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Altered Haematological Parameters in Gasoline Station Workers Due to Benzene Exposure

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Abstract: Benzene is harmful to human health and early detection of haematological alterations is important in preventing adverse health effects. This study aimed to investigate the biomarkers of benzene exposure and its effects due to haematological alterations. Gasoline station workers with potential risks according to the biomatrix concerning benzene exposure underwent blood and urine evaluation for the biological monitoring of urinary trans, trans-muconic acid (tt-MA), and haematological and biochemical parameter evaluation. The results were analysed for correlations between biological and haematological effects. The tt-MA biomarker was detected in some workers and approximately 50% of workers had a blood profile that showed abnormal parameters with respect to the haemoglobin (Hb), haematocrit (Hct) and white blood cell parameters, which were outside the normal range. A significant correlation was observed between the tt-MA biomarker's level and the levels of the haematological and biochemical parameters, which were Hb, Hct, eosinophil, neutrophil, SGOT and blood creatinine. The level of urinary tt-MA as a marker of benzene exposure correlated with haematological and biochemical changes in the blood, suggesting that the gasoline station workers were affected by benzene exposure. Moreover, the current study suggests that early detection of haematological abnormalities may be possible by analysing biomarkers of their effects through regular health surveillance of workers.

Keywords: trans, trans-muconic acid; benzene; biomatrix; haematological effect; gasoline station

Citation: Chaiklieng, S.; Tongsantia, U.; Suggaravetsiri, P.; Autrup, H. Altered Haematological Parameters in Gasoline Station Workers Due to Benzene Exposure. *Safety* **2024**, *10*, 38. https://doi.org/10.3390/safety10020038

Academic Editor: Raphael Grzebieta

Received: 12 October 2023
Revised: 28 March 2024
Accepted: 10 April 2024
Published: 12 April 2024

1. Introduction

Benzene is found in ambient air at fuel service stations due to fuel evaporation from gasoline filling operations [1]. It has been found that workers have increased health risks, including the risk of cancer from occupational benzene exposure [2–4] and health effects caused by long-term exposure to benzene [5]. The symptoms of benzene poisoning include headaches, fatigue, throat irritation, nose irritation, nausea, dizziness, and depression [3]. The amount of exposure to benzene at gasoline stations can be assessed by measuring the concentration of benzene in the working ambient air and biomarker monitoring [2,6]. A health risk assessment performed using the detected benzene concentration indicated a risk of cancer among gasoline station workers [2].

In this study, biomarkers of exposure, namely, trans, trans-muconic acid (tt-MA) and S-phenylmercapturic acid (S-PMA), were assessed [7] after work finished; their standards had been set by the American Conference of Governmental Industrial Hygienists (ACGIH) [8], which set the biological exposure index (BEI) of tt-MA detected after shift work to be not over 500 µg/g creatinine (Cr) [8]. Gasoline station workers in Thailand who took part in one study had tt-MA levels which were in the 3.0–1127 µg/g Cr range [8], while another study of Indonesian gasoline workers found that they had levels of 480.74 (219.7) µg/g Cr [9]. In a study of tt-MA levels and the effect of DNA oxidative stress among

fuelling workers and university staff in Egypt, tt-MA was found to be high among the fuelling workers [10], and effects on the haematology system were also present [7,11]. In another study involving the effects of benzene exposure at gasoline stations, several abnormalities, such as a decrease in almost all blood cell counts, were identified as a phenotypic effect of benzene [12].

Long-term effects on the haematological system have been found [5,11]. A previous study reported the activation of proto-oncogenes, the loss of heterozygosity, and the inactivation of tumour suppressor genes [13]. In the case of blood cell abnormalities, alterations in serum liver enzyme levels, especially serum glutamic pyruvic transaminase (SGPT) and serum glutamic-oxaloacetic transaminase (SGOT), have been found [14]. In addition, one study demonstrated genotoxic damage related to occupational exposure among gasoline station workers [15]. In one analysis of hepatotoxic responses, the levels of bilirubin, alanine aminotransferase, aspartate aminotransferase, blood urea and plasma creatinine were found to be significantly elevated in exposed individuals compared to workers not exposed to benzene [16]. A study in India supported these observations in that the serum liver enzyme levels, namely those of SGPT and SGOT, were found to be significantly higher among gasoline station workers in comparison to workers not exposed to benzene [17].

