

ORIGINAL ARTICLE

Biomatrix of health risk assessment of benzene-exposed workers at Thai gasoline stations

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Abstract

Objective: This study assessed the health risk of benzene exposure among Thai gasoline station workers through biomarker detection and experience of adverse symptoms.

Methods: Trans, trans-muconic acid (tt-MA) metabolites of benzene were analyzed from spot urine sampled among gasoline station workers after shift work using HPLC-UV. Air benzene monitoring was done with an active sampler connected to a charcoal sorbent tube, and analyzed by GC-FID. The health risk was calculated by using the biomatrix of the likelihood of benzene exposure and the severity of adverse symptoms.

Results: The tt-MA concentration, among 235 workers, ranged from less than 10–2159 $\mu\text{g/g Cr}$, which corresponded to the air benzene concentration range of <0.1 to 65.8 ppb. In total, 32.3% of workers had a higher than acceptable risk level and there was a significant association between gasoline station work zones and the likelihood of benzene exposure as well as the health risk of workers. The health risk levels estimated from the biomarker monitoring were consistent with the risk matrix of air benzene monitoring.

Conclusion: This tt-MA biomarker monitoring and biomatrix of health risk assessment is suggested as useful for health surveillance of gasoline station workers exposed to benzene.

KEYWORDS

benzene, biomatrix, health risk matrix, Thai gasoline station workers, tt-muconic acid

1 | INTRODUCTION

Benzene is classified as a human carcinogen by the International Agency of Research for Cancer, or IARC.¹ It is found in petrol for automobiles and allowed as a one percent component by volume of gasoline in Thailand.² Benzene can evaporate into the air during refueling at

gasoline stations and it is also found in vehicle exhaust fumes. Previous Thai studies have found ambient air benzene concentrations at gasoline stations, ranging from 0.03 to 65.71 ppb in the city of Khon Kaen,³ and averaging 92.75 ± 16.67 ppb in Bangkok, Thailand.⁴ These concentrations are lower than the occupational exposure limit (OEL) of the 8-h time weighted average (TWA) recommended

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by the American Conference of Governmental Industrial Hygienists (ACGIH) as 0.5 ppm, or 500 ppb,⁵ and the recommended exposure limit (REL-TWA) set by the National Institute for Occupational Safety and Health (NIOSH) at 0.1 ppm, or 100 ppb.⁶

Exposure to low concentrations of benzene, however, could still expose gasoline station workers to the risk of cancer via inhalation uptake.⁷ Potential health risk could be modified by gasoline station workers' profile, i.e., station locations and job functions as well as any preventive behaviors they may exhibit. Trans, trans-muconic acid (tt-MA), a metabolite of an internal dose of benzene, has been detected in benzene-exposed workers at concentrations lower than the OEL-TWA⁸; however, other studies report that tt-MA has been detected at concentrations exceeding the biological exposure index (BEI), or above 500 µg/g creatinine (Cr).^{9,10} Exposure to different levels of benzene could be a reason for the varying tt-MA metabolite release concentrations shown in the studies.¹¹ The predicted percentage of workers exposed to concentrations of benzene in air between 0.001 and 0.25 ppm was 88%, based upon detection of the urinary tt-MA metabolite.

The use of a biological marker for calculation of the likelihood of exposure levels from the internal dose marker was reliable, and was similar to the external dose marker when compared to the BEI⁵ in occupational exposure screening of workers.¹² Previous studies found over 60% of Thai gasoline station workers had experienced adverse symptoms related to benzene toxicity and over 36% had ongoing potential health risks via air benzene in their work environments.³ Refueling and fuel tank loading workers had the highest potential health risk from benzene exposure of all gasoline station workers.^{7,13} Risk reduction through preventive actions could be measured by occupational health surveillance programs using exposure biomarkers. This study was designed to investigate gasoline station workers' health risk by application of a health risk matrix of benzene exposure made up of detected tt-MA biomarkers and adverse symptom reports.

