Statistical Analysis of Long-term Health Effects of Thailand's Oil Spill on the Health of Spill Cleaners

Benjamin Atta Owusu

A Thesis Submitted in Fulfillment of the Requirements for the Degree of

Doctor of Philosophy in Research Methodology

Prince of Songkla University

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ABSTRACT

The Rayong oil spill was caused by a ruptured pipeline and leaked over 50,000 litres of crude oil into Thailand's Gulf. The clean-up activities included personnel from the PTT Global Chemical (PTTGC), the Thai Navy and civilian volunteers. Annual follow-up visits were conducted in which the oil spill clean-up workers visited the Rayong hospital for health assessment from 2014 to 2018. However, no longitudinal study has been conducted to evaluate the possible long-term adverse effects of the Rayong oil spill exposure on the workers who participated in the clean-up activities. This study aimed to investigate the long-term health effects of the Rayong oil spill on haematological, renal, and hepatic indices of the clean-up workers using the data from Rayong hospital's 5-year health follow-up protocol. Data for this study was obtained from the Rayong hospital and included the haematological, hepatic, and renal indices of 869 workers who participated in the oil spill clean-up and attended at least one follow-up visit between 2014 and 2018. Haemoglobin (HB), haematocrit (HCT), white blood cell (WBC) count, red blood cell (RBC) count, and platelet count for haematological function. Other haematological indices were mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), polymorphonuclear neutrophils (PMN), and Lymphocytes

(LYM). Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were assessed for hepatic function, creatinine (Cr) and blood urea nitrogen (BUN) for renal function. An endpoint analysis was conducted using analysis of variance (ANOVA) to determine the annual changes of the haematological, hepatic, and renal indices between the baseline in 2013 and the final follow-up in 2018, using the level of exposure to differentiate between subjects. The generalised estimating equations (GEEs) were used to determine the longitudinal trends of the indices, while latent class trajectory analyses were used to assess the presence of latent clusters based on the longitudinal trends. The results showed increasing trends of WBC ($0.02 \pm 0.01 \times 10^3$ cells/μL per year), RBC count (0.008 ± 0.01 cells/μL per year), platelet count ($3.44 \pm 0.39 \times 10^3$/μL per year), BUN ($0.22 \pm 0.03$ mg/dL per year) and CR (0.01 ± 0.00 mg/dL per year).

On the other hand, the average trends of LYM ($-0.14 \pm 0.$ 07% per year) and AST (-1.63 ± 0.20 IU/L per year) were decreasing. The level of exposure showed no significant effects on the trends of all but one of the haematological, hepatic, and renal indices. Gender and occupation were significantly associated with HB, platelets, MCHC and BUN trends. Clean-up workers from the PTTGC (0.31 ± 0.10) and military personnel (0.42 ± 0.18) had significantly lower trends of HB than civilians. The HB trend among men was 1.94 ± 0.12 times higher than women. The findings from this study indicate significant differences between the levels of some haematological, hepatic, and renal indices at baseline and final follow-up. Long-term trends found in this study, coupled with the significant increasing latent trends of some clean-up workers, indicate worsening renal functions due to oil spill exposure. Furthermore, results from this study show the possibility of cardiovascular effects among some of the oil spill clean-up workers 5 years after the clean-up.

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Benjamin Atta Owusu

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Chapter 1

Introduction

1.1 Background and Rationale

Over the last five decades, increased numbers and sizes of marine oil spills have been observed. The notable spills were the Prestige oil spill in Galicia, Spain, which occurred in 2002, the Hebei Spirit Oil Spill (HEROS) in Korea in 2007, the Deepwater Horizon (BP) oil spill in the United States of America in 2010, as well as the 2019 Northeast Brazil oil spill, which is ongoing. Correspondingly, the number of oil spill-related research has increased over the same period (Murphy et al., 2016). Crude oil comprises volatile organic compounds (VOCs), polycyclic aromatic hydrocarbons (PAHs) and other chemical compounds. These chemical compounds are released into marine and terrestrial ecosystems during oil spill incidents (Beyer et al., 2010; de Hoop et al., 2011). PAHs and VOCs in crude oil, such as benzo[a]pyrene, have been classified as human carcinogens by the International Agency for Research on Cancer (Baan et al., 2009; IARC, 1988). After every oil spill, citizens voluntarily participate in the clean-up activities and are directly and indirectly exposed to these toxicants. Hence, they risk developing health problems (Hildur et al., 2015; Peres et al., 2016; Suárez et al., 2005). Various studies have found that intake of these compounds, primarily through inhalation, may cause severe adverse health effects. Some of the adverse health effects of oil spill exposure include alterations in haematological, hepatic, and renal functioning (Choi et al., 2017; D'Andrea and Reddy, 2018; Murphy et al., 2016), chromosomal damage due to genetic mutations (Hildur et al., 2015) and acute health symptoms including itchy eyes, headache, dizziness, throat irritation, nausea, and cough

(Lee et al., 2010; Peres et al., 2016; Song et al., 2009; Suárez et al., 2005). Other studies have also documented chronic respiratory diseases, allergic rhinitis and reduced pulmonary functioning as possible effects of oil spill exposure (Park et al., 2019; Zock et al., 2012). The nature and severity of oil spill health effects are determined by factors such as proximity to the oil spill site, the number of days of clean-up, time between an oil spill and human contact (Ha et al., 2012; Ingviya et al., 2020; Peres et al., 2016).

The Rayong oil spill incident occurred in July 2013 in the Rayong province. The spill incident was caused by a ruptured pipeline owned and operated by PTT Global Chemical (PTTGC). Based on official reports, more than 54,000 litres of crude oil leaked into the Gulf of Thailand during transportation from the oil well into an oil vessel. The spill site was 20 km from the shores of Map Ta Phut and approximately 35 km from the Samet tourist island. Within 48 hours, the oil had spread and deposited at the Ao Phrao Bay on Samet island, covering an area of 20 km^2 (Casarotto et al., 2014; Laemun et al., 2014; PTTGC., 2013). The aerial image of the oil spill site is shown in Figure 1.1. Oil spill clean-up activities started on July 29, 2013, spearheaded by the PTTGC and Thai Navy. Clean-up activities were conducted by a combination of employees from the PTTGC, territorial defence volunteers, personnel from the Royal Thai Navy and civilian volunteers. The Rayong oil spill clean-up lasted more than a month, and over 2,000 clean-up workers were involved. These workers undertook various procedures in the clean-up. Dispersants such as Superdispersant-25 and Slickgone-NS were used to dissolve the offshore oil slick with seawater and sink the oil to the bottom of the sea (Casarotto et al., 2014; Laemun et al., 2014).

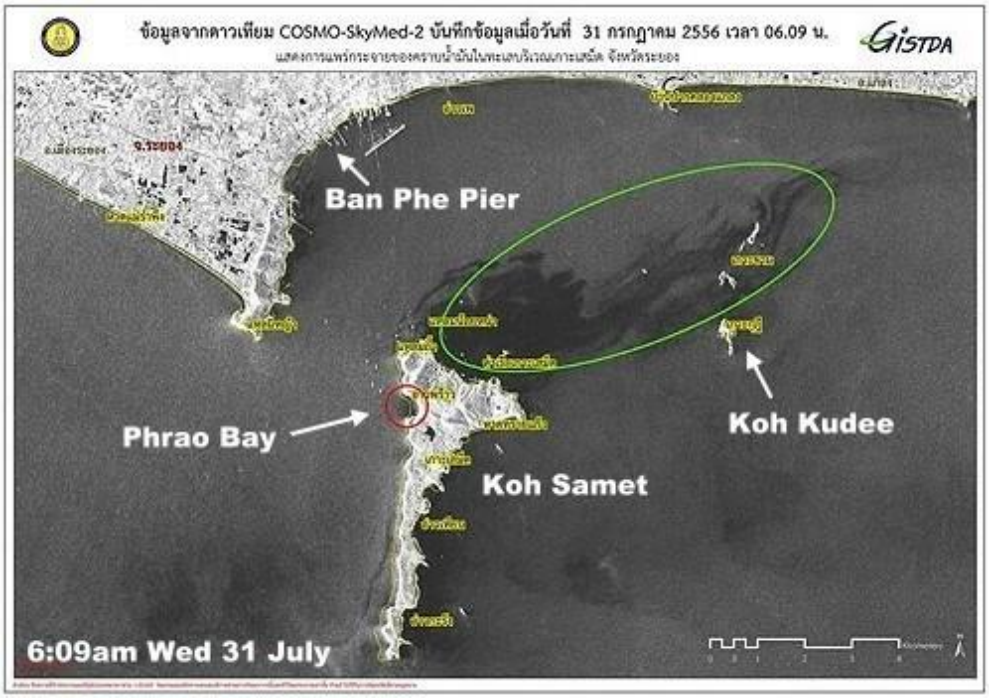


Figure 1.1 Satellite image of the spill oil on July 31, 2013

Source: Barrow (2013)

During the first 24 hours after the oil leakage was detected, primary respondents closed oil transport valves and began containment with booms. The skimmers were used to collect the spilt oil from the sea's surface. The dispersants were used to dissolve a large quantity of the oil offshore. On the second day, floating booms were used to contain oil while responders cleaned the oil slick. Onshore clean-up activities began after 48 hours of the spill and continued through August 27. The full schedule for the oil spill clean-up is shown in Table 1.1.

Table 1.1 Sequence of events during the Rayong oil spill clean-up

Activity/Day	2013					
	July 27	**July 28**	**July 29**	**July 29 – Aug 2**	**Aug 3**	**Aug 27**
Spill detected Dispersants sprayed on the oil						
Floating booms to contain oil on water						
On-land clean-up starts						
Intensive on-land clean-up with vacuums, shovels, and absorbent booms						
Clean-up continues while moving oil debris from spill sites to PTTGC refineries						
Clean-up officially ends						

Source: Adapted from Ingviya et al. (2017)

In the aftermath of the Rayong oil spill, the Rayong hospital collaborated with the Rayong provincial public health office and designed a health surveillance protocol to monitor the metabolites PAHs and VOCs and the haematological, hepatic, and renal profiles of the clean-up workers post shifts. Blood and urine samples were collected from the clean-up workers during the health surveillance. The Rayong hospital measured internal dose biomarkers of PAHs and VOCs from the urine samples, known as 1-hydroxypyrene-glucuronide (1-OHPG) and trans, trans-muconic acid (t,t-MA), respectively. Additionally, questionnaires were used to record demographic information, allergic reactions, and acute symptoms due to the clean-up. Recently, Ingviya et al. (2020) assessed the level of exposure of these clean-up workers to the VOCs and PAHs based on their metabolite levels using the left-over specimen from the Rayong hospital health surveillance. The study reported evidence of increased benzene metabolites during the first 72 hours of clean-up. The Rayong hospital conducted annual follow-up visits as part of the health surveillance program. All the oil spill clean-up workers were eligible for the follow-up visits, subject to availability. The follow-up program continued for 5 years, from 2014 to 2018. However, no longitudinal study has been conducted to evaluate the possible long-term adverse effects of the Rayong oil spill exposure on the workers who participated in the clean-up activities.

1.2 Objectives of Research

This study seeks to assess the long-term health effects of the 2013 Rayong oil spill exposure on clean-up workers using the data from Rayong hospital's 5-year health follow-up protocol. Specifically, this study targets the following objectives:

1. To assess and compare the changes in haematological, hepatic, and renal indices during each follow-up year by exposure level
2. To assess the trends and trajectories of these indices over 5 years after the exposure

1.3 Expected Advantages

The analysis of this study shed some light on how the level of oil spill exposure affects the haematopoietic and renal systems longitudinally. Findings from the research are expected to guide policy decisions and policy implementations. Finally, this research proposes recommendations for managing future oil spill disasters.

1.4 Scope of the Study

This study investigated the long-term health effects of oil spill exposure among clean-up workers. The level of exposure to crude oil was assessed by 1) using the number of hours between the oil spill and human contact and 2) using the urinary concentration of the internal dose biomarkers of exposure (1-OHPG and t,t-MA). Baseline data collected in 2013 and the follow-up data collected from 2014 to 2018 by the Rayong Hospital oil spill surveillance programme were used. Descriptive analysis was conducted to provide an overview of the data and examine the characteristics of the oil spill clean-up workers. The analysis of variance (ANOVA) for repeated measures was used to assess changes in haematological, hepatic and renal indices due

to oil spill exposure. Finally, multivariate models such as generalised estimating equations (GEE), the generalised linear mixed models (GLMM) and latent class analysis were used to examine the longitudinal trajectories and factors associated with the long-term changes in the haematological, hepatic, and renal indices of the oil spill clean-up workers.

This thesis dissertation is divided into five chapters according to the following structure. The detailed background on oil spills and the rationale for the study have been presented in chapter 1 above. Chapter 2 entails the review of relevant literature related to oil spills, the mode of assessing exposure, the acute and long-term health effects, and the methods of analysing longitudinal data. Information related to the study area, the data source, the outcome measurement, and statistical models have been presented in chapter 3. Chapter 4 outlines the results of the statistical analysis. A description of the characteristics of the oil spill clean-up workers, longitudinal trajectories, and trends from latent class analysis, as well as the longitudinal changes in haematological, hepatic, and renal indices, are presented in chapter 4. Chapter 5 presents the discussion and conclusion of the study results.

Chapter 2

Literature Review

2.1 General background of oil spills

An oil spill refers to the discharge of petroleum products into an environment and can be terrestrial or marine (Laemun et al., 2014). A significant concern for human health from an oil spill is the exposure to levels of different hazardous chemicals such as benzene, ethylbenzene, and other PAHs and VOCs. Marine oil spills considered major marine disasters, can result from factors such as human error, as in the case of the Exxon Valdez oil spill or mechanical failure, as in the BP oil spill (Beyer et al., 2010; NRCC, 2003). There are different procedures to respond to an oil spill disaster. These procedures usually depend on several factors, including the size, location and type of spill. At the occurrence of each oil spill, the organisations responsible for the spill usually spearhead the response activities. Internationally recognised response procedures are adhered to during the containment and clean-up of the oil slick. During an oil spill, the primary response measures include containment and recovery, while dispersants are occasionally applied when the tidal wave power is adequate (Laemun et al., 2014; NIEHS, 2010).

Crude oil is composed of high levels of hazardous VOCs such as benzene, ethylbenzene, and other PAHs, some of which are classified as human carcinogens (IARC, 1985). Thus, exposure to crude oil could lead to adverse health consequences (Bosetti et al., 2007; D'Andrea and Reddy, 2013). Oil spill pollutes the sea, alters marine

ecosystems, and contaminates beaches with high levels of PAHs, benzene, toluene and other chemicals (Piatt et al., 1990; Tronczyński et al., 2004).

2.2 Toxicokinetics of VOCs and PAHs

Volatile organic compounds (VOCs) and polycyclic aromatic hydrocarbons (PAHs) are chemical components of crude oil. The primary mode of exposure to benzene and other chemical elements in crude oil is inhalation.

2.2.1 Exposure, absorption, distribution and metabolism of benzene

After inhalation and oral exposure, benzene is immediately absorbed into the body. Different modes, such as skin contact and oral ingestion, are also significant. Available literature shows that humans absorb benzene rapidly after inhalation exposure. The absorption rate of benzene could be as high as 70-80% within 5 minutes of inhalation exposure; however, the rate reduces with time (Srbova et al. 1950). When absorbed, benzene distributes rapidly in the body and accumulates in fatty tissues. Benzene and benzene metabolites have been found in tissues and biological fluids of subjects exposed to benzene-contained substances, either accidentally or intentionally (Pekari et al. 1992; Bechtold et al. 1992). After exposure to crude oil, benzene chemicals are metabolized within various biological organs such as the liver and kidney. Furthermore, benzene and its metabolites are deposited in the placenta and umbilical cords of pregnant women (Dowty et al. 1976), as well as in the lungs, liver, and bone marrow of animals (Sabourin et al. 1988). However, benzene-related toxicity is expressed in the bone marrow (Powley and Carlson 2002).

Benzene is a toxicant carcinogen to the haematological profile. Exposure to lower concentrations of benzene has subtle effects on blood cells and adverse

reproductive and developmental effects (Lan et al., 2004; Xing et al., 2010). The half-life of benzene in the environment and benzene in the human body is measured in days, and it is not known to bio-accumulate (Goldstein et al., 2011). Studies have found that both spill clean-up volunteers and people who live near oil spill sites could suffer from adverse health effects due to their exposure to benzene (Doherty et al., 2017; Tanyanont and Vichit-Vakadan, 2012). The possible adverse health effects reported include prolonged respiratory impairments, chromosomal damage, and changes in haematological and hepatic biomarkers (D'Andrea and Reddy, 2018; Hildur et al., 2015; Zock et al., 2012). According to the Centre for Disease Control and Prevention (CDC), oil spill clean-up volunteers are directly exposed to the benzene components in crude oil (King et al., 2011), and these workers are at high risk of developing acute and long-term health complications (Suárez et al., 2005).

2.2.2 Exposure, absorption, distribution and metabolism of PAHs

PAHs such as benzo[a]pyrene are significant compounds in crude oil. The absorption of PAHs occurs mainly after inhalation, oral or dermal exposure. The absorption rate of PAHs into the body differs by exposure mechanism. After absorption, over 50% of the concentration of PAHs is cleared from the lungs within a few hours, while more than 94% is cleared within 24 hours (Bevan et al., 1991; Sun et al., 1982). Significant quantities of PAHs are deposited in the lung, liver and kidney tissues immediately after absorption. However, after 6 hours, fat tissues contain the highest concentration of PAH metabolites (Withey et al., 1993), indicating that PAHs are distributed and deposited in fat tissues. The metabolism of PAHs occurs in several organs, including the liver, lung and kidneys.

2.3 Health effects of oil spills

There have been numerous studies that discuss the health effects of oil spills. Over the last three decades, more than 70 studies have investigated the health impacts of 10 oil spills (Laffon et al., 2016; Murphy et al., 2016).

The nature and severity of the health effects of oil spill exposure depend on many factors. Among these factors are the proximity to the spill site (Lyons et al., 1999), the number of days of clean-up work and the number of different clean-up activities performed (Suárez et al., 2005) and the availability and usage of PPEs (Carrasco et al., 2006). Also, the health effects of oil spills are due to the type and quantity of dispersants used and the level of exposure. Oil spill clean-up workers who work during the first week of the spill are likely to have the highest exposure to benzene and PAHs and are more likely to experience significant health effects (D'Andrea and Reddy, 2014; Ingviya et al., 2020).

Generally, exposure to benzene has been linked with abnormal haematological indices such as WBC, RBC, neutrophil counts, HCT, MCHC and lymphocytes. For instance, workers exposed to low levels of benzene had reduced HB, WBC, platelet counts and lymphocytes (Lan et al., 2004). A different study has also reported significant reductions in various haematological parameters, including WBC, LYM, platelet count, RBC count and HCT (Rothman et al., 1996). Exposure dose of benzene exposure also showed positive associations with haematological parameters such as HCT and MCHC (Zhang et al., 2020). Among oil spill exposed subjects, exposure to crude oil could cause significant alterations in the haematological, hepatic, and renal indices. Indices such as WBC count, HB, HCT, platelet count, CR, AST, and ALT

significantly increased after oil spill exposure, as in the BP oil spill case (D'Andrea and Reddy, 2013; D'Andrea and Reddy, 2014). Physiological (physical) impacts due to oil spill exposure have also been documented. Oil spill-exposed subjects report an increased rate of sore throat, sore eyes, cough, runny nose, nausea, and headache (D'Andrea and Reddy, 2013; Lyons et al., 1999, Meo et al., 2009). Other physical effects among clean-up workers are respiratory tract problems (Suárez et al., Zock et al., 2007; Carrasco et al., 2006). Women exposed to petrochemicals, such as benzene, have an increased risk of spontaneous abortion (Xu et al., 1998).

There is enough evidence to suggest that oil spill exposure causes significant mental stress disorders. Residents exposed to the Exxon Valdez oil spill reported a higher prevalence of general anxiety disorder and post-traumatic stress disorder, which were higher in women than in men (Palinkas et al., 1993; Palinkas et al., 2004). High anxiety scores have also been observed among exposed subjects (Lyons et al., 1999). Chromosomal damage could also occur due to oil spill exposure as in the case of the *Prestige* oil spill (Biern et al., 2015; Hildur et al., 2015). The health effects of oil spill exposure can be classified as acute or long-term.

Some studies on oil spill disasters have used questionnaires to investigate the acute symptoms of oil spill exposure among clean-up workers and residents. For instance, women and children residing in Louisiana and exposed to the BP oil spill suffered from burning in the throat, nose or lungs, sore throat as well as dizziness. Other symptoms such as itchy eyes, headaches and runny nose were also present (Peres et al., 2016). Similar acute health effects were also observed among the residents of Taean province (the heavily affected coast after the HEROS) and the clean-up workers of the *Prestige* oil spill in Spain. Aside from these symptoms, nausea, skin symptoms and

fatigue were also observed. These acute health effects are primarily attributed to proximity to the spill incident, the nature of clean-up work, the days of work and skin contact with oil (Lee et al., 2010; Suárez et al., 2005). While a sizable number of studies have discussed acute health effects, just a handful of studies have investigated the long-term effects of oil spill exposure. Evidence from some of these studies suggests that local fishers and individuals who participated in the clean-up activities of the *Prestige* oil spill suffered from prolonged lower respiratory tract symptoms. These respiratory symptoms were directly associated with the risk of persistent respiratory complications (Zock et al., 2012). High exposure to the oil spill disaster could also cause significant changes in the haematological and hepatic indices. Chronic diseases could also result from exposure to oil spill disasters (Choi et al., 2017; D'Andrea and Reddy, 2018). Aside from the effects above, subjects exposed to oil spill disaster risk have lower pulmonary functioning and allergic rhinitis in the long-term as evident among the residents of HEROS-affected communities (Park et al., 2019).

2.4 Statistical models

Longitudinal studies are frequently designed to investigate changes over time. Such studies are widely used in the social, behavioural and health sciences. Longitudinal studies are mainly observational and involve the collection of repeated measurements on the same subject at different times. Longitudinal studies allow the characterisation of changes over time and determine factors responsible for the changes (Fitzmaurice et al., 2012). Analysing the long-term effects of oil spill exposure involves conducting follow-up studies and collecting longitudinal data. One important characteristic of longitudinal data is that repeated measures on the same subject at different times are highly correlated. Therefore, any models that analyse longitudinal

data must account for such a correlation. Analysis of longitudinal (follow-up) data requires robust statistical techniques.

The multivariate log-binomial regression model has been used to investigate the cross-sectional association between oil spill exposure and respiratory health outcomes among clean-up workers of the *Prestige* oil spill. Longitudinal data were collected on oil spill exposed subjects at different times, and the presence of and type of respiratory disorders were assessed. Four distinct outcomes (lower respiratory tract symptoms; nasal symptoms; inhaled medication; oral medication) were measured using smoking status, gender, age and exposure level as predictors. Multinomial regression analysis was also used to account for changes in these respiratory health outcomes at baseline and follow-up. These models provided statistically significant results that explained the long-term effects of oil spill exposure (Zock et al., 2012).

Although the multivariate models described in Zock et al. (2012) can provide significant analyses of longitudinal data, these models summarised all the data for each subject into one value (Twisk, 2013). Other methods exist to analyse all the longitudinal data at the same time. Two models that have gained much significance in analysing longitudinal data are the generalised estimating equation (GEE) and generalised linear mixed models (GLMM).

