UNIVERSITI PUTRA MALAYSIA

CONTAMINATION OF ISOPROTHIOLANE, PROPICONAZOLE AND PRETILACHLOR IN PADDY WATER AND THE HEALTH RISK TO FARMERS IN TANJUNG KARANG, KUALA SELANGOR

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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 and Health Sciences, Universiti Putra Malaysia

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ABSTRACT

CONTAMINATION OF ISOPROTHIOLANE, PROPICONAZOLE AND PRETILACHLOR IN PADDY WATER AND THE HEALTH RISK TO FARMERS IN TANJUNG KARANG, KUALA SELANGOR

ASNA SYAHIRAH BINTI MOHAMAD SALIHIN

Introduction: Tanjung Karang is well known for its rice cultivation activities and is the third largest paddy field in Malaysia. The use of pesticides in rice cultivation activities to reduce yield loss and to maintain the quality of crop yield is inevitable and this has turned into a worry as pesticides can cause undesirable effects to human health and to the environment. Paddy farmers are routinely exposed to high levels of pesticides and this makes them at a very high risk of occupational diseases. **Objective:** This study aims to determine the concentration of pesticides (isoprothiolane, propiconazole and pretilachlor) in paddy water and to assess the potential health risk among paddy farmers in Tanjung Karang, Selangor. **Methodology:** Selected pesticides in the water samples were extracted by using solid-phase extraction (SPE) method and analysed by using ultra performance liquid chromatography tandem mass spectrometry (UPLC-MS/MS). A total of 152 questionnaires were distributed among paddy farmers to obtain information such as demographic and pesticides exposure of paddy farmers. The hazard quotient (HQ) value was calculated to estimate the potential dermal health risk of paddy farmers due to occupational exposures in paddy water. **Results and Discussion:** The results revealed that isoprothiolane (51.542 μg/L) was the highest mean concentration in paddy water followed by propiconazole (12.434 μg/L) and pretilachlor (0.109 μg/L). There was no significant relationship between pesticides concentrations with *in situ* water quality parameters. There was no significant non-carcinogenic dermal health risk due to exposure of propiconazole in paddy water as HQ did not exceed the value of one. **Conclusion:** Although calculated HQ showed there was no significant non-carcinogenic dermal health risk, there are concerns towards the danger of long-term exposure to the mixture of pesticides which was not assessed in this study.

Keywords: pesticides contamination, paddy water, hazard quotient (HQ), ultra performance liquid chromatography tandem mass spectrometry (UPLC-MS/MS), solid-phase extraction (SPE)

ABSTRAK

PENCEMARAN ISOPROTHIOLANE, PROPICONAZOLE DAN PRETILACHLOR DALAM AIR PADI DAN RISIKO KESIHATAN KEPADA PESAWAH DI TANJUNG KARANG, KUALA SELANGOR

ASNA SYAHIRAH BINTI MOHAMAD SALIHIN

Pengenalan: Tanjung Karang terkenal dengan aktiviti penanaman padi dan merupakan sawah padi yang ketiga terbesar di Malaysia. Penggunaan racun perosak dalam aktiviti penanaman padi untuk mengurangkan kehilangan hasil tanaman dan mengekalkan kualiti hasil tanaman tidak dapat dielakkan dan ini telah menjadi kebimbangan kerana racun perosak boleh menyebabkan kesan yang tidak diingini kepada kesihatan manusia dan alam sekitar. Pesawah secara rutin terdedah kepada tahap racun serangga yang tinggi dan ini menjadikan mereka sangat berisiko mendapat penyakit pekerjaan. **Objektif:** Kajian ini bertujuan untuk menentukan kepekatan racun perosak *(isoprothiolane, propiconazole dan pretilachlor)* dalam air padi dan untuk menilai potensi risiko kesihatan dalam kalangan pesawah di Tanjung Karang, Selangor. **Metodologi:** Racun perosak terpilih dalam sampel air telah diekstrak dengan menggunakan kaedah *solid-phase extraction* (SPE) dan dianalisis dengan menggunakan *ultra performance liquid chromatography tandem mass spectrometry* (UPLC-MS / MS). Sebanyak 152 soal selidik diedarkan dalam kalangan pesawah untuk mendapatkan maklumat seperti demografi dan pendedahan pesawah kepada racun perosak. *Hazard quotient* (HQ) dikira untuk menganggarkan potensi risiko kesihatan dermal pesawah akibat pendedahan pekerjaan dalam air padi. **Keputusan dan Perbincangan:** Hasil kajian menunjukkan bahawa isoprothiolane (51.542 μg/L) adalah kepekatan tertinggi dalam air padi diikuti oleh propiconazole (12.434 μg/L) dan pretilachlor (0.109 μg/L). Tiada hubungan yang signifikan antara kepekatan racun perosak dengan parameter kualiti air in situ. Tiada risiko kesihatan dermal bukan karsinogenik yang disebabkan oleh pendedahan kepada *propiconazole* dalam air padi kerana HQ tidak melebihi nilai satu. **Kesimpulan:** Walaupun HQ yang dikira menunjukkan tiada risiko kesihatan dermal bukan karsinogenik, terdapat kebimbangan terhadap bahaya pendedahan jangka panjang terhadap campuran racun perosak yang tidak diukur dalam kajian ini.

