



UNIVERSITI PUTRA MALAYSIA

***CONTAMINATION OF ISOPROTHIOLANE, PROPICONAZOLE AND
PRETILACHLOR IN PADDY SOIL AND POTENTIAL DERMAL
HEALTH RISK TO PADDY FARMERS IN TANJUNG KARANG, KUALA
SELANGOR***

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FPSK4 2018 3**

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PRETILACHLOR IN PADDY SOIL AND POTENTIAL DERMAL HEALTH
RISK TO PADDY FARMERS IN TANJUNG KARANG, KUALA SELANGOR**



BY

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**Thesis submitted in fulfilment of the requirement for the degree of Bachelor
Science (Environmental and Occupational Health) from the Faculty of Medicine
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ABSTRACT

CONTAMINATION OF ISOPROTHIOLANE, PROPICONAZOLE AND PRETILACHLOR IN PADDY SOIL AND POTENTIAL DERMAL HEALTH RISK TO PADDY FARMERS IN TANJUNG KARANG, KUALA SELANGOR

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Introduction: Tanjung Karang, Kuala Selangor is the third largest paddy field in Peninsular Malaysia. The pesticide can give a positive impact on destroying pest in agricultural activities but it can also contribute to the adverse environmental and health consequences. Besides, the paddy farmers in Tanjung Karang, Kuala Selangor are not using proper personal protective equipment (PPE) when working in paddy field. Therefore, this would increase their exposure to the pesticides, particularly through dermal exposure. **Objective:** To determine the concentration of isoprothiolane, propiconazole and pretilachlor in paddy soil and their potential dermal health risk among paddy farmers at Tanjung Karang, Kuala Selangor. **Methodology:** This study was conducted at Kampung Sawah Sempadan, Tanjung Karang, Kuala Selangor which consists of 24 paddy blocks (Block A to Block X). Questionnaires were distributed among 152 paddy farmers to obtain the information regarding the socio-demographic, the personal hygiene and usage of personal protective equipment and their exposure to the pesticides. 72 soil samples were randomly collected from 24 paddy blocks. The samples were extracted using dispersive solid phase extraction (dSPE) and analysed by ultra performance liquid chromatography – tandem mass spectrometry (UPLC-MS/MS). Dermal health risk assessment of the paddy farmers was calculated using Hazard Quotient (HQ). **Result and discussion:** The findings showed that the concentration of isoprothiolane, propiconazole and pretilachlor in paddy soil samples were ranged from 0.546 mg kg⁻¹ to 42.917 mg kg⁻¹, 0.773 mg kg⁻¹ to 25.502 mg kg⁻¹ and 0.435 mg kg⁻¹ to 9.942 mg kg⁻¹, respectively. The HQ calculated for propiconazole was less than one for all of the paddy blocks. There were significant correlations between (i) pretilachlor and C/N ratio ($r = -0.664$ $p = 0.007$) and between (ii) pretilachlor and total organic carbon (TOC) ($r = -0.677$, $p = 0.006$). **Conclusion:** There was no significant non-carcinogenic dermal health risks due to occupational exposure of propiconazole in paddy soil ($HQ < 1$) for paddy farmers that worked in Block A to Block X.

Keywords: Pesticides contamination, hazard quotient (HQ), dermal health risk assessment, ultra performance liquid chromatography – tandem mass spectrometry (UPLC-MS/MS), dispersive solid phase extraction (dSPE)

ABSTRAK

KESAN PENDEDAHAN KEPADA TANAH PADI YANG DICEMARI OLEH ISOPROTHIOLANE, PROPICONAZOLE DAN PRETILACHLOR DAN PENILAIAN RISIKO KESIHATAN KULIT DALAM KALANGAN PESAWAH PADI DI KAWASAN SAWAH PADI TANJUNG KARANG, KUALA SELANGOR

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Pengenalan: Tanjung Karang, Kuala Selangor adalah kawasan sawah padi ketiga terbesar di Semenanjung Malaysia. Racun perosak boleh memberi kesan positif terhadap pemusnahan serangga perosak dalam aktiviti pertanian tetapi ia juga boleh menyumbang kepada kesan buruk kepada alam sekitar dan kesihatan manusia. Selain itu, pesawah padi di Tanjung Karang, Kuala Selangor tidak menggunakan peralatan pelindung diri (PPE) dengan betul ketika bekerja di sawah padi. Oleh itu, hal ini akan meningkatkan pendedahan mereka terhadap racun perosak, terutamanya melalui pendedahan kulit. **Objektif:** Untuk menentukan kandungan *isoprothiolane*, *propiconazole* dan *pretilachlor* dalam sampel tanah padi dan menilai potensi risiko kesihatan kulit dalam kalangan pesawah padi di Kampung Sawah Sempadan, Tanjung Karang, Kuala Selangor. **Metodologi:** Kajian ini telah dijalankan di Kampung Sawah Sempadan, Tanjung Karang, Kuala Selangor yang terdiri daripada 24 blok padi (Blok A hingga Blok X). Soal selidik telah diedarkan di kalangan 152 pesawah padi untuk mendapatkan maklumat mengenai sosio-demografi, kebersihan diri, penggunaan peralatan pelindung diri dan pendedahan kepada racun perosak. 72 sampel tanah telah diambil secara rawak daripada 24 blok padi. Sampel tanah telah diekstrak dengan menggunakan *dispersive solid phase extraction (dSPE)* dan dianalisis dengan menggunakan *ultra performance liquid chromatography – tandem mass spectrometry (UPLC-MS/MS)*. Penilaian risiko kesihatan kulit pesawah padi dikira menggunakan *Hazard Quotient (HQ)*. **Keputusan dan Perbincangan:** Kandungan *isoprothiolane*, *propiconazole* dan *pretilachlor* dalam sampel tanah padi adalah dari 0.546 mg kg⁻¹ hingga 42.917 mg kg⁻¹, 0.773 mg kg⁻¹ hingga 25.502 mg kg⁻¹ and 0.435 mg kg⁻¹ hingga 9.942 mg kg⁻¹, masing-masing. *Hazard Quotient (HQ)* telah dikira hanya untuk *propiconazole* adalah kurang daripada satu untuk kesemua blok padi. Terdapat korelasi yang signifikan antara (i) *pretilachlor* dan nisbah C/N ($r = -0.664$, $p = 0.007$) dan antara (ii) *pretilachlor* dan jumlah karbon organik ($r = -0.677$, $p = 0.006$). **Kesimpulan:** Tidak terdapat risiko kesihatan kulit yang bukan karsinogenik disebabkan pendedahan pekerjaan kompaun *propiconazole* dalam tanah padi ($HQ < 1$) untuk pesawah padi yang bekerja di Blok A hingga Blok X.

Kata kunci: Pencemaran racun perosak, *hazard quotient (HQ)*, penilaian risiko kesihatan kulit, *ultra performance liquid chromatography – tandem mass spectrometry (UPLC-MS/MS)*, *dispersive solid phase extraction (dSPE)*

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LIST OF ABBREVIATIONS

CAS	Chemical abstract service
C.E.	Collision Energy
CEC	Cation exchange capacity
dSPE	Dispersive solid phase extraction
FAO	Food and Agriculture Organizational
HCL	Hydrochloric acid
HPLC	High Performance Liquid Chromatography
HQ	Hazard Quotient
IDL	Instrumental Detection Limit
IQL	Instrumental Quantification Limit
IS	Internal Standards
MDL	Method Detection Limit
MQL	Method Quantification Limit
MRM	Multiple reaction monitoring
PPE	Personal Protective Equipment
Rfd	Reference dose
TN	Total Nitrogen
TOC	Total Organic Carbon
UPLC-MS/MS	Ultra Performance Liquid Chromatography-Tandem Mass Spectrometry
USEPA	United State Environmental Protection Agency
WHO	World Health Organization

CHAPTER 1

INTRODUCTION

1.1 Introduction

Rice is the important food source for Malaysian and the third most important crop after rubber and palm oil. It becomes the most common grain in Malaysia, both in production and consumption. Rice production in Malaysia need to raise up to feed a growing population. Based on Department of Statistics Malaysia (2017), the total population in Malaysia is estimated at 31.7 million person in 2016, an increase of 0.5 million persons as compared 31.2 million persons in 2015 with 1.5 per cent population growth rate for the same period. In order to meet the food demands, the productivity of agricultural activity should be increased. The increased pressure to maintain high quality of rice output for consumption has resulted in increased the usage of pesticides on rice fields (Selvadurai & Azima, 2012).

Based on World Health Organization (2018), pesticides can be defined as chemical compounds that are used to kill pests, including insects, rodents, fungi and unwanted plants (weeds). Pesticides are used in public health to kill vectors of disease, such as mosquitoes, and in agriculture, to kill pests that damage crops. By their nature, pesticides are potentially toxic to other organisms, including humans, and need to be used safely and disposed of properly. Pesticides can be grouped according to their class of intended action such as insecticide, herbicide or fungicide. They can also be grouped by type with compounds of similar chemical properties (Sanford et al., 2015).

In this study, the targeted pesticides that are selected from the common pesticides used by the paddy farmers in Kampung Sawah Sempadan, Tanjung Karang, Kuala Selangor are isoprothiolane, propiconazole and pretilachlor. Isoprothiolane and propiconazole are classified as fungicides that are used to prevent or eradicate fungal infections from plants or seeds. The application of fungicides is usually intensive in different crops, and the presence of these compounds and their residues has frequently been detected in recent years in waters and soils (Álvarez-martín et al., 2017). Meanwhile, pretilachlor is categorized as herbicides that are used for destroying various weeds or inhibiting their growth. They have variable degrees of specificity. The worldwide use of herbicides is almost 48% of the total pesticide usage. (Gupta, 2011).

Moreover, pesticides are considered as important component of modern farming and playing a major role to maintain high agricultural productivity. The widespread overuse of pesticides in agriculture has generated increasing concerns about the negative effects of pesticides on human health and the environment (Jallow et al., 2017). There are several severe diseases such as cancer, chronic obstructive pulmonary disease, birth defects, infertility and more damages of human health are associated with the exposure of pesticides (Samsidar & Shaarani, 2018). A major group who are constantly handling with pesticides are the farmers and high risk of exposure to pesticides (Hamsan et al., 2017). A study by Sankoh et al. (2016), reported that there was few cases of health problems among farmers such as nausea, respiratory disorders and blurred vision had significantly higher among the farmers who are used pesticides than those who do not use pesticides. Farmers were found to have the highest prevalence of allergies, nasal congestion, wheezing, and acute symptoms after pesticide use (Kongtip et al., 2018).

On other hands, paddy cultivation has become the main activity in the community in the district of Tanjung Karang, Selangor where the study area has been selected because it is the third largest area of paddy farmers in Peninsular Malaysia. It is also known as 'the rice bowl of Selangor'. The paddy farms in Kampung Sawah Sempadan cover an area of approximately 2,300 hectares of Tanjung Karang. The selection was also made because there are many cases of pesticide poisoning were reported by the paddy farmers in the area (Hashim & How, 2016).

Some of the common pesticides used in Kampung Sawah Sempadan, Tanjung Karang, Kuala Selangor which focused on this study are isoprothiolane, propiconazole and pretilachlor. Information of the target compounds is summarized in Table 1.1.

Table 1.1: Information of the compounds chosen.

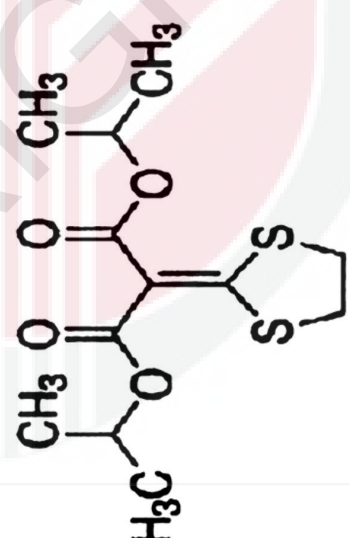
Common Name ^a	Pesticide Type ^a	Chemical Class ^a	IUPAC Name ^a	CAS Number ^a	Molecular formula ^a	Molecular structure ^a	Molecular weight (g mol ⁻¹) ^a	Log Kow ^a	Cancer classification ^b
Isoprothiolane	Fungicide	Phosphorothiolate	dipropan-2-yl 2-(1,3-dithiolan-2-ylidene)propanedioate	50512-35-1	C ₁₂ H ₁₈ O ₄ S ₂		290.4	3.3	NA

Table 1.1 Continued...

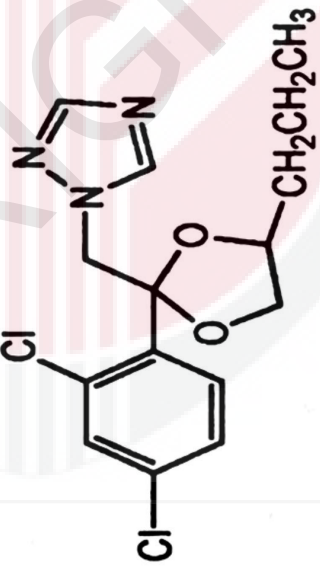
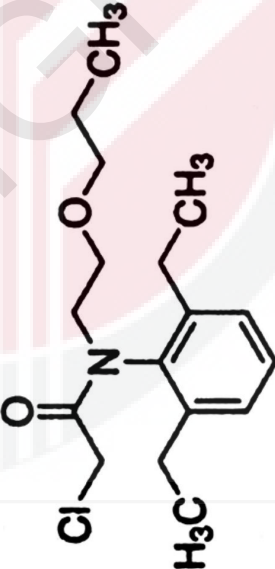
Common Name ^a	Pesticide Type ^a	Chemical Class ^a	IUPAC Name ^a	CAS Number ^a	Molecular formula ^a	Molecular structure ^a	Molecular weight (g mol ⁻¹) ^a	Log Kow ^a	Cancer classification ^b
Propiconazole	Fungicide	Triazole	1-[[2(2,4-dichlorophenyl)-4-propyl-1,3-dioxolan-2-yl]methyl]1-H-1,2,4-triazole	60207-90-1	C ₁₅ H ₁₇ Cl ₂ N ₃ O ₂		342.23	3.72	Group C. Possible Human Carcinogenic

Table 1.1 Continued...

Common Name ^a	Pesticide Type ^a	Chemical Class ^a	IUPAC Name ^a	CAS Number ^a	Molecular formula ^a	Molecular structure ^a	Molecular weight (g mol ⁻¹) ^a	Log Kow ^a	Cancer classification ^b
Pretilachlor	Herbicide	Amide	2-chloro-N-(2,6-diethylphenyl)-N-(2-propoxyethyl)acetamide		C ₁₇ H ₂₆ ClNO ₂		311.89	4.08	NA

^a Pubchem, (2018). National Center for Biotechnology Information (NCBI). <https://pubchem.ncbi.nlm.nih.gov/> (accessed March, 2018).
^b USEPA, (2017). Chemicals Evaluated for Carcinogenic Potential (Annual Cancer Report 2017). http://npic.orst.edu/chemicals_evaluated.pdf (accessed March, 2018)
 NA: Not available

1.2 Problem Statement

The significant increase in the worldwide need for food demands higher agricultural productivity, which can be achieved only by an extensive use of pesticides. Pesticides are one of the most hazardous contaminants of the environment. Through overuse and misuse there is considerable waste contributing to the adverse environmental and health consequences. Inappropriate application of pesticides affects the whole ecosystem by entering the residues in the food chain and polluting the soil, air, and ground and surface waters (Caldas et al., 2011).

Many studies have been discussed that there is a number of accidents, cases of poisoning and occurrence of environmental hazards because of the increasing usage of pesticide in agricultural activity (Idayu, Anis, Akmal, & Esa, 2014). The World Health Organization, (2018) had mentioned that nearly a million people die each year as a result of chemicals account for a significant number of these deaths. For example, it is estimated that 370,000 deaths each year cause by deliberated ingestion of pesticides. By limiting the availability of and accessibility to highly toxic pesticide, the number of these deaths can be reduced.

A study by Idayu et al., (2014) stated that small-scale farmers in the agriculture sector tend to use the high toxicity of the pesticides without adequate knowledge of safe pesticide management practices and failed to wear appropriate personal protective equipment (PPE). Besides, the farmers' health are at high risk because of the pesticides being dispersed, spilled or leaked and entering the human body either directly or indirectly and its application is continuously being used. There are some studies argued that the improper and excessive use of pesticides, especially in developing countries,

is linked to the lack of education and training in pesticide use, lack of alternatives to pesticides, inadequate information on related hazards and the unwillingness of farmers to accept the risk of crop loss (Galt, 2008). However, understanding farmers' perceptions of risk of pesticides and the determinants of pesticide overuse is important to modifying their behavior towards reducing pesticide use.

Kampung Sawah Sempadan, Tanjung Karang was selected as study location because most of the communities in the area are involved in rice farming and exposed to the pesticides. Interview with the farmers were conducted in this study in order to know the compounds of the commonly used pesticides. The interview was done by enquiring the farmers and the local pesticides supplier on which brands that the farmers most commonly used during spraying season. Based on the interview, there are thirteen compounds of the commonly used pesticides in Tanjung Karang paddy fields. From the thirteen compounds, three of the active compounds were selected. The three selected compound of the pesticides were isoprothiolane, propiconazole and pretilachlor.

Based on the field observation in Kampung Sawah Sempadan, the usage of personal protective equipment (PPE) seems neglected. It became worst when the farmers directly in contact with the pesticides and paddy soil without any protection as shown in **Figure 1.1**.



Figure 1.1: Farmers that do not wear PPE while mixing the pesticides

1.3 Research Justification

Agricultural activities have been arising along with the use of pesticides. Without realised, pesticides can affect not only pest but also environment and human health. Based on the interview conducted by Sallehuddin (2017), it was found that some of the farmers did not wear appropriate and complete personal protective equipment (PPE) such as gloves and boots while handling with the paddy soil, hence this may increase the risk of exposure to the pesticides that present in the environment especially through dermal contact. According to Ossain et al., (2010), there are some studies showed that the significant exposure pathway of absorption of chemicals are through the skin which can occur without being noticed by the paddy farmer. Direct skin effects also can occur when the exposure of the pesticides produces a local effect such as irritation, necrosis, or corrosion (Stacey E Anderson and B Jean Meade, 2014).

