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Oxidative DNA damage and influence of genetic polymorphisms among urban and rural schoolchildren exposed to benzene

Nantaporn Buthbumrung [a], Chulabhorn Mahidol [b], Panida Navasumrit [a], Jeerawan Promvijit [a], Potchanee Hunsonti [a], Herman Autrup [c], Mathuros Ruchirawat [a,d,*]

[a] *Laboratory of Environmental Toxicology, Chulabhorn Research Institute, Vipavadee Rangsit Highway, Lak Si, Donmuang, Bangkok 10210, Thailand*
[b] *Laboratory of Chemical Carcinogenesis, Chulabhorn Research Institute, Bangkok, Thailand*
[c] *Department of Environmental and Occupational Medicine, Institute of Public Health, University of Aarhus, Aarhus, Denmark*
[d] *Department of Pharmacology, Faculty of Science, Mahidol University, Bangkok, Thailand*

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Abstract

Traffic related urban air pollution is a major environmental health problem in many large cities. Children living in urban areas are exposed to benzene and other toxic pollutants simultaneously on a regular basis. Assessment of benzene exposure and oxidative DNA damage in schoolchildren in Bangkok compared with the rural schoolchildren was studied through the use of biomarkers.

Benzene levels in ambient air at the roadside adjacent to Bangkok schools was 3.95-fold greater than that of rural school areas. Personal exposure to benzene in Bangkok schoolchildren was 3.04-fold higher than that in the rural schoolchildren. Blood benzene, urinary benzene and urinary muconic acid (MA) levels were significantly higher in the Bangkok schoolchildren. A significantly higher level of 8-hydroxy-2′-deoxyguanosine (8-OHdG) in leukocytes and in urine was found in Bangkok children than in the rural children. There was a significant correlation between individual benzene exposure level and blood benzene ($r_s = 0.193$, $P < 0.05$), urinary benzene ($r_s = 0.298$, $P < 0.05$), urinary MA ($r_s = 0.348$, $P < 0.01$), and 8-OHdG in leukocyte ($r_s = 0.130$, $P < 0.05$). In addition, a significant correlation between urinary MA and 8-OHdG in leukocytes ($r_s = 0.241$, $P < 0.05$) was also found. Polymorphisms of various xenobiotic metabolizing genes responsible for susceptibility to benzene toxicity have been studied; however only the *GSTM1* genotypes had a significant effect on urinary MA excretion.

Our data indicates that children living in the areas of high traffic density are exposed to a higher level of benzene than those living in rural areas. Exposure to higher level of benzene in urban children may contribute to oxidative DNA damage, suggesting an increased health risk from traffic benzene emission.

Keywords: Environmental benzene; Air pollution; Urinary MA; 8-OHdG; Genetic polymorphisms

1. Introduction

Traffic is a major source of urban air pollution as a result of the emission of many toxic substances e.g., particulate matter, polycyclic aromatic hydrocarbons and benzene. Significant concentrations of benzene in

* Corresponding author at: Laboratory of Environmental Toxicology, Chulabhorn Research Institute, Vipavadee Rangsit Highway, Lak Si, Donmuang, Bangkok 10210, Thailand. Tel.: +662 5740615; fax: +662 5740616.
E-mail address: mathuros@cri.or.th (M. Ruchirawat).

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ambient air have been reported in Metropolitan Bangkok ambient air, due in large part to vehicle emissions [1]. Exposure to benzene can be occurred from gasoline refueling of vehicles, burning of coal and oil [2,3]. Benzene exposure poses a threat to human health, especially in children. The major toxicological consequence of chronic exposure to benzene is myelotoxicity leading to leukemia [4], and many studies have shown an increased risk of childhood cancer from benzene exposure either directly or indirectly through parental exposure [5–7]. Inhaled benzene is readily absorbed into the blood by passive diffusion through alveolar capillary membrane. The measurement of benzene in blood, therefore, provides a sensitive means of detecting low level benzene exposure. Unmetabolized benzene is also excreted in urine after passive diffusion from blood to urine [8]. Benzene is oxidized by cytochrome *P*-450 enzymes (*CYP2E1*) to a reactive intermediate benzene oxide-oxepin. This highly reactive species is further metabolized by different metabolic pathways, generating various metabolites such as muconic acid (MA), *S*-phenyl mercapturic acid (SPMA), hydroquinone, phenol and other compounds which are excreted in the urine. Among the urinary metabolites of benzene, MA is considered an appropriate biomarker of exposure because of its high specificity and sensitivity [9,10]. It has been shown that benzene generates reactive oxygen species that induce oxidative DNA damage [11–13]. Oxidative damage to DNA may be important in benzene-induced carcinogenesis as DNA base lesions such as 8-hydroxy-2′-deoxyguanosine (8-OHdG) are highly mutagenic [14]. 8-OHdG, one of the major oxidative DNA base products, is not a specific marker for benzene exposure but a general biomarker for oxidative damage to DNA. Most 8-OHdG is removed by the DNA repairing enzyme system [15]. Some studies reported that exposure to urban pollutants in young children and adolescents caused significant increased levels of 8-OHdG [16,17].

Genetic polymorphisms in genes encoding *CYP2E1*, *NQO1*, and *GSTs* might be responsible for human susceptibility to benzene toxicity [18]. *CYP2E1* polymorphisms have not been found to have a significant effect on urinary MA [19]; however a case-control study has indicated a role for *CYP2E1* genotype in benzene toxicity, gene expression and disease risk [20]. Also, detoxification enzymes such as *NQO1* could protect cells against oxidative stress and toxic quinones by converting benzene to less toxic hydroxy metabolize. It has been reported that *GSTM1*2/*2* and *GSTT1*2/*2* genotypes in workers increased susceptibility to benzene, causing DNA damage and myelodysplastic syndrome [21].

This study aimed to evaluate the potential health risk of children living in a megacity environmentally exposed to benzene. Ambient benzene exposure, blood and urinary benzene, urinary MA and 8-OHdG levels were measured. In order to obtain a better understanding of inter-individual variation in susceptibility, the influence of polymorphisms in metabolic activation and detoxification genes such as *CYP2E1*, *NQO1*, *GSTA1*, *GSTM1* and *GSTT1* was also examined.

2. Materials and methods

2.1. Study population

The schoolchildren recruited in this study consisted of groups of 109 and 62 boys (ages of 9 and 13 years, mean ± S.E., 10.72 ± 0.11), for urban and rural groups, respectively. The urban children selected for this study live and attend schools in the Bangkok Metropolitan Area (BMA). The rural group was selected from schools located in the province of Chonburi. A consent form was signed by the parents who were also requested to complete a questionnaire about personal history, health history and their routine lifestyle activities and food habits. This study was approved by the institutional ethical committee in agreement with the Helsinki declaration.