In one previous study in Thailand on benzene, exposure was assessed primarily based on the detection of tt-MA as an internal dose of exposure [6], and this was useful for the biomatrix of health risk assessment (HRAB), which is used in order to assess the occupational risk of gasoline workers in terms of their exposure to benzene. Long-term exposure to benzene appeared to initiate a high risk of cancer among gasoline station workers [2]. Therefore, the objective of this study was to identify putative biomarkers using the haematological and hepatological endpoints that were indicative of adverse health effects due to benzene exposure within a high-risk group of gasoline station workers.

2. Materials and Methods

2.1. Sample Size

The study was conducted on 20 gasoline station workers in Khon Kaen, Thailand, who were sampled from 10% of all participants from 47 stations that had participated in a prior study on risk assessment [18]. The sample of 20 workers in this study was a representative sample of workers in this secondary study for blood and urine sampling with adverse symptom monitoring and a biomatrix of health risk assessment (HRAB) analysis. These workers had participated in the screening phase, which had a sample size of 151 gasoline station workers who underwent a tt-MA biomarker screening and a benzene exposure determination with air benzene monitoring, and completed an adverse symptoms questionnaire in the primary study of the year 2021 [18]. There was a significant relationship between external benzene exposure and the levels of the tt-MA biomarker in the urine of each gasoline station worker who participated in that primary study ($R^2 = 0.127$, p-value < 0.05) [18].

The 20 subjects included into this secondary phase were representative of workers at all levels of the HRAB matrix (level 1–3), as shown in Figure 1.

The inclusion criteria were as follows: (1) there was data on the worker showing previous exposure to benzene with a tt-MA detection in urine of more than 50 μg/g Cr, (2) the worker had worked at their current gasoline station for longer than 3 months and was not pregnant or menstruating, and (3) the worker had entered voluntarily into the study. This study was approved by the Khon Kaen University Ethics Committee for Human Research (No. HE612030). Before entering into the study, participants were given a consent form to sign. Smokers were excluded, as were alcohol drinkers as alcohol induces an enzyme involved in the metabolism of benzene. In addition, the consumption of food containing sorbic acid was not allowed 24 h before the day of urine and blood collection.

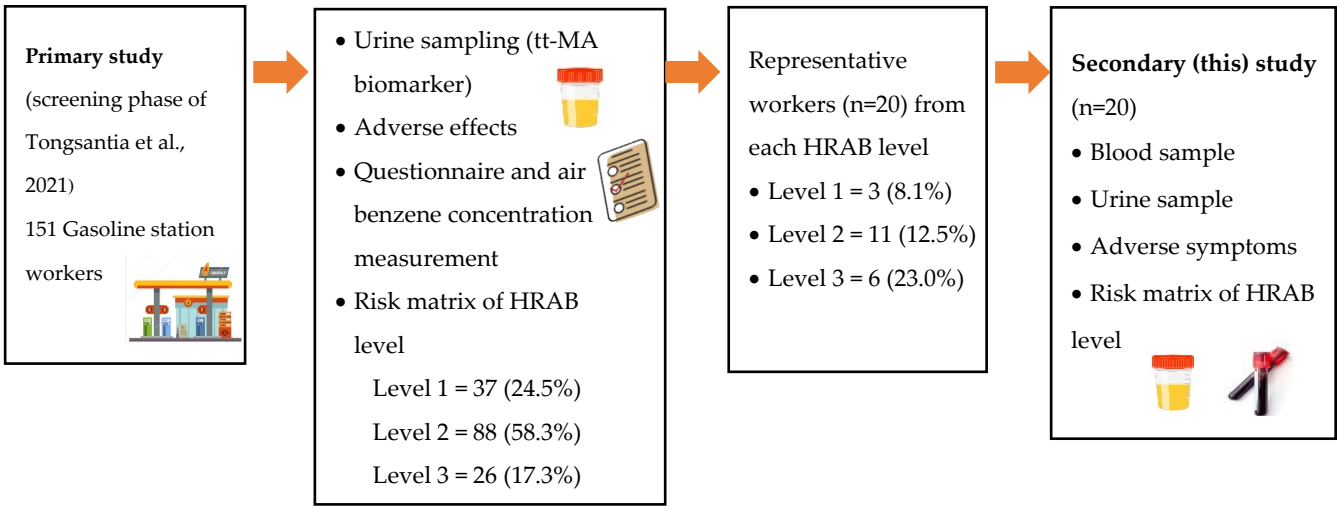

Figure 1. A flow diagram illustrating the methodology, including the sample size and the input of each stage in the primary study [18] and the secondary (this) study for data collection.