Kata kunci: pencemaran racun perosak, air padi, *hazard quotient* (HQ), *ultra performance liquid chromatography tandem mass spectrometry* (UPLC-MS/MS), *solid-phase extraction* (SPE)

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LIST OF ABBREVIATIONS

CAS	Chemical Abstracts Service
DAD	Dermal Absorbed Dose
DDT	Dichloro-diphenyl-trichloroethane
EPA	Environmental Protection Agency
GFF	Glass Fibre Filter
HQ	Hazard Quotient
IDL	Instrumental Detection Limit
IQL	Instrument Quantitation Limit
ISO	International Organization for Standardization
IUPAC	International Union of Pure and Applied Chemistry
MDL	Method Detection Limit
MQL	Method Quantitation Limit
MRM	Multiple Reaction Monitoring
PPE	Personal Protective Equipment
RfD	Reference Dose
S/N	Signal-to-noise Ratio
SPE	Solid-phase Extraction
SPSS	Statistical Package for Social Sciences
UPLC-MS/MS	Ultra Performace Liquid Chromatography – Tandem Mass Spectrometry
USEPA	United States Environmental Protection Agency

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CHAPTER 1

INTRODUCTION

1.1 Background

Paddy and rice industry plays an important role in the agriculture economy of Malaysia as rice is the staple food for the country (Adnan et al., 2017). Rice is primarily grown in Peninsular Malaysia with total area around 300,500 hectares. Many paddy fields are planted in Peninsular Malaysia on the West Coast particularly in the Northern part which include Kedah, Perak and Perlis. In addition, Sabah and Sarawak also play a role in Malaysia's agriculture sector as a whole to produce the staple rice. There are approximately 190,000 hectares of lands are cultivated for rice in Sabah and Sarawak.

As a developing country, paddy cultivation activities need to be improved in order to fulfill the demands of rice production due to population increment. Nevertheless, there are challenges arise due to the presence of pests, for instance, birds, insects, rodents and other living organisms such as fungi and bacteria in the paddy field. Pest outbreaks can cause the rice production to decrease and also threaten food security. In an effort to overcome the presence of pests and to increase rice productivity, pesticides are widely used in rice farming to kill pest organisms (Idayu et al., 2014) and it is reported that over 90% of the pesticides used are utilized in the paddy field (Ahmad et al., 2017). Other than being utilized in the cultivation of paddy as one of the basic beneficence to enhance rice productivity, the use of pesticides are inevitably helps paddy farmers to increase their income with increased yields.

The World Health Organization (2017) has defined pesticides as chemical compounds that are used to kill pests such as insects, rodents, fungi and weeds. Based on a report by USEPA (2011), it was estimated that 5.2 billion pounds of pesticides have been used worldwide in 2006 as well as in 2007. These pesticides can be classified according to the types of chemicals with more than eight hundred active ingredients and manufactured in different formulations (Ahmad et al., 2017). The types of pesticides are usually divided based on its purpose or target organisms they are intended to control or kill such as insecticides, herbicides, rodenticides, fungicides and larvicides. Insecticides are utilized to control insects, herbicides kill or restrain the development of undesirable plants, for instance weeds, rodenticides are utilized to execute rodents like mice and rats, fungicides are utilized to control fungal problems, such as molds and larvicides are utilized to kill larvae of insects.

In spite of benefits that could come from the usage of pesticides in ensuring food supplies, they still have potentially hazardous effects to surrounding environment as well as post threats to human health (Ahmad, 2016) depending on their specific distribution and use. Because of that, many countries have banned and restricted the utilization of some pesticides.

The application methods of pesticides in rice farming may have different fates following release into the environment. Based on a study by Senoro et al. (2016), about 95-98% of the applied pesticides go to non-target species or areas. Therefore, numerous amount of pesticides will be moved and transferred to the environment, and according to Yogo (2009), soil, water and air or their combinations are major media for pesiticides behaviour in the environment. Pesticides that are sprayed and volatilized would transfer in air and may eventually transfer to the soil and water during runoff and leaching (Senoro et al., 2016). Meanwhile, pesticides which are applied directly to the soil may be washed off the soil into nearby bodies of surface water or may percolate through the soil to lower soil layers and groundwater (Kamrin & Montgomery, 2000). Other than that, the application of pesticides indirectly to water as a result of cleaning the spraying instruments causes both surface and groundwater to build up of pesticides and which can cause serious damages to the ecosystem (Jin et al., 2015). Therefore, pesticides movement in the environment are affected by the methods of pesticides application whether they are applied directly or indirectly to their target organisms.