2.2. Determination of benzene in the air and individual exposure

Air sampling of benzene was carried out during the dry season (January–March, 2004). Due to a small variation from day to day and a slight increase from Monday to Friday, the exposure was measured on Wednesday as representative of 5-day monitoring data. Both area and personal air sampling of benzene was conducted by using the passive air sampler (3M^{TM} Organic Vapor Monitor No. 3500) which was set in the breathing zone of the children for a period of 8 h. The sampling conditions including location, air volume, meteorology, temperature, and wind speed and direction were also recorded. After the collection, samples were transported to the laboratory and kept refrigerated (4 °C) until analysis. The absorbed benzene vapors were desorbed by carbon disulfide (CS_2) (Merck, Cat. No. 1.02213.0500) and the solution was analyzed using gas chromatograph with flame ionization detection (Hewlett Packard Model 6890) according to the standard method of the U.S. National Institute for Occupational Safety and Health [22].

2.3. Determination of blood benzene level

Blood samples were collected at the end of study time, preserved with 10% EDTA solution, then transported to the laboratory in a container with freeze pack and processed on the next day. Briefly, a 1 mL of blood sample was placed in a 7-mL sealed head-space vial with 2 mL of prepurged distilled water. Deuterated benzene solution (10 μL of 100 ppb) was added to the sample as an internal standard. The vial septum was pieced with the solid phase micro extraction (SPME) fiber (Carboxen^{TM}/Polydimethylsiloxane), the fiber exposed directly to the head-space above the sample for 30 min to allow the analytes to come to equilibrium between the gas and liquid phases [23]. Then fiber was desorbed by heat and analyzed by GC–MS (Agilent GC model 6890N with capillary column model No. 19091S-433 and Mass selective detector model 5973 N). Standard curves were prepared by spiking benzene and deuterated benzene into purged blood over the concentration range of 0–2 μg/L. They were plotted between concentration of benzene and the ratio of peak areas of benzene to deuterated benzene. The calibration curves were consistently linear with $R^2 > 0.98$. Benzene concentrations in the samples were determined from standard curve.

2.4. Determination of urinary benzene metabolite, MA

Urine samples from each subject were collected three times, day 0, morning (7.30–9.00 a.m.), day 0, afternoon (2.30–4.00 p.m.), and day 1, morning (6.00 a.m., next day) and stored frozen until analysis. The urine samples were purified by extracting through strong anion exchange (SAX) column (Supelco). Then urinary MA was determined by HPLC (Hewlett Packard series 1100) with reversed phase (C_{18}) ODS2 column 150 mm × 4.6 mm 5 μm (Prodigy, Phenomenex, USA) and equipped with diode array detector, adopted from [24]. The calibration curves were prepared by spiking standard MA into 2 mL of prepurged urine samples over the concentration range of 0–200 ng/mL. Standard curves were consistently linear with $R^2 > 0.98$. Levels of urinary MA in the samples were determined from standard curve. The levels of urinary MA was expressed as mg/g creatinine. Urinary creatinine was analyzed using a Sigma Diagnostic creatinine kit.

2.5. Determination of urinary benzene

Unmetabolized benzene in urine was analyzed in samples collected in the afternoon by using purge and trap (Tekmar model LSC 2000, Cincinnati, Ohio, USA) equipped with Gas Chromatography (GC 3400; Varian) and mass spectrometry (Fininigan Mat ITS40 ion trap mass spectrometer) [25]. Deuterated benzene (10 μL of 100 ppb) was added to the sample as internal standard before they were attached to the purge and trap sampler. Prepurged urine was spiked with benzene and deuterated benzene over the concentration range of 0–700 ng/L. They were consistently linear with $R^2 > 0.98$. The ratio of peak area of benzene at *m/z* 78 to deuterated benzene at *m/z* 84 was compared with the standard curve. The value of benzene in urine samples was expressed as creatinine ratio.

2.6. Determination of 8-oxo-7, 8-dihydro-2′-deoxyguanosine (8-OHdG) by HPLC-electrochemical detection (ECD)

2.6.1. 8-OHdG in leukocytes

All blood samples were prepared and analyzed within 24 h after collection. Genomic DNA was isolated from peripheral blood by using the NucleoSpins® Blood XL kit according to the recommendations of the manufacturer. The NucleoSpin® Blood Kit, which has been shown to give a lower background level of 8-OHdG than the conventional methods, has been used for DNA purification. The kit allowed an average yield of 20 μg DNA/mL blood. DNA was enzymatically digested to the deoxynucleoside. Briefly, 60 μg of DNA sample was incubated with 8U of nuclease P1 at 37 °C for 10 min. Subsequently, 5 U of alkaline phosphatase was added and the mixture was incubated at 37 °C for 1 h. Following digestion, the hydrolysate was filtered through a 0.22-mm syringe filter before analysis. The levels of dG and 8-OHdG in DNA hydrolysate were analyzed by an HPLC-ECD system (Agilent 1100 series) by a modification of the method by [26] by using the following conditions: mobile phase of 5% (v/v) methanol in 100 mM sodium acetate buffer, pH 5.2; 3.0 mm × 150 mm, 3 mm guanine adducts column (ESA, Inc., USA); flow rate of 0.5 mL/min; and a 100 mL injection volume. Deoxyguanosine (dG) was determined by a UV detector operated at 254 nm. Detection of 8-OHdG was achieved on the Coulochem III detector (ESA, Inc., USA) equipped with a model 5020 Guard Cell, a model 5011 Analytical Cell, and the Agilent 3900E Interface Module. The detection potentials were set at 400 and 720 mV for electrodes 1 and 2, respectively. The guard cell potential was set at 850 mV. Standard lines were used for determination of 8-OHdG in leukocytes in the concentration range of 0–0.5 pmol. They were consistently linear with $R^2 > 0.98$. Levels of 8-OHdG in leukocytes

were determined from standard curve. Results were expressed as 8-OHdG/10^5 dG.

2.6.2. 8-OHdG in urine

Four milliliters of urine samples were adjusted pH to 4.5 by addition of 2 M HCl and stored at −20 °C until assay. The urine samples were purified through Bond Elut LCR (C_{18}/OH, 500 mg) solid phase extraction column (Varian, USA). The detection of urinary 8-OHdG in urine was carried out by the two analytical columns, ESA guanine adduct column (125 mm × 3.0 mm. I.D.) and Phenomenax prodigy 5 μm ODS2 (150 mm × 4.6 mm). The system was operated at 50 nA full range deflection and the ECD potential was 500 mV. The HPLC separation condition used was based on the methods described by [27,28]. Concentration of 8-OHdG was measured by HPLC with electrochemical detection. Standard lines were prepared in the concentration range of 0–220 nmol/L. They were consistently linear with $R^2 > 0.98$. Levels of urinary 8-OHdG were determined from standard curve. Level of urinary 8-OHdG excretions was expressed as μmol/mol creatinine.

