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ORIGINAL ARTICLE

COMPARISON OF URINARY BIOMARKERS CONCENTRATIONS IN EXPOSED AND NON-EXPOSED PETROL STATION WORKERS IN THE EASTERN ECONOMIC CORRIDOR (EEC), THAILAND

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ABSTRACT

Background. The Thai government has been developing its Eastern Economic Corridor (EEC), which spans three provinces, with the aim of improving connections with other Asian nations. Since this strategic development, the number of trucks, private car, and passenger car registrations have continued to grow, with a corresponding increase in related to benzene, toluene, ethylbenzene, and xylene (BTEX).

Objectives. This study aims to compare the levels of trans, trans-muconic acid (t, t MA); toluene (TU); mandelic acid (MA); and methyl hippuric acid (MHA) in the urine of gas station employees, considering demographic and occupational factors.

Material and methods. These employees worked either near or away from the fuel dispenser, and there 100 people in each group. Data were collected using interviews and testing environmental air and urine samples for benzene, toluene, ethyl benzene, and xylene (BTEX).

Results. The results showed that BTEX concentrations were just detectable in all 200 cases (100%). The mean (±SD) urine level of t, t MA was 449.28 (±213.32) μg/g creatinine, while the median (min-max) was 428.23 (95.58-1202.56) μg/g creatinine. The mean TU was 0.011 (0.001) mg/L, while the median (min-max) was 0.011 (0.010-0.013) mg/L. MA levels were higher inside the pollution control zone than they were outside the zone (p=.009). Employees who practiced poor personal hygiene had relatively high urinary toluene and MHA levels (p=.009) and those who did not wear personal protective equipment (PPE) had relatively high MA levels (p=.040).

Conclusion. The results of this study revealed statistically significant biomarkers influencing the levels of t, t MA; TU; MA; and MHA in urine. The recommendations of this study are that employers should provide their employees with suitable PPE, check regularly to ensure that it is worn, and strongly encourage employees to take care of their sanitation. Employees should take appropriate breaks and days off to minimize their exposure to BTEX.

Key words: *exposure to gasoline, trans trans-muconic acid, toluene, mandelic methyl hippuric acid, urinary biomarkers*

INTRODUCTION

Since 2018, the Thai government has been developing its Eastern Economic Corridor (EEC), which spans three provinces (Chachoengsao, Chonburi, and Rayong) [1]. As a result, the EEC region has experienced high population growth. According to the EEC office, the number of residents is projected to grow from 4 million to more than 6 million by 2037 [2]. This national policy has led to a greater demand for energy, including demand for transportation fuels, in this region than in others [3, 4, 5].

Petrol stations exist in many settings, including in car wash facilities and adjacent to convenience stores, and are manned by attendants, cashiers, and fuel loading personnel [6, 7]. Pump attendants providing fuels are at risk of benzene, toluene, ethylbenzene, and xylene (BTEX) exposure, with benzene being the most toxic to humans [8]. Volatile organic compounds (VOC) can enter the human body *via* inhalation [9, 10],

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the skin, and ingestion [11]. The most common form of occupational exposure to chemical vapors is inhalation, with workers at gas stations across the world all being at risk [12].

BTEX exposure affects multiple body systems [13, 14, 15], leading to both serious health and cognitive and behavioral problems in occupationally exposed groups [8]. Benzene is the most dangerous substance. Even at low concentrations, long-term exposure to benzene increases the risk of leukemia and aplastic anemia in humans. Benzene has been classified as carcinogenic to humans (IARC group 1) [16]. The assessment of biomarkers of exposure, such as urinary levels of benzene and toluene [17, 18], would facilitate occupational health surveillance. For example, trans, trans-muconic acid (t, t MA) and s-phenyl mercapturic acid (SPMA) [18] can be used as markers for benzene, mandelic acid (MA) can be used as a marker for ethylbenzene, and methyl hippuric acid (MHA) can be used as a marker for xylene [17]. The biological monitoring of urinary excretion levels of such solvents or products is of fundamental importance in terms of the health surveillance of fuel distributors.

Employees working at fuel service stations may be exposed to BTEX, with their risk level dependent on factors such as the working environment (e.g., whether they work inside or outside a building), the nature of their work, and their work duration [19]. Additionally, personal behaviors, such as the consumption of alcohol and smoking, can aggravate health problems [20], while the use of personal protective equipment (PPE) and wearing a facemask can reduce substance exposure [21]. Demographic characteristics such as sex, age [22], and educational level [23] may also be related to the level of BTEX in the body.

