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Low level occupational exposure to styrene: Its effects on DNA damage and DNA repair

Sirilak Wongvijitsuk[a,b,c], Panida Navasumrit[a,c], Udomratana Vattanasit[a,b,c], Varabhorn Parnlob[a], Mathuros Ruchirawat[a,b,c,d,*]

[a] Laboratory of Environmental Toxicology, Chulabhorn Research Institute, Bangkok 10210, Thailand
[b] Center of Excellence on Environmental Health, Toxicology and Management of Chemicals (ETM), Bangkok 10400, Thailand
[c] Inter-University Program in Environmental Toxicology, Technology and Management, Chulabhorn Research Institute, Asian Institute of Technology and Mahidol University, Thailand
[d] Department of Pharmacology, Faculty of Science, Mahidol University, Phayathai, Bangkok, Thailand

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ABSTRACT

The present study aimed to evaluate the effects of styrene exposure at levels below the recommended standards of the Threshold Limit Value (TLV-TWA_8) of 20 ppm (ACGIH, 2004) in reinforced-fiberglass plastics workers. Study subjects comprised 50 exposed workers and 40 control subjects. The exposed workers were stratified by styrene exposure levels, i.e. group I (<10 ppm, <42.20 mg/m^3), group II (10–20 ppm, 42.20–84.40 mg/m^3), and group III (>20 ppm, >84.40 mg/m^3). The mean styrene exposure levels of exposed workers were significantly higher than those of the control workers. Biomarkers of exposure to styrene, including blood styrene and the urinary metabolites, mandelic acid (MA) and phenylglyoxylic acid (PGA), were significantly increased with increasing levels of styrene exposure, but were not detected in the control group. DNA damage, such as DNA strand breaks, 8-hydroxydeoxyguanosine (8-OHdG), and DNA repair capacity, were used as biomarkers of early biological effects. DNA strand breaks and 8-OHdG/10^5 dG levels in peripheral leukocytes of exposed groups were significantly higher compared to the control group ($P < 0.05$). In addition, DNA repair capacity, determined by the cytogenetic challenge assay, was lower in all exposed groups when compared to the control group ($P < 0.05$). The expression of *CYP2E1*, which is involved in styrene metabolism, in all styrene exposed groups, was higher than that of the control group at a statistically significant level ($P < 0.05$). Levels of expression of the DNA repair genes *hOGG1* and *XRCC1* were significantly higher in all exposed groups than in the control group ($P < 0.05$). In addition to styrene contamination in ambient air, a trace amount of benzene was also found but, the correlation between benzene exposure and DNA damage or DNA repair capacity was not statistically significant. The results obtained from this study indicate an increase in genotoxic effects and thus health risk from occupational styrene exposure, even at levels below the recommended TLV-TWA_8 of 20 ppm.

Introduction

Styrene is an aromatic hydrocarbon and a monomer used in the production of a variety of polymers and rubbers. The major uses of styrene are in the reinforced-fiberglass plastics industry, the butadiene–styrene rubber industry, and in polystyrene manufacturing.

Styrene, a known mutagen and possible human carcinogen (group 2B; IARC, 1994), requires metabolic activation to exert genotoxic and carcinogenic effects. Styrene is metabolized by cytochrome P450 2E1 (*CYP2E1*) to styrene-7,8-oxide (SO), which is further metabolized to mandelic acid (MA) and phenylglyoxylic acid (PGA) as the major end products (Bardodej and Bardodejova, 1970). MA and PGA are the main urinary metabolites that are used as biomarkers of exposure in biological monitoring of styrene exposure (Apostoli et al., 1983; De Rosa et al., 1993). Reactive oxygen species (ROS) can be generated during the metabolic activation of *CYP2E1* through SO. ROS can cause a variety of genotoxic effects, such as formation of DNA adducts, e.g. 8-hydroxydeoxyguanosine (8-OHdG) (Marczynski et al., 1997), as well as DNA strand breaks, sister-chromatid exchanges, micronuclei and chromosomal aberrations in human lymphocytes (Migliore et al., 2006; Vodicka et al., 2004). ROS can also cause lipid peroxidation, protein oxidation and alter defense mechanisms such as the antioxidant system and DNA repair process. DNA strand breaks and oxidative DNA damage, such as 8-hydroxydeoxyguanosine (8-OHdG), can be used as biomarkers

* Corresponding author at: Laboratory of Environmental Toxicology, Chulabhorn Research Institute, Vipavadee Rangsit Highway, Lak Si, Bangkok 10210, Thailand. Tel.: +66 2 574 0615; fax: +66 2 574 0616.
E-mail address: mathuros@cri.or.th (M. Ruchirawat).

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of early biological effects (De Zwart et al., 1998). XRCC1 is required for the repair of DNA single strand breaks (Taylor et al., 2002) which, in mammalian cells, involves four basic steps, i.e. damage detection, end processing, gap filling and DNA ligation (Caldecott, 2007). When DNA single strand breaks are present, poly [ADP-ribose] polymerase 1 (PARP-1) rapidly binds to DNA strand breaks and is activated. This step is required for cellular single strand breaks (SSBs) to recruit, stabilize or accumulate the scaffold protein, X-ray repair cross-complementing group I (XRCC1), which then mediates multiple interactions with enzymatic components of the repair process. XRCC1 appears to interact with APE1/DNA polymerase β and DNA polynucleotide kinase (PNK)/PCNA, which play important roles in end processing and gap filling, respectively. XRCC1 also interacts with DNA ligase IIIa, which seals single nucleotide, nicks in the process of base excision repair (BER) (Petermann et al., 2006). In the case of oxidative damage, 8-OHdG has its own glycosylase, 8-oxoguanine glycosylase (OGG1), which is involved in base excision repair of 8-oxoguanine caused by the effects of ROS on DNA (Memisoglul and Samson, 2000). Alterations in the expression of these repair genes can affect the ability of cells to repair DNA damage. Inefficient or incorrect repair of DNA damage can eventually lead to mutagenesis and carcinogenesis.

In the present study, we aimed to evaluate the effects of styrene exposure on DNA damage and DNA repair in workers in a reinforced-fiberglass plastics factory through the use of various biomarkers. The biomarkers of exposure to styrene employed in this study included unmetabolized styrene in blood, and the urinary metabolites PGA and MA. The potential health effects from exposure to styrene were assessed through DNA damage levels (DNA strand breaks and 8-OHdG) and DNA repair capacity. Styrene has been shown to induce xenobiotic metabolizing enzymes (Marczynski et al., 1997). We therefore investigated the effect of such exposure on the expression of genes associated with the metabolism, such as *CYP2E1*, that subsequently lead to oxidative DNA damage, as well as genes associated with DNA repair, such as *XRCC1* and *hOGG1*. Since benzene is a precursor for styrene in resin production, the reinforced-fiberglass plastics workplace may be contaminated with benzene, therefore benzene exposure levels in these workers was also investigated and assessed as to whether trace levels of benzene could also contribute to DNA damage caused by styrene exposure at levels below the occupational standard setting (TLV-TWA_8 of 20 ppm) (ACGIH, 2004).

Materials and methods

Study location and subjects

The study was conducted in a styrene reinforced-fiberglass plastics factory. The job related activities of the exposed workers included resin laminating and trimming. This factory produces many kinds of products, such as parts for ventilation hoods, fiberglass roofs, storage tanks, boats, and vehicle parts. The working day is 8 h per day and 5 days per week.

The exposed population consisted of 50 workers at a reinforced-fiberglass plastics factory and 40 control subjects recruited from a mail sorting service center located about 5 km from the main road and who should therefore not be occupationally exposed to styrene. These workers were requested to complete a questionnaire about personal information, work history, accommodation, diet and life style, such as alcohol consumption and smoking habits. The average age of study subjects was 36.5 years (31–40 years) for exposed workers and 33.6 years (21–45 years) for control workers. All participants were healthy and non-smokers. Consumption of coffee and alcohol, were not statistically different among groups. The local ethics committee, in accordance with the Helsinki declaration, approved the study and the volunteers signed an informed consent form prior to the study.

