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Exposure to benzene in various susceptible populations: Co-exposures to 1,3-butadiene and PAHs and implications for carcinogenic risk

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ABSTRACT

Exposure to benzene in human populations can occur in various work-related settings in which benzene is used or produced, or from traffic emissions resulting from incomplete combustion of fossil fuel, or from other sources. Two scenarios of benzene exposure were studied in 4 susceptible groups in Thailand. The first scenario is work-related exposures primarily to benzene, with the study subjects consisting of petrochemical laboratory workers and gasoline service station attendants, who are exposed at levels of 78.32 and 360.84 μg/m^3, respectively. The second scenario is traffic-related exposure and exposure to incense smoke, where co-exposures to other pollutants occurs, with the study groups consisting of school children attending schools in the city center and exposed to traffic emissions, and temple workers exposed to incense smoke. The individual benzene exposure levels were approximately 19.38 μg/m^3 in city school children and 45.90 μg/m^3 in temple workers. Co-exposures to 1,3-butadiene and polycyclic aromatic hydrocarbons (PAHs) generated from the same sources occurred in the second exposure scenario. 8-OHdG, DNA strand breaks and DNA repair capacity were measured as biomarkers of early effects of carcinogenic compound exposure. Petrochemical laboratory workers and gasoline service stations attendants had significantly higher levels of DNA strand breaks and significantly lower DNA repair capacity compared to controls, while gasoline service station attendants also had significantly higher levels of 8-OHdG than controls. City school children had significantly higher levels of PAH–DNA adducts, 8-OHdG, and DNA strand breaks and significantly lower levels of DNA repair capacity compared to rural children. Temple workers also had significantly higher levels of 8-OHdG and DNA strand breaks and significantly lower levels of DNA repair capacity compared to controls. In all of the study groups, the levels of benzene exposure correlated significantly with 8-OHdG levels, DNA strand breaks, and DNA repair capacity. In school children, PAH levels also correlated significantly with 8-OHdG levels, DNA strand breaks and DNA repair capacity. In temple workers, 1,3-butadiene levels correlated significantly with 8-OHdG and DNA strand breaks, but not with DNA repair capacity, while in the school children they did not correlate significantly with 8-OHdG or DNA strand breaks, and correlated marginally significantly with DNA repair capacity (deletions per metaphase). Multivariate regression analysis identified total PAHs concentrations converted to B[a]P equivalents as the only factor significantly affecting 8-OHdG levels, and total PAHs concentrations converted to B[a]P equivalents, as well as 1,3-butadiene concentrations as the factors significantly affecting DNA repair capacity in the school children. PAHs concentration was identified as the factor most significantly affecting DNA strand breaks in temple workers, followed by benzene concentrations, while DNA repair capacity was also significantly influenced by PAHs concentrations.

1. Introduction

Exposure to benzene in human populations can occur in various work-related settings in which benzene is used or produced, or from traffic emissions or other combustion processes, such as coal and incense burning, as well as cigarette smoke. Combustion-derived emissions contain a mixture of volatile compounds and particles that varies in composition depending on the source, weather, topography and other variables [1–4]. Other carcinogenic

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compounds, such as 1,3-butadiene and certain polycyclic aromatic hydrocarbons (PAHs), are also found in combustion-derived emissions.

Benzene, 1,3-butadiene and certain PAHs are carcinogens [5–7], which can cause DNA damage. These compounds require metabolic activation for their carcinogenic activities. Their reactive metabolites, which are electrophilic, can bind to DNA, causing DNA damage, e.g. DNA adducts. Certain pathways in their metabolism lead to the generation of reactive oxygen species (ROS), e.g. through redox cycling [8,9], and these can interact with DNA and induce oxidative DNA damage. An association between exposure to benzene and the development of leukemia is well established [5]. Benzene and its metabolites induce oxidative DNA damage, such as 8-hydroxy-2′-deoxyguanosine (8-OHdG) and DNA strand breaks, possibly through the generation of these ROS [10–12]. The carcinogenicity of 1,3-butadiene has been linked to its metabolic activation to genotoxic epoxides, which cause DNA adducts and chromosomal aberrations [13]. It has been reported that the most potent metabolite of 1,3-butadiene, diepoxybutane (DEB), can damage DNA through the generation of ROS and 8-OHdG [14,15]. PAHs exposure has been associated with human cancers in various organs, including skin, lung and bladder [16]. Certain PAHs are metabolized into reactive intermediates that can bind DNA and cause DNA adducts/damage, as well as lead to the formation of ROS [9].

Oxidative damage is involved throughout the multi-staged development of cancer. During the initiation stage, oxidative DNA damage, e.g. 8-OHdG, can lead to genetic alterations that result in a heritable mutation. During the promotion stage, ROS may contribute to persistent oxidative stress, resulting in an increase in cell proliferation and clonal expansion of the initiated cells to preneoplastic local lesions. In the progression stage, oxidative stress plays a direct role in the development of cancer characteristics, such as uncontrolled growth, genomic instability, invasion and metastasis [8,17].

In the assessment of health impacts in exposed populations, it is important to establish clear linkages between exposure to these carcinogens, individually or collectively, and disease outcome. Exposure to the aforementioned carcinogens, as well as the resultant early effects, can be monitored through the use of biomarkers. Earlier studies by our laboratory have shown a relationship between ambient exposure levels and levels of urinary metabolites of benzene, as well as early effects such as DNA damage in various susceptible populations [18–20]. We have also observed that certain susceptible populations in certain exposure scenarios are co-exposed to several other pollutants, e.g. PAHs and 1,3-butadiene, which may also contribute significantly to DNA damage. The present study investigates benzene exposure in 2 different scenarios: a work-related setting where people are exposed primarily to benzene, i.e. gasoline service station attendants and petrochemical laboratory workers, and other exposure settings where people are exposed to benzene concurrently with other carcinogens, such as 1,3-butadiene and PAHs, i.e. city school children attending schools in the city center and exposed to traffic emissions, and temple workers exposed to incense smoke. This study also attempts to determine which of the carcinogen(s) contributes significantly to DNA damage and repair capacity, which are indicative of risk for cancer development.

Biomarkers of effect, which reflect early biological changes in response to pollutant exposures that may ultimately progress and manifest in diseases such as cancer, including DNA damage, such as PAH–DNA adducts, 8-OHdG and DNA strand breaks, as well as DNA repair capacity, were employed in our study. 8-OHdG, DNA strand breaks, and DNA repair capacity are non-specific and can result from exposure to many different carcinogens. Thus, the measurements reflect a combined effect of all exposures. Since cancer development is a consequence of a balance between DNA damage and DNA repair, a reduction in DNA repair capacity resulting from exposure to these carcinogens may increase the risk of cancer development.

In the study of potential health effects in these different benzene-exposed groups, the statistical correlation between biomarkers of early effects and exposure levels has been assessed and compared within the same study groups. Additionally, an analysis of the comparative contributions of the 3 pollutants of interest in the co-exposed groups to the various early effects has been done.