Previous studies in Thailand have examined factors affecting urinary t, t MA levels in gas station laborers [6] assessed health risks related to benzene; assessed [24] associations between BTEX and biological parameters in petrol attendants; and conducted urinary t, t MA assessments in gas station attendants [25]. Previous studies from other countries include Egyptian research on urinary t, t MA levels in gas station workers [26] and BTEX exposure assessments before and after work in gas station attendants [18, 26]. BTEX levels in ambient air in Sri Lanka have also been studied and related research has been conducted in Iran in petrol pumps station workers [27] and Brazil in gas station attendants [26]. In addition, research based in Portugal found high BTEX exposures in residents near fuel service stations [28], and in Malaysia BTEX exposure was linked to adverse health effects in petrol attendants [29].

There remains a lack of comparative studies in this field that have measured the levels of t, t MA; TU; MA; and MHA in fuel service workers based both inside and outside dispenser areas. The aim of this study is to look for comparisons between the levels of urine t, t MA; TU; MA; and MHA and the personal and occupational factors of fuel service employees in EEC gas stations. This fundamental knowledge would help us to create healthcare policies for employees in high-risk groups.

MATERIAL AND METHODS

Study site and population

This analytical study included an exposure group of 100 Rayong Province fuel service attendants who were exposed to BTEX for at least three months and a non-exposure group of 100 employees who worked in fuel stations but were not involved directly in fuel service duties.

Each of the participants was recruited from one of three provinces - Chonburi, Rayong, and Chachoengsao - with 100 locations in total. A multistage sampling method was used. Initially, data from the Thai Department of Energy Business were used to select the top three large fuel service companies and several of their stations [5]. Following this, a survey revealed that company employees' performance characteristics were similar in all three EEC provinces, so the analysts chose to use the sampling group in Rayong. The researchers then communicated with the municipal authorities for public relations and randomly selected fuel service stations near main roads in Rayong province using simple random sampling. The stations were located on main roads (n=2), on by-pass roads (n=3), and on interdistrict roads (n=3). The owners of each of the eight stations were contacted to obtain permission to collect data in the operation area and to recruit filling attendant employees using cluster random sampling. All employees who agreed to participate and met the inclusion criteria were included in the study. Rayong city is currently divided into two sections: a pollution control zone and the surrounding area [30].

Sample size determination

The researchers used the G*Power software to calculate the sample size using an effect size of 0.20 in Cohen's formula and a statistical power of 0.84. G*Power calculation [31] indicated a sample size of 176 people. Twenty-four (15%) extra people were added to this sample to allow for attrition, thus making the total sample size 200. Gas station employees aged between 18 and 60 years old who were able to read, listen, write Thai, and work on the day of data collection for 8 hours were eligible for inclusion in the study.

Research ethics

This study was conducted in accordance with the Declaration of Helsinki. Burapha University Institutional Review Board for Protection of Human Subjects in Research (BUU-IRB) approved this research under certificate no. 019/2020.

Data collection tools

The research tools and equipment used in this study included interview forms, and urine specimen collection equipment. These are described as follows:

The interview form

The interview form was divided into four parts, with 30 questions in total, and was completed by selecting responses and adding text. In part 1, ten demographic questions concerning gender, age, weight, height, marital status, smoking history, and alcohol use were included. A total of nine questions in part 2 were on personal hygiene care, five of which were of a positive nature - such as hand washing before eating. Four questions of a negative nature such as drinking water in the workplace, wearing un-washed work clothes, and others. Part 3 (six items) focused on whether the respondent practiced personal protective behaviors such as wearing a nose mask, gloves, boots or sneakers, pants, or long-sleeved shirts. Part 4 asked about work history, with five items on the number of years of work experience the employee had, the hours of work they completed per day, their days of work per week, their hours of overtime per week, and their hours sleep each night. The quality of the interview questions was assessed based on the content validity, structure, alignment with the objectives of the research, and suitability of the language using the tools created for this purpose by three experts. The item–objective congruence index (IOC) showed that each item had an IOC greater than 0.5 and a *Cronbach's* alpha coefficient of 0.88, indicating their reliability.

Biological collection

Biological collection cards with 50 mL polyethylene tubes were used for urine specimen collection. The purpose of urine collection was to evaluate the levels of t, t MA; TU; MA; and MHA using the data interpretation criteria recommended by the American Conference of Governmental Industrial Hygienists [32].