The GEE introduced is a semi-parametric model for the mean observed value and a model for the correlation due to repeated measurement Liang and Zeger (1986). The GEE method is based on multivariate quasi-likelihood theory and is able to overcome the complex nature of longitudinal data (Twisk, 2013). GEE comprises a

mean model and a model for the longitudinal correlation within the same subject. Some important assumptions of the GEE are:

- There is no between-subject correlation.
- Measures on the same subject are correlated.
- The homogeneity of variance does not need to be satisfied.
- Errors are correlated.
- Missing data, if present, are missing completely at random.

The GEE provides valid estimates and standard errors for regression parameters of interest even if the correlation model is incorrectly specified.

The GLMM is a model for analysing continuous outcomes as functions of fixed effects. The GLMM assumes that each subject has a regression model characterised by subject-specific parameters; a combination of fixed-effects parameters common to all individuals in the population and random-effects parameters unique to each subject. An important assumption of the GLMM is that missing data is considered missing at random. Edwards (2000) has noted that the GEE cannot be used for subject-specific estimation and hypothesis testing. The study by Edwards (2000) shows that the GEE and the GLMM are two statistically robust techniques to estimate regression coefficients while using longitudinal data.

Chapter 3

Research Methodology

3.1 Study design and population

This study is a longitudinal cohort study to assess the long-term health effects among participants of the Rayong oil spill clean-up in 2013. Data for this study was obtained from the electronic records of the Rayong hospital. All clean-up workers exposed to the spill were examined at the baseline visit in 2013. Information from 2,376 spill cleaners was obtained with a structured questionnaire. These spill cleaners consisted of 531 civilian volunteers (22.22%), 375 PTTGC workers (15.78%) and 1473 personnel from the Royal Thai Army (61.99%). All the oil spill clean-up workers who attended at least one follow-up visit were included in the analysis of this study. Table 3.1 shows the number of subjects who participated in each visit during the follow-up from 2014 to 2018. The newly enrolled subjects are the subjects who had come for a follow-up for the first time.

Table 3.1 Number of clean-up workers for the baseline study and the 5-year follow-up for the Rayong oil spill research

Year/Group	Rayong oil spill study				Total
	PTTGC workers	Civilians	Thai Royal Army	No group	
Baseline (2013)	N = 375	N = 528	N = 1473		2,376
1st Follow-up (2014)	-	61	32		N = 128
2nd Follow-up	-	179	139	-	N = 352
(2015)	-	125*	126*	30*	N* = 281
3rd Follow-up	-	56	108	-	N = 164
(2016)	-	3*	38*		N* = 41
4th Follow-up	329	53	122	31	N = 535
(2017)	329*	5*	42*	31*	N* = 407
5th Follow-up	324	152	95	45	N = 616
(2018)	8*	65*	36*	27*	N* = 136
Total	337	259	274	123	

Note: N* represents the newly admitted subjects in a year.

3.2 Baseline survey and follow-up protocol

According to the follow-up protocol, the oil spill exposed subjects were invited to the Rayong hospital every year to undertake health examinations, including blood examinations for CBC, liver, and renal function tests. The Rayong hospital collected demographic information and possible adverse effects at baseline, including respiratory, skin, and neurological symptoms. Blood and urine samples were also collected to assess the impact of exposure to crude oil on the haematopoietic and renal systems. In a complete blood count (CBC) analysis, 22 blood components were assessed during the baseline study. The information recorded at baseline and follow-up was gender, age, workgroup, number of days of work, specific duties, and hours of work per day. The haematological, hepatic, and renal indices assessed during the baseline and follow-up protocol are presented in Table 3.2.

Table 3.2 Haematological, hepatic, and renal indices measured at baseline and follow-up surveys

Index	Description	Unit	Normal range	Measured
Glomerular filtration rate (GFR)	Measures the overall index of kidney function	Millilitres per min (mL/min)	90 – 130	Baseline
Mean platelet volume (MPV)	Measures the average size of platelets in the blood	Femto Litres (fL)	9.4 – 12.3	Baseline
Absolute neutrophil count (ANC)	Neutrophils are a type of white blood cell that protect from infections	Cells per litre (cells/L)	1500 – 8000	Baseline and Follow-up
Absolute eosinophil count (AEC)	Measures the number of one type of white blood cell called eosinophils	cells/L	350 – 500	Baseline and Follow-up
Eosinophils count (EOS)	Measures the components in the immune system that fight multicellular parasites	Percentage	0 – 7%	Baseline and Follow-up
White blood cell (WBC)	White blood cells	cells/L	$3.5 – 10.5 \times 10^5$ /L	Baseline and Follow-up
Platelet count (PLATECOUNT)	Number of platelets	Cells per microliter (cells/μL)	$150 – 450 \times 10^3$/μL	Baseline and Follow-up
Basophils (BASO)	Basophil cells measure the body's response to allergic stimuli.	Percentage (%)	0.0 – 2.0%	Baseline and Follow-up
Blood urea nitrogen (BUN)	Measures the amount of urea nitrogen in the blood to determine kidney function	Milligram per decilitre (mg/dL)	5.0 – 25.0 mg/dL.	Baseline and Follow-up
Creatinine (CR)	A by-product of muscle metabolism to determine kidney function	mg/dL	0.5 – 1.5 mg/dL	Baseline and Follow-up
Haemoglobin (HB)	Measure the haemoglobin in the red blood cells	Grams per decilitre (g/dL)	13.8-17.2 (Male) 12.1-15.1 (Female)	Baseline and Follow-up

Table 3.2 cont'd

Index	Description	Unit	Normal range	Measured
Haematocrit (HCT)	Measures the percentage of red blood cells	Percentage	40.0 – 50.3 (Male) 36.0–45.0(Female)	Baseline and Follow-up
Lymphocytes (LYM)	Percentage of Lymphocytes	Percentage	16.0 – 45.0%	Baseline and Follow-up
Mean corpuscular haemoglobin (MCH)	Measures the average amount of HB per red blood cell	Picograms/cell	27–31 pg	Baseline and Follow-up
MCH concentration (MCHC)	Measures the average concentration of HB per unit volume of red blood cells	g/dL	32–36 g/dL	Baseline and Follow-up
Mean corpuscular volume (MCV)	Measures of the average volume of a red blood cell	fL	80–100 fL	Baseline and Follow-up
Monocytes (MONO)	Percentage of Monocytes	Percentage	2–8%	Baseline and Follow-up
Neutrophil (PMN)	Measures the body's ability to fight against foreign invaders	Percentage	45–74%	Baseline and Follow-up
Red blood cell (RBC)	Red blood cells	cells/µL	4.2 – 6.1 million cells/µL	Baseline and Follow-up
Red cell distribution width (RDW)	Measures the variability of the size of the red blood cells in circulation.	Percentage	11.8 – 15.6%	Baseline and Follow-up
Aspartate Aminotransferase (AST)	Enzymes that contribute to liver functioning.	International units/litre (IU/L)	6 – 40 IU/L	Baseline and Follow-up
Alanine Aminotransferase (ALT)	Enzymes that monitor liver damage.	International units/litre (IU/L)	7 – 56 IU/L	Baseline and Follow-up

Adapted from. Kasper et al., 2015.

Throughout the five years, 993 unique subjects reported to the hospital at least once to undergo a health examination. Out of these 993,869 clean-up workers were part of the baseline study. The remaining 124 subjects had no records in the baseline study. Therefore, this study focused on the 869 subjects included in the baseline study and reported for at least one follow-up visit. The flowchart of data management is shown in the Figure 3.1 below.

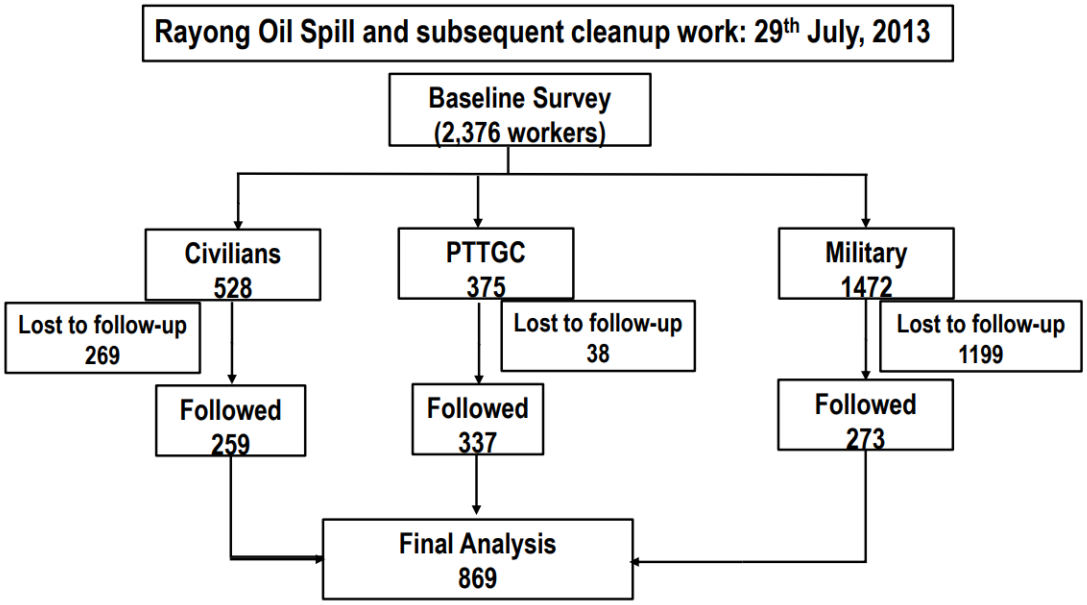


Figure 3.1 Flowchart for data management and subject inclusion criteria

3.3 Exposure assessment

PAHs and VOCs known biomarkers of exposure to benzene or benzene-containing products, such as crude oil. The level of exposure to crude oil or the chemical components of crude oil has been previously assessed using urinary metabolites of PAH and VOCs. Urinary metabolites such as 1-OHPG and t,t-MA have been quantified in various studies to assess the level of environmental exposure to PAHs and VOCs (Aprea et al. 2008; Bechtold et al. 1991; Jongeneelen 2001; Kamal et al. 2015; Sithisarankul and Intawong 2015; Wiwanitkit et al. 2001; Zhang et al. 2014). The urinary concentrations of 1-OHPG and t,t-MA indicate the quantifiable levels of exposure to benzene from different sources – for example, from oil spills and cigarette smoke (Jain 2015; Strickland et al. 1996).

3.3.1 Quantification of 1-OHPG and t,t-MA concentrations

The concentrations of 1-OHPG and t,t-MA were quantified in the urine samples that were collected during the clean-up in 2013. Immunoaffinity chromatography and synchronous fluorescence spectroscopy were used to measure the 1-OHPG concentration at Paul Strickland laboratory in John Hopkins University, (limit of detection 0.04 pmol/mL; coefficient of variation 5.6%) (Ingviya et al. 2020). The baseline 1-OHPG concentration was categorised as high (>5.0 pmol/mL), moderate (1.0–5.0 pmol/mL), or low (<1.0 pmol/mL), based on a study by Kang et al. (1995). The urinary t,t-MA concentration was analysed using high-performance liquid chromatography with fluorescence detection in three laboratories, including the Rayong Hospital laboratory and two other private laboratories. The agreement among t,t-MA measurements reported by the three laboratories was 99.99% (Intawong et al. 2015).

The t,t-MA concentration was classified as detectable or undetectable because a large proportion of the samples had levels below the limit of detection (0.01 mg/dL) of the assay. An initial assessment by Ingviya et al. (2020) quantified the urinary concentrations of these metabolites to determine the level of exposure among the Rayong oil spill clean-up workers. Findings from that study correlated lower concentrations of urinary metabolites of benzene with increased time after oil spill.

Among the oil spill clean-up workers investigated in this study, more than 80% did not provide urine samples. Therefore, urinary concentrations of 1-OHPG and t,t-MA could not be used to quantify all subjects' exposure to crude oil. The levels of exposure to the oil spill were classified using the days of clean-up work. The clean-up workers were grouped as high exposure, low exposure, or unknown exposure based on Ingviya et al. (2020). Workers in the first 72 hours of the spill were classified as the high exposure group, and those who worked on subsequent days were classified as the low exposure group. Oil spill clean-up workers who did not provide data on the exact clean-up dates were grouped as 'unknown exposure'. We decided against excluding them from the analysis to avoid potential selection bias. Due to the nature of the sentinel questionnaire data collection, the information on the hours of work, protective equipment, and duration of clean-up work was not available as sufficient details for the analysis.

3.4 Data management and variable description

3.4.1 Independent variables

During the baseline health monitoring, the urinary concentrations of 1-hydroxypyreneglucuronide (1-OHPG), cotinine and urinary trans, trans-muconic acid (t,t-MA) were measured from urine samples. 1-OHPG and t, t-MA are metabolites of PAHs and benzene, able to measure the level of exposure to crude oil and benzene, respectively. Cotinine is a metabolite of tobacco exposure and a surrogate laboratory measure of smoking status (Benowitz, 1996; Cerniglia; 1984; Inoue et al., 1989). In an analysis of changes in haematological, hepatic, and renal indices over time, information such as age at baseline, gender, level of exposure, smoking status, duty, and background occupations were employed as independent variables. Age was categorised into five groups: 20-29 years, 30-39 years, 40-49 years, 50 years or above, and unknown. Unknown included subjects who did not declare their age at baseline or any follow-up visit. The clean-up group was categorised into 3 categories based on the background occupation of the subjects. These categories were PTTGC workers, civilian volunteers, and military personnel. Duty was grouped into 5 categories depending on the exact responsibilities at the clean-up. The categories included vacuum cleaning, sand removal, healthcare, and supervision, supporting staff and others. Since the level of exposure was not reported at baseline, it was calculated based on the date the subjects started clean-up. Benzene is known to have high stability and minimum reactivity, with an atmospheric half-life of 3-10 days (Rich and Orimolye, 2016). Based on this half-life, subjects who worked from the first day of the clean-up (July 29, 2013) to August 1, 2013, were classified as high exposure subjects. Subjects who reported to work on subsequent days were classified as low exposure subjects and subjects who did not

report the date of work were classified as unknown. Smoking status was estimated from the measured cotinine and categorised as non-smokers (cotinine < 5 ng/ml), active smokers (6-50 ng/ml), or heavy smokers (> 50 ng/ml).

3.4.2 Dependent variables

The outcome variables in the study were the haematological, hepatic, and renal function indices measured during the baseline survey and the follow-up visits. These indices were analysed using medically approved protocols. The outcome variables were extracted from the Rayong Hospital laboratory data. The haematological, hepatic, and renal indices included haemoglobin (HB), haematocrit level (HCT), red blood cell count (RBC), white blood cell count (WBC), absolute neutrophil count (ANC) and platelet count. Other indices included mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), mean corpuscular volume (MCV), polymorphonuclear neutrophils (PMN), and Lymphocytes (LYM). The measurement of complete blood count assessed not only the haematological function but also the possibility of the underlying inflammation process observable from the increase in WBC and platelet numbers. Liver enzymes (aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were measured to assess hepatic function. Assessing AST and ALT levels among oil spill-exposed workers could help in the early detection of liver diseases. The renal function indices measured were creatinine (CR) and blood urea nitrogen (BUN). Although not sufficient, persistent elevation of BUN and CR levels is a warning sign of chronic kidney failure (Macedo and Mehta 2013).

3.5 Statistical analysis

3.5.1 Descriptive analysis and analysis of variance

Measures of central tendency and dispersion were used to understand the distribution of the haematological, hepatic, and renal indices. Analysis of variance (ANOVA) for repeated measures was used to compare the measures of the high-exposure subjects, low exposure subjects and subjects with unknown exposure. Specifically, the repeated-measures ANOVA was used to test the hypothesis that there was no significant difference in the haematological, hepatic, and renal indices measured at baseline and at the final follow-up. Such a method accounts for the within-subject correlation between the measured haematological, hepatic, and renal indices. The difference between the indices for each exposure group was estimated, and the significance of the differences was assessed. The comparison was made between the baseline (2013) and the fifth-year follow-up visit (2018).

3.5.2 Specifying the GEE model

Generalised linear models (GLMs), by assumption, model normally distributed outcomes with expectations being a set of independent variables. However, many response variables are not necessarily continuous and may not even be normally distributed, and GLMs enable the analysis of such diverse types of univariate responses. In general, when repeated response variables in longitudinal studies do not necessarily have a multivariate normal distribution but the correlation of the responses is considered, one can make use of the generalised estimating equations (GEEs), proposed by Liang and Zeger (1986), for the estimation of parameters, which is a generalisation of multivariate linear models.

Definition:

Let the vector $Y_i = \left(Y_{i1}, Y_{i2}, \ldots, Y_{ij}\right)^T$ be the measured outcomes, in this case, blood components for the i^{th} subject. Let $x_{ij} = \left(1, x_{ij1}, x_{ij2}, \ldots x_{ijk}\right)$ be covariates measured on all subjects at a particular visit. Let $X_i = \left(1, x_{i1}, x_{i2}, \ldots x_{ij}\right)^T$ be a design matrix. This design matrix is a collection of covariates measured on subject i. Let $\beta = \left(\beta_0, \beta_1, \ldots, \beta_k\right)^T$ be regression parameters, each corresponding to a measured covariate.

The GEE assumes no correlation between subjects, but the within-subject correlation cannot be ignored. Thus, the GEE models the mean expected observation as a regression line and models the correlation within observations on the same subject.

The model for the mean is given by equation (1) below

$$\begin{aligned} E\left[Y_{ij} \mid x_{ij}\right] &= \mu_{ij} \\ g(\mu_{ij}) &= X_{ij}\beta \end{aligned} \tag{1}$$

Where $g(\mu_{ij})$ is a link function based on the structure of the outcome. The outcomes of this study are continuous variables. Therefore, no link function is used. The longitudinal relationship between a continuous outcome variable Y and one or more covariates X is given as shown in equation (2)

$$Y_{ij} = \beta_0 + \sum_{k=1}^{K} \beta_{1k} X_{ijk} + \varepsilon_{ij} \tag{2}$$

From equation (2), Y_{ij} are measured blood components for the i^{th} subject at time j. β_0 is a regression intercept, β_{ik} is the regression coefficient for covariate k. The total number of covariates is represented by K and ε_{ij} is the error for the i^{th} subject at j^{th}.

Equation (2) is used to model the mean observed blood components. It remains to determine a correlation structure for the repeated measurements and an appropriate correlation matrix. The possible correlations to be considered are independence, exchangeable, auto-regressive and unstructured.

For the i^{th} subject, if Y_{ij} and $Y_{ij'}$ are measured blood components at time j and j' respectively, then the correlation between Y_{ij} and $Y_{ij'}$ is represented by equation (3)

$$Corr\left[Y_{ij}, Y_{ij'} \mid X_i\right] \tag{3}$$

Independence correlation assumes that there is no correlation between two measured outcomes on the same subject at two different times. The correlation matrix is given by

$$\begin{bmatrix} 1 & 0 & 0 & \cdots & 0 \\ 0 & 1 & 0 & \cdots & 0 \\ 0 & 0 & 1 & \cdots & 0 \\ \vdots & \vdots & \vdots & \ddots & \vdots \\ 0 & 0 & 0 & \cdots & 1 \end{bmatrix}$$

Exchangeable correlation assumes that correlation is a constant

$$Corr\left[Y_{ij}, Y_{ij'} \mid X_i\right] = \alpha$$

The correlation matrix is given as

$$\begin{bmatrix} 1 & \alpha & \alpha & \cdots & \alpha \\ \alpha & 1 & \alpha & \alpha & \alpha \\ \alpha & \alpha & 1 & \alpha & \alpha \\ \vdots & \vdots & \vdots & \ddots & \vdots \\ \alpha & \alpha & \alpha & \cdots & 1 \end{bmatrix}$$

The assumption for auto-regressive correlation assumes that correlation between Y_{ij} and $Y_{ij'}$ depends on the time (distance) between the measurement. That is to say that the correlation one measurement apart Y_{ij} and Y_{ij+1}, for $j = 1$ is assumed to be α, the correlation between two measurements apart Y_{i1} and Y_{ij+2} for $j = 1$ is assumed to be α^2. Thus,

$$Corr\left[Y_{ij}, Y_{ij'} \mid X_i\right] = \alpha^{|j-j'|}$$

The correlation matrix is given as

$$\begin{bmatrix} 1 & \alpha & \alpha^2 & \cdots & \alpha^{j-1} \\ \alpha & 1 & \alpha & \cdots & \alpha^{j-2} \\ \alpha^2 & \alpha & 1 & \cdots & \alpha^{j-3} \\ \vdots & \vdots & \vdots & \ddots & \vdots \\ \alpha^{j-1} & \alpha^{j-2} & \alpha^{j-3} & \cdots & 1 \end{bmatrix}$$

An unstructured correlation means that each Y_{ij} and $Y_{ij'}$ has a different correlation. Therefore, $Corr\left[Y_{ij}, Y_{ij'} \mid X_i\right] = \alpha_{jj'}$ and

$$\begin{bmatrix} 1 & \alpha_{21} & \alpha_{31} & \cdots & \alpha_{m1} \\ \alpha_{12} & 1 & \alpha_{32} & \cdots & \alpha_{m2} \\ \alpha_{13} & \alpha_{23} & 1 & \cdots & \alpha \\ \vdots & \vdots & \vdots & \ddots & \vdots \\ \alpha_{1m} & \alpha_{2m} & \alpha_{3m} & \cdots & 1 \end{bmatrix}$$

The robustness of GEE stems from the use of Huber-White sandwich estimator as a parameter estimation technique. Such robustness is against a wrong correlation

structure (Liang and Zeger, 1986; Zeger and Liang, 1986). However, when there is significant missing data, correlation structure could have a significant effect on the results from GEE (Twisk et al., 1997; Twisk, 2004). The within-subject correlation is accommodated in the GEE as shown below

$$Y_{ij} = \beta_0 + \sum_{k=1}^{K} \beta_{1k} X_{ijk} + Corr\left[Y_{ij}, Y_{ij'} \mid X_i \right] + \varepsilon_{ij} \qquad (3)$$

In this study, ANOVA for repeated measures was used to assess changes in the levels of blood components at each follow-up year among 570 clean-up workers who reported for the final follow-up. These changes were compared based on the level of exposure. The GEEs were used to fit mathematical models explaining the repeated measurements of haematological, hepatic, and renal indices, using gender, age, level of exposure, background, and duties as independent variables. Traditional regression models are based on ordinary least squares and maximum likelihood for parameter estimations. Evaluation of these models are based on the Akaike information criteria (AIC). However, the AIC cannot be directly applied to evaluate models for repeated and correlated measures (example GEEs), because such methods are quasi-likelihood-based and have no assumptions related to the distributions of the repeatedly measured responses (Cui 2007). The quasi-likelihood under the independence model criterion (QIC) proposed by Pan (2001) is more appropriate to evaluate models for correlated responses. Also, the QIC can be used to determine the working correlation for GEEs (Cui, 2007). Therefore, this study used the QIC to determine the best GEE and working correlation structure for each haematological, hepatic and renal index.

Latent class trajectory analyses were used to identify the presence of latent trajectories and trends in haematological, hepatic, and renal indices among the oil spill clean-up workers, using the level of indices at baseline and the direction of change over

the study period. The observed means of the haematological and hepatic indices were labelled based on their level at baseline (high, low, and normal) and trends throughout the study (stable, increasing, decreasing). The definitions of high, low, and normal were based on the standard medical reference ranges for each haematological, hepatic, and renal index (Kasper et al., 2015).