2. Materials and methods

2.1. Study locations and study subjects

All adult subjects were non-smoking, healthy volunteers between the ages of 18 and 45. Mean levels of urinary cotinine lower than 28 μg/mmol were used to affirm non-smoking status [21]. The control subjects were age-, gender- and lifestyle (non-smoking status, medication, type of diet, etc.)-matched workers. All workers were requested to complete a questionnaire with their personal information, work history, accommodation, diet and life style, such as alcohol consumption and smoking habits. The local ethics committee, in accordance with the Helsinki declaration, approved the study, and the adult volunteers signed an informed consent form prior to the initiation of the study. The parents of all school children recruited to the study signed a consent form.

2.1.1. Petrochemical laboratories and gasoline service stations

The quality control laboratories (air-conditioned) of an olefin factory, a petroleum refinery, and a petroleum distributor, as well as 7 gasoline service stations in Bangkok were selected as study sites. A mail sorting service center, which is not related to the use of benzene, is located about 5 km from the main road, and is air-conditioned and thus somewhat isolated from the air outside, was selected as the control site. Benzene exposure was studied in 62 exposed male workers (31 petrochemical factory workers and 31 gasoline service attendants) and 34 non-exposed, gender-matched controls recruited from the control site.

2.1.2. Schools and temples

Four city schools located on or near main roads in Bangkok were selected as the study sites for traffic-related exposures, while two rural schools situated in Chonburi province (approximately 110 km from Bangkok) were selected as the control sites. School children consisted of 165 and 111 boys (9–13 years old) from city and rural schools, respectively. The city children selected for this study lived and attended schools in the Bangkok Metropolitan Area. The rural children were selected from schools located in Chonburi province. Parents were requested to complete a questionnaire about their personal history, health history, and their routine lifestyle, activities, and eating habits.

Three temples located away from main roads in Samutprakarn, Chachoengsao and Ayutthaya provinces (approximately 60 km from Bangkok) were selected as study sites for exposures to incense smoke in order to minimize exposure to carcinogenic air pollutants from other sources, such as from traffic emissions. An office building with no incense burning was used as the control site. Temple workers consisted of 40 study subjects of both genders. Twenty-five control subjects conducting work unrelated to benzene, butadiene or PAH exposure were recruited from the control site.

2.2. Sample collection

For ambient monitoring, air samples were collected at a height of approximately 150 cm off the ground. The air samples were

collected from various sampling areas in the study locations. Individual air samples were collected in the breathing zone of study subjects throughout the entire 8-h work shift or school day. After air sampling was completed, samples were capped, transported to the laboratory, and stored at −20 °C until analysis. Blood samples were collected at the end of work or school day. Whole blood samples were processed for analysis immediately upon arrival at the laboratory.

2.3. Air sample collection and analysis

2.3.1. Benzene and 1,3-butadiene

Benzene and 1,3-butadiene were collected using passive air samplers for 8 h using thermal desorption tubes (Tenax TA, Markes International Ltd., UK for benzene; Carbopack X, Markes International Ltd. for butadiene). For quantification, benzene and 1,3-butadiene were desorbed from the tubes using a thermal desorption unit (UNITY, Markes International Ltd.) and analyzed by gas chromatography equipped with mass spectrometry (GC–MS, Agilent 6890, USA) as described previously [22].

2.3.2. PAHs

Particle-associated PAHs in ambient air and in the breathing space of test subjects were collected on glass fiber filters (37 mm, 0.6 μm) using personal air samplers attached to a battery operated SKC air check sampler (model 224). Analysis of total PAHs and the associated B[a]P equivalents was carried out as described previously [19,23].

2.4. Determination of PAH–DNA adducts

The level of DNA adducts was determined in DNA extracted from peripheral mononuclear white blood cells using the butanol enrichment procedure as previously described [19]. The adduct spots were quantified by phosphorimage analysis (Molecular Imager, Bio-Rad GS-363). A B[a]P-diolepoxide DNA adduct standard was included in the analysis to correct for assay variability. The reported PAH–DNA adduct level is the average of at least two completely independent assays, and the results are given as the mean of these assays, and the adduct level is expressed as adducts/10^8 nucleotides.

2.5. Determination of 8-OHdG in leukocytes

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 by HPLC equipped with an ECD as previously described [22,24].

2.6. DNA strand breaks

DNA strand breaks were determined by way of the alkaline Comet assay as previously described [25,26] with minor modifications [27]. The extent of DNA damage was measured quantitatively using CometScan image analysis software (MetaSystems) and expressed as Tail Length and Olive Tail Moment.

2.7. DNA repair capacity

The challenge assay used in this study was carried out according to methods that have been previously described [28,29]. At 24 h after blood culture, the cells were irradiated with 100 cGy using a ^{137}Cs-source at a dose rate of 5 Gy/min. Fifty hours after culture initiation, cells were blocked with Colcemid and harvested using the standard procedure. The presence of dicentric chromosomes and chromosome deletions per metaphase cell were determined.

2.8. Statistical analysis

Mean exposure levels of carcinogens, and levels of all study biomarkers, were compared between study subjects and their respective controls by way of the Mann–Whitney *U*-test using the SPSS v.12 software package. A *p*-value ≤0.05 was considered as statistically significant. Statistical correlation between study parameters was assessed by the Spearman rank correlation coefficient using SPSS v.12. Multivariate regression analysis was performed to identify the relative contributions of the carcinogenic air pollutants to levels of biomarkers of early effects in school children and temple workers using the Stata software package (v.10.1).

3. Results

3.1. Exposure to benzene in petrochemical laboratory workers and gasoline service attendants

The ambient concentrations of benzene, 1,3-butadiene and PAHs in the workplace are summarized in Table 1. The mean levels of benzene in petrochemical factories and gasoline service stations were approximately 36- and 89-fold higher than those of the control workplace, respectively ($p < 0.001$). However, 1,3-butadiene was non-detectable in the petrochemical factories or the control site, while only a very low concentration (0.006 μg/m^3) was detected in gasoline service stations. The ambient level of PAHs detected in these workplaces was also very low: 1.40 ng/m^3 at the control site, 1.08 ng/m^3 in the petrochemical factories, and 3.81 ng/m^3 in the gasoline service stations. In our previous studies in various study populations, it was observed that individual exposure levels corresponded with ambient levels.

Table 1
Ambient concentrations of benzene, 1,3-butadiene, and total PAHs in the workplace.