Data collection

Following ethical approval (see below) and prior to data collection, the researchers communicated with managers at each site to request cooperation with the study and to clarify the study objectives and data collection details and gain permission to collect the data. The study analysts then met with the managers to confirm their permission to collect data and to schedule the data collection. The research team then conducted data collection, which included interviews, and urine specimen collection, as described below.

Interview

The researchers explained the questions carefully to the research assistants to ensure that they understood. Arrangements were made with the managers at each gas station to interview each participating individual in the private office area of each gas station. The interview form was collected by the researcher upon the completion of the interview, which lasted approximately 15 minutes.

Urine sample collection

The researchers provided urine sample containers to employees to carry during their working day and explained that they should be used to collect urine after work. The employees were instructed to collect a mid-stream urine sample in the plastic cup provided. At least 50 ml of this sample was placed into cold storage immediately. The urine specimen collections were sent to the laboratory each day and stored at -20 C to analyze the t, t MA concentration for benzene; TU concentration for toluene; MA concentration for ethylbenzene; and MHA concentration for xylene [33, 34]. The urine samples were analyzed using HPLC following the method described as these studies [33, 34].

Statistical analysis

The researchers checked the accuracy of the data and coded for them analysis using the Statistical Package for The Social Sciences/Personal Computer (SPSS/PC) software version 20.

The statistical data were divided into two sections: 1) descriptive statistics in tabular form, including frequency, percentage, mean, standard deviation, geometric mean and standard deviation GM (GSD), median, minimum, and maximum values. 2) Inferential statistics including comparative analyses of urine results based on 17 independent variables were collected from interview data, while dependent variables were the urinary t, t MA; TU; MA; and MHA volumes. Comparisons between the two groups were made using an independent samples t-test, while comparisons between more than two groups were made using a one-way analysis of variance (ANOVA). P values less than 0.05 were considered to be statistically significant.

RESULTS

Demographic data

Of the 200 subjects, most (68.5%) were female. The mean age (SD) of the sample was 30.25 (11.02) years, with the majority of the employees, 73%, being ≤35

Table 1. Demographic information

Factors	N (200)	%
Gender		
Male	63	31.5
Female	137	68.5
Age (years)		
≤ 35	146	73.0
> 35	54	27.0
Mean ± standard deviation	30.25±11.015	100
Body Mass Index (kg/m^3)		
≤22.9	112	56.0
>22.9	88	46.0
Mean ± standard deviation	23.63±2.259	100
Marital status		
Single	105	52.5
Partner/Married	86	43.0
Widowed / Divorced / Separated	9	4.5
Education level		
Illiterate	6	3.0
Primary	58	29.0
Secondary	105	52.5
Higher than secondary	31	15.5
Income (baht) (n = 169)		
Less or equal to 10,000	56	33.1
10,001 – 20,000	98	58.0
More than 20,000	15	8.8
Mean ± standard deviation	12053.25±4406.189	
Smoking		
Non-smoking	68	34.0
Currently smoke	132	66.0
Alcohol consumption		
Do not drink alcoholic beverages	91	45.5
Currently drink	109	54.5

years old. In terms of education, the majority (52%) had completed secondary school. Of the 200 subjects, 34% were non-smokers and 66% were smokers, as detailed in Table 1.

Personal hygiene and work history

Interview data showed the frequency of personal hygiene practices in workers stationed at and away from fuel dispensers, with 84% practicing personal hygiene in the workplace and 30.5% wearing personal protective equipment (PPE) for 3 hours or more. Most subjects (73.5%) had work experience of 1 year or more, 56% worked >8 hours per day, and 13.5% worked ≥six days per week. These data are shown in Table 2.

Table 2**.** Employment history and occupational exposure of gasoline station workers to BTEX compounds at fuel service stations