Chapter 4

Results

4.1 Descriptive Analysis

4.1.1 Characteristics of the study subjects

Based on the 869 subjects included in this study, there were 119 (13.69%) females and 750 (86.36%) males. Most of the subjects (38.34%) who attended at least one follow-up visit were staff of PTTGC, while 274 (31.49%) were personnel from the Thai navy. The characteristics of the study subjects according to the exposure level are shown in Table 4.1 below. During the oil spill clean-up, most workers were between 40-49 years (31.72%), while the least represented age group was 50 years and above (10.69%). A total of 193 clean-up workers did not report their age during the clean-up activities. Based on the days of work, 128 (14.73%) workers had high exposure, 112 (12.89%) had low exposure, and 629 (72.38 %) workers were of unknown exposure. Using the concentration of urinary cotinine at baseline, 39 (4.49%) of the clean-up workers were active smokers, 70 (8.06%) were heavy smokers, and 60 (6.90 %) were non-smokers.

4.1.2 Distribution of haematological, hepatic, and renal indices

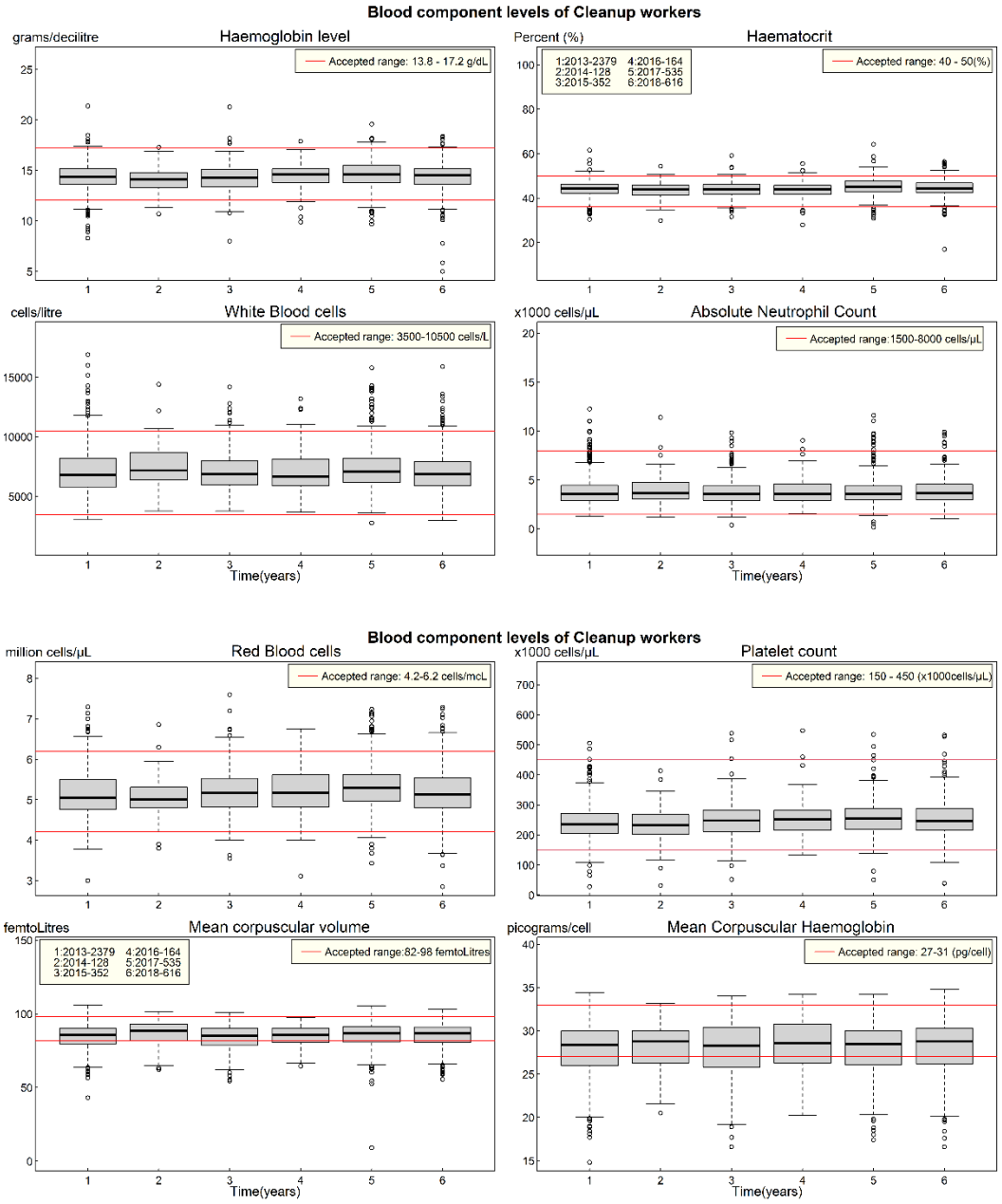
Preliminary analysis of the follow-up visits revealed that the haematological, hepatic, and renal indices measured at each follow-up visit differed. At baseline, the recorded measurements indicated that most subjects had higher than normal WBC, AST, and ALT levels. The levels of HB, HCT, platelet count, BUN and CR were within the normal range for most clean-up workers at baseline. Figure 4.1 and Figure 4.2 show

the distribution of blood components of the clean-up workers who reported to each follow-up at the Rayong hospital.

Throughout the follow-up, the serum liver enzymes (AST and ALT) levels were consistently above the normal range for most clean-up workers. During the baseline and follow-up studies, there was little difference in the mean level of ANC, BUN, and CR. Most of the respondents who reported for follow-up had above-normal levels. Most of the clean-up workers had normal levels of platelets and lymphocytes. The blood component levels for the baseline and follow-up surveys are illustrated in Figures 4.1 and 4.2.

Table 4.1 Characteristics of the study subjects, stratified by level of exposure

Variable	High exposure (%)	Low exposure (%)	Unknown exposure (%)	Total (%)
Oil clean-up group				
Civilian	59 (22.78)	40 (15.44)	160 (61.78)	259 (29.80)
Military	43 (15.75)	36 (13.19)	195 (71.06)	273 (31.41)
PTTGC	26 (7.72)	36 (10.68)	275 (81.60)	337 (38.78)
Age group				
20-29	14 (10.00)	30 (21.43)	96 (68.57)	140 (16.11)
30-39	40 (23.95)	21 (12.57)	106 (63.47)	167 (19.22)
40-49	52 (18.84)	40 (14.49)	184 (66.67)	276 (31.76)
50+	22 (23.66)	21 (22.58)	50 (53.76)	93 (10.70)
Unknown	0	0	193 (100)	193 (22.21)
Gender				
Female	10 (8.40)	11 (9.24)	98 (82.35)	119 (13.69)
Male	118 (15.73)	101 (13.47)	531 (70.80)	751 (86.30)
Smoking status				
Active smokers	13 (33.33)	26 (66.67)	0	39 (4.49)
Heavy smokers	45 (64.29)	25 (35.71)	0	70 (8.06)
Nonsmokers	43 (71.67)	16 (26.67)	1 (1.66)	60 (6.90)
Unknown	27 (3.86)	45 (6.43)	628 (89.71)	700 (80.55)


Blood component levels of Cleanup workers
grams/decilitre
Haemoglobin level
Accepted range: 13.8 - 17.2 g/dL
Percent (%)
Haematocrit
1:2013-2379 4:2016-164
2:2014-128 5:2017-535
3:2015-352 6:2018-616
Accepted range: 40 - 50(%)
cells/litre
White Blood cells
Accepted range: 3500-10500 cells/L
x1000 cells/µL
Absolute Neutrophil Count
Accepted range:1500-8000 cells/µL
Time(years)
Blood component levels of Cleanup workers
million cells/µL
Red Blood cells
Accepted range: 4.2-6.2 cells/mcL
x1000 cells/µL
Platelet count
Accepted range: 150 - 450 (x1000cells/µL)
femtoLitres
Mean corpuscular volume
1:2013-2379 4:2016-164
2:2014-128 5:2017-535
3:2015-352 6:2018-616
Accepted range:82-98 femtoLitres
picograms/cell
Mean Corpuscular Haemoglobin
Accepted range: 27-31 (pg/cell)
Time(years)

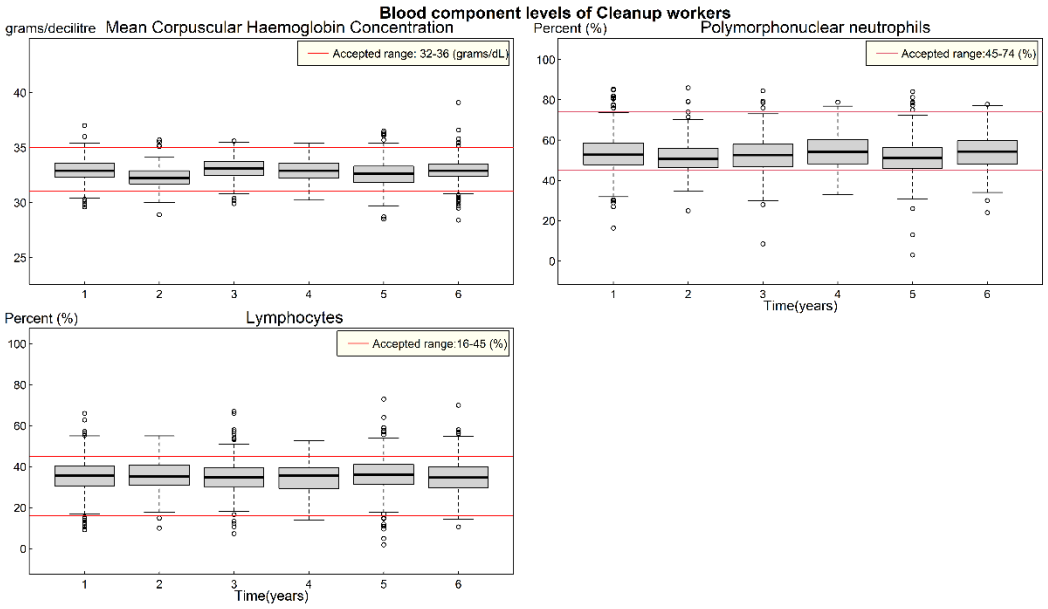


Figure 4.1 Levels of haematological indices among the Rayong oil spill clean-up workers at baseline and each follow-up visit

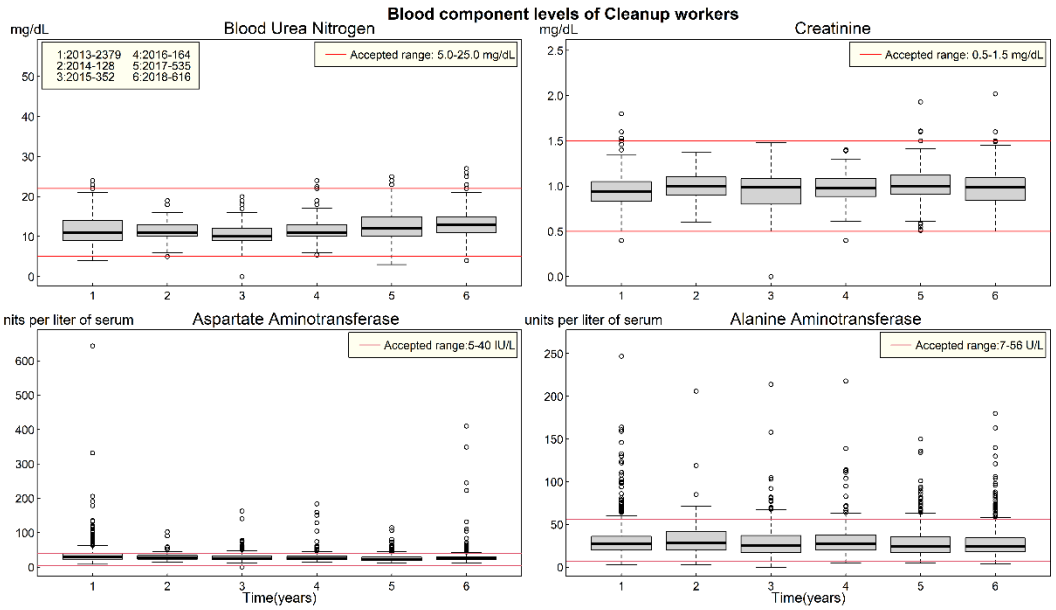


Figure 4.2 Levels of hepatic and renal indices among the Rayong oil spill clean-up workers at baseline and each follow-up visit

4.2 Changes in haematological, hepatic, and renal indices

4.2.1 Annual differences in haematological, hepatic, and renal indices by exposure level

The ANOVA for repeated measures was used to determine the yearly differences between the haematological, hepatic, and renal indices of high, low, and unknown exposure workers of the Rayong oil spill clean-up.

During the oil spill clean-up activities in 2013, there were differences in some of the haematological, hepatic, and renal indices of the clean-up subjects based on exposure level. For instance, the average percentage of HCT was significantly higher among high-exposure workers than among unknown exposure workers (difference = 1.43 ± 0.89 %, p-value < 0.001). The clean-up workers with low exposure had a higher average MCHC level than workers with high exposure (difference = 0.33 ± 0.29 g/dL, p-value < 0.001). Also, the average level of MCHC was higher among workers with unknown exposure than among workers with high exposure (difference = 0.55 ± 0.22 g/dL, p-value < 0.001). The average BUN level of workers with unknown exposure was significantly higher than workers with high exposure to the oil spill (difference = 0.839 ± 0.827 mg/dL, p-value=0.046). The average differences between the levels of haematological, hepatic, and renal indices at the baseline (2013) by exposure level are summarised in Table 4.2 below

Table 4.2: Differences in haematological, hepatic, and renal indices at the baseline according to exposure level (2013)

Indices	Baseline (2013) Exposure level	Difference ± SD	p-value
HB (g/dL)	Unknown exposure - Low exposure	0.02 ± 0.33	0.988
	High exposure-Low exposure	0.26 ± 0.42	0.321
	High exposure- Unknown exposure	0.24 ± 0.31	0.182
HCT (%)	Low exposure- Unknown exposure	0.27 ± 0.94	0.778
	High exposure- Unknown exposure	**1.43 ± 0.89**	**< 0.001**
WBC ($\times 10^3$ cells/L)	High exposure-Low exposure	1.16 ± 1.18	0.055
	Low exposure- Unknown exposure	0.02 ± 0.45	0.994
	High exposure - Unknown exposure	0.37 ± 0.43	0.101
ANC ($\times 10^3$cells/µL)	High exposure-Low exposure	0.35 ± 0.57	0.314
	Low exposure- Unknown exposure	0 ± 0.34	0.999
	High exposure - Unknown exposure	0.18 ± 0.32	0.391
	High exposure - Low exposure	0.17 ± 0.43	0.603
RBC (cells/µL)	Unknown Exposure -Low exposure	0.01 ± 0.18	0.996
	High exposure-Low exposure	0.13 ± 0.22	0.309
	High exposure- Unknown exposure	0.13 ± 0.16	0.148
Platelets ($\times 10^3$ cells/µL)	Low exposure-High exposure	7.59 ± 17.09	0.55
	Unknown exposure -High exposure	8.45 ± 12.81	0.269
	Unknown exposure -Low exposure	0.86 ± 13.55	0.988
MVC (femtolitres)	Low exposure- Unknown exposure	0.93 ± 2.03	0.534
	High exposure- Unknown exposure	1.39 ± 1.92	0.206
	High exposure-Low exposure	0.47 ± 2.57	0.904
MCH (pg/cell)	Unknown exposure -High exposure	0.02 ± 0.69	0.998
	Low exposure-High exposure	0.13 ± 0.93	0.938
	Low exposure- Unknown exposure	0.12 ± 0.73	0.927

Table 4.2 cont'd.

Indices	Baseline (2013) Exposure level	Difference ± SD	p-value
	Low exposure-High exposure	**0.33 ± 0.29**	**< 0.001**
MCHC (g/dL)	Unknown exposure -High exposure	**0.55 ± 0.22**	**< 0.001**
	Unknown exposure -Low exposure	0.21 ± 0.23	0.073
	Low exposure-High exposure	0.60 ± 2.63	0.852
PMN (%)	Unknown exposure -High exposure	0.60 ± 1.97	0.752
	Unknown exposure -Low exposure	0.00 ± 2.09	0.999
	High exposure-Low exposure	0.36 ± 2.32	0.929
LYM (%)	Unknown exposure -Low exposure	0.55 ± 1.84	0.765
	Unknown exposure - High exposure	0.19 ± 1.74	0.966
	Unknown exposure -High exposure	**0.84 ± 0.83**	**< 0.001**
BUN (mg/dL)	Low exposure-High exposure	0.96 ± 1.14	0.121
	Low exposure- Unknown exposure	0.12 ± 0.93	0.951
	Low exposure- Unknown exposure	0.00 ± 0.04	0.992
CR (mg/dL)	High exposure- Unknown exposure	0.03 ± 0.04	0.121
	High exposure-Low exposure	0.03 ± 0.05	0.353
	Low exposure-High exposure	0.53 ± 9.07	0.990
AST (IU/L)	Unknown exposure -High exposure	0.67 ± 6.8	0.970
	Unknown exposure -Low exposure	0.15 ± 7.19	0.999
	Low exposure - Unknown exposure	0.07 ± 4.81	0.999
ALT (IU/L)	High exposure- Unknown exposure	2.46 ± 4.55	0.412
	High exposure-Low exposure	2.39 ± 6.06	0.626

A year after the oil spill clean-up, the average platelet count among workers with unknown exposure was significantly higher than the average platelet counts among workers with high (difference = $36.81 \pm 30.98 \times 10^3$ cells/µL, p-value < 0.001) and low (difference = $37.59 \pm 34.36 \times 10^3$ cells/µL, p-value < 0.001) exposure. Also, unknown exposure workers had a higher average MCHC level than workers with high exposure to the oil spill (difference = 0.82 ± 0.58 g/dL, p-value < 0.001). The analyses found significant differences in BUN levels among the clean-up workers. The average BUN level was highest among workers with low exposure, with a difference of 1.88 ± 1.78 mg/dL between workers with high exposure and 2.84 ± 1.74 mg/dL between unknown

exposure workers. During the second follow-up visit, the average HB, WBC, and ANC levels were higher among workers with high exposure than among workers with unknown exposure. However, no significant differences were observed for other haematological, hepatic, and renal indices. The average differences between the levels of haematological, hepatic, and renal indices at the first follow-up year (2014) by exposure level are summarised in Table 4.3 (Refer to Table A1- Table A4 in the appendix for similar tables for every follow-up year between 2015 and 2018).

Table 4.3: Differences in haematological, hepatic, and renal indices at the first follow-up in 2014.

	First follow-up visit (2014)		
Indices	**Exposure level**	**Difference ± SD**	**p-value**
HB (g/dL)	Low Exposure - Unknown Exposure	0.01 ± 0.76	0.999
	High Exposure - Unknown Exposure	0.15 ± 0.69	0.854
	High Exposure -Low Exposure	0.14 ± 0.78	0.898
HCT (%)	Low Exposure - Unknown Exposure	0.18 ± 2.35	0.982
	High Exposure - Unknown Exposure	1.33 ± 2.12	0.296
	High Exposure - Low Exposure	1.15 ± 2.40	0.490
WBC ($\times 10^3$ cells/L)	High Exposure - Low Exposure	0.28 ± 1.12	0.823
	Unknown Exposure - Low Exposure	0.96 ± 1.09	0.099
	Unknown Exposure - High Exposure	0.68 ± 0.98	0.236
ANC ($\times 10^3$cells/µL)	Low Exposure - High Exposure	0.01 ± 0.92	0.999
	Unknown Exposure - High Exposure	0.81 ± 0.81	0.051
	Unknown Exposure - Low Exposure	0.8 ± 0.90	0.092
RBC (cells/µL)	High Exposure - Low Exposure	0.13 ± 0.34	0.648
	Unknown Exposure - Low Exposure	0.19 ± 0.33	0.366
	Unknown Exposure -High Exposure	0.06 ± 0.3	0.872
Platelets ($\times 10^3$ cells/µL)	High Exposure - Low Exposure	0.79 ± 35.15	0.998
	Unknown Exposure -Low Exposure	**37.59 ± 34.36**	< 0.001
	Unknown Exposure - High Exposure	**36.81 ± 30.98**	< 0.001
MVC (femtolitres)	Low Exposure - Unknown Exposure	3.53 ± 5.19	0.242
	High Exposure - Unknown Exposure	3.76 ± 4.68	0.141
	High Exposure - Low Exposure	0.22 ± 5.31	0.994
MCH (pg/cell)	High Exposure - Unknown Exposure	0.56 ± 1.62	0.691
	Low Exposure - Unknown Exposure	0.94 ± 1.79	0.427
	Low Exposure - High Exposure	0.38 ± 1.83	0.872

Table 4.3 cont'd.

Indices	First Follow-up visit (2014) Exposure level	Difference ± SD	p-value
	Low Exposure - High Exposure	0.51 ± 0.66	0.165
MCHC (g/dL)	Unknown Exposure - High Exposure	**0.82 ± 0.58**	< 0.001
	Unknown Exposure - Low Exposure	0.31 ± 0.65	0.495
	Low Exposure - High Exposure	1.77 ± 6.45	0.791
PMN (%)	Unknown Exposure - High Exposure	4.82 ± 5.68	0.113
	Unknown Exposure - Low Exposure	3.05 ± 6.30	0.483
	Low Exposure - Unknown Exposure	3.62 ± 5.21	0.228
LYM (%)	High Exposure - Unknown Exposure	3.82 ± 4.70	0.134
	High Exposure - Low Exposure	0.20 ± 5.33	0.995
	High Exposure - Unknown Exposure	0.96 ± 1.57	0.316
BUN (mg/dL)	Low Exposure - Unknown Exposure	**2.84 ± 1.74**	< 0.001
	Low Exposure - High Exposure	**1.88 ± 1.78**	< 0.001
	High Exposure - Unknown Exposure	0.05 ± 0.09	0.367
CR (mg/dL)	Low Exposure - Unknown Exposure	0.08 ± 0.10	0.157
	Low Exposure - High Exposure	0.03 ± 0.10	0.811
	High Exposure - Unknown Exposure	1.50 ± 7.47	0.881
AST (IU/L)	Low Exposure - Unknown Exposure	5.26 ± 8.28	0.289
	Low Exposure - High Exposure	3.76 ± 8.47	0.542
	High Exposure - Unknown Exposure	0.77 ± 14.20	0.991
ALT (IU/L)	Low Exposure - Unknown Exposure	10.07 ± 15.74	0.285
	Low Exposure - High Exposure	9.30 ± 16.11	0.358

4.2.2 Differences in haematological, hepatic, and renal indices between the baseline and final follow-up by exposure level

The ANOVA for repeated measures was used to determine the changes in haematological, hepatic, and renal indices between the baseline in 2013 and the final follow-up in 2018. The endpoint analysis was restricted to 570 clean-up workers who were part of the clean-up and attended the follow-up health check-up. The effects of high and low exposures were compared between the baseline and the final follow-up.