Locations	Benzene (μg/m^3)	1,3-Butadiene (μg/m^3)	Total PAHs (ng/m^3)	Temperature (°C)	Relative humidity (%)
Control workplace	2.34 ± 0.22 2.09 (0.77–4.91) (*n* = 10)	nd	1.40 ± 0.14 0.72 (0.78–1.99) (*n* = 7)	28.40 ± 0.20 28.40 (27.10–29.7) (*n* = 10)	69.0 ± 1.8 69.7 (55.8–89.5) (*n* = 10)
Petrochemical factories	85.26 ± 17.46*** 42.79 (3.34–576.71) (*n* = 14)	nd	1.08 (0.76–1.39) (*n* = 2)	27.0 ± 0.1 27.0 (24.6–27.5) (*n* = 14)	60.4 ± 0.8 64.8 (55.8–74.0) (*n* = 14)
Gasoline service stations	207.94 ± 66.77*** 191.03 (5.57–764.03) (*n* = 24)	0.006 ± 0.003 nd (nd-0.012) (*n* = 4)	3.81 ± 0.17 3.87 (3.34–4.16) (*n* = 4)	29.4 ± 0.30 30.0 (27.50–31.0) (*n* = 7)	65.3 ± 1.20 66.8 (51.8–72.2) (*n* = 7)

Values are expressed as the mean ± SE on the first line and the median (min–max) on the second line of each parameter. nd, non-detectable.
*** Statistically significant difference from controls at $p < 0.001$.

Table 2
Individual benzene exposure, DNA damage and DNA repair capacity in gasoline service station attendants and petrochemical laboratory workers.

Parameters	Control workers	Petrochemical laboratory workers	Gasoline service station attendants
Individual benzene exposure ($\mu g/m^3$)	4.46 ± 0.54 3.27 (0.55–9.66) (n = 34)	78.32 ± 18.68*** 23.37(6.77–384.43) (n = 31)	360.84 ± 44.68*** 287.90(8.99–1001.42) (n = 31)
8-OHdG/10^5 dG	0.09 ± 0.01 0.05 (0.01–0.33) (n = 34)	0.12 ± 0.02 0.13 (0.01–0.59) (n = 31)	2.61 ± 0.36*** 1.93 (0.14–7.89) (n = 31)
DNA strand breaks			
Tail Length (μm)	1.25 ± 0.09 1.29 (nd-2.09) (n = 35)	1.33 ± 0.15 1.13(0.64–3.20) (n = 31)	1.93 ± 0.19*** 1.61(0.16–6.47) (n = 31)
Olive Tail Moment (μm)	0.24 ± 0.01 0.25 (0.17–0.31) (n = 35)	0.33 ± 0.02** 0.32 (0.00–0.57) (n = 31)	0.41 ± 0.04*** 0.32 (0.23–1.17) (n = 31)
DNA repair capacity			
Dicentrics/metaphase	0.12 ± 0.01 0.13 (0.04–0.22) (n = 34)	0.17 ± 0.01*** 0.16(0.06–0.31) (n = 31)	0.19 ± 0.01*** 0.18 (0.06–0.36) (n = 31)
Deletions/metaphase	0.16 ± 0.01 0.16(0.06–0.26) (n = 34)	0.22 ± 0.02*** 0.20 (0.10–0.46) (n = 31)	0.39 ± 0.03*** 0.38 (0.16–0.84) (n = 31)

Values are expressed as the mean ± SE on the first line and the median (min–max) on the second line of each parameter.
** Statistically significant difference from controls at $p < 0.01$.
*** Statistically significant difference from controls at $p < 0.001$.

Individual benzene exposure and early biological effects, i.e. DNA damage and DNA repair capacity, were assessed in the benzene-exposed workers and summarized in Table 2. Individual exposure to benzene throughout the 8-h work shift was 17-fold higher in petrochemical factory workers (78.32 $\mu g/m^3$, $p < 0.001$) and 80-fold higher in gasoline service station attendants (360.84 $\mu g/m^3$, $p < 0.001$) than in workers from the control site (4.46 $\mu g/m^3$), while levels were approximately 5-fold higher in gasoline service station attendants than in the petrochemical factory workers.

DNA damage was assessed as 8-OHdG and DNA strand breaks. The increase in 8-OHdG in petrochemical factory workers was not statistically significant when compared to workers at the control site, whereas it was 29-fold higher in gasoline service station attendants (2.61/10^5 dG vs 0.09/10^5 dG in gasoline service station attendants and control workers, respectively; $p < 0.001$). DNA strand breaks, measured as Tail Length and Olive Tail Moment were significantly higher in the gasoline service attendants (1.5- and 1.7-fold for Tail Length and Olive Moment, respectively; $p < 0.001$), while in petrochemical factory workers a 1.3-fold higher Olive Tail Moment ($p < 0.01$), but only a slight increase in Tail Length was observed.

DNA repair capacity was assessed through the cytogenetic challenge assay. Petrochemical factory workers and gasoline service station attendants had a significant lower DNA repair capacity, as observed by the increased frequencies of dicentrics and deletions per metaphase, compared to control workers ($p < 0.001$). Petrochemical factory workers had a 1.4-fold higher frequency of dicentrics and deletions per metaphase, compared to the control workers. Gasoline service station attendants had higher frequencies of dicentrics and deletions per metaphase than workers in the petrochemical factories as well as those from the control site, indicating a lower DNA repair capacity compared to these two groups.

Correlations between the level of individual benzene exposures and early biological effects are shown in Fig. 1. The levels of individual benzene exposure correlated significantly with the levels of

Table 3
Ambient concentrations of benzene, 1,3-butadiene, and total PAHs at the roadside and inside schools.

School locations	Benzene ($\mu g/m^3$)	1,3-Butadiene ($\mu g/m^3$)	Total PAHs (ng/m^3)	Temperature (°C)	Relative humidity (%)
Rural (Chonburi)					
Roadside	14.41 ± 1.89 13.48 (7.06–23.45) (n = 10)	0.93 ± 0.09 0.92 (0.74–1.14) (n = 4)	1.50 ± 0.28 1.35 (0.31–2.92) (n = 12)	30.35 ± 0.50 30.85 (29.05–31.55) (n = 5)	65.99 ± 2.18 67.15 (59.30–70.09) (n = 5)
School area	8.70 ± 1.22 7.06 (4.82–14.12) (n = 9)	0.52 ± 0.04 0.55 (0.36–0.72) (n = 9)	1.12 ± 0.19 1.07 (0.28–2.31) (n = 14)	30.95 ± 0.39 30.93 (26.65–32.55) (n = 6)	63.99 ± 1.09 63.38 (61.05–68.00) (n = 6)
City (Bangkok)					
Roadside	56.98 ± 7.16*** 47.67 (21.19–134.18) (n = 20)	10.09 ± 2.17** 7.83 (0.36–28.02) (n = 19)	30.39 ± 5.80*** 19.59 (4.63–99.95) (n = 22)	30.68 ± 0.31 30.71 (29.05–34.30) (n = 17)	69.56 ± 1.77 70.10 (60.90–85.50) (n = 17)
School area	26.48 ± 2.50*** 24.56 (14.45–44.94) (n = 12)	4.64 ± 1.62* 1.00 (0.36–28.02) (n = 20)	5.78 ± 1.08*** 4.79 (2.10–25.54) (n = 22)	30.77 ± 0.31 30.35 (26.65–32.55) (n = 11)	67.16 ± 1.86 64.35 (58.50–80.00) (n = 11)

Values are expressed as the mean ± SE on the first line and the median (min–max) on the second line of each parameter.
* Statistically significant difference from rural schools at $p < 0.05$.
** Statistically significant difference from rural schools at $p < 0.01$.
*** Statistically significant difference from rural schools at $p < 0.001$.