Sample collection

A preliminary study was conducted to determine if there was any variation in the concentrations of styrene and benzene in ambient air during the work week. Air sampling was conducted for 8 h (8.00 a.m. to 4.00 p.m.) at various locations on working days: Monday, Wednesday and Friday of the work week. This factory is closed on the weekend. The data from the preliminary study showed that the mean ambient levels of styrene and benzene in the workplace at the same locations were not significantly different from day to day. Therefore, in the subsequent study air and biological sample collection was conducted on the first working day of the week. Sample collection consisted of air sampling, both ambient and individual, throughout the 8 h work shift. Biological samples, including urine and blood, were collected on the same day as individual air samples.

Ambient air samples were collected into Tenex® tubes attached to personal air samplers at a flow rate of 200 ml/min according to NIOSH 1514 (OSHA, 1980). Air sampling was carried out throughout the entire 8 h work shift. After sampling was completed, the Tenex® tube samples were capped, transported to the laboratory, and stored at −20 °C until analysis.

Individual air samples were collected in Tenex® tubes attached near the breathing zone of subjects for an entire work shift (8 h). After air sampling was completed, the Tenex® tubes were capped, transported to the laboratory, and stored at −20 °C until analysis.

Urine samples were collected from workers before (pre-shift) and after (post-shift) the work shift and stored at −20 °C until the analysis. Whole blood samples (25 ml) were collected after the work shift and were processed for DNA strand breaks, lymphocyte isolation and DNA extraction immediately upon arrival at the laboratory.

Measurement of styrene and benzene in air samples

The Tenex® tubes were then subjected to the thermal desorption unit and analyzed by gas chromatography equipped with flame ionization detector (GC-FID, Agilent model 5975C, USA). Styrene and benzene were desorbed from the adsorbent media with a thermal desorption unit that was heated at 250 °C for 10 min, and analyzed by GC with the following conditions: 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. Calibration curves for styrene and benzene were generated with various concentrations of standard solutions of styrene and benzene. Peak areas of styrene and benzene obtained from the chromatograms of various standard levels were plotted between peak area and concentration of standards. Concentrations of styrene or benzene in the air samples were determined from the calibration curve and expressed as ppm for styrene and ppb for benzene. The detection limit was 3.245 ppb for styrene and 0.355 ppb for benzene. The linearity of the calibration curve was 0.9994 for styrene and 0.9990 for benzene.

Measurement of blood styrene and benzene

Venous blood samples were collected in tubes containing EDTA and stored at 4 °C until analysis. Concentrations of styrene or benzene in blood samples were measured by solid phase micro extraction (SPME) and detection was carried out through gas chromatography–mass spectrometry (GC–MS, Agilent model 6890N, USA). Analysis was completed within 24 h after sample collection according to the method previously described (Ruchirawat et al., 2005). The detection limit was 0.144 ppb for styrene and

1.655 ppt for benzene. The linearity of the calibration curve was 0.9985 for styrene and 0.9930 for benzene.

Measurement of urinary metabolites and creatinine

Measurement of mandelic acid (MA) and phenylglyoxylic acid (PGA)

Urine samples were thawed at room temperature for 15 min with frequent stirring, and then centrifuged at 3000 × *g* for 10 min. An aliquot of 20 μl of 6 N HCl was added for acidification followed by extraction with 800 μl ethyl acetate. Subsequently, the extracts (0.5 ml) was transferred to a glass tube and dried. After drying, the residue was resuspended with 0.5 ml of the mobile phase (30% methanol in water containing 1% acetic acid) The suspension was filtered and analyzed by high performance liquid chromatography (HPLC, Agilent model 1100 series, USA) equipped with a UV detector. A variable wavelength UV detector was used for determination of MA at 225 nm and PGA at 254 nm. Chromatography was performed using a LUNA 5 μm C18(2), 150 mm × 4.60 mm, column type (Phenomenex, USA). The detection limit was 33.24 μg/l for both MA and PGA. The linearity of the calibration curve was 0.9992 for MA, and 0.9999 for PGA, respectively. The concentrations of MA and PGA were expressed as μg/l and then converted to mg/g creatinine.

Measurement of trans-trans-muconic acid (t,t-MA)

Urine samples were thawed at room temperature for 15 min with frequent string, and then centrifuged at 3000 × *g* for 10 min. Aliquots of 2 ml alkalinized urine were applied to a strong anion exchange (SAX) column and sequentially washed with 2 ml H_2O and 1 ml 1% (v/v) acetic acid. The *t,t*-MA acid was eluted with 1 ml 10% (v/v) aqueous acetic acid and then analyzed by HPLC equipped with a UV detector as previously described (Ruchirawat et al., 2005). The detection limit was 3.309 μg/l. The linearity of the calibration curve was 0.9998. The concentrations of *t,t*-MA was expressed as μg/l and then converted to mg/g creatinine.

Measurement of S-phenylmercapturic acid (S-PMA)

Urinary *S*-PMA was analyzed using high performance liquid chromatography equipped with a triple quadrupole mass spectrometer (HPLC/MS–MS). The urine samples were thawed and centrifuged at 2500×g for 5 min. A fraction of 5 ml of supernatant was acidified with 150 μl HCl and extracted with 6 ml ethyl acetate. After centrifugation at 4000 rpm for 5 min, the extracted fraction was collected and dried using a speedvac concentrator. The residue was dissolved in a solution containing 1% acetic acid and methanol (50:50, v/v), filtered, and then analyzed by HPLC (Agilent model 1100 series, USA) equipped with a reverse-phase column and mass spectrometry (Micromass Quattro micro™ API, Micromass, Waters Ltd., United Kingdom) as previously described (Navasumrit et al., 2008). The detection limit was 0.106 μg/l. The linearity of the calibration curve was 0.9978. The concentrations of *S*-PMA was expressed as μg/l and then converted to μg/g creatinine.

Measurement of urinary creatinine

Urinary creatinine levels were measured using the picric acid reaction in alkaline conditions (Sigma Diagnostics kit 555-A, Sigma–Aldrich, St. Louis, MO, USA).

Measurement of 8-hydroxydeoxyguanosine (8-OHdG) and deoxyguanosine (dG)

Genomic DNA was isolated from peripheral blood by using the NucleoSpins® Blood XL kit according to the recommendations of the manufacturer. Analysis of leukocyte DNA was carried out as previously described (Sudprasert et al., 2006). Briefly, 60 μg of DNA sample was incubated with 8 U 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 μm syringe filter before analysis. The levels of dG and 8-OHdG in DNA hydrolysate were analyzed by HPLC (Agilent model 1100 series) equipped with an electrochemical detector (ECD) (Coulochem III, ESA, Inc., USA). The results are expressed as 8-OHdG per 10^5 dG.

Measurement of DNA strand breaks

DNA strand breaks were determined by way of the alkaline Comet assay, as described in detail elsewhere (Navasumrit et al., 2008). Briefly, 20 μl of whole blood was mixed with LMP agarose, and embedded in an agarose pre-coated slide. Slides were submerged in cold lysis solution for at least 1 h at 4 °C. Subsequently, slides were transferred to an 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 duplicate slides were examined randomly under an epi-fluorescence microscope (Axioplan 2, Zeiss, Germany). The extent of DNA damage was measured quantitatively using the Comet Scan image analysis software (Meta Systems), and expressed as Tail length, Olive tail moment and %DNA in Tail.

Measurement of DNA repair capacity

The DNA repair capacity was determined by using the cytogenetic challenge assay developed by Au (1993) and Au et al. (2003), in which blood cells were irradiated with ionizing radiation to induce chromosome damage and then allowed to repair this damage. The increase in radiation-induced chromosome aberrations, measured as dicentric chromosomes and chromosome deletions per metaphase cells, reflected reduced DNA repair capacity. Experimentally, at 24 h after blood culture, 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 standard procedures. Cytological preparations were made, coded and stained with a 10% Giemsa solution for 15 min. Fifty metaphase cells were analyzed from each of the duplicate slides under a microscope. The presence of radiation-induced dicentric chromosomes and chromosome deletions per metaphase were determined.