Work history and exposure to BTEX compounds	N	%
Working area		
At fuel dispenser	100	50
Outside the fuel dispenser	100	50
Personal hygiene		
Do not practice	32	16.0
Practice	168	84.0
Wearing personal protective equipment (PPE)		
Do not use / use for 1-3 hours	139	69.5
Use for >3 hours or more	61	30.5
Wearing safety glasses		
Do not use	190	95.0
Use	10	5.0
Wearing a mask		
Do not use	32	16.0
Use	168	84.0
Wearing gloves		
Do not use	158	79.0
Use	42	21.0
Using boots / shoes		
Do not use	102	51.0
Use	98	49.0
Wearing a long-sleeved shirt		
Do not use	142	71.0
Use	58	29.0
Wearing long pants		
Do not use	39	19.5
Use	161	80.5
Work experience (years)		
Less than 1 year	53	26.5
1 year or more	147	73.5
Mean ± standard deviation	2.44±4.063	
Hours of work per day		
8 hours	88	44.0
> 8 hours	122	56.0
Mean ± standard deviation	9.05±1.568	
Number of workdays per week		
6 days	111	55.5
7 days	89	44.5
Mean ± standard deviation	6.31±0.477	
Overtime (Hour / week)		
<6 hours	173	86.5
≥6 hours	27	13.5

Metabolite concentration

The results of the analysis of the t, t MA concentration showed that the mean (SD) was 449.28 (213.32) µg/g creatinine, with a median (min-max) value of 428.23 (95.58-1202.56) µg/g. The TU concentration showed a mean (SD) of 0.011 (0.001) mg/L, with a median (min-max) of 0.011 (0.010-0.013) mg/L. The mean (SD) MA concentration was 0.061 (0.012) g/g creatinine and the median (min-max) was 0.060 (0.04-0.09) g/g creatinine, both calculated from all 200 samples (100%).

These levels are below the standard (<0.15 g/g creatinine). The results of the analysis of the concentration of MHA levels of workers at and away from fuel dispensers were below the standard of 1.5 g/g creatinine, with a mean (SD) value of 0.43 (0.112) g/g creatinine and a median (min-max) value of 0.410 (0.25-0.88) g/g creatinine, as detailed in Table 3.

Comparison of various factors with urinary BTEX levels

The results of a comparative analysis focusing on a range of factors and the urinary t, t MA; TU; MA; and MHA values of gas station workers showed statistically significant ($p<0.05$) associations in urinary t,t MA; TU; MA; and MHA levels. Being male, having a low body mass index, having a working duration of more than 8 hours per day, and having a level of overtime of 6 hours per week or more were the factors that raised the t, t MA levels. Ineffective hygiene practices were linked to a higher TU level. The absence of PPE was related to higher MA, while male gender and body

Table 3**.** Occupational data and level of exposure to t, t muconic acid; toluene; mandelic acid; and methyl hippuric acid. SD=standard deviation; IQR=inter-quartile range; GM=geographic mean; GSD=geographic standard deviation

Factor	t,t- muconic acid (µg/g creatinine)	Toluene in urine (mg/L)	Mandelic acid (g/g creatinine)	Methyl hippuric acid (g/g creatinine)
Work in the area of the fuel dispenser (n=100)				
- Mean (SD)	449.28 (213.323)	0.0016 (0.002)	0.061 (0.012)	0.43 (0.112)
- GM (GSD)	407.38 (1.595)	0.0011 (1.798)	0.060 (1.233)	0.41 (1.279)
- Median (IQR)	428.23 (256.973)	0.0010 (0.0000)	0.060 (0.167)	0.41 (0.150)
- Max-Min	1202.56 -95.58	0.0138 - 0.0010	0.091- 0.035	0.88 - 0.25
Work outside area of fuel dispenser (n=100)				
- Mean (SD)	413.17 (252.200)	0.0020 (0.003)	0.063 (0.017)	0.40 (0.094)
- GM (GSD)	346.73 (1.803)	0.0013 (2.094)	0.060 (1.333)	0.38 (1.267)
- Median (IQR)	375.57 (256.983)	0.0010 (0.0000)	0.062 (0.260)	0.39 (0.110)
- Max-Min	1482.46 -59.71	0.0133 - 0.0010	0.108 - 0.030	0.72 - 0.20
Total (n = 200)				
- Mean (SD)	431.23 (233.68)	0.0018 (0.002)	0.062 (0.015)	0.41 (0.015)
- GM (GSD)	380.18 (1.706)	0.0012 (1.949)	0.060 (1.285)	0.398 (1.276)
- Median (IQR)	393.40 (244.59)	0.0010 (0.0000)	0.061 (0.228)	0.40 (0.130)
- Max-Min	779.98 - 95.58	0.0138 - 0.0010	0.108 -0.030	0.88 - 0.20
Gas station locations in the pollution control zone (n = 137)				
- Mean (SD)	450.87 (260.571)	0.0015 (0.002)	0.064 (0.015)	0.41 (0.095)
- GM (GSD)	389.04 (1.778)	0.001 (1.733)	0.061 (1.276)	0.398 (1.256)
- Median (IQR)	406.93 (253.905)	0.0010 (0.0000)	0.063 (0.210)	0.40 (0.125)
- Max-Min	1482.46-59.71	0.0133 - 0.0010	0.108 - 0.030	0.72 - 0.22
Gas station locations outside the pollution control zone (n = 63)				
- Mean (SD)	388.51 (153.734)	0.0024 (0.003)	0.058 (0.014)	0.42 (0.120)
- GM (GSD)	354.81 (1.545)	0.0014 (2.3550)	0.056 (1.288)	0.41 (1.315)
- Median (IQR)	378.05 (219.660)	0.0010 (0.0000)	0.056 (0.230)	0.41 (0.150)
- Max-Min	779.98 - 95.58	0.0138 - 0.0010	0.090 - 0.035	0.88 - 0.20
Total (n = 200)				
- Mean (SD)	431.23 (233.686)	0.0018 (0.002)	0.062 (0.015)	0.41 (0.015)
- GM (GSD)	380.18 (1.706)	0.0012 (1.949)	0.060 (1.285)	0.398 (1.276)
- Median (IQR)	393.40 (244.59)	0.0010 (0.0000)	0.061 (0.228)	0.40 (0.130)
- Max-Min	1482.46 - 59.71	0.0138 - 0.0010	0.108 - 0.030	0.88 - 0.20