There are many studies reported on pesticides contamination in soil (Nishina et al., 2010; Zhang et al., 2015; Liu et al., 2011). However, the pesticides contamination of the targeted compounds (isoprothiolane, propiconazole and pretilachlor) have never been reported in Malaysia. A study by Nishina et al. (2010) in Vietnam reported that the detection results of the residue of isoprothiolane in the soil was $0.0166 \text{ mg kg}^{-1}$. The Vietnam government has set the maximum allowable concentration (MAC) of isoprothiolane in the soil was 0.1 mg kg^{-1} and the result shown was below than the MAC standard in Vietnam. Meanwhile, Zhang et al. (2015) reported that the maximum concentration of propiconazole residues in soil samples of China was 0.45 mg kg^{-1} . Other than that, Liu et al., (2011) reported that the concentration of pretilachlor in the soil samples in China were ranged from 0.082 to 0.791 mg kg^{-1} . Studies above shown that the target compounds have been widely used by farmers and contaminated the soil in other countries.

Therefore, it is essential to study the concentration of pesticide residues in paddy soil and their associated dermal health risk among the paddy farmers in Tanjung Karang, Kuala Selangor. The non-carcinogenic health risk to the paddy farmers were determined by calculating the Hazard Quotient (HQ).

1.4 Conceptual Framework

This study is focused on two parts which are the concentration of pesticides in soil and the effect of pesticide residues toward human health. The independent variable of this study is the physico-chemical parameters of the soil (pH-H₂O, total organic carbon (TOC), total nitrogen (TN), C/N ratio, cation exchange capacity (CEC) and moisture) and the dependent variables of this study is the concentration of isoprothiolane, propiconazole and pretilachlor. To assess the effect of pesticide residues toward human health, the concentration of isoprothiolane, propiconazole and pretilachlor in paddy soil become the independent variable of this study while the dependent variables of this study is hazard quotient. **Figure 1.2** shows the conceptual framework of the study.

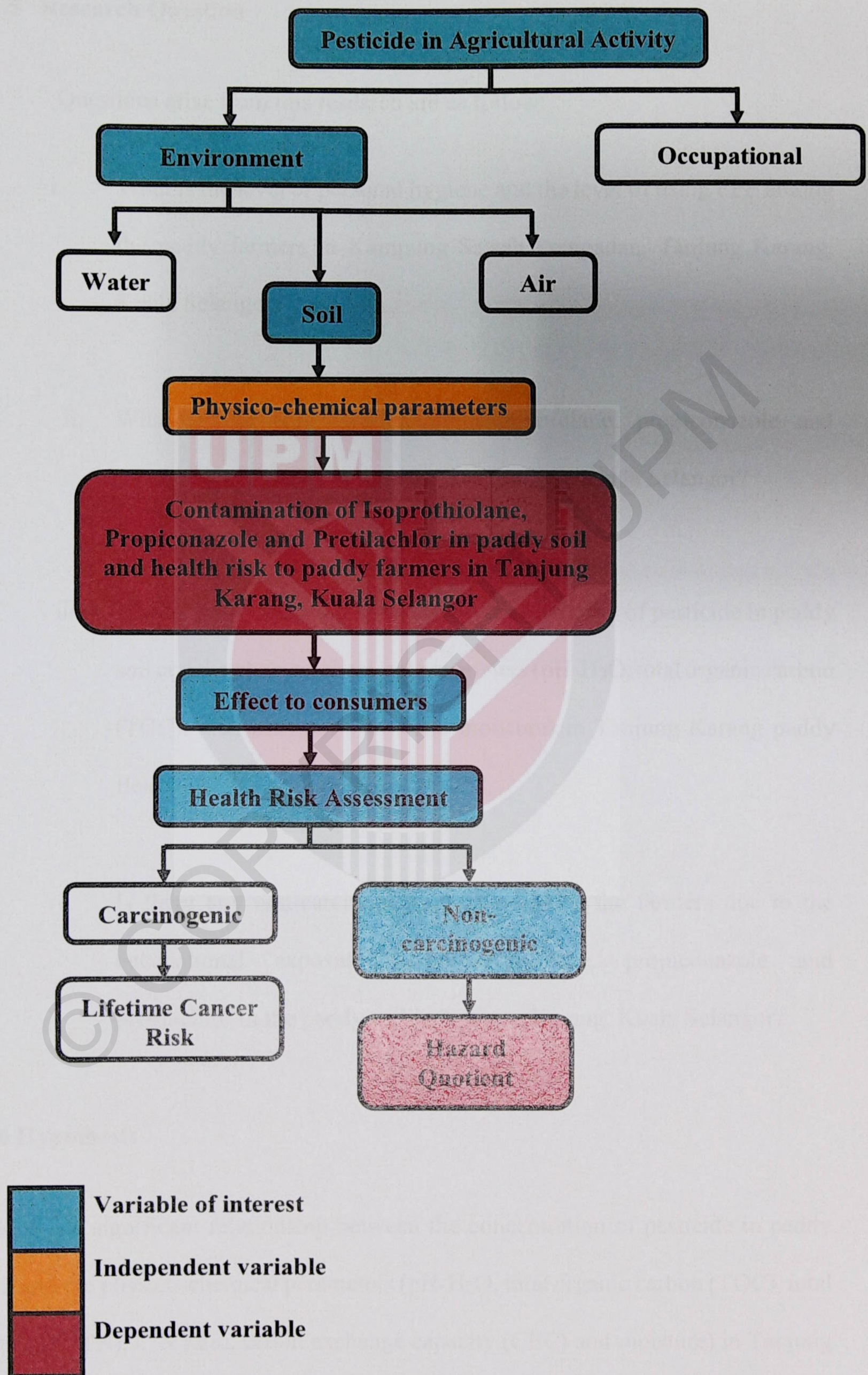


Figure 1.2: Conceptual Framework

1.5 Research Question

Questions arise from this research are as follow:

- i. What is the level of personal hygiene and the level of using PPE among the paddy farmers in Kampung Sawah Sempadan, Tanjung Karang, Kuala Selangor?
- ii. What is the concentration of isoprothiolane, propiconazole and pretilachlor in paddy soil in Tanjung Karang, Kuala Selangor?
- iii. Is there any correlation between the concentration of pesticide in paddy soil and the physico-chemical parameters (pH-H₂O, total organic carbon (TOC), total nitrogen (TN), and moisture) in Tanjung Karang paddy field?
- iv. Is there any non-carcinogenic health risk to the farmers due to the occupational exposure of isoprothiolane, propiconazole and pretilachlor in the paddy soil in Tanjung Karang, Kuala Selangor?

1.6 Hypothesis

There is a significant relationship between the concentration of pesticide in paddy soil and the physico-chemical parameters (pH-H₂O, total organic carbon (TOC), total nitrogen (TN), C/N ratio, cation exchange capacity (CEC) and moisture) in Tanjung Karang paddy field.

1.7 Research Objective

1.7.1 General Objective

To determine the concentration of isoprothiolane, propiconazole and pretilachlor in paddy soil and their potential dermal health risk among farmers at Tanjung Karang, Kuala Selangor.

1.7.2 Specific Objective

- i. To identify the level of personal hygiene and the level of using PPE among the paddy farmers in Kampung Sawah Sempadan, Tanjung Karang, Kuala Selangor.
- ii. To quantify the concentration of isoprothiolane, propiconazole and pretilachlor residue in paddy soil samples collected from Tanjung Karang, Kuala Selangor.
- iii. To determine the correlation between the concentration of isoprothiolane, propiconazole and pretilachlor in paddy soil samples with the physico-chemical parameters (pH-H₂O, total organic carbon (TOC), total nitrogen (TN), C/N ratio, cation exchange capacity (CEC) and moisture) in Tanjung Karang paddy field.
- iv. To assess the non-carcinogenic health risk of the farmers due to the occupational exposure of isoprothiolane, propiconazole and pretilachlor in soil in Tanjung Karang paddy field.

1.8 Definition of terms

1.8.1 Operational Definition

I Pesticide Contamination

Pesticide may contaminate the paddy soil due to the usage of pesticides in paddy field where in this study three targeted compound of pesticides has been selected (Isoprothiolane, Propiconazole and Pretilachlor) out of thirteen compounds. Then, those compounds were analysed through Ultra Performance Liquid Chromatography-tandem Mass Spectrometry (UPLC-MS/MS).

II Ultra Performance Liquid Chromatography-tandem Mass Spectrometry (UPLC-MS/MS)

The UPLC was used to analyse pesticide compound in the soil samples using electro spray ionization in positive method with optimized operating condition.

III Health Risk Assessment

This study focuses on the risk assessment through dermal exposure of the paddy farmers. The assessment through the Hazard Quotient for non-carcinogenic effects were used to estimate the health risk of paddy farmers who were exposed to pesticides through dermal contact.

CHAPTER 2

LITERATURE REVIEW

2.1 Pesticide Contamination in Soil

2.1.1 Fate of Pesticides

Pesticides are chemicals that are function to destroy or prevent the unwanted organisms including insects, weeds, fungi and nematodes. Most sectors of the agricultural production are dominantly used pesticide since the 1950s to control or to minimize the losses made by the pests and in order to raise the quality of the production. These pesticides can be classified based on the organisms they are intended to kill such as fungicides, insecticides, herbicides, molluscicides, nematocides, rodenticides and other. Besides, in developing countries, the application of pesticides have been promoted among the agriculture farmers to raise their economic life and productivity (Idayu et al., 2014).

Many of the adverse effects of pesticides on the environment depend on the interactions between the physicochemical properties (vapour pressure, stability, solubility, pKa) of the pesticide, soil adsorption and soil persistence, the soil factors (pH, organic components, inorganic surfaces, soil moisture, soil microflora, soil fauna), the plant species, and the climatic variation (Damalas & Eleftherohorinos, 2011). Besides, some of the adverse effects on the environment are the toxicity, the dosage applied, the weather conditions prevailing after the pesticide application, and how long the pesticide persists in the environment. The important factors that can

affect the fate of pesticide in the environment are soil factors and weather conditions and it can cause adverse effects on the environment (Monaco et. Al, 2002).

When the extraneous compounds accumulate and remain in the environment for a very long period of time, there will be a significant effect on soil and water quality. The distribution of pesticide depends on the characteristics of soils (such as type of soil, temperature, moisture, soil structure and the availability of oxygen) and the chemical structure of the pesticide compound (their half-life, toxicity, solubility, and soil-water partition coefficient) (Harrison, Bull, & Michaelides, 2013).

2.2 Target compounds

One of the popular pesticides active ingredients used by farmers in Kampung Sawah Sempadan, Tanjung Karang is isoprothiolane (Sallehuddin, 2017). ISO common name for isoprothiolane is diisopropyl 1,3-dithiolan-2-ylidenemalonate (IUPAC). It has been categorized as a fungicide and a plant growth regulator. The function of isoprothiolane on rice plants is used to control the fungi such as *Pyricularia oryzae*, *Helminthosporium sigmoideum*, *Fusarium nivale* (Food & Authority, 2012)

Propiconazole (1-[2-(2,4-dichlorophenyl)-4-propyl-1,3-dioxolan-2-ylmethyl]-1H-1,2,4-triazole) is in the triazole class of fungicides. It is a fungicide and antimicrobial that used to protect grass grown for seed and it was first registered in 1981. According to Findland Competent Authorities (2015), propiconazole is classified as skin sensitizer according to the Regulation (EC) No 1272/2008. Qualitative local risk characterization was performed for local dermal effects. Propiconazole targets fungi, bacteria, and viruses for affecting the plants. It is less in

organic matter but moderately mobile in soil. Propiconazole has some potential to contaminate water (S.C. Gad & T. Pham, 2014). Based on the research, propiconazole (fungicides) remained mainly in surface soil. It will formed a large fraction of residue in soil after the application of propiconazole and it is because of its persistence in the soil for a long period of time. (I. S. Kim, Beaudette, Han Shim, Trevors, & Tack Suh, 2002). Based on Zhang et al., (2015), it reported that the half-lives of propiconazole was 6.1 - 8.4 days in soil in Chinese fields.

One of the common pesticides used in Malaysia is pretilachlor. Pretilachlor [2-chloro-2,6-diethyl-N-(propyloxyethyl) acetanilide] is widely used to control the annual weeds in rice fields. Pretilachlor is classified as herbicides to kill unwanted plants. The use of herbicides has increased the crop yield in agricultural production. However, with the increasing application of herbicides in modern agriculture, a large portion of these herbicides accumulate in surface water and pose environmental risks to aquatic organisms and human health (Jiang et al., 2016). Currently, the researches on pretilachlor are mainly concentrated on the fate of pretilachlor after it goes into the environment. However, there are few reports on removal of pretilachlor from the environment (Wei, Feng, Sun, Liu, & Zhu, 2011).

2.3 Health Risk Assessment

2.3.1 Route of exposure

Pesticide exposure to human may occur through occupational exposure especially for the workers that are handling the pesticides in the industry and the agricultural workers in open fields. However, the pesticides are contribute to the potential occupational exposure even though the occupation does not involves to the

pesticide. The highest risk for possible acute intoxications goes to the workers who are mix, load transport and apply formulated pesticides are normally considered to be the group that will receive the greatest exposure because of the nature of their work for example from accidental spills of chemicals, leakages, or faulty spraying equipment. The exposure of workers increases are because of they are not paying attention to the instructions on how to use the pesticides and particularly when they ignore basic safety guidelines on the use of personal protective equipment and fundamental sanitation practices such as washing hands after pesticide handling or before eating (Damalas & Eleftherohorinos, 2011).

Humans are exposed to pesticides that is found in environmental media such as soil, water, air and food by different routes of exposure such as inhalation, ingestion and dermal contact. Exposure of pesticides to human contact can come directly or indirectly and it can results in acute and chronic health problems (Abhilash & Singh, 2009).

Dermal exposure is one of the most common and effective routes through which pesticide applicators are exposed to pesticides (Anderson & Meade, 2014). Dermal absorption may occur as a result of a splash, spill, or spray drift, when mixing, loading, disposing, and cleaning of pesticides. Absorption may also result from exposure to large amounts of residue. Pesticide formulations vary broadly in physicochemical properties and in their capacity to be absorbed through the skin (Beard et al., 2014), which can be influenced by the amount and duration of exposure, the presence of other materials on the skin, temperature and humidity, and the use of personal protective equipment (Macfarlane et al., 2013). In general, solid forms of

pesticides are not as readily absorbed through the skin and other body tissues as liquid formulations. However, the hazard from skin absorption increases when workers are handling the concentrated pesticides. Certain areas of the body such as the genital areas and ear canal are more susceptible to pesticide (Hoque, 2014).

2.4 Health effect

Pesticides consist of an active ingredient, the actual poison and a variety of additives which can improve the efficacy of its application and action. Pesticides can be classified or grouped depending to the target organisms (eg. insecticides, fungicides, and herbicides), chemical structure of the compound or type of health hazard involved. Health effects resulting from pesticide exposure vary according to the individual pesticide involved and may be the result of exposure via the dermal, oral, or inhalational routes, however, dermal exposure is the most relevant route of exposure for pesticide applicators. Health effects may be classified as acute or chronic, based on the period it takes for symptoms of toxicity to develop (Macfarlane et al., 2013).

A study by K. Kim, Kabir, & Ara (2017) had mentioned that exposure to pesticides that are poisonous can be extremely hazardous to human health and other living organisms even though in low doses of exposure. According to Fuad et al., (2012), a survey was conducted among 219 farmers at Kampung Sungai Burung and Kampung Sawah Sempadan, Tanjung Karang, Kuala Selangor has resulted about 51.5% of the farmers' complaints they have difficulty in breathing after spraying pesticide, 26% had itchiness and sore and the other 13.7% had rashes and hand skin peel off. In addition, they also reported that there are effect of hazardous usage of modern agriculture that has been proven according to the recorded data by District

Hospital of Tanjung Karang in which there are farmers getting poisoned each year, for instance the highest case was in 2009 with a number of 24 cases and involving 2 deaths.

According to Damalas & Eleftherohorinos (2011), the impact of pesticides has become a serious concern when they mixed and applied the pesticides in their work. All these activities can cause accidental poisoning and expose major health risks to farmers whether in short term or long term. Exposure to pesticides tends to pose various disease including cancer. A susceptible group such as children, pregnant women, or aging populations may be at a high risk to the effect of pesticides. The relationship between exposure to pesticides and the incidence of some disease cannot be ignored because there is statistical association between those two (K. Kim et al., 2017).

2.4 Legislation and Regulation

2.4.1 International legislation and regulation

There are law, legislation and regulation that have been established around the world to ensure the safety of pesticides usage. The Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) is provides for federal regulation of pesticide distribution, sale, and use in the United States. Besides, all pesticides that is distributed or sold in the United States must be registered (licensed) by Environmental Protection Agency. The process of registering a pesticide is a scientific, legal, and administrative procedure. They will examine the ingredients of the pesticide, the particular site or crop where it is to be used, the amount, frequency, and timing of its use, and the storage and disposal practices of the pesticides. They will evaluate the pesticide registration

application by assessing the potential human health and environmental effects associated with use of the product.

Food and Agriculture Organization of the United Nations (FAO) and World Health Organization also have implemented The International Code of Conduct on Pesticide Management on 2014. The main objective of the Code of Conduct to be developed is to maximize the benefits of pesticides to effectively control pests in public health and agriculture. It is also to protect human and animal health and the environment from their harmful effects. This Code also mentioned that they need all the collaboration from many sectors such as governments, pesticide industry, all stakeholders, including farmers and farmer associations, extension agents, crop consultants, food industry, manufacturers of biological and chemical pesticides in order to control the management of the pesticide usage.