2 | MATERIALS AND METHODS

2.1 | Study population and study area

The study was conducted in Khon Kaen Province, Thailand, among 235 gasoline station workers from 98 gasoline stations in Mueang Khon Kaen, the capital district of Khon Kaen Province, Thailand. Subjects recruited for this study worked in different gasoline stations along Mittraparp road, the major highway bisecting Khon Kaen city and linking it to Bangkok, the national capital. Gasoline stations were categorized into three zones based

upon their geographic location: 'urban', 'suburban' and 'rural'. The gasoline stations in the urban zone were defined as those located in the Nai Mueang sub-district of the city of Khon Kaen and where the majority of the residents' occupations were not in agriculture. The suburban gasoline stations were located around Mueang district and within 5 km of either side of the main highway. The rural gasoline stations were those situated outside the Nai Mueang sub-district and where the majority of the residents worked in agriculture.

This research was reviewed and approved by Khon Kaen University Human Ethics Research Committee (HE 562237 and HE 592330). Participants gave informed consent prior to taking part in the study.

2.2 | Air benzene concentration

Air benzene monitoring was done by personal sampling with a low flow rate control pump connected to a coconut charcoal sorbent tube. The monitor was fixed in the breathing zone for each worker following the standard protocol of the National Institute for Occupational Safety and Health (NIOSH) number 1501.⁶ Benzene sampling was carried out twice (the first data collection was performed between November 2013 and March 2014 and the second was performed between January and April 2016, during northeastern Thailand's dry season. A single 8-h continuous measurement was done, which covered the peak service period during each worker's shift. The temperature ranged from 21.9 to 35.5°C, the humidity from 52% to 94.6%, and the wind velocity from 0.63 to 5.75 km/h. Samples were analyzed by gas chromatography equipped with a flame ionization detector (Hewlett Packard 1996, Germany). The limit of detection (LOD) was <0.03 ppb.

2.3 | tt-MA biomarker

Urine samples were collected on the same day as the air benzene monitoring took place. A 30 ml urine sample was collected from each of the 235 workers in a sterile container after they had completed their shift, and was sealed and stored at 4°C until analysis. tt-MA concentration was analyzed by using high-performance liquid chromatography with UV detector (HPLC-UV) operated at 264 nm with the following conditions; a mobile phase of aqueous acetic acid: methanol (82:18), and a reversed phase C18 column at 20°C. Urine samples were extracted by solid-phase extraction C18-LP 100 mg and eluted with 1% aqueous acetic acid. Urinary tt-MA concentrations were expressed as micrograms per gram creatinine (µg/g Cr). The limit of the detectable level (LOD) of tt-MA was 10 µg/g Cr.

Additional data on the enrolled workers were collected through an interview questionnaire and observation of all subjects who met the inclusion criteria: (1) at least 18 years of age, (2) workday of at least 8 h, (3) employed at the gasoline station for more than 6 months, and (4) non-smoker. Alcohol drinking and passive smoking were prohibited for 24 h before urine sample collection from the included subjects. The questionnaire had three parts: demographic information, work characteristics and environment, and a final part which asked about adverse symptoms related to benzene toxicity.

2.4 | Biomatrix tool for health risk assessment

The health risk assessment was performed by using a risk matrix (5 × 5) of the likelihood of exposure levels and the adverse symptoms severity levels, as shown in Table 1.

2.4.1 | Classification of the likelihood of exposure level

This study used the level of tt-MA biomarker of benzene exposure and the frequency of exposure level, applying them from the previous model³ which was used to calculate likelihood of benzene exposure levels.

- Following the previous model of using air benzene concentration,³ the measured concentration was divided into five levels according to OEL-TWA standards (NIOSH-TLV = 100 ppb).⁶ The five levels of benzene concentration were; (1) Benzene <10.0 ppb (<10.0% OEL-TWA), (2) 10.0–49.9 ppb (10.0%–49.9% OEL-TWA) (3) 50.0–74.9 ppb (50.0%–74.9% OEL-TWA), (4) 75.0–100.0 ppb (75.0%–100.0% OEL-TWA) and (5) benzene >100 ppb (>100.0% OEL-TWA). Applying this to the use of the tt-MA biomarker,

tt-MA detected in urine was divided into five levels according to the BEI of 500 µg/g Cr, recommended by ACGIH.⁵ The five tt-MA levels were as follows; (1) tt-MA <50 µg/g Cr (<10.0% BEI), (2) tt-MA 50.0–249.9 µg/g Cr (10.0%–49.9% BEI), (3) tt-MA 250.0–374.9 µg/g Cr (50.0%–74.9% BEI), (4) tt-MA 375–500 µg/g Cr (75.0%–100.0% BEI) and (5) tt-MA >500 µg/g Cr (>100% BEI).