Table 4. Comparison of various factors and BTEX exposure in urine (benzene, toluene, ethylbenzene, xylene, BTEX). GM=geographic mean; GSD=geographic standard deviation

Factor	t,t-muconic acid (t,tMA) (µg/g creatinine)		Toluene in urine(TU) (mg/L)		Mandelic acid (MA) (g/g creatinine)		Methyl hippuric acid (MHA) (g/g creatinine)	
	GM(GSD)	*p*-value	GM(GSD)	*p*-value	data	GM(GSD)	*p*-value	GM(GSD)
Gender								
Male	416.87 (1.714)	0.048	0.0012 (1.854)	0.598	0.062 (1.271)	0.407	0.43 (1.297)	0.031
Female	354.81 (1.694)		0.0012 (1.995)		0.060 (1.294)		0.39 (1.259)	
Age (years)								
≤35	380.19 (1.694)	0.624	0.0012 (1.862)	0.322	0.060 (1.291)	0.533	0.40 (1.276)	0.166
> 35	363.08 (1.746)		0.0013 (2.183)		0.062 (1.271)		0.42 (1.274)	
BMI (kg/m^3)								
≤22.9	407.38 (1.663)	0.009	0.0012 (1.950)	0.995	0.060 (1.291)	0.986	0.42 (1.265)	0.005
>22.9	338.84 (1.734)		0.0012 (1.959)		0.060 (1.279)		0.38 (1.276)	
Marital status								
Single	398.11 (1.652)	0.049	0.0011 (1.782)	0.213	0.060 (1.276)	0.689	0.40 (1.265)	0.106
Partner/Married	363.08 (1.766)		0.0013 (2.051)		0.062 (1.291)		0.40 (1.288)	
Widowed/divorced/Separ.	257.04 (1.563)		0.0017 (2.838)		0.058 (1.358)		0.48 (1.208)	
Income (baht)								
≤10,000	331.13 (1.644)	0.051	0.0012 (1.901)	0.740	0.060 (1.274)	0.918	0.40 (1.274)	0.803
>10,001 – 20,000	407.38 (1.702)		0.0011 (1.770)		0.060 (1.279)		0.41 (1.276)	
>20,000	371.54 (1.462)		0.0010 (1.000)		0.062 (1.358)		0.41 (1.180)	
Smoking								
Do not smoke	380.19 (1.690)	0.696	0.0013 (2.109)	0.411	0.062 (1.256)	0.318	0.42 (1.276)	0.254
Currently smoke	371.54 (1.718)		0.0012 (1.871)		0.059 (1.300)		0.40 (1.274)	
Alcohol drinking								
Do not drink	398.11 (1.675)	0.245	0.0011 (1.750)	0.229	0.060 (1.276)	0.625	0.41 (1.291)	0.256
Currently drink	363.08 (1.734)		0.0013 (2.109)		0.060 (1.294)		0.40 (1.259)	
Personal sanitation								
Do not practice	371.54 (1.637)	0.756	0.0010 (1.000)	0.002	0.049 (1.318)	0.060	0.47 (1.169)	0.009
Practice	354.81 (1.531)		0.0015 (2.506)		0.057 (1.276)		0.39 (1.327)	
Wearing personal protective equipment								
Do not use / use 1-3 hours	380.19 (1.706)	0.581	0.0012 (1.884)	0.511	0.062 (1.282)	0.040	0.41 (1.274)	0.786
Use 4 hours or more	363.08 (1.718)		0.0013 (2.104)		0.058 (1.288)		0.40 (1.279)	
Work experience								
Less than 1 year	389.05 (1.626)	0.570	0.0011 (1.786)	0.481	0.060 (1.239)	0.962	0.39 (1.285)	0.237
Over 1 year	371.54 (1.738)		0.0012 (2.009)		0.060 (1.303)		0.41 (1.271)	