In our preliminary investigations, the background level of chromosomal aberration-detected as the frequencies of dicentrics and deletions per metaphase was measured before challenging the cells from styrene-exposed and control workers with gamma radiation. However, the levels of dicentrics and deletions prior to challenging cells with gamma radiation were non-detectable. Therefore, in all subsequent challenge assays, the incidence of dicentrics and deletions was not measured before challenging blood culture with gamma radiation.

Measurement of mRNA gene expression

Lymphocytes were separated using Ficoll-Pague PLUS (Amersham Biosciences) according to the recommendations of the manufacturer with slight modification. The lymphocyte-rich layer was removed and washed in RPMI 1640 medium (Gibco). The concentration of viable cells was established by Trypan blue dye exclusion. Purified lymphocytes were resuspended at 5×10^5 cells/ml, the cell suspension was transferred to cryovials and storage as described previously (Sudprasert et al., 2006).

Table 1
Preliminary study on the ambient levels of styrene and benzene in the reinforced-fiberglass plastics factory.

Fiberglass plastics factory	Date of sampling	Number of sites (no. of sampling point)	Temp (°C)	Relative humidity (%)	Ambient levels of styrene (ppm)	Ambient levels of benzene (ppb)
Resin laminating	Monday	2(6)	30.27	67.82	9.67 ± 1.12 8.34(4.09–16.59)	15.87 ± 1.63 15.87(6.98–25.41)
	Friday	2(6)	30.50	70.20	9.93 ± 1.22 9.14(2.38–18.04)	14.99 ± 1.77 12.35(5.15–30.11)
Trimming process	Monday	3(6)	31.90	67.82	2.94 ± 0.43 2.18(0.32–7.07)	4.21 ± 0.30 4.23(1.69–6.98)
	Wednesday	3(6)	31.70	69.50	2.92 ± 0.26 3.36(0.34–4.66)	5.60 ± 2.55 5.52(1.69–9.79)
	Friday	3(6)	30.50	70.20	2.34 ± 0.28 2.31(0.26–4.72)	6.63 ± 0.69 5.45(0.50–13.14)

The values are expressed as mean ± s.e. on the first line and the median (minimum–maximum) on the second line.

Finally, the supernatant was discharged, and cells were prepared for RNA extraction treatment.

Total mRNA was isolated from 1×10^5 lymphocytes using a Versagene TM kit (cat. no. VGA-0050D, Gentra systems Co., USA), then chloroform extracted, isopropanol precipitated and quantified by measuring its absorbance at 260 nm. Reverse transcription was carried out using 5 ng of random hexamers (Promega) and 5 μl of DNases I treated-RNA in a total volume of 20 μl. Initially, the total RNA was denatured at 65 °C for 10 min and immediately chilled on ice, and then 1 mM dNTPs, 1× ExpandTM reverse transcriptase buffer (Roche), 10 mM dithiothreitol (DTT) solution and 25 U of ExpandTM reverse transcriptase were added into the mixture. First-strand cDNA was obtained after incubation at 30 °C for 10 min and at 42 °C for 48 min, followed by 95 °C for 2 min. Each cDNA was amplified using primers for *CYP2E1*, *hOGG1*, *XRCC1* and beta-actin (β-actin). The mRNA expression was measured by relative quantification real-time PCR using the LightCycle® system (Roche Diagnostics, Mannheim, Germany).

CYP2E1 mRNA expression

Primers sequences for *CYP2E1* were 5′-TTG AAG CCT CTC GTT GAC CC-3′ and 5′-CGT GGT GGG ATA CAG CCA A-3′ (Piton et al., 2005). The PCR reaction was carried out in a final volume of 20 μl, containing 1 μl of 5 μM LC-hCYP2E1-Fs, 1 μl of 5 μM LC-hCYP2E1-Rs, 10 μl of 2× QuantiTect SYBR Green RT-PCR Master Mix and 0.2 μl of QuantiTect RT mix in a 1× PCR buffer. The samples were amplified for 50 cycles at 94 °C for 15 s, 62 °C for 15 s and 72 °C for 20 s. The mRNA expression of *CYP2E1* was normalized to that of β-actin.

hOGG1 mRNA expression

Primers sequences for *hOGG1* were 5′-ACA CTG GAG TGG TGT ACT AGC G-3′and 5′-GCG ATG TTG TTG TTG GAG G-3′ (Jüngst et al., 2004). The PCR reaction was carried out in a final volume of 20 μl, containing 1 μl of 5 μM LC-h hOGG1-Fs, 1 μl of 5 μM LC-hCYP2E1-Rs, 10 μl of 2× QuantiTect SYBR Green RT-PCR Master Mix and 0.2 μl of QuantiTect RT mix in a 1× PCR buffer. The samples were amplified for 50 cycles at 95 °C for 15 s, 64 °C for 15 s and 72 °C for 30 s. The mRNA expression of *hOGG1* was normalized to that of β-actin.

XRCC1 mRNA expression

Primers sequences for *XRCC1* were 5′-CCC CTG AAG AGA CCA AAG CA-3′ and 5′-CCA TTG AAG GCT GTG ACG TA-3′ (Willey et al., 1998). The PCR reaction was carried out in a final volume of 20 μl, containing 0.5 μl of 4 μM LC-hXRCC1-Fs, 1 μl of 4 μM LC-hXRCC1-Rs, 10 μl of 2× QuantiTect SYBR Green RT-PCR Master Mix and 0.2 μl of QuantiTect RT mix in a 1× PCR buffer. The samples were amplified for 45 cycles at 95 °C for 10 s, 60 °C for 25 s and 72 °C for 30 s. The mRNA expression of *XRCC1* was normalized to that of β-actin.

Statistical analysis

The Mann–Whitney *U* test was used to determine non-parametric statistical differences of the test parameters between study groups. The correlations between two variables were determined by Pearson correlation coefficients. A *P*-value less than 0.05 were considered to be statistically significant.

Results

Ambient levels of styrene and benzene in the workplace of reinforced-fiberglass plastics factory

Initially, ambient air monitoring was conducted on Monday, Wednesday and/or Friday of the same working week at various sites in the factory at which temperature, humidity, styrene and benzene levels were measured. The preliminary study showed that the mean concentrations of styrene during the work week at the same location were not significantly different. The mean concentrations of styrene in ambient air at the same location varied slightly between sampling days on Monday and Friday. Ambient levels of styrene were 9.67 ppm (40.81 mg/m^3) on Monday and 9.93 ppm (41.90 mg/m^3) on Friday in resin laminating areas, and 2.94 ppm (12.41 mg/m^3) on Monday, 2.92 ppm (12.32 mg/m^3) on Wednesday and 2.34 ppm (9.87 mg/m^3) on Friday in trimming areas. The benzene concentration in ambient air at the same workplace location varied slightly in the resin laminating areas with the mean levels of 15.87 ppb (50.78 μg/m^3) on Monday and 14.99 ppb (47.97 μg/m^3) on Friday, but the difference was not statistically significant. The mean benzene levels in the trimming area were 4.21 ppb (13.74 μg/m^3) on Monday, 5.60 ppb (17.92 μg/m^3) on Wednesday and 6.63 ppb (21.22 μg/m^3) on Friday (Table 1). Therefore, ambient air and biological sample collection throughout the subsequent study was conducted on the first working day of the week.

The results of this study showed that styrene concentrations in the resin laminating area (6.22 ppm, 26.25 mg/m^3) were 2.2-fold higher than that in the trimming process area (2.78 ppm, 11.73 mg/m^3, $P < 0.05$), while not detectable in the control area. Atmospheric benzene concentrations were in the same range in the resin laminating (5.58 ppb, 17.86 μg/m^3), trimming area (8.78 ppb, 28.10 μg/m^3), and the control workplace area (6.95 ppb, 22.24 μg/m^3) (Table 2).