According to European Food Security & Authority (2012), it is reported that isoprothiolane and pretilachlor is an active substance that are not registered in Europe and the manufacturer does not intend to apply for an approval of both compounds under Regulation (EC) No 1107/2009. This situation can be dangerous to the public who are going to use this pesticides. However, propiconazole is approved to be used in European country. European Commission Implementing Regulation (EU) 2018/84 of 19 January 2018 amending Implementing Regulation (EU) No 540/2011 as regards the extension of the approval periods of the active substances.

2.4.2 Malaysia legislation and regulation

In Malaysia, the Department of Agriculture, Ministry of Agriculture and Agro-based Industry has implemented and enforced the Pesticide Act 1974 to cover the management of all pesticides and other chemicals used in agriculture including the targeted pesticides (isoprothiolane, propiconazole and pretilachlor). The Act also control the quality, efficacy and adverse effects of pesticides imported and manufactured in this country. The company or persons who want to import or manufacture a pesticide must apply to the Pesticides Board for registration of the pesticide and follow all the guidelines that are provided. Based on Department of Agriculture (2018), all the targeted pesticides (isoprothiolane, propiconazole and pretilachlor) are registered to be used in Malaysia.

There are other related regulations on the management of pesticides in Malaysia which are the Environmental Quality Act 1974 and Food Act 1983. The primary objective of Environmental Quality Act 1974 is to control the discharge of chemical and industrial wastes including pesticides into the environment, so that there will be no adverse effects on human health and the environment. Meanwhile, the Food Act 1983 prescribes the maximum residue levels of certain pesticides in food.

CHAPTER 3

METHODOLOGY

3.1 Chemicals and Standards

HPLC-grade methanol, HPLC-grade dichloromethane, HPLC-grade acetone and HPLC-grade acetonitrile were purchased from Fisher Scientific (New Hampshire, USA). Analytical Reagent Grade dichloromethane, acetone and methanol were purchased from Fisher Scientific (New Hampshire, USA). Hydrochloric acid (HCl), formic acid, anhydrous magnesium sulfate and sodium acetate were purchased from R&M Chemicals (Malaysia). Decon 90 was purchased from Decon Laboratories Limited (England). Ammonium formate solution was purchased from Sigma-Aldrich (Switzerland). Dichlorodimethyl-silane was purchased from EMSURE (Germany). QuEChERS Dispersive kit 15mL AOAC was purchased from Agilent Technologies (CA, USA). Reference standards of isoprothiolane (97.8%), propiconazole (99%) were purchased from Dr. Ehrenstorfer (Germany) while pretilachlor (98.7%) and internal standard imidacloprid-d₄ (99%) were sourced from Sigma-Aldrich (MO, USA). Individual stock standard solutions (1000 mg/L) of target compounds were prepared monthly in HPLC grade methanol. The stock solution was diluted (10mg/L) in HPLC grade 25% MeOH as working standard solutions and kept in flame-sealed amber-glass ampoules before storing at -20°C.

3.2 Study Design

The study design is cross-sectional study design where it can measures the outcome and the exposures in the study participants at the same time. This study also involved laboratory analysis as the concentration of three compounds (isoprothiolane, propiconazole and pretilachlor) were quantified. No intervention program in this study. The concentration of the pesticides were quantified to assess the health risk to the farmers.

3.3 Study Location

This study was carried out in Tanjung Karang, Kuala Selangor which widely known for its paddy cultivation activity and the third largest paddy field in Peninsular Malaysia. Kampung Sawah Sempadan, Tanjung Karang consist of 24 blocks of paddy namely as Block A until Block X. The study location is depicted in Figure 3.1. There is a few consideration that needs to be taking care of before selecting the location of study. The criteria of the study location are:

Agriculture area with paddy cultivation as the main activity.

- i. Study location must be nearby or in a short distance from the laboratory which preservation and analysis were conducted.
- ii. The willingness and cooperation of the residents throughout the study.

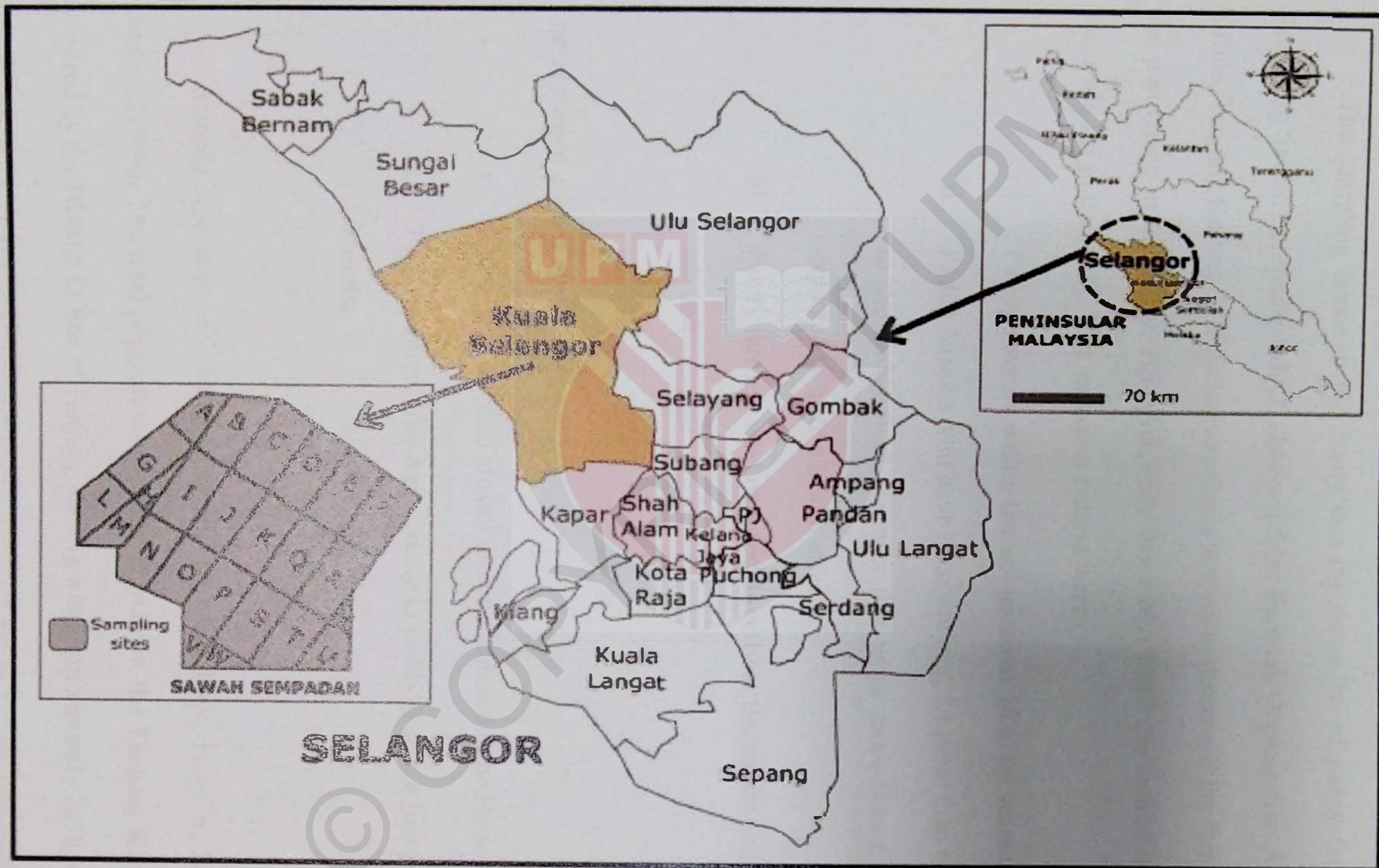


Figure 3.1: Sampling locations in Kampung Sawah Sempadan, Tanjung Karang, Kuala Selangor

3.4 Sample Population

The sampling frame in this study was the name list of paddy farmers in Kampung Sawah Sempadan which obtained from Farmers Organization Authority Office, Tanjung Karang. The inclusive criteria was set to determine the farmers that can participate in this study. However, only those who fulfil the inclusion criteria was included in the study. The inclusion criteria were:

- i. Paddy farmers within the age group of 18 to 60 years old.
- ii. Paddy farmers who have been working for more than 6 months in the paddy field. Farmers that work more than 6 months expected to have a health effect.
- iii. Paddy farmers who exposed directly to the paddy soil in their job task.

The exclusion criteria are:

- i. Paddy farmers who are not directly exposed to paddy soil.
- ii. Paddy farmers who are working in paddy field not more than 6 months.

Sample size was calculated according to Lemeshow, Hosmer, Klar, & Lwanga (1990). The total population of paddy farmers in the Tanjung Karang as acquired by the District Office of Tanjung Karang was approximately 7679, and the population of the paddy farmers in the study area (Kampung Sawah Sempadan) was 1147. The sample size was calculated using the following equation:

$$n = \frac{Z^2_{1-\frac{\alpha}{2}} P (1-P)}{d^2} \quad (\text{Equation 3.1})$$

Where,

n = Sample size

$Z^2_{1-\frac{\alpha}{2}}$ = Standard errors associated with confidence intervals, 95% (1.96)

P = Prevalence, 0.1

d = Desired precision, 0.05

The value of prevalence, P is 0.1 from the study on dermatitis among farmers engaged in peri-urban aquatic food production in Hanoi, Vietnam by Anh et al., (2007). Below is the sample size calculation for this study.

$$n = \frac{1.96^2 \times (0.1) (1 - 0.1)}{(0.05)^2} \quad (\text{Equation 3.2})$$

$$n = 138$$

In order to recover the loss of respondent, 10% of the minimum sample size was added, which is:

$$\begin{aligned} 10\% \text{ of } 138 &= 13.8 \approx 14 \\ &= 14 + 138 \\ &= 152 \text{ respondents} \end{aligned}$$

3.5 Sampling Technique

3.5.1 Soil Sample

All of the paddy blocks (Block A – Block X) in Kampung Sawah Sempadan were included in this study. Each block consists of few plots. Simple random sampling was used to select different plots of each block. The samples were taken in triplicates, in order to increase the accuracy. Then, 72 soil samples were randomly collected from 24 different blocks of paddy plots consist of Block A to Block X.

Composite soil samples were collected from all of the blocks in Kampung Sawah Sempadan. Five different points in each block were taken and the samples were mixed as a composite sample. Figure 3.2 shows the example of the composite sample collected from five different points in a paddy block. Paddy soil samples were collected at the depth of 0-20 cm from each block according to the method by Fenoll J, Hellín P & Martínez CM, (2009). Then, those composite samples were mixed up and approximately 1 kg of composite soil samples were collected in the zip-lock bag. The zip-lock bags then were labelled, wrapped with aluminium foil and kept in ice box at 0-4 °C.

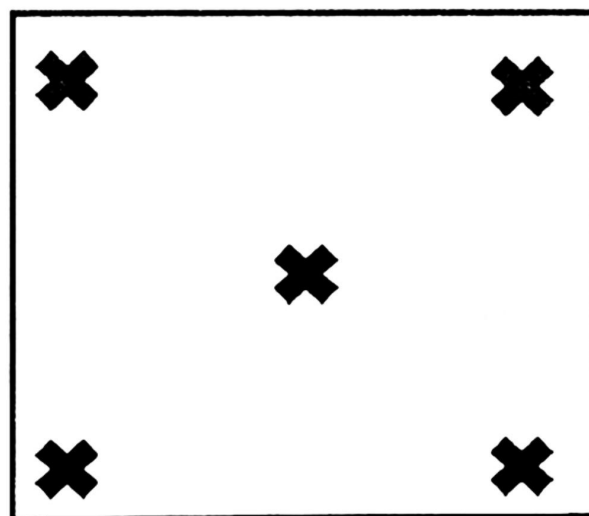


Figure 3.2: Example of composite soil sampling in one paddy block

3.5.2 Determination of Physico-chemical Properties of Soil Samples

Soil samples were characterized by using different physico-chemical parameters to obtain comprehensive data and to establish a relationship between sample concentration and sample characteristics. Special attention was paid to analyse parameters such as pH-H₂O, total organic carbon (TOC), total nitrogen (TN), C/N ratio, cation exchange capacity (CEC) and moisture. The analysis of all the parameters was provided by Espek Research & Advisory Services (E.R.A.S), RISDA Estates Sdn. Bhd.

3.5.3 Soil Extraction and Preparation

Soil extraction and preparation procedure was modified according to Caldas et al., (2011). Upon the arrival to the laboratory, 0.5 kg of soil sample was homogenized and passed through a 2 mm sieve. Then, 10 g of soil sieved was placed into a polypropylene centrifuge tube (50 mL). 100 µL of acetic acid and 50 µL of 1 mg/L internal standard (imidacloprid-d4) were spiked into the polypropylene centrifuge tube (50 mL). Next, 15 mL of acetonitrile was added and the mixture was shaken for 15 seconds. Six gram of anhydrous magnesium sulfate and 1.5 g of sodium acetate were added. The tube was shaken for 1 minutes and vortex for 15 seconds. Then, the extract was centrifuged for 10 minutes at 3000 rpm. Six milliliter of supernatant extract was transferred into dispersive solid phase extraction (dSPE) clean up tube, shaken and vortex for 15 seconds. The tube was centrifuged at 3000 rpm for 10 minutes. The purified extract was evaporated to dryness using N₂ over water bath at 40°C, then it was reconstituted with 1:3, methanol: water for UPLC-MS/MS analysis.

3.6 Selection of Targeted Compounds

The target pesticides were selected based on the interview with the farmers. The popular pesticides were selected as the target compounds in this study. Table 3.1 summarized the frequency of the usage of pesticides active ingredients.

Table 3.1: Frequency of the usage of pesticide’s active ingredients by farmers in Kampung Sawah Sempadan, Tanjung Karang.

Pesticide active ingredients	Frequency	Percentage (%)	Remark
Chlorantraniliprole	44	36	The compound have been reported by Sallehuddin, (2017)
Difenoconazole	44	36	
Pymetrozine	43	36	
Isoprothiolane	41	34	Target compound
Propiconazole	38	32	
Pretilachlor	31	26	
Tebuconazole	29	24	
Trifloxystrobin	20	24	
Azoxystrobin	27	23	
Fipronil	22	18	
Tricyclazole	21	18	
Imidacloprid	17	14	
Buprofezin	13	11	
N = 152			

3.7 Study Instrument

3.7.1 Questionnaire

A set of questionnaire was distributed among the farmers to obtain the information for health risk assessment. The questionnaire form can be found in **Appendix 1**. The questionnaire was divided into four parts which include Section A: information regarding personal information, Section B: exposure to pesticides and personal hygiene and Section C: usage of personal protective equipment (PPE).

The information obtained from this questionnaire was used to assess the dermal risk exerted by the farmers. The questionnaire was adapted based on Nordic Occupational Skin Questionnaire (2002) and Taneepanichskul et al. (2010).

Fishbowl Draw technique was used to get the human sampling which selected from a list of farmers in Kampung Sawah Sempadan provided by Farmers Organization Authority Office, Tanjung Karang.

Pre-test study of the questionnaire were conducted in Kampung Sungai Burung, Tanjung Karang, Kuala Selangor. The questionnaire was distributed to the farmers in Kampung Sungai Burung, Tanjung Karang, Kuala Selangor. The result of Cronbach alpha in the pre-test was 0.8.

3.7.2 Ultra Performance Liquid Chromatography-tandem Mass Spectrometry (UPLC-MS/MS)

The UPLC-MS/MS (Agilent, USA) method conditions were as follows: chromatographic separation was performed on an Eclipse Plus C18 column (2.1 mm × 50 mm I.D., 1.8 µm particle size) (Agilent, USA). The mobile phase compositions were as follows: A, ultrapure water with 0.1% formic acid and 5 mM ammonium formate, and B, methanol with 0.1% formic acid and 5 mM ammonium formate. The mobile phase was mixed as follows: 0 min 94% A, 6% B; 15 min 2% A, 98% B; 18 min 2% A, 98% B; 18.01 min 94% A, 6% B; and 20 min 94% A, 6% B. The flow rate was 0.5 mL min⁻¹, and the total runtime was 20 minutes. The column temperature was set at 40 °C, and the injection volume was 2.0 µL. All pesticides were detected using electrospray ionization (ESI) in positive ion mode. The optimized operating conditions were as follows: capillary voltage, 3500 V; gas temperature, 220 °C; gas flow, 11 L min⁻¹; and nebulizer, 30 psi. The optimised ESI and MS/MS parameters, comprising of precursor ions, product ions, collision energy (C.E.) and fragmentation voltage are shown in **Table 3.2**. The optimized ESI and MS/MS parameters were subsequently used for multiple reaction monitoring (MRM) in UPLC-MS/MS analysis. Data collection, peak integration and linear regression were performed using the QQQ Quantitative Analysis software from Agilent (USA).

Table 3.2: MRM conditions

Compound	Precursor ion, <i>m/z</i>	Product, <i>m/z</i> (Collision energy, V)	Fragmentation, V
Isoprothiolane	291.10	231.00 (8), 188.80 (20)	380
Propiconazole	342.10	159.00 (32), 69.10 (16)	380
Pretilachlor	312.17	252.10 (17), 176.10 (29)	380
Internal standards	Precursor ion, <i>m/z</i>	Product, <i>m/z</i> (Collision energy, V)	Fragmentation, V
Imidacloprid-d ₄	260.10	212.90 (21)	380

3.8 Quality Control

3.8.1 Cleaning of glassware

All glassware were undergo acid wash before used to ensure they are free of any contaminants based on USEPA Method 1699 (EPA, 2007). All glassware were soaked with 5-10% hydrochloric acid (HCL) overnight followed by washed with Decon 90. Then, the glassware was rinsed immediately, first with methanol, then with hot tap water. After that, it was rinsed with methanol again, followed by acetone, and then dichloromethane. After washing, all glassware were dried at 60 °C and capped with solvent rinsed aluminium foil to prevent any accumulation of dust or other contaminants.