Work per day								
8 hours	380.19 (1.722)	0.690	0.0011 (1.578)	0.026	0.059 (1.279)	0.401	0.41 (1.262)	0.475
More than 8 hours	371.54 (1.702)		0.0013 (2.198)		0.062 (1.291)		0.40 (1.285)	
Overtime								
Less than 6 hs	363.08 (1.702)	0.007	0.0012 (2.004)	0.364	0.060 (1.291)	0.686	0.40 (1.285)	0.466
6 hs or more	489.78 (1.626)		0.0011 (1.574)		0.059 (1.259)		0.42 (1.211)	
Sleep duration								
Less than 8 hs	371.54 (1.698)	0.762	0.0012 (1.897)	0.709	0.062 (1.300)	0.162	0.41 (1.262)	0.844
Over 8 hours	380.19 (1.722)		0.0012 (2.018)		0.059 (1.268)		0.40 (1.294)	
Work Area								
At fuel dispenser	407.38 (1.596)	0.054	0.0011 (1.799)	0.301	0.060 (1.233)	0.625	0.42 (1.279)	0.110
Away from fuel dispenser	349.74 (1.803)		0.0013 (2.094)		0.060 (1.334)		0.39 (1.268)	
Gasoline station location								
Inside control zone (in city)	389.05 (1.778)	0.343	0.0011 (1.734)	0.031	0.062 (1.276)	0.009	0.40 (1.256)	0.872
Outside control zone	354.81 (1.545)		0.0014 (2.355)		0.056 (1.288)		0.41 (1.315)	
Number of trucks refueled								
≤ 10 cars	371.54 (1.718)	0.239	0.0013 (2.042)	0.058	0.060 (1.288)	0.513	0.40 (1.268)	0.146
> 10 cars	407.38 (1.656)		0.0011 (1.496)		0.059 (1.274)		0.43 (1.306)	

mass index were related to MHA. Details are shown in Table 4.

DISCUSSION

Our finding that the majority of fuel station employees (68.5%) were female contradicts the findings of Tunsaringkasm et al [35], who studied the characteristics of gas station attendants in Bangkok and found most to be male workers, as well as the findings of a study conducted in Brazil, which found that the vast majority of employees were male (90.5%) [19].

The average age of participants in the present study, 30.25 (± 11.015) years, is in better agreement with the results of a previous study [35], which investigated the characteristics of gas station attendants in Bangkok and discovered that the average age was 29.8 years, as well as the results of similar research conducted in Indonesia [36] and Brazil [19]. In the present study, 52.5% of workers had completed secondary school, which is consistent with the results of a study conducted in Brazil, which found that 50.2% had graduated with secondary education [19].

Our finding that around half of the gas station employees in this sample were smokers or alcohol drinkers is similar to previous findings in Thailand [35] and highlights potential factors that increase body exposure to BTEX. Similarly, *Chambers* et al [37] stated that smoking was a crucial source of benzene, styrene, toluene, and xylene exposure in US citizens. Our results could not identify a link between smoking and BTEX compound levels, contradicting a study by *Lovrglio* et al [38] which evaluated benzene exposure in 137 people. The estimated benzene exposure in smokers was 10% higher than that in non-smokers [39]. The blood levels of BTEX in 151 fuel pump attendants exposed to BTEX were statistically significantly higher after an average of five cigarettes smoked within five hours than those in non-smokers [40].