Based on the repeated measures ANOVA results, the average HCT from the baseline survey (44.70 ± 1.55 %) was significantly higher than at the final follow-up

(43.04 ± 1.73 %) visit only in the high exposure group. The platelet counts among workers with high exposure and unknown exposure to the oil spill were significantly higher at the final follow-up than at the baseline. Among the high exposure workers, the platelet count was 227.20 ± 17.04 $\times 10^3$ cells/µL and 239.54 ± 13.91 $\times 10^3$ cells/µL at the baseline and final follow-up, respectively. Workers with unknown exposure had an average platelet count of 243.32 ± 5.08 $\times 10^3$ cells/µL in 2013 and 255.16 ± 5.48 $\times 10^3$ cells/µL in 2018. The average RBC count among the high exposure group had reduced significantly at the final follow-visit (5.22 ± 0.23 cells/µL) compared to the baseline measurement (5.37 ± 0.33 cells/µL). The average levels of CR and BUN were significantly higher at final follow-up than at baseline for all exposure groups. The CR levels increased significantly from 0.96 ± 0.05 mg/dL in 2013 to 1.00 ± 0.05 mg/dL among high exposure workers. There was a significant reduction in AST levels from baseline to the final follow-up. The most pronounced reduction of 8.40 IU/L was among workers with unknown exposure. However, significant ALT differences between the baseline and final follow-up measures were observed only among unknown exposure workers with lower ALT in the final follow-up visit. Table 4.4 shows the haematological, renal, and hepatic indices of each group at baseline and the fifth-year follow-up visit based on exposure levels.

Workers with unknown exposure to the oil spill had significantly lower HCT at the baseline than the high-exposure workers. The difference between these two groups was 1.25 ± 1.38 %. Clean-up workers with high exposure had significantly higher RBCs than unknown exposure workers at baseline. The average platelet count of low-exposure workers was 25.06 × 10^3 µL higher than that of high-exposure workers at baseline. At the 5^{th} year follow-up, there were no significant differences between all

haematological, renal, and hepatic indices by exposure levels, except BUN. The BUN level among low-exposure workers was 0.98 mg/dL, higher than the BUN among unknown exposure workers. No significant differences were observed by exposure level to the oil spill for other haematological, hepatic, and renal indices. The comparison of haematological, renal, and hepatic indices between the high exposure, low exposure, and unknown study groups for the baseline and final follow-up visit is shown in Table 4.4.

Table 4.4 Comparison of haematological, renal, and hepatic indices between the baseline and final follow-up visit among 570 Rayong oil spill clean-up workers

Index	High exposure workers (N =46)			Low exposure workers (N =67)			Unknown exposure workers (N = 457)		
	Baseline (2013)	Follow-up visit (2018)	p-value[a]	Baseline (2013)	Follow-up visit (2018)	p-value[a]	Baseline (2013)	Follow-up visit (2018)	p-value[a]
HB (g/dL)	14.64 ± 0.55	14.15 ± 0.21	0.090	14.37 ± 0.37	14.15 ± 0.47	0.214	14.33 ± 0.11	14.37 ± 0.11	0.359
HCT (%)	44.70 ± 1.55	43.04 ± 1.73	**0.045***	43.83 ± 1.11	43.29 ± 1.13	0.158	43.44 ± 0.32	43.62 ± 0.35	0.191
WBC ($\times 10^3$cells/µL)	7.13 ± 0.54	7.11 ± 0.46	0.945	7.26 ± 0.43	6.97 ± 0.31	0.111	6.95 ± 0.16	7.01 ± 0.16	0.400
ANC ($\times 10^3$cells/µL)	3.81 ± 0.41	3.86 ± 0.46	0.935	3.92 ± 0.33	3.84 ± 0.45	0.126	3.76 ± 0.122	3.95 ± 0.22	0.868
Platelets ($\times 10^3$ cells/µL)	227.20 ± 17.04	239.54 ± 13.91	**0.041***	252.25 ± 13.98	256.49 ± 14.25	0.493	243.32 ± 5.08	255.16 ± 5.48	**< 0.001**
MCV (femtolitres)	84.28 ± 2.90	83.16 ± 2.95	0.235	83.89 ± 2.42	84.37 ± 2.36	0.424	84.29 ± 0.72	85.57 ± 0.70	**< 0.001**
MCH (pg/cell)	25.78 ± 1.00	26.46 ± 1.13	0.727	27.53 ± 0.85	27.87 ± 0.92	0.164	27.82 ± 0.26	28.28 ± 0.27	**< 0.001**
MCHC (g/dL)	32.70 ± 0.26	32.93 ±0.30	0.209	32.80 ± 0.28	32.96 ± 0.37	0.425	32.98 ± 0.08	32.90 ± 0.08	0.101
PMN (%)	52.63 ±2.36	53.61 ± 3.71	0.642	53.47 ± 1.96	53.36 ± 4.17	0.947	53.47 ± 0.78	54.29 ± 1.26	0.187
LYM (%)	35.18 ± 2.02	34.53 ± 2.07	0.492	35.25 ± 1.69	34.47 ± 2.21	0.435	35.83 ± 0.71	34.91 ± 0.71	0.020
RBC (cells/µL)	5.37 ± 0.33	5.22 ± 0.23	**0.026***	5.17 ± 0.23	5.26 ± 0.19	0.393	5.06 ± 0.08	5.15 ± 0.05	0.127
BUN (mg/dL)	11.60 ± 1.23	13.24 ± 0.90	**< 0.001**	12.85 ± 1.07	14.11 ± 1.029	**< 0.001**	12.08 ± 0.32	13.13 ± 0.32	**< 0.001**
CR (mg/dL)	0.96 ± 0.05	1.00 ± 0.05	**0.028***	0.91 ± 0.03	0.979 ± 0.04	**< 0.001**	0.93 ± 0.02	0.96 ± 0.02	**< 0.001**
AST (IU/L)	36.80 ± 5.11	30.02 ± 8.99	**< 0.001**	37.00 ± 3.95	30.22 ± 3.28	**< 0.001**	37.27 ± 3.22	28.87 ± 2.55	**< 0.001**
ALT (IU/L)	32.17 ± 4.10	29.70 ± 5.93	0.302	31.10 ± 4.05	31.44 ± 4.44	0.881	31.28 ± 1.82	29.30 ± 1.86	**0.029***

[a]p-values were calculated by ANOVA for repeated measures

Table 4.5 Comparison of haematological, renal, and hepatic indices between the high exposure, low exposure, and unknown study groups (N=570)

Index	Exposure level	Baseline		Follow-up	
		Difference	**p-value**	**Difference**	**p-value**
HB (g/dL)	Low Exposure - High Exposure	-0.27 ± 0.61	0.557	0.01 ± 0.64	0.999
	Unknown - High Exposure	-0.31 ± 0.49	0.312	0.21 ± 0.52	0.598
	Unknown - Low Exposure	-0.04 ± 0.42	0.976	0.21 ± 0.44	0.489
HCT (%)	Low Exposure-High Exposure	-0.86 ± 1.70	0.459	0.24 ± 1.85	0.948
	Unknown-High Exposure	**-1.25 ± 1.38**	**0.022***	0.575 ± 1.49	0.637
	Unknown-Low Exposure	-0.39 ± 1.16	0.709	0.33 ± 1.26	0.811
WBC ($\times 10^3$cells/µL)	Low Exposure-High Exposure	0.12 ± 0.78	0.925	-0.15 ± 0.76	0.891
	Unknown-High Exposure	-0.18 ± 0.63	0.774	-0.10 ± 0.61	0.920
	Unknown-Low Exposure	-0.31 ± 0.53	0.365	0.05 ± 0.52	0.976
ANC ($\times 10^3$cells/µL)	Low Exposure-High Exposure	0.11 ± 0.60	0.898	-0.02 ± 0.928	0.999
	Unknown-High Exposure	-0.05 ± 0.49	0.964	0.09 ± 0.74	0.957
	Unknown-Low Exposure	-0.17 ± 0.41	0.610	0.10 ± 0.66	0.924
Platelets ($\times 10^3$/µL)	Low Exposure-High Exposure	**25.06 ± 25.08**	**0.039***	16.95 ± 26.36	0.287
	Unknown-High Exposure	16.13 ± 20.26	0.148	15.61 ± 21.30	0.198
	Unknown-Low Exposure	-8.93 ± 17.13	0.439	-1.34 ± 18.01	0.983
MCV (femtolitres)	Low Exposure-High Exposure	-0.39 ± 3.74	0.967	1.21 ± 3.64	0.714
	Unknown-High Exposure	0.01 ± 3.02	0.999	2.42 ± 2.94	0.131
	Unknown-Low Exposure	0.399 ± 2.55	0.928	1.20 ± 2.49	0.492
MCH (pg/cell)	Low Exposure-High Exposure	-0.05 ± 1.34	0.996	0.41 ± 1.39	0.766
	Unknown-High Exposure	0.24 ± 1.08	0.862	0.81 ± 1.12	0.205
	Unknown-Low Exposure	0.29 ± 0.91	0.739	0.40 ± 0.95	0.580
MCHC (g/dL)	Low Exposure-High Exposure	0.10 ± 0.42	0.832	0.03 ± 0.44	0.984
	Unknown-High Exposure	0.28 ± 0.34	0.124	-0.03 ± 0.36	0.975
	Unknown-Low Exposure	0.18 ± 0.29	0.307	-0.06 ± 0.30	0.871
PMN (%)	Low Exposure-High Exposure	0.84 ± 3.77	0.861	-0.26 ± 5.86	0.994
	Unknown-High Exposure	0.84 ± 3.05	0.793	0.68 ± 4.99	0.938
	Unknown-Low Exposure	0.01 ± 2.53	0.999	0.93 ± 4.14	0.856
LYM (%)	Low Exposure-High Exposure	0.06 ± 3.39	0.998	-0.06 ± 3.54	0.999
	Unknown-High Exposure	0.65 ± 2.74	0.841	0.38 ± 2.87	0.949
	Unknown-Low Exposure	0.59 ± 2.31	0.822	0.44 ± 2.42	0.906
RBCs	Low Exposure-High Exposure	-0.19 ± 0.39	0.470	0.05 ± 0.28	0.914
	Unknown-High Exposure	**-0.30 ± 0.31**	**0.027***	-0.06 ± 0.23	0.784
	Unknown-Low Exposure	-0.11 ± 0.27	0.670	-0.11 ± 0.19	0.353

Table 4.5 Cont'd

Index	Exposure level	Baseline		Follow-up	
		Difference	p-value	Difference	p-value
BUN (mg/dL)	Low Exposure-High Exposure	1.25 ± 1.64	0.172	0.87 ± 1.60	0.408
	Unknown-High Exposure	0.48 ± 1.28	0.648	-0.11 ± 1.29	0.978
	Unknown-Low Exposure	-0.77 ± 1.17	0.270	**-0.98 ± 1.09**	**0.029***
CR (mg/dL)	Low Exposure-High Exposure	-0.05 ± 0.08	0.289	-0.02 ± 0.09	0.851
	Unknown-High Exposure	-0.03 ± 0.06	0.443	-0.04 ± 0.07	0.424
	Unknown-Low Exposure	0.02 ± 0.05	0.443	-0.02 ± 0.06	0.772
AST (IU/L)	Low Exposure-High Exposure	0.20 ± 14.53	0.999	0.20 ± 12.02	0.999
	Unknown-High Exposure	0.46 ± 11.73	0.995	-1.16 ± 9.71	0.958
	Unknown-Low Exposure	0.27 ± 9.92	0.998	-1.35 ± 8.21	0.920
ALT (IU/L)	Low Exposure-High Exposure	-1.07 ± 8.55	0.954	1.75 ± 8.99	0.891
	Unknown-High Exposure	-0.90 ± 6.91	0.950	-0.40 ± 7.26	0.991
	Unknown-Low Exposure	0.17 ± 5.84	0.997	-2.15 ± 6.14	0.690

4.3 The GEE models

The QIC presents an assessment for models for repeated and correlated measurements. The generalised estimating equations were fitted to each haematological, hepatic, and renal index. The GEEs for each index were evaluated using the QIC to determine the best correlation structure. The GEEs were fitted to the data by assuming three correlation structures: exchangeable, independence and autoregressive (AR1). The best correlation structure was selected based on the smallest QIC. The QIC of the correlation structures for each GEE is shown in Table 4.6.

Table 4.6: QIC and working correlation structure for GEEs of each index

The QIC for each correlation Structure				
		QIC		**Selected correlation**
Index	**Exchangeable**	**Independence**	**AR1**	
HB	**3627.96**	3651.61	3633.71	Exchangeable
HCT	31256.88	**31235.06**	31413.48	Independence
WBC	**7920.34**	7924.96	7933.66	Exchangeable
ANC	**4234.44**	4244.88	4235.05	Exchangeable
RBC	**686.1**	709.25	688.66	Exchangeable
Platelets	7.97×10^6	$\mathbf{7.95 \times 10^6}$	7.99×10^6	Independence
MCV	170696.00	**169136.20**	170912.60	Independence
MCH	22926.70	**22795.50**	22975.00	Independence
MCHC	**2591.90**	2600.10	2599.00	Exchangeable
PMN	175071.00	**175009.50**	175209.00	Independence
LYM	152340.80	**152219.40**	152455.80	Independence
BUN	23145.90	**23135.00**	23165.00	Independence
CR	**77.00**	98.30	78.40	Exchangeable
AST	1376020.40	**1375243.80**	1377413.20	Independence
ALT	1000426.70	**999243.00**	1000628.30	Independence

4.4 Factors associated with changes in haematological, hepatic, and renal indices

This study used the GEE to determine the demographic factors significantly associated with changes in haematological, hepatic, and renal indices. The GEE estimated the overall trend of the indices throughout the study. Also, the trend for each demographic factor was estimated according to the categories of the factor. The GEE showed no significant trends for HB, HCT, WBC, ANC, MCV, MCH, MCHC, and PMN over the study period. However, the results showed significant increasing trends of $3.44 \pm 0.39 \times 10^3$ cells/μL for platelet count and a significant decreasing trend of 0.14 ± 0.07 % for LYM among the oil spill cleaners. Renal function indices increased at 0.22 ± 0.03 mg/dL per year for BUN and 0.02 ± 0.001 mg/dL per year for Cr. The serum liver enzyme AST also decreased significantly (-1.63 ± 0.20 IU/L per year) over the study period. The results from the GEE analysis are summarised in Table 4.7. The factors associated with significant changes in the haematological, hepatic, and renal indices varied based on the index. Gender and occupation were significantly associated with HB, platelets, MCHC and BUN trends. Clean-up workers from the PTTGC (0.31 ± 0.10) and military personnel (0.42 ± 0.18) had significantly lower trends of HB than civilians. The HB trend among men was 1.94 ± 0.12 times higher than women.

Table 4.7: Trends of haematological, renal, and hepatic indices from generalised estimating equations

Index	HB (g/dL)	HCT (%)	WBC ($\times 10^3$µL)	ANC	RBC (cells/µL)	Platelets ($\times 10^3$/µL)	MCV (femtolitres)	MCH (pg/cell)
Average mean	**12.34 ± 0.18***	**38.1 ± 0.51***	**7.47 ± 0.25***	**4.18 ± 0.18***	**4.72 ± 0.09***	**255.99 ± 8.78***	80.11 ± 1.36	**26.04 ± 0.5***
Trend	-0.01 ± 0.01	0.02 ± 0.03	**0.02 ± 0.01***	0.01 ± 0.01	**0.008 ± 0.01***	**3.44 ± 0.39***	0.01 ± 0.05	0.02 ± 0.02
Variable	Average trend for each category ± Standard error							
Age								
20 – 29	Reference							
30 – 39	0.13 ± 0.08	0.54 ± 0.28	-0.08 ± 0.15	-0.10 ± 0.12	-0.05 ± 0.05	-1.73 ± 5.91	2.83 ± 0.85	**0.82 ± 0.31***
40 – 49	0.10 ± 0.10	0.16 ± 0.35	-0.31 ± 0.16	-0.20 ± 0.13	**-0.13 ± 0.06***	-0.54 ± 5.85	4.21 ± 0.89	**1.39 ± 0.33***
50 +	0.15 ± 0.11	0.25 ± 0.39	**-0.36 ± 0.18***	**-0.32 ± 0.14***	**-0.24** ± 0.06*	**-16.33 ± 6.47***	5.4 ± 0.98	**1.73 ± 0.37***
Unknown age	**-0.41 ± 0.18***	**-1.36 ± 0.61***	-0.45 ± 0.28	-0.30 ± 0.21	-0.17 ± 0.10	**-18.18 ± 9.21***	0.41 ± 1.43	0.26 ± 0.55
Occupation	**<0.001**							
Civilian	Reference							
Military	**0.42 ± 0.18***	0.96 ± 0.63	0.25 ± 0.24	0.21 ± 0.18	0.12 ± 0.09	**18.29 ± 7.84***	1.11 ± 1.27	0.77 ± 0.48
PTTGC	**0.31 ± 0.10***	**1.21 ± 0.28***	**-0.27 ± 0.13***	-0.18 ± 0.10	0.10 ± 0.05	**9.39 ± 4.86***	0.11 ± 0.77	-0.13 ± 0.29
Gender	**<0.005**							
Female	Reference							
Male	**1.94 ± 0.12***	**5.49 ± 0.31***	0.18 ± 0.17	-0.07 ± 0.12	**0.59 ± 0.05***	**-29.4 ± 6.43***	0.74 ± 0.92	0.40 ± 0.34
Exposure level								
High exposure	Reference							
Low exposure	-0.17 ± 0.16	-0.77 ± 0.49	-0.37 ± 0.20	-0.17 ± 0.15	-0.07 ± 0.08	2.38 ± 7.15	0.56 ± 1.13	0.3 0± 0.43
Unknown exposure	0.16 ± 0.14	0.22 ± 0.40	-0.21 ± 0.18	-0.11 ± 0.13	0.01 ± 0.07	7.11 ± 5.79	0.88 ± 1	0.41 ± 0.38

Table 4.7 cont'd.

Index	MCHC (g/dL)	PMN (%)	LYM (%)	BUN (mg/dL)	CR (mg/dL)	AST (IU/L)	ALT (IU/L)
Average mean	**32.45 ± 0.13***	**55.43 ± 1.14***	**35.02 ± 1.03***	**8.28 ± 0.36***	**0.65 ± 0.02***	**25.12 ± 2.55***	**21.59 ± 2.67***
Trend	-0.01 ± 0.01	-0.10 ± 0.09	**-0.14 ± 0. 07***	**0.22 ± 0.03***	**0.01 ± 0.00***	**-1.63 ± 0.20**	-0.29 ± 0.16
Variable	Average trend for each category ± Standard error						
Age							
20 – 29	Reference						
30 – 39	-0.11 ± 0.09	-0.76 ± 0.82	0.69 ± 0.73	0.32 ± 0.27	**0.03 ± 0.01***	3.04 ± 1.91	0.70 ± 1.79
40 – 49	0.11 ± 0.09	-0.15 ± 0.86	-0.14 ± 0.76	**0.50 ± 0.26***	0.02 ± 0.01	**2.96 ± 1.31**	1.96 ± 1.72
50 +	0.01 ± 0.10	-1.00 ± 0.95	-0.25 ± 0.82	**1.05 ± 0.32***	**0.06 ± 0.01***	**4.00 ± 1.91**	1.88 ± 2.54
Unknown age	0.01 ± 0.15	-0.51 ± 1.23	0.56 ± 1.09	0.33 ± 0.42	0.02 ± 0.02	1.81 ± 2.64	1.74 ± 3.00
Occupation							
Civilian	Reference						
Military	**0.3 ± 0.14***	0.86 ± 1.11	-1.22 ± 1.01	**-0.83 ± 0.33***	0.03 ± 0.02	-4.23 ± 2.28	-5.04 ± 2.59
PTTGC	**-0.16 ± 0.07***	-0.73 ± 0.64	**1.56 ± 0.57***	**1.60 ± 0.23***	**0.04 ± 0.01***	-1.99 ± 2.51	**-5.58 ± 1.98**
Gender							
Female	Reference						
Male	**0.23 ± 0.08***	**-2.48 ± 0.77***	0.65 ± 0.71	**1.97 ± 0.26***	**0.29 ± 0.01***	**11.13 ± 1.99**	**13.93 ± 1.59**
Exposure level							
High exposure	Reference						
Low exposure	**0.21 ± 0.11***	0.72 ± 0.98	-0.28 ± 0.87	**0.97 ± 0.3***	-0.01 ± 0.02	1.20 ± 1.96	1.02 ± 2.27
Unknown exposure	**0.23 ± 0.09***	0.28 ± 0.79	-0.35 ± 0.72	0.44 ± 0.26	-0.01 ± 0.01	1.04 ± 2.67	-0.05 ± 2.41

4.4 Latent trends

The latent trajectory analyses showed at least two latent classes for each haematological, hepatic, and renal index. The HB level showed three distinct latent trends. Among the oil spill cleaners, 3.68 % had a low HB level at the baseline and a stable trend over time (low-stable), 95.86% had a normal-stable trend of HB, and 0.46% had a high-decreasing trend of HB. There were two latent trends for HCT: normal-stable (99.65%) and high-decreasing (0.35 %). Furthermore, two latent trends were observed for the WBC count. Most clean-up workers (96.89%) had a normal WBC count with a stable trend, while 3.11% had a high WBC count with decreasing trend. Based on the latent trajectory analyses, 3.91% of the clean-up workers had normal ANC with decreasing trend, 92.29% had normal ANC with a stable trend, and 3.80% had normal ANC with an increasing trend. The RBC and platelet counts had four and three latent trends, respectively. The distinct latent trends of RBC count were normal-increasing (68.89%), normal-decreasing (19.59%), normal-stable (7.60%) and high-stable (3.92%). Of the 869 workers, 98.51% had a normal-increasing platelet count trend. However, 1.04% had a very high increasing trend (39.58 $\times 10^3$ per year), while 97.47 had a relatively low trend (3.34 $\times 10^3$ per year). The latent trends of BUN and CR were two and three, respectively. More than 90% of the clean-up workers had a normal-increasing BUN trend, while 5.10% had a normal-stable trend. The average level of CR was normal for all the clean-up workers. However, 10.90% had a stable trend, 88.30% had an increasing trend, and 0.81% had a decreasing trend. Figure 4.3 shows the latent trends of all haematological, hepatic, and renal indices of the Rayong oil spill clean-up workers.