2.7. DNA isolation and identification of metabolic genotype

Gene encoding five benzene-metabolizing enzymes, *CYP2E1*, *NQO1*, *GSTA1*, *GSTM1* and *GSTT1*, were studied for polymorphisms. DNA purification was carried out using NucleoSpin® Blood XL Kits (Machery-Nagel, Germany). DNA concentration was determined spectrophotometrically. All primers were synthesized by the Bioservice Unit of the National Center for Genetic Engineer and Biotechnology (BIOTEC, Thailand).

CYP2E1: *CYP2E1* genotypes were characterized by PCR-RFLP in a slight modification of a method previously described [29]. The amplification of *CYP2E1* promotor region was performed using the following primers: 5′-CCC GTG AGC CAG TCG AGT-3′ (−1380 to −1363) and 5′-ATA CAG ACC CTC TTC CAC-3′ (−870 to −887). Individual homozygous for *CYP2E1*1/*1* exhibited two bands of 360 and 150 bp, *CYP2E1*5/*5* homozygotes were not cleavaged by RsaI, and a band at 510 bp was observed, while PCR products from *CYP2E1*1/*2* heterozygous produced a digestion of all three bands, of 510, 360, and 150 bp long.

NQO1: Primers used in the amplification were 5′-TCC TCA GAG TGG CAT TCT GC-3′ and 5′-TCT CCT CAT CCT GTA CCT CT-3′ [30]. The complete digestion of the 230-bp PCR product gave a cut fragment of 195 bp for wild type or **1/*1*, two fragments of 195 and 151 bp for heterozygous or **1/*2* and 151 bp for mutant or **2/*2*.

GSTA1: Human *GSTA1* genotyping was performed as described previously with slightly modifications [31]. The sequences of primers used were 5′-TGT TGA TTG TTT GCC TGA AAT T-3′ and 5′-GTT AAA CGC TGT CAC CGT CCT-3′. *GSTA1*A/*A* (wild type) samples were undigested 480 bp band, *GSTA1*B/*B* (homozygous mutation) samples produced a fragment of 380 bp, while *GSTA1*A/*B* (heterozygous) samples gave two bands of 480 and 380 bp.

GSTM1: The polymorphic deletion of the *GSTM1* gene was determined as previously described [32]. Primers used were as follows: (P1), 5′-CGC CAT CTT GTG CTA CAT TGC CCG-3′; (P2), 5′-ATC TTC TCC TCT TCT GTC TC-3′; and (P3), 5′-TTC TGG ATT GTA GCA GAT CA-3′. P1 and P3 amplified a 230-bp product specific to *GSTM1*, while P1 and P2 amplified a 157-bp fragment that served as an internal control for each PCR reaction. The *GSTM1* genotype was classified as *GSTM1*2/*2* (both alleles deleted) or *GSTM1*1/* (at least one undeleted allele).

GSTT1: The polymorphism deletion of *GSTT1* gene was determined by a modification of a PCR protocol previously described [33]. The primers used were 5′-TTC CTT ACT GGT CCT CAC ATC TC-3′ and 5′-TCA CCG GAT CAT GGC CAG CA-3′. The genotypes are classified as *GSTT1*2/*2* (deletion of both alleles) given a fragment of 334 bp, and *GSTT1*1/* (presence of one or two *GSTT1* alleles) identified by a fragment of 480 and 334 bp.

2.8. Statistics

All statistical tests were performed using the SPSS program statistical software package (Version 12.0). The non-parametric Mann–Whitney *U*-test was applied to detect the difference between urban and rural groups with differences considered statistically significant at $\alpha < 5\%$ level. The quantitative relationships between the levels of external exposure and the concentrations of the various markers were examined with Pearson's coefficient of rank correlation. Differences in the distribution of gene polymorphisms between the groups were tested by X^2-test with $\alpha = 5\%$ as the significant level. Statistical analysis was performed on combined heterozygous and homozygous variant genotypes as in previous studies [13,34] due to the small number of subjects with heterozygous and homozygous variant genotypes. The analysis of heterozygous and homozygous variant geno-

types for *CYP2E1*, *NQO1* and *GSTA1* was also tested separately. The genetic polymorphism effect on blood benzene, urinary benzene and its metabolite, 8-OHdG was investigated by general linear model (GLM) analysis with differences considered statistically significant at $\alpha < 5\%$ level, and post hoc analysis as least significant difference (LSD) at $\alpha < 5\%$ level.

3. Results

3.1. Benzene exposure and biomarkers

Benzene concentrations in the environment at the roadside and inside school areas at 4 locations in Bangkok and 2 locations in the rural area (Chonburi) are shown in Table 1. In Bangkok, the mean level of benzene at roadside from all sites was 2.15-fold higher than inside school area. In the rural area, ambient benzene level on the roadside varied from 2.20 to 7.40 ppb. Mean level of benzene in ambient air at roadsides adjacent to the Bangkok schools (17.75 ± 2.23 ppb) was approximately 4-fold greater than that of the rural school (4.49 ± 0.59, $P < 0.001$). Benzene level inside the rural school areas was about 2.71 ppb which was 3.04-fold lower than those of Bangkok school.

Assessment of personal benzene exposure was carried out by measuring the concentrations of benzene in the breathing zone and biomarkers of exposure including blood benzene, urinary benzene and a urinary metabolite of benzene (MA). Results on benzene exposure levels and biological markers were presented in Table 2. The average benzene exposure level in Bangkok schoolchildren was 2.17-fold higher than that in the rural schoolchildren (5.50 ± 0.40 ppb versus 2.54 ± 0.23 ppb, $P < 0.001$). Blood benzene and urinary benzene level in Bangkok schoolchildren was found to be significantly higher ($P < 0.001$) than those in the rural schoolchildren and correlated well with personal exposure ($P < 0.05$).

On day 0, urinary MA excretion increased after the school period and thus reflected the benzene exposure during school hour. The level of urinary MA in the morning of day 1 was slightly higher than that of day 0, morning but lower than those in day 0, afternoon. In the after class period (day 0, afternoon), there was a greater increase of urinary MA levels in Bangkok children compared to that of the rural children (0.12 ± 0.01 mg/g creatinine versus 0.06 ± 0.01 mg/g creatinine; $P < 0.001$). Urinary MA at day 0, afternoon appeared to correlate well with personal exposure level ($P < 0.005$). However, no association could be found between blood benzene level and urinary MA ($P > 0.05$).

