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Exposure to BTEX and Assessment of the Potential Risk among Pregnant Residents Living in the Vicinity of a Petro-chemical Industrial Estate

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Abstract

This research was conducted to estimate exposure and risk of exposure to BTEX (benzene, toluene, ethylbenzene and xylene) by a cross-sectional studying among pregnant women who lived in the vicinity of a petro-chemical industrial estate area. Personal exposure was monitored by environmental and biological sampling. Other relevant data were gathered by a structured questionnaire for analysis. The relationship between BTEX exposure and potential risk factors was determined by multiple linear regression. Average BTEX exposure levels were 18.89, 29.40, 10.26, and 17.85 μ g m⁻³, respectively, which were significantly higher than the control group. Urinary metabolite levels of muconic acid (*t*, *t*-MA), hippuric acid (HA), mandelic acid (MA), and methyl-hippuric acid *(m*HA) were significantly higher in pregnant residents living in the vicinity of the petro-chemical industrial estate area. The correlation between women's individual exposure to BTEX and their urinary metabolites showed significantly difference between groups. Risks of benzene and ethylbenzene exposure for these pregnant subjects were approximately 4.37×10^{-7} and 1.92×10^{-8} lower than the acceptable limits of United States Environmental Protection Agency (US.EPA.) guidelines (10^{-6}) . The hazard index (HI) of these pregnant subjects a lifetime rate was 0.555 which was below the reference level (HI < 1). Multiple linear regression analysis found that *t, t*-MA, HA, MA, and *m*HA in the urine of all models were significantly ($p < 0.05$) and positively related with BTEX in ambient air. The study showed that there was a low cancer risk for pregnant women in the petro-chemical industrial estate zone. However, when compared with the control area, a statistically significant difference was found in the relationship between BTEX content in ambient air and metabolites in the urine of pregnant women. The implication is that the responsible government agencies should implement health surveillance continuously.

Keywords: Pregnant women; Health risk assessment; BTEX

Introduction

The petro-chemical industry is a major employer in Rayong Province, Thailand. Air pollution in this area has been monitored by many sectors, e.g. the Office of the National Environment Board, universities and public sector agencies. Volatile organic compounds (VOC) commonly found in petro-chemical industrial areas such as BTEX (benzene, toluene, ethylbenzene, and xylene) are highly hazardous air pollutants. Benzene is classified as a class A human carcinogen by the Inter-national Agency for Research on Cancer, whereas ethylbenzene is classified as class 2B as a possible carcinogen [1]. Toluene and xylene were not classifiable as to human carcinogenicity (class D). However, these may nevertheless cause health risks, as reported by many studies of air pollution related to BTEX. Benzene presents serious risks for the local population, especially its association with increased cases of cancer [2]. Moreover, adverse reproductive effects have been correlated with maternal exposure to air pollutant combustion emissions in Eastern Europe and North America [3]. Pregnant women may be particularly susceptible to the adverse effects of ambient air pollution. Female workers exposed to organic solvents during pregnancy were found to have an increased risk of major fetal malformations and spontaneous abortion [4]. The estimated cancer risk from exposure to carcinogenic compounds is calculated as the product of unit risk and the concentrations of the carcinogens. The estimated concentrations are usually based on measurements of individual hazardous air pollutants [5].

The cluster of industrial estates in Map Ta Phut form the largest petro-chemical Industrial hub of Thailand. With increasing production capacity, these place increasing numbers of the population at risk of BTEX exposure. The biomonitoring of BTEX, complementary to air monitoring, can be used to improve

assessment of personal uptake related to body burden, and estimate the internal dose for all exposure routes. The purpose of this study was to evaluate exposure and potential risks of BTEX, and to identify demographic factors among pregnant residents living in the vicinity of the petro-chemical industrial estate area studied.