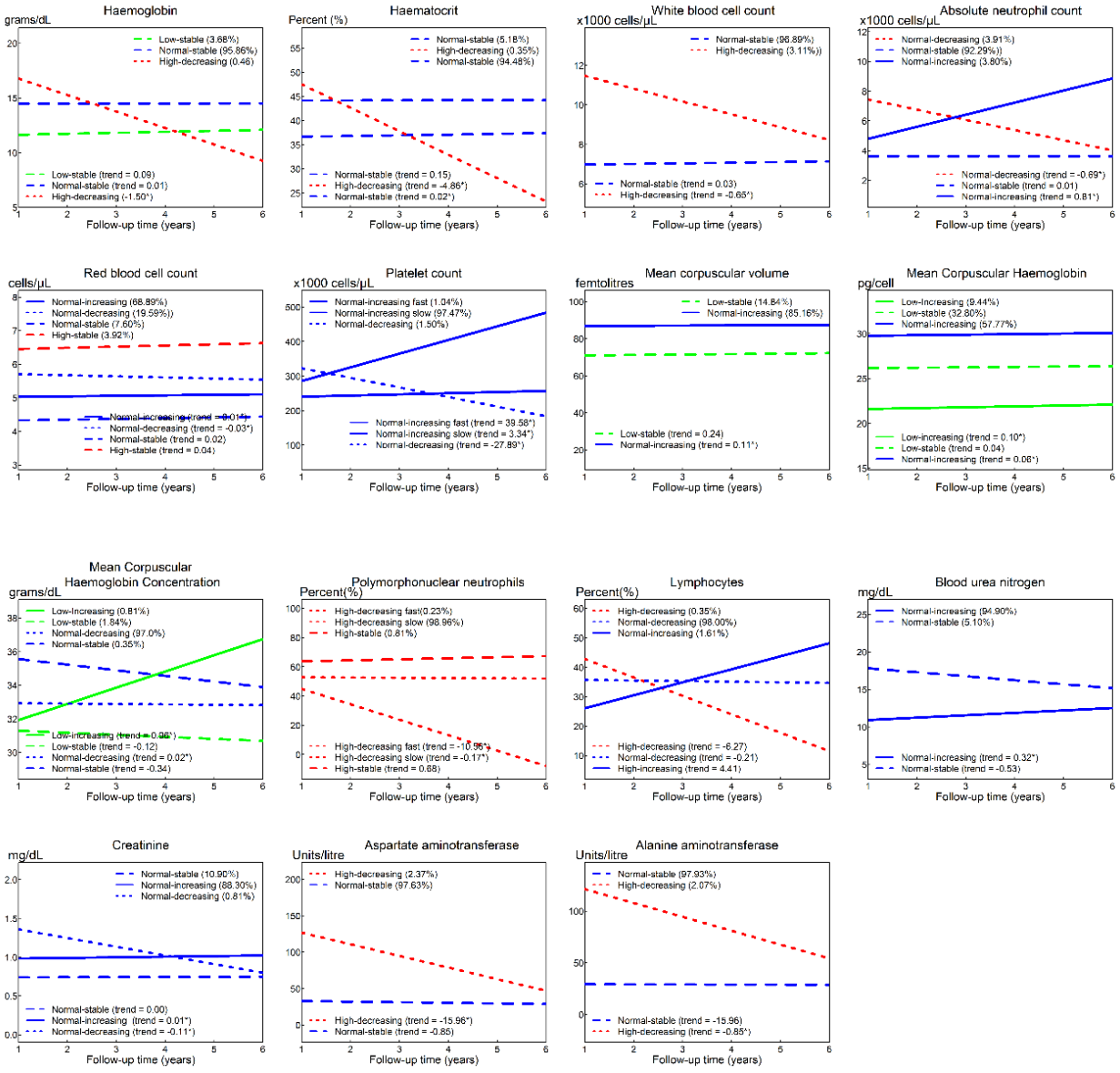


Figure 4.3: Latent trends of the haematological, hepatic, and renal indices

Chapter 5

Discussion and Conclusion

5.1 Summary of findings

The Rayong oil spill disaster occurred in 2013 and, until 2022, was the largest marine disaster in Thailand. More than two thousand workers were mobilised to undertake the onshore and offshore post-disaster clean-up, which lasted about a month (PTTGC, 2013). During the clean-up activities, the workers were exposed to various chemicals at varying levels (Ingviya et al., 2020). Exposure to these hazardous chemicals increases the risk of adverse health effects. This research has examined the annual changes in haematological, hepatic, and renal indices among 869 oil spill clean-up workers who attended follow-up visits at the Rayong hospital from 2013 to 2018. The haematological, hepatic, and renal indices investigated in this study are very important for the early screening of diseases (D'Andrea and Reddy 2018). This study suggests that significant alterations in the haematological, hepatic, and renal functions could occur from exposure to the Rayong oil spill. The longitudinal trajectories analysis conducted in this study has identified different latent trends for the haematological, hepatic, and renal profiles of the oil spill clean-up workers. Furthermore, the results indicate that endpoint analyses of longitudinal data using t-test and ANOVA could present different information from GEE to account for subject-specific correlation.

Evidence of long-term haematological alterations has been reported after 3 years (Choi et al. 2017; Doherty et al. 2017) and 7 years (D'Andrea and Reddy 2018) post-oil spill exposure. Five years after the Rayong oil spill, the results of this study

show significant increasing trends in WBC count, RBC counts, platelet count, BUN, and CR among the workers of the oil spill. Significant decreasing trends were found for LYM, AST and ALT. The background occupation before the oil spill clean-up and gender were significantly associated with changes in HB, HCT, RBC count MCHC, BUN, and CR. Different studies among oil spill-exposed subjects in Canada and Korea have reported significantly increased WBC counts associated with various demographic factors (Cakmak et al., 2020; Choi et al., 2017). This study reports increasing average WBC counts among the oil spill clean-up workers. This positive trend indicates the possibility of chronic elevated WBC due to oil spill exposure. A high WBC count is a biological stress response mechanism after oil spill exposure and is restored to pre-exposure levels when exposure ends (Lutcavage et al., 1995). However, elevated WBC count over an extended period is a significant factor associated with cardiovascular complications, stroke, and impulsivity-related traits (Lee et al., 2001; Sutin et al., 2012).

Our study found that the average platelet count of the Rayong oil spill clean-up workers was higher after 5 years than at the baseline. Furthermore, the platelet count had a significantly increasing trend throughout the study. This result is consistent with that of Watson et al. (2021). Among subjects not exposed to crude oil or chemicals in crude oil, the increased platelet count is considered an acute reaction to various infections and inflammation, as well as tumours (Jenne and Kubes 2015; Lippi and Franchini 2015; Vora and Lilleyman 1993). Five years after the oil spill clean-up, the higher platelet count among the clean-up workers may result from a reduced platelet count during the clean-up in 2013 as a short-term biological response to oil spill exposure (Choi et al. 2017; D'Andrea and Reddy 2013, 2018; Ibrahim et al. 2014).

Crude oil contains PAHs, and exposure to these compounds can cause oxidative stress and inflammatory response, as reported by Choi and Kim (2021). Different studies have found that elevated WBC and platelet counts are significant factors associated with diabetes mellitus and cardiovascular diseases (Twig et al., 2012).

The renal functioning indices (BUN and CR) among the oil spill clean-up workers were significantly higher in 2018 than in 2013**;** the changes were significantly associated with age, gender and background occupation. The GEE results showed that BUN and CR trends were significantly higher among older clean-up workers and men. The CR level usually increases with ageing and is higher among men (Tiao et al., 2002). However, other studies have documented increased CR levels after exposure to benzene (Al-Helaly and Ahmed, 2014; D'Andrea and Reddy, 2016), a chemical component of crude oil. The subjects exposed to the Hebei Spirit oil spill in Korea had reduced CR and BUN levels after 3 years (Choi et al., 2017), while the subjects exposed to the BP oil spill had no change in CR and decreased BUN levels after 7 years (D'Andrea and Reddy 2018). The findings of these studies contradict the results from the Rayong oil spill clean-up workers. The differences could be attributed to the length of oil spill exposure and the presence of different heavy metals in different crude oil. The increased levels of BUN and CR found in this study indicate worsening renal functioning among the Rayong oil spill clean-up workers. Increased CR might be partially due to exposure to components in the oil spill.

The findings from this study indicate the effects of oil spill exposure on the haematopoietic system of the oil spill clean-up workers. Aside from the oil spill exposure, smoking status, age and gender were significantly associated with changes in haematological, hepatic, and renal indices. Specifically, gender was significantly

associated with changes in HB, HCT, RBC and platelet count (Mandala et al., 2017). Also, smoking status was adjusted as a confounding variable since cigarette smoke was associated with increased WBC and HCT (Malenica et al., 2017). The background occupation was significantly associated with the increase in HCT and platelets. This associated could be due to the healthy worker effect.

5.2 Limitations of the study

The present study identified possible changes in the haematopoietic and renal systems of the Rayong oil spill clean-up workers. Despite these significant findings, the study had some limitations. Firstly, the level of exposure was classified based on the day of first contact with the oil spill. Therefore, non-differentiated misclassification error is possible, which can bias the results. Secondly, other exposure information such as the duration (number of days or hours) of clean-up and personal protective equipment (PPE) usage were not included in the analysis due to the unavailability of data. However, Ingviya et al. (2020) indicate that a few clean-up workers had access to and used complete PPEs during the clean-up work. Additionally, PPEs did not provide significant protection against acute effects and allergic symptoms of the Rayong oil spill exposure, partly due to inadequate and improper use. Hence, the confounding effects of not accounting for the usage of PPEs are expected to be minimal. Finally, the follow-up protocol of the Rayong oil spill clean-up workers was voluntary and subject to availability, convenience and proximity to the Rayong hospital. There is a possibility of volunteer bias because most clean-up workers from the Thai military personnel had been reassigned and lost to follow-up.

5.3 Conclusions

This longitudinal study has analysed the haematological, hepatic, and renal indices of the 869 clean-up workers of the Rayong oil spill. The study used ANOVA to analyse annual changes and conducted endpoint analyses to determine the changes in haematological, hepatic, and renal indices between the baseline in 2013 and the final follow-up in 2018, using the level of exposure to differentiate between subjects. Additionally, longitudinal analyses were performed using the GEEs and latent class trajectory analysis to (1) assess the trends of the haematological, hepatic, and renal indices, (2) determine factors associated with the trends and (3) assess the presence of latent clusters based on trends of the indices over five years after the oil spill clean-up.

The results indicate significant differences between the baseline and fifth-year follow-up measurements of some haematological, hepatic, and renal indices. Long-term trends of these indices indicate that exposure to the Rayong oil spill could lead to cardiovascular health problems. Crude oil contains benzene and other harmful chemicals. After exposure and xenobiotic metabolism, benzene metabolites can lead to bone marrow suppression. This haematopoietic damage usually manifests as significant alterations in the haematological indices found in this study.

5.4 Recommendations and further research

The findings of this dissertation give indication of possible significant haematological alterations due to exposure to the Rayong oil spill of 2013. Many exposure factors could have contributed to these changes. Five years after the oil-spill clean-up, the alterations in the haematopoietic, hepatic and renal systems from the exposure to PAHs and VOCs were still observable among the oil-spill workers.

Exposure to the Rayong oil spill could have triggered many xenobiotic reactions in the body to suppress haematopoietic, hepatic, and renal functioning. Therefore, various activities undertaken during oil-spill clean-up should be classified as potentially dangerous jobs which expose workers to harzadous chemicals such as carcinogens and haematotoxins. These chemicals induce hepatotoxicity. Health care management and policymakers need to plan health monitoring protocols of clean-up workers before they participate in future oil spill clean-up activities. Also, health monitoring of the clean-up workers should be frequent (possibly more than once a year) and extend beyond five years. It is also important that pre-exposure health monitoring is conducted among clean-up workers. This assessment provides a better understanding of the effects of oil spill exposure.

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APPENDICES

Appendix I

Annual differences in haematological, hepatic, and renal indices by exposure level

The annual changes in haematological, hepatic, and renal indices by exposure level for second, third, fourth- and fifth-year follow-ups are presented in the tables below. Table A1: Differences in haematological, hepatic, and renal indices at the second follow-up according to exposure level (2015)

Second Follow-up visit (2015)			
Indices	**Exposure level**	**Difference ± SD**	**p-value**
HB (g/dL)	Low exposure-High exposure	-0.41 ± 0.65	0.307
	Unknown exposure-High exposure	**-0.54 ± 0.46**	**< 0.001**
	Unknown exposure-Low exposure	-0.14 ± 0.56	0.831
HCT (%)	Low exposure-High exposure	-1.1 ± 1.88	0.352
	Unknown exposure-High exposure	-1.1 ± 1.34	0.133
	Unknown exposure-Low exposure	0.01 ± 1.63	1
WBC ((×10^3 cells/L)	Low exposure-High exposure	-0.62 ± 0.78	0.143
	Unknown exposure-High exposure	**-0.59 ± 0.56**	**< 0.001**
	Unknown exposure-Low exposure	0.03 ± 0.68	0.993
ANC (×10^3cells/µL)	Low exposure-High exposure	-0.4 ± 0.6	0.269
	Unknown exposure-High exposure	**-0.52 ± 0.43**	**< 0.001**
	Unknown exposure-Low exposure	-0.12 ± 0.52	0.854
RBC (cells/µL)	Low exposure-High exposure	-0.21 ± 0.27	0.154
	Unknown exposure-High exposure	-0.06 ± 0.19	0.711
	Unknown exposure-Low exposure	0.15 ± 0.23	0.299
Platelets (×10^3 cells/µL)	Low exposure-High exposure	4.83 ± 26.68	0.905
	Unknown exposure-High exposure	3 ± 19.04	0.927
	Unknown exposure-Low exposure	-1.82 ± 23.18	0.981
MVC (femtolitres)	Low exposure-High exposure	0.65 ± 3.93	0.919
	Unknown exposure-High exposure	-1.7 ± 2.81	0.33
	Unknown exposure-Low exposure	-2.35 ± 3.42	0.239
MCH (pg/cell)	Low exposure-High exposure	0.47 ± 1.52	0.75
	Unknown exposure-High exposure	-0.44 ± 1.08	0.601
	Unknown exposure-Low exposure	-0.91 ± 1.32	0.241
MCHC (g/dL)	Low exposure-High exposure	**0.51 ± 0.46**	**< 0.001**
	Unknown exposure-High exposure	0.17 ± 0.33	0.458
	Unknown exposure-Low exposure	-0.34 ± 0.4	0.115
PMN (%)	Low exposure-High exposure	-0.75 ± 4.21	0.907
	Unknown exposure-High exposure	-2.54 ± 3	0.116
	Unknown exposure-Low exposure	-1.79 ± 3.66	0.483
LYM (%)	Low exposure-High exposure	1.98 ± 3.64	0.407
	Unknown exposure-High exposure	1.24 ± 2.59	0.497
	Unknown exposure-Low exposure	-0.73 ± 3.16	0.848

Table A1 cont'd

Second Follow-up visit (2015)			
Indices	**Exposure level**	**Difference ± SD**	**p-value**
BUN (mg/dL)	Low exposure-High exposure	0.49 ± 1.31	0.65
	Unknown exposure-High exposure	-0.34 ± 0.93	0.674
	Unknown exposure-Low exposure	-0.83 ± 1.14	0.201
CR (mg/dL)	Low exposure-High exposure	-0.02 ± 0.09	0.843
	Unknown exposure-High exposure	-0.06 ± 0.06	0.052
	Unknown exposure-Low exposure	-0.04 ± 0.08	0.408
AST (IU/L)	Low exposure-High exposure	-0.87 ± 6.61	0.948
	Unknown exposure-High exposure	-3.13 ± 4.72	0.263
	Unknown exposure-Low exposure	-2.26 ± 5.74	0.624
ALT (IU/L)	Low exposure-High exposure	-1.38 ± 9.57	0.939
	Unknown exposure-High exposure	-3.09 ± 6.84	0.537
	Unknown exposure-Low exposure	-1.71 ± 8.32	0.879

Table A2: Differences in haematological, hepatic, and renal indices at the third follow-up according to exposure level (2016)

Indices	Third Follow-up visit (2016) Exposure level	Difference ± SD	p-value
HB (g/dL)	Low exposure-High exposure	-0.57 ± 0.68	0.117
	Unknown exposure-High exposure	-0.31 ± 0.49	0.308
	Unknown exposure-Low exposure	0.26 ± 0.61	0.565
HCT (%)	Low exposure-High exposure	-2.07 ± 2.16	0.064
	Unknown exposure-High exposure	-0.34 ± 1.57	0.866
	Unknown exposure-Low exposure	1.73 ± 1.96	0.097
WBC ($\times 10^3$ cells/L)	Low exposure-High exposure	**-1.17 ± 1.02**	**< 0.001**
	Unknown exposure-High exposure	-0.37 ± 0.74	0.468
	Unknown exposure-Low exposure	0.8 ± 0.93	0.108
ANC ($\times 10^3$cells/µL)	Low exposure-High exposure	-0.6 ± 0.81	0.187
	Unknown exposure-High exposure	-0.21 ± 0.59	0.683
	Unknown exposure-Low exposure	0.39 ± 0.73	0.412
RBC (cells/µL)	Low exposure-High exposure	-0.06 ± 0.36	0.924
	Unknown exposure-High exposure	0.2 ± 0.27	0.183
	Unknown exposure-Low exposure	0.26 ± 0.31	0.12
Platelets ($\times 10^3$ cells/µL)	Low exposure-High exposure	-26.52 ± 34.82	0.172
	Unknown exposure-High exposure	-23.99 ± 25.31	0.067
	Unknown exposure-Low exposure	2.53 ± 31.61	0.98
MVC (femtolitres)	Low exposure-High exposure	-1.75 ± 4.14	0.58
	Unknown exposure-High exposure	-2.61 ± 3.01	0.103
	Unknown exposure-Low exposure	-0.87 ± 3.76	0.849
MCH (pg/cell)	Low exposure-High exposure	-0.88 ± 1.82	0.484
	Unknown exposure-High exposure	-1.33 ± 1.39	0.064
	Unknown exposure-Low exposure	-0.45 ± 1.55	0.773
MCHC (g/dL)	Low exposure-High exposure	0.41 ± 0.64	0.289
	Unknown exposure-High exposure	-0.22 ± 0.49	0.539
	Unknown exposure-Low exposure	**-0.63 ± 0.55**	**< 0.001**
PMN (%)	Low exposure-High exposure	1.28 ± 5.07	0.823
	Unknown exposure-High exposure	0.4 ± 3.7	0.965
	Unknown exposure-Low exposure	-0.88 ± 4.59	0.894
LYM (%)	Low exposure-High exposure	1 ± 4.31	0.848
	Unknown exposure-High exposure	-0.4 ± 3.13	0.951
	Unknown exposure-Low exposure	-1.4 ± 3.91	0.676

Table A2 cont'd

Third Follow-up visit (2016)			
Indices	**Exposure level**	**Difference ± SD**	**p-value**
BUN (mg/dL)	Low exposure-High exposure	0.81 ± 1.91	0.578
	Unknown exposure-High exposure	0.17 ± 1.39	0.956
	Unknown exposure-Low exposure	-0.64 ± 1.74	0.659
CR (mg/dL)	Low exposure-High exposure	-0.02 ± 0.1	0.888
	Unknown exposure-High exposure	0.01 ± 0.07	0.979
	Unknown exposure-Low exposure	0.02 ± 0.09	0.782
AST (IU/L)	Low exposure-High exposure	-5.29 ± 13.61	0.629
	Unknown exposure-High exposure	-5.78 ± 9.89	0.352
	Unknown exposure-Low exposure	-0.49 ± 12.35	0.995
ALT (IU/L)	Low exposure-High exposure	-6.55 ± 15.57	0.581
	Unknown exposure-High exposure	-5.43 ± 11.31	0.493
	Unknown exposure-Low exposure	1.11 ± 14.13	0.981

Table A3: Differences in haematological, hepatic, and renal indices at the fourth follow-up according to exposure level (2017)

Fourth Follow-up visit (2017)			
Indices	**Exposure level**	**Difference ± SD**	**p-value**
HB (g/dL)	Low exposure-High exposure	-0.22 ± 0.51	0.579
	Unknown exposure-High exposure	0.23 ± 0.35	0.283
	Unknown exposure-Low exposure	**0.44 ± 0.43**	**< 0.001**
HCT (%)	Low exposure-High exposure	-0.05 ± 1.57	0.997
	Unknown exposure-High exposure	**1.57 ± 1.09**	**< 0.001**
	Unknown exposure-Low exposure	**1.61 ± 1.32**	**< 0.001**
WBC ($\times 10^3$ cells/L)	Low exposure-High exposure	-0.19 ± 0.71	0.804
	Unknown exposure-High exposure	-0.29 ± 0.5	0.362
	Unknown exposure-Low exposure	-0.1 ± 0.6	0.923
ANC ($\times 10^3$cells/µL)	Low exposure-High exposure	0.05 ± 0.55	0.970
	Unknown exposure-High exposure	-0.1 ± 0.39	0.813
	Unknown exposure-Low exposure	-0.16 ± 0.47	0.714
RBC (cells/µL)	Low exposure-High exposure	-0.11 ± 0.24	0.514
	Unknown exposure-High exposure	0.04 ± 0.17	0.865
	Unknown exposure-Low exposure	0.15 ± 0.2	0.178
Platelets ($\times 10^3$ cells/µL)	Low exposure-High exposure	10.33 ± 22.58	0.530
	Unknown exposure-High exposure	**18.65 ± 15.76**	**< 0.001**
	Unknown exposure-Low exposure	8.32 ± 19.08	0.561
MVC (femtolitres)	Low exposure-High exposure	1.39 ± 3.39	0.602
	Unknown exposure-High exposure	2.03 ± 2.37	0.11
	Unknown exposure-Low exposure	0.64 ± 2.87	0.858
MCH (pg/cell)	Low exposure-High exposure	0.21 ± 1.21	0.912
	Unknown exposure-High exposure	0.29 ± 0.86	0.707
	Unknown exposure-Low exposure	0.08 ± 1	0.980
MCHC (g/dL)	Low exposure-High exposure	-0.41 ± 0.45	0.088
	Unknown exposure-High exposure	-0.66 ± 0.32	< 0.001
	Unknown exposure-Low exposure	-0.26 ± 0.37	0.243
PMN (%)	Low exposure-High exposure	2.26 ± 3.58	0.301
	Unknown exposure-High exposure	0.63 ± 2.5	0.823
	Unknown exposure-Low exposure	-1.62 ± 3.03	0.418
LYM (%)	Low exposure-High exposure	-1.75 ± 3.22	0.410
	Unknown exposure-High exposure	0.05 ± 2.25	0.999
	Unknown exposure-Low exposure	1.79 ± 2.72	0.268

Table A3 cont'd

Fourth Follow-up visit (2017)			
Indices	**Exposure level**	**Difference ± SD**	**p-value**
BUN (mg/dL)	Low exposure-High exposure	**1.52 ± 1.32**	**< 0.001**
	Unknown exposure-High exposure	**1.16 ± 0.92**	**< 0.001**
	Unknown exposure-Low exposure	-0.37 ± 1.12	0.720
CR (mg/dL)	Low exposure-High exposure	0.02 ± 0.07	0.707
	Unknown exposure-High exposure	0.04 ± 0.05	0.146
	Unknown exposure-Low exposure	0.01 ± 0.06	0.818
AST (IU/L)	Low exposure-High exposure	0.19 ± 4.17	0.994
	Unknown exposure-High exposure	-2.58 ± 2.91	0.095
	Unknown exposure-Low exposure	-2.77 ± 3.52	0.156
ALT (IU/L)	Low exposure-High exposure	2.83 ± 7.24	0.629
	Unknown exposure-High exposure	-2.61 ± 5.05	0.446
	Unknown exposure-Low exposure	-5.44 ± 6.12	0.093

Table A4: Differences in haematological, hepatic, and renal indices at the fifth follow-up according to exposure level (2018)

Fifth Follow-up visit (2018)			
Indices	**Exposure level**	**Difference ± SD**	**p-value**
HB (g/dL)	Low exposure-High exposure	0.01 ± 0.64	**0.999**
	Unknown exposure-High exposure	0.21 ± 0.52	0.598
	Unknown exposure-Low exposure	0.21 ± 0.44	0.489
HCT (%)	Low exposure-High exposure	0.24 ± 1.85	0.948
	Unknown exposure-High exposure	0.58 ± 1.49	0.637
	Unknown exposure-Low exposure	0.33 ± 1.26	0.811
WBC ($\times 10^3$ cells/L)	Low exposure-High exposure	-0.15 ± 0.76	0.891
	Unknown exposure-High exposure	-0.10 ± 0.62	0.920
	Unknown exposure-Low exposure	0.05 ± 0.52	0.976
ANC ($\times 10^3$ cells/µL)	Low exposure-High exposure	-0.02 ± 0.93	0.999
	Unknown exposure-High exposure	0.09 ± 0.74	0.957
	Unknown exposure-Low exposure	0.10 ± 0.66	0.925
RBC (cells/µL)	Low exposure-High exposure	0.05 ± 0.28	0.914
	Unknown exposure-High exposure	-0.06 ± 0.23	0.784
	Unknown exposure-Low exposure	-0.11 ± 0.19	0.353
Platelets ($\times 10^3$ cells/µL)	Low exposure-High exposure	16.95 ± 26.36	0.287
	Unknown exposure-High exposure	15.61 ± 21.3	0.198
	Unknown exposure-Low exposure	-1.34 ± 18.01	0.983
MVC (femtolitres)	Low exposure-High exposure	1.21 ± 3.64	0.714
	Unknown exposure-High exposure	2.42 ± 2.94	0.131
	Unknown exposure-Low exposure	1.20 ± 2.49	0.492
MCH (pg/cell)	Low exposure-High exposure	0.41 ± 1.39	0.766
	Unknown exposure-High exposure	0.81 ± 1.12	0.205
	Unknown exposure-Low exposure	0.40 ± 0.95	0.58
MCHC (g/dL)	Low exposure-High exposure	0.03 ± 0.44	0.984
	Unknown exposure-High exposure	-0.03 ± 0.36	0.975
	Unknown exposure-Low exposure	-0.06 ± 0.30	0.871
PMN (%)	Low exposure-High exposure	-0.26 ± 5.86	0.994
	Unknown exposure-High exposure	0.68 ± 4.7	0.938
	Unknown exposure-Low exposure	0.93 ± 4.14	0.856
LYM (%)	Low exposure-High exposure	-0.06 ± 3.54	0.999
	Unknown exposure-High exposure	0.38 ± 2.86	0.949
	Unknown exposure-Low exposure	0.44 ± 2.42	0.906

Table A4 cont'd

Fifth Follow-up visit (2018)			
Indices	**Exposure level**	**Difference ± SD**	**p-value**
BUN (mg/dL)	Low exposure-High exposure	0.87 ± 1.60	0.408
	Unknown exposure-High exposure	-0.11 ± 1.29	0.978
	Unknown exposure-Low exposure	-0.98 ± 1.09	0.089
CR (mg/dL)	Low exposure-High exposure	-0.02 ± 0.09	0.851
	Unknown exposure-High exposure	-0.04 ± 0.07	0.424
	Unknown exposure-Low exposure	-0.02 ± 0.06	0.772
AST (IU/L)	Low exposure-High exposure	0.20 ± 12.02	0.999
	Unknown exposure-High exposure	-1.16 ± 9.71	0.958
	Unknown exposure-Low exposure	-1.36 ± 8.21	0.920
ALT (IU/L)	Low exposure-High exposure	1.75 ± 8.99	0.891
	Unknown exposure-High exposure	-0.40 ± 7.26	0.991
	Unknown exposure-Low exposure	-2.15 ± 6.14	0.689

Appendix II

Manuscript I

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ORIGINAL ARTICLE

Haematological, renal, and hepatic function changes among Rayong oil spill clean-up workers: a longitudinal study

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Abstract

Purpose The Rayong oil spill incident of 2013 leaked over 50,000 barrels of crude oil into the Gulf of Thailand. This study assessed trends and changes in the haematological, renal, and hepatic indices among the Rayong oil spill clean-up workers 5 years after the spill.