2.2. tt-MA Detection and Risk Assessment

General characteristics, working characteristics, and adverse symptoms from benzene exposure were collected from 20 workers using a structured questionnaire (general information, working experience, PPE usage, and working behaviour) before blood and urine sampling. The severity of the health effect symptoms experienced in the past 3 years, referenced from the study of Chaiklieng [18,19], was indicated according to 5 levels: no symptoms, mild symptoms, moderate symptoms, severe symptoms, and very severe symptoms.

2.3. Urine Sampling and Analysis

Spot urine was collected from workers at the end of the work shift and analysed for tt-MA (in μg/g Cr). Urine samples were kept in a container at a temperature of 4 °C and sent to the laboratory for analysis using high performance liquid chromatography with a UV detector (HPLC-UV). The limit of detection was <0.01 mg/g Cr. The level of tt-MA in urine (the detection level was 13.69 μg/g Cr) was noted from the first round of screening in the previous study as an inclusion criterion of the subjects to be blood sampled.

The tt-MA levels in urine after a work shift were categorized by referencing the BEI value and dividing them into 5 levels for risk assessment, which were as follows: level 1: <50 μg/g Cr; level 2: 50.0–249.9 μg/g Cr; level 3: 250.0–374.9 μg/g Cr; level 4: 375–500 μg/g Cr; and level 5: >500 μg/g Cr [20]. The likelihood of exposure to benzene was calculated from the tt-MA level and the frequency of benzene exposure according to the classification of working hours. The previous risk assessment via the biomatrix of opportunity levels multiplied by the severity level of benzene exposure was followed, and for this group of workers, the risk levels were as follows: an acceptable risk level (score 1–3), a low risk level (score 4–9), a moderate risk level (score 10–16), a high risk level (score 17–20), and a very high risk level (score 21–25) [19].

2.4. Blood Sampling and Analysis

Blood samples (20 mL) were collected and kept in a container at 4 °C for haematological analysis according to the following parameters: complete blood count: CBC; haemoglobin: Hb (grams per decilitre g/dL); haematocrit: Hct (%); white blood cell count: WBC (cells per cubic millimetre: cells/mm^3); neutrophils (%); lymphocytes (%); monocytes (%); eosinophils (%); platelets (cells/mm^3); and mean corpuscular volume: MCV (microlitres: μL). Biochemical analysis was performed to determine blood creatinine (milligrams per decilitre: mg/dL), and SGOT (units per litre; U/L) levels. The parameters

of haematological and biochemical analysis were compared with the normal parameter values [20] for defining the workers who had abnormalities in their blood analysis.

2.5. Statical Analysis

Data were analysed using STATA version 10.0 software for the continuous distribution of haematological parameters. The hepatological parameters' abnormality distribution was analysed via a Kruskal–Wallis test by ranks and the hepatological parameters were analysed with the benzene exposure parameters (tt-MA levels, adverse health effects) using Spearman's rank correlation coefficient, which showed that the medians significantly differed at a p-value < 0.05. The correlation between the haematological parameters and the biological markers, measured using multiple linear regression analysis, was also found to be significant at a p-value < 0.05.

3. Results

3.1. Sample Characteristics

The workers (N = 20) had an average age of 32 years old (min:max 20:53) and the work experience of one year, on average (min = 1, max = 30); there were eight male and twelve female workers, of whom fifteen were fuelling workers and five were cashiers. The highest proportion of workers worked in a suburban area (55%), followed by those working in urban stations (25%) and those working in rural stations (20%), respectively (Table 1).

Table 1. Characteristics of gasoline station workers who underwent blood sampling (N = 20).

