider, A simple technique for quantitation of low levels of DNA damage in individual cells, Exp. Cell. Res. 175 (1988) 184–191.
[9] F. Marcon, C. Andrioli, S. Rossi, A. Verdina, R. Galati, R. Crebelli, Assessment of individual sensitivity to ionizing radiation and DNA repair efficiency in a healthy population, Mutat. Res. 541 (2003) 1–8.
[10] W.W. Au, D.M. Walker, J.B. Ward, E. Whorton, M.S. Legator, V. Singh, Factors contributing to chromosome damage in lymphocytes of cigarette smokers, Mutat. Res. 260 (1991) 137–144.
[11] W.W. Au, Abnormal chromosome repair and risk to develop cancer, Environ. Health Perspect. 101 (S3) (1993) 303–308.
[12] V.M. Weaver, C.T. Davoli, P.J. Heller, A. Fitzwillium, H.L. Peters, J. Sunyer, S.E. Murphy, G.W. Goldstein, J.D. Groopman, Benzene exposure, assessed by urinary *trans,trans*-muconic acid in urban children with elevated blood lead level, Environ. Health Perspect. 104 (1996) 318–323.
[13] F. Brugnone, L. Perbellini, L. Romeo, M. Cerpelloni, M. Bianchin, A. Tonello, Benzene in blood as a biomarker of low level occupational exposure, Sci. Total Environ. 235 (1999) 247–252.
[14] S. Waidyanatha, N. Rothman, G. Li, M.T. Smith, S. Yin, S.M. Rappaport, Rapid determination of six urinary benzene metabolites in occupationally exposed and unexposed subjects, Anal. Biochem. 327 (2004) 184–199.
[15] C.N. Ong, P.W. Kok, H.Y. Ong, C.Y. Shi, B.L. Lee, W.H. Phoon, K.T. Tan, Biomarkers of exposure to low concentrations of benzene: a field assessment, Occup. Environ. Med. 53 (1996) 328–333.
[16] G. Sherrer, T. Renner, M. Meger, Analysis and evaluation of *trans,trans*-muconic acid as a biomarker for benzene exposure, J. Chromatogr. B 717 (1998) 179–199.
[17] American Conference of Governmental Industrial Hygienists (ACGIH), Benzene, in: Documentation of Threshold Limit Values and Biological Indices, seventh ed., ACGIH, Cincinnati, OH, 2001.
[18] National Institute for Occupational Safety and Health (NIOSH), Manual of analytical methods, No. 1501: Hydrocarbons, Aromatics, fourth ed., NIOSH, Cincinnati, OH, 1994.
[19] B. Turkel, U. Egeli, Analysis of chromosomal aberrations in shoe-workers exposed long term to benzene, Occup. Environ. Med. 51 (1994) 50–53.
[20] L. Zhang, N. Rothman, Y. Wang, R.B. Hayes, W. Bechtold, P. Venkatesch, S. Yin, Y. Wang, M. Dosemeci, G. Li, W. Lu, M.T. Smith, Interphase cytogenetics of workers exposed to benzene, Environ. Health Perspect. 104 (1996) 1325–1329.
[21] C. Andreoli, P. Leopardi, R. Crebelli, Detection of DNA damage in human lymphocytes by alkaline single cell gel electrophoresis after exposure to benzene or benzene metabolites, Mutat. Res. 377 (1997) 95–104.
[22] T.H. Lam, C.Q. Zhi, C.Q. Jiang, Lymphocyte DNA damage in elevator manufacturing workers in Guangzhou, China, Mutat. Res. 515 (2002) 147–157.
[23] L.M. Hallberg, R. El Zein, L. Grossman, W. Au, Measurement of DNA repair deficiency in workers exposed to benzene, Environ. Health Perspect. 104 (Suppl. 3) (1996) 529–534.