3.2. Oxidative DNA damage marker

Levels of leukocyte 8-OHdG and urinary 8-OHdG in schoolchildren in Bangkok and rural area are summarized in Table 2. The level of 8-OHdG in leukocyte DNA was 3-fold higher in Bangkok schoolchildren compared with the rural schoolchildren ($0.25 \pm 0.02/10^5$ dG versus $0.08 \pm 0.06/10^5$ dG, $P < 0.001$). The study showed that the level of 8-OHdG in leukocytes was statistically associated with benzene exposure level ($P < 0.05$), and urinary MA at day 0, afternoon ($r_s = 0.24$, $P < 0.05$). A significant increase in urinary 8-OHdG levels was also observed in Bangkok schoolchildren (2.16 ± 0.28 μmol/mol creatinine) compared to rural schoolchildren (1.32 ± 0.22 μmol/mol creatinine, $P < 0.05$). However, no statistical correlation was found between urinary 8-OHdG and benzene exposure levels. A poor ($r_s = 0.07$) but statistically significant ($P < 0.05$) correlation between urinary 8-OHdG and urinary MA was observed. Levels of 8-OHdG in leukocytes was not significantly correlated with urinary 8-OHdG.

3.3. Influence of genetic polymorphisms on biomarkers of benzene exposure and oxidative DNA damage

In this study, polymorphisms of *CYP2E1*, *NQO1* and *GSTs* were investigated as potential markers of genetic susceptibility to benzene toxicity. The frequencies of combined variant genotypes were 26.92, 60.99, and 35.17% for *CYP2E1*, *NQO1*, and *GSTA1*, respectively. The observed distribution of these genotypes are in agreement with the expected genotype distributions calculated by the Hardy–Weinberg equilibrium. For *GSTM1* and *GSTT1* polymorphisms, 63.19 and 32.42% of children had the variant genotypes, respectively. The heterozygous and homozygous variant genotypes of *CYP2E1RsaI*, *NQO1*, and *GSTA1* were combined in the statistical analysis, because of the small number of subjects with homozygous genotypes in these genes.

The influence of polymorphisms of certain metabolizing genes on levels of blood benzene, urinary MA, and on 8-OHdG in leukocytes and urine, was summarized in Table 3. Benzene exposure level was not significantly different in the subjects grouped by genotype for any of the studied genes. There was no significant effect of *CYP2E1* genotype on blood benzene level. *CYP2E1*, *NQO1*, *GSTA1* and *GSTT1* genotypes did not affect significantly urinary MA and 8-OHdG level measured in leukocytes or urine. However, our results showed that levels of urinary MA in the combined *CYP2E1*1/*5*

Table 1
Ambient benzene concentrations at the roadside and inside school areas

School locations	Sampling locations	Benzene concentration (ppb)		Temperature (°C)	Relative humidity (%)	Wind speed (km/h)
		Roadside	Inside school			
Urban (Bangkok)	Site 1	26.18 ± 5.51, 25.55 [6]***	6.93 ± 1.66, 6.20 [3]*	27.9	63	4.24
	Site 2	14.77 ± 0.67, 14.85 [6]***	8.43 ± 1.63, 6.80 [3]*	28.4	73	3.70
	Site 3	10.27 ± 1.49, 9.30 [6]**	8.77 ± 2.62, 6.20 [3]*	27.8	80	4.63
	Site 4	23.85 ± 0.45, 23.85 [2]	8.87 ± 0.20, 8.90 [3]*	26.8	61	3.33
	Average	17.75 ± 2.23, 14.85 [20]***	8.25 ± 0.78, 7.65[12]***	27.73 ± 0.33	69.25 ± 4.44	3.15 ± 0.78
Rural (Chonburi)	Site 5	5.01 ± 0.61, 5.30 [8]	2.97 ± 0.56, 3.00 [6]	29.1	73	4.70
	Site 6	2.40 ± 0.20, 2.40 [2]	2.17 ± 0.03, 2.20 [3]	28.6	74	3.70
	Average	4.49 ± 0.59, 4.20 [10]	2.71 ± 0.38, 2.20 [9]	28.85 ± 0.25	73.5 ± 0.5	4.91 ± 1.21

Values are expressed as mean ± S.E., median [*n*].
* Statistically significant difference from rural at $P < 0.05$.
** Statistically significant difference from rural at $P < 0.005$
*** Statistically significant difference from rural at $P < 0.001$.

Table 2
Benzene exposure levels and study biomarkers in schoolchildren

Parameters	School locations	
	Urban	Rural
Individual exposure (ppb)	5.50 ± 0.40, 4.60 [43]***	2.54 ± 0.23, 2.20 [32]
Blood benzene level (ppt)	77.97 ± 11.67, 65.63 [40]***	46.23 ± 4.32, 47.24 [30]
Urinary benzene (μg/g creatinine)	0.10 ± 0.01, 0.07 [43]***	0.04 ± 0.01, 0.03 [32]
Urinary MA (mg/g creatinine)		
Day 0, morning	0.08 ± 0.01, 0.07 [109]***	0.04 ± 0.003, 0.04 [62]
Day 0, afternoon	0.12 ± 0.01, 0.09 [109]***,§§	0.06 ± 0.005, 0.06 [62]§§
Day 1, morning	0.09 ± 0.01, 0.07 [109]**	0.06 ± 0.004, 0.05 [62]§
8-OHdG		
Leukocyte 8-OHdG (/10^5 dG)	0.25 ± 0.02, 0.24 [40]***	0.08 ± 0.06, 0.06 [32]
Urinary 8-OHdG (μmol/mol creatinine)	2.16 ± 0.28, 1.69 [43]*	1.32 ± 0.22, 0.95 [32]

Values were expressed as mean ± S.E., median [*n*].
* Statistically significant difference from rural at $P < 0.05$.
** Statistically significant difference from rural at $P < 0.005$.
*** Statistically significant difference from rural at $P < 0.001$.
§ Statistically significant difference from day 0, morning at $P < 0.05$.
§§ Statistically significant difference from day 0, morning at $P < 0.005$.

and *CYP2E1*5/*5* genotype group were slightly lower than in the *CYP2E1*1/*1* genotype group. In addition, schoolchildren who carried variant genotypes of *NQO1* and *GSTA1* had lower levels of urinary MA. The carriers of the variant *CYP2E1* and *NQO1* genotypes also had a lower levels of 8-OHdG in leukocytes and urine. Only the *GSTM1*2/*2* genotype had a significant effect on urinary MA excretion at day 0, afternoon ($P < 0.05$). Schoolchildren who carried the *GSTM1*1/* genotype excreted more urinary MA than those with the *GSTM1*2/*2* genotype. However, there was no effect of *GSTM1* genotypes on 8-OHdG level both in leukocytes and urine.