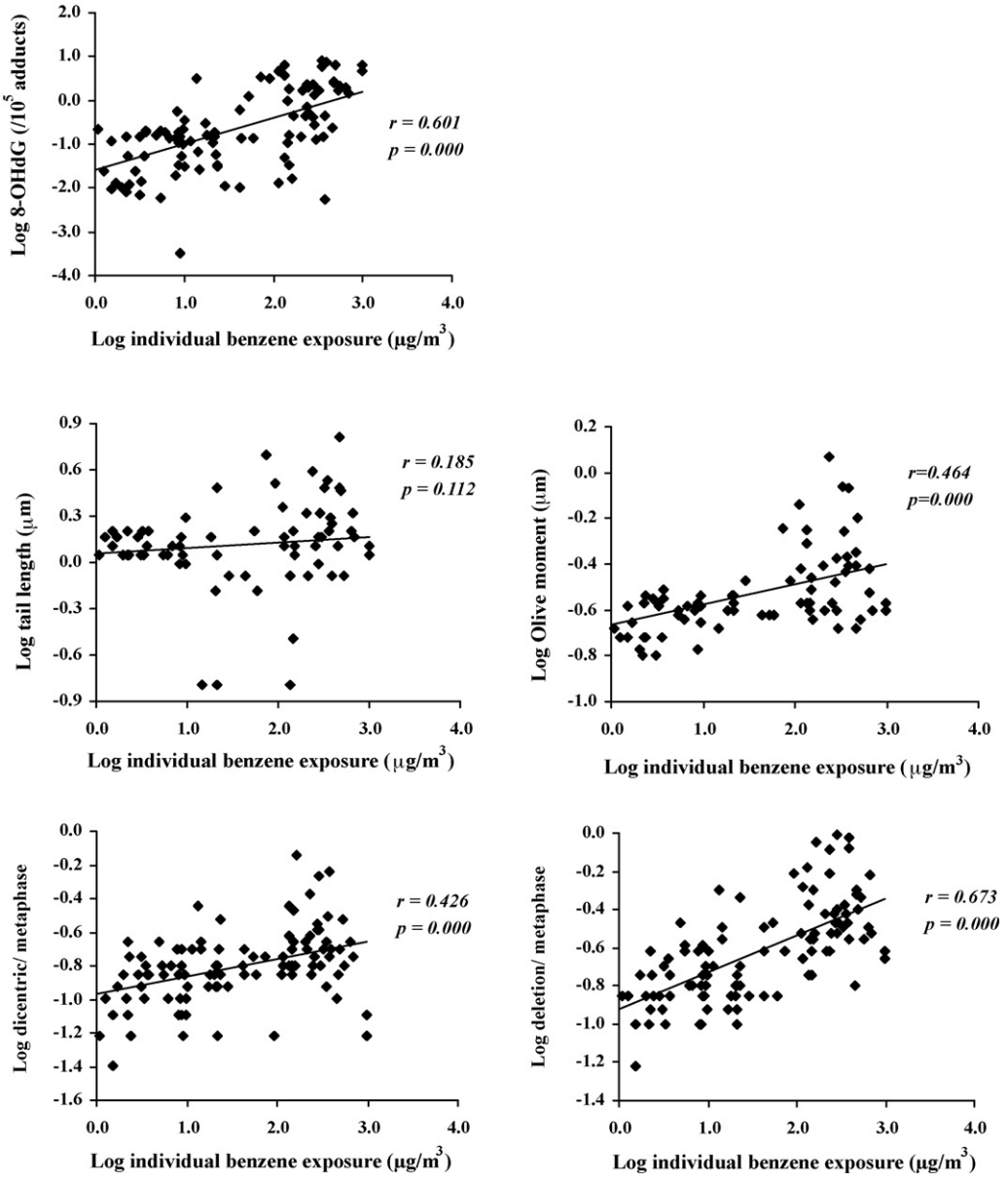

Fig. 1. Correlation between benzene exposure and biomarkers of early effects in benzene-exposed workers.

8-OHdG ($r = 0.601$, $p < 0.001$), DNA strand breaks ($r = 0.464$, $p < 0.001$ for Olive Moment), and DNA repair capacity ($r = 0.426$, $p < 0.001$ and $r = 0.673$, $p < 0.001$ for dicentrics and deletions per metaphase, respectively).

3.2. Exposures to benzene, PAHs and 1,3-butadiene in school children and temple workers

3.2.1. School children—traffic-related exposures

City school children are exposed to traffic-related benzene concurrently with 1,3-butadiene and PAHs in their immediate environment. Ambient levels of benzene, 1,3-butadiene and PAHs measured in front (at the roadside) and inside of city (Bangkok) and rural (Chonburi) schools are summarized in Table 3. At the roadside, ambient levels of benzene, 1,3-butadiene and total PAHs were approximately 4-, 10- and 20-fold higher, respectively, in the city than in the rural areas. Roadside levels of benzene, 1,3-butadiene and PAHs were approximately 2-, 2- and 5-fold higher, respectively, than those measured inside the city schools. Ambient levels of benzene, 1,3-butadiene and PAHs inside the city schools were also significantly higher than those in the rural schools.

Biomarkers of early effects measured in the school children are summarized in Table 4. City school children were exposed to significantly higher levels of benzene (2.3-fold, $p < 0.001$), 1,3-butadiene (3.7-fold, $p < 0.01$) and PAHs, (3.5-fold, $p < 0.001$; both total PAHs and B[a]P equivalents) than the rural school children. Levels of PAH–DNA adducts were found to be 5-fold higher in the city school children than in the rural school children (0.45 vs 0.09 adducts/10^8 nucleotides). Levels of 8-OHdG measured in leukocytes were also 2.5-fold higher in the city school children than in the rural school children ($0.25/10^5$ dG vs $0.10/10^5$ dG, $p < 0.001$). DNA strand breaks measured as Tail Length and Olive Tail Moment were 1.5-fold higher in the Bangkok school children ($p < 0.001$), and DNA repair capacity in these children was significantly lower than in the rural school children ($p < 0.001$), as observed by the significantly higher frequencies of dicentrics and deletions per metaphase.

Exposure to benzene and PAHs (total PAHs and B[a]P equivalents) correlated significantly with levels of 8-OHdG ($r = 0.398$,

Table 4
Individual exposure, DNA damage and DNA repair capacity in school children.