Exposure to styrene and biomarkers of exposure in workers

The exposed workers were divided into 3 groups based on styrene exposure level: group I (<10 ppm, <42.20 mg/m^3),

Table 2
Ambient levels of styrene and benzene in the reinforced-fiberglass plastics factory.

Workplace	Number of sites (no. of sampling point)	Temp (°C)	Relative humidity (%)	Ambient levels of styrene (ppm)	Ambient levels of benzene (ppb)
Control	8(20)	28.73	60.12	nd	6.95 ± 0.66 6.52(3.08–14.35)
Reinforced-fiberglass factory	16(32)	31.73	69.21	4.55 ± 0.58 4.35(0.23–12.86)	7.38 ± 1.00 5.15(1.24–29.56)
Resin laminating	6(16)	31.27	70.02	6.22 ± 0.79 5.70(2.22–12.86)	5.58 ± 1.24 5.01(1.24–25.17)
Trimming process	10(16)	31.90	67.93	2.78 ± 0.53[a] 2.56(0.32–7.07)	8.78 ± 1.15 7.04(0.71–29.56)

The values are expressed as mean ± s.e. on the first line and the median (minimum–maximum) on the second line. nd: non-detectable.
[a] Statistically significant difference from resin laminating at $P < 0.05$.

Table 3
Styrene exposure levels and biomarkers of styrene exposure in exposed workers.

Parameters	Study groups			
	Control ($n = 40$)	Exposed workers ($n = 50$)		
		Group I <10 ppm styrene ($n = 16$)	Group II 10–20 ppm styrene ($n = 13$)	Group III >20 ppm styrene ($n = 14$)
Individual styrene exposure (ppm)	nd	3.81 ± 0.67 2.77(0.30–7.61)	12.52 ± 1.40[a] 14.36(11.38–19.81)	36.23 ± 3.08[a,b] 32.31(24.26–66.53)
Blood styrene (ppb)	nd	25.33 ± 5.71 14.40(1.24–83.92)	69.79 ± 9.46[a] 70.59(30.06–113.24)	117.43 ± 11.28[a,b] 118.16(63.29–166.30)
Urinary MA (mg/g creatinine)				
Pre-shift	nd	5.97 ± 1.79 4.59(nd to 22.10)	7.26 ± 2.39 3.69(nd to 26.67)	5.56 ± 2.13 nd(nd to 31.30)
Post-shift	nd	26.51 ± 2.39[c] 23.55(6.03–39.43)	56.11 ± 8.55[a,c] 60.10(12.93–84.28)	260.70 ± 43.42[a,b,c] 220.40(112.20–535.10)
Urinary PGA (mg/g creatinine)				
Pre-shift	nd	9.89 ± 2.34 6.78(1.14–30.83)	24.63 ± 8.53[a] 8.87(nd to 95.49)	29.99 ± 8.32[a] 32.36(nd to 132.57)
Post-shift	nd	17.93 ± 10.31[c] 5.71(nd to 21.10)	52.72 ± 10.87[a] 41.90(26.08–97.63)	54.85 ± 7.8[a,c] 45.30(23.52–112.10)

The values are expressed as mean ± s.e. on the first line and the median (minimum–maximum) on the second line. nd: non-detectable.
[a] Statistically significant difference from group I at $P < 0.05$.
[b] Statistically significant difference from group II at $P < 0.05$.
[c] Statistically significant difference from the corresponding pre-shift at $P < 0.05$.

group II (10–20 ppm, 42.20–84.40 mg/m^3) and group III (>20 ppm, >84.40 mg/m^3). The group with low levels of exposure (group I) were workers in the trimming section, while the higher exposed groups (groups II and III) were workers in the resin laminating section. As shown in Table 3, the mean levels of individual styrene exposure in groups I, II and III were 3.81 (16.08 mg/m^3), 12.52 (52.83 mg/m^3) and 36.23 ppm (152.89 mg/m^3), respectively. Resin laminators were exposed to significantly higher levels of styrene than trimming workers ($P < 0.05$). Biomarkers of styrene exposure, including blood styrene and urinary MA and PGA, are shown in Table 3. Blood styrene concentrations were significantly increased with increasing levels of exposure, although the relationship was non-linear. The slope of the concentration–response curve increased sharply from individual styrene exposure levels of 3.81–12.52 ppm (16.08 to 52.83 mg/m^3) ($r = 0.621$), and then appeared to decrease when it reached an exposure level of 12.52–36.23 ppm (52.83–152.89 mg/m^3) ($r = 0.024$), indicating that clearance of styrene from the blood may be enhanced by a possibly increased level of metabolism (Fig. 1). A statistically significant correlation between blood styrene concentration and individual exposure levels was observed in group I ($r = 0.763$, $P < 0.05$) and group II ($r = 0.471$, $P < 0.05$), but not in group III ($r = 0.138$) (data not shown). In the exposed groups, urinary MA levels before the work shift (pre-shift) were not statistically different among exposed groups, but post-shift MA was significantly increased with increasing exposure levels (group I < group II < group III). Post-shift levels of urinary MA increased significantly from the corresponding pre-shift levels, by approximately 4-fold, 7-fold and 46-fold for groups I, II and III, respectively. In the case of urinary PGA, post-shift levels in exposed workers of all groups were significantly increased compared to their corresponding pre-shift level. Styrene exposure and the urinary metabolites were not detected in the control group. Fig. 1 shows the excretion pattern of urinary MA and PGA with

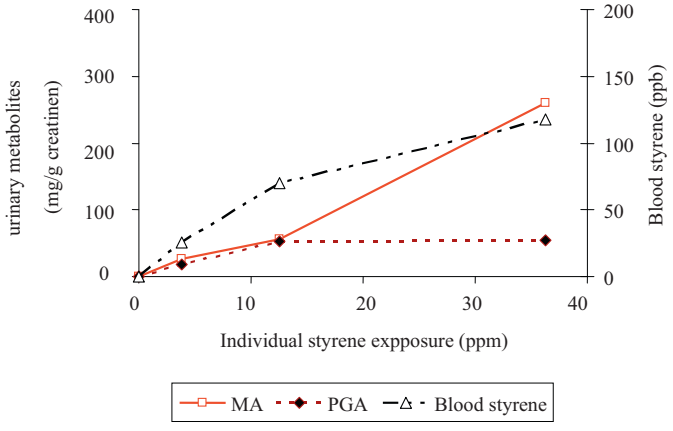

Fig. 1. Each point represents the mean levels of urinary excretion of mandelic acid (MA), phenylglyoxylic acid (PGA) and blood styrene of the control and styrene-exposed groups at the mean level of individual styrene exposure at 3.81, 12.52 and 36.23 ppm (16.08, 52.83, and 152.89 mg/m^3, respectively).

Table 4
Benzene exposure levels and biomarkers of benzene exposure in expose workers.