Methods Haematological, renal, and hepatic indices measured for 570 oil spill clean-up workers at baseline and annually during 5-year follow-ups were analysed. Haemoglobin (Hb), haematocrit (Hct), white blood cell (WBC) count, red blood cell (RBC) count, and platelet count for haematological function, aspartate aminotransferase (AST) and alanine aminotransferase (ALT) for hepatic function, and creatinine (Cr) and blood urea nitrogen (BUN) for renal function were assessed. The longitudinal measures of haematological, renal and hepatic indices were analysed using analysis of variance for repeated measures. The generalised estimating equations (GEE) were used to assess trends of these indices and associated factors, including exposure level.

Results Increasing trends were observed per year for WBC ($0.52 \pm 0.03 \times 10^3$ cells/μL), Cr (0.01 ± 0.00 mg/dL), platelet (0.31×10^3 μL per year), and BUN (0.24 ± 0.03 mg/dL). Decreasing trends of aspartate aminotransferase (AST) were observed (1.54 ± 0.21 IU/L per year). Clean-up workers with high exposure to the oil spill had a significantly higher average of WBC and lower average of BUN than low-exposure and unknown-exposure workers. Gender and age were significantly associated with creatinine changes.

Conclusion Results of this study show the differences between baseline and follow-up haematological, renal, and hepatic indices and trends of these indices. The long-term changes in the indices in this study show worsening renal functions after oil spill and possibility of cardiovascular effects. These findings contribute to expanding knowledge on the long-term health effects of oil spills.

Keywords Clean-up · Exposure · Follow-up · Haematological indices · Oil spill

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Introduction

Crude oil comprises volatile organic compounds (VOCs), polycyclic aromatic hydrocarbons (PAHs), and other carcinogens (IARC 1984). Oil spill clean-up volunteers are directly and indirectly exposed to these toxic chemicals in crude oil; hence, they are at risk of developing health problems (Hildur et al. 2015; Peres et al. 2016; Suárez et al. 2005). Various studies have examined the health effects of oil spill exposure (Choi et al. 2017; D'Andrea and Reddy 2014; Murphy et al. 2016). The main adverse health effects reported have been significant alterations in haematological, hepatic, and renal indices (D'Andrea and Reddy 2013, 2014), chromosomal damage (Hildur et al. 2015), and acute health complications, including headache, itchy eyes, throat irritation, dizziness, nausea, and cough (Lee et al. 2010; Peres et al. 2016; Song et al. 2009; Suarez et al. 2005). Chronic respiratory complications, reduced pulmonary functioning, and allergic rhinitis have also been reported (Park et al. 2019; Zock et al. 2012). Studies have shown that the nature and severity of acute health effects are related to the level of exposure (Ingviya et al. 2020; Peres et al. 2016). Factors, such as proximity to an oil spill site, the time between an oil spill and human contact, and the number of days of clean-up, are related to the level of exposure (Ha et al. 2012; Ingviya et al. 2020).

The Rayong oil spill of 2013 leaked over 50,000 L of crude oil into the Gulf of Thailand. The oil clean-up process was spearheaded by a collaboration between the Thai government and the state-owned PTT Global Chemical (PTTGC) company responsible for the spill. Over two thousand personnel were mobilised to perform different roles during clean-up. These clean-up workers included personnel from the PTTGC company, the Thai Navy, and civilian volunteers (PTTGC 2013). The Rayong oil spill clean-up lasted for more than a month and involved workers of different backgrounds.

Globally, oil spill-related research has been conducted extensively over the last five decades. However, only a small fraction of these researches have focused on public health effects of oil spills on humans (Murphy et al. 2016). Few longitudinal studies have been conducted to highlight the long-term effects of oil spill exposure (Choi et al. 2017; D'Andrea and Reddy 2018; Gam et al. 2018; Park et al. 2019). Most of these studies were endpoint analyses, comparing physiological changes between baseline and a follow-up after years of clean-up. Considering the toxicodynamics of the VOCs and PAHs, changes in haematological, renal, and hepatic functions should be assessed at multiple periods to obtain a more comprehensive understanding of the long-term trends and possible longitudinal changes. This study analysed the baseline and five-year longitudinal data from the Rayong oil spill follow-up visits to assess the long-term effects of exposure among the oil spill-exposed workers. Specifically, long-term changes and trends of haematological, renal, and hepatic indices of the oil spill clean-up workers were analysed at baseline and over five years after the disaster. We hypothesised that exposure to the Rayong oil spill could cause significant long-term alterations of human organ systems in the dose–response pattern. As shown by various studies, exposure to PAHs and VOCs included in crude oil has demonstrated the possibility of long-term effects in haematological renal and hepatic systems (ATSDR 1995). The results from this study not only present the haematological, kidney, and hepatic changes due to oil spill exposure after a specific period but also assess the trends of the changes that might help in early detection of severe health complications.

Data and methods

Study design and participants

During the Rayong oil spill clean-up, the Rayong Hospital designed a baseline and 5-year follow-up surveillance protocol to monitor the health of the oil spill clean-up workers. Post-shift blood and urine samples were taken from the workers following the clean-up period. Questionnaires were used to record demographic information, allergic reactions and acute symptoms which developed during the clean-up. The baseline levels of exposure of the clean-up workers to VOCs and PAHs were assessed based on urinary metabolite levels (Ingviya et al. 2020). As part of the health surveillance programme, the Rayong Hospital conducted annual follow-up visits to monitor the oil spill-exposed clean-up workers' health every year for five years, from 2014 to 2018. During these visits, blood samples were taken to assess the oil spill-exposed workers' haematological, renal, and hepatic indices.

Data on the follow-ups and baseline survey for this study were obtained from the Rayong Hospital. The baseline study and characteristics of the baseline workers have been described in other studies (Ingviya et al. 2020; Sithisarankul and Intawong 2013). All clean-up workers and other staff exposed to the spill were asked to come for yearly follow-up. The data for this study were selected based on the information contained in the baseline survey from Ingiviya et al. (2020) and Sithisarankul and Intawong (2013). Throughout the follow-up visits period, 869 workers reported to the hospital at least once for an annual health examination. The analysis of this study focused on 570 workers who reported for the final-year follow-up in 2018. This is to provide a consistent time endpoint assessment of the effects of oil spill exposure.

Exposure assessment

The levels of exposure were classified using days of clean-up works. A previous study by Ingviya et al. (2020) has quantified the level of exposure to the Rayong oil spill using the urinary concentration of 1-hydroxypyrene glucuronide (1-OHPG), a metabolite of polycyclic aromatic hydrocarbons. In this study, the clean-up workers were grouped as high exposure, low exposure, or unknown exposure based on the findings from Ingviya et al. (2020). Workers in the first 72 h of the spill were classified as high exposure group, and those who worked on subsequent days were classified as low exposure group. Oil spill clean-up workers who did not provide data on the exact clean-up dates were grouped as 'unknown exposure'. To avoid potential selection bias, we decided against excluding them from the analysis.

Due to the nature of the sentinel questionnaire data collection, the information on the hours of works, protective equipment, and duration of clean-up work was not available as details enough for the analysis.

Outcome measurement

The outcomes were analysed based on the haematological, renal, and hepatic indices extracted from the Rayong Hospital Laboratory Data. Blood samples collected from the oil spill clean-up workers were analysed with medically approved protocol at the Rayong hospital.

The analysis of blood samples involved assessment of complete blood count parameters, such as indices for haematological function, including haemoglobin (Hb), haematocrit (Hct), white blood cell (WBC) count, red blood cell (RBC) count, and platelet count. The measuring of complete blood count did assess not only the haematological function but also the possibility of the underlying inflammation process observable from the increase in WBC and platelet numbers.

Liver enzymes (aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were measured to assess hepatic function. Various liver disorders were associated with elevated levels of AST and ALT (Dhillon and Steadman 2012). Assessing AST and ALT levels among oil spill-exposed workers could help in early detection of liver diseases.

The renal function indices measured were creatinine (Cr) and blood urea nitrogen (BUN). Although not sufficient, persistent elevation of BUN and Cr levels is warning signs of chronic kidney failure (Macedo and Mehta 2013) Table 1.

Statistical analysis

The demographic characteristics of the workers were compared with descriptive statistics and Fisher's exact test. An endpoint analysis was conducted to assess the differences between the haematological, renal, and hepatic indices at baseline (2013) and the final follow-up visit (2018) using paired *t*-test (Table 2). Analysis of variance (ANOVA) for

Table 1 Distribution of demographic factors and exposure levels

Variable	Total = 570	High exposure $N=46$	Low exposure $N=67$	Unknown exposure $N=457$	p-value[a]
	Number (%)	Number (%)	Number (%)	Number (%)	
Gender					< 0.001
Male	510 (89.5%)	44 (95.7%)	63 (94.0%)	403 (88.2%)	
Female	60 (10.5%)	2 (4.3%)	4 (6.0%)	54 (11.8%)	
Age group					< 0.001
20–29	100 (17.5%)	7 (15.2%)	16 (23.9%)	77 (16.9%)	
30–39	114 (20.0%)	9 (19.7%)	13 (19.4%)	92 (20.1%)	
40–49	225 (39.5%)	26 (56.5%)	28 (41.8%)	171 (37.4%)	
50 +	56 (9.8%)	4 (8.7%)	10 (14.9%)	42 (9.2%)	
Not stated	75 (13.2%)	0 (0.0%)	0 (0.0%)	75 (16.4%)	
Occupation					0.022
Civilian	152 (26.7%)	21 (45.7%)	17 (25.4%)	114 (24.9%)	
Military	94 (16.5%)	3 (6.5%)	15 (22.4%)	76 (16.7%)	
PTTGC Staff	324 (56.8%)	22 (47.8%)	35 (52.2%)	267 (58.4%)	
Smoking status					< 0.001
Non-smokers	48 (8.4%)	18 (39.1%)	29 (43.3%)	1 (0.2%)	
Active smokers	23 (4.1%)	13 (28.3%)	10 (14.9%)	0 (0.0%)	
Not available	499 (87.5%)	15 (32.6%)	28 (41.8%)	456 (99.8%)	

p-values were calculated by Fisher's exact test

Table 2 Comparison of haematological, renal, and hepatic indices between the baseline and final follow-up visit among 570 Rayong oil spill clean-up workers

Index	High exposure workers ($N=46$)			Low exposure workers ($N=67$)			Unknown exposure workers ($N=457$)		
	Baseline (2013)	Follow-up visit (2018)	p-value[b]	Baseline (2013)	Follow-up visit (2018)	p-value[b]	Baseline (2013)	Follow-up visit (2018)	p-value[b]
Hb (g/dL)	14.64 ± 0.55	14.15 ± 0.21	0.090	14.37 ± 0.37	14.15 ± 0.47	0.214	14.33 ± 0.11	14.37 ± 0.11	0.359
Hct (%)	44.70 ± 1.55	43.04 ± 1.73	0.045*	43.83 ± 1.11	43.29 ± 1.13	0.158	43.44 ± 0.32	43.62 ± 0.35	0.191
RBC (cells/μL)	5.37 ± 0.33	5.22 ± 0.23	0.026*	5.17 ± 0.23	5.26 ± 0.19	0.393	5.06 ± 0.08	5.15 ± 0.05	0.127
WBC ($\times 10^3$ cells/μL)	7.13 ± 0.54	7.11 ± 0.46	0.945	7.26 ± 0.43	6.97 ± 0.31	0.111	6.95 ± 0.16	7.01 ± 0.16	0.400
Platelets ($\times 10^3$ cells/μL)	227.20 ± 17.04	239.54 ± 13.91	0.041*	252.25 ± 13.98	256.49 ± 14.25	0.493	243.32 ± 5.08	255.16 ± 5.48	< 0.001
BUN (mg/dL)	11.60 ± 1.23	13.24 ± 0.90	< 0.001	12.85 ± 1.07	14.11 ± 1.029	< 0.001	12.08 ± 0.32	13.13 ± 0.32	< 0.001
Cr (mg/dL)	0.96 ± 0.05	1.00 ± 0.05	0.028*	0.91 ± 0.03	0.979 ± 0.04	< 0.001	0.93 ± 0.02	0.96 ± 0.02	< 0.001
AST (IU/L)	36.80 ± 5.11	30.02 ± 8.99	< 0.001	37.00 ± 3.95	30.22 ± 3.28	< 0.001	37.27 ± 3.22	28.87 ± 2.55	< 0.001
ALT (IU/L)	32.17 ± 4.10	29.70 ± 5.93	0.302	31.10 ± 4.05	31.44 ± 4.44	0.881	31.28 ± 1.82	29.30 ± 1.86	0.029*

*p-value < 0.05 [b]p-values were calculated by ANOVA for repeated measures

repeated measures was used to compare the changes in the haematological, renal, and hepatic indices by exposure level at baseline and final follow-up (Table 3). Then, generalised estimating equations (GEE) were used to determine the long-term trends of the 5-year repeated measurements for each haematological, renal, and hepatic index while adjusting for pre-selected factors, including age, gender, and occupational and smoking status (Table 4). Smoking status was determined using the urinary cotinine level of more than 50 ng/ml. Quasi-likelihood under the independence model criterion (QIC) was used to determine the correlation structure for each GEE model as independence or exchangeable (Cui 2007). The R statistical program (version 4.0.5) was used for all data analysis (R Core Team 2020). This study was approved by the Research Ethics Committee of Prince of Songkla University (Institutional Review Board [IRB] approval number psu.pn.1–001/64).

Results

Information on all 570 workers who reported for at least one follow-up visit was analysed in this study. Based on the exposure assessment from Ingviya et al. (2020), 46 (8.1%) workers were in the high exposure group, and 67 (11.8%) workers were in the low exposure group. There were 510 (89.5%) males and 60 (10.5%) females. The highest number was the staff of PTTGC (56.8%), 16.5% were from the military, and 26.7% were civilian volunteers. The largest number of the sample were aged 40–49 years (39.5%), while 37.5% were below 40 years. The distributions of demographic factors and exposure levels are shown in Table 1.

Table 2 shows the haematological, renal, and hepatic indices of each group at baseline and the fifth-year follow-up visit based on exposure levels. The average Hct from the baseline survey (44.70 ± 1.55) was significantly higher than the final follow-up (43.04 ± 1.73) visit only in the high exposure group. The average RBC count among high exposure group had reduced significantly at the final follow-visit (5.22 ± 0.23) compared to the baseline measurement (5.37 ± 0.33). The Cr levels increased significantly among all the clean-up workers between 2013 and 2018. The average levels of both Cr and BUN were significantly higher at final follow-up than at baseline for all exposure groups. There was a significant reduction of AST level from baseline to the last follow-up. The most pronounced reduction of 8.40 IU/L was among workers with unknown exposure. However, significant ALT differences between the baseline and final follow-up measures were observed only among unknown-exposure workers with lower ALT in the final follow-up visit. Platelets were significantly higher in the final follow-up visit for both the high exposure and unknown exposure groups.

Table 3 shows the changes in the haematological, renal, and hepatic profiles of the oil spill-exposed workers according to the level of exposure. Unknown-exposure workers had significantly lower Hct at the baseline than the high-exposure workers. Clean-up workers with high exposure had significantly higher RBCs than unknown-exposure workers at baseline. The average platelet count of low-exposure workers was 25.06×10^3 μL higher than that of high-exposure workers at baseline. At the 5th-year follow-up, there were no significant differences between all haematological, renal, and hepatic indices by exposure levels, except BUN. The BUN level among low-exposure workers was 0.98 mg/dL higher than BUN among unknown-exposure workers.

From the analysis with GEE, no significant trends of Hb and Hct were observed during the period from 2013 to 2018. However, a significantly increasing trend of 0.52 ± 0.03

Table 3 Comparison of haematological, renal, and hepatic indices between the high exposure, low exposure, and unknown study groups ($N = 570$)

Index	Exposure level	Baseline		Follow-up	
		Difference	*p*-value	Difference	*p*-value
Hb (g/dL)	Low exposure–high exposure	−0.27 ± 0.61	0.557	0.01 ± 0.64	0.999
	Unknown–high exposure	−0.31 ± 0.49	0.312	0.21 ± 0.520	0.598
	Unknown–low exposure	−0.04 ± 0.42	0.976	0.21 ± 0.44	0.489
Hct (%)	Low exposure–high exposure	− 0.86 ± 1.70	0.459	0.24 ± 1.85	0.948
	Unknown–high exposure	− 1.25 ± 1.38	0.022*	0.575 ± 1.49	0.637
	Unknown–low exposure	− 0.39 ± 1.16	0.709	0.33 ± 1.26	0.811
RBCs (cells/μL)	Low exposure–high exposure	−0.19 ± 0.39	0.470	0.05 ± 0.28	0.914
	Unknown–high exposure	−0.30 ± 0.31	0.027*	−0.06 ± 0.23	0.784
	Unknown–low exposure	−0.11 ± 0.27	0.670	−0.11 ± 0.19	0.353
WBC ($\times 10^3$cells/μL)	Low exposure–high exposure	0.12 ± 0.78	0.925	−0.15 ± 0.76	0.891
	Unknown–high exposure	−0.18 ± 0.63	0.774	−0.10 ± 0.61	0.920
	Unknown–low exposure	−0.31 ± 0.53	0.365	0.05 ± 0.52	0.976
Platelets ($\times 10^3$/μL)	Low exposure–high exposure	25.06 ± 25.08	0.039*	16.95 ± 26.36	0.287
	Unknown–high exposure	16.13 ± 20.26	0.148	15.61 ± 21.30	0.198
	Unknown–low exposure	−8.93 ± 17.13	0.439	−1.34 ± 18.01	0.983
BUN (mg/dL)	Low exposure–high exposure	1.25 ± 1.64	0.172	0.87 ± 1.60	0.408
	Unknown–high exposure	0.48 ± 1.28	0.648	− 0.11 ± 1.29	0.978
	Unknown–low exposure	−0.77 ± 1.17	0.270	−0.98 ± 1.09	0.029*
Cr (mg/dL)	Low exposure–high exposure	−0.05 ± 0.08	0.289	−0.02 ± 0.09	0.851
	Unknown–high exposure	−0.03 ± 0.06	0.443	−0.04 ± 0.07	0.424
	Unknown–low exposure	0.02 ± 0.05	0.443	−0.02 ± 0.06	0.772
AST (IU/L)	Low exposure–high exposure	0.20 ± 14.53	0.999	0.20 ± 12.02	0.999
	Unknown–high exposure	0.46 ± 11.73	0.995	−1.16 ± 9.71	0.958
	Unknown–low exposure	0.27 ± 9.92	0.998	−1.35 ± 8.21	0.920
ALT (IU/L)	Low exposure–high exposure	−1.07 ± 8.55	0.954	1.75 ± 8.99	0.891
	Unknown–high exposure	−0.90 ± 6.91	0.950	−0.40 ± 7.26	0.991
	Unknown–low exposure	0.17 ± 5.84	0.997	−2.15 ± 6.14	0.690

*p-value < 0.05

per year was observed for WBC count, while the average platelet count among the Rayong oil spill clean-up workers significantly increased at an average rate of 254.83×10^3/μL per year. Results from the GEE also showed that renal function indices significantly increased at 0.01 ± 0.00 mg/dL and 0.24 ± 0.03 per year for Cr and BUN, respectively. There were different trends for liver function indices AST and ALT, as the AST decreased at 1.54 ± 0.21 IU/L per year, while ALT remained stable through the study period. The results from the GEE analysis are summarised in Table 4.

During the final follow-up, the platelet counts among both the 46 high-exposure workers and the 457 unknown-exposure workers were also higher than baseline. Also, the low-exposure workers had a higher average platelet count than the high-exposure worker at baseline. However, the GEEs showed no difference between platelet counts by exposure. Occupation, gender, and age were associated with levels of haematological indices, including Hb level, Hct percentages, RBC count, and platelet count. However, there is no significant association between the trends of those indices with the factors. The clean-up workers with high exposure to the oil spill had significantly higher average WBC count than the low-exposure and unknown-exposure workers.