Biological monitoring and assessment

We assessed exposure to urinary t, t MA; TU; MA; and MHA in an exposed group and a non-exposed group after they finished their shifts, according to the recommendations of the ACGIH [32]. The average concentration of t, t MA was higher in employees working at the fuel dispenser than in those working away from it. These levels were lower than those of the *Chaiklieng* et al. [6] study, with 25% of t, t MA exceeding the standard μg/g creatinine. The *Geraldino* et al. [26] study conducted in Brazil found t, t MA to be higher in fuel station workers than in office workers, with lower levels still found in convenience store workers. The results of this study were consistent with research on workers at gas stations in Iran with a low risk of TU exposure [8, 32]. Therefore, the regular health surveillance of fuel service attendants should include analyses of BTEX exposure.

The comparison of various factors with levels of urinary t, t muconic acid; toluene; mandelic acid; and methyl hippuric acid. A range of factors were found to be significantly linked to higher t, t MA; TU; MA; an MHA levels, as discussed below. Near or away from the fuel dispenser. The concentrations of t, t MA; TU; MA; and MHA were statistically similar in employees working at or away from the fuel dispenser, although the comparison was close to significance (p=0.054). This finding is inconsistent with that of *Chaiklieng* et al. [6], who found that the risk was 93.7 times higher in the exposed group than in a control group. Differences in the study settings and designs may account for this lack of agreement.

Gas station workers working near to or away from the fuel dispenser are exposed to a variety of solvents at different levels [10]. Employees working outside the dispenser area, such as in office employees, coffee shop staff, and convenience store workers, are often in a closed building environment, but the high frequency of door opening and closure in these environments allows BTEX compounds to enter. Employees in other areas, such as car wash facilities, were further away from the source of the emissions but were still at risk of BTEX due to the spread of vapor from the emissions [26].

Employees engaged in fueling service activities were unavoidably exposed to fuel sources, although operators outside the dispenser were more likely to be exposed to BTEX. *Fakhrinnur* et al. [36] found that exposed employees' urine t, t MA levels were significantly higher than those of office workers. This result was similar to the findings of *the Geraldino* et al. [26] study, which discovered that the exposed group had statistically significantly higher t, t MA levels than the control group.

Within and outside of the pollution control zone, Rayong province districts are currently divided into two groups: those within and those outside of the pollution control zone [30]. While the concentrations of t, t MA were higher within than outside of the pollution control zone, the difference was not quite statistically significant (p-value = 0.054). The concentrations of urine MA and TU in individuals within the pollution control zone, however, were significantly higher than in those outside the zone. This finding is inconsistent with the study of *Tunsaringkarn* et al. [14].

Personal factors

Gender. In this study, males had higher t, t MA levels than females, which is inconsistent with the results *Fakhrinnur* et al. [36], who found that sex was not associated with urinary t, t MA levels. In addition, the present study found higher MHA in males than in females, consistent with the findings of *Ernstgrd* et al. [41].

Marital Status. The relationship between marital status and work has attracted interest from researchers and national policy leaders [42]. Research has reported that single parents tend to work more than married couples to increase their income and due to family responsibilities [41]. Thus, it is important to include single status as well. In addition, *McManus* et al. [43] found that family income might vary depending on marital status. This study found that a single status factor affecting t, t MA arises because single people had more free time and needed income to take care of family members. These factors encouraged them to complete more work over long working hours, putting them at greater risk of exposure to benzene in the body. However, this study not only examined the metabolites of benzene as t, t MA but also looked at the levels of toluene in urine; the metabolites of ethyl benzene and xylene were found to be mandelic acid and methyl hippuric acid in urine.

Body mass index (BMI). The results showed that low body mass index (BMI) was a factor affecting t, t MA and MHA in urine. The case study group had an average BMI of just over 23 kg/m^3, with 44% considered overweight/obese [44, 45, 46]. This finding is in agreement with previous research [44] carried out in gas stations in Bangkok and suggests that employees should receive healthcare promotions encouraging weight control and the prevention of non-communicable diseases [45, 47]. The present findings show that a BMI within the standard range was a factor in the urinary elevation of t, t MA and MHA. It is possible that participants with a standard body mass index and without obesity were able to move their body and perform more activities in fuel services than those with a high BMI, therefore increasing their chance of accumulating benzene and xylene in their bodies. The results of this study are consistent with those of *Fakhrinnur* et al. [36], indicating that individuals within the standard BMI range were more likely to have increased urinary t, t MA levels, although the results were not statistically significant.