Materials and methods Study subjects

This study's subjects included 110 pregnant women from Map Ta Phut (80 women) and Nong Rai (30 women), with governmental sub-provincial hospitals as the exposed and control groups, respectively. The homes of women in the exposed group were located within approximately 5 km from Map Ta Phut Industrial Estate. We divided these residence locations into three zones: < 1 km, 1-3 km, and 3-5 km. The following equation was used to calculate the sample size needed for each zone.

$$
n = \left[\left(Z_{\alpha/2} + Z_{\beta} \right) \sigma^2 \right] / \Delta^2
$$

Because of no data regarding BTEX exposure in pregnant women were available at the time of our study, preliminary data on benzene exposure $(n = 10)$, as the human carcinogen, was used to calculate the sample size for the BTEX study, where average benzene levels in exposed and control groups were 19.16 \pm 2.74 and 14.14 \pm 2.22 μ g/m³, α $= 0.01$, $\beta = 0.15$. The resulting sample size (n) was less than 10, which was too small for parametric statistical analysis. Therefore, a sample size of 30 per zone was used (30 cases x 3 zones $= 90$ for exposed group, 30 cases for control group). However, we lost 10 cases during data collection for the exposed group. Thus, our sampling included 80 exposed subjects and 30 subjects. Pregnant women were selected randomly by hospital number

(HN) from Obstetric and Gynecological Departments. Criteria for case selection were as follows: age not less than 18 years old, nonsmoking, not occupationally related to volatile organic compounds, living more than one year in the study area, and gestation age between three to nine months. This study was approved by the Ethics Review Committee for Research on Human Subjects, Burapha University.

Sampling period

Air sampling of BTEX was carried out during the dry season each Thursday from November 2010 to April 2012. Air samples were collected by using Carbopack B^{TM} packed into a stainless steel tube attached for eight hours at the breathing zone of each of the exposed and control subjects. The analyses were carried out according to the Instruction Manual TO-17 [6].

Determination of BTEX

Air samples were analyzed at the Environmental Research Technology Centre in Pathum Thani Province. Four-step thermal desorption was used. First, purse tube type diffusive sampler by nitrogen at 25°C, for 5 minutes; next the samples were heated at 300 °C, using helium gas to carry the sample to a cold trap at -10 $^{\circ}$ C; finally heating at 310 $^{\circ}$ C for 10 minutes. Samples were passed to a gas chromatograph mass spectrometer(GC-MS) with DB-624 column Serial No. US9471912H with film thickness: 1.8 μ m length: 60 m, diameter: 0.32 mm, connected to MS-QP5000 (SHIMADZU, Japan). The status of MS was detector temperature 300 ºC, ion source (EI mode), transfer line temperature 230 ºC, and filament multiplier delay 3 minutes. The calibration curves were prepared by diluting air standard benzene, toluene, ethylbenzene, and xylene with mobile phase. The concentration range of 0-500 µg/mL standard curves were

consistently linear for R^2 benzene, toluene, ethylbenzene, and $xylene = 0.9919, 0.9924,$ 0.9961, and 0.9984, respectively. LOD of benzene, toluene, ethylbenzene, and xylene were 0.488, 0.487, 0.756, and 0.651 μ g/m³, respectively.

Urine sample collection

Spot urine samples were collected from the exposed and control subjects at two times (morning between 06:00 and 08:00 a.m. and afternoon between 2:00 p.m. and 4:00 p.m.) every Thursday from November 2010 to April 2012. Urine samples were collected in plastic tubes and closed tightly. The samples were stored at -20 ºC until analysis.

Determination of urinary metabolites *1) Determination of urinary t, t-MA*

Urine samples were analyzed at the Rayong Occupational Health and Environmental Development Center. Thawed samples were extracted through strong anion exchange (VertiPakTM SAX column). Extracted samples were determined by HPLC (Hewlett Packard series 1100, USA.) using C18 reversed phase column equipped with UV detector and measured at wavelength 264 nm. The samples were centrifuged at 10,000 x g for 10 min. Then 1.0 mL of the supernatant was loaded onto SAX column and washed with 2.0 mL of distilled water followed by 2.0 mL of 1% (v/v) acetic acid. The urine samples were next eluted with 1.0 mL 10% (v/v) acetic acid and then analyzed by HPLC. Twenty mL of extracted sample was injected onto Hypersil ODS C18 column 250 mm x 4.6 mm x 5 µm, with a mobile phase of 1% acetic acid in $H_2O/MeOH$ (90:10, v/v) and a flow rate of 1.0 mL/min under isocratic conditions. The column temperature was 35° C and the UV detector was used at 264 nm. Calibration curves were prepared by diluting standard with mobile phase over the concentration range of 0-5 µg/mL. Standard curves were consistently linear with $R^2 > 0.9991$, and limit of detection of *t, t*-MA was 0.031 mg/l. Relative standard deviation was less than 20%, and recovery was greater than 0.999.