There were significant differences between the BUN levels of low-exposure, unknown-exposure, and high-exposure clean-up workers. Such differences in exposure levels found with GEE were not observed in the endpoint analysis with ANOVA. Changes in Cr were associated with occupation, gender, and age group, while BUN changes were associated with occupation, gender, and exposure level. Changes in liver function indices were significantly associated with gender, male workers having significantly higher levels than females. Although the t-test showed lower AST at the final follow-up year by exposure levels, such differences were not found by the GEE.

Table 4 Trends of haematological, renal, and hepatic indices from generalised estimating equations (GEE) ($N = 570$)

Index	Trends and mean differences from generalised estimating equations								
	Hb (g/dL)	Hct (%)	RBC (cells/μL)	WBC (x10^3μL)	Platelets (×10^3/μL)	BUN (mg/dL)	Cr (mg/dL)	AST (IU/L)	ALT (IU/L)
Average mean	12.44 ± 0.18*	38.20 ± 0.48*	4.83 ± 0.09*	2.40 ± 0.24*	254.83 ± 8.87*	8.23 ± 0.36*	0.65 ± 0.02*	24.89 ± 2.38*	20.74 ± 2.63*
Trend	0.001 ± 0.01	0.01 ± 0.02	0.004 ± 0.04	0.52 ± 0.03*	3.01 ± 0.31*	0.24 ± 0.03*	0.01 ± 0.00*	− 1.54 ± 0.21*	− 0.25 ± 0.16
Variable									
Exposure level	*Average trend for each category ± Standard error*								
High exposure	Ref								
Low exposure	− 0.18 ± 0.16	− 0.48 ± 0.45	− 0.08 ± 0.07	− 1.05 ± 0.21*	5.13 ± 7.09	0.96 ± 0.31*	− 0.01 ± 0.02	0.94 ± 2.01	1.06 ± 2.24
Unknown exposure	0.14 ± 0.14	0.34 ± 0.38	− 0.02 ± 0.07	− 1.62 ± 0.18*	8.99 ± 5.60	0.47 ± 0.27*	− 0.01 ± 0.01	0.93 ± 2.63	− 0.08 ± 2.38
Age									
20–29	Ref								
30–39	0.07 ± 0.11	0.30 ± 0.31	− 0.18 ± 0.06*	− 0.05 ± 0.17	2.59 ± 6.51	0.35 ± 0.28	0.01 ± 0.01	3.86 ± 2.16	3.07 ± 1.80
40–49	0.02 ± 0.11	0.05 ± 0.32	− 0.19 ± 0.06*	− 0.24 ± 0.16	0.06 ± 5.85	0.65 ± 0.25*	0.04 ± 0.01*	1.57 ± 0.98	0.91 ± 1.42
50 +	− 0.08 ± 0.16	− 0.43 ± 0.46	− 0.44 ± 0.07*	− 0.28 ± 0.23	− 17.40 ± 7.52*	0.93 ± 0.38*	0.05 ± 0.02*	6.18 ± 3.03*	4.04 ± 3.50
Unknown age	− 0.44 ± 0.19	− 1.42 ± 0.56	− 0.22 ± 0.10*	0.65 ± 0.29*	− 15.09 ± 9.25	0.33 ± 0.42	0.02 ± 0.02	1.63 ± 2.59	2.28 ± 3.00
Occupation									
Civilian	Ref								
Military	0.35 ± 0.18*	0.71 ± 0.53	0.07 ± 0.09	− 0.13 ± 0.24	17.75 ± 8.10*	− 0.78 ± 0.35*	0.03 ± 0.02	− 3.72 ± 2.26	− 4.77 ± 2.59
PTTGC	0.29 ± 0.10*	1.08 ± 0.28*	0.05 ± 0.06	− 1.14 ± 0.15*	7.76 ± 5.22	1.61 ± 0.23*	0.03 ± 0.01*	− 0.91 ± 2.28	− 4.77 ± 1.82*
Gender									
Female	Ref								
Male	1.95 ± 0.12*	5.63 ± 0.32*	0.61 ± 0.05*	0.76 ± 0.18*	− 30.22 ± 6.48	1.90 ± 0.26	0.28 ± 0.01*	10.84 ± 1.90*	13.88 ± 1.55*

*p-value < 0.001

Discussion

The Rayong oil spill of 2013 is the most recent and largest in Thailand. Over two thousand workers participated in various activities during the month-long clean-up (PTTGC 2013). These workers were exposed to different levels of hazardous chemicals in crude oil (Ingviya et al. 2020) and were at risk of adverse health effects. This study examined the haematological, renal, and hepatic indices among 869 workers who participated in the Rayong Oil spill clean-up activities over the 5 years after the oil spill. Haematological, renal, and hepatic indices are important indices for early screening of diseases (D'Andrea and Reddy 2018). This study suggests that exposure to the Rayong oil spill could induce significant haematological alterations. The results from this study showed that information from endpoint analysis using t-test and ANOVA could be different from using GEE to account for within-subject correlation.

Earlier studies have provided evidence of haematological alterations at three years (Choi et al. 2017; Doherty et al. 2017) and seven years (D'Andrea and Reddy 2018) after exposure to an oil spill. This study found significantly increasing trends of WBC count, platelet count, and Cr and BUN among all the clean-up workers five years after exposure to the Rayong oil spill. Higher average WBC counts were significantly associated with gender, occupation before the oil spill clean-up, and exposure level. Studies among VOC-exposed subjects in Korea and Canada have also reported elevated WBC counts associated with varying demographic factors (Cakmak et al. 2020; Choi et al. 2017). The significantly increasing average WBC counts found in this study represent the possibility of chronically elevated WBCs from oil spill exposure. Elevated WBC counts are considered a stress response to oil exposure; however, WBC counts are expected to return to pre-exposure counts in several months or years after the end of exposure (Lutcavage et al. 1995). Chronic WBC elevation is a risk factor for cardiovascular disease, stroke, and mortality related to cardiovascular disease, and impulsivity-related traits (Lee et al. 2001; Sutin et al. 2012). The higher average platelet count among Rayong oil spill clean-up workers is consistent with the results from Watson et al. (2021) among subjects exposed to VOCs. Increased platelet count among non-exposed subjects is considered an acute-phase response to infections, tumours, or inflammation (Jenne and Kubes 2015; Lippi and Franchini 2015; Vora and Lilleyman 1993).

However, the significantly increasing trend and higher platelet count found after 5 years may result from reduced platelet count as an acute haematologic response to oil spill exposure (Choi et al. 2017; D'Andrea and Reddy 2013, 2018; Ibrahim et al. 2014). Exposure to PAHs can result in the inflammatory response and oxidative stress found in studies (Choi and Kim 2021). The persistent elevated WBC and platelet are independent risk factors for developing cardiovascular diseases and diabetes mellitus (Twig et al. 2012, 2013).

The present study found significantly increased serum creatinine among the clean-up workers associated with age and gender. The GEE results found that older workers and men had higher Cr levels 5 years after participating in the oil spill clean-up. Studies from D'Andrea and Reddy (2016) and Al-Helaly and Ahmed (2014) have also documented increased creatinine levels among benzene-exposed subjects, and Cr concentrations are usually higher among the elderly and men (Tiao et al. 2002). However, subjects of the Hebei Spirit oil spill in Korea had decreased Cr levels after 3 years, while those of the BP oil spill had no change in the Cr levels after 7 years (Choi et al. 2017; D'Andrea and Reddy 2018). Such disparities could be attributed to the duration of exposure and the varying levels of heavy metals present in different types of crude oil. The average BUN level had an increasing trend five years following the oil spill clean-up among all the workers. These findings contradict the studies by Choi et al (2017) and D'Andrea and Reddy (2018), which reported that their subjects exposed to oil spills had decreased BUN levels after 3-year and 7-year follow-ups, respectively. The results indicate that the worsening of renal function as indicated by the increases in the levels of Cr might not be solely caused by older age but might be partially caused by exposure to certain components of the oil in the spill. The active metabolites of PAHs by activating cytochrome P450s were associated with kidney damage in animal models due to oxidative stress (Farzan et al. 2016). The possibility of causing chronic kidney disease of PAHs, including 2-naphthalene, was observed in large epidemiological studies, including the National Health and Nutrition Examination Survey (Rahman et al. 2021).

Although the results from this study indicate possible adverse health effects due to oil spill exposure, the study has some limitations. Firstly, the classification of exposure was solely based on the day of clean-up. Therefore, there might be some potential non-differentiate misclassification error of the exposure which can bias the results towards the null hypothesis. Nonetheless, significant trend of indices was still observable. Second, information, such as the duration (number of days or hours) of clean-up and personal protective equipment (PPE) usage, was unavailable for the analysis. However, Ingviya et al (2020) have shown that only a small proportion of the workers used complete protective equipment during the clean-up. In addition, PPE usage did not protect against acute effects of the Rayong oil spill due to the possibility of improper or inadequate use. Therefore, the confounding effect by not adjusting for PPE use should be minimal. Third, our GEE model accounted for age, gender, and smoking status. However, other factors, such as underlying health conditions, diet, and environmental monitoring data, which are not available for the analysis, could confound the results of this study. Finally, based on the baseline data, most Rayong oil spill clean-up workers were from Thai military (Ingviya et al. 2020). However, the follow-up protocol was voluntary and included all the clean-up workers based on their availability and proximity to the Rayong hospital. Therefore, there is the potential for volunteer bias, as many military personnel had been reassigned or retired from the military service and lost to follow-up. Nonetheless, this study is one of the few studies that analyse and assess oil spill workers' long follow-up data using rigorous statistical methods for longitudinal data adjusting for covariates.

Conclusion

In Conclusion, this longitudinal study evaluated haematologic, renal, and hepatic profiles of Rayong oil spill clean-up workers over the 5 years after the disaster. This study employed endpoint and longitudinal analyses to determine changes in haematological indices during the 5 years. The study results not only show the differences between baseline and 5 years haematological, renal, and hepatic indices but also examine the trends of these indices. Despite the study's limitations, long-term changes in haematologic profiles found in this study show the possibility of cardiovascular health complications and worsening renal functions after exposure to oil spills. The findings from this study contribute to `expanding knowledge on the long-term health effects of oil spills.

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Declarations

Conflict of interest The authors have no conflicts of interest to declare that are relevant to the content of this article.

Ethics approval This study was approved by the Research Ethics Committee of Prince of Songkla University (Institutional Review Board [IRB] approval number psu.pn.1–001/64).

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Appendix III

Manuscript II

Association between urinary metabolites of polycyclic aromatic hydrocarbons and volatile organic compounds and long-term changes in haematological, hepatic, and renal profiles: a longitudinal analysis

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Declarations

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Conflicts of interest

The authors have no conflicts of interest to declare relevant to this article's content.

Availability of data and material

The data, codes, and materials generated and analysed in this published article are available from the corresponding author upon reasonable request.

Ethics approval

The clean-up workers verbally consented regarding the use of their data for further analysis to improve the quality of health services and follow-up protocols. The Research Ethics Committee of Prince of Songkla University approved this study (Institutional Review Board [IRB] approval number psu.pn.1-001/64).

Abstract

Purpose

Oil spills release polycyclic aromatic hydrocarbons (PAHs) and volatile organic compounds (VOCs) into ecosystems. Despite known carcinogenic effects, the long-term effects of PAHs and VOCs on haematological, hepatic, and renal functions remain controversial.

Methods

We investigated the effects of crude-oil-based PAHs and VOCs on the human haematopoietic, hepatic, and renal systems by evaluating the association between the urinary metabolites of PAHs and VOCs [i.e., 1-hydroxypyrene-glucuronide (1-OHPG) and trans, trans-muconic acid (t,t-MA)] and haematological, hepatic, and renal function indices in 169 workers of the 2013 Rayong oil-spill clean-up. Latent class trajectory analyses and generalised linear mixed models were used for the analyses after adjusting for demographics and smoking status.

Results

The mean age was 42 years, and 94.7% of participants were male; 10% and 57.4% of the clean-up workers had high and low concentrations of 1-OHPG, respectively. In most urine samples (71.6%), t,t-MA was not detected. Higher 1-OHPG concentrations were associated with significantly reducing trends of haemoglobin (−0.39 ± 0.12 g/dL) and haematocrit (−1.05 ± 0.29%). The t,t-MA concentration was associated with significant reductions in the haemoglobin and haematocrit levels (trend: −0.12 ± 0.04 g/dL and −0.38 ± 0.12%, respectively; both $p<0.05$). The average blood urea nitrogen level increased significantly among all clean-up workers but more profoundly among workers with high 1-OHPG and detectable t,t-MA concentrations (trend: 0.58 ± 0.12 and 0.36 ± 0.12 mg/dL, respectively; both $p<0.05$).

Conclusion

This study demonstrated postexposure changes in the haematopoietic, renal, and hepatic profiles, indicating long-term health complications and worsening renal function following oil-spill exposure.

Keywords: Polycyclic aromatic hydrocarbons, haematological profile, kidney function, liver function, volatile organic compounds.

Introduction

Polycyclic aromatic hydrocarbons (PAHs) and volatile organic compounds (VOCs) from crude oil have continued to be released into the marine, and terrestrial ecosystems from oil spill for decades after the invention of liquid propellant (Beyer et al. 2010; de Hoop et al. 2011). For most oil spills, local citizens voluntarily participate in the clean-up activities and thus get exposed to various levels of toxicants. PAHs and VOCs in crude oil, such as benzo[a]pyrene, are human carcinogens classified as Group I by the International Agency for Research on Cancer (Baan et al. 2009; IARC 1988). Intake of these compounds, primarily by inhalation, may induce severe adverse health effects, including alterations in the haematological, hepatic, and renal functions (Choi et al. 2017; D'Andrea and Reddy 2018).

The quantification of urinary metabolites of PAH and VOCs to estimate the exposure levels has been previously investigated. Especially, the levels of 1-hydroxypyrene-glucuronide (1-OHPG) and trans, trans-muconic acid (t,t-MA) have been widely used to assess the environmental exposure to PAHs (Jongeneelen 2001; Kamal et al. 2015; Zhang et al. 2014). and VOCs (e.g., benzene), respectively (Aprea et al. 2008; Bechtold et al. 1991; Sithisarankul and Intawong 2015; Wiwanitkit et al. 2001). The levels of urine metabolites represent quantifiable levels of exposure from multiple routes and sources – for instance, from both cigarette smoke and oil spills (Jain 2015; Strickland et al. 1996). In the human, complete blood count (CBC) and serum levels of renal and liver biomarkers are popular biomarkers for assessing the effects of PAH and VOC exposure, and several studies have used these haematological, hepatic, and renal parameters to assess the effects of PAH and VOC exposure on human health (Abou-ElWafa et al. 2015; D'Andrea and Reddy 2018; Kamal et al. 2016; Samadi et al. 2019). However, the effects on the parameters vary by the level and duration of exposure as well as by the influence of other environmental factors.

The Rayong oil spill of 2013 was caused by a ruptured pipeline whereby more than 50,000L of crude oil spilled into the Gulf of Thailand and, until January 2022, this was the most recent marine disaster in Thailand. More than 2,000 workers with various backgrounds participated in the onshore and offshore clean-up activities (PTTGC 2013). An observational baseline study by Ingviya et al. (2020) assessed the urinary 1-OHPG and t,t-MA concentrations to determine the internal exposure to PAHs and VOCs among the clean-up workers of the Rayong oil spill on different clean-up days. The study showed that workers who participated during the initial days of the clean-up had significantly higher levels of 1-OHPG, whilst the t,t-MA concentration was undetectable amongst the majority of clean-up workers throughout the

clean-up period (Ingviya et al. 2020). Similarly, in another observational study, Owusu et al. (2022) assessed the 5-year changes in the haematological, hepatic, and renal indices among the Rayong oil spill clean-up workers and differentiated the exposure levels based on their work on different clean-up days. We hypothesised that different concentrations of urinary metabolites induce different non-linear trends of the haematological, hepatic, and renal profiles. Therefore, assessing these trends can guide and encourage the implementation of health follow-up protocols for workers who clean up oil spills.

This study aimed to examine the long-term effects of the toxicants (PAHs and VOCs) by assessing the changes in the haematological, hepatic, and renal indices measured at baseline and during a 5-year follow-up period after the 2013 Rayong oil spill. Primarily, we examined the longitudinal changes of these indices by levels of exposure determined using urinary concentration of the toxicants' metabolites. Secondly, we classified the patterns of changes of these indices by assessing the latent trends of haematological, hepatic, and renal indices among the oil spill clean-up workers.

Data and Methods

Study design and setting

In this retrospective longitudinal cohort study, we analysed the haematological indices, including CBC and liver and kidney function parameters, that were measured at baseline and during a 5-year follow-up period after the 2013 Rayong oil spill. We used the exposure data from a cohort of the Rayong oil spill clean-up workers, and the urinary concentrations of 1-OHPG and t,t-MA that were quantified by Ingviya et al. (2020) as an exposure assessment at baseline. The data from the oil spill clean-up workers was originally collected by the Rayong hospital as part of health services.

Ethical approval

The clean-up workers verbally consented regarding the use of their data for further analysis to improve the quality of health services and follow-up protocols. The Research Ethics Committee of Prince of Songkla University approved this study (Institutional Review Board [IRB] approval number psu.pn.1-001/64).

Study population and measurements

During and after the Rayong oil spill clean-up activities, the Rayong Provincial Health Office collaborated with Rayong Hospital to undertake 5-year health surveillance of the clean-up workers. The workers were invited to undergo annual health assessments, including laboratory tests for CBC and liver and renal function parameters, after the oil spill. The analysis dataset included 169 oil-spill clean-up workers who had provided urine samples for the measurement of the concentrations of urinary 1-OHPG and t,t-MA during the clean-up and had attended at least one of the annual health follow-ups. Information on the workers' demographics, including age, sex, and background occupation, clean-up activities, and haematological, hepatic, and renal parameters at baseline and follow-up were retrieved from the electronic records of the Rayong Provincial Health office and Rayong Hospital. A urinary cotinine level cut-off of 50 ng/mL was used to differentiate between non-smokers and passive or active smokers, according to the method described by Zielinska-Danch et al. (2007)

Quantification of 1-OHPG and t,t-MA concentrations

The concentrations of 1-OHPG and t,t-MA were quantified in the urine samples that were collected during the clean-up in 2013. Immunoaffinity chromatography and synchronous fluorescence spectroscopy were used to measure the 1-OHPG concentration at Paul Strickland laboratory in John Hopkins University, (limit of detection 0.04 pmol/mL; coefficient of variation 5.6%) (Ingviya et al. 2020). The baseline 1-OHPG concentration was categorised as high (>5.0 pmol/mL), moderate (1.0–5.0 pmol/mL), or low (<1.0 pmol/mL), based on a study by Kang et al. (1995). The urinary t,t-MA concentration was analysed using high-performance liquid chromatography with fluorescence detection in three laboratories, including the Rayong Hospital laboratory and two other private laboratories. The agreement among the t,t-MA measurements reported the three laboratories was 99.99% (Intawong et al. 2015). The t,t-MA concentration was classified as detectable or undetectable because a large proportion of the samples had levels below the limit of detection (0.01 mg/dL) of the assay.

Measurement of haematological, hepatic, and renal indices

Blood samples from the 169 oil-spill clean-up workers were analysed using approved medical protocols in standardised laboratory in the Royal Hospital. The CBC parameters, including haemoglobin (Hb), haematocrit level (Hct), red blood cell count (RBC), white blood cell count (WBC), absolute neutrophil count (ANC), platelet count, mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), mean

corpuscular volume (MCV), polymorphonuclear neutrophil count (PMN), and lymphocyte count (LYM), were measured using an automatic CBC analyser, and hepatic [serum aspartate transaminase (AST) and alanine transaminase (ALT)] and renal indices [blood urea nitrogen (BUN) and serum creatinine (Cr)] were determined using an enzymatic assay.

Statistical analysis

Descriptive statistics (mean, standard deviation, and percentages) were used to describe the demographic of the oil-spill clean-up workers, and to summarise the haematological and hepatic profiles. Latent class trajectory analyses were used to classify the latent groups based on the level of the indices at baseline and the changes over time (Dayimu et al. 2019; Farooqui et al. 2020; Randall et al. 2019). Polynomial specifications as functions of time, with different numbers of latent classes ranging from 2 to 5, were used to assess the different trends. The observed means of the haematological and hepatorenal indices were labelled based on their concentrations at baseline (high, low, and normal) and their trends during the study (stable, increasing, and decreasing). The definitions of high, low, and normal concentrations were based on the standard medical reference ranges for each haematological, hepatic, and renal index (Kasper et al. 2015).

The ***"lcmm"*** package in ***R*** (Proust-Lima et al. 2021) was used for the latent class trajectory analysis. The optimal numbers of latent classes for each model were determined using the Bayesian information criterion and posterior probabilities above the 0.7 threshold (Proust-Lima et al. 2017). Sex, age at the time of oil spill clean-up, and smoking status were adjusted as covariates for assessing the adjusted trends of each profile based on the 1-OHPG concentration. Next, the effects of baseline urinary concentrations of 1-OHPG and t,t-MA on the overall trajectories and trends of haematological and hepatorenal profiles were investigated by using age-centred generalised linear mixed models. Sensitivity analysis was conducted with a subgroup of non-smokers to examine the robustness of the study's results for understanding the effects of PAH and VOC exposure in the cohort and to assess the exact effects of smoking on the parameters that were evaluated in the study (Thabane et al. 2013). All statistical analyses and graphical displays were undertaken with ***R*** version 4.0.5 (R Core Team, 2020).

Results

Demographics of the oil-spill clean-up workers

During the clean-up, 2,118 workers were engaged in various activities. The demographics of these workers have been reported previously (Ingviya et al. 2020;

Sithisarankul and Intawong 2015). During the 5-year follow-up surveillance, 869 clean-up workers reported to the Rayong Hospital at least once. However, only 169 of the workers had provided urine samples for assessing the levels of 1-OHPG and t,t-MA at the baseline, wherein the workers were actively participating in the clean-up. The demographics of the 169 workers are summarised in Table 1. The mean (±SD) age of the clean-up workers at baseline was 39.72 ± 9.91 years, and 94.10% of the study population comprised male participants. Civilian volunteers accounted for 44.40% of all clean-up workers. Based on the cotinine levels, 109 (64.50%) workers were non-smokers (<50 ng/mL). At baseline, 17 (10.10%) clean-up workers had high levels of 1-OHPG (>5.0 pmol/mol), 55 (32.50%) had moderate levels (1.00–5.00 pmol/ml), and 97 (57.40%) had low levels (<1.0 pmol/mol).