3.8.2 Calibration of UPLC-MS/MS

At a five-point calibration curve, the instrument was calibrated with each analyte. Each calibration point were added the labelled internal standards (IS) corresponding to the analytes at a concentration of 50 ng mL^{-1} to generate relative response factors (RRF). A calibration curve was obtained by injecting the standards of isoprothiolane, propiconazole, and pretilachlor range from instrument quantification limit (IQL) to $500 \mu\text{g kg}^{-1}$.

3.8.3 Instrumental Detection Limit (IDL), Instrumental Quantification Limit (IQL), Method Detection Limit (MDL) and Method Quantification Limit (MQL) in paddy soil

IDL and IQL were determined by preparing different concentrations of mixture of pesticide standards from $0.05 \mu\text{g kg}^{-1}$ down to $0.001 \mu\text{g kg}^{-1}$. Each concentration was directly injected into UPLC-MS/MS instrument, and the IDL was determined based on chromatographic response that gave out signal-to-noise ratio (S/N) 3 while IQL was determined at S/N 10. Meanwhile, MDL and MQL determined by signal-to-noise method. This method measures the peak-to-peak noise exhibited by the target analyte and estimates the concentration of analyte that will produce a certain value of signal-to-noise ratio (S/N). S/N of 3 and 10 can be estimated for MDL and MQL respectively. (Shrivastava, Alankar, & Gupta, 2011).

3.8.4 Extraction Recovery

The extraction recovery was determined in order to validate the analytical procedure by spiking the samples with varied amounts of standard solutions of each compound (Zhuang, McBride, Xia, Li, & Li, 2009). The percentage of recovery was calculated according to Ho, Pauzi, Abdul, & Saari (2012), the percentage of recovery was calculated by comparing the concentration of each compound spiked before extraction (C_p) to its concentration spiked after extraction (C_a) in the same sample matrix using Equation 3.4, where C_{qc} is the concentration of analyte in the blank sample.

$$\text{Recovery (\%)} = \frac{C_p - C_{qc}}{C_a - C_{qc}} \times 100 \quad (\text{Equation 3.4})$$

3.8.5 Procedural Blank and Field Blank

In this study, the blank soil samples were collected from Ayer Hitam Forest Reserve Trail, Puchong, Selangor. In order to keep away from any interference or contamination, a procedural blank (except no sample was added, the other experimental procedures were exactly the same as the samples from the extraction until the instrumental analysis) was analysed for each batch of samples.

3.9 Health Risk Assessment

3.9.1 Non-carcinogenic Risk

The non-carcinogenic chronic health risk of farmers was assessed using hazard quotient (HQ). Hazard Quotient (HQ) is a ratio of exposure dose to the compounds-specified reference dose (RfD). USEPA (2001) stated that if the value of HQ is less than one, then there is no significant risk of non-carcinogenic effects. Whereas, when the HQ value exceeds one, then there is a significant non- carcinogenic risk that may increase as the HQ increases.

HQ was calculated based on USEPA (2004) as shown in **Equation 3.5**:

$$HQ = \frac{DAD}{Rfd_{dermal}} \quad \text{(Equation 3.5)}$$

Based on USEPA (2004), DAD and DAevent were calculated using the following equation:

$$DAD = \frac{DA_{event} \times EV \times ED \times EF \times SA}{BW \times AT} \quad \text{(Equation 3.6)}$$

$$DA_{event} = C \times CF \times AF^a \times ABS_d \quad \text{(Equation 3.7)}$$

$$Rfd_{dermal} = \frac{Rfd_{oral}}{AF^b} \quad \text{(Equation 3.8)}$$

$$AF^b = 1 - e^{-K_a \cdot ET} \quad (\text{Equation 3.9})$$

$$\log K_a = 0.233 \cdot \log K_{ow} - 0.00566 \cdot MW - 1.49 \quad (\text{Equation 3.10})$$

The values of each parameter stated in Equation 3.5, Equation 3.6, Equation 3.7, Equation 3.8, Equation 3.9 and Equation 3.10 are shown in Table 3.3.



Table 3.3: Variable used in risk assessment

Name	Acronyms	Unit	Value	References								
Dermal Absorbed Dose	DAD	mg ⁻¹ kg ⁻¹ day ⁻¹	Based on calculation	From this study								
Reference dose	Rfd _{oral}	mg ⁻¹ kg ⁻¹ day ⁻¹	<table><tr><th>Compound</th><th>cRf^d</th></tr><tr><td>Isoprotiolane</td><td>NA</td></tr><tr><td>Propiconazole</td><td>0.0694</td></tr><tr><td>Pretilachlor</td><td>NA</td></tr></table>	Compound	cRf ^d	Isoprotiolane	NA	Propiconazole	0.0694	Pretilachlor	NA	USEPA (2004)
Compound	cRf ^d											
Isoprotiolane	NA											
Propiconazole	0.0694											
Pretilachlor	NA											
			Based on calculation									
Absorbed Dose	DA _{event}	hr event ⁻¹	Based on calculation	From this study								
Concentration of pesticides	C	mg kg ⁻¹	Based on laboratory analysis	From this study								
Conversion factor	CF	kg mg ⁻¹	10 kg mg ⁻¹	USEPA (2004)								
Dermal absorption factor	ABS _d	-	<table><tr><th>Compound</th><th>ABS^d</th></tr><tr><td>Isoprotiolane</td><td>NA</td></tr><tr><td>Propiconazole</td><td>0.4</td></tr><tr><td>Pretilachlor</td><td>NA</td></tr></table>	Compound	ABS ^d	Isoprotiolane	NA	Propiconazole	0.4	Pretilachlor	NA	USEPA (2004)
Compound	ABS ^d											
Isoprotiolane	NA											
Propiconazole	0.4											
Pretilachlor	NA											
NA – Not Available												

Table 3.3 *continue...*

Event frequency	EV	events day ⁻¹	1	Questionnaire
				(Question 16)
Exposure duration	ED	years	27	Questionnaire
				(Question 9)
Exposure frequency	EF	days year ⁻¹	220	Questionnaire
				(Question 9)
Adherence factor of soil to skin	AF ^a	mg cm ⁻²		USEPA (2004)
		Surface area	Recommend Values for	
			Mean Solids Adherence	
			(mg cm ⁻²)	
		Forearms	0.175	
		Hands	0.533	
		Lower legs	0.04	
Absorption factor	AF ^b	-	Based on calculation	Kegley et al., (2014)
Total exposure time	ET	hour	24	Kegley et al., (2014)

Table 3.3 continue...

First-order dermal absorption		K _a	h ⁻¹	Based on calculation		Kegley et al., (2014)
coefficient						
Octanol/water partition		K _{ow}	-		3.72	PubChem, (2018)
coefficient						
Molecular weight		MW	g/mol		342.22	PubChem, (2018)
Averaging time (days)		AT	days		5940	From this study
50 th Percentile Skin Area				Surface area	50 th Percentile of Surface Area for Adults Male (cm ²)	USEPA (2004)
				Forearms	1310	
				Hands	990	
				Lower legs	2560	
Average body weight		BW	kg		70.19	Questionnaire (Question 4)

3.10 Statistical Analysis

Statistical analysis was carried out by using IBM SPSS Statistics Version 22. Spearman Rank Order correlation was used to find the association between the concentration of pesticide in paddy soil and the physico-chemical parameters (pH-H₂O, total organic carbon (TOC), total nitrogen (TN), C/N ratio, cation exchange capacity (CEC) and moisture) in Tanjung Karang paddy field.

3.11 Ethical Consideration

The ethical approval and consent was obtained from the University Research Ethical Committee of Universiti Putra Malaysia (JKEUPM) for research involving human subject and can be refer with JKEUPM-2017-204. Besides that, the consent form and the questionnaire used in this study was explained to the respondents and they were guided during fill up the questionnaire. All information given by the respondents was kept private and confidential and only used for research and scientific publication purposes.

CHAPTER 4

RESULTS

4.1 Socio-demographic background of respondents

One hundred and fifty-two farmers who work in the Kampung Sawah Sempadan paddy field were involved in this study. The socio-demographic information of respondents that obtained from the questionnaire was summarized in Table 4.1.

Table 4.1: Socio-demographic information of respondents (n=152)

Variables	Category	Frequency	Percentage (%)
Gender	Male	152	100
Race	Malay	152	100
Use of pesticides	Yes	150	98.7
	No	2	1.3
Smoking habit	Smoker	96	63.2
	Non – smoker	56	36.8

Variables	Mean (SD)	Minimum	Maximum
Age (years)	51.29 (12.604)	18	60
Weight (kg)	70.16 (12.425)	45	120

All 152 respondents that involved in this study were male and Malay farmers as the majority of the population in Kampung Sawah Sempadan, Tanjung Karang, Kuala Selangor were Malay. Most of the respondents (98.7%) that worked in the Kampung Sawah Sempadan paddy field use the pesticide to control the pests while the rest (1.3%) did not use the pesticides during working in the paddy field. The result also showed that 96 out of 152 respondents (63.2%) were smoker and only (36.8%) were non-smoker. Based on the results, the respondent's age was ranged from 18 to 60 years old, with a mean of 51 years old. The average weight of respondents was 70.16 kg and ranged from 45 to 120 kg.

4.2 Personal hygiene and Use of Personal Protective Equipment (PPE)

The paddy farmers need to use the personal protective equipment (PPE) in order to protect and minimize the dermal exposure of pesticides that in contact with paddy soils. The information regarding personal hygiene and the usage of personal protective equipment (PPE) were obtained from the questionnaire as recorded in **Table 4.2.**

Table 4.2: Personal Hygiene and Use of Personal Protective Equipment (PPE) among paddy farmers (n=152)

Variables	Categories	Frequency	Percentage (%)
Wash up / shower after handling pesticides	Yes	127	83.6
	No	25	16.4
Change clothes after handling pesticides	Yes	145	95.4
	No	7	4.6
Use full PPE during pesticides application	Yes	71	46.7
	No	81	53.3

From the results shown in **Table 4.2**, 127 respondents (83.6%) take a shower after handling with the pesticides, while another 25 respondents (16.4%) do not immediately wash up after handling with the pesticides. About 145 respondents (95.4%) changes the clothes they wear after pesticides application every day while 7 respondents (4.6%) of the farmers did not change the clothes. Out of 152 respondents, only 71 respondents (46.7%) use the full set of PPE which consist of boots and gloves, while the remaining 81 respondents (53.3%) did not wear a complete of PPE.

Table 4.3: Type of PPE used by paddy farmers (n=152)

Type of PPE	Number of paddy farmers	Percentage (%)
Boots	63	41.4
Apron	3	2.0
Overalls	17	11.2
Gloves	52	34.2
Goggles	37	24.3
Face Shield	56	36.8
Hat or hood	68	44.7

As shown in **Table 4.3**, majority of the paddy farmers wear hat or hood (44.7%) and followed by boots (41.4%), face shield (36.8%) and gloves (34.2%). Additionally, 37 out of 152 paddy farmers used goggles (24.3%) and only 17 paddy farmers used overalls (11.2%) during the paddy cultivation activities in paddy field. Meanwhile, only 2 out of 152 of paddy farmers wear apron (2.0%) during working in paddy fields.

4.3 Physico-chemical parameters of paddy soil samples

The results of physico-chemical parameters of paddy soil samples for each block include pH-H₂O, total organic carbon (TOC), total nitrogen (TN), C/N ration, cation exchange capacity (CEC) and moisture are listed in **Table 4.4**. The analysis of all the parameters was provided by Espek Research & Advisory Services (E.R.A.S), RISDA Estates Sdn. Bhd.

Table 4.4: The physico-chemical parameters of paddy soil for each of the block

Block	pH-H ₂ O	Moisture	C/N ratio	Total Organic Carbon (%)	Total nitrogen (%)	Cation exchange capacity (CEC) (meq/100 g)
A	6.1	22.0	8	1.56	0.20	8.30
B	6.2	27.4	9	2.08	0.22	14.67
C	6.3	60.3	7	1.36	0.21	6.87
D	6.2	44.9	90	3.36	0.04	11.69
E	6.5	43.0	19	4.54	0.24	11.58
F	6.3	45.3	22	5.34	0.25	17.63
G	6.2	48.6	12	2.68	0.23	12.11
H	6.8	25.2	7	1.17	0.17	13.98
I	6.3	39.2	12	2.56	0.22	11.67
J	6.6	34.6	11	2.32	0.22	16.25
K	6.5	120.4	20	4.53	0.23	8.33
L	6.7	48.5	24	6.26	0.26	20.13
M	6.5	26.5	17	3.52	0.20	5.28
N	6.8	38.0	26	4.79	0.18	20.16
O	5.9	36.6	6	0.95	0.17	20.79
P	5.7	36.6	15	3.50	0.23	16.94

Table 4.4 continue...

Q	5.9	42.4	13	3.05	0.24	19.46
R	6.0	41.4	19	4.23	0.22	16.34
S	5.9	40.2	18	3.98	0.22	17.28
T	6.1	36.3	8	1.85	0.23	21.51
U	6.3	46.7	19	4.79	0.26	20.96
V	6.3	42.4	10	2.10	0.21	17.36
W	6.1	39.3	15	3.71	0.24	14.39
X	6.0	33.7	16	3.83	0.24	16.35

Based on Table 4.4, the pH readings in paddy soil samples were ranged from 5.7 to 6.8 which are neutral. The highest readings of moisture of the paddy soil was found in Block K (120.4) and the lowest readings of moisture was 22.0 that found in Block A. The results for C/N ratio, total organic carbon (TOC), total nitrogen (TN) and cation exchange capacity (CEC) were ranged from 6 to 90, 0.95% to 6.26%, 0.04% to 0.26% and 5.28 meq/100 g to 21.51 meq/100g, respectively.

4.4 Quality Control

Calibration curves were obtained for all target compounds to determine the concentration of pesticides in the soil samples. Linear calibration graphs were constructed by plotting the peak areas against concentration of pesticides. All target compounds showed good linearity in the range between 0.01 $\mu\text{g kg}^{-1}$ and 500 $\mu\text{g kg}^{-1}$ with regression coefficients (R^2) ranging from 0.9995 to 0.9997. The instrument detection limit (IDL) and instrument quantification limit (IQL) for all target compounds ranged from 0.001 $\mu\text{g/kg}$ to 0.050 $\mu\text{g kg}^{-1}$. The method detection limit

(MDL) and method quantification limit (MQL) for all target compounds ranged from 0.040 $\mu\text{g kg}^{-1}$ to 0.500 $\mu\text{g kg}^{-1}$ respectively. Results for IDL, IQL, MDL, MQL, linear range, coefficient of determination (R^2) and recovery (RSD) are summarized in **Table 4.5**.

Table 4.5: IDL, IQL, MDL, MQL, linear range, coefficient of determination (R^2) and recovery (RSD)

Analyte	IDL ($\mu\text{g kg}^{-1}$)	IQL ($\mu\text{g kg}^{-1}$)	MDL ($\mu\text{g kg}^{-1}$)	MQL ($\mu\text{g kg}^{-1}$)	Linear Range ($\mu\text{g kg}^{-1}$)	R^2	Recovery % (mean $\pm$ SD), (100 $\mu\text{g kg}^{-1}$) n=3
Isoprothiolane	0.001	0.01	0.04	0.1	0.01-500	0.9996	96.7 $\pm$ 16
Propiconazole	0.001	0.1	0.2	0.5	0.01-500	0.9995	90.5 $\pm$ 11
Pretilachlor	0.01	0.05	0.1	0.4	0.05-500	0.9997	82.8 $\pm$ 12

4.5 Pesticides concentration in paddy soil samples

The concentration of three pesticides (isoprothiolane, propiconazole and pretilachlor) were detected in twenty four paddy block from Block A to X in Kampung Sawah Sempadan. The summarized results for the concentration of the selected compounds in paddy soil samples are listed in **Table 4.6**.

Table 4.6: Pesticides concentrations in paddy soil samples (mg kg^{-1}) (n=3)

Block	Isoprothiolane Mean $\pm$ SD	Propiconazole Mean $\pm$ SD	Pretilachlor Mean $\pm$ SD
A	2.831 $\pm$ 0.877	2.643 $\pm$ 0.818	3.864 $\pm$ 0.727
B	34.806 $\pm$ 4.248	5.607 $\pm$ 0.844	3.453 $\pm$ 0.842
C	21.139 $\pm$ 1.751	12.742 $\pm$ 1.993	9.942 $\pm$ 2.683
D	0.546 $\pm$ 0.162	7.760 $\pm$ 4.281	0.860 $\pm$ 0.531
E	1.395 $\pm$ 1.142	11.395 $\pm$ 4.682	0.435 $\pm$ 0.169
F	0.956 $\pm$ 0.096	1.346 $\pm$ 0.273	< MQL
G	42.917 $\pm$ 1.893	13.271 $\pm$ 2.499	< MQL

Table 4.6 continue...