- The frequency of exposure level, based on hours of exposure,³ was extracted from face-to-face interview data and classified into five levels: (1) once a month, (2) once a week, (3) once per work shift (less than 2 h), (4) continuously for between 2 and 7 h per work shift, and (5) continuously for 8 h or more per day.
- The outcome scores were calculated by multiplying the frequency of exposure by tt-MA concentration levels or benzene concentration levels, and were classified into five likelihood of exposure levels: level 1—non exposure (score: 1–5), level 2—low exposure (score: 6–8), level 3—medium exposure (score: 9–15), level 4—high exposure (score: 16–20), and level 5—the highest exposure (score: 21–25).

2.4.2 | Classification of the severity of adverse symptoms

Benzene toxicity symptoms were extracted from the face-to-face interview data based on symptoms experienced by workers during or after work shifts over the previous 6 months. Additionally, workers could choose more than one of the symptoms, which differed in severity and were grouped according to a five-level classification based on a previous study³; level 1 (score 1): non-symptomatic, which indicated that workers had no experience of symptoms; level 2 (score 2): mild symptoms; level 3 (score 3) moderate symptoms; level 4 (score 4): severe symptoms; and level 5 (score 5): very severe or chronic disease, i.e., leukemia or cancer.

TABLE 1 Biomatrix of health risk assessment among gasoline station workers exposed to benzene

Adverse symptom level	Likelihood of exposure level (5 levels of tt-MA x 5 levels of exposure hours)					Health risk		
	1	2	3	4	5	Score	Risk	Level
5: Very high	5	10	15	20	25	21–25	Very high	5
4: High	4	8	12	16	20	17–20	High	4
3: Moderate	3	6	9	12	15	9–16	Medium	3
2: Low	2	4	6	8	10	4–8	Low	2
1: Non-symptomatic	1	2	3	4	5	1–3	Acceptable	1

Note: Applied from Chaiklieng et al.³

2.4.3 | Criteria for health risk assessment in the biomatrix risk assessment

Health risk assessment was performed by calculating risk from the matrix of the adverse symptom levels and the likelihood of exposure levels, and classified into five levels: (1) level 0, or acceptable risk (score: 1–3), (2) level 1, low risk (score: 4–9), (3) level 2, moderate risk (score: 9–16), (4) level 3, high risk (score: 17–20), and level 5, the highest risk (score: 21–25). Scores were grouped from lowest, as acceptable risk, to highest, as unacceptable risk, with preventive action recommended when the biomatrix calculation indicated at least a moderate risk (see details in Table 1).

2.5 | Statistical analysis

STATAversion 10.0 software (StataCorp LLC) was used to analyze the data. Adverse symptom levels, likelihood of exposure levels and risk levels were analyzed by descriptive statistics and displayed as percentages and frequency. Pearson's correlation coefficient was used to examine the correlation between tt-MA concentrations and benzene concentrations, and the associations of zones or job functions; and the exposure profile affecting health effects (adverse effects and health risk levels) was assessed by Pearson's chi-squared test. To indicate the relationships of predicted health risk from inhaled benzene monitoring and the tt-MA biomarker of exposure, a linear regression model adjusted by covariates was used, with statistical significance set at a $P < .05$.

3 | RESULTS

3.1 | Gasoline station workers' characteristics

There were 108 male (46.0%) and 127 female workers (54.0%), with an average age of 32.2 years (SD ± 9.97 years) (range: 18–60 years). Most were aged between 21 and 40 years (63.0%); 40.4% had attended high school and 35.7% had finished primary school. There were 220 fueling workers (93.6%) and 15 cashiers (6.4%). Most worked in the suburban zone (124 workers, 52.8%), followed by the urban zone (67 workers, 28.5%), and the rural zone (44 workers, 18.7%). The majority worked six days per week (78.7%) and had work training experience (144 workers, 61.3%). Personal air monitoring indicated that the average benzene concentration in their work environments was 32.0 ppb (SD ± 23.4) and the median concentration was 49.9 ppb, which was about 50.0% of the OEL-TWA, or 50.0 ppb.