Parameters	Groups	
	Rural (Chonburi)	City (Bangkok)
Individual benzene exposure ($\mu g/m^3$)	8.40 ± 0.61 8.04 (0.68–19.03) ($n = 62$)	$19.38 \pm 1.11^{***}$ 14.90 (0.69–48.78) ($n = 128$)
Individual 1,3-butadiene exposure ($\mu g/m^3$)	0.65 ± 0.02 0.66 (0.31–0.93) ($n = 45$)	$2.42 \pm 0.25^{**}$ 1.08 (0.40–10.19) ($n = 79$)
Individual PAHs exposure		
Total PAHs (ng/m^3)	1.18 ± 0.09 1.03 (0.11–3.67) ($n = 68$)	$4.13 \pm 0.21^{***}$ 3.48 (1.01–10.57) ($n = 114$)
B[a]P equivalents (ng/m^3)	0.43 ± 0.05 0.23 (0.00–1.85) ($n = 68$)	$1.50 \pm 0.12^{***}$ 1.31 (0.15–8.76) ($n = 114$)
PAH–DNA adducts (adducts/10^8 nucleotides)	0.09 ± 0.00 0.09 (0.05–0.16) ($n = 69$)	$0.45 \pm 0.03^{***}$ 0.34 (0.13–1.04) ($n = 107$)
8-OHdG		
Leukocyte 8-OHdG/10^5 dG	0.10 ± 0.02 0.07 (0.01–0.55) ($n = 37$)	$0.25 \pm 0.01^{***}$ 0.23 (0.05–0.72) ($n = 76$)
DNA strand breaks		
Tail Length (μm)	1.28 ± 0.12 1.13 (0.00–6.29) ($n = 69$)	$1.93 \pm 0.09^{***}$ 1.94 (0.65–6.77) ($n = 115$)
Olive Tail Moment (μm)	0.16 ± 0.01 0.13 (0.00–0.52) ($n = 69$)	$0.23 \pm 0.01^{***}$ 0.21 (0.03–0.81) ($n = 115$)
DNA repair capacity		
Dicentrics/metaphase	0.21 ± 0.00 0.20 (0.12–0.28) ($n = 55$)	$0.34 \pm 0.01^{***}$ 0.36 (0.14–0.48) ($n = 91$)
Deletions/metaphase	0.26 ± 0.01 0.26 (0.18–0.36) ($n = 55$)	$0.45 \pm 0.01^{***}$ 0.46 (0.18–0.76) ($n = 91$)

Values are expressed as the mean ± SE on the first line and the median (min–max) on the second line of each parameter.
** Statistically significant difference from rural children at $p < 0.01$.
*** Statistically significant difference from rural children at $p < 0.001$.

$p < 0.001$ for benzene; $r = 0.411$, $p < 0.001$ for total PAHs; $r = 0.441$, $p < 0.001$ for B[a]P equivalents), DNA strand breaks (Tail Length) ($r = 0.311$, $p < 0.001$ for benzene; $r = 0.265$, $p < 0.001$ for total PAHs; $r = 0.280$, $p < 0.001$ for B[a]P equivalents), DNA strand breaks (Olive Tail Moment) ($r = 0.182$, $p < 0.01$ for benzene; $r = 0.343$, $p < 0.001$ for total PAHs; $r = 0.178$, $p < 0.01$ for B[a]P equivalents), DNA repair capacity (dicentrics per metaphase) ($r = 0.536$, $p < 0.001$ for benzene; $r = 0.291$, $p < 0.001$ for total PAHs; $r = 0.401$, $p < 0.001$ for B[a]P equivalents), and DNA repair capacity (deletions per metaphase) ($r = 0.523$, $p < 0.001$ for benzene; $r = 0.291$, $p < 0.001$ for total PAHs; $r = 0.368$, $p < 0.001$ for B[a]P equivalents). In contrast, 1,3-butadiene exposure did not correlate significantly with levels of 8-OHdG and DNA strand breaks, while correlation with DNA repair capacity was marginally statistically significant ($r = 0.377$, $p < 0.052$ for deletions per metaphase). It is possible that the observed non-statistical significance of the correlation of these study parameters with 1,3-butadiene exposure in children is due to the smaller number of study subjects. Multivariate regression analysis (Table 6) identified total PAHs concentrations converted to B[a]P equivalents as the only factor significantly affecting 8-OHdG levels ($r = 0.895$, $p < 0.05$), and 1,3-butadiene concentrations ($r = 1.399$, $p < 0.001$ and $r = 1.734$, $p < 0.01$ for dicentrics and deletions per metaphase, respectively) as well as total PAHs concentrations converted to B[a]P equivalents ($r = 1.591$, $p < 0.001$ and $r = 1.806$, $p < 0.01$ for dicentrics and deletions per metaphase, respectively) as the factors significantly affecting DNA repair capacity.

3.2.2. Temple workers—exposure to incense smoke

Temple workers were exposed to benzene concurrently with 1,3-butadiene and PAHs in incense smoke. We have previously reported [22] that the mean concentrations of these carcinogens emitted from burning of incense inside temples were significantly higher than those in a control workplace, with levels of benzene ($98.82\ \mu g/m^3$), 1,3-butadiene ($10.46\ \mu g/m^3$) and PAHs ($18.02\ ng/m^3$) being approximately 4- ($p < 0.01$), 261- ($p < 0.001$) and 12.5-fold ($p < 0.01$) higher, respectively. Exposure to incense smoke in temple workers and biomarkers of early biological effects (8-OHdG, DNA strand breaks and DNA repair capacity) are summarized in Table 5. Compared to control workers, temple workers were exposed to higher levels of benzene (1.7-fold; $45.90\ \mu g/m^3$ vs $26.49\ \mu g/m^3$, $p < 0.001$), 1,3-butadiene (280-fold; $11.29\ \mu g/m^3$ vs $0.04\ \mu g/m^3$, $p < 0.001$), and total PAHs (9-fold, $19.56\ ng/m^3$ vs $2.09\ ng/m^3$, $p < 0.001$). Levels of B[a]P (63-fold; $2.52\ ng/m^3$ vs $0.04\ ng/m^3$, $p < 0.001$) were also higher in these temple workers. Temple workers had significant higher levels of DNA damage, observed as approximately 2-fold higher levels of 8-OHdG (0.18 vs 0.09 per 10^5 dG; $p < 0.001$). They also had sig-

Table 5
Individual exposure, DNA damage and DNA repair capacity in temple workers.