2) Determination of urinary HA, MA, and mHA

After thawing urine samples to room temperature, HPLC was used for direct determination of urinary concentrations of HA, MA, and *m*HA. Samples were centrifuged at 10,000 x g for 10 min. Then 10 µL of the supernatant was injected for HPLC (Hewlett Packard series 1100, USA) onto an Ultra Aqueous C18 column (250 mm x 4.6 mm x 5 µm by Restek Corpolation, USA) equipped with UV detector measuring at wavelength 210 nm. The mobile phase was 10 mm $KH₂PO₄$ (adjusted to pH 3 with phosphoric acid)/ tetrahydrofuran (95:5, v/v) with a flow rate of 1.5 mL/min under isocratic conditions, and the column temperature was $35\degree$ °C. Calibration curves were prepared by diluting HA, MA, and *m*HA standards with mobile phase over the concentration range of 0-500 µg/mL. The standard curves were consistently linear with $R^2 > 0.9994$. LOD of metabolites; HA, MA, 2-*m*HA, 3-*m*HA, and 4-*m*HA were 1.11, 1.46, 1.10, 1.86, and 2.22 mg/L, respectively. Relative standard deviation was greater than 20% and recovery was greater than 0.999. The levels of all urinary metabolites were expressed as mg/g creatinine. Urinary creatinine was measured by an analyzer for clinical chemistry; model Stradust MC 15 (Dia Sys diagnostic system, Germany) at the Reference Laboratory and Toxicology Center in Nonthaburi Province.

Assessment of chemical exposure and the potential risks

Factors that should be considered in the determination of air contaminant intake include frequency, duration of exposure and body weight. The following equation was used to calculate the inhalation intake.

$$
I_{inh} = \frac{C \times IR \times ET \times EF \times ED \dots (1)}{BW \times AT}
$$

where C is the individual concentration $(mg/m³)$; IR is the inhalation rate, 0.875 m 3 /hr assumed for adult; ET is the exposure time, 24 hr/day [7]; EF is the exposure frequency, 365 days/yr; ED is the exposure duration average, 51.48 yrs for pregnant women in Map Ta Phut; BW is the body weight average (61.22 kgs for pregnant women in Map Ta Phut, and 62.22 kgs for pregnant women in Nong Rai); AT is the average time exposed, 76.08 years for Thai women [8]. Potential health risk was calculated in terms of non-cancer risk and cancer risk. Hazard quotient (HQ) was used to estimate the non-cancer health effects as expressed in Equation 3. If the quotient is less than 1, then the systemic effects are assumed not to be of concern; if the hazard quotient is greater than 1, then the systemic effects are assumed to be of concern.

$$
EC = [C \times ET \times EF \times ED] / AT \qquad (2)
$$

$$
HQ = EC / RFC
$$
 (3)

where EC is the exposure concentration $(mg/m³)$, RfC is the non-cancer reference concentration (μ g/m³). In addition, the sum of hazard quotients which target the same organ was estimated as shown in Equation 4 (HQ₁= benzene, $HQ_2 =$ toluene, $HQ_3 =$ ethylbenzene, and $HQ_4 = xylene$). This hazard index (HI) was calculated as:

$$
HI = HQ1 + HQ2 + HQ3 + HQ4
$$
 (4)

The cancer risk was calculated by the following equation in Equation 5:

$$
Cancer Risk = I_{inh} x \text{ CSF} \tag{5}
$$

where I_{inh} is the air contaminant intake of a certain chemical and CSF is the Cancer Slope Factor value of the corresponding chemical. A cancer risk of $> 10⁶$ was considered a "carcinogenic effect of concern"; a value $\leq 10^{-6}$ was considered an "acceptable level" [9].