Characteristic	N (%)
Gender	
Male	8 (40.0)
Female	12 (60.0)
Age (years)	
≤30 years	9 (45.0)
>30 years	11 (55.0)
Average ± SD	32.6 ± 9.4
Median (min–max)	32.3 (19.5–52.6)
Work experience (months)	
≤12	10 (50)
>12	10 (50)
Average ± SD	3.8 ± 6.7
Median (min–max)	12.5 (3–36)
Job function	
Fuelling worker	15 (75.0)
Cashier	5 (25.0)
Zone	
Urban station	5 (25.0)
Suburban station	11 (55.0)
Rural station	4 (20.0)

3.2. tt-Muconic Acid Biomarker Detection and Adverse Effects Found using the Health Risk Assessment of Benzene Exposure

The tt-MA detected in the urine samples of gasoline station workers, taken after shift work, ranged from 13.69 to 301.47 μg/g Cr (median = 132.92 μg/g Cr). There were no workers who had a detected tt-MA exceeding the BEI (500 μg/g Cr). The percentage of workers with levels of tt-MA at a 10%BEI or greater (50–249.9 μg/g Cr) was 75% and the percentage of workers with levels of tt-MA at a 50%BEI or greater (250–500 μg/g Cr) was 20% (Table 2).

Table 2. The internal dose of exposure risk level from the biomatrix of gasoline station workers (N = 20).

Level	Symptom	N (%)	tt-MA Level (μg/g Cr)	N (%)	Health Risk	N (%)
1	Asymptomatic	3 (15%)	<50	5 (25.0%)	Acceptable	8 (40.0%)
2	Mild	7 (35%)	50.0–249.9	11 (55.0%)	Low	11 (55.0%)
3	Moderate	9 (45%)	250.0–374.9	4 (20.0%)	Moderate	1 (5.0%)
4	Severe	1 (5%)	375–500	0	High	0
5	Very severe	0	>500	0	Very high	0

According to the adverse symptoms related to benzene toxicity that were self-reported by these gasoline workers, the top five symptoms conveyed were itchy skin; headaches; exhaustion and dry/sore throat; and conjunctivitis or itchy eyes, itching in the nose, and a cough, which were mild symptoms. There were three workers (15%) who were found to have inhaled fuel based on the smell they had noticed without experiencing any symptoms. In total, 50% of workers had moderate and severe symptoms from benzene exposure at work, which can be explained by the specific symptoms that were found: unconsciousness, shortness of breath, a tight chest, blurred vision, epistaxis, numbness, vomiting and burning skin. A mild level of symptoms was reported by seven workers (35%), a moderate level was reported by nine workers (45%), and a severe level (unconsciousness) was reported by one worker (5%). From the biomatrix of risk assessment using tt-MA as a biomarker of exposure, 16 workers (80%) had a low risk level of adverse health effects, two workers (10%) had a moderate risk level, and another two workers (10%) had an acceptable risk level (Tables 2 and 3).

Table 3. Adverse health symptoms of gasoline station workers.

Moderate	N (%)	Mild	N (%)
Shortness of breath	3 (15%)	Itchy skin	11 (55%)
Tight chest	2 (10%)	Headache	6 (30%)
Blurred vision	2 (10%)	Exhaustion	5 (25%)
Epistaxis	2 (10%)	Dry/Sore throat	5 (25%)
Numbness	1 (5%)	Conjunctivitis, itchy eyes	4 (20%)
Vomiting	1 (5%)	Itching in the nose	4 (20%)
Burning skin	1 (5%)	Cough	4 (20%)
		Suffocation	3 (15%)
Severe/Unconsciousness	1 (5%)	Runny nose	3 (15%)
		Dizziness	1 (5%)

3.3. Haematological Analysis of Benzene Exposure

This study presents parameter values in terms of the median (min–max) value. Some results of blood analysis did not exceed the normal reference values, but some workers had results which did exceed those values. The following parameters were altered to be above or below the normal range: WBC, neutrophil, Hct, and Hb levels. Regarding those abnormalities found via analysis (Table 4), the median Hct result (min–max) was 39.6% (33.8–50.8%), which was outside the normal range (40–54%), as was the median Hb, which was 2.9 g/dL. The examination of WBCs indicated that fuelling workers had a higher WBC than the normal value (4000–10,000 cells/mm^3), with a maximum value of 12,100 cells/mm^3. When considering the group with tt-MA detections of <50%BEI, or <250 μg/g Cr, and the group with tt-MA detections of a 50%BEI or higher (250 μg/g Cr), a significant difference between median eosinophil levels was found.