H	0.820 ± 0.253	< MQL	< MQL
I	1.692 ± 0.2776	1.081 ± 0.455	< MQL
J	24.544 ± 3.131	25.502 ± 4.713	0.547 ± 0.246
K	21.295 ± 2.031	0.985 ± 0.457	< MQL
L	13.733 ± 0.824	3.324 ± 1.375	< MQL
M	10.254 ± 0.217	2.616 ± 0.659	< MQL
N	18.293 ± 0.611	< MQL	1.240 ± 0.189
O	4.522 ± 0.480	7.059 ± 0.857	3.389 ± 1.959
P	1.696 ± 0.357	1.430 ± 0.037	1.681 ± 0.409
Q	0.873 ± 0.307	< MQL	0.742 ± 0.074
R	2.134 ± 0.204	< MQL	0.581 ± 0.329
S	3.430 ± 0.170	< MQL	< MQL
T	2.179 ± 0.519	< MQL	1.467 ± 0.147
U	7.450 ± 0.647	4.602 ± 0.426	1.350 ± 0.279
V	11.696 ± 1.146	0.773 ± 0.631	2.628 ± 0.480
W	1.727 ± 0.612	1.884 ± 0.414	0.567 ± 0.057
X	0.565 ± 0.208	< MQL	< MQL

MQL - method quantification limit

Based on the table above, the results showed that the concentration of isoprothiolane were ranged from 0.546 mg kg⁻¹ to 42.917 mg kg⁻¹. The highest concentration of isoprothiolane was found in Block G (42.917 mg kg⁻¹) while the lowest concentration was found in Block D (0.546 mg kg⁻¹). Meanwhile, the concentration of propiconazole were ranged from 0.773 mg kg⁻¹ to 25.502 mg kg⁻¹. The paddy soil samples in block J showed the highest concentration of propiconazole (25.502 mg kg⁻¹) while sample for block V recorded the lowest concentration (0.773 mg kg⁻¹). On the other hand, the concentration of pretilachlor in paddy soil were ranged

from 0.435 mg kg⁻¹ to 9.942 mg kg⁻¹. The highest concentration of pretilachlor was 9.942 mg kg⁻¹ from paddy soil samples at block C and the lowest concentration was 0.435 mg kg⁻¹ from paddy soil samples at block E.

4.6 Correlation between the concentration of targeted compound and physico-chemical parameters

Spearman Rank Order correlation was used to determine the significant correlation coefficient between the concentration of isoprothiolane, propiconazole and pretilachlor in paddy soil samples and the physico-chemical parameters (pH-H₂O, total organic carbon (TOC), total nitrogen (TN), C/N ratio and cation exchange capacity (CEC) as summarized in **Table 4.7**.

As shown in the table, the result showed that there were significant correlations between (i) pretilachlor and C/N ratio ($r = -0.664$ $p = 0.007$) and between (ii) pretilachlor and total organic carbon (TOC) ($r = -0.677$, $p = 0.006$). Therefore, there were no significant correlation between isoprothiolane and propiconazole and the physico-chemical parameters in paddy soil samples.

Table 4.7: Correlation between the targeted compounds for all paddy blocks and the physico-chemical parameters

Physico-chemical parameters	Isoprothiolane		Propiconazole		Pretilachlor	
	Coefficient	p-value	Coefficient	p-value	Coefficient	p-value
	correlation (r) ^a		correlation (r) ^a		correlation (r) ^a	
pH-H ₂ O	0.310	0.141	0.045	0.864	-0.195	0.487
Moisture	0.181	0.396	0.045	-0.204	-0.190	0.498
C/N ratio	-0.152	0.477	-0.204	0.433	-0.664**	0.007
Total Organic Carbon (TOC)	-0.071	0.741	-0.260	0.314	-0.677**	0.006
Total nitrogen (TN)	-0.076	0.723	-0.136	0.603	-0.438	0.103
Cation Exchange Capacity (CEC)	-0.045	0.834	-0.059	0.823	-0.032	0.909

a-Spearman Rank Order Correlation

** Correlation is significant at the 0.01 level (2-tailed)

4.7 Exposure of pesticides

In order to assess the dermal health risk assessment of the farmers, information on exposure of the farmers to pesticides were needed. This information was obtained from the interview by using the questionnaire. All of the information of farmer’s exposure to pesticides is tabulated in **Table 4.8**.

Table 4.8: Information of farmer’s exposure to pesticides (n=152)

Parameters	Average
t_{event} (hr event ⁻¹)	2
Event frequency (events day ⁻¹)	1
Exposure duration (years)	27
Exposure frequency (days year ⁻¹)	220
Averaging time (days)	5940

Based on **Table 4.8**, the average of t_{event} was 2 hours that refer to the hour of the farmers in contact with the pesticides in paddy soil per event. Event frequency (EV) is how many times the farmers involve in paddy cultivation activities in a day and the result showed that they work in paddy field once a day. Meanwhile, the exposure duration (ED) is refer to the number of years that the farmers have involved in the activity. The result showed that the average ED for the respondents was 27 years. The number of days per year of exposure is refer to exposure frequency (EF) which is 220 days and the averaging time (AT) is the product of ED and EF which is 5940 days.

4.8 Dermal health risk assessment

The assessment through the Hazard Quotient for non-carcinogenic effects was used to estimate the health risk of paddy farmers who were exposed to pesticides through dermal contact. The HQ was calculated only for propiconazole as depicted in **Table 4.9**. Therefore, the certain possible body surfaces that were considered to estimate the health risk of the paddy farmers by calculating HQ include forearms, hands and lower legs.

Table 4.9: Hazard Quotient (HQ) for targeted compound Propiconazole

BLOCK	HQ			HQ (Total)
	Forearm	Hands	Lower Legs	
A	1.578 x 10 ⁻⁵	3.705 x 10 ⁻⁵	7.289 x 10 ⁻⁶	6.012 x 10 ⁻⁵
B	3.740 x 10 ⁻⁵	8.608 x 10 ⁻⁵	1.67 x 10 ⁻⁵	1.402 x10 ⁻⁴
C	8.499 x 10 ⁻⁵	1.956 x 10 ⁻⁴	3.796 x 10 ⁻⁵	3.186 x 10 ⁻⁴
D	5.176 x 10 ⁻⁵	1.191 x 10 ⁻⁴	2.312 x 10 ⁻⁵	1.939 x 10 ⁻⁴
E	7.601 x 10 ⁻⁵	1.749 x 10 ⁻⁴	3.395 x 10 ⁻⁵	2.849 x 10 ⁻⁴
F	8.978 x 10 ⁻⁶	2.067 x 10 ⁻⁵	4.01 x 10 ⁻⁶	3.366 x 10 ⁻⁵
G	8.852 x 10 ⁻⁵	2.038 x 10 ⁻⁴	3.954 x 10 ⁻⁵	3.319 x 10 ⁻⁴
I	7.211 x 10 ⁻⁶	1.66 x 10 ⁻⁵	3.221 x 10 ⁻⁶	2.703 x 10 ⁻⁵
J	1.701 x 10 ⁻⁴	3.915 x 10 ⁻⁴	7.598 x 10 ⁻⁵	6.376 x 10 ⁻⁴
K	6.570 x 10 ⁻⁶	1.471 x 10 ⁻⁵	2.935 x 10 ⁻⁶	2.421 x 10 ⁻⁵
L	2.217 x 10 ⁻⁵	5.103 x 10 ⁻⁵	9.903 x 10 ⁻⁶	8.31 x 10 ⁻⁵
M	1.745 x 10 ⁻⁵	4.016 x 10 ⁻⁵	7.794 x 10 ⁻⁶	6.54 x 10 ⁻⁵

Table 4.9 continue...

O	4.709×10^{-5}	1.084×10^{-4}	2.103×10^{-5}	1.765×10^{-4}
P	9.538×10^{-6}	2.195×10^{-5}	4.261×10^{-6}	3.575×10^{-5}
U	3.069×10^{-5}	7.065×10^{-5}	1.371×10^{-5}	1.151×10^{-4}
V	5.156×10^{-6}	1.187×10^{-5}	2.303×10^{-6}	1.933×10^{-5}
W	1.257×10^{-5}	2.893×10^{-5}	5.613×10^{-6}	4.711×10^{-5}

Based on the result tabulated in **Table 4.9**, the HQ of propiconazole for forearm, hands and lower leg exposure were ranged from 1.933×10^{-5} to 6.376×10^{-4} . The highest HQ of propiconazole for forearm, hands and lower leg exposure was recorded at block J with 6.376×10^{-4} . Meanwhile, the lowest HQ of propiconazole among all the blocks was found in block V with 1.933×10^{-5} for all of the exposures. Therefore, HQ calculated for forearm exposure, hands exposure and lower legs exposure were less than 1 for each of the block. Thus, there were no significant health risks due to dermal exposure to pesticides.

CHAPTER 5

DISCUSSION

5.1 Socio-demographic background of respondents

In this study, one hundred and fifty-two farmers who work in the Kampung Sawah Sempadan paddy field were involved in this study. All of the information of the paddy farmers was obtained from the questionnaire. Based on the results obtained, all the paddy farmers that involved in this study were male farmers as reported by Galiè, Jiggins, & Struik (2013), the farm activity is usually dominated by male-related characteristics such as productive and able to do heavy work. The race of all the paddy farmers were Malay as they were the majority of the population in Kampung Sawah Sempadan. Most of the respondents that worked in the Kampung Sawah Sempadan paddy field use the pesticide to control the pests while the rest did not use the pesticides during working in the paddy field. From the results obtained in **Table 4.1**, half of the paddy farmers were smokers. A study by Antle & Pingali (2018) found that the risk of respiratory abnormalities is significantly related to pesticide exposure even when the confounding effect of smoking is accounted for.

5.2 Personal hygiene and Use of Personal Protective Equipment (PPE)

Personal protective equipment (PPE) can substantially reduce the risk exposure from pesticide spraying (Sharif, Damalas, & Abdollahzadeh, 2017). In this study, the

information regarding the personal hygiene and usage of personal protective equipment (PPE) were obtained from the questionnaire as it is important to reduce the dermal health risk of the paddy farmers that in contact directly with the pesticides. Most of the paddy farmers immediately take a shower and changes the clothes after handling with the pesticides indicated that they are practiced and aware of their personal hygiene after worked in paddy fields. However, there are half of the paddy farmers did not wear a complete PPE. A study by Sharif et al., (2017) reported that the main reasons why the farmers did not wear PPE because of the discomfort (50.6%), the unavailability of PPE when needed (23.4%), the extra time required for using it (15.6%), and the non-necessity of PPE for every situation (15.6%).

Based on Table 4.3, majority of the paddy farmers wear hat or hood (44.7%) and followed by boots (41.4%), face shield (36.8%) and gloves (34.2%). Additionally, 37 out of 152 paddy farmers used goggles (24.3%) and only 17 paddy farmers used overalls (11.2%) during the paddy cultivation activities in paddy field. Meanwhile, only 2 out of 152 of paddy farmers wear apron (2.0%) during working in paddy fields. In this study, Malaysian Standard Code of recommended practice (MS 479:2012) was used as a guideline for the safe handling of agricultural pesticides and to determine the adequacy of personal protective equipment for dermal exposure. MS 479:2012 stated that when handling the pesticides, the following articles of clothing should be worn during mixing or loading of the pesticides and spraying of the pesticides: (i) regular work clothes (long pants and sleeves; heavyweight and tightly woven fabrics of cotton or polyester/cotton blends); (ii) overalls (durable cotton fabric and must be washed immediately after used); (iii) aprons (rubber or polyvinyl chloride (PVC) aprons); (iv) boots (rubber or PVC boots); (v) gloves (PVC, rubber, nitrile or neoprene gloves

should be used in handling concentrates but are unsuitable for continuous wear); and (vi) face shield (face shields are intended for face protection); and (vii) wide-brimmed hat or hood (may be a hat with a broad brim or a full hood that drapes to cover the shoulders) (Department of Standards Malaysia, 2012).

5.3 Pesticides concentration in paddy soil samples

Among these three targeted compounds in paddy soil, the highest mean concentration was isoprothiolane (9.646 mg kg^{-1}) and followed by propiconazole (6.113 mg kg^{-1}). A study reported by Nishina et al. (2010), the Vietnamese government has set the maximum allowable concentration (MAC) in soils at 0.1 mg kg^{-1} for isoprothiolane and fenobucarb. Nishina et al. (2010) reported that the detection results of the residue of isoprothiolane in the soil was $0.0166 \text{ mg kg}^{-1}$ and the result was below than the MAC standard in Vietnam. In this study, the concentration of isoprothiolane (9.646 mg kg^{-1}) in the paddy soil was higher compared to the study by Nishina et al. (2010).

Based on the previous study by Zhang et al. (2015) in Chinese field, it has been reported that the maximum concentration of propiconazole residues in soil was 0.45 mg kg^{-1} which is lower as compared to this study (6.113 mg kg^{-1}). The main factors for pesticide residue in crops are based on the pesticides application times, rates and pre-harvest intervals (Zhang et al., 2015). Meanwhile, pretilachlor was the lowest mean concentration (2.183 mg kg^{-1}) among the three targeted compounds in the paddy soil for all the paddy blocks. However, the concentration of pretilachlor found in this study was higher compared to study by Liu, Yu, Fang, Fan, & Wang (2011) reported

that the concentration of pretilachlor in the soil samples in China were ranged from 0.082 to 0.791 mg kg⁻¹.

Based on the result shown in **Table 4.6**, most of the concentration of pretilachlor and propiconazole were below than MQL that might be due to the low frequency of pesticides application during the sampling period. The paddy soil samples were collected one week before the harvest season which means they are not using the herbicides (pretilachlor) to kill the weeds. However, isoprothiolane was detected in every paddy block due to the paddy farmers tend to use fungicide (isoprothiolane) to protect the production by controlling the fungi.

5.4 Correlation between the concentration of targeted compound and physico-chemical parameters

The physico-chemical parameters of the paddy soil may affect the concentration of the targeted compound (isoprothiolane, propiconazole and pretilachlor) in paddy field at Kampung Sawah Sempadan, Tanjung Karang, Kuala Selangor. The results obtained from this study reported that there were significant correlations between pretilachlor and C/N ratio. The C:N ratio is refer to the mass of carbon to the mass of nitrogen in a particular substance. It is important because due to the fact that it has a direct impact on residue decomposition and also nitrogen cycling in the paddy soils. Thus, C:N ratio can affect the concentration of pretilachlor in paddy soil eventhough it was the lowest mean concentration among the three targeted compounds.

According to USEPA (2002), the naturally-occurring organic carbon sources are the sources that are derived as a result of contamination through anthropogenic activities. The spills or releases of contaminants into the environment can increase the total carbon content present in the soil or sediment. Based on the **Table 4.7**, there were negative correlation for both physico-chemical parameters between pretilachlor and total organic carbon (TOC) and pretilachlor and C/N ratio. It can be described when the number of total organic carbon (TOC) and C/N ratio increases, it can causing the decreasing number of concentration of pretilachlor in paddy soils as pretilachlor was the lowest mean concentration among the three targeted compounds in this study. To the best of author's knowledge, the correlation between pretilachlor and TOC has never been reported in paddy soil. However, a study by Gong et al. (2004) reported that there was positive correlation between TOC and DDT ($r = 0.371$, $p < 1.5 \times 10^{-7}$) in soil at China. On the other hand, there were no significant correlation between isoprothiolane and propiconazole with the physico-chemical parameters in paddy soil samples.

5.5 Dermal health risk assessment

According to USEPA (2016), human health risk assessment is the process of estimating the nature and probability of adverse health effects in humans who may be exposed to chemicals in contaminated environmental media. This study focuses on the risk assessment through dermal exposure of the paddy farmers that in contact with paddy soils. In order to estimate the dermal health risk of paddy farmers, the Hazard Quotient for non-carcinogenic effects was calculated. The HQ was calculated only for propiconazole because of the reference dose value for isoprothiolane and pretilachlor were not available in the risk assessment database.

The certain possible exposures of body surfaces were considered to estimate the dermal health risk of the paddy farmers that exposed to propiconazole by calculating HQ including forearms, hands and lower legs in which it can be describe as body-part-weighted adherence factors to be calculated and used in estimating exposure via dermal contact with soil based on assumed exposed body parts. As reported in **Table 4.9**, the highest HQ of propiconazole was found at block J with 6.376×10^{-4} for forearm, hands and lower legs exposure. Meanwhile, the lowest HQ of propiconazole among all the blocks was found at block V with 1.933×10^{-5} for all of the exposures. However, some of the paddy blocks did not have HQ values due to the concentrations of propiconazole in paddy soil were below than MQL. The HQ calculated for forearm exposure, hands exposure and lower legs exposure were less than one for every paddy block indicating that the risk of pesticides exposure was not significant. Thus, there were no significant chronic non-carcinogenic dermal health risks due to occupational exposure of propiconazole in paddy soil ($HQ < 1$) for paddy farmers that worked in Block A to Block X.

To the best of author's knowledge, there has been no publication on the non-carcinogenic dermal health risk due to the occupational exposure of propiconazole in paddy soil. However, a study by Hamsan et al. (2017) reported that HQ value for propiconazole through inhalation exposure was 7.17×10^{-5} which was less than one ($HQ < 1$) indicating the risk of inhalation exposure to mix pesticides was not significant.

CHAPTER 6

CONCLUSION AND RECOMMENDATION

6.1 Conclusion

This study showed that the targeted compound of pesticides which are isoprothiolane, propiconazole and pretilachlor in the paddy soils at Kampung Sawah Sempadan present in low concentration. Among the three target compounds, the highest mean concentration in the paddy soil was isoprothiolane with 9.646 mg kg^{-1} , followed by propiconazole (6.113 mg kg^{-1}) and pretilachlor (2.183 mg kg^{-1}).

Based on the results obtained, there were significant correlations between (i) pretilachlor and C/N ratio ($r = -0.664$ $p = 0.007$) and between (ii) pretilachlor and total organic carbon (TOC) ($r = -0.677$, $p = 0.006$). Therefore, there were no significant correlation between isoprothiolane and propiconazole and the physico-chemical parameters in paddy soil samples.

The HQ calculated only for compounds propiconazole was below the value of 1, indicating the chronic non-carcinogenic dermal health risk to propiconazole in paddy soil among farmers was not significant.