4. Discussion

Benzene concentrations inside 4 school areas in Bangkok were not much different from one another, although the levels on the roadside in front of the schools varied significantly. In comparison with other cities, benzene concentrations at Bangkok roadsides (17.75 ppb) were higher than the levels reported in Copenhagen (0.78 ppb, [35]) and Mexico city (13.5 ppb, [36]), but still lower than those reported in Cotonou, Benin (23.8 ppb, [11]), Kathmandu, Nepal (24.06 ppb, [37]), Tehran, Iran (39.88 ppb, [38]) and Lagos, Nigeria (78.1 ppb, [39]) where traffic density might be higher than Bangkok.

Table 3
Influence of genetic polymorphisms in metabolism on biomarker of exposure and oxidative damage

Parameters	Genotypes	Urban	Rural
Blood benzene (ppt)	*CYP2E1Rsa1 *1/*1*	74.18 ± 14.16, 56.14 [31]	43.17 ± 4.38, 43.91 [22]
	*1/*5 + *5/*5	91.43 ± 17.57, 78.55 [9]	48.27 ± 11.36, 56.54 [9]
Urinary benzene (μg/g creatinine)	*CYP2E1Rsa1 *1/*1*	0.10 ± 0.02, 0.07 [34]	0.04 ± 0.01, 0.03 [23]
	*1/*5 + *5/*5	0.09 ± 0.03, 0.06 [9]	0.03 ± 0.01, 0.02 [9]
Urinary MA (mg/g creatinine)	*CYP2E1Rsa1 *1/*1*	0.12 ± 0.01, 0.09 [77]	0.06 ± 0.01, 0.05 [44]
	*1/*5 + *5/*5	0.10 ± 0.02, 0.08 [27]	0.07 ± 0.01, 0.06 [17]
	*NQO1 *1/*1*	0.11 ± 0.01, 0.11 [33]	0.06 ± 0.01, 0.06 [28]
	*1/*2 + *2/*2*	0.12 ± 0.01, 0.07 [71]	0.06 ± 0.01, 0.05 [33]
	*GSTA1 *A/*A*	0.13 ± 0.02, 0.09 [65]	0.06 ± 0.01, 0.05 [42]
	*A/*B + *B/*B*	0.09 ± 0.01, 0.08 [39]	0.08 ± 0.01, 0.07 [19]
	*GSTM1 *1/*	0.14 ± 0.02, 0.10 [38]	0.06 ± 0.01, 0.05 [22]
	*2/*2	0.10 ± 0.01, 0.07 [66]*	0.07 ± 0.01, 0.06 [39]
	*GSTT1 *1/*	0.11 ± 0.01, 0.08 [69]	0.06 ± 0.01, 0.05 [41]
	*2/*2	0.13 ± 0.02, 0.09 [35]	0.07 ± 0.01, 0.07 [20]
Leukocytic 8-OHdG (/10^5 dG)	*CYP2E1Rsa1 *1/*1*	0.25 ± 0.01, 0.24 [52]	0.09 ± 0.02, 0.07 [23]
	*1/*5 + *5/*5	0.23 ± 0.02, 0.21 [22]	0.05 ± 0.01, 0.05 [9]
	*NQO1 *1/*1*	0.26 ± 0.02, 0.24 [29]	0.09 ± 0.03, 0.06 [17]
	*1/*2 + *2/*2*	0.24 ± 0.01, 0.23 [45]	0.07 ± 0.01, 0.07 [15]
	*GSTA1 *A/*A*	0.24 ± 0.01, 0.22 [46]	0.09 ± 0.02, 0.09 [20]
	*A/*B + *B/*B*	0.25 ± 0.02, 0.24 [28]	0.06 ± 0.01, 0.05 [12]
	*GSTM1 *1/*	0.25 ± 0.03, 0.23 [24]	0.10 ± 0.04, 0.06 [12]
	*2/*2	0.24 ± 0.01, 0.24 [50]	0.07 ± 0.01, 0.06 [20]
	*GSTT1 *1/*	0.24 ± 0.01, 0.24 [58]	0.07 ± 0.02, 0.05 [24]
	*2/*2	0.25 ± 0.03, 0.22 [16]	0.10 ± 0.03, 0.09 [8]
Urinary 8-OHdG (μmol/mol creatinine)	*CYP2E1Rsa1 *1/*1*	1.84 ± 0.23, 1.69 [29]	1.17 ± 0.20, 0.91 [23]
	*1/*5 + *5/*5*	1.57 ± 0.45, 0.93 [14]	1.09 ± 0.27, 0.87 [9]
	*NQO1 *1/*1*	1.96 ± 0.45, 1.27 [12]	1.23 ± 0.19, 1.03 [17]
	*1/*2 + *2/*2*	1.67 ± 0.19, 1.58 [31]	1.05 ± 0.28, 0.62 [15]
	*GSTA1 *A/*A*	1.87 ± 0.31, 1.68 [23]	1.11 ± 0.18, 0.89 [20]
	*A/*B + *B/*B*	1.63 ± 0.28, 1.39 [20]	1.20 ± 0.33, 0.99 [12]
	*GSTM1 *1/*	1.79 ± 0.37, 1.58 [15]	1.06 ± 0.28, 0.78 [12]
	*2/*2	1.74 ± 0.26, 1.59 [28]	1.19 ± 0.20, 1.01 [20]
	*GSTT1 *1/*	1.68 ± 0.25, 1.42 [30]	1.27 ± 0.20, 0.95 [24]
	*2/*2	1.95 ± 0.40, 2.05 [13]	0.76 ± 0.21, 0.71 [8]

Values were expressed as mean ± S.E., median [*n*].
* Statistically significant difference at $P < 0.05$ between wild type and variant type in the same children group.