In regard to the details of gasoline workers who had haematological alterations, there were 12 persons (60.0%) who had a haematological analysis result out of the normal range. Abnormal alterations were found in workers with regard to the following parameters: RBC, two persons (16.6%); WBC, two persons (16.6%); Hct, eight persons (67.7%); eosinophil, five persons (41.7%); MCV, five persons (41.7%); MCH, six persons (50.0%); and MCHC, three persons (5.0%).

Table 4. Haematological alterations and their associations with detected tt-Muconic acid levels (>50%BEI, or 250 μg/g creatinine) in gasoline station workers (N = 20).

Parameters	Normal Range	Median (Min–Max)	IQR (25th–75th)	*p*-Value
Haemoglobin (g/dL)	13.0–18.0	12.9 (11.0–16.3)	13.25 (12.25–14.05)	0.681
Haematocrit (%)	40–54	39.6 (33.8–50.8)	39.37 (36.75–42.15)	0.957
WBC (cell/mm^3)	4000–10,000	7100 (4400–12,100)	7000 (5800–8200)	0.539
RBC (cell/mm^3)	4.5–6.0 × 10^6	4.84 × 10^6 (3.97 × 10^6–6.06 × 10^6)	4.92 × 10^6 (4.57 × 10^6–5.26 × 10^6)	0.293
Neutrophil (%)	40–74	66.5 (52–79)	67 (65–69)	0.434
Lymphocyte (%)	19–48	28 (18–40)	27.5 (25–30.5)	0.260
Monocyte (%)	3–9	3 (1–6)	3 (2–4)	0.260
Eosinophil (%)	0–7	3 (2–4)	2 (1–3)	0.013 *
Platelet (cell/mm^3)	150,000–450,000	317,500 (216,000–403,000)	304,000 (264,000–345,000)	0.362
MCV (μL)	80–100	83.5 (63–96)	82.5 (75.5–89.5)	0.084
MCH (pg)	27–33	27 (21–33)	26.75 (24–29.5)	1.000
MCHC (g/dL)	31–35	33 (31–35)	33 (32–34)	1.000

Remark: g/dL = grams per decilitre, cell/mm^3 = cells per cubic millimetre, μL = 1 microlitre, pg = picograms. * significant difference between the two groups of tt-MA levels: >50% BEI (250 μg/g creatinine) and ≤50% BEI at a *p*-value < 0.05, found via a Kruskal–Wallis test by ranks.

3.4. Biochemical Analysis of Blood Enzymes to Evaluate Benzene Exposure

From the biochemical analysis, it was found that the median values of creatinine, SGOT, and SGPT had not exceeded their normal values. The result of the median value of creatinine in blood was 1.02 mg/dL for males and 0.685 mg/dL for females, while three workers showed creatinine levels exceeding the normal value. Four workers had SGOT values exceeding the normal value; the median was 33.0 U/L for male workers and 20.5 U/L for female workers. Meanwhile, five workers had SGPT values exceeding the standard level; the median was 36.5 U/L for male workers and 14.5 U/L for female workers.

When comparing the results between genders with job function consideration, it was found that there was a significant difference in the SGOT values between female and male fuelling workers (*p*-value = 0.005), and a significant difference in the SGPT levels between male and female fuelling workers at a *p*-value = 0.014. In addition, it was found that the SGOT levels in the fuelling workers were higher compared to the SGPT levels of the cashiers (Table 5).

Table 5. A comparison of biological marker detection between fuelling workers and cashiers.

Parameters	Normal Range	Total (N = 20) Median (Min–Max)	Fuelling (N = 15) Median (Min–Max)	Cashier (N = 5) Median (Min–Max)
Blood creatinine (mg/dL)				
Total		0.76 (0.56–1.30)	0.76 (0.56–1.18)	0.77 (0.63–1.30)
Male	0.67–1.17	1.02 (0.63–1.30)	1.02 (0.63–1.18)	1.06 (0.81–1.30)
Female	0.51–0.95	0.68 (0.56–0.98)	0.68 (0.56–0.98)	0.69 (0.63–0.73)
SGOT (U/L) *				
Total		25.5 (16–104)	28 (17–104)	19.5 (16–34)
Male	0–40	33.0 (22–104)	38.5 (28–104)	27.5 (22–33)
Female	0–32	20.5 (16–34)	22 (17–34)	17 (16–17)
SGPT (U/L) *				
Total		24 (3–86)	25.5 (7–86)	19.5 (3–55)
Male	0–41	36.5 (28–86)	36.5 (31–86)	41.5 (28–55)
Female	0–33	14.5 (3–47)	18 (7–47)	9 (3–11)
tt-Muconic acid (μg/g Cr)				
Total	≤500	132.92 (13.69–301.47)	118.75 (13.69–301.47)	206.35 (15.48–294.01)
Male	≤500	113.88 (20.00–291.54)	113.88 (20.00–291.54)	156.07 (57.48–254.65)
Female	≤500	132.92 (13.69–301.47)	132.92 (13.69–301.47)	137.12 (15.48–294.01)