6.2 Limitation of this study

There are several limitations of this study that should be considered to improve on further research. One of them is the Hazard Quotient value for isoprothiolane and propiconazole cannot be calculated due to unavailable data of reference dose value in any database. Even though the hazard quotient for propiconazole was less than one ($HQ < 1$), but the actual risk could be greater than the result in this study because of the mixture of other pesticides used by the paddy farmers in paddy field. The health risk of the paddy farmers was only assessed by dermal exposure of pesticides. Another possible exposure pathways for pesticides in paddy soil are oral exposure. As most of the paddy farmer worked in paddy field using bare hands, the farmers may accidentally ingest the pesticides if they are not taking care of good personal hygiene. However, this route of exposure was not assessed in this study as the likelihood of the accidental ingestion of the soil was low compared to the dermal exposure in this case. However, if the oral exposure happens, the concern could be higher than the dermal exposure. Thus, the consideration of this pathway in the further study is recommended.

6.3 Recommendation

6.3.1 Recommendation for future studies

Future studies on the health risk of other pesticides are recommended since the paddy farmers are using other popular pesticides that are not listed in this study. Different route of exposures such as inhalation and ingestion should be considered for further study as the paddy farmers may accidentally ingest the pesticides if they are not taking care of good personal hygiene and do not wear a complete set of PPE while spraying pesticides.

6.3.2 Recommendation for farmers

The paddy farmers of Kampung Sawah Sempadan should be trained on how to use a proper PPE in order to reduce the risk of exposure to pesticide. The health promotion or campaign on the effect of the pesticides also should be provided by the employer to create awareness among the paddy farmers. Higher levels of education, gives farmers better access to information about pesticides and more knowledge of how much pesticides to use. While less educated farmers may be hampered in their ability to access information about pesticides and follow recommended safety and application guidelines (Galt, 2008).

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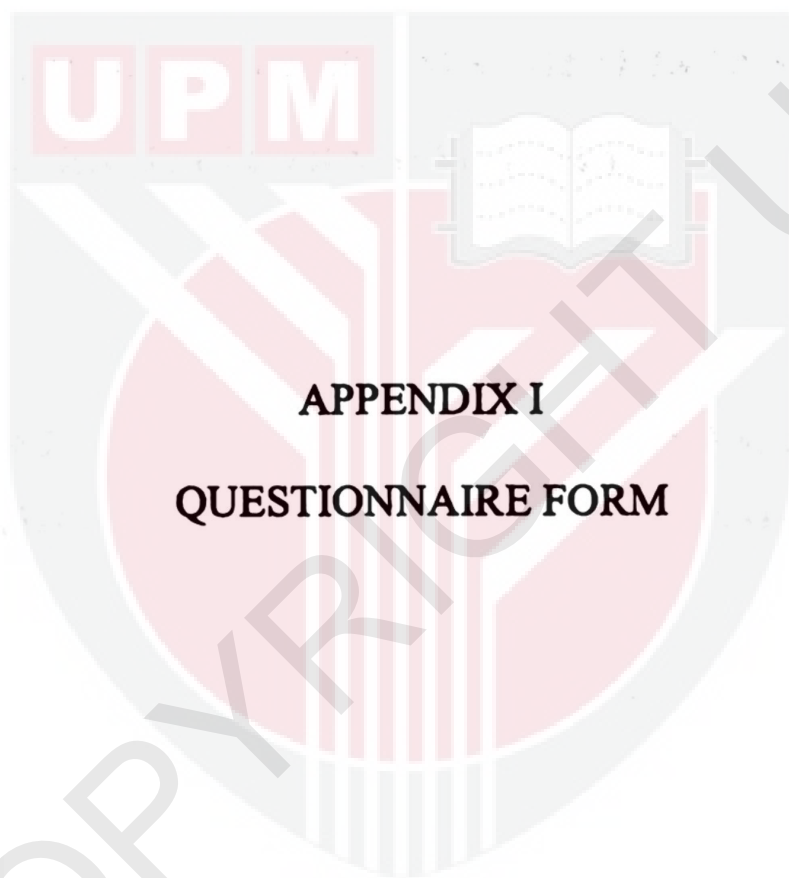
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APPENDIX I
QUESTIONNAIRE FORM



JABATAN KESIHATAN PEKERJAAN & PERSEKITARAN

FAKULTI PERUBATAN DAN SAINS KESIHATAN

UNIVERSITI PUTRA MALAYSIA

**PENDEDAHAN KEPADA RACUN PEROSAK DALAM KALANGAN
PESAWAH DI KAMPUNG SAWAH SEMPADAN, TANJUNG KARANG.**

ARAHAN SOALAN:

1. Borang soal selidik ini mengandungi **Tiga (3)** bahagian iaitu:

Bahagian A : Maklumat Diri

Bahagian B : Pendedahan kepada Racun Perosak

Bahagian C : Penggunaan Kelengkapan Pelindung Diri

2. Anda diminta menjawab semua soalan yang ada dalam buku soalan ini

3. Buku soalan ini hendaklah dikembalikan kepada pengkaji setelah selesai menjawab

PENDEDAHAN KEPADA RACUN PEROSAK DALAM KALANGAN
PESAWAH DI KAMPUNG SAWAH SEMPADAN, TANJUNG KARANG

KEGUNAAN
PENYELIDIK

BAHAGIAN A: MAKLUMAT DIRI

1. Umur: Tahun

2. Jantina:

☐
☐

Lelaki

Perempuan

3. Bangsa:

☐
☐
☐
☐

Melayu

Cina

India

Lain-lain (sila nyatakan):

4. Berat: kg

5. Tinggi: m

6. Jisim berat badan: kg/m^2

7. Adakah anda merokok?

☐
☐

Ya

Tidak

8. Pekerjaan sekarang:

SULIT

PENDEDAHAN KEPADA RACUN PEROSAK DALAM KALANGAN
PESAWAH DI KAMPUNG SAWAH SEMPADAN, TANJUNG KARANG

KEGUNAAN
PENYELIDIK

BAHAGIAN B: PENDEDAHAN KEPADA RACUN PEROSAK

9. Tempoh bekerja di tempat sekarang: bulan/ tahun

10. Pekerjaan terdahulu:

11. Tempoh bekerja di tempat dahulu: bulan/ tahun

12. Adakah anda mandi selepas ke tempat kerja?

☐	Ya
☐	Tidak

13. Adakah anda menukar pakaian kerja setiap hari?

☐	Ya
☐	Tidak

14. Adakah anda menggunakan racun perosak pada tanaman anda?

☐	Ya
☐	Tidak

15. Nyatakan kekerapan anda menggunakan racun perosak:

..... kali/ hari

atau

..... kali/ minggu

atau

..... kali/bulan

atau

..... kali/ tahun

SULIT

BAHAGIAN C: PENGGUNAAN KELENGKAPAN PELINDUNG DIRI

16. Adakah anda menggunakan kelengkapan pelindung diri semasa bekerja?

☐	Ya
☐	Tidak

17. Jika “Ya”, apakah jenis kelengkapan pelindung diri yang digunakan semasa bekerja?

- a. But
- b. Apron
- c. Baju kalis air
- d. Alat penafasan
- e. Sarung tangan
- f. Cermin mata
- g. Topeng
- h. Penutup kepala

18. Jika “Ya”, berapa kerapkah anda memakai kelengkapan pelindung diri?

- a. Sentiasa (setiap kali turun ke sawah)
- b. Hampir sentiasa (2-4 kali seminggu)
- c. Jarang (sekali seminggu)
- d. Sangat jarang (kurang daripada 4 kali sebulan)

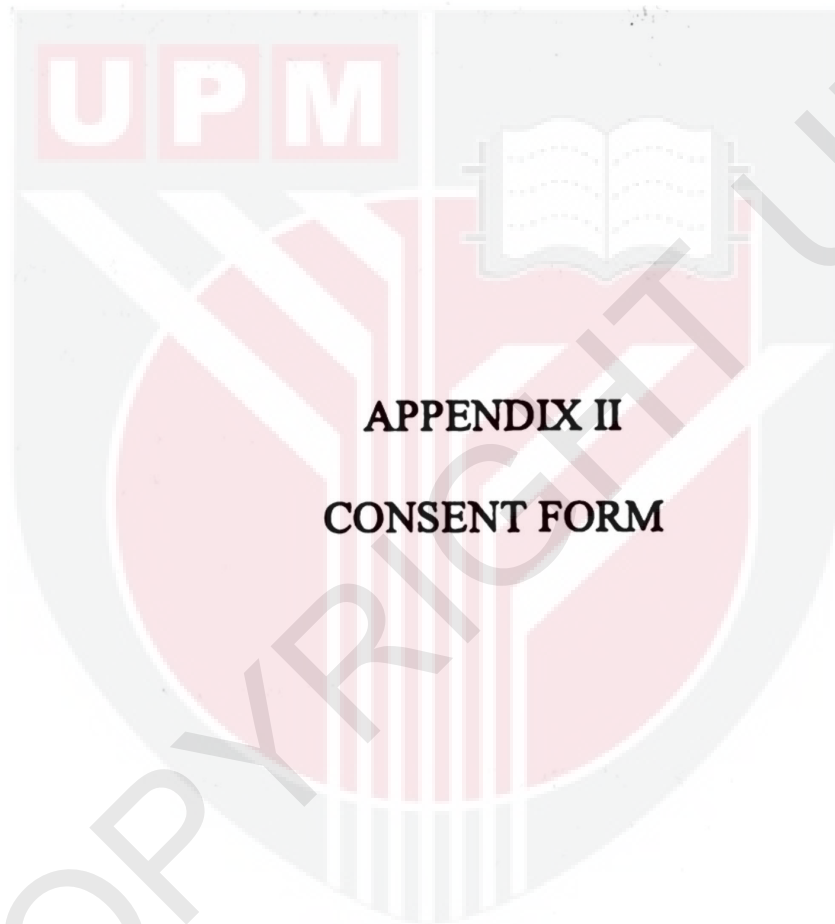
SULIT

19. Kekerapan anda menukar kelengkapan pelindung diri:

- a. Setiap hari
- b. Seminggu sekali
- c. Dua minggu sekali
- d. Sebulan sekali
- e. Lain-lain (sila nyatakan)

-Tamat soal-selidik-

SULIT



APPENDIX II
CONSENT FORM

UPM



**JAWATANKUASA ETIKA UNIVERSITI UNTUK
PENYELIDIKAN MELIBATKAN MANUSIA (JKEUPM)
UNIVERSITI PUTRA MALAYSIA, 43400 UPM SERDANG,
SELANGOR, MALAYSIA**

BORANG 2.4: PENERANGAN DAN PERSETUJUAN RESPONDEN

Sila baca maklumat berikut dengan teliti. Sekiranya anda mempunyai sebarang pertanyaan, sila kemukakan kepada penyelidik.

1.TAJUK KAJIAN

Kesan pendedahan kepada tanah padi yang dicemari oleh *isoprothiolane*, *propiconazole* dan *pretilachlor* dan penilaian risiko kesihatan kulit di kalangan pesawah padi di kawasan sawah padi Tanjung Karang, Kuala Selangor.

2. PENGENALAN

Isoprothiolane, *propiconazole* dan *pretilachlor* adalah racun perosak. Pesawah adalah kumpulan yang berpotensi tinggi terdedah kepada racun perosak kerana mereka menggunakan racun perosak dalam aktiviti penanaman padi. Terdapat kajian sebelum ini didapati bahawa racun perosak menyebabkan masalah kesihatan kepada manusia. Penilaian risiko kesihatan dijalankan untuk menganggarkan kebarangkalian berlakunya penyakit kulit kronik jika terdedah kepada bahan kimia. Kajian ini dijalankan untuk mengenal pasti risiko penyakit kulit kronik yang mungkin berlaku di kalangan pesawah padi kerana penggunaan sisa racun perosak dalam aktiviti penanaman padi. Oleh itu, para pesawah padi akan dinasihatkan untuk mengambil langkah berhati-hati semasa musim meracun padi.

3. APAKAH YANG PERLU ANDA LAKUKAN?

Responden dikehendaki untuk menjawab semua soalan yang terdapat di dalam borang kaji selidik yang dibekalkan oleh penyelidik. Selain itu, berat badan responden juga akan diambil oleh penyelidik. Pengukuran berat badan diukur oleh penyelidik menggunakan skala berat badan peribadi setelah responden menjawab borang kaji selidik di Kampung Sawah Sempadan, Tanjung Karang, Kuala Selangor. Walau bagaimanapun, penyertaan adalah sukarela. Keengganan untuk menyertai tidak akan melibatkan penalti atau tindakan apapun. Para responden yang mungkin tidak buta huruf atau tidak berpendidikan juga akan dibantu dan dijelaskan oleh penyelidik untuk menjawab semua soalan dalam borang kaji selidik. Di samping itu, penyelidik akan menggunakan pembiayaan yang disediakan oleh Geran Putra Vot. nombor 9478100 untuk tujuan penyelidikan ini.

4. SIAPA YANG TIDAK BOLEH MENYERTAI KAJIAN INI?

Penduduk Kampung Sawah Sempadan yang tidak terlibat dalam aktiviti pertanian padi dan pesawah yang tidak menggunakan racun perosak dalam tanaman. Selain itu, kanak-kanak yang di bawah umur 18 tahun juga tidak dibenarkan terlibat dalam kajian ini. Kajian ini melibatkan pesawah yang berumur antara 18 tahun sehingga 60 tahun.

5. APAKAH FAEDAH MENYERTAI KAJIAN INI?

a) KEPADA ANDA SEBAGAI PESERTA?

Kajian ini akan menilai sama ada terdapat risiko pendedahan kesihatan kronik semasa aktiviti penanaman padi adalah penting atau tidak penting. Di samping itu, anda akan menerima sejumlah RM10.00 sebagai tanda terima kasih atas penyertaan anda dalam kajian ini. Penyelidik akan memberikan pampasan kepada anda selepas menerima borang persetujuan yang anda telah dimaklumkan dan borang kaji selidik anda yang lengkap.

b) KEPADA PENYELIDIK?

Penyelidik akan dapat mengumpul maklumat mengenai kehadiran *isoprothiolane*, *propiconazole* dan *pretilachlor* dalam tanah di kawasan sawah padi Kampung Sawah Sempadan dan seterusnya mengkaji kesan kehadiran *isoprothiolane*, *propiconazole* dan *pretilachlor* ini kepada kesihatan pesawah padi yang terdedah kepada tanah yang dicemari dengan racun perosak ini.

6. ADAKAH IA BERISIKO?

Terdapat risiko minimum. Proses penyelidikan hanya memerlukan responden untuk menjawab borang kaji selidik sahaja.

7. ADAKAH MAKLUMAT DAN IDENTITI SAYA KEKAL RAHSIA?

Ya, segala maklumat dan identiti responden adalah sulit dan akan kekal rahsia dan hanya akan digunakan untuk tujuan kajian sahaja. Setelah selesai kajian, semua maklumat peribadi responden akan dibuang dan identiti tidak akan didedahkan.

8. SIAPA YANG SAYA PERLU HUBUNGI SEKIRANYA SAYA MEMPUNYAI SOALAN TAMBAHAN SEMASA MENGIKUTI PENYELIDIKAN INI?

Responden boleh menghubungi penyelidik kajian ini:

0167090029

ZAHIRA BINTI ISHAK @ MOHD FARID

JABATAN KESIHATAN PERSEKITARAN DAN PEKERJAAN,

FAKULTI PERUBATAN DAN SAINS KESIHATAN,

UNIVERSITI PUTRA MALAYSIA,

43400 UPM SERDANG,

SELANGOR, MALAYSIA.

0126140221 / 0389472396

HO YU BIN, Ph.D

JABATAN KESIHATAN PERSEKITARAN DAN PEKERJAAN,

FAKULTI PERUBATAN DAN SAINS KESIHATAN,

UNIVERSITI PUTRA MALAYSIA,

43400 UPM SERDANG,

SELANGOR, MALAYSIA.

Sila tandatangan di sini sekiranya anda telah membaca dan memahami kandungan halaman ini _____

9. PERSETUJUAN

Saya..... No Kad Pengenalan.
beralamat.....
.....dengan ini bersetuju untuk mengambil bahagian secara sukarela dalam penyelidikan
yang tersebut di atas *(kajian klinikal/percubaan ubat-ubatan/rakaman video/kumpulan sasaran/temuduga/
soal selidik).

Saya telah diberi penjelasan secara menyeluruh mengenai penyelidikan ini dari segi metodologi, risiko dan
komplikasi (seperti tertulis pada Helaiian Penerangan Responden). Saya memahami bahawa saya berhak
menarik diri dari penyelidikan ini pada bila-bila masa tanpa memberi sebarang alasan.Saya juga
memahami bahawa sebarang maklumat yang berkaitan identiti saya akan dirahsiakan.

Saya* berminat / tidak berminat untuk mengetahui keputusan kajian yang melibatkan saya.

I setuju/tidak bersetuju untuk imei/gambar/rakaman video/ rakaman suara digunakan dalam apa jua
bentuk penerbitan atau pembentangan. (sekiranya berkaitan).

*potong yang tidak berkenaan

Tandatangan Tandatangan
(Responden) (Saksi)

Tarikh :..... Nama :.....
No. K/P:

Saya mengesahkan bahawa saya telah menerangkan kepada responden ini sifat dan tujuan
penyelidikan yang tersebut di atas.