Statistical analysis

All statistical tests were performed using a statistical software program. BTEX in ambient air and their metabolites were compared to find the difference between those in the petro-chemical industrial area and those in the control area by using Independent Ttest.Stepwise linear regression was applied to explore the relationship of BTEX in ambient air and their metabolites with demographic factors.

Results

Demographic factors

Pregnant women whose average age was 24.60 years were surveyed. Based on weight and height information in the questionnaires, Body Mass Index (BMI) was calculated. For the subjects in this study, 18.75% had low BMI, 46.25% had normal BMI, and 35.0% had high BMI. For women in the exposed group, the average distance from the Industrial estate was 2.01 km. The average time spent outdoors for women in the exposed group (2.74 hours per day) was significantly higher $(p<0.05)$ than women in the control group (2.15 hours per day). With regard to passive smoking in the families of pregnant women, there were 77.50% of those in the petro-chemical industrial estate; this waslower

than for the control area (83.30%), but not significantly different. For the exposed group, 10.00 percent reported drinking alcohol during pregnant, 77.50 percent cooked with petroleum-based fuels, and 18.80 percent consumed preserved foods (Table 1).

Personal BTEX concentration exposure

Concentrations of ambient benzene, toluene, ethylbenzene, (*m+p*) xylene, and (*o*) xylene were 18.89, 29.40, 10.26, 9.79, and 8.06 μ g/m³, respectively, for subjects living near the petro-chemical industrial estate(exposed group) and 13.03, 17.49, 8.18, 7.65, and 6.88 μ g/m³ respectively, for those in the control area. The differences were statistically significant for all four chemicals (Figure 1).

Figure 1 BTEX exposure levels in pregnant women.

, ** Statistically significant difference from controlled area at *p*<0.05 and 0.001, respectively.

Urinary BTEX concentrations of *t***,** *t***-MA, HA, MA,** *2-m***HA and** *3,4-m***HA**

The comparison of metabolites in the spot urine samples from the women in the exposed group and those in the control area showed that concentration levels of *t*, *t*-MA, HA, MA, *2-m*HA and *3,4-m*HA were significantly different (*p*<0.05, 0.001) (Figure 2).

Pregnant women Information	Petro-chemical	Control area	p -value*
	industrial estate	$(n=30)$	
	$(n=80)$		
Age (yrs)	24.60 ± 5.79	26.13 ± 5.72	NS
	$24.00(18.00 - 41.00)$	$26.00(18.00 - 39.00)$	
BMI (kg/m^2)			
Low	15 (18.75%)	$6(20.0\%)$	NS
Normal	37 (46.25%)	$15(50.0\%)$	NS
High	28 (35.0%)	$9(30.0\%)$	NS
Distance from a main road (km)	0.11 ± 0.16	0.08 ± 0.12	NS
	$0.05(0.10 - 0.50)$	$0.05(0.10 - 0.55)$	
Time spent outdoors (hr/day)	2.74 ± 1.58	2.15 ± 1.27	0.04
	$2.00(0.50 - 6.00)$	$2.00(0.50 - 5.00)$	
Passive smoking	62 (77.50%)	25 (83.30%)	NS
Alcohol drinking	$8(10.00\%)$	$1(3.30\%)$	NS
Cooked with petroleum-based fuels	62 (77.50%)	23(76.70%)	NS
Consumption of preserved food	15 (18.80%)	$5(16.70\%)$	NS

Table 1 Demographic factors of pregnant women

Values were expressed as mean \pm S.D., and median (minimum-maximum)

***** Difference of mean statistical independent t-test; between petro-chemical industrial estate and control area (*p*< 0.05)

NS; Not significant

Relationship between BTEX in ambient air with metabolites of exposure for pregnant women living near the petro-chemical industrial estate area and control area