Parameters	Study groups			
	Control ($n=40$)	Exposed workers ($n=50$)		
		Group I <10 ppm styrene ($n=16$)	Group II 10–20 ppm styrene ($n=13$)	Group III >20 ppm styrene ($n=14$)
Individual benzene exposure (ppb)	6.96 ± 0.66	16.23 ± 2.78[a]	16.13 ± 2.95[a]	18.14 ± 2.67[a]
	6.52(3.08–11.37)	11.70(7.33–47.70)	12.19(4.39–41.20)	17.07(7.01–37.76)
Blood benzene (ppt)	39.48 ± 2.88	93.26 ± 10.52[a]	60.34 ± 5.56[a,b]	54.02 ± 3.96[a,b]
	40.23(9.42–69.77)	86.35(41.53–171.29)	61.84(26.20–81.19)	51.03(29.62–77.85)
Urinary *t*,*t*-MA (mg/g creatinine)				
Pre-shift	0.02 ± 0.01	0.02 ± 0.01	0.02 ± 0.01	0.02 ± 0.01
	0.005(nd to 0.13)	0.04(nd to 0.04)	0.01(nd to 0.06)	0.02(nd to 0.09)
Post-shift	0.04 ± 0.01	0.07 ± 0.02[a]	0.04 ± 0.01[b]	0.03 ± 0.01[b]
	0.02(nd to 0.15)	0.04(0.01–0.20)	0.04(0.01–0.09)	0.03(nd to 0.05)
Urinary *S*-PMA (μg/g creatinine)				
Pre-shift	0.32 ± 0.05	0.33 ± 0.05	0.21 ± 0.04	0.24 ± 0.04
	0.25(0.14–0.69)	0.25(0.10–0.75)	0.157(0.10–0.46)	0.264(0.10–0.45)
Post-shift	0.39 ± 0.03	0.56 ± 0.67[a,c]	0.51 ± 0.06[a,c]	0.48 ± 0.08[a,c]
	0.38(0.21–0.67)	0.51(0.12–0.93)	0.53(0.18–0.74)	0.41(0.21–0.86)

The values are expressed as mean ± s.e. on the first line and the median (minimum–maximum) on the second line. nd: non-detectable.
[a] Statistically significant difference from the corresponding control at $P<0.05$.
[b] Statistically significant difference from group I at $P<0.05$.
[c] Statistically significant difference from the corresponding pre-shift at $P<0.05$.

respect to styrene exposure. Urinary excretion of MA showed a linear response at mean styrene exposures from 3.81 to 12.52 ppm (16.08–52.83 mg/m^3) with a positive correlation ($r=0.492$), and then increased sharply between 12.52 and 36.23 ppm (52.83 to 152.89 mg/m^3) with a better correlation ($r=0.872$). In contrast, the urinary excretion pattern for PGA increased linearly with increasing mean styrene exposures from 3.81 to 12.52 ppm (16.08 to 52.83 mg/m^3) with a correlation ($r=0.721$), and then leveled off slightly toward 36.23 ppm (152.89 mg/m^3) with a lower correlation ($r=0.333$).

Co-exposure to benzene and biomarkers of exposure

The ambient levels of benzene in the factory and in the control workplace did not differ significantly. However, individual exposure to benzene in groups I, II and III was significantly higher than in the control group ($P<0.05$). Blood benzene concentrations of control workers (39.48 ppt) were significantly lower than those of the exposed groups which were 93.26, 60.34 and 54.02 ppt for groups I, II, and III, respectively, at $P<0.05$. While benzene exposure levels were similar in all styrene-exposed groups, blood benzene decreased with increasing levels of styrene exposure. The levels of *t*,*t*-MA, a urinary metabolite of benzene, were the same in all groups before the work shift. After the work shift, only the *t*,*t*-MA levels of group I were significantly different from those of the control group at $P<0.05$. The post-shift levels of urinary *S*-PMA of all exposed groups were significantly higher than those of the control group at $P<0.05$. Post-shift levels of *S*-PMA increased significantly from the corresponding pre-shift levels by approximately 2-fold for exposed workers but levels in the control group were not significantly different from the corresponding pre-shift levels (Table 4).

Effects of styrene exposure on DNA damage and DNA repair capacity

DNA strand breaks and 8-OHdG in peripheral leukocytes, as well as DNA repair capacity in exposed workers are shown in Table 5. The mean levels of DNA strand breaks measured by the comet assay in all exposed groups were significantly increased ($P<0.05$) with

Table 5
DNA damage and DNA repair capacity in exposed workers.

Parameters	Study groups			
	Control ($n=37$)	Exposed workers ($n=43$)		
		Group I <10 ppm styrene ($n=16$)	Group II 10–20 ppm styrene ($n=13$)	Group III >20 ppm styrene ($n=14$)
DNA strand breaks				
Tail length (μm)	1.73 ± 0.09	2.19 ± 0.11[a]	2.63 ± 0.20[a,b]	3.14 ± 0.16[a,b,c]
	1.72(0.63–2.81)	2.24(1.29–2.79)	2.74(1.37–3.94)	3.21(2.19–4.38)
Olive moment (μm)	0.18 ± 0.01	0.22 ± 0.03[a]	0.25 ± 0.03[a,b]	0.36 ± 0.03[a,b,c]
	0.171(0.05–0.33)	0.18(0.12–0.64)	0.24(0.16–0.46)	0.36(0.20–0.56)
Percentage of DNA in Tail	1.48 ± 0.09	1.87 ± 0.27	1.93 ± 0.19[a]	2.65 ± 0.16[a,b,c]
	1.40(0.20–2.84)	1.71(0.52–3.15)	1.79(0.49–3.18)	2.71(1.69–3.77)
8-OHdG(per 10^5 dG)	0.46 ± 0.03	0.97 ± 0.34[a,b]	1.21 ± 0.17[a]	1.41 ± 0.22[a,b,c]
	0.46(0.18–0.85)	0.42(0.07–4.28)	1.25(0.52–2.21)	1.32(0.47–3.58)
DNA repair capacity				
Dicentric/metaphase	0.10 ± 0.004	0.20 ± 0.02[a]	0.20 ± 0.03[a]	0.24 ± 0.03[a]
	0.1(0.08–0.16)	0.18(0.04–0.38)	0.17(0.12–0.40)	0.22(0.10–0.54)
Deletion/metaphase	0.11 ± 0.03	0.18 ± 0.02[a]	0.14 ± 0.03	0.15 ± 0.03
	0.1(0.04–0.28)	0.18(0.06–0.34)	0.11(0.04–0.36)	0.10(0.02–0.40)

The values are expressed as mean ± s.e. on the first line and the median (minimum–maximum) on the second line. nd: non-detectable.
[a] Statistically significant difference from the corresponding control at $P<0.5$.
[b] Statistically significant difference from group I at $P<0.05$.
[c] Statistically significant difference from group II at $P<0.05$.