Parameters	Groups	
	Control workers	Temple workers
Individual benzene exposure ($\mu g/m^3$)	26.49 ± 1.32 25.70 (11.12–38.41) ($n = 25$)	$45.90 \pm 2.58^{***}$ 41.55 (23.17–82.26) ($n = 35$)
Individual 1,3-butadiene exposure ($\mu g/m^3$)	0.04 ± 0.004 0.04 (0.01–0.12) ($n = 25$)	$11.29 \pm 1.48^{***}$ 11.03 (2.03–41.14) ($n = 31$)
Individual PAHs exposure		
Total PAHs (ng/m^3)	2.09 ± 0.65 0.85 (0.23–12.43) ($n = 24$)	$19.56 \pm 2.78^{***}$ 15.16 (2.91–65.74) ($n = 40$)
B[a]P (ng/m^3)	0.04 ± 0.01 ND (ND-0.06) ($n = 24$)	$2.52 \pm 0.83^{***}$ 1.73 (ND-6.41) ($n = 40$)
B[a]P equivalents (ng/m^3)	0.29 ± 0.12 ND (ND–2.84) ($n = 24$)	$4.60 \pm 1.35^{***}$ 1.91 (0.01–57.33) ($n = 40$)
8-OHdG		
Leukocyte 8-OHdG/10^5 dG	0.09 ± 0.03 0.07 (0.001–0.66) ($n = 24$)	$0.18 \pm 0.01^{***}$ 0.19 (0.03–0.32) ($n = 40$)
DNA strand breaks		
Tail Length (μm)	1.56 ± 0.09 1.53 (0.66–2.27) ($n = 25$)	$3.23 \pm 0.16^{***}$ 3.22 (1.57–5.07) ($n = 41$)
Olive Tail Moment (μm)	0.16 ± 0.01 0.16 (0.06–0.24) ($n = 25$)	$0.26 \pm 0.01^{***}$ 0.25 (0.16–0.38) ($n = 40$)
DNA repair capacity		
Dicentrics/metaphase	0.15 ± 0.01 0.16 (0.10–0.22) ($n = 23$)	$0.20 \pm 0.01^{**}$ 0.18 (0.10–0.36) ($n = 36$)
Deletions/metaphase	0.21 ± 0.01 0.20 (0.14–0.28) ($n = 23$)	$0.27 \pm 0.01^{***}$ 0.26 (0.14–0.48) ($n = 36$)

Values are expressed as the mean ± SE on the first line and the median (min–max) on the second line of each parameter. Data obtained from [22].
** Statistically significant difference from controls at $p < 0.01$.
*** Statistically significant difference from controls at $p < 0.001$.

Table 6
Multivariate regression analysis of individual exposure to carcinogenic air pollutants and their impacts on levels of biomarkers of biological effects.

	Oxidative DNA damage		DNA strand breaks				DNA repair capacity			
	8-OHdG		Tail length		Olive moment		Dicentrics/metaphase		Deletions/metaphase	
	R	*p*-value	*R*	*p*-value	*R*	*p*-value	*R*	*p*-value	*R*	*p*-value
School children										
Benzene	−0.028	0.887	0.078	0.785	0.140	0.390	−0.134	0.175	−0.183	0.542
1,3-Butadiene	0.690	0.055	−0.215	0.628	−0.228	0.366	1.399	0.000	1.734	0.006
Total PAHs	−1.395	0.078	1.258	0.233	−0.178	0.749	−0.448	0.193	−1.455	0.189
B[a]P equivalent	0.895	0.011	0.316	0.423	−0.325	0.160	1.591	0.000	1.806	0.002
Temple workers										
Benzene	0.187	0.260	0.233	0.015	0.209	0.068	0.051	0.747	0.043	0.743
1,3-Butadiene	0.298	0.226	0.028	0.833	0.250	0.138	−0.137	0.566	0.059	0.766
Total PAHs	0.080	0.816	1.024	0.000	0.765	0.002	0.495	0.146	0.622	0.031
B[a]P equivalent	0.152	0.743	0.146	0.573	0.086	0.784	0.090	0.842	−0.667	0.083

nificantly higher levels of DNA strand breaks, measured as Tail Length (3.23 μm vs 1.56 μm; $p < 0.001$) and Olive Tail Moment (0.26 μm vs 0.16 μm; $p < 0.001$). A significantly lower DNA repair capacity in temple workers was also observed as significantly higher levels of dicentrics ($p < 0.01$) and deletions ($p < 0.001$) per metaphase. Levels of 8-OHdG and DNA strand breaks correlated significantly ($p < 0.01$) with exposure levels of the three carcinogenic compounds. A statistically significant correlation was also

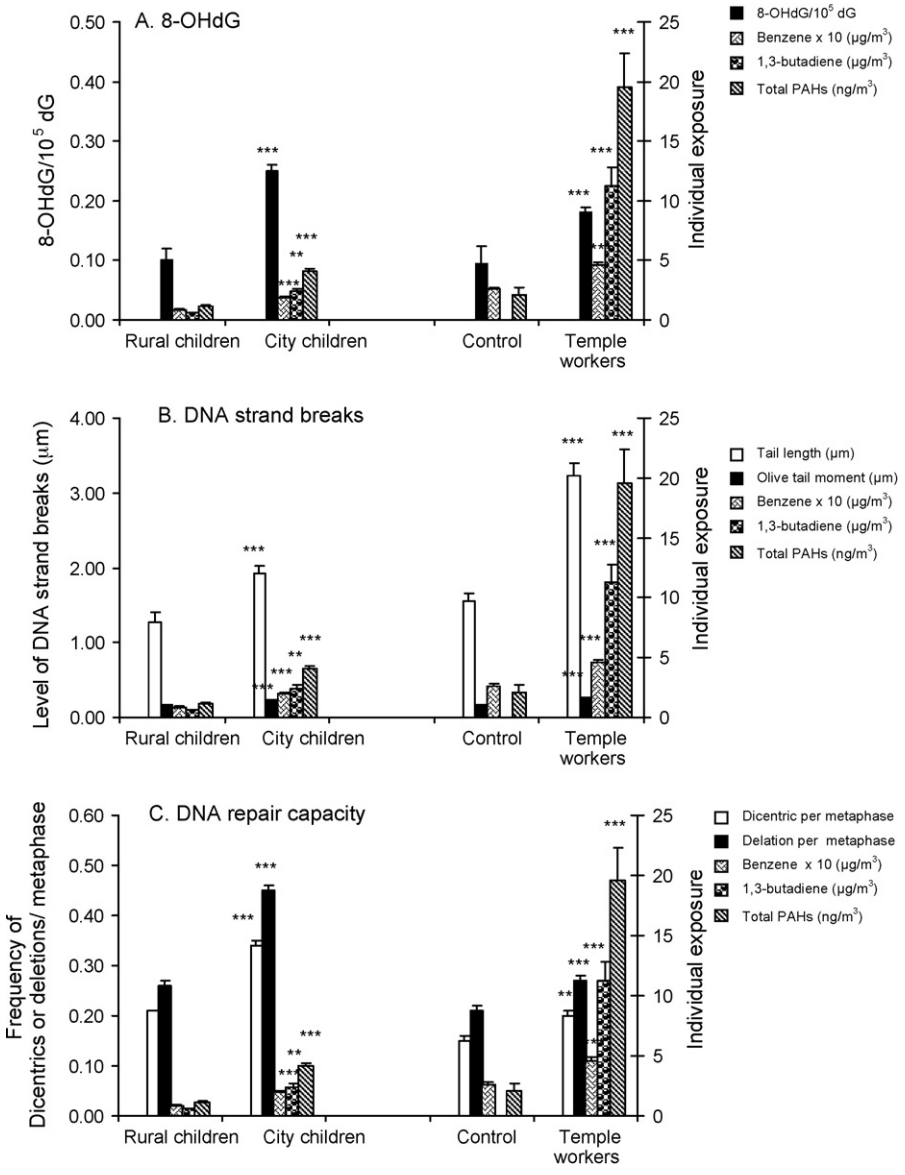

Fig. 2. Comparison of levels of exposure and biomarkers of early biological effects in children and adult (temple workers).