Work history factors

Working hours per day and overtime. Our results show that most employees in our sample worked more than 40 hours per week and over 6 days per week, exceeding the limits set by the Labor Protection Act, 1998, section [28]. Employees should prioritize adequate sleep and rest to reduce their risk of toluene exposure. The participants worked more hours per day than those assessed in a Brazilian study, which found that most fuel service employees worked 6 hours per day, or 8 hours per day for those working overtime [19].

In this study, a higher number of working hours per day contributed to higher urinary toluene exposure, while working six or more hours of overtime per week contributed to higher t, t MA levels. A previous report found that longer working hours are associated with higher BTEX exposure and an increased risk of benzene in the body [49], among other findings. *Fakhrinnur* et al. [36] also found that the duration of the fueling service affected the urinary t, t MA levels (p=0.000), suggesting that workers should have reasonable break periods to limit their BTEX absorption [12].

In this study, the time taken to refuel each vehicle was not evaluated, but the number of cars refueled per day was recorded. The latter was not a significant factor in the urinary levels of t, t MA, TU, MA, and MHA. As benzene is known to be a carcinogen [16], developing strategies to assist employees in reducing benzene exposure in fuel is critical, since this study found that the duration of exposure to chemicals may be a factor associated with chemical accumulation in employees. The duration of chemical exposure may also be a factor in chemical accumulation in living organisms.

Personal hygiene. We found that inappropriate personal hygiene practices influenced urinary TU and MHA levels. These habits may increase BTEX exposure due to the unintentional contamination of water and food, as well as through dermal benzene exposure, which can occur if fuel spills on the employee's skin [50]. Therefore, fuel service employees working both within and away from the dispenser area should be advised about methods to maintain personal cleanliness, such as washing hands before eating or drinking water, avoiding drinking water or eating in the work area, and not repeatedly wearing used clothes. The findings of this study emphasize the importance of PPE and establish a standard for personal hygiene guidance based on the research of *Wiwanitkit* et al. [25]. Additionally, the BTEX exposure level may be reduced by providing sinks near the fueling area where employees can wash their hands.

Wearing personal protective equipment

The results revealed that wearing PPE caused urine MA levels to rise more than they rose when not wearing PPE (p=0.040). As a result, employers should advise their staff to wear a mask at all times while working to avoid BTEX exposure, including exposure to ethylbenzene, consistent with the results of *Chang* et al. [21], who found that the concentration of solvent was different in air outside compared to inside the mask.

The results revealed that many employees performed multitasking operations while wearing PPE. Most (84%) employees wore masks, while 49% wore sneakers rather than protective boots. This study collected data during the coronavirus 19 pandemic, so the study group wore fabric masks more than usual, and these may have been ineffective against BTEX exposure. The study collected samples between October and December 2020, during which Thailand launched a campaign for the universal wearing of PPE. This is consistent with research carried out in other areas of Thailand [6] that studied the relationship between the use of personal protective equipment and t, t MA in gas station employees in Khonhaen province before the coronavirus pandemic.

The findings of this study are inconsistent with those of a study conducted in Brazil, which found that almost all employees wore boots, while only 6.3% wore short-sleeved t-shirts and trousers [19]. According to one study, using all forms of PPE during all work shifts reduced BTEX contamination [46]. Since BTEX, particularly benzene, is a highly hazardous chemical, employees should take precautions while performing work-related tasks. In addition to the control of BTEX using various technologies, exposure reduction could be accomplished by ensuring that PPE is worn to reduce BTEX exposure and transmission into the body [51].

CONCLUSION AND RECOMMENDATIONS

The results of this study revealed statistically significant biomarkers influencing the levels of t, t MA; TU; MA; and MHA in urine. The recommendations of this study are that employers should provide their employees with suitable PPE, check regularly to ensure that it is worn, and strongly encourage employees to take care of their sanitation. Employees should take appropriate breaks and days off to minimize their exposure to BTEX. Future research should investigate strategies to prevent BTEX exposure among employees working as fuel service attendants in EEC areas.

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Authors' contributions

Anamai Thetkathuek, Chan Pattama Polyong, and Jintana Sirivarasai decided to conduct this study and *collected data. Anamai Thetkathuek wrote the first draft of the manuscript. Anamai Thetkathuek and Wanlop Jaidee planned the design of the study. Wanlop Jaidee also helped with the research methodology. All authors read and approved the final manuscript.*

Disclosure statement

All authors declare that they have no competing interests in this work.

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