Remark: U/L = units per litre, mg/dL= milligrams per decilitre, μg/g Cr = micrograms/gram of creatinine. * significant difference in SGOT and SGPT between genders of fuelling workers at *p*-value = 0.005 and 0.014, respectively, found by Kruskal–Wallis test by ranks.

Among the 12 workers who had altered haematological parameters which were out of range, there were increased SGPT and SGOT values in two workers and one worker, respectively.

3.5. The Correlation between Biological Exposure Markers and Haematological and Biochemical Parameters of Benzene Exposure

As shown in Table 6, a correlation between the tt-MA levels at the end of the work shift and the haematological and biochemical parameters of benzene exposure among gasoline station workers was observed. Specifically, haematological parameters and tt-MA level correlations were found for the Hct (p-value = 0.014), Hb (p-value = 0.016), and MCHC (p-value = 0.001) levels, while a significant correlation was found between tt-MA levels and the biochemical parameter, blood creatinine (p-value < 0.001). Four gasoline station workers (20.0%) had tt-MA levels of a more than 50%BEI (250 μg/g Cr), and of those workers, two workers and one worker had Hct and Hb values that were lower than the normal range, respectively. The non-parametric method analysis using Spearman's rank correlation coefficient showed a significant correlation between the tt-MA (ug/g Cr) levels and the haematological parameters, which were eosinophil (rho = 0.5767, p-value = 0.007) and neutrophil (rho = 0.1173, p-value = 0.022). Correlations between the HRA biomatrix (HRAB) level and biochemical parameters (SGOT, MCV) were found as well as a significant correlation between adverse effects and blood haematocrit (p-value = 0.045) and haemoglobin(p-value = 0.020) levels, as shown in Figure 2. A positive correlation was found with the WBCs and a negative correlation was found with the haematocrit and haemoglobin levels, and the MCV and SGOT levels (haematological and hepatological endpoints).

Table 6. The correlation between the haematological parameters and biological markers among gasoline station workers (N = 20).

Parameters	tt-MA	WBC	RBC	Hct	Hb	MCHC	SGOT	SGPT	Creatinine
tt-Muconic acid	1	0.054	0.107	0.014 -*	0.016 -*	0.001 *	0.051	0.252	0.000 *
WBC	0.054	1	0.187	0.040 *	0.039 -*	0.042 *	0.163	0.317	0.086
RBC	0.107	0.187	1	0.034 *	0.109	0.082	0.081	0.068	0.122
Hct	0.014 -*	0.040 *	0.034 *	1	0.000 *	0.003 *	0.008 *	0.111	0.016 *
Hb	0.016 -*	0.039 -*	0.109	0.000 *	1	0.003 *	0.012 *	0.176	0.017 *
MCHC	0.001 *	0.042 *	0.082	0.003 *	0.003 *	1	0.018	0.175	0.001 *
SGOT	0.051	0.163	0.081	0.008 *	0.012 *	0.018	1	0.050 *	0.040 *
SGPT	0.252	0.317	0.068	0.111	0.176	0.175	0.050	1	0.302
Creatinine	0.000 *	0.086	0.122	0.016 *	0.017 *	0.001 *	0.050	0.302	1

* significant correlation between haematological and biological markers at a p-value < 0.05, found via multiple linear regression analysis. -* significant correlation in a negative direction, found via multiple linear regression analysis.