From our questionnaire, schoolchildren have to travel between home and school 10–20 min on average or 20–30 min during the rush hour, potentially increasing their the exposure to benzene and other ambient air pollutants, and thus increasing the risk of developing adverse health effects due to environmental exposure to genotoxic compounds. Blood and urinary unmetabolized benzene levels in schoolchildren correlated with personal exposure levels of benzene, however poor correlation between blood and urinary benzene was found. In Bangkok schoolchildren, higher levels of unmetabolized benzene confirm a greater level of benzene exposure. Sexton et al. [40] reported that blood benzene level in children was comparable with those measured in adults who were exposed to similar levels. Urinary MA that was significantly higher in samples of Bangkok schoolchildren collected three times: day 0, morning, day 0, afternoon; and day 1, morning ($P < 0.001$) confirmed higher exposure levels in these children at all times i.e., in the school and in their home environment. Moreover, a significantly higher level of benzene exposure in Bangkok schoolchildren in the morning (day 0) would seem to indicate exposure to benzene was occurring outside of school, e.g. on the way to school. However, outliers were found in some schoolchildren who had a long transportation time to school. The formation of urinary MA in the body is believed to be mainly through benzene metabolism with a possible small contribution from metabolism of sorbic acid from foods; thus urinary MA is fairly specific to benzene exposure [41]. The low

background levels of urinary MA in the rural schoolchildren demonstrated that the contribution to MA formation from other sources was minor. In order to evaluate the confounding effects of smoking which contribute to benzene exposure, cotinine, a biomarker for nicotine was measured in all urine samples. The results indicated that schoolchildren in Bangkok and in the rural areas did not differ with regard to cotinine levels (data not shown).

Oxidative damage to DNA may indicate an increased risk of cancer and it is associated with exposure to polluted urban air [42]. The main pathway of benzene toxicity is thought to involve redox cycling of quinones which induce oxidative damage in particular to DNA in the bone marrow [43]. Our results demonstrated that oxidative damage to DNA was significantly higher in Bangkok schoolchildren compared with the rural schoolchildren, suggesting that urban children were exposed to higher level of polluted air. The concentration of 8-OHdG in leukocytes correlated well with benzene exposure level ($r_s = 0.411$, $P = 0.000$), suggesting that exposure to benzene in schoolchildren may contribute to oxidative damage to DNA. This relationship appeared to be stronger in subjects with the *GSTM1*2/*2* genotype. This finding was in accordance with some previous reports which indicate a significant increase in 8-OHdG, and DNA strand breaks in subjects exposed to environmental benzene in urban areas [13,44]. Furthermore, an experimental study found increased 8-OHdG levels in the bone marrow after benzene administration [45]. Resulting oxidative damage to DNA may be important in benzene exposure related carcinogenesis as the DNA base lesions such as 8-OHdG are highly mutagenic [14]. An earlier study by Tuntawiroon et al. [46] on children attending the same schools as in this study demonstrated that levels of DNA strand breaks and the number of radiation-induced aberrant chromosomes in Bangkok schoolchildren were about 1.5-fold higher than those in rural schoolchildren. Bangkok schoolchildren may be more susceptible to genetic damage, a potential first step in carcinogenesis due to greater exposure to genotoxic compounds in polluted air. Other urban air pollutants may contribute to the damage to DNA such as PM 2.5 [44]. Although measurement of 8-OHdG in urine to represent oxidative damage in DNA offers some advantages due to the fact that these assays are non invasive, involve no artefactual formation of 8-OHdG during the sample preparation, and do not degrade through incubation in urine [15], our results showed that there was no correlation between the 8-OHdG levels in leukocytic DNA and in urine. This is in agreement with Foksinski et al. [47] who found the lack of correlation between biomarkers of oxidative damage in leukocyte and urine among 81 healthy subjects [47].

An individual's health risk from exposure to environmental toxicants is modified by several host susceptibility factors which are based on genetic polymorphisms and environmental influences. In the present study, it was found that Bangkok schoolchildren who carried **5* allele of *CYP2E1* had a higher level of blood benzene than those with the *CYP2E1*1/*1* genotype, but this was not statistically significant. The results were similar to those found in the study in gasoline service attendants [48]. There was no effect of the polymorphisms on the urinary MA, except for *GSTM1* polymorphism. Schoolchildren who carry the *GSTM1*1/* genotype excreted higher concentrations of urinary MA than schoolchildren who carry *GSTM1*2/*2* genotype, suggesting that *GSTM1*2/*2* genotype may be a susceptibility factor in children who are exposed regularly to environmental benzene. These results were in accordance with the study of Knudsen et al. [49] who found that *GSTM1*2/*2* genotype in bus drivers and postal workers associated with reduced detoxification ability. However, it is difficult to explain how *GST* genotype could have a direct effect on urinary MA excretion, as there is no obvious role for *GST* in the formation of urinary MA, but in a competing pathway the excretion of *S*-phenyl mercapturic acid, that is considered a minor metabolite.

Genetic polymorphism of *CYP2E1*, *NQO1* and *GSTs* did not have any significant effect on 8-OHdG level. This finding is in accordance with other studies [50,51], who reported that the *GSTM1* and *GSTT1* genotypes did not significantly influence the 8-OHdG level. However, our results were not in agreement with those reported by Sorensen et al. [35] who has shown significantly higher levels of oxidative damage and excretion of urinary MA in university students carrying at least one *NQO1*2* allele compared with the *NQO1*1/*1* genotype. This may be due to a difference in level of exposure and other genetic polymorphisms among Thai children which are important factors determining benzene metabolism pathways.

In conclusion, our results show that children living in the urban areas of high traffic density are exposed to benzene at levels higher than those living in rural areas. Exposure to higher levels of benzene in urban children would contribute to oxidative DNA damage, suggesting an increased health risk in these children. However, oxidative damage could also be induced by numerous environmental pollutants besides benzene (i.e., PAHs, and 1,3-butadienes). Young people are very vulnerable to many environmental carcinogens and their protection is

an important public health challenge. Future assessment of health risk among children should take into consideration co-exposures as well as interactions among exposure of the other air pollutants.

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References

[1] M. Ruchirawat, P. Navasumrit, D. Settachan, J. Tuntaviroon, N. Buthbumrung, S. Sharma, Measurement of genotoxic air pollutant exposures in street vendors and school children in and near Bangkok, Toxicol. Appl. Pharmacol. 206 (2005) 207–214.

[2] P. Navasumrit, S. Chanvaivit, P. Intarasunanont, M. Arayasiri, N. Lauhareungpanya, V. Parnlob, D. Settachan, M. Ruchirawat, Environmental and occupational exposure to benzene in Thailand, Chem. -Biol. Interact. 30 (153–154) (2005) 75–83.

[3] F. Meneses, I. Romieu, M. Ramirez, S. Colome, K. Fung, D. Ashley, M.H. Avila, A survey of personal exposures to benzene in Mexico city, Arch. Environ. Health 54 (1999) 359–363.

[4] R. Snyder, T. Chepiga, C.S. Yang, Benzene metabolism by reconstituted cytochromes P450, 2B1, and 2E1 and its modulation by cytochrome b5, microsomal epoxide hydrolase, and glutathione transferases: evidence for an important role of microsomal epoxide hydrolase in the formation of hydroquinone, Toxicol. Appl. Pharmacol. 122 (1993) 172–181.