Exposure levels of BTEX were significantly influenced by each subject's ambient air level at *p*<0.05 (Table 2). The analysis also showed effect of the personal variables on the urinary metabolites of BTEX. Demographic factors did not show any relationship with urinary metabolites among pregnant women (data not shown). In our study, multiple linear regression found that *t, t*-MA in urine was positively related with benzene in ambient air with a statistically significant difference at *p*<0.05; the level of benzene in ambient air explained 96.6 percent of variation in *t, t*-MA. HA in urine was positively related with toluene in ambient air with statistically significant difference at *p*<0.001, and explained 22.0 percent of variation in HA. MA in urine was positively related with ethylbenzene in ambient air with statistically significant difference at *p*<0.001, and explained 12.7 percent of variation in MA among pregnant subiects. *m*HA in urine was positively correlated with xylene in ambient air, with statistically significant difference at $p<0.05$, and explained 4.9 percent of variation in *m*HA.

Figure 2 Urinary metabolite levels of BTEX in pregnant women. Columns represent geometric means±SD for: (a) *t*, *t*-muconic acid (µg/g creatinine), (b) Hippuric acid (mg/g creatinine) (c) Mandelic acid (mg/g creatinine) and (d) Methyl-hippuric acid (mg/g creatinine) ***** Statistically significant difference from controlled area at *p*<0.05, 0.001, respectively

#, ### Statistically significant difference from spot urine samples in the morning at *p*<0.05, 0.001, respectively.

Exposure evaluation in relation to risk assessment of the concentration of BTEX

The non-cancer risks for BTEX exposure, as assessed by HQ, were within acceptable limits $(HQ<1)$, meaning that the systemic effects were assumed not to be of concern. Furthermore, the hazard index (HI), determined by adding the HQ of BTEX for the petro-chemical industrial estate and control groups, were 0.554 and 0.338, respectively. The HI for each target organ or system was smaller than 1 and neurological systems showed greater HI values than other organs or systems (Table 3). The cancer risk of two compounds which were confirmed or suspected carcinogens (IARC, 2009) are shown in Table 4**.** Risks of benzene exposure for pregnant women residing near the petrochemical industrial estate (4.37×10^{-7}) were greater than for the of control group (2.86 x 10-7). The average lifetime cancer risk for ethylbenzene exposure was lower than benzene exposure for both the petro-chemical industrial estate and control area (1.92×10^{-8}) and 1.54 x 10^{-8}). However, the cancer risk for both compounds in the petro-chemical industrial estate and control group were lower than the acceptable limit of 10^{-6} .

 $a \overline{Cal / US.EPA. (2011)}$

 b OEHHA. [18]

Discussion

As the diffusive samplers were small, silent, and easy to handle and needed no field calibration, they were used for monitoring BTEX for subjects in our study. The pollutants of BTEX in ambient air result from their many uses in petro-chemical industrial estates. Our study found that levels of benzene, toluene, ethylbenzene, *(m+p)* xylene, and *(o)* xylene were 18.89, 29.40, 10.26, 9.79, and 8.06 μ g/m³, respectively. These levels were higher than those reported in a study of people working and living in the city of Milan [10], which reported benzene, toluene, ethylbenzene, *(m+p)* xylene, and *(o)*xylene levels of 4.00, 25.30, 3.80, 9.30, and 3.40 μ g/m³, respectively. Petrochemical industrial estates have very high concentrations of air pollutants. Toluene was found to be the most abundant component, followed by benzene (29.40 and 18.89 μ g/m³, respectively). This is similar to the study of [11] which found high concentrations of toluene and benzene in ambient air of the metropolitan city of Kolkatta, India. Biomarkers of urinary BTEX metabolites (*t, t*-MA*,* hippuric acid, mandelic acid, and methylhippuric acid) were measured HPLC analysis. These were in general more readily available for testing in laboratories and easier to measure, and believed to be mainly through benzene metabolites. In comparison, the concentration levels of *t*, *t*-MA, HA, MA, and *m*HA in urine of pregnant subjects on Thursday mornings in the zone of petro-chemical industrial estate were statistical significantly different $(p<0.01)$ from those in the control area. Similarly, concentrations of *t*, *t*-MA, HA, MA, and *m*HA in urine of pregnant subjects on Thursday afternoons in the zone of petro-chemical industrial estate were significantly different (*p*<0.005) from those in the control area. *m*HA concentrations were also significantly different (*p*<0.05) from the control area. This is similar to the study of Yimrungruang *et al.* [12] who found that