Based on a study by Kim et al. (2017) of exposure to pesticides and the associated human health effects have demonstrated that the unintended exposure to pesticides can be extremely hazardous to humans and living organisms when exposed as they are designed to be poisonous. Moreover, human population might be exposed to pesticides either through occupational use of pesticides, consumption of food that contained pesticide residue or inhalation of air that contaminated with pesticides (Kim et al., 2017). Studies suggested that pesticides may be linked with various diseases and their effects can be classified as acute or chronic effects based on the period of exposures or symptoms of their toxicity to develop. Acute exposure refer to a single exposure that cause immediate health effects and this type of exposure differs from chronic exposure, which the adverse health effects are resulting from long-term exposure. The acute effects of pesticides exposures are including skin and eye irritation, headaches, nausea and dizziness, while chronic effects are asthma, diabetes and cancer (Hamsan et al., 2017). These health effects resulting from pesticides exposure may vary according to the individual and may be the result of exposures either through dermal contact, inhalation or oral consumption.

Pesticides usage in agricultural activity, particularly in rice cultivation are of growing concern due to their possible adverse effects on farmers' health when exposed. A study by Hamsan et al. (2017) in Malaysia had demonstrated that farmers are the major group of workers to be exposed to pesticides as they are constantly handling pesticides during rice cultivation activities. Thus, exposing the farmers to a high exposure risk. However, the wellbeing of an individual is a subjective matter where one's level of health is different from one to another. This would mean that not every individual will have the same potential effects when exposed to pesticides. More over, the routine use of pesticides would still pose significant health risks to an individual both in the short and the long run. A study by Asghar et al. (2016) suggested that even though pesticides are very harmful if they are exposed to human, they cannot be completely banned or restricted their use, but their exposure and effects can be reduced. Therefore, the management of pesticides and their safety measures are important as these would help to reduce human health risk to pesticides exposure.

1.2 Problem statement

Pesticides are widely used in agricultural sector to kill pests or unwanted organisms, however, since pesticides can be transported almost everywhere in the surrounding environment such as air, soil, water and living organisms, their occurrence in the environment can cause many adverse effects on ecosystem and human health due to exposure to pesticides toxicity (Li, 2017) which resulting in both acute and chronic health effects. Pesticides shown acute or chronic and short-term or long-term effects on nervous system by the high or low-level exposure which eventually lead to a very chronic nervous disorder, for instance Parkinson disease (Asghar et al., 2016). Therefore, concerns over the environment polluted with pesticides as well as their effects on human health cause pesticides to be important.

Paddy farmers are at a high risk of poisoning by the pesticides as they are in contact with pesticides on a daily basis. It was indicated that skin problems were significantly higher among farmers who use pesticides than those who do not (Sankoh et al., 2016). In relation to these, many researchers have conducted studies pertaining to pesticides particularly on dermal exposure assessment (Mazlan et al., 2016) and occupational health risk assessment (Ahmad, 2016). Occupational dermal exposure can lead to numerous negative health effects among paddy farmers. The likelihood of developing possible health outcomes from pesticides exposures are affected by various factors, for instance, type of pesticides or chemicals, application duration, dose applied, route of exposure, usage of personal protective equipment (PPE) as well as individual health status.

Tanjung Karang in the State of Selangor lives up to its name as 'the rice bowl of Selangor' is the third largest area of paddy field in Peninsular Malaysia (Mazlan et al., 2016) and Kampung Sawah Sempadan is located in the district of Tanjung Karang. The residents of Kampung Sawah Sempadan performed agricultural activities, particularly rice cultivation as their main source of income. They have been using pesticides in rice cultivation activities in order to maintain rice production. The type of compounds in the commonly used pesticides were identified during the interview with the paddy farmers in Tanjung Karang. Through the interview, it was found that there were 13 compounds existed in the active ingredients of commonly utilized pesticides. The 13 compounds of pesticides are listed in Table 1.1. From the 13 compounds, only three of them have been selected for this study, which were isoprothiolane, propiconazole and pretilachlor that were used in order to control pests such as fungi and weeds.

Table 1.1: List of pesticides active ingredients, frequency and percentage of usage by paddy farmers in Kampung Sawah Sempadan

Pesticides Active Ingredients	Frequency (N=152)	Percentage	Remark
Chlorantrinoprole	44	36	Previously reported by Junaidi, (2017).
Difenoconazole	44	36	
Pymetrozine	43	36	
Isoprothiolane	**41**	**34**	There were no papers have been published on the simultaneous analysis of these selected compounds.
Propiconazole	**38**	**32**	
Pretilachlor	**31**	**26**	
Tebuconazole	29	24	
Trifloxystrobin	29	24	
Azoxystrobin	27	23	
Fipronil	22	18	
Tricyclazole	21	18	
Imidacloprid	17	14	
Buprofezin	13	11	

In addition, several observations were also made on worker operative modalities and the use of PPE during their working in paddy field. Based on the observations that have been made, some of the paddy farmers did not use PPE, for example, gloves and boots during mixing of pesticides and working in the paddy field. These eventually cause the paddy farmers to be exposed via dermal absorption of the pesticides from their daily activities in the paddy field.

Dermal contact is one of the most common route among three main pathways (inhalation, ingestion and dermal contact) of pesticides exposure, which normally occurs during pesticides mixing or loading, application, harvesting and other farming activities (Nurulain et al., 2017). Thus, this research focused on the potential health risk of paddy farmers when exposed to pesticides via dermal.