Tarikh Tandatangan
(Penyelidik)



APPENDIX III
ETHICAL APPROVAL

UPM

**ETHICS COMMITTEE FOR RESEARCH INVOLVING HUMAN SUBJECTS
(JKEUPM)
UNIVERSITI PUTRA MALAYSIA**

Research title	: Contamination of Isoprotiolane, Propiconazole and Pretilachlor in Paddy Soil and Their Associate Health Risk to Paddy Farmers in Tanjung Karang, Kuala Selangor
Study Site	: Tanjung Karang, Kuala Selangor
JKEUPM Ref No.	: JKEUPM-2017-204
Researcher	: Zahira bt Ishak @Mohd Farid
Supervisor	: Dr. Ho Yu Bin

Documents received and reviewed with reference to the above study:

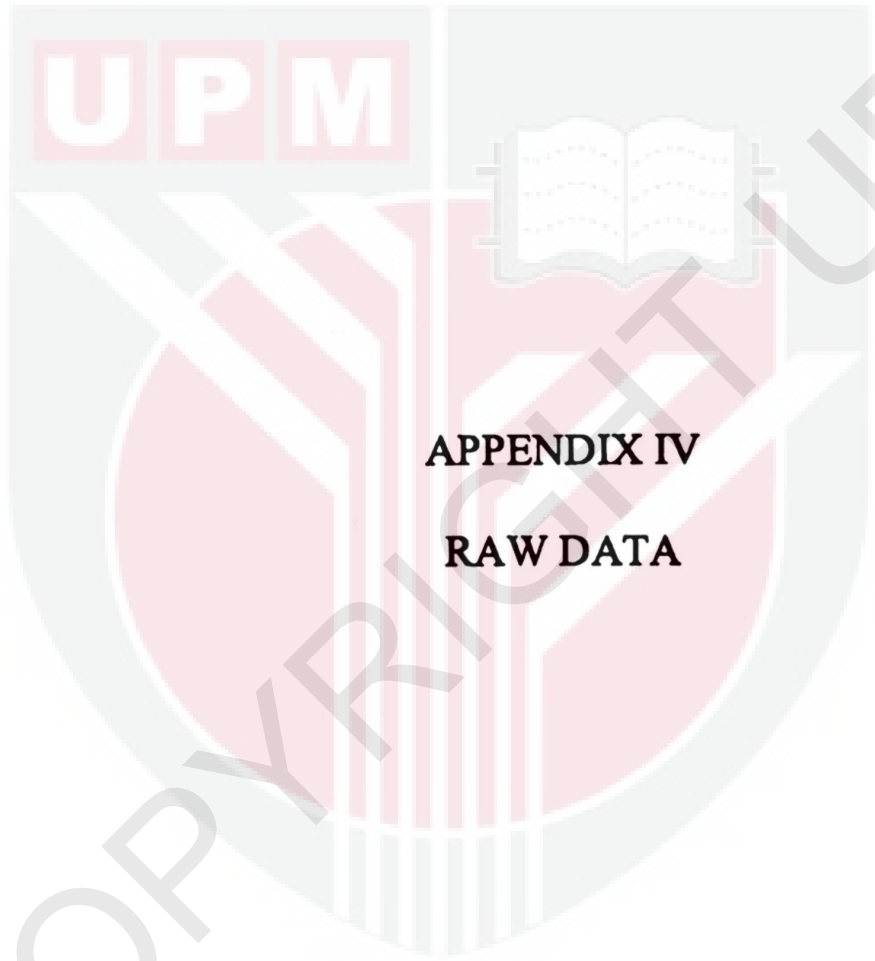
1. Ethics Application Form, Version 1 dated 31/10/2017
2. Respondent Information Sheet & Consent (English), Version 3 dated 21/2/2018
3. Respondent Information Sheet & Consent (Malay), Version 1 dated 31/1/2017
4. Proposal (English), Version 3 dated 9/1/2018
5. Questionnaire (Malay), Version 1 dated 31/10/2017
6. Curriculum Vitae of:
 - a. Dr. Ho Yu Bin
 - b. Assoc Prof. Dr. Sarva Mangala Praveena

The University Research Ethics Committee, Universiti Putra Malaysia (JKEUPM) operates in accordance to the ICH-GCP Guidelines.

Decision by JKEUPM:

- ☒ Approved
- ☒ **Permission MUST BE OBTAINED** from the respective hospitals/ institutions before conducting the research
- ☐ Disapproved

Please note that the approval is **VALID UNTIL 19 MARCH 2019**



APPENDIX IV
RAW DATA

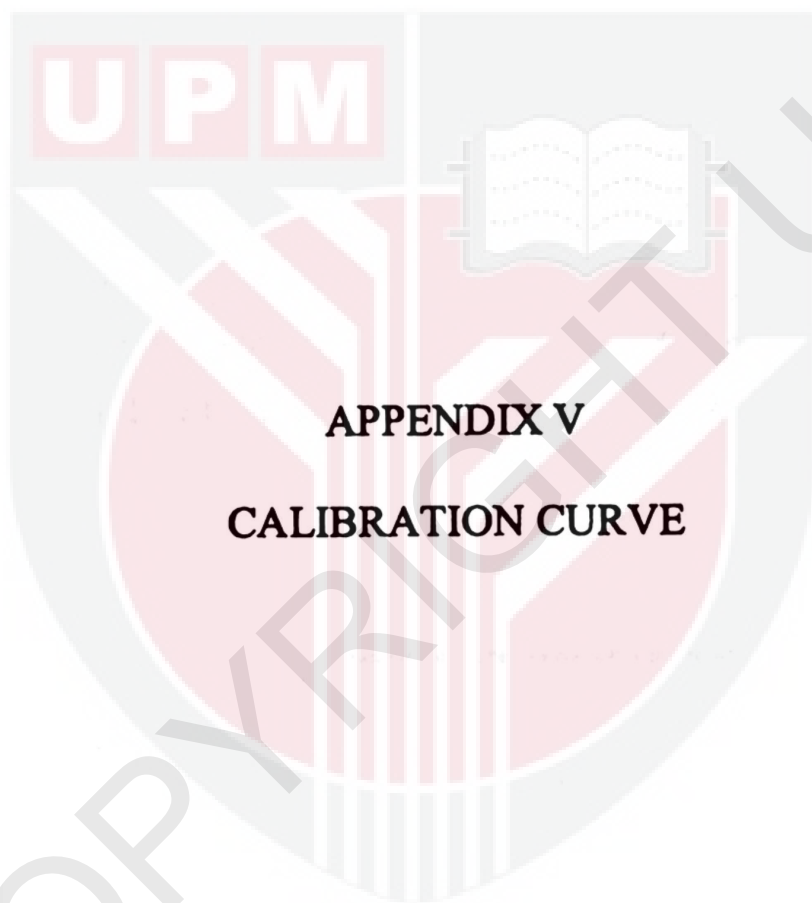
Table 1: Pesticides concentration in paddy soil sample for each replication

Block	Isoprophthiolane (mg kg ⁻¹)	Propiconazole (mg kg ⁻¹)	Pretilachlor (mg kg ⁻¹)
A1	2.024	2.481	3.026
A2	3.763	1.917	4.332
A3	2.702	3.529	4.233
B1	29.940	5.128	4.186
B2	37.773	5.110	2.534
B3	36.705	6.581	3.638
C1	19.117	10.860	7.146
C2	22.150	12.534	12.495
C3	22.150	14.830	10.183
D1	0.683	6.048	0.595
D2	0.367	4.600	0.513
D3	0.586	12.632	1.470
E1	0.877	16.501	0.477
E2	2.704	10.377	0.249
E3	0.603	7.305	0.578
F1	1.005	1.609	0.202
F2	1.016	1.361	0.185
F3	0.845	1.065	0.185
G1	40.730	16.156	0.221
G2	44.010	11.827	0.181
G2	44.010	11.827	0.181
H1	0.611	0.687	0.265
H2	0.747	0.375	0.128

H3	1.101	0.255	0.114
I1	1.592	0.632	0.104
I2	2.005	1.541	0.232
I3	1.477	1.069	0.152
J1	26.567	29.728	0.272
J2	26.125	20.418	0.616
J3	20.938	26.357	0.750
K1	18.964	0.771	0.428
K2	22.689	1.509	0.156
K3	22.230	0.673	0.312
L1	14.127	2.456	0.280
L2	12.785	4.909	0.304
L3	14.285	2.604	0.229
M1	10.397	2.457	0.286
M2	10.004	2.051	0.294
M3	10.360	3.339	0.548
N1	18.241	< MQL	1.454
N2	18.927	< MQL	1.095
N3	17.709	< MQL	1.170
O1	4.430	6.631	5.648
O2	4.094	8.045	2.157
O3	5.042	6.500	2.362
P1	1.676	1.426	2.049
P2	2.062	1.394	1.752
P3	1.349	1.468	1.240

Q1	0.853	< MQL	0.708
Q2	0.576	< MQL	0.826
Q3	1.189	< MQL	0.689
R1	1.985	< MQL	0.413
R2	2.367	< MQL	0.961
R3	2.049	< MQL	0.369
S1	3.370	< MQL	< MQL
S2	3.622	< MQL	< MQL
S3	3.298	< MQL	< MQL
T1	2.648	< MQL	1.617
T2	2.240	< MQL	1.322
T3	3.270	< MQL	1.462
U1	6.741	4.929	1.028
U2	7.601	4.120	1.493
U3	8.008	4.756	1.528
V1	12.842	1.464	3.179
V2	10.549	0.627	2.410
V3	11.697	0.228	2.297
W1	1.355	1.598	0.574
W2	1.392	1.697	0.506
W3	2.434	2.359	0.621
X1	0.798	< MQL	0.173
X2	0.397	< MQL	0.119
X3	0.499	< MQL	0.496