3.2 | Classification of adverse health effects

There were 200 workers (85.1%) who had experienced adverse symptoms. The highest reported symptoms for each severity level were: mild symptoms, headache (54.0%), fatigue (52.5%), and dizziness (38.0%); moderate symptoms, muscle weakness (32.0%), drowsiness (23.0%), and tight chest (19.0%); severe or high level of symptoms, tachycardia (5.5%), petechia (0.5%) and unconsciousness (0.5%). Very severe or chronic disease was not detected in this study. Fuller details are shown in Table 2.

Regarding the highest severity level of detected symptoms of each worker, the highest symptom severity level for most workers (132 workers, 56.2%) was level 3 (moderate symptom severity). There were no symptom complaints from 35 workers (14.9%) and the highest severity level for adverse symptoms from benzene toxicity was level 4 (severe symptom severity) for about 10% ($n = 23$) of workers, as shown in Table 3.

Suburban workers had the highest symptom frequency (114 workers, 88.7%), followed by workers in the urban zone (56 workers, 83.6%), and then the rural zone (34 workers, 77.3%), respectively. There was a higher adverse symptom frequency in fueling workers ($n = 189$, 85.9%) compared to cashiers ($n = 11$, 73.3%).

3.3 | Benzene exposure levels

The average detectable tt-MA from spot urine was 299.7 $\mu\text{g/g Cr}$ (min-max: non-detectable ($<10 \mu\text{g/g Cr}$)—2159 $\mu\text{g/g Cr}$). The minimum of detected tt-MA was 23.0 $\mu\text{g/g Cr}$, median was 197.9 $\mu\text{g/g Cr}$. The 95th percentile of the tt-MA value was 900.0 $\mu\text{g/g Cr}$, which was above the BEI (500 $\mu\text{g/g Cr}$). The tt-MA metabolite was detected in 77 workers (32.8%) and 16 workers (6.8%) had the highest level of more than 500 $\mu\text{g/g Cr}$ (13 fueling workers and three cashiers). The highest benzene concentration found in monitoring of the breathing zone of workers was 65.8 ppb, which was below the OEL-TWA standard set by NIOSH.⁶ The air benzene concentrations range of <0.03 to 65.8 ppb were classified into four criteria levels: Level 1 (<10.0 ppb) was 71.9%; Level 2 (10.0–49.9 ppb) was 18.3%; Level 3 (50.0–74.9 ppb) was 8.1% and Level 4 (75.0–100.0 ppb) was 1.7%. Details are presented in Table 3.

From the results of the workers with detected tt-MA from benzene exposure at the gasoline stations, the tt-MA metabolite's correlation with air benzene concentration was clearly illustrated (Pearson's correlation coefficient (r) = .666, $P < .001$) as shown in Figure 1. There was a significant indication of correlation between concentration levels of inhaled benzene and tt-MA concentrations

TABLE 2 Adverse health effects related to benzene toxicity among 200 gasoline workers experiencing symptoms

Mild symptoms (low level), <i>n</i> (%)	Moderate symptoms (moderate level), <i>n</i> (%)	Severe symptoms (high level), <i>n</i> (%)
Cough/hoarseness 28 (14.0)	Anorexia 24 (12.0)	Petechia 1 (0.5)
Burning nose/congestion 60 (30.0)	Blurred vision 32 (16.0)	Tachycardia 11 (5.5)
Sore throat 63 (31.5)	Tight chest 39 (19.5)	Unconsciousness 1 (0.5)
Breathlessness 33 (16.5)	Vomiting 28 (14.0)	Anemia 12 (6.0)
Dizziness 76 (38.0)	Muscle weakness 64 (32.0)	
Headache 108 (54.0)	Cramp/ Nausea 36 (18.0)	
Sleeplessness 14 (7.0)	Drowsiness 46 (23.0)	
Cracked skin 47 (23.5)	Depression 5 (2.5)	
Skin rashes 46 (23.0)	Confusion 6 (3.0)	
Burning sensation 16 (8.0)	Unusual tiredness 18 (9.0)	
Burning eyes 50 (25.0)	Tremor 7 (3.5)	
Fatigue 105 (52.5)	Scurvy 9 (4.5)	
Numbness 20 (10.0)		
Palpitations 6 (3.0)		
Runny nose 16 (8.0)		

Note: There were 35 workers (14.9%) who had no experience of adverse symptoms.