Figure 1.1: Paddy farmers working in the paddy field do not wear PPE while mixing the pesticides

Figure 1.1 shows the paddy farmers do not wear PPE during mixing of pesticides. Previous study by Atabila et al. (2017) revealed that the lower body parts (hands, upper legs, lower legs and feet) were more prone to pesticides exposure compared to upper body parts (head, upper arms, lower arms, front abdomen and back abdomen).

According to "MSDS Solutions Center" (2018), the three selected compounds are found to have potential health effects to human, which cause harmful effects if absorbed through skin and this may contribute to skin irritation and details of information of the chosen compounds are summarized in Table 2.1. Paddy farmers who do not wear proper PPE will be at high risk to be exposed to pesticides through dermal contact. With this context, this study focuses on the potential of dermal health risk of paddy farmers related to pesticides exposure in paddy water.

1.3 Study justification

Previous study by Ouyang et al., (2017) had detected three concentrations of pesticides (atrazine, oxadiazon and isoprothiolane) in surface runoff of paddy land in northern China and the results showed isoprothiolane was detected at the maximum value of 1.082 μg/L. Another study by Edwards et al. (2016) reported that high concentrations of azoxystrobin and propiconazole were found in water samples in year 2014 when compared to previous year due to the significant rainfall event that contribute to the runoff pesticides into the water in agricultural fields located in Moultrie County, Illinois, United States. There was also a previous study of selected compounds of isoprothiolane, propiconazole and pretilachlor that has been conducted simultaneously. The study resulted that the concentration of isoprothiolane, propiconazole and pretilachlor of pesticides pollution in drinking water sources in Mekong Delta, Vietnam were 8.49 μg/L, 4.76 μg/L and 0.85 μg/L respectively (Chau et al., 2015). Although many specific case studies regarding pesticides concentration were reported across the world, there have been only limited studies that have pinpointed on isoprothiolane, propiconazole and pretilachlor in Malaysia.

To a certain extent, there have been many studies regarding pesticides conducted in Tanjung Karang, Selangor. Most previous studies that were focusing on pesticides exposure in personal air samples (Hamsan et al., 2017), reported on the risk of imazapic herbicide posed towards farmers using dermal exposure assessment (Mazlan et al., 2016) and the study of exposure to herbicides and skin symptoms among paddy farmers (Chokeli et al., 2016). Despite all the past studies, there is no research conducted to determine the concentration of selected pesticides in paddy water due to occupational exposure in Malaysia. Therefore, in this study, an attempt has been made to resolve the gap of knowledge. The analysis of the target pesticides in this study can provide essential data on the concentration of isoprothiolane, propiconazole and pretilachlor in the paddy water and help other researchers to continue studying on pesticides used in paddy cultivation activities.

In general, there are three types of routes for pesticides exposures, which include inhalation, ingestion and dermal contact. For the purpose of this study, only dermal exposure was assessed. According to Anderson & Meade (2014), the most important routes of exposure in the agriculture industry is through dermal. Occupational end users of pesticides may experience bodily absorption of the pesticides products they utilize and this put them at risk of possible health effects related with pesticides exposure (Macfarlane et al., 2013), and therefore, the present study is focusing on dermal health risk among paddy farmers as they are working directly with pesticides in agriculture field. For this purpose, hazard quotient (HQ) value was calculated to estimate the potential dermal health risk that may be imposed to paddy farmers through occupational exposure. Therefore, hazard risks data for the three selected pesticides can be used as references for future risk assessment which contain similar active ingredients.

As rice is the most important staple food in Malaysia, studies investigating the effects of pesticides usage during paddy cultivation activities are strongly recommended. It is essential to study the concentration of pesticides in paddy water as many studies reported that a huge number of paddy farmers are not wearing appropriate PPE during handling of pesticides (Mazlan et al., 2016, Chokeli et al., 2016) and eventually, this may imposed risk to the health of paddy farmers via body absorption from their working activities in the paddy water.

The main purpose of this research study is to determine the concentration of isoprothiolane, propiconazole and pretilachlor in the paddy water and assess the non-carcinogenic dermal health risk that may be imposed on the paddy farmers through occupational exposure. Overall, the outcome of this study can be used in terms of developing agricultural practices which provide a fundamental basis towards a good management of pesticides usage in agricultural activities. In addition, the data on the concentration of selected pesticides can be used as reference for other researchers to conduct deeper research on the presence of pesticides in paddy water through other types of exposure pathways.