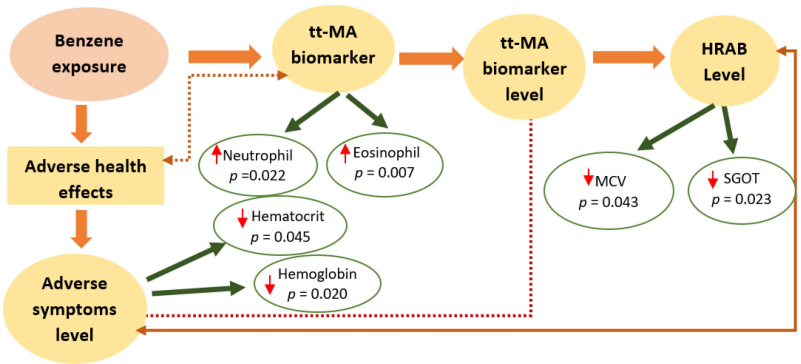

Figure 2. The effects of benzene exposure, according to the levels of the detected tt-MA biomarker in urine and the observed adverse symptoms, on significant alterations in the haematological and hepatological endpoints.

4. Discussion

Of the fuelling station workers who participated in this study, 50 percent showed specific symptoms: unconsciousness, shortness of breath, a tight chest, blurred vision, and epistaxis, or bleeding from the nose, which can be caused by abnormal blood clotting and is a disorder of the body which is a specific symptom of benzene exposure. When considering the internal dose of benzene exposure, 75% of workers had a tt-MA level higher than a 10%BEI, which was consistent with the adverse health symptoms that had been reported by 85% of workers. A previous study reported adverse effects, such as breathing difficulty or shortness of breath, among workers who had been exposed to benzene [21]. These effects are confirmed by this study as a specific symptom of benzene exposure. Workers who had a detected tt-MA level higher than a 50%BEI had a significantly different eosinophil level compared to those who had a detected tt-MA level lower than a 50%BEI.

The WBC and eosinophil levels found were similar to those of gasoline station workers in Sudan. In a group of 87 benzene-exposed workers and a control group of 87 persons who were in good health and were not exposed to benzene, it was found that the gasoline station workers had a higher WBC level (11×10^3 cell/L) than workers not exposed to benzene [22]. The number of gasoline station workers who had eosinophil levels greater than 4% was found to be greater than that of those who were not exposed to benzene, with this study also finding that eosinophil and neutrophil levels were higher when workers had higher tt-MA levels which were equivalent to having been exposed to benzene in ambient working air. A significant increase in the eosinophil counts of gasoline station workers in Southwest Ethiopia was also observed [23], which supports our finding in this study of a significant alteration in the levels of detected tt-MA in workers, which were higher than a 50%BEI.

Regarding the RBC analysis, workers had Hb and Hct levels that were lower than normal values. Reduced levels of Hb are indicative of anaemia, which can occur for many reasons, such as blood loss, malnutrition, and genetic blood diseases such as thalassaemia and bone marrow diseases that reduce red blood cell production. This is in accordance with a past study of petrol station workers where Hb and Hct levels were significantly decreased [24]. Most of the workers in this study had an RBC count close to the minimal value, similar to the results reported in a study of Korean workers who had been exposed to low-level benzene concentrations [25].

In a previous study of petrochemical factory workers in China, it was found that some workers had lower than standard Hct levels (less than 40.0%), and the Hct levels of workers exposed to benzene were greater than those of the control group, who did not work with benzene, with statistical significance (p-value < 0.001) [26].

The liver function test found that workers, especially male fuelling workers, had SGPT and SGOT results outside the normal range. In a study of workers exposed to petroleum fumes with similar working characteristics to the workers in this study, a significant elevation in SGOT and SGPT levels indicated a predisposition to hepatotoxicity [27]. A study of Nigerian petrol attendants found that fuel attendants employed for a period of 27–36 months in the petrol station showed a significant increase in SGPT and SGOT activities compared with other petrol attendants that had spent less time in the petrol station [28]. Additional support from our findings is given in that the workers who had SGPT and SGOT values out of the normal range were all from the risk group which was based on the surveillance biomatrix [19].