MQL – Method quantification limit



APPENDIX V
CALIBRATION CURVE

UPM

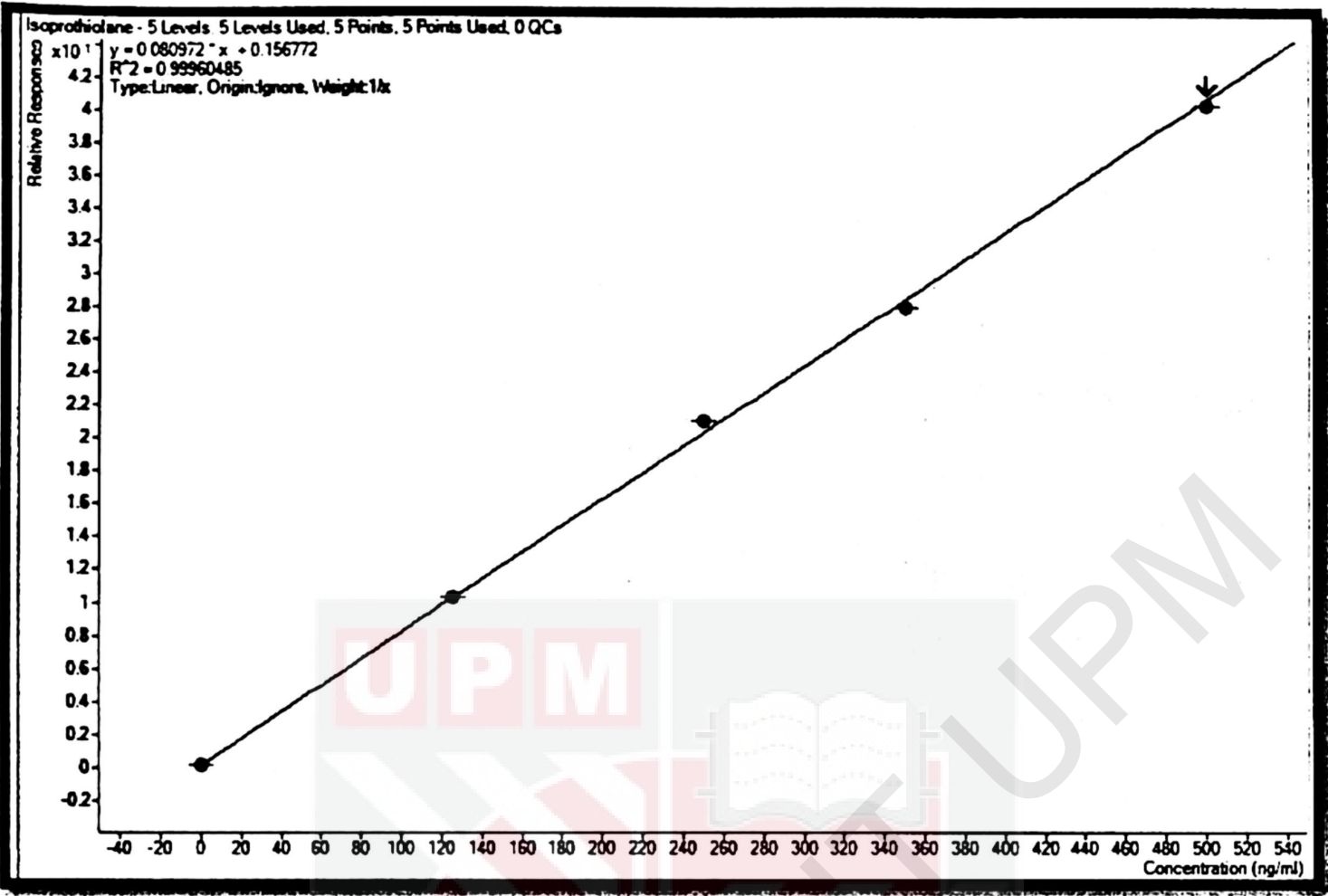


Figure 1: The calibration curve for isoprothiolane

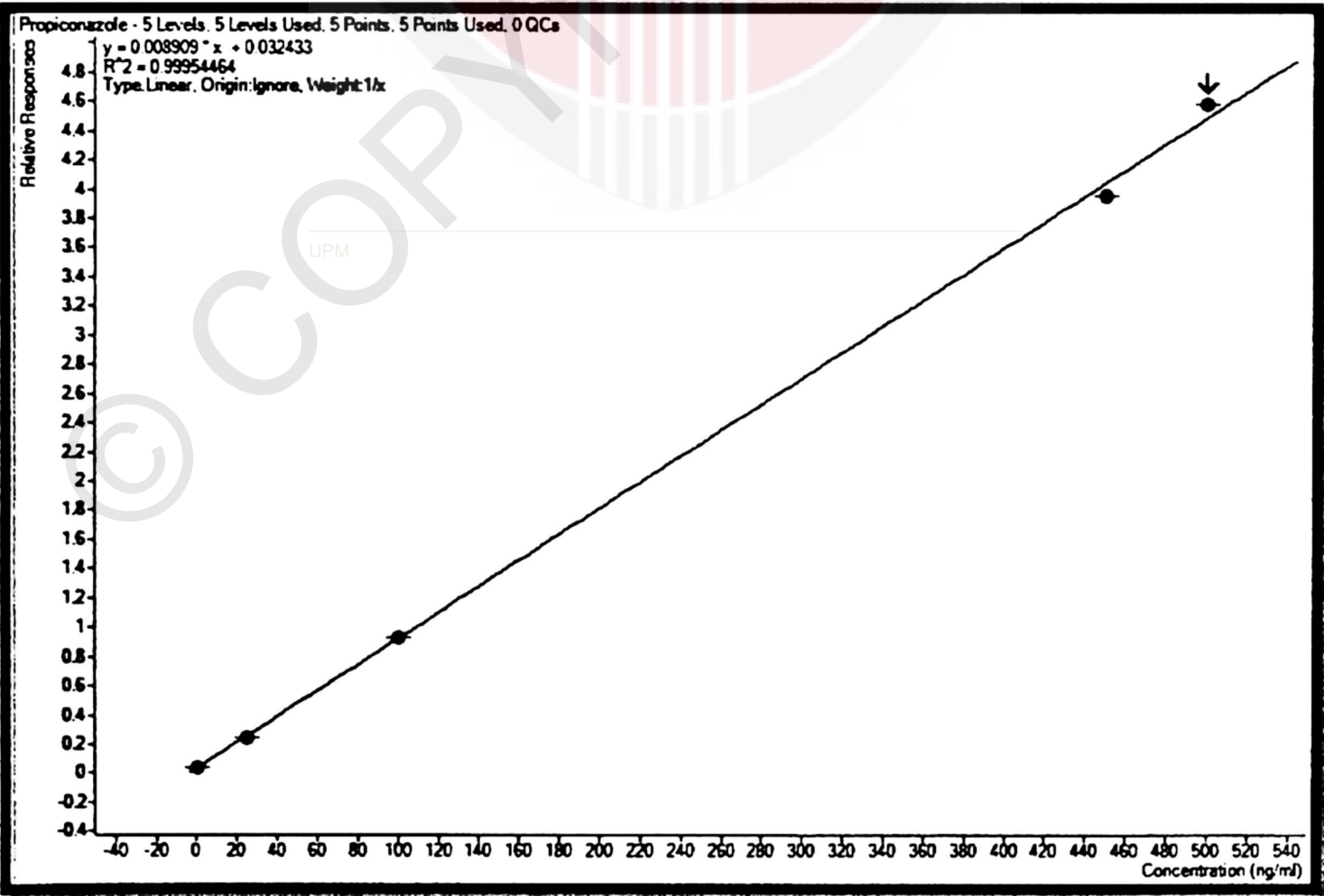


Figure 2: The calibration curve for propiconazole

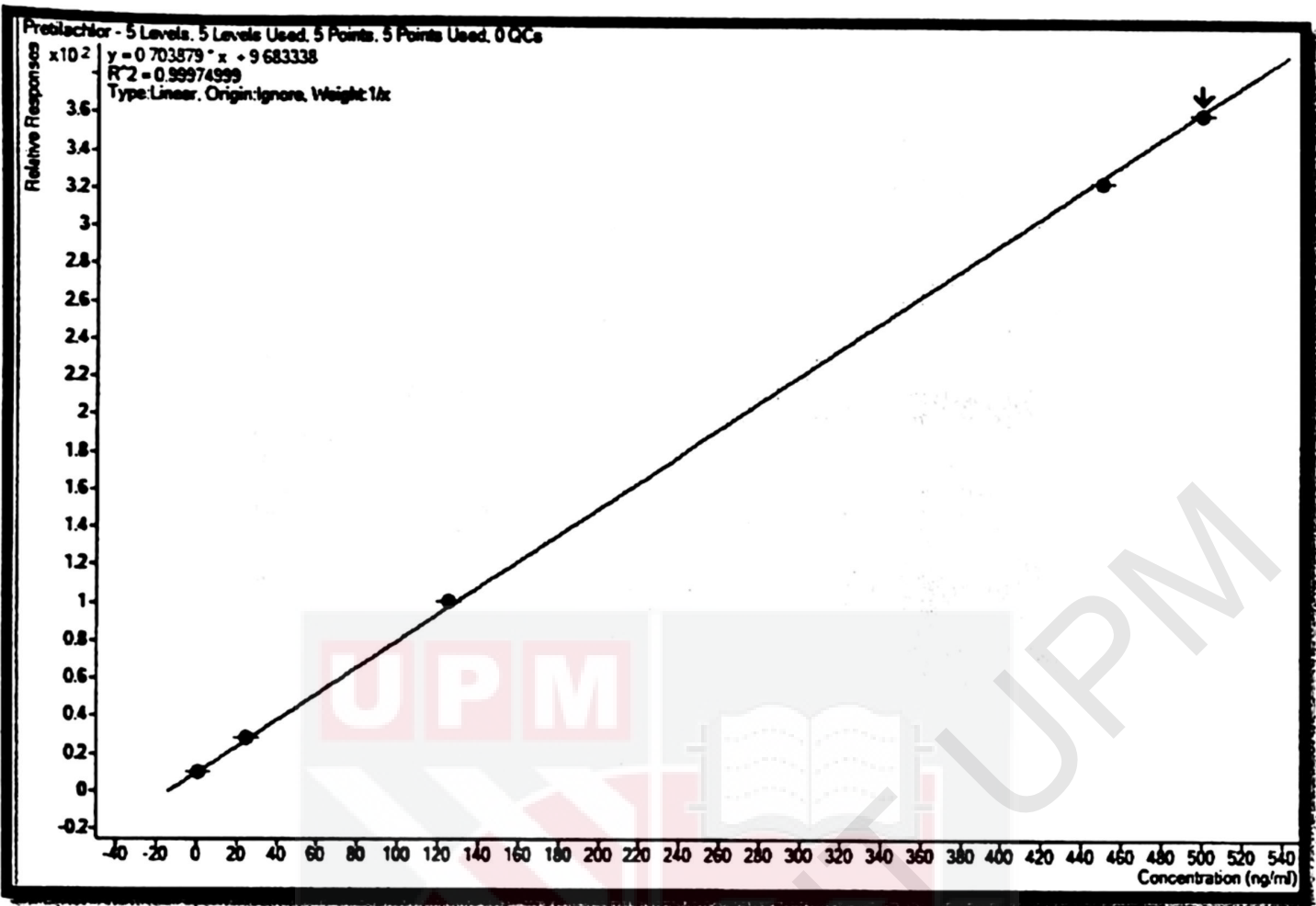
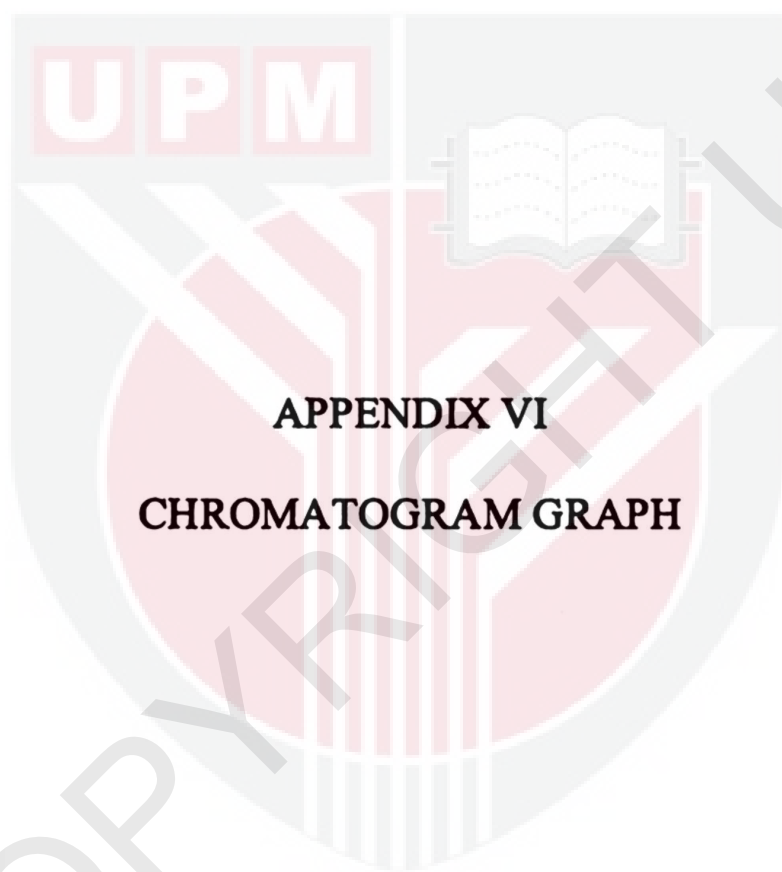


Figure 3: The calibration curve for pretilachlor



APPENDIX VI
CHROMATOGRAM GRAPH

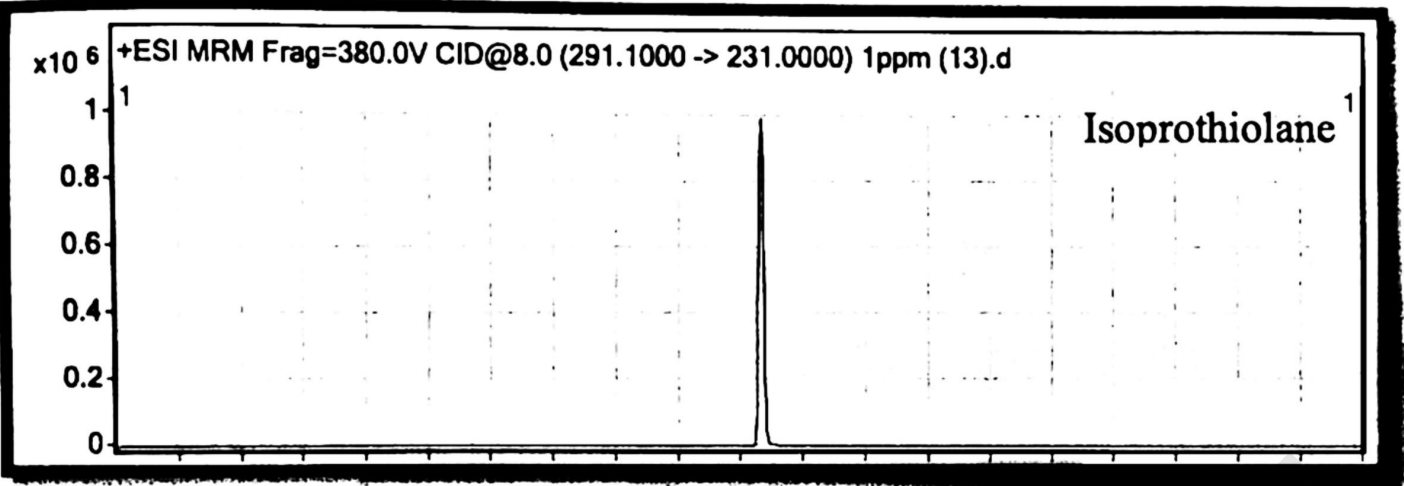


Figure 4: Chromatogram for isoprothiolane

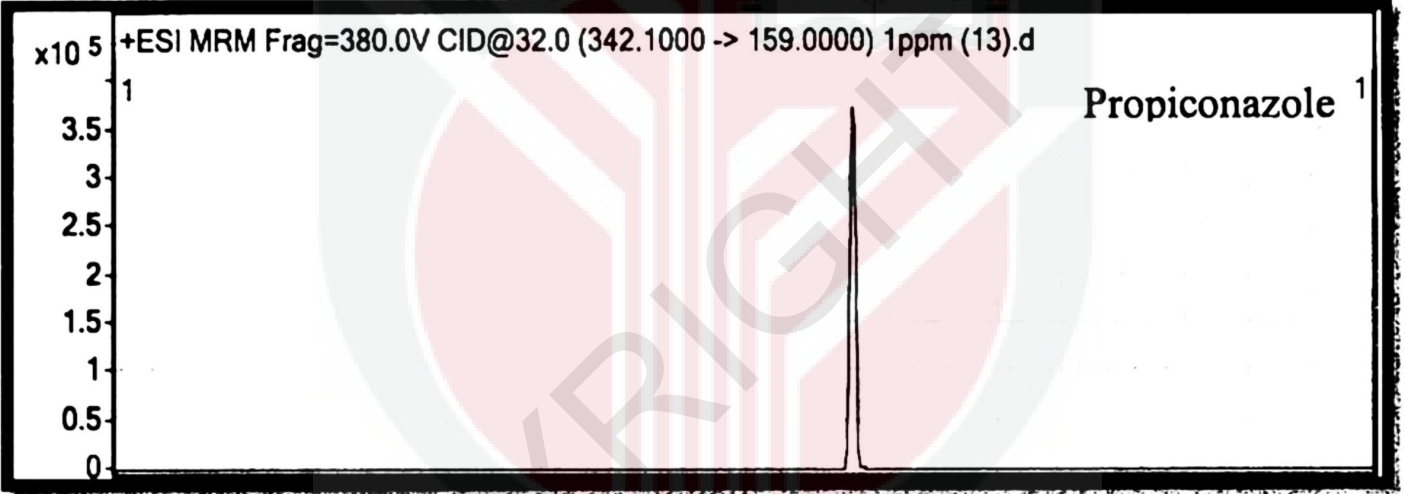


Figure 5: Chromatogram for propiconazole

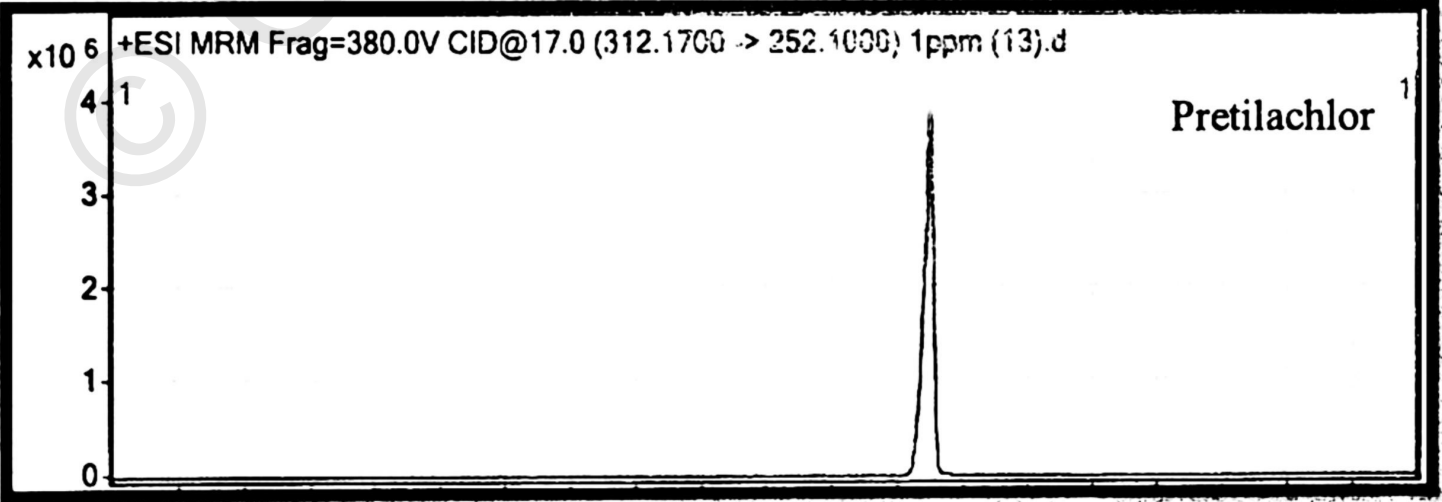
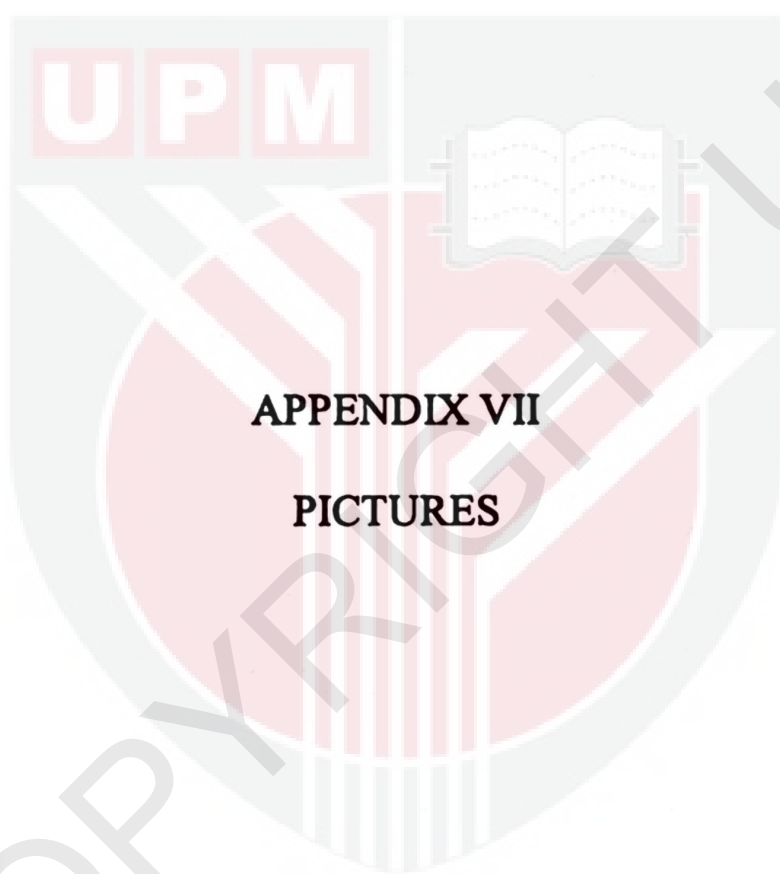


Figure 6: Chromatogram for pretilachlor



APPENDIX VII
PICTURES

UPM



Figure 7: Collection of paddy soil

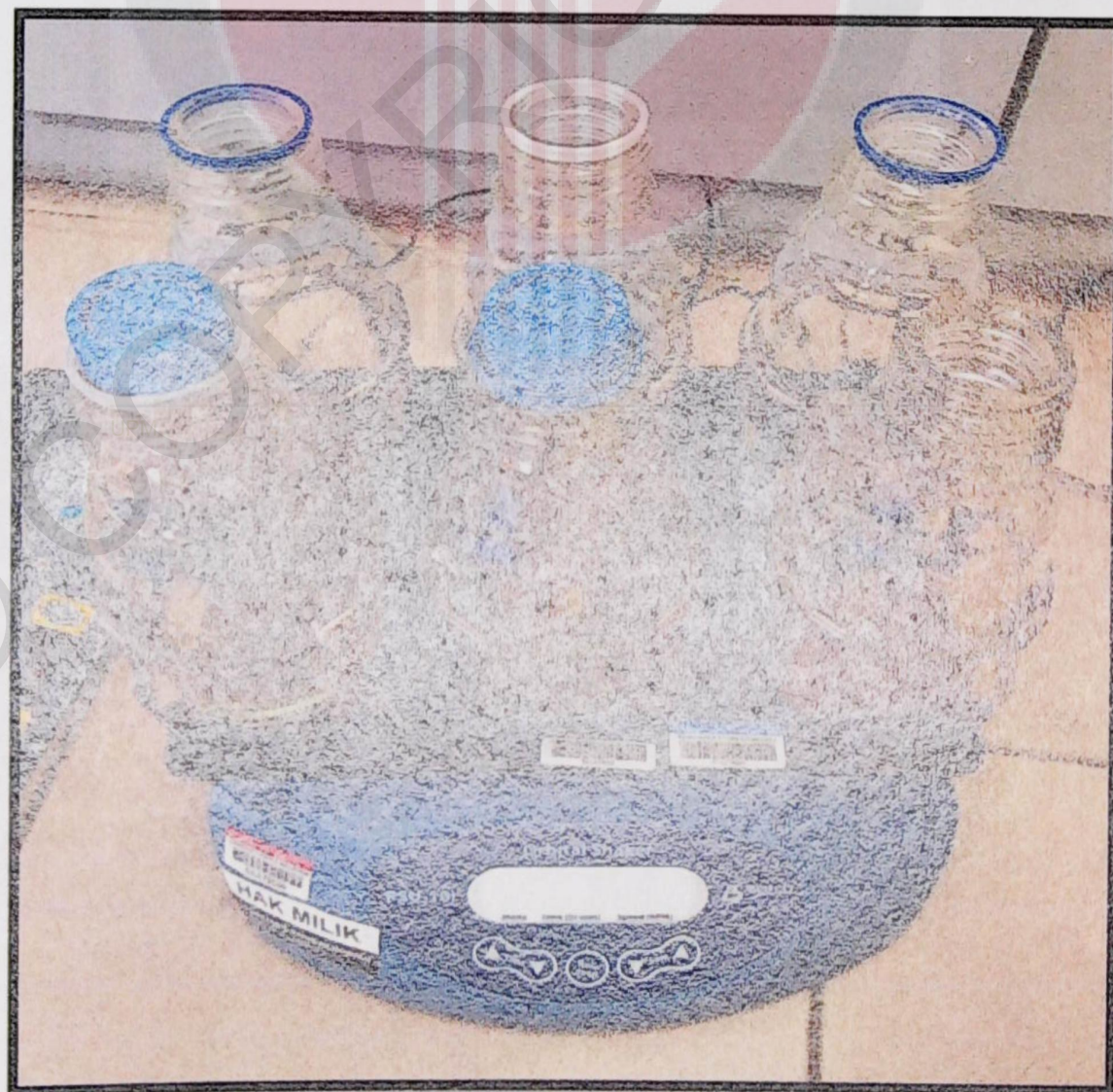


Figure 8: The tubes were shaken for 1 minutes



Figure 9: Centrifuged at 3000 rpm at 10 minutes



Figure 10: Analysed with UPLC-MS/MS