observed between DNA repair capacity and benzene, as well as with PAHs exposure ($p < 0.05$), but not with 1,3-butadiene exposure. Multivariate regression analysis (Table 6) identified PAHs concentrations as the factor most significantly affecting DNA strand breaks ($r = 1.024$, $p < 0.05$ and $r = 0.765$, $p < 0.01$ for Tail length and Olive tail moment, respectively), followed by benzene concentrations ($r = 0.233$, $p < 0.05$ for Tail length). DNA repair capacity was also significantly influenced by PAHs concentrations ($r = 0.622$, $p < 0.05$ for deletions per metaphase).

3.2.3. Age-related difference in school children and temple workers in response to co-exposure to benzene, PAHs and 1,3-butadiene

Fig. 2 compares levels of 8-OHdG, DNA strand breaks and DNA repair capacity in school children with those in adult temple workers. Both groups are co-exposed to benzene, PAHs and 1,3-butadiene. While the levels of 8-OHdG reflected the levels of exposure to benzene, 1,3-butadiene and PAHs in both the school children and the temple workers, the levels of 8-OHdG in the school children were higher despite the lower levels of exposure compared to those in the adult temple workers. DNA strand breaks reflected exposure levels in school children and adult temple workers quite well, except for the 1,3-butadiene exposure levels in control workers, where Tail Length was pronounced despite the very low levels of 1,3-butadiene exposure (0.04 $\mu g/m^3$). Similar to the case of 8-OHdG, the effect on DNA repair capacity was more pronounced in the school children (i.e. lower, as observed by the higher levels of dicentrics and deletions per metaphase) compared to the adult temple workers.

4. Discussion

In our work-related exposure scenario, i.e. in petrochemical factories and gasoline service stations, it has been shown that benzene is the major carcinogenic pollutant detected in the ambient air, and that levels of 1,3-butadiene and PAHs were very low or non-detectable. It should be noted that all activities related to burning are prohibited in the petrochemical factory, which is also located far from the main road (and thus traffic-related combustion). Additionally, the working area of the petrochemical factory workers is air-conditioned and thus somewhat isolated from air outside the laboratory. Therefore, the study groups in these two settings were exposed primarily to benzene. The other exposure scenario is different since benzene is a by-product of combustion, from which 1,3-butadiene and PAHs are also emitted. Our other exposed study groups, i.e. school children and temple workers, were therefore exposed concurrently to these 3 carcinogenic compounds. Interestingly, levels of B[a]P emitted from incense smoke was found to be approximately 12% of total PAHs, compared to approximately 7% in traffic emissions [22,30], which is significant due to the fact that B[a]P is considered to be the most potent carcinogenic PAHs.

The potential health risk from exposure to chemical carcinogens can be assessed through biomarkers of early biological effects, i.e. DNA damage, such as 8-OHdG and DNA strand breaks, as well as DNA repair capacity. 8-OHdG and DNA strand breaks are non-specific biomarkers that can be induced from the metabolism of benzene, 1,3-butadiene, as well as PAHs, and have been associated with an increased risk of diseases such as cancer [31]. An increased number of DNA single-strand breaks, micronuclei and chromosomal aberrations have been reported in benzene-exposed workers [23,32]. A reduction in DNA repair capacity has also been shown in the different susceptible populations exposed to benzene or PAHs in air [18,19,23]. Application of the cytogenetic challenge assay, where whole blood cultures are irradiated to challenge cells to repair the radiation-induced DNA damage, for investigating induced repair deficiency in populations has been reported [33]. The effect is an abnormal DNA repair response [34].

DNA strand breaks can result from interaction with ROS at the sugar phosphate diester backbone of DNA or by direct modification of DNA by chemicals and their metabolites leading to DNA strand cleavage [35]. Accumulation of DNA strand breaks may block transcription and subsequently lead to cell death [36]. The alkaline comet assay, therefore, has been used as a sensitive biomarker that reveals DNA damage caused either directly by reactive oxidizing agents, or indirectly by substances that can generate free radicals [37–39].

A significant increase in 8-OHdG was observed in gasoline service attendants, while a significant increase in DNA strand breaks was observed in both groups of benzene-exposed workers; however, neither DNA strand breaks nor DNA repair capacity were dramatically changed with increasing benzene exposure levels as observed in gasoline service attendants. An 80-fold higher level of benzene exposure resulted in a 29-fold higher level of 8-OHdG than that found in the control group. However, DNA strand breaks and DNA repair capacity were only 1.5–2-fold different, when compared to controls. In petrochemical workers who were exposed to much lower concentrations of benzene (17-fold higher than in the controls), levels of 8-OHdG and DNA strand breaks were 1.3-fold higher and DNA repair capacity was 1.4-fold lower.

These results indicate that higher levels of benzene exposure lead to a remarkable increase in the formation of 8-OHdG, out of proportion with the levels formed at lower benzene exposure levels. This could be due to (1) a stimulation of the specific metabolic pathway that leads to 8-OHdG formation by benzene itself, or (2) a decrease in the 8-OHdG repair mechanism that leads to accumulation of 8-OHdG. Benzene is known to induce CYP2E1 expression at both the transcriptional and translational levels, as well as in terms of enzymatic activity [40]. Therefore, continuous exposure to benzene at certain levels could lead to an increase in CYP2E1 activity, which could result in increased ROS generation and, subsequently, 8-OHdG formation. There are serious implications for health as the oxidized base is highly mutagenic and, if unrepaired, its presence in DNA causes GC → TA transversions, which are amongst the most frequent somatic mutations found in human cancers [31]. This is also why analysis of 8-OHdG is a useful approach to assess individual cancer risk due to oxidative stress [41]. Several studies conducted by other laboratories have found not only that benzene is linked to oxidative DNA damage, i.e. 8-oxoG formation, but also that polymorphisms in genes encoding enzymes that repair oxidative DNA damage, e.g. the variant genotype of hOGG1 326Cys/Cys, can decrease DNA repair capacity and increase risk for chronic benzene poisoning [11,42]. At the same time, results from our study seem to indicate that DNA strand breaks and DNA repair capacity are not as sensitive to increasing benzene exposure levels as 8-OHdG.