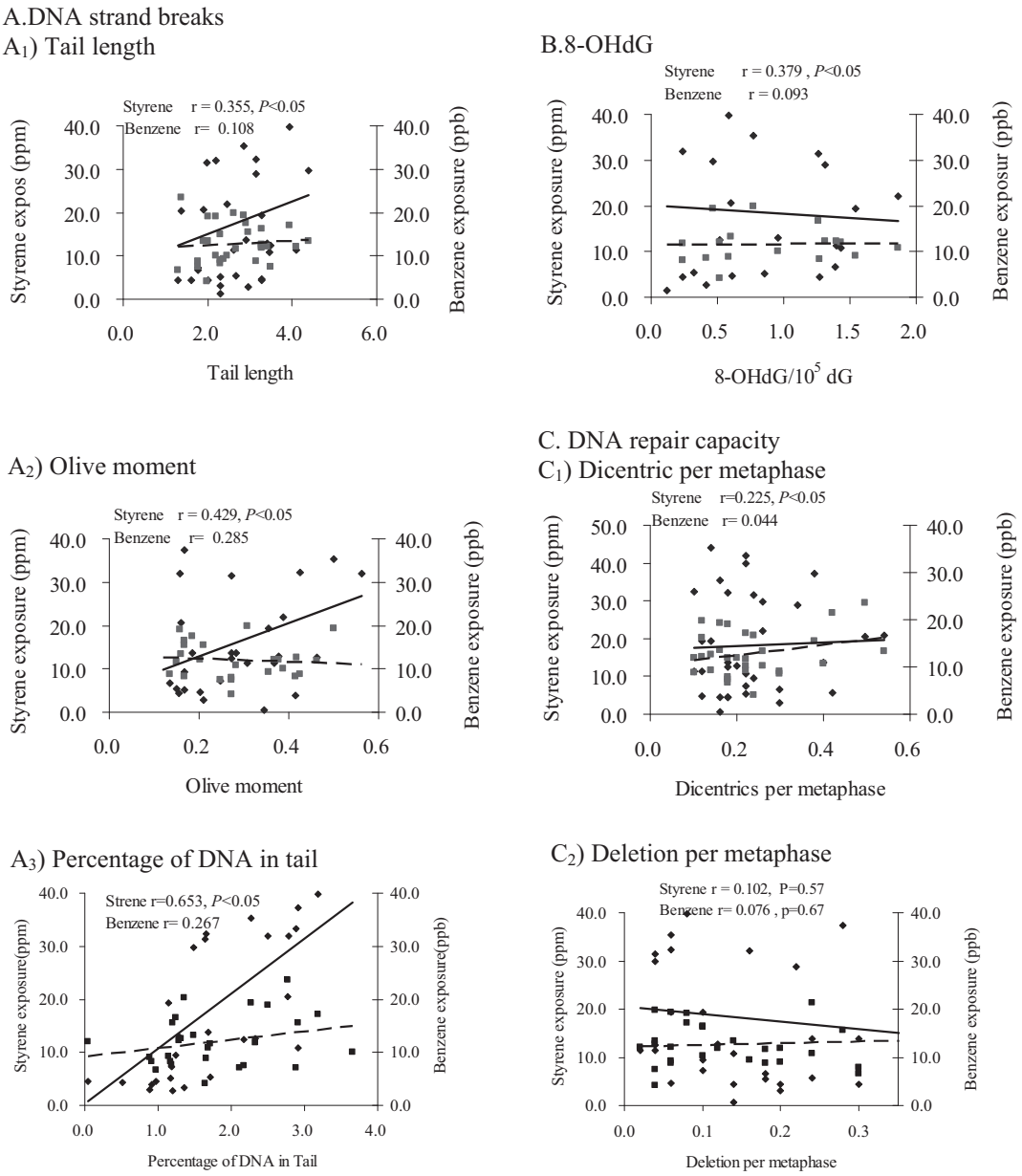

Fig. 2. Pearson correlation coefficients were used to determine the correlation between mean level of styrene exposure (—◊—) or benzene exposure (- - -■- -) and biomarkers of effects including DNA strand breaks (A), 8-OHdG (B) and DNA repair capacity (C). The '*r*' represents the correlation coefficient and '*P*' represents the *P*-value for the correlation.

increasing levels of styrene exposure. Similarly, the levels of 8-OHdG in styrene-exposed groups increased significantly ($P < 0.05$) in a dose dependent manner, by 2.11-, 2.63- and 3.06-fold for groups I, II and III, respectively.

Radiation-induced chromosome aberrations measured as dicentrics and deletions per metaphase in the challenge assay were used to assess DNA repair capacity in study subjects. As shown in Table 5, lower DNA repair capacity, which was observed as an approximately 2-fold increase in the frequency of radiation-induced dicentrics per metaphase ($P < 0.05$), was found in all exposed groups when compared to the controls. The level of radiation-induced chromosome deletions per metaphase was also increased in all styrene-exposed groups but this was statistically significant only in group I (<10 ppm, <42.20 mg/m^3) at $P < 0.05$. Statistical analysis showed that styrene exposure correlated significantly with DNA damage [DNA strand breaks ($r = 0.355$, $P < 0.05$ for Tail length, $r = 0.429$, $P < 0.05$ for Olive moment, $r = 0.653$, $P < 0.05$ for %DNA in tail) and $r = 0.379$, $P < 0.05$ for 8-OHdG]. Benzene exposure did not correlate with DNA strand breaks and 8-OHdG ($r = 0.108$ for Tail length, $r = 0.285$ for Olive moment, $r = 0.267$ for %DNA in tail and $r = 0.093$, for 8-OHdG) (Fig. 2A and B). In the case of DNA repair capacity, the level of dicentrics per metaphase significantly correlated with styrene exposure ($r = 0.225$, $P < 0.05$), but not with benzene exposure ($r = 0.044$) (Fig. 2C).

CYP2E1, hOGG1 and XRCC1 expression in styrene-exposed workers

In an attempt to elucidate the mechanism underlying the increased level of DNA damage and decreased DNA repair capacity in styrene-exposed workers, the expression of a metabolizing gene (i.e. *CYP2E1*) and DNA repair genes (i.e. *hOGG1* and *XRCC1*) in these workers were examined.

As shown in Table 6, mRNA expression of *CYP2E1* increased in a dose-dependent manner in all styrene-exposed groups. A sta-

Table 6
Induction of *CYP2E1*, *hOGG1* and *XRCC1* mRNA expression in peripheral lymphocytes of exposed workers.

Relative mRNA expression[a]	Study groups			
	Control ($n=25$)	Exposed workers ($n=22$)		
		Group I <10 ppm styrene ($n=7$)	Group II 10–20 ppm styrene ($n=7$)	Group III >20 ppm styrene ($n=8$)
CYP2E1	4.20 ± 0.59	5.65 ± 0.80	8.35 ± 2.99[b]	10.11 ± 1.47[b]
	3.79(0.18–8.95)	4.89(3.77–11.05)	5.66(3.48–17.09)	8.35(5.26–18.72)
hOGG1	4.14 ± 0.58	8.77 ± 2.19[b]	8.79 ± 1.67[b]	9.03 ± 2.37[b]
	4.29(0.25–11.42)	8.26(0.60–17.62)	7.08(2.89–15.83)	5.53(1.54–25.19)
XRCC1	4.51 ± 0.56	12.20 ± 2.60[b]	11.27 ± 3.17[b]	7.92 ± 1.87[b]
	4.48(0.31–11.55)	10.16(7.04–29.19)	7.72(2.84–22.15)	6.15(3.74–23.27)

The values are expressed as mean ± s.e. on the first line and the median (minimum–maximum) on the second line. nd: non-detectable.
[a] mRNA expression of each gene is normalized to the mRNA expression of β-actin.
[b] Statistically significant difference from the corresponding control at $P<0.05$.

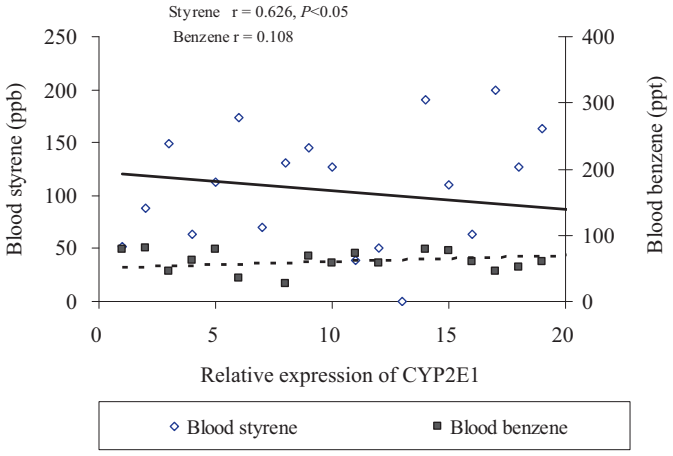

Fig. 3. Correlation between mean level of blood styrene or blood benzene and *CYP*2E1 mRNA expression was determined by Pearson correlation coefficients. The '*r*' represents the correlation coefficient and '*P*' represents the *P*-value for the correlation.