1.4 Conceptual framework

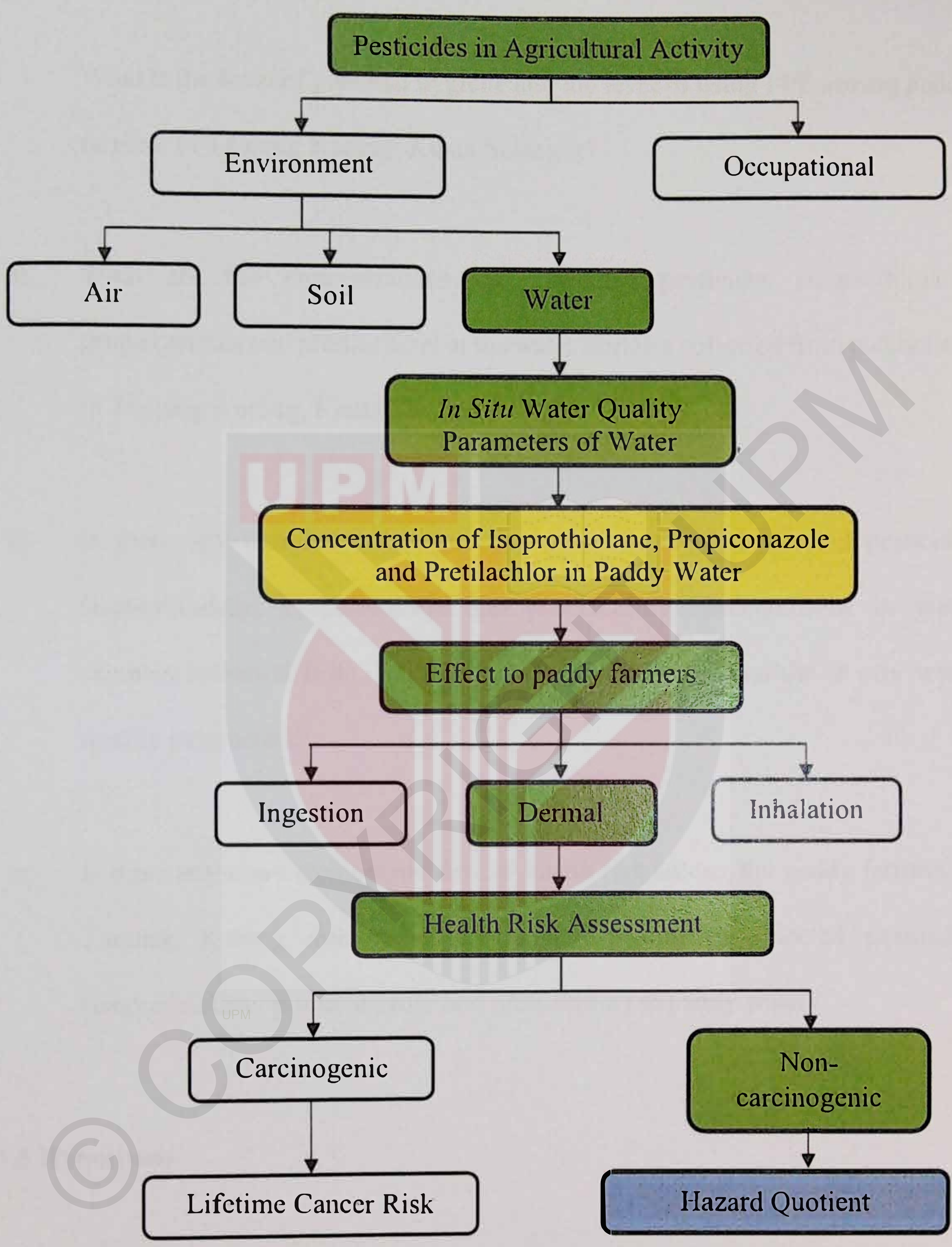
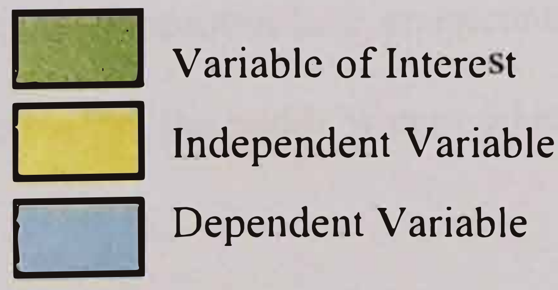


Figure 1.2: Conceptual framework for this study



1.5 Research questions

i. What is the level of personal hygiene and the level of using PPE among paddy farmers in Tanjung Karang, Kuala Selangor?

ii. What are the concentrations of selected pesticides (isoprothiolane, propiconazole and pretilachlor) in the water samples collected from paddy field in Tanjung Karang, Kuala Selangor?

iii. Is there any correlation between the concentrations of selected pesticides (isoprothiolane, propiconazole and pretilachlor) concentrations in water samples collected from Tanjung Karang paddy water and the *in situ* water quality parameters?

iv. Is there any non-carcinogenic dermal health risk among the paddy farmers in Tanjung Karang due to occupational exposure of selected pesticides (isoprothiolane, propiconazole and pretilachlor) in paddy water?

1.6 Hypothesis

There is a significant relationship between the concentrations of selected pesticides (isoprothiolane, propiconazole and pretilachlor) and the *in situ* water quality parameters in the paddy water collected from Tanjung Karang, Kuala Selangor.

1.7 Objectives

1.7.1 General objective

To determine the concentration of selected pesticides (isoprothiolane, propiconazole and pretilachlor) in paddy water and their associated dermal health risks among paddy farmers in Tanjung Karang, Selangor.