This study also demonstrated the possible health risk in school children exposed to traffic emissions and temple workers exposed to incense smoke, where benzene exposure occurs concurrently with 1,3-butadiene and PAHs. PAHs and 1,3-butadiene contribute to oxidative stress induction via their metabolism and subsequent formation of reactive metabolites and ROS [9,14]. ROS can be generated by metabolites of 1,3-butadiene, e.g. DEB, which is a highly reactive compound that acts as a bifunctional alkylating agent exhibiting both inter-strand and intra-strand DNA cross-linking ability. DEB also generates ROS that can damage DNA or produce H_2O_2 [14]. It has been observed that DEB significantly increased 8-OHdG levels in a dose-dependent manner in sea urchin embryos [15]. 1,3-butadiene has also been found to induce DNA adduct formation in humans, with mean adduct levels of 0.8 ± 1.2 adducts/10^9 nucleotides detected at a 1,3-butadiene concentration

range of 5–150 μg/m^3 [13]. In this study, individual exposure to 1,3-butadiene was much lower (2.42 and 11.29 μg/m^3 in school children and temple workers, respectively), and it is therefore unlikely that adduct formation would be detected in these study subjects. As for PAHs, it has been postulated that, in addition to DNA adduct formation, another contributing factor for their carcinogenicity may also be the production of ROS following oxidative stress, leading to DNA damage. There is evidence linking the metabolism of PAHs by CYP450 1A1 with the generation of ROS and oxidative stress through (1) the redox cycling processes associated with PAH metabolism involved in the formation of quinone metabolites, and (2) the catechol metabolite generated by the NADPH-dependent two electron reduction of the quinone metabolite resulting in redox cycling [9]. Therefore, exposure to benzene, 1,3-butadiene and PAHs can cause an increase in ROS formation contributing to DNA damage. It has been reported that 8-OHdG increased in a dose-dependent manner when calf thymus DNA reacted with cooking oil fumes containing relatively high levels of benzene, 1,3-butadiene and benzo[a]pyrene [43].

The present study showed that city school children and temple workers, who were concurrently exposed to significantly higher levels of benzene, 1,3-butadiene and PAHs, had significantly higher levels of DNA damage, observed as elevated levels of 8-OHdG and DNA strand breaks, and significant lower DNA repair capacity compared to their respective controls. The decrease in repair efficiency of the radiation-induced dicentrics and deletions in the challenge assay suggests that exposure to carcinogenic air pollutants may significantly decrease the cellular capacity to repair DNA damage. Manifestation of an abnormal DNA repair response may be indicative of health risk for diseases and cancer. Additionally, school children from the city also had significantly higher levels of PAH–DNA adducts than rural school children. This is in agreement with another previous study on traffic-related exposures to PAHs, where Bangkok traffic policemen had significantly higher levels of DNA adduct levels than office police [44]. It has been reported that peripheral blood DNA adducts are an acceptable surrogate for target tissues and are predictive for the risk of cancer [45,46].

Levels of 8-OHdG and DNA strand breaks in the city school children and temple workers significantly correlated with benzene, 1,3-butadiene and PAHs, suggesting that the observed increase in DNA damage may, in part, be due to the effects of these 3 carcinogenic compounds, although other carcinogenic substances may also be emitted from traffic emissions and incense burning. Multivariate regression analysis would seem to indicate, however, that PAHs concentrations, converted to B[a]P equivalents or as total PAH concentrations, as the major factor significantly influencing resultant effects in the co-exposed study subjects, i.e. 8-OHdG levels and DNA repair capacity in children and DNA strand breaks and repair capacity in temple workers, respectively. Humans exposed to urban air with vehicle emissions have been observed to have elevated levels of oxidative DNA damage in blood cells and urine [2]. Epidemiological studies have reported an increased risk of leukemia in children whose parents burn incense in the home [47]. Recently, it has been reported that long-term use of incense is associated with an increased risk of squamous cell carcinoma of the respiratory tract [48].

In this study, it was observed that the city school children, who were exposed to lower levels of benzene, 1,3-butadiene and PAHs than adult subjects from other studies, e.g. temple workers, had higher levels of 8-OHdG and DNA strand breaks. This is possibly due to either a lower DNA repair capacity in children or increasing formation of 8-OHdG through oxidative stress as well as through the different rates of metabolism of these carcinogens. In agreement with other studies [49,50], we have found that oxidative damage may be influenced by age. High levels of oxidative DNA damage in children, determined as 8-OHdG, may reflect their higher metabolic rates, high levels of mitochondrial respiration, and subsequent elevated production of ROS [49]. In addition, levels of 8-OHdG are possibly affected by the growth and the development of the lung, and the maturity of the metabolic systems and host defenses, which may be the reason for the observed negative correlation of 8-OHdG with age in children [50]. While we cannot completely rule out the possibility of different exposures to other pollutants that were not measured in this study, particularly in school children since they were exposed to lower levels of the 3 pollutants of interest and yet had higher levels of 8-OHdG and DNA strand breaks, the results of our study do suggest a higher risk for development of cancer if exposure occurs during childhood, when increased DNA damage and reduced DNA repair capacity have been observed. This is a potentially significant finding and should be noted for further study into the influence of age on effects from exposure to environmental pollutants.

The results from this study are indicative of relationships between exposure levels to individual levels of benzene, PAHs and 1,3-butadiene and resultant early biological effects, with implications for health risks. Results from the multivariate regression analysis also are indicative of a major influence of PAHs concentrations over benzene and 1,3-butadiene, particularly for particular measured endpoints. However, further studies are required to better our understanding of the effects that result from these very low levels of exposure. Additional measurements, for example alternative assays for DNA damage, such as the binucleate micronucleus test, and for DNA repair capacity such as the host cell reactivation assay, may provide additional useful information and should thus be considered for future studies.

5. Conclusion

It is clear from these studies that relatively low levels of benzene exposure, alone or concurrently with other carcinogens, result in early biological effects in the study populations that have implications for the risk of cancer development. At the same time, it is important to assess the usefulness of biomarkers of exposure and effects across different susceptible populations with different exposure levels in terms of consistency in the response. This would help validate the biomarkers for use in generating sound scientific evidence that can be used to initiate preventive and corrective measures such that the health risks from these exposures may be minimized. From our study comparing the use of biomarkers of early effects in school children and temple workers exposed to benzene, 1,3-butadiene and PAHs in ambient air, as well as petrochemical factory workers and gasoline service station attendants exposed primarily to benzene in their workplace, we have observed that: (1) 8-OHdG is a biomarker of DNA damage which responds to increasing levels of exposure to benzene as observed in the gasoline service station attendants, (2) total PAHs concentrations converted to B[a]P equivalents was identified as the factor significantly influencing both 8-OHdG levels and DNA repair capacity in school children, while 1,3-butadiene significantly influenced DNA repair capacity, (3) PAHs concentrations significantly influenced both DNA strand breaks and DNA repair capacity in temple workers, while benzene significantly influenced DNA strand breaks, and (4) children may be physiologically more susceptible to carcinogen exposures as observed from the higher levels of biomarkers of early effects compared to those in the adult study groups who had higher exposure levels.

Conflict of interest

None.

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