Considering the self-reported data on the period of occupational exposure to petroleum-related benzene in our study, which ranged from 3 months to 30 years, there should be a follow-up for the haematological analysis of workers who are beginning employment at gasoline stations. To date, only one study has been conducted addressing biochemical parameters among gasoline station workers compared to male adult workers who were ward boys (control group). The SGPT and SGOT levels were found to be significantly higher among the petrol filling attendants in India in comparison to a control group [17]. This study, which confirmed that benzene exposure altered hepatic function, also found a significant difference in the median SGOT values between male and female fuelling

workers, which was likely due to the higher benzene exposure of fuelling workers compared to cashiers. This is consistent with a previous study of gasoline station workers in Thailand, which found that SGPT and SGOT values differed between male workers and female workers [19]. In this study, most of the fuelling workers were males who exhibited risky behaviour, like standing near dispensers while performing fuel services, as seen in our previous report [18].

There was a significant correlation between the biological marker of tt-MA levels and haematological alterations (Hct, Hb). Benzene exposure among gasoline workers resulted in a decrease in their blood cell counts, confirming a previous study of workers who were exposed to benzene [12]. This study also found that 96.6% of workers (11 in 12 workers) in the unacceptable risk group from the surveillance risk matrix had abnormal haematological changes that are supported by previous reports [12,19]. The discovered HRAB matrix in this study serves as a useful tool, as discussed in a previous report [19], based on the use of the significant haematological endpoint as a marker of benzene exposure in health surveillance programs for gasoline workers.

A significant correlation between the tt-MA biomarker and biochemical change in terms of blood creatinine was observed among gasoline station workers. This could be explained by the hepatoxic effects of benzene, as suggested by elevated SGPT and SGOT values in petrol filling attendants in comparison to workers not exposed to benzene [17]. Therefore, it may be important to continue monitoring workers for potential adverse health effects resulting from occupational exposure to gasoline fumes. The hepatological endpoint serves as one of the useful biomarkers of effects in benzene-exposed workers.

There are some suggestions for the improvement of this study. Regarding the adverse effect symptoms from benzene exposure in this study, it was reported that the interviewees' symptoms could sometimes remain unrecognized. Therefore, adverse health effects should be routinely screened for during health examinations by physicians in parallel with haematological analysis, and such effects should be recorded in workers' records. In addition, this study should be additionally conducted as a longitudinal study, and urine and blood samples should be repeatedly collected from each worker.

5. Conclusions

Among workers who had previously been concerned with the potential health risk of benzene exposure due to levels of tt-MA being higher than 50 μg/g Cr, in this study, the urinary tt-MA after shift work was 13.69 to 301.47 μg/g Cr (median = 132.92 μg/g Cr). It was found that 50% of the workers who had an increased level of adverse health effects (85%) showed specific symptoms of moderate to severe levels of benzene toxicity. The haematological alterations observed in the exposed workers included alterations in their Hb and Hct levels, which were below normal levels, while their WBC values were found to be outside the normal range. Eosinophil and neutrophil levels were significantly higher in those with a detected tt-MA level higher than 50%BEI, compared to those workers who had a <50%BEI. Fuelling workers specifically exhibited hepatological blood enzyme alterations, demonstrated by elevated levels of SGOT that were higher than those of the cashiers. The levels of SGOT and SGPT enzymes were modestly elevated above what is considered to be normal, particularly in male workers. The correlation between tt-MA, the biological marker, and the haematological or biochemical parameters, which were Hb, Hct, eosinophil, neutrophil, blood creatinine, SGOT, and MCV, was significant. This study strongly suggests that gasoline station employees should undergo regular health evaluations for abnormal haematological parameters in combination with measurements of the biomarkers of exposure and effects.

Author Contributions: Conceptualization, S.C.; methodology, S.C.; validation, P.S. and U.T.; formal analysis, U.T.; investigation, U.T., S.C. and P.S.; data curation, U.T. and P.S.; writing—original draft preparation, U.T. and S.C.; writing—review and editing, S.C. and H.A.; visualization, S.C. and H.A.; supervision, S.C.; project administration, S.C.; funding acquisition, S.C. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding, and the research was supported by the National Research Council of Thailand (NRCT 6100007).

Institutional Review Board Statement: The study was conducted according to the guidelines of the Declaration of Helsinki and approved by the Khon Kaen University Ethics Committee for Human Research (protocol no. HE612030 and date of approval 12 March 2018).

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: Data are available upon personal request.

Acknowledgments: The authors are very grateful to Norbert E. Kaminski from the Institute for Integrative Toxicology, Michigan State University, USA, for his kind and helpful discussion of the paper.

Conflicts of Interest: The authors declare no conflicts of interest.

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