1.7.2 Specific objectives

i. To determine the demographic background, personal hygiene and the use of PPE among paddy farmers in Tanjung Karang, Kuala Selangor.

ii. To measure the concentrations of selected pesticides (isoprothiolane, propiconazole and pretilachlor) in the water samples collected from Tanjung Karang paddy field.

iii. To determine the relationship between the concentrations of selected pesticides (isoprothiolane, propiconazole and pretilachlor) and the *in situ* water quality parameters in the paddy water collected from Tanjung Karang.

iv. To evaluate the non-carcinogenic dermal health risks among paddy farmers in Tanjung Karang due to occupational exposure of selected pesticides (isoprothiolane, propiconazole and pretilachlor) in paddy water.

CHAPTER 2

LITERATURE REVIEW

2.1 Pesticides and agricultural development

Agriculture remains as an important economic sector in Malaysia. Referring to Department of Statistics Malaysia (2016), agriculture sector in 2015 continued to expand with a contribution of 8.9% to the Gross Domestic Product. In order to obtain high yield of crops, a large amount of pesticides are used in agricultural activities to kill pests. However, excessive application of pesticides have become a major issue. The eagerness to gain profit through improved crop yields by utilizing pesticides during planting activities has affected the environment as well as living things. The use of pesticides is increasing in parallel with the increasing of plant consumption.

Farmers in the olden days used traditional agricultural practices such as crop rotation method to protect their plants against pests. According to Singh and Singh (2017), crop rotation is a traditional practice of cultivation that has been used for thousands of years. Crop rotation is the practice of rotation of cultivation in which changing a sequence of plant species. For example, rice plants in rotation with corn cultivation on the same land. Crop rotation is recognized to help reduce and prevents accumulation of pests population that often attack one species only by intruding pests' life cycles and interfering with pests' habitat. Nevertheless, traditional agricultural practices were inadequate for pest control and because of that human have been looking for other solutions to manage problem of crop damaged by pests. A variety of modern agricultural practices have been introduced and these include the use of chemical pesticides. Human struggles for food supplies and therefore, pesticides are used in their farming activities. Pesticides usage in cultivation activities has helped improve agricultural productivity by controlling various pest populations such as rodents, insects, fungus and weeds. Pesticides that are widely known include insecticides, herbicides, rodenticides and fungicides.

According to Oberemok et al. (2015), human used pesticides in plants to control the presence of pests was recorded since 1000 BC. Sulphur was the first chemical used as pesticides to get rid of lice and subsequently followed by arsenic in the year 900s AD. The use of pesticides was increasingly widespread where they have been used in the control of malaria vectors. Dichloro-diphenyl-trichloroethane (DDT), a well-known chemical at that time was used to control malaria and also used as insecticides for control of insect populations in crop production. DDT was a very effective pesticides. However, it was eventually banned as chemists found that DDT not only threaten pests but also dangerous to other living organisms such as mammals, reptiles and birds. The use of DDT for pests control was then had been replaced by other chemicals. At this point, there are regulations that have been established to control the use of pesticides which assist in the selection of safe and appropriate chemicals to acquire greatest efficiency and negligible risk from a pesticides applications.

2.2 Properties of target pesticides

2.2.1 Isoprothiolane

Isoprothiolane or diisopropyl 1,3-dithiolan-2-ylidenemalonate is an agricultural fungicide that has broadly utilized as protectant against fungi in rice planting since 1975. Isoprothiolane was first introduced in China in 1981 as a protective and remedial fundamental fungicide for rice blast control (Hu et al., 2014). It is belongs to the family of phospholipid biosynthesis inhibitor which influence a cell's ability to synthesize lipids. Isoprothiolane can cause restraint of phospholipid synthesis in cells (Kaonga et al., 2017) where the activity of isoprothiolane is acknowledged to be interference with transmethlation in the biosynthesis of a major membrane lipid in eukaryotic cells, which is phosphatidylcholine, an essential part of the fungal cell membrane (Zhang et al., 2016).

Although the use of isoprothiolane is beneficial in rice productivity, but its extensive usage can cause environmental problems as well as adverse effects to human health. A study by Selvi and Manonmani (2013) in India, reported that isoprothiolane bringing in severe acute toxicity prompting to sweating, cerebral pain, vomiting, giddiness, unconsciousness, eye irritation and serious eye damage to humans. In a study regarding pesticides concentrations in river water from agricultural area in northern China, Ouyang et al (2017) found that isoprothiolane was detected with the highest concentrations among the pesticides studied. Further information of isoprothiolane is summarized in Table 2.1.

2.2.2 Propiconazole

Fungicide propiconazole or its International Union of Pure and Applied Chemistry (IUPAC) name, 1-[[2-(2,4-dichlorophenyl)-4-propyl-1,3-dioxolan-2-yl]methyl]-1,2,4-triazole has been developed in 1979 and marketed worlwide in a range of fungicide treatment products for preventive and control of plant diseases and fungi purposes. Propiconazole acts as an ergosterol biosynthesis inhibitor in which it was reported that fungal growth was significantly affected by the addition of propiconazole for doses more than 50 mg/kg (Fernández-Calviño et al., 2017). Propiconazole's mode of action is particularly targets demethylation of C-14 in the midst of ergosterol biosynthesis. The biosynthesis of these ergosterols is essential to the development of cell dividers of living organisms. This nonappearance of ordinary sterol creation slows or stops the development of the growth, effectively avoiding further contamination as well as intrusion of host tissues. Consequently, propiconazole is believed to be fungistatic or growth repressing rather than fungicidal or killing.