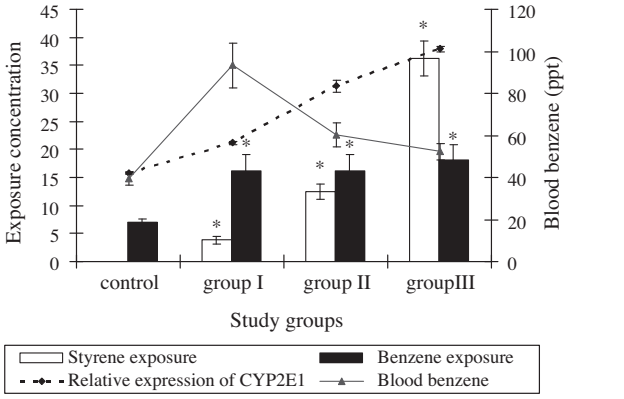

Fig. 4. The mean levels of exposure to styrene or benzene in each group are presented as the bar graph. Each data points in dashed line and solid line represents the mean levels of relative mRNA expression of *CYP2E1* and blood benzene, respectively. The error bar represents the standard errors of mean. * represents statistically significantly different from the corresponding control at $P<0.05$.

tistically significant increase in expression of *CYP2E1* was found in group II (1.99-fold, $P<0.05$) and group III (2.41-fold, $P<0.05$), when compared to the control group. The correlation between *CYP2E1* expression and styrene exposure was statistically significant ($r=0.401$, $P<0.05$), but *CYP2E1* expression did not correlate significantly with benzene exposure ($r=0.108$). The increase in *CYP2E1* mRNA expression was inversely correlated with concentration of blood styrene in all exposed groups ($r=-0.626$, $P<0.05$) (Fig. 3). In the case of benzene exposure, the observed blood benzene levels decreased as *CYP2E1* expression increased, although the levels of benzene exposure were about the same in all groups. This result indicated that styrene-induced expression of *CYP2E1* mRNA expression influenced blood benzene concentrations as shown in Fig. 4.

Table 6 also shows the expression of the DNA repair genes, *hOGG1* and *XRCC1*, which were significantly increased in all styrene-exposed groups. When compared to the control group, the relative expression levels of *hOGG1* for all exposed groups were increased approximately 2-fold ($P<0.05$), and the relative expression levels of *XRCC1* in exposed workers were also significantly increased ($P<0.05$), by approximately 3-, 3- and 2-fold in groups I, II and III, respectively. However, the correlation between *XRCC1* expression and DNA strand breaks, as well as *hOGG1* expression and 8-OHdG, were not statistically significant (data not shown).

Discussion

In an attempt to evaluate DNA damage and DNA repair from occupational exposure to styrene in the workplace, particularly at levels below the recommended standards of TLV-TWA_8 at 20 ppm., workers from a reinforced-fiberglass plastics factory were divided into 3 groups based on their levels of styrene exposure. Resin laminating workers (groups II and III) were exposed to a significantly higher level of styrene than trimming workers (group I). This is possibly due to their job-related activity involving the resin spraying process, of which styrene is the main component. It has been reported that the commercial unsaturated polyester resins contain 40% styrene monomer and nearly 10% of it evaporates during laminating or spraying (IARC, 2002). In the general environment, airborne concentrations of styrene are approximately 1 ppb or lower and the main source of styrene in ambient air is emissions from motor vehicle exhaust and cigarette smoking (Cohen et al., 2002). The control workplace was far from the roadside. Therefore, styrene was not detected in the control workplace or the control subjects. Post-shift levels of urinary MA and PGA in exposed workers were higher than pre-shift levels, indicating that styrene exposure occurred during the work shift. However, post-shift urinary MA and PGA in exposed workers did not exceed the biological exposure index (BEI) values of MA and PGA, which are 300 and 100 mg/g creatinine, respectively, corresponding to the TLV-TWA_8 of styrene at 20 ppm. It can be observed that blood styrene concentrations and the urinary metabolites of styrene, MA and PGA, were detected in only styrene-exposed workers, even at exposure levels below 10 ppm (42.20 mg/m^3), but they were not detectable in the control group.

The results in Table 3 are presented as the mean and median levels. It was observed that there were inconsistencies in the individual exposure and blood styrene concentrations and the levels

of urinary metabolites when the results were presented as mean levels. However, when the results are presented as median levels, the 10-fold difference in styrene exposures between the low and high exposure groups is consistent with the difference in styrene blood concentrations (8-fold) and MA (10-fold) and PGA excretion (8-fold). Therefore, the median level seems to be more appropriate than the mean levels for presenting data in human studies in which the inter-individual variation, e.g. in the biotransformation process, is large. Many human studies have reported results using median levels (Gong et al., 2002; Johnson et al., 2006).

The excretion pattern of urinary MA increased with increasing styrene exposure levels but demonstrated a different slope and correlation coefficient at higher concentrations. In contrast, the excretion pattern of PGA increased sharply at styrene concentrations between 3.81 and 12.52 ppm (16.08 and 52.83 mg/m^3), and then seemed to reach a plateau toward 36.23 ppm (152.89 mg/m^3) (Fig. 1). This phenomenon demonstrated a difference in the kinetics of the MA and PGA pathways. Styrene is metabolized to styrene oxide, which is then converted to styrene glycol and further metabolized to MA or to benzoic acid and hippuric acid, and excreted in urine (Laffon et al., 2001). MA is oxidized to PGA with an excretion ratio of MA to PGA after exposure to styrene of 8.5 (Bardodej and Bardodejova, 1970). The half-life for rapid and slow clearance phase of MA and PGA depends on the concentration of exposure (Droze and Wu, 1990). It has been reported that subjects exposed to styrene at low levels (11 ppm) in a reinforced-fiberglass plastic plant had a half-life of 1.9 h and 5.1 h for urinary MA and PGA, respectively (Shi et al., 1994). On the other hand, at higher exposure level (50–200 ppm), the half-life of MA is biphasic (the first phase half-life is 4 h and the second phase is 25 h), whereas the half-life of PGA was 11 h (Apostoli et al., 1983). In our study the rate of MA excretion in the urine was faster than that of PGA and MA correlated better with styrene exposure level than PGA.

A number of studies have shown that various xenobiotics can induce *CYP2E1* expression (Mendoza-Cantú et al., 2006) and thereby result in an increase in its metabolic activity. Rats treated with styrene (600 mg/kgBW) have been shown to have an induced expression of *CYP2E1* (Hirasawa et al., 2005). In our study, styrene-exposed workers had higher levels of *CYP2E1* expression compared to controls. It was also observed that expression of *CYP2E1* was inversely correlated with blood styrene concentration (Fig. 3). As observed in Fig. 1, blood styrene concentration increased with increasing levels of styrene exposure from 3.81 to 36.23 ppm (16.08–152.89 mg/m^3), but the relationship was non-linear, with the slope and correlation coefficient of the curve decreasing at higher concentrations (12.52–36.23 ppm, 52.83–152.89 mg/m^3), indicating that metabolism may take place at a higher rate as styrene exposure levels increase. This observation is in accordance with an increase in *CYP2E1* expression with increasing levels of styrene exposure. The results suggested that the induction of *CYP2E1* expression in styrene-exposed workers enhanced the metabolism of styrene, resulting in a faster blood styrene clearance. A recent study also showed that an increase of *CYP2E1* expression enhanced the activity of styrene metabolism determined as an increase in urinary excretion of styrene metabolites (Prieto-Castello et al., 2010). Expression of *CYP2E1* mRNA was also found in occupational exposure to styrene at 27 ± 5 ppm and this was associated with MA excretion in urine during the 24 h (Teixeira et al., 2004).

The individual styrene exposure in this study corresponds to the exposure level reported by Vodicka et al. (2004); however, the levels of urinary metabolites and DNA damage are different. The discrepancy in the results is possibly due to the variation in styrene metabolism, susceptibility to genotoxic effects of styrene exposure in different populations and the difference in analytical techniques used for of MA and PGA. The analysis of urinary MA and PGA reported by Vodicka et al. (2004) was done using GC and HPLC, while in this study we used HPLC with a UV detector. Fracasso et al. (2009) reported that occupational exposure to styrene at 46.74 ppm had levels of urinary metabolites of styrene (MA combined with PGA) analyzed by HPLC–UV detection in the same range as those found in this study.