[5] P. Crosignani, A. Tittarelli, A. Borgini, T. Codazzi, A. Rovelli, E. Porro, P. Contiero, N. Bianchi, G. Tagliabue, R. Fissi, F. Rossitto, F. Berrino, Childhood leukemia and road traffic: a population-based case-control study, Int. J. Cancer 10 (2004) 596–599.

[6] O. Raaschou-Nielsen, O. Hertel, B.L. Thomsen, J.H. Olsen, Air pollution from traffic at the residence of children with cancer, Am. J. Epidemiol. 153 (2001) 433–443.

[7] E.G. Knox, E.A. Gilman, Hazard proximities of childhood cancers in Great Britain from 1953–80, J. Epidemiol. Commun. Health 51 (1997) 151–159.

[8] S. Ghittori, M.L. Fiorentino, L. Maestri, Urinary excretion of unmetabolized benzene as an indicator of benzene exposure, J. Toxicol. Environ. Health 38 (1993) 233–243.

[9] R.R. Lauwerys, J.P. Buchet, F. Andrien, Muconic acid in urine: a reliable indicator of occupational exposure to benzene, Am. J. Ind. Med. 25 (1994) 297–300.

[10] S. Ghittori, L. Maestri, M.L. Fiorentino, M. Imbriani, Evaluation of occupational exposure to benzene by urinalysis, Int. Arch. Occup. Environ. Health 67 (1995) 195–200.

[11] F.L. Ayi, T.A. Mobio, E.E. Creppy, B. Fayomi, S. Fustoni, P. Moller, S. Kyrtopoulos, P. Georgiades, S. Loft, A. Sanni, H. Skov, S. Ovrebo, H. Autrup, Survey of air pollution in Cotonou, Benin—air monitoring and biomarkers, Sci. Total Environ. 358 (2005) 85–96.

[12] J. Wan, H.J. Badham, L. Winn, The role of c-MYP in benzene-initiated toxicity, Chem. -Biol. Interact. 153/154 (2005) 171–178.

[13] P.H. Avogbe, L. Ayi-Fanou, H. Autrup, S. Loft, B. Fayomi, A. Sanni, P. Vinzents, P. Møller, Ultrafine particulate matter and high-level benzene urban air pollution in relation to oxidative DNA damage, Carcinogenesis 26 (2005) 613–620.

[14] S. Loft, H.E. Poulsen, Cancer risk and oxidative DNA damage in man, J. Mol. Med. 74 (1996) 297–312.

[15] M.K. Shigenaga, C.J. Gimeno, B.N. Ames, Urinary 8-hydroxy-2-deoxyguanosine as a biological marker of in vivo oxidative DNA damage, Proc. Natl. Acad. Sci. U.S.A. 86 (1989) 9697–9701.

[16] J.A. Staessen, T. Nawrot, E.D. Hond, L. Thijs, R. Fagard, K. Hoppenbrouwers, G. Koppen, V. Nelen, G. Schoeters, D. Vanderschueren, E.V. Hecke, L. Verschaeve, R. Vlietinck, H.A. Roels, Renal function, cytogenetic measurements, and sexual development in adolescents in relation to environmental pollutants: a feasibility study of biomarkers, Lancet 357 (2001) 1660–1669.

[17] L. Calderon-Garciduenas, L. Wen-Wang, Y.J. Zhang, A. Rodriguez-Alcaraz, N. Osnaya, A. Villarreal-Calderón, R.M. Santella, 8-Hydroxy-2′-deoxyguanosine, a major mutagenic oxidative DNA lesion, and DNA strand breaks in nasal respiratory epithelium of children exposed to urban pollution, Environ. Health Perspect. 107 (1999) 469–474.

[18] A. Puga, D.W. Nebert, R.A. Mckinnon, A.G. Menon, Genetic polymorphisms in human drug-metabolizing enzymes: potential uses of reverse genetics to identify genes of toxicological relevance, Crit. Rev. Toxicol. 27 (1997) 199–222.

[19] A. Verdina, R. Galati, G. Falasca, S. Ghittori, M. Imbriani, F. Tomei, L. Marcellini, A. Zijno, V.D. Vecchio, Metabolic polymorphism and urinary biomarkers in subjects with low benzene exposure, J. Toxicol. Environ. Health A 64 (2001) 607–618.

[20] J. Wan, J. Shi, L. Hui, D. Wu, X. Jin, N. Zhao, W. Huang, Z. Xia, G. Hu, Association of genetic polymorphisms in *CYP2E1*, *MPO*, *NQO1*, *GSTM1*, and *GSTT1* genes with benzene poisoning, Environ. Health Perspect. 110 (2002) 1213–1218.

[21] H. Autrup, Genetic polymorphisms in human xenobiotica metabolizing enzymes as susceptibility factors in toxic response, Mutat. Res. 464 (2000) 65–76, Review.

[22] NIOSH, NIOSH Manual of Analytical Methods No. 1501: Hydrocarbons, Aromatic, 2003 (Cited May 10, 2007). Available from: URL: http://www.cdc.gov/niosh/nmam/pdfs/1501.pdf.

[23] E. Schimming, K. Levsen, C. Köhme, W. Schürman, Biomonitoring of benzene and toluene in human blood by headspace solid phase microextraction, Fresnius J. Anal. Chem. 363 (1999) 88–91.

[24] G. Scherer, 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.

[25] D.L. Ashley, M.A. Bonin, F.L. Cardinali, J.M. McCraw, J.S. Holler, L.L. Needham, D.G. Patterson, Determining volatile organic compounds in human blood from a large sample population by using purge and trap gas chromatography–mass spectrometry, Anal. Chem. 64 (1992) 1021–1029.

[26] T. Takeuchi, M. Nakajima, Y. Ohta, K. Mure, T. Takeshita, K. Morimoto, Evaluation of 8-hydroxydeoxyguanosine, a typical oxidative DNA damage, in human leukocytes, Carcinogenesis 15 (1994) 1519–1523.

[27] D. Germadnik, A. Pilger, H.W. Rudiger, Assay for the determination of urinary 8-hydroxy-2′-deoxy guanosine by high-performance liquid chromatography with electrochemical detection, J. Chromatogr. B Biomed. Sci. Appl. 21 (1997) 399–403.

[28] S. Loft, K. Vistisen, M. Ewertz, A. Tjønneland, K. Overvad, H.E. Poulsen, Oxidative DNA damage estimated by 8-hydroxydeoxyguanosine excretion in humans: influence of smoking, gender and body mass index, Carcinogenesis 13 (1992) 2241–2247.