Table 1 Description of the participants' demographics

Demographic factor	Description	Number of workers	Percentage
Sex	Men	160	94.70%
	Women	9	5.30%
Age group at baseline (years)	20–29	22	13.00%
	30–39	40	23.90%
	40–49	73	43.10%
	50+	34	20.00%
Background occupation	PTTGC staff	29	17.20%
	Civilian	75	44.40%
	Military	65	38.40%
Cotinine level (ng/mL)	Median (1st–3rd quartiles)	7.51 (3.11–1040.79)	
Non-smokers	Cotinine <50 ng/mL	109	64.50%
Smokers	Cotinine >50 ng/mL	60	35.50%
1-OHPG (pmol/mL)	Median (1st–3rd quartiles)	0.76 (0.31–2.27)	
High	1-OHPG >5.0 pmol/mL	17	10.10%
Low	1-OHPG <1.0 pmol/mL	97	57.40%
Moderate	1.0–5.0 pmol/mL	55	32.50%
t,t-MA	Median (1st–3rd quartiles)	0. 00 (0.00–36.40)	
	Detectable	48	28.40%
	Undetectable	121	71.60%

Latent trends

The trajectory analysis showed that each index had at least two latent trends. Based on the intercept and slope, two distinct trends (high-decreasing and normal-stable) were identified for the Hb level among the oil-spill clean-up workers. Four (2.37%) clean-up workers had a high-decreasing Hb trend. There were three distinct trends for the Hct: low-stable (3.55%) with no significant trend, normal-decreasing (1.18%) with a significant trend of −4.92% per year,

and normal-stable (95.30%). The WBC count of 5.92% of the clean-up workers demonstrated a high-decreasing trend (average trend for the high-decreasing WBC group, -0.47×10^3/year). Of the 169 oil-spill clean-up workers, 8.88% showed a normal-decreasing trend for ANC, whereas 2.96% had a normal increasing average trend. The latent trends for BUN and Cr were two and four, respectively. A normal-increasing BUN trend was identified in 87.40% of the workers. The latent trends identified for Cr were low-stable (5.92%), high-stable (1.18%), normal-stable (92.31%), and high-decreasing (0.59%). The latent trends for all haematological and hepatic indices are shown in Figure 1.

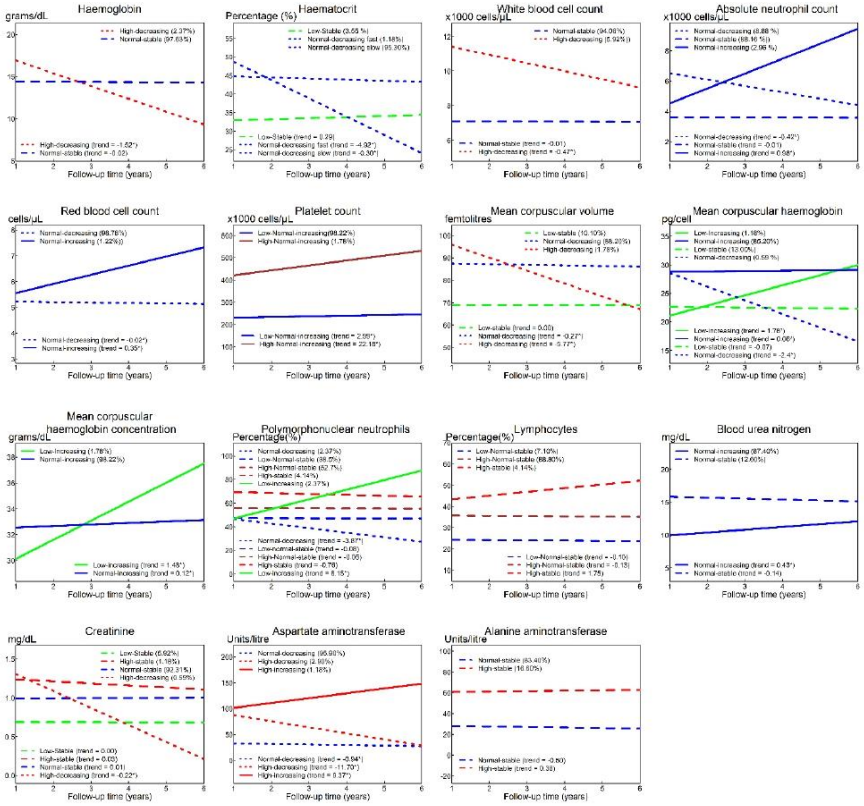

Figure 1 Latent trends of the haematological and hepatic indices

Longitudinal trajectories according to the urinary 1-OHPG concentration

Most of the haematological indices showed varying trajectories. With a sigmoid pattern, the Hb trajectory among workers with high urinary 1-OHPG concentrations decreased over time, whereas the Hct exhibited a linear decreasing trajectory. Workers with low 1-OHPG concentrations had a stable trajectory of Hb and Hct over time. The trajectories of WBC, ANC, and platelet count were almost linearly stable during the 5 years. However, the RBC count of the oil-spill clean-up workers with low 1-OHPG concentrations demonstrated a hormesis trend, which was characterised by a decrease within the first 2 years and an increase after the third year. The trajectories of BUN and Cr generally remained stable, though BUN demonstrated an upward trajectory among all the workers. The serum liver enzymes AST and ALT showed linear trajectories during the study period. Figure 2 depicts the trajectories of all indices based on the urinary 1-OHPG concentrations.

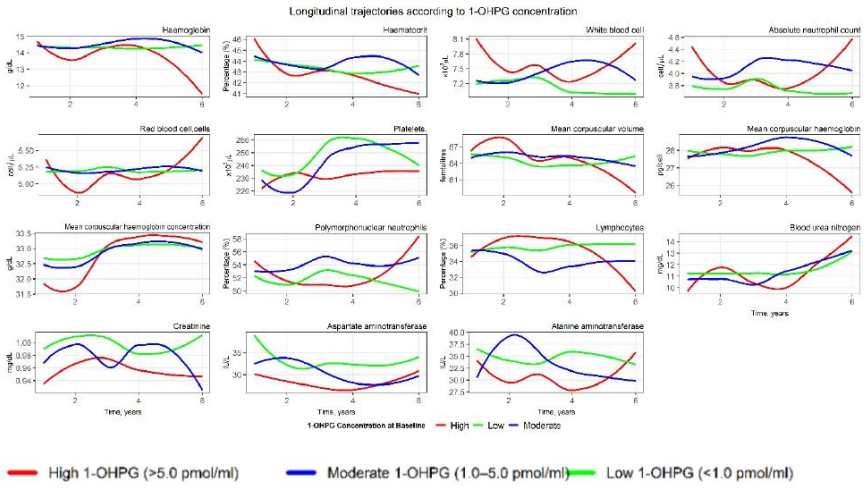

Figure 2 Longitudinal trajectories of the haematological, hepatic, and renal indices by the baseline concentration of 1-hydroxypyrene-glucuronide (1-OHPG)

Longitudinal trends according to the urinary 1-OHPG concentration

The trends determined from the results of mixed-effects models showed that the oil-spill clean-up workers with high 1-OHPG concentrations showed higher Hb levels at baseline, followed by a rapid, significant decline (rate 0.42 ± 0.12 g/dL per year) in the 5 years after

exposure to the oil spill. Trends of Hb among workers with low-to-moderate levels of 1-OHPG were relatively stable. At baseline, the Hct was 47.71% and 44.28% among workers with high and moderate concentrations of 1-OHPG, respectively. The Hct percentage was 44.95% among workers with a low 1-OHPG concentration. In the high 1-OHPG concentration group, the Hct reduced significantly (average rate 1.14 ± 0.28% per year). A high 1-OHPG concentration during the clean-up was associated with higher WBC levels at baseline. However, no significant trends were found for WBC among the workers during the follow-up study. At baseline, the average RBC count was 5.71 × 10^3/μL among workers with elevated levels of 1-OHPG and remained nearly unchanged during the study; thus, no significant trends over time were observed. The trend of platelet count was the highest among workers with a moderate 1-OHPG concentration (average trend 4.52 ± 1.38 ×10^3/year), whereas the average trend among workers with a low 1-OHPG concentration was 2.60 ± 1.29 ×10^3/year.

In the follow-up period, the average BUN level increased significantly among all workers, but more profoundly among workers with a high 1-OHPG concentration (0.58 ± 0.19 mg/dL). Creatinine levels demonstrated no significant trends among all oil-spill clean-up workers. The levels of AST in workers with moderate and low concentrations of 1-OHPG showed a reducing trend during the study period. The age-adjusted longitudinal trends of all haematological, renal, and hepatic indices according to the 1-OHPG concentration are shown in Figure 3.

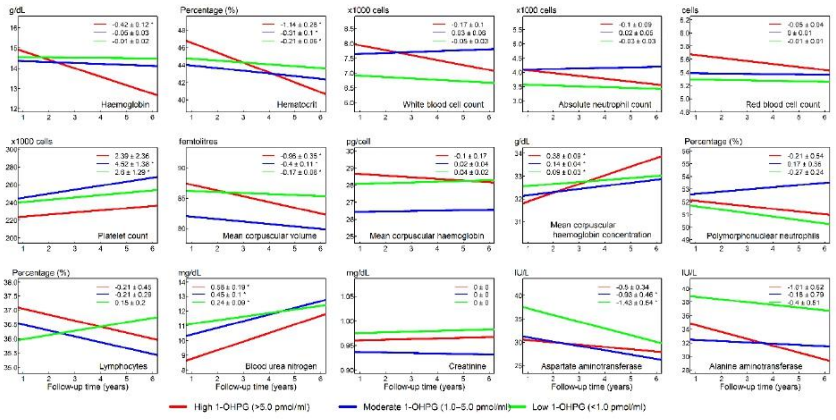

Fig 3 Age-adjusted longitudinal trends of the haematological, hepatic, and renal indices by the baseline concentration of 1-hydroxypyrene-glucuronide (1-OHPG)

Longitudinal trajectories according to the urinary t,t-MA concentration

In samples from the majority of the clean-up workers, t,t-MA was not detected. The longitudinal trajectories among workers with detectable and undetectable concentrations of t,t-MA differed for most of the indices. The Hct percentage for a detectable t,t-MA concentration had a U-shaped trajectory, and decreased gradually until the fourth follow-up and then increased. The WBC count among all the clean-up workers was stable until the third follow-up, with a higher WBC count in the undetectable group than in the detectable group. After the third follow-up, the WBC count in workers with detectable t,t-MA concentrations increased, whereas that in workers without a detectable t,t-MA concentration decreased. The longitudinal trajectories of the platelet count, MCV, MCH, and MCHC were similar for both the t,t-MA groups. The MCV and MCH maintained a relatively stable trajectory among all the clean-up workers, irrespective of the urinary t,t-MA concentration. The BUN level was stable during the first 4 visits and assumed an increasing trajectory after the fourth follow-up. The longitudinal trajectory of the Cr level was shaped like a sinewave, which increased at the first follow-up visit, and then decreased until the fifth follow-up. The longitudinal trajectories of the haematological, hepatic, and renal indices according to the baseline t,t-MA concentrations are shown in Figure 4.

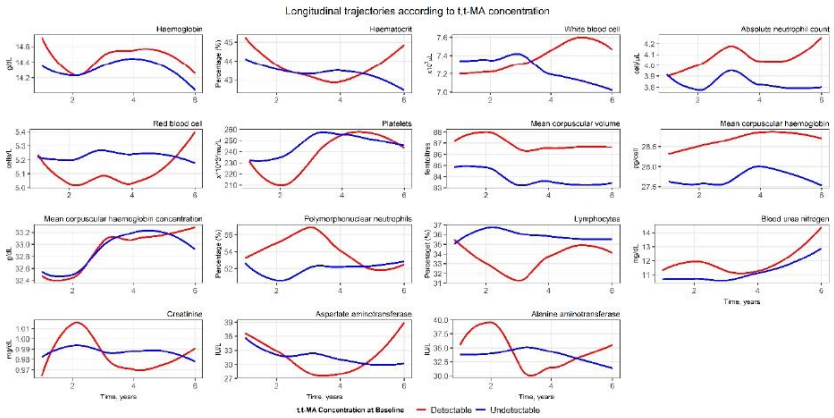

Fig 4 Longitudinal trajectories of the haematological, hepatic, and renal indices by the baseline concentration of trans, trans-muconic acid (t,t-MA)

Longitudinal trends according to the urinary t,t-MA concentration

The results from the mixed-effects model indicated that the average Hb level was the same at the baseline for workers with detectable and undetectable t,t-MA concentrations. Throughout the follow-up, clean-up workers in the t,t-MA detectable group showed a decreasing trend for the Hb level (0.12 ± 0.04 g/dL per year). At the baseline, the percentage of Hct was higher in clean-up workers with higher t,t-MA concentrations. Over time, decreasing trends of Hct percentage were observed in workers with detectable concentrations of t,t-MA (0.40 ± 0.12% per year) as well as in the group without detectable t,t-MA concentrations (0.31 ± 0.08% per year). During the first 3 years after the oil-spill clean-up, the AST level was consistently higher among workers with undetectable t,t-MA compared with that in workers with detectable t,t-MA levels. The longitudinal trends of all haematological, renal, and hepatic indices according to the t,t-MA concentration are shown in Figure 5.

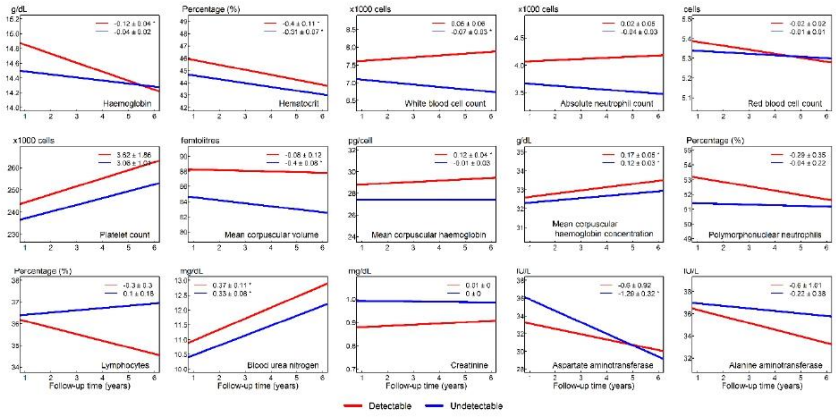

Fig 5 Age-adjusted longitudinal trends of the haematological, hepatic, and renal indices by the baseline concentration of trans, trans-muconic acid (t,t-MA)

Discussion

By using urinary concentrations of 1-OHPG and t,t-MA as biomarkers of exposure to PAHs and VOCs, respectively, this longitudinal study identified different latent trends for haematological, hepatic, and renal profiles of 169 Rayong oil-spill clean-up workers. Our findings indicate possible long-term haematopoietic function alterations occurred among the oil-spill clean-up workers. Two latent classes with different trends of the Hb level were observed. The average Hb levels at baseline in the high-decreasing class decreased significantly, and, at the final follow-up, the Hb level in the high-decreasing latent class had decreased to anaemic levels (<10 g/dL) (Tefferi 2003). The longitudinal trajectories and trends indicated that high urinary concentrations of 1-OHPG and t,t-MA at baseline were associated with reduced Hb levels over time. Similarly, Kponee et al. (2015) and McLoone et al. (2021) found that PAHs induced haemolytic anaemia in humans and other organisms. Compared with undetectable t,t-MA levels at baseline, a detectable urinary t,t-MA concentration at baseline was associated with higher Hb levels over time. An initial increase in the Hb levels has been reported among a Canadian sub-population exposed to VOCs (Cakmak et al. 2020). Consistently, the Hct level exhibited a decreasing trend among all clean-up workers. The Hct levels decreased with increasing 1-OHPG concentration. The concentration of t,t-MA was also associated with significant reductions in the Hct level. Similarly, Kamal et al. (2016) and D'Andrea and Reddy (2018) found significant reductions in the Hct among subjects exposed to PAHs from combustion emission in Pakistan and subjects exposed to the Gulf oil spill.

Our study found distinct latent trends for the WBC, RBC, and platelet counts. In most clean-up workers, the WBC count was normal at baseline and remained stable over time. Significant positive trends in the platelet count were found for both low and moderate concentrations of 1-OHPG as well as for those without detectable t,t-MA levels. A negative association between an undetectable concentration of urinary t,t-MA and WBC count was observed. However, there was no significant association between ANC and RBC count trends and the concentrations of 1-OHPG and t,t-MA. Following exposure and xenobiotic metabolism, benzene metabolites, including tt-muconaldehyde, 1,4-benzoquinone, hydroquinone, and catechol, can suppress bone marrow (Ross 2000; Shahsavani et al. 2021; Wang et al. 2012). Such haematopoietic damage can manifest in many ways, including alterations in the haematologic indices. Therefore, the alterations in haematological functions identified in this study may be attributable to the suppression of bone marrow function by the effects of PAH and VOC.

The present study observed two and four latent trends for BUN and Cr, respectively. The majority of the oil-spill clean-up workers had normal BUN at baseline, followed by significantly increasing latent trends during the follow-up. Conversely, the Cr level was normal and remained stable among most clean-up workers. Longitudinal trajectories of BUN showed an upward trajectory after the third follow-up visit. A consistent increase in the BUN levels with a significant trend suggests that exposure to the oil spill caused elevated BUN levels, which is consistent with the results of other studies (Choi et al. 2017; D'Andrea and Reddy 2018). A higher 1-OHPG concentration correlated with higher longitudinal trends of BUN over time, whereas detectable and undetectable concentrations of t,t-MA correlated with increasing BUN trends at the same rate. A study by Li et al. (2020) documented that higher exposure to PAHs significantly increased the BUN. Among the oil-spill clean-up workers, the significantly increasing trends indicate the possibility that BUN levels increased beyond the normal levels over time. Metabolites of PAHs, such as 1-OHPG, can cause oxidative stress and induce kidney damage, as reported by Palackal et al. (2002) and Sun et al. (2021). However, only an increase in the BUN level without changes in Cr may not suffice to support the renal adverse effects of exposure to PAHs and VOCs.

There were three and four latent trends for AST and ALT, respectively. Most workers had normal-decreasing latent trends, whereas the remaining had high AST at baseline, followed by increasing or decreasing trends. The longitudinal trajectories of AST showed a downward trend within the first 4 years after the clean-up and a slight upward trend in the fifth year. These trajectories of AST were consistent for all levels of urinary 1-OHPG and t,t-MA. Higher long-term decreasing trends were found in workers with low and moderate concentrations of 1-OHPG and workers with undetectable t,t-MA. Levels of AST and ALT are reflections of hepatocellular injury and are highly sensitive to PAH exposure (Wu et al. 1997). Though studies have reported possible liver damage from PAH exposure, the levels of PAHs or detectable levels of t,t-MA in our study were not associated with liver damage that would manifest as a high-decreasing trend. Other environmental toxicants or factors for which data were unavailable might be related to the damage.

Smoking is a known risk factor for changes in the cardiovascular, haematopoietic, renal, and other organ systems (Bullen 2008). After excluding smoking, the subgroup analysis in 109 non-smokers was performed to assess the haematological, renal, and hepatic alterations due to PAHs and VOCs. The observed effects among the non-smokers were similar to those found in the overall study group (Supplementary Figure 1 and Figure 2). The magnitude of the baseline

biomarkers was slightly smaller. However, the direction of the changes in the biomarkers remained the same. These analyses further supported the possible impact of PAHs and VOCs on the haematological, renal, and hepatic systems.

Strength and limitations of the study

This study found possible alterations in the haematopoietic system among oil spill clean-up workers. The findings of this study should be interpreted with credence to the following limitations. Firstly, most clean-up workers who attended the follow-up visits did not provide urinary samples at the baseline. Therefore, less than 10% of the total number of oil-spill clean-up workers were included in this study. Secondly, the urinalysis could not quantify very low levels of t,t-MA (<0.01 mg/dL). Thus, the t,t-MA concentration in many clean-up workers was undetectable.

Conclusion

More than 5 years after the oil-spill clean-up, the alterations in the haematological system from the exposure to PAHs and VOCs were still observable among the oil-spill workers. Therefore, oil-spill clean-up activities should be classified as potentially dangerous jobs exposing workers to carcinogens and haematotoxins, which can induce haematotoxicity. Health monitoring of workers should be planned before they participate in oil-spill clean-ups, and assessments should be performed at pre-placement and then periodically for years after the clean-up.

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Appendix IV

Conference

Adverse Health Effects of Oil Spill Exposure on First Responders of Rayong Oil Spill

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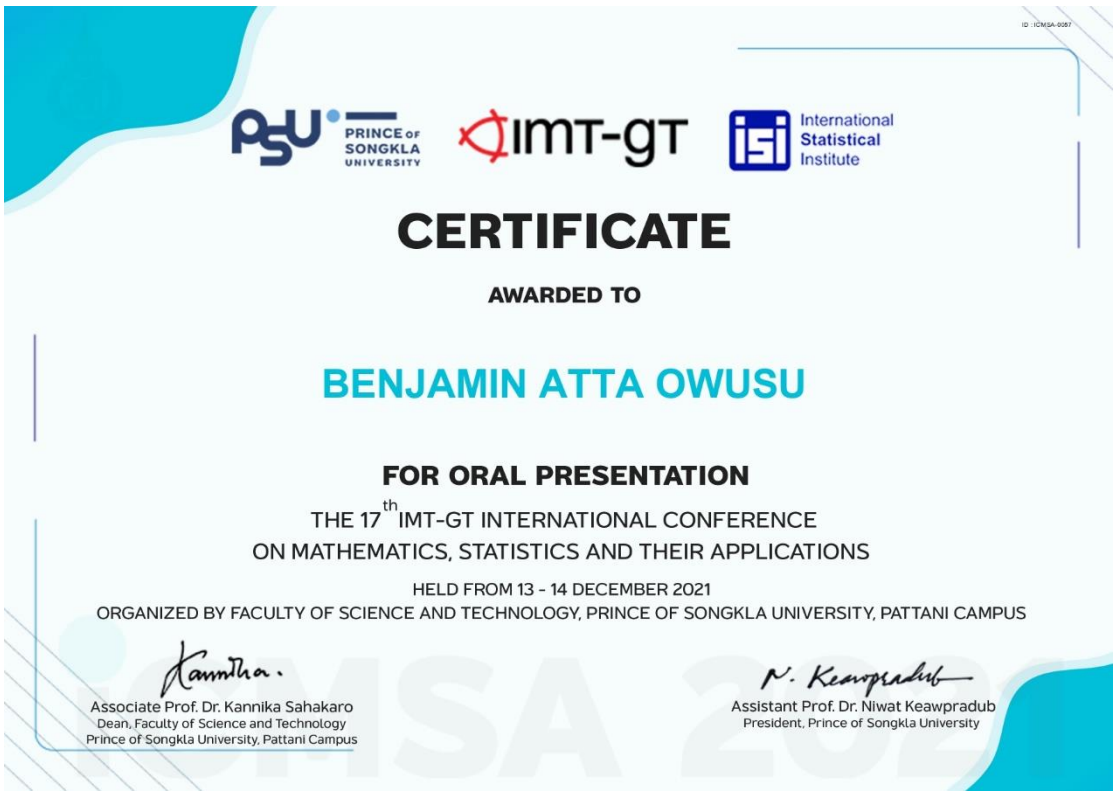
Abstract: Workers who participated in the Rayong oil spill clean-up within the first 72 hours showed elevated levels of urinary 1-hydroxypyreneglucuronide at the time of clean-up. This study aimed to assess the long-term health effects among the first responders, five years after the oil spill clean-up. Data from 75 oil spill first responders who participated in the oil spill clean-up with the first 48 hours and attended at least one follow-up visit from 2014-2018 are included in this study. Blood components on haematological and hepatic indices were measured at each follow-up visit. The trends of the haematological and hepatic parameters were assessed over time and adjusted using exposure conditions during the oil spill clean-up. Among the 75 subjects, 37 were civilian volunteers, while the rest were personnel from the Thai military. 24 subjects (32%) reported at least one acute health symptom during the clean-up. The analyses showed a significantly decreasing trend of haematocrit levels (0.5 % per year) and significantly increasing trend of blood urea nitrogen levels (0.505 mg/dL per year). Also, the platelet count had increasing trend of 4.16 ($\times 10^3/\mu L$). This longitudinal study showed that the first respondents of the Rayong oil spill incident experienced significant haematological changes 5 years after exposure to the spill.

Keywords: hepatotoxicity, oil spill, longitudinal analysis



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