Since a trace amount of benzene is known to occur ubiquitously in reinforced-fiberglass plastics factories, we also measured ambient benzene levels and individual exposure in our study. While there was no difference in ambient benzene levels in the control workplace and in the factory, the mean levels of individual benzene exposure were higher than the level in the control subjects [16.23 ppb (51.94 μg/m^3), 16.13 ppb (51.62 μg/m^3) and 18.14 ppb (58.05 μg/m^3)] in styrene-exposed groups I, II and III, respectively, and 6.96 ppb (22.27 μg/m^3) in the control subjects]. These benzene exposure levels were below the recommended standard TLV-TWA$_8$ of 500 ppb (ACGIH, 2004) and were much lower than those observed in exposed workers who work with petrochemicals, such as gasoline service attendants (121.67 ppb, 389.43 μg/m^3) and petrochemical factory workers (73.55 ppb, 235.36 μg/m^3) (Navasumrit et al., 2005). The observed levels of benzene exposure in these reinforced-fiberglass workers were in the same range as those living in the city, such as in monks and nuns (12.95 ppb, 41.44 μg/m^3) residing in temple areas in Bangkok (Navasumrit et al., 2005).

In this study, benzene exposure in the all exposed workers was in the same range, but levels of blood benzene, urinary *t,t*-MA and *S*-PMA were lower in subjects with increasing styrene exposure, indicating the influence of styrene on benzene metabolism, possibly through the effects of styrene-induced *CYP2E1* expression. Benzene is metabolized by CYP2E1 to benzene oxide and then to phenol (Kim et al., 2006). A second pathway is oxidation of oxepin, followed by ring opening to produce the muconaldehydes and ultimately conversion to *t,t*-MA (Snyder and Hedli, 1996). The major phenolic metabolites are more easily excreted in urine (Sabourin et al., 1989; Seaton et al., 1995; Kenyon et al., 1995) than those of the minor pathways of benzene metabolism, such as *S*-PMA following reaction of BO with glutathione (Kenyon et al., 1995).

In our study, a significant increase in DNA damage, measured as 8-OHdG and DNA strand breaks, was observed in all exposed groups when compared to the control group. This is in agreement with Marczynski et al. (1997) who reported that styrene and styrene-7,8-oxide (SO), the major metabolite of styrene, increased 8-OHdG in white blood cells of boat building workers. Elevated levels of DNA strand breaks in white blood cells of styrene-exposed workers were also reported in workers who had been occupationally exposed to styrene for an average of 10.5 years (Vodicka et al., 1999). The mechanisms of styrene-induced DNA damage possibly involve oxidative stress resulting from either increased production of ROS and/or decreased antioxidant defenses leading to DNA damage as suggested by Marczynski et al. (2002), who reported that SO may disrupt the pre-existing oxidative status in white blood cells. This disruption would alter the balance between oxidants and antioxidants in cells through depletion of glutathione and increase lipid peroxidation. It has been reported that ROS can be generated by CYP2E1, which has high capacity to produce O_2^- and H_2O_2 in microsomes during NADPH-dependent electron transfer (Zhukov and Ingelman-Sundberg, 1999). CYP2E1 is the primary isozyme responsible for the bioactivation of styrene (Gadberry et al., 1996). In human liver microsomes, CYP2E1 was mainly responsible for the metabolism of styrene at low concentrations, but at higher concentrations *CYP2B6* seems to be the main isoform involved in the metabolism (Kim et al., 1997). An increase in oxidative DNA damage in styrene-exposed workers may be mediated through an induction of *CYP2E1*, which generates SO, a reactive metabolite of styrene that is potentially carcinogenic in humans. The aforementioned obser-

vations are in agreement with the result of the present study in which *CYP2E1* expression in styrene-exposed workers increased with increasing levels of styrene exposure. Consequently, oxidative DNA damage occurred as the result of the production of ROS.

In our study, we were able to detect a significant increase in 8-OHdG and DNA strand breaks in all workers exposed to styrene, with a positive correlation between styrene exposure and DNA damage. These workers were also exposed to trace amounts of benzene, which is known to cause DNA damage. However, exposure to styrene should be the main cause of DNA damage, since styrene exposure was better correlated with DNA damage than benzene. In addition, the exposure to low levels of styrene (below 10 ppm, 42.20 mg/m^3) caused a higher level of DNA damage compared to controls. The results obtained from this study indicate that styrene exposure below a TLV-TWA$_8$ 20 ppm can cause DNA damage measured as 8-OHdG and DNA strand breaks.

In this study, it was found that expression levels of *XRCC1* and *hOGG1* increased in styrene-exposed groups when compared to the unexposed group. Increased expression of both DNA repair genes may be in response to styrene-induced DNA damage. The types of DNA damage induced by styrene can be repaired by the BER pathway (Vodicka et al., 2002; Au et al., 2003). The BER process is controlled by a specific set of genes encoding the enzymes that catalyze cellular responses to DNA damage. The repair enzymes that are involved in the BER process are multiple enzymes such as DNA glycosylase i.e. hOGG1, XRCC1, DNA ligases I, II, III and polymerase beta. The *hOGG1* gene encodes a DNA glycosylase/apurinic (AP) lyase activity which removes 8-oxoguanine from DNA as part of the BER pathway. The XRCC1 has a central role in the main pathway to repair DNA strand breaks and chromosome aberrations. At the earliest stage of single strand breaks repair, PARP rapidly binds to SSBs and is thereby activated, leading to the rapid formation of poly (ADP-ribose) polymer (PAR), followed by recruiting of XRCC1 at the same sites (Caldecott, 2007). However, radiation-induced chromosome aberrations (dicentric and deletion) measured by the cytogenetic challenge assay, which is a sensitive method to determine DNA repair capacity, was increased in styrene-exposed workers (Au et al., 1995). The reduction in DNA repair capacity was observed in all styrene-exposed groups, including trimming workers who were exposed to low levels of styrene at a mean level of 3.81 ppm (16.08 mg/m^3) for more than 10 years. In contrast, a previous study reported that a low level of styrene exposure (47.1–112.4 mg/m^3) and short duration of exposure (approximately 2.5–3.4 years) resulted in a significant increase in capacity rate to repair irradiation-specific DNA damage in exposed workers compared to unexposed subjects (Vodicka et al., 2004). This discrepancy in results may be due to the difference in levels and duration of styrene exposure. Increased levels of *hOGG1* mRNA expression were also observed in workers who were chronically exposed to chemical carcinogens such as arsenic (Mo et al., 2006). In this study, decreased DNA repair capacity but increased *hOGG1* and *XRCC1* mRNA expression in styrene-exposed workers was observed. This may be due to the fact that many repair enzymes are involved in the repair process (Caldecott, 2007) and an increased expression of *hOGG1* and *XRCC1* may not be able to increase the overall capacity of DNA repair. It has been reported that deficiency in DNA repair capacity may be caused by mutations in DNA repair genes which can be induced by genotoxic substances (Au et al., 1996). Decreased DNA repair capacity will cause cells from affected individuals to make more mistakes in the repair of DNA damage, especially from further exposure to mutagens (Au, 1993). Therefore, long-term occupational styrene exposure can cause an induction of DNA damage and reduction of DNA repair capacity. This abnormality may cause an increase in health risk for cancer development in styrene-exposed workers.

Benzene has been suggested to be a mutagen via an indirect mechanism, leading to oxidative DNA damage through the formation of hydroxyl radicals via hydrogen peroxide (Andreoli et al., 1997) and the lesions of benzene-induced DNA damage can be repaired by the BER pathway that is similar to the repair process of styrene-induced DNA damage. In this study, benzene exposure levels were not statistically significantly correlated with expression levels of *XRCC1* ($r = 0.197$) or *hOGG1* ($r = 0.187$), unlike styrene exposure ($r = 0.227$, $P < 0.05$ for *XRCC1* and $r = 0.321$, $P < 0.05$ for *hOGG1* (data not shown). The non-significant correlation between benzene exposure with expression of either DNA repair genes, or with DNA damage, indicated the response to benzene exposure was less than to styrene exposure.

In conclusion, the present study reports for the first time that chronic exposure to styrene at levels below TLV-TWA$_8$ at 20 ppm increases genotoxic risk observed as increased oxidative DNA damage and induction of expression of DNA repair genes, as well as decreased DNA repair capacity. Therefore, occupational exposure to styrene below the recommended level of TLV could still potentially result in effects that may increase the risk for development of various diseases such as cancer in the future.

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