[29] S.I. Hayashi, J. Watanabe, K. Kawajiri, Genetic polymorphism in 5′-flanking region change transcriptional regulation of the human cytochrome P450 IIE1 gene, J. Biochem. 110 (1991) 559–565.

[30] H. Chen, A. Lum, A. Seifried, L.R. Wilken, L.L. Marchand, Association of the NAD(P)H: quinone Oxidoreductase $^{609}C \rightarrow T$ polymorphism with disease lung cancer risk, Cancer Res. 59 (1999) 3045–3048.

[31] B.F. Coles, F. Morel, C. Rauch, W.W. Huber, M. Yang, C.H. Teitel, B. Green, N.P. Lang, F.F. Kadlubar, Effect of polymorphism in the human glutathione *S*-transferase A1 promoter on hepatic *GSTA1* and *GSTA2* expression, Pharmacogenetics 11 (2001) 663–669.

[32] H. Okkels, T. Sigsgaard, H. Wolf, H. Autrup, Glutathione *S*-transferase mu as a risk factor in bladder tumours, Pharmacogenetics 6 (1996) 251–256.

[33] H.H. Nelson, J.K. Wiencke, D.C. Christiani, T.J. Cheng, Z.F. Zuo, B.S. Schwartz, B.K. Lee, M.R. Spitz, M. Wang, X. Xu, Ethnic differences in the prevalence of the homozygous detected genotype of glutathione *S*-transferase theta, Carcinogenesis (Lond.) 16 (1995) 1243–1245.

[34] S. Fustinoni, D. Consonni, L. Campo, M. Buratti, A. Colombi, A.C. Pesatori, Monitoring low benzene exposure: comparative evaluation of urinary biomarkers, influence of cigarette smoking, and genetic polymorphisms, Cancer Epidemiol. Biomarkers Prev. 14 (2005) 2237–2244.

[35] M. Sorensen, H. Skov, H. Autrup, O. Hertel, S. Loft, Urban benzene exposure and oxidative DNA damage: influence of genetic polymorphisms in metabolism genes, Sci. Total Environ. 20 (309) (2003) 69–80.

[36] E. Ortiz, E. Alemon, D. Romero, J.L. Arriaga, P. Olaya, F. Guzman, et al., Personal exposure to benzene, toluene and xylene in different microenvironment at Mexico city metropolitan zone, Sci. Total Environ. 287 (2002) 241–248.

[37] A.K. Raut, Position Paper on Benzene Concentration in Kathmandu's air, Clean Energy Nepal (CEN), 2002 (Cited January 20, 2007). Available from: URL: http://www.adb. org/Vehicle-Emissions/NEP/docs/Benzene_position.pdf.

[38] A.R. Bahrami, Distribution of volatile organic compounds in ambient air of Tehran, Arch. Environ. Health 56 (2001) 380–383.

[39] G. Baumbach, U. Vogt, K.R.G. Hein, A.F. Oluwole, O.J. Ogunsola, H.B. Olaniyi, et al., Air pollution in a large tropical city with a high traffic density: results of measurements in Lagos, Nigeria, Sci. Total Environ. 169 (1995) 25–31.

[40] K. Sexton, J.L. Adgate, T.R. Church, D.L. Ashley, L.L. Needham, G. Ramachadran, et al., Children's exposure to volatile organic compounds as determined by longitudinal measurements in blood, Environ. Health Perspect. 113 (2005) 342–349.

[41] V.M. Weaver, C.T. Davoli, P.J. Heller, Benzene exposure, assessed by urinary trans, trans-muconic acid, in urban children with elevated blood lead levels, Environ. Health Perspect. 104 (1996) 318–323.

[42] S. Loft, H.E. Poulsen, Markers of oxidative damage to DNA: antioxidants and molecular damage, Methods Enzymol. 300 (1999) 166–184.

[43] J. Tuo, X. Deng, S. Loft, H.E. Poulsen, Dexamethasone ameliorates oxidative DNA damage induced by benzene and LPS in mouse bone marrow, Free Radic. Res. 30 (1999) 29–36.

[44] M. Sørensen, H. Autrup, O. Hertel, H. Wallin, L.E. Knudsen, S. Loft, Personal exposure to PM 2.5 and biomarkers of DNA damage, Cancer Epidemiol. Biomarkers Prev. 12 (2003) 191–196.

[45] J. Tuo, S. Loft, M.S. Thomsen, H.E. Poulsen, Benzene-induced genotoxicity in mice in vivo detected by the alkaline comet assay: reduction by *CYP2E1* inhibition, Mutat. Res. 368 (1996) 213–219.

[46] J. Tuntawiroon, C. Mahidol, P. Navasumrit, H. Autrup, M. Ruchirawat, Increased health risk in Bangkok children exposed to polycyclic aromatic hydrocarbons from traffic-related sources, Carcinogenesis 28 (2007) 816–822.

[47] M. Foksinski, D. Gackowski, R. Rozalski, R. Olinski, Cellular level of 8-oxo-2′-deoxyguanosine in DNA does not correlate with urinary excretion of the modified base/nucleoside, Acta Biochim. Pol. 50 (2003) 549–553.

[48] S. Chanvaivit, P. Navasumrit, P. Hunsonti, H. Autrup, M. Ruchirawat, Exposure assessment of benzene in Thai workers, DNA-repair capacity and influence of genetic polymorphisms, Mutat. Res. 626 (2007) 79–87.

[49] L.E. Knudsen, H. Norppa, M.O. Gamborg, P.S. Nielsen, H. Okkels, H. Soll-Johanning, E. Raffn, H. Järventaus, H. Autrup, Chromosomal aberrations in humans induced by urban air pollution: influence of DNA repair and polymorphisms of glutathione *S*-transferase M1 and *N*-acetyltransferase 2, Cancer Epidemiol. Biomarkers Prev. 8 (1999) 303–310.

[50] M. Sørensen, J. Poole, H. Autrup, V. Muzyka, A. Jensen, L. Loft, L.E. Knudsen, Benzene exposure assessed by metabolite excretion in Estonian oil shale mineworkers: influence of glutathione *S*-transferase polymorphisms, Cancer Epidemiol. Biomarkers Prev. 13 (2004) 1729–1735.

[51] B. Marczynski, H.P. Rihs, B. Rossbach, J. Holzer, J. Angerer, M. Scherenberg, G. Hoffmann, T. Bruning, M. Wilhelm, Analysis of 8-oxo-7,8-dihydro-2′-deoxyguanosine and DNA strand breaks in white blood cells of occupationally exposed workers: comparison with ambient monitoring, urinary metabolites and enzyme polymorphisms, Carcinogenesis 23 (2002) 273–281.