TABLE 3 Classification of gasoline station workers according to adverse health effects, and tt-MA and inhaled benzene concentrations (*n* = 235)

Levels	Adverse effects, <i>n</i> (%)	tt-MA ^a		Benzene ^a	
		Concentration (µg/g Cr)	Workers, <i>n</i> (%)	Concentration (ppb)	Workers, <i>n</i> (%)
1	35 (14.9)	<50.0	163 (69.4)	<10.0	169 (71.9)
2	45 (19.2)	50.0–249.9	42 (17.9)	10.0–49.9	43 (18.3)
3	132 (56.2)	250.0–374.9	12 (5.1)	50.0–74.9	19 (8.1)
4	23 (9.8)	375.0–500.0	2 (0.9)	75.0–100.0	4 (1.7)
5	0	>500.0	16 (6.8)	>100.0	0

^aA significant relationship between levels of inhaled benzene concentration and tt-MA concentration by a linear regression model ($t = 13.65$, $R^2 = 0.444$, coefficient = 0.044, 95%CI = 0.038–0.051, $P < .001$).

according to a linear regression model ($R^2 = .444$, coefficient = .044, 95%CI = 0.038–0.051, $P < .001$).

The longest hours of exposure among all gasoline workers was eight hours or more per shift, which was classified as a level 5 likelihood of exposure. There were 72 workers (30.6%) who had a medium to high likelihood of exposure to benzene. The likelihood of exposure levels are shown in Table 4.

3.4 | Health risk assessment

The biomatrix health risk assessment with the internal dose marker of benzene exposure, tt-MA, categorized 159 workers (67.7%) as being at an acceptable health risk and

76 (32.3%) with higher than acceptable risk levels. The highest risk level was level 4 (high risk). According to our previous risk assessment model using an external dose of exposure to inhaled benzene,³ 30.2% of the workers were exposed to higher than acceptable risk levels. About 18.3% of workers had potential benzene exposure health risks requiring intervention. In addition, a linear regression adjusted model revealed a significant health risk correlation between levels of external benzene exposure and the internal tt-MA biomarker with high predictability ($R^2 = .826$, adjusted $R^2 = .825$, 95%CI = 0.798–0.898, $P < .001$). The Pearson's correlation coefficient (r) was .909. This is presented in Table 4.

There was a significant association between the likelihood of exposure level and the location of the gasoline

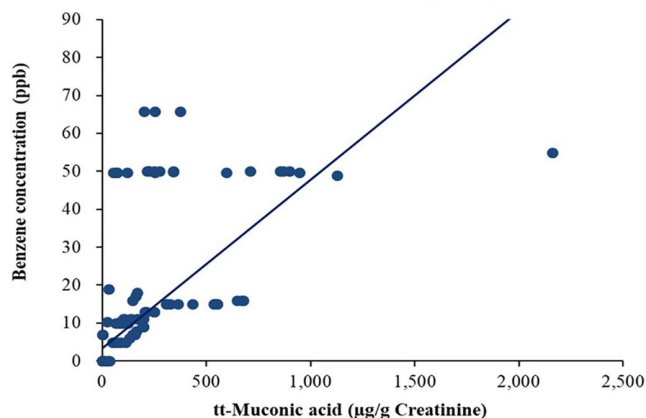


FIGURE 1 The correlation between the trans, trans-Muonic acid (tt-MA) metabolite and air benzene concentration. The significance was identified at a $P < .001$, and the Pearson's correlation coefficient (r) = .666

stations. Workers in the suburban zone had significantly higher levels of potential exposure as well as the higher health risk than their urban and rural zones, counterparts. Those potential benzene exposure health risks were not significantly related to the function of workers. The linear regression model could predict the health risk levels of fueling workers and cashiers with significance, but was a poor predictor of health risk when considering the differences between those functions ($R^2 = .019$, adjusted $R^2 = .015$; 95%CI = 0.004–0.093) as shown in Table 5.