average concentrations of *t, t*-MA, HA, MA and *m*HA metabolites in the urine of gas service station workers were significantly higher than those in a control group of office workers (*p*<0.05).

The multiple linear regressions employed to determine factors influencing BTEX exposure among the pregnant residents living near the petro-chemical industrial estate found that the influence of toluene in ambient air had the strongest effect on HA in the urine of pregnant exposed subjects. This is consistent with the study of [13] who reported a correlation between toluene in ambient air and HA in the urine. Our study also found that the model explained the effect of benzene on the *t, t*-MA in urine for exposed subjects. This is consistent with the study of Ou *et al.* [14] who found benzene in ambient air to be correlated with exposure levels to *t, t*-MA in the urine**.** Table 1 shows that time spent outdoors (hr/day) differed significantly (*p*<0.05) between pregnant subjects living near petro-chemical industrial estate and those living in the control area. Time spent outdoors and amount of BTEX in ambient air corresponded to metabolites in the urine of pregnant subjects.

In this study, the estimation of risk has focused on the inhalation pathway which is the main route of exposure [15]. Pregnant women living near the petro-chemical Industrial estate had an elevated risk of benzene exposure (4.37×10^{-7}) and ethylbenzene exposure (1.92×10^{-8}) , but both were lower than the acceptable limit of 10^{-6} . These figures are lower than those found in a study in Turkey [16] which conducted a health risk assessment of benzene at an estimated level of 5.03×10^{-5} . In determining the risk assessment ET value, the mean age of Thai women throughout the countrywas used because data on the mean of age of women living in Map Ta Phut were not available. According to the

hazard index and hazard quotient, a risk rate lower than for general diseases in pregnant women living near the petro-chemical industrial estate was 0.554 (below the reference level). This is similar to the study of Tunsaringkarn et al. [17] who found HQ of BTEX at locations of gasoline stations and HQ along the roadside were 0.617 and 0.369, respectively, both which were < 1. These values suggest no adverse health effects to the bone marrow, hema-tological and neurological systems. Based on the results of this study, a low cancer risk was found for pregnant women in the zone of the petro-chemical industrial estate. However, when compared with the control area, significant differences were found in BTEX in the ambient air and metabolites in the urine of the pregnant women. The implication is that responsible government agencies should implement continuous health monitoring. Besides using *t*, *t*-MA as a carcinogenic index in the urine, other indices should be applied to detect low level exposure to benzene in the vicinity of the petro-chemical industrial estate.

Conclusion

In this study, air sampling of BTEX exposure levels of pregnant subjects was carried out during the dry season from November 2010 to April 2012. The geometric means in the exposure area were 18.89, 29.40, 10.26, and 17.85 μ g/m³ for BTEX, respectively. The correlation between indi-vidual exposure to BTEX and the urinary metabolites showed significant differences (*p*<0.05). Cancer risk was evaluated by the exposure to benzene [2] and ethylbenzene. In this present study, the pregnant women who lived near the petrochemical industrial estate had an evaluated cancer risk of benzene (4.37×10^7) and ethylbenzene (1.92×10^{-8}) . According to the hazard index, the lifetime risk rate for general diseases in the pregnant women living near the petro-chemical industrial estate was 0.554 which

was below the reference level. The government sector should implement continuous health surveillance and monitoring programmes. Relevant agencies should plan well in monitor health of pregnant women systematically, for example, to provide know-ledge of how to avoid exposure to BTEX in ambient air, especially near the petro-chemical industrial estate, and to make regular follow up examinations during pregnancy.

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