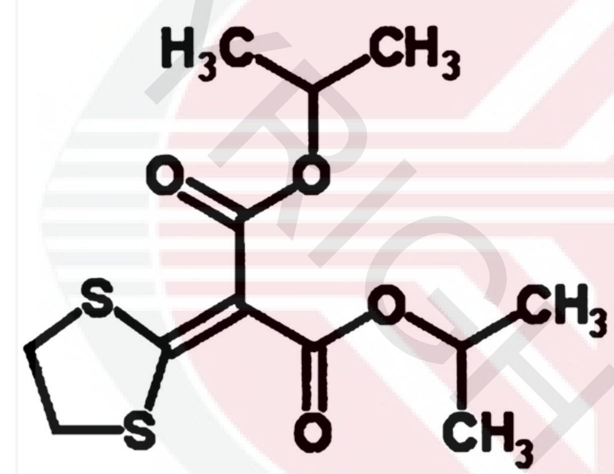
Propiconazole is applied at late growth stages in plant crops requiring the utilization of air and soil application procedures respectively and further causing a potential route of transport to adjacent aquatic environments by means of drift as well as runoff (Edwards et al., 2016). The continuous use of propiconazole in cultivation activities may result in fungicides reaching the soil surface by drifting during application and subsequently, soil will act as sorbent carriers for these fungicides following significant rainy days. Rain washing propiconazole to the ground will reaching the aquatic environment and has potential to contaminate the groundwater. Table 2.1 summarized the properties of propiconazole.

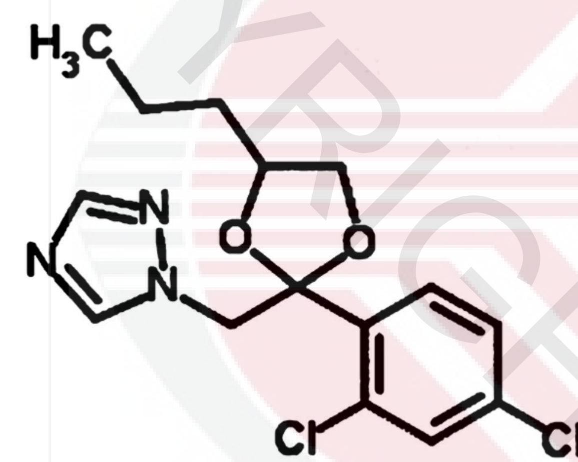
2.2.3 Pretilachlor

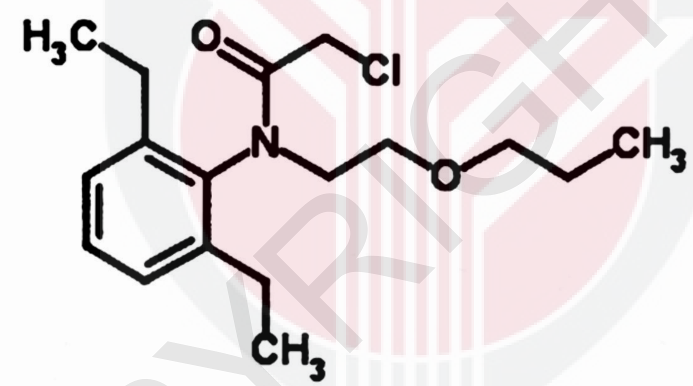
The active ingredient of 2-chloro-N-(2,6-diethylphenyl)-N-(2-propoxyethyl) acetamide or known as pretilachlor was produced as an effective herbicide to control weeds and grasses found in rice field especially for early season weed control. Pretilachlor is a herbicides that belongs to the chloroactamide group which restrains growth and halts cell division through preventing the synthesis of long chain fatty acids in plant cells (Maryam et al., 2013).

A previous study by Mo et al. (2015) claimed that pretilachlor is highly toxic to the aquatic organisms but has a low toxicity to humans and mammals. Pretilachlor reaching into the water body through these herbicides residues in the soil by the running water and prone to accumulate in water bodies and has a potential risk to aquatic organisms, for example, fish and shellfish. Due to their continuous use and chemical stability, the accumulation of these herbicides in water bodies also bring a potential risk to the human health. There is a previous study indicates that pretilachlor is easy to disappear in rice fields by photodecomposition, microbial degradation and volatilization and prescribed rates do not influence the soil properties or poses a serious problem to the environment (Kumar et al., 2018). Nevertheless, it was discovered that pretilachlor persists on the water surface of the laboratory microscope following ten days of treatment. Table 2.1 summarized the properties of pretilachlor.