4 | DISCUSSION

Previous research on metabolite detection among benzene-exposed workers, i.e., fuel tanker drivers,

Levels	Likelihood of exposure levels, n (%)	Health risk levels of workers ^a		
		tt-MA biomatrix, n (%)	Benzene matrix, n (%)	Risk level
1	163 (69.4)	159 (67.7)	164 (69.8)	Acceptable
2	0	33 (14.0)	32 (13.6)	Low
3	54 (23.0)	40 (17.0)	39 (16.6)	Medium
4	2 (0.8)	3 (1.3)	0	High
5	16 (6.8)	0	0	Very high

TABLE 4 The likelihood of exposure levels (via tt-MA biomarker) and the health risk levels of workers according to the tt-MA biomatrix and the matrix of inhaled benzene ($n = 235$)

^aA significant relationship between the health risk level of the external dose of benzene and the internal dose of tt-MA by a linear regression model ($t = 33.23$, $R^2 = .826$, coefficient = .848, 95% CI = 0.798–0.898, $P < .001$, and the Pearson's correlation coefficient (r) = .909, $P < .001$).

TABLE 5 Zones of gasoline stations and job functions in relation to the likelihood of exposure levels, adverse effects and risk levels ($n = 235$)

Characteristic	Likelihood of exposure levels		Adverse health effects		Health risk levels from biomatrix ^a	
	Non-exposure	Medium to highest exposure	Non-symptomatic	Mild to severe	Acceptable	Low to high risk
Zone		0.007*		0.172		0.009*
Urban (67)	54 (80.6)	13 (19.4)	11 (16.4)	56 (83.6)	51 (76.1)	16 (23.9)
Suburban (124)	75 (60.5)	49 (39.5)	14 (11.3)	110 (88.7)	73 (58.9)	51 (41.1)
Rural (44)	34 (77.3)	10 (22.7)	10 (22.7)	34 (77.3)	35 (79.6)	9 (20.4)
Job function		0.416		0.186		0.512
Cashier (15)	9 (60.0)	6 (40.0)	4 (26.7)	11 (73.3)	9 (60.0)	6 (40.0)
Fueling (220)	154 (70.0)	66 (30.0)	31 (14.1)	189 (85.9)	150 (68.2)	70 (31.8)

^aThe significant relationship by the linear regression model adjusted by covariates of health risk levels of fueling workers and cashiers ($P = .033$), but poor prediction of health risk when considering the differences between functions ($R^2 = .019$, adjusted $R^2 = .015$; 95%CI = 0.004–0.093).

*Significant association at a $P < .05$ by Pearson's chi-squared test.

filling-station attendants, and office workers, found differing concentration levels of detected tt-MA,¹⁴ confirming metabolite sensitivity to benzene exposure at gasoline stations. Similar studies^{9,10} on gasoline station workers have found a range of tt-MA concentrations exceeding the recommended safe value (BEI: 500 $\mu\text{g/g Cr}$) set by ACGIH.⁵ Rahimpoor et al.¹⁵ reported air benzene concentrations above the OEL set by ACGIH⁵ corresponding to a mean tt-MA of 1431 $\mu\text{g/g Cr}$ exceeding the BEI in male workers exposed to benzene. Our previous study⁹ showed similar correspondence of a detected tt-MA concentration exceeding the BEI in some gasoline station workers at an air benzene concentration of 0.05 ppm, or 50 ppb, as 50% OEL set by NIOSH.⁶ In this study, concentrations of the tt-MA metabolite significantly correlated with inhaled benzene concentrations of gasoline workers. A detectable tt-MA level above the BEI was indicated in both cashiers and fueling workers. This exposure was explained in the previous report, which showed that it was based on fueling service activities having similar characteristics. Fueling workers can frequently handle the fuel nozzles during refueling, depending upon the amount of service provided, and the cashier works in a one-side-opened booth located in the center of the station, which is not that far from the dispensing area. Additionally, some of the fueling workers also do the cashier's task at the cashier desk. Hence, this confirmed the previous studies in showing that tt-MA biomarker detection is one aspect of biological screening for low levels of benzene exposure among workers involved with gasoline or petroleum products.^{9,16} Therefore, cashiers as well as fueling workers have to be warned about hazardous conditions at gasoline stations for protection against benzene exposure.

A previous study⁷ found that benzene concentrations in the suburban gasoline stations were higher than in the urban stations because of higher amounts of service provided. Another finding of that study was that fueling workers who worked longer hours in service had a health risk linked to benzene exposure which was higher than that of workers with fewer hours of exposure.⁷ Our study supported the idea that in the potential exposure to benzene according to fueling service activities and zones of stations, the association between workers in the suburban stations and a higher level of benzene exposure was significant, when compared to the associations between workers in the urban and the rural stations and a higher level of benzene exposure.

Furthermore, our previous report showed that tt-MA biomarker levels increased during shift work (post-shift work levels compared to pre-shift work levels) and were significantly higher in fueling workers than in the cashiers.¹² In addition to gasoline station workers being at risk of benzene exposure, those in other occupations—for

example, policemen in areas of traffic congestion—are also at high risk from benzene exposure.¹⁷ This study confirms that the direct source of the benzene exposure was the high level of refueling service provided. This was shown by the higher adverse symptom frequency in fueling workers compared to cashiers, which was particularly evident in the suburban workers. Consistent with the previous report on benzene concentrations above the 50% OEL,¹⁸ the developed biomatrix could explain the health risk levels of fueling workers and cashiers at Thai gasoline stations. The similarities in the characteristics of those exposed to benzene and other volatile organic compounds (VOCs) from fuel dispensing activities in a polluted health hazardous zone has previously been explained for cashiers and fueling workers.¹⁸

Most workers in the previous studies also had moderate adverse health effects similar to those found in this study, and gasoline maintenance workers have also previously been found to have tt-MA levels at similar ranges of benzene concentrations.⁸ US.EPA guides have quantified the benzene-exposure health risk related to the frequency of exposure and benzene concentrations at levels confirmed by our previous studies.^{3,7,19} These workers' routine shifts (eight or more continuous hours per day) put them at unacceptable risk levels according to our biomatrix. Previous studies among benzene-exposed gasoline station workers have also revealed carcinogenic health risks from lifetime exposure.^{4,7,20} Moreover, gasoline station attendants or customers coming to gasoline stations should be aware of the likelihood of exposure related to frequency and duration of benzene inhalation from ambient air in the hazardous area.²¹

A linear regression model revealed a significant correlation of the health risk levels from the biomatrix of the tt-MA biomarker with that of the matrix of benzene concentrations. About 32% of workers from the biomatrix, as same as from the benzene matrix, had health risks higher than acceptable levels, and about 18% of workers were required to take preventive action due to their moderate to high health risk levels. The use of risk levels from the biomatrix risk assessment would be a useful model for health surveillance programs of Thai gasoline station workers in compulsory health monitoring.³ As there was a significant correlation between the linearity of the external dose of exposure and the internal dose of benzene or its health risk affecting the workers, carrying out health surveillance with benzene exposure biomonitoring and health screening can be an important aspect of a strategy to reduce other exposures to VOCs. The previous reports on the likelihood of exposure to BTEX (benzene, toluene, ethyl benzene and xylene) of gasoline workers^{18,21-23} could recommend further study of the biomatrix risk assessment model on BTEX exposure at Thai gasoline stations.

5 | CONCLUSIONS

The health risk assessment matrix based on air benzene exposure levels and detectable tt-MA among benzene-exposed workers was developed in this study. The tt-MA concentrations, which ranged from non-detectable to 2159 $\mu\text{g/g Cr}$, corresponded to air benzene concentrations ranging from less than 0.1–65.8 ppb. The biomatrix revealed that 32.3% of workers had health risks which were at a higher than acceptable level, and 18.3% were therefore in need of preventive action. There was a significant association between gasoline station work zones and the likelihood of exposure as well as the health risk of workers. The health risk levels found by biological monitoring of detected tt-MA levels was consistent with the risk matrix of air benzene monitoring. Therefore, this biomatrix could be a useful tool for occupational health surveillance programs serving benzene-exposed workers. Annual health check-ups, and the monitoring of the tt-MA biomarker and air benzene concentration are recommended for gasoline station workers in order to prevent their health effects and cancer from benzene toxicity.

DISCLOSURE

Ethical approval: The study obtained ethical approval from Khon Kaen University Ethics Committee, Thailand, no. HE 562237 and HE 592330. **Informed Consent:** All participants gave informed consent prior to taking part in the study. **Registry and the Registration No. of the Study/Trial:** N/A, **Animal Studies:** N/A, **Conflict of Interest:** The authors declare no conflicts of interest in writing this article.

AUTHOR CONTRIBUTIONS

Sunisa Chaiklieng invented and conceptualized the study, performed the investigation, and wrote and edited the manuscript; Pornnapa Suggaravetsiri performed data curation and participated in the discussion; and Herman Autrup participated in the discussion and reviewed the manuscript.

DATA AVAILABILITY STATEMENT

Data available on request due to privacy/ethical restrictions.

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