Table 2.1: Information of the targeted compounds

Common Name[a] Pesticide Type[a] IUPAC Name[a] CAS Number[a]	Molecular Formula[a]	Molecular Structure[b]	Molecular Weight (g/mole)[a]	Log Kow[a]	Cancer Classification[d]
Isoprothiolane Fungicide dipropan-2-yl 2-(1,3-dithiolan-2-ylidene)propanedioate 50512-35-1	$C_{12}H_{18}O_4S_2$		290.392	2.88	N/A

Common Name[a] Pesticide Type[a] IUPAC Name[a] CAS Number[a]	Molecular Formula[a]	Molecular Structure[b]	Molecular Weight (g/mole)[a]	Log Kow[a]	Cancer Classification[c]
Propiconazole Fungicide 1-[[2-(2,4-dichlorophenyl)-4-propyl-1,3-dioxolan-2-yl]methyl]-1,2,4-triazole 60207-90-1	$C_{15}H_{17}Cl_2N_3O_2$		342.22	3.72	Group C – Possible Human Carcinogen

Common Name[a] Pesticide Type[a] IUPAC Name[a] CAS Number[a]	Molecular Formula[a]	Molecular Structure[b]	Molecular Weight (g/mole)[a]	Log Kow[a]	Cancer Classification[d]
Pretilachlor Herbicide 2-chloro-N-(2,6-diethylphenyl)-N-(2-propoxyethyl)acetamide 51218-49-6	$C_{17}H_{26}ClNO_2$		311.85	4.1	N/A

[a]Pubchem, 2016. National Center for Biotechnology Information (NCBI). https://pubchem.ncbi.nlm.nih.gov/ (accessed October 2017)

[b]Chemspider, 2016. Royal Society of Chemistry. http://www.chemspider.com/ (accessed October 2017)

[c]USEPA (2016). Chemicals Evaluated for Carcinogenic Potential (Annual Cancer Report 2016). http://npic.orst.edu/chemicals_evaluated.pdf (accessed October 2017)

[d]Not available

2.3 Migration of pesticides into water

The problem of pesticides pollute the water bodies has been widely identified for a long time and it is an important issue that is often said when it comes to agricultural activities. The agricultural sector has been massively developed in tandem with rising need for source of nutrition by the growing population. In order to meet growing demand for foods, pesticides is used for pests control has helped improve crop yields. The main route of pesticides to move into water from treated fields is that the residues in the soil go into the water body through running water, leakage, washing out and so on. Wu et al. (2017) claimed that most pesticides are in the form of droplets and roughly sprayed on crops, of which just around 10% are attached to crops, while the greater part of which are spread in the air and the rest ends up in the soil. The measure of pesticides deposited and subordinated in soil would increase and genuinely pollute the soil. The pesticides in the soil will stream to the lake, the waterway and the ocean, which then pollute the water. A study conducted by Affum et al., (2018) in Ankobra Basin, Ghana showed that the surface water was seriously polluted with the pesticides used on cocoa crops. Therefore, the use of pesticides in agricultural activities move into surface water and this causes water contamination by pesticides.

2.4 Pesticides effects on human health

There are various degrees of pesticides toxicity. The widespread use of pesticides in agricultural activities help to control pests but at the same time can cause adverse effects to human health as their toxicity is not always limited to the target organisms. As the farmers mix the concentrated pesticides substances and apply sprays in the field, they have a tendency to be a group at higher risk to pesticides exposure. A study conducted by Kasambala Donga and Eklo (2018), have determined that the potential pathways of pesticides exposure for farmers are through pesticides storage, mixing and spraying. The main route of pesticides absorption among agricultural workers is through skin (Colosio et al., 2017) and most of them knew that pesticides could enter the human body through skin (Kasambala Donga & Eklo, 2018).

Health effects of pesticides are divided into acute (short-term) and chronic effects (long-term) (Hamsan et al., 2017). Pesticides are usually present in low concentrations in water bodies which may cause acute health effects such as eye irritation, headaches, dizziness and nausea (Kim et al., 2017). However, its concern is for the potential of chronic effects such as central nervous-, reproductive- and immune system disorders as well as cancer (Manyilizu et al., 2017). It was reported by Sugeng et al. (2013) the diseases such as cancer, endocrine disruption and reproductive toxicity have been linked with the pesticides applications. A study by Sankoh et al. (2016) in Sierra Leone, reported that the health problems (nausea, respiratory disorders and blurred vision) of rice farmers who use pesticides are significantly higher when compared to who do not use pesticides.

2.5 Legislations and regulations related to pesticides

2.5.1 International legislation and regulation related to pesticides

The use of pesticides has threatened both the environment quality and human health even though they were originally thought to be safe. It was found that the accumulation of pesticides in environment build toxicity. However, the hazards that may arise due to pesticides usage can be prevented by controlling its use. Therefore, legislations and regulations on the usage of pesticides have been implemented in many countries. With the existence of such legislations and regulations, the pesticides to be used must be registered first and the levels of pesticides in the environment could be monitored. Most countries require such pesticides to be approved before they can be used and sold by the government agency. Referring to European Commission (2016), the status for isoprothiolane and pretilachlor under Regulation (EC) No. 1107/2009 are not approved. This means that these pesticides hazardous are being considered for its use and sale to the general public. However, propiconazole is approved under European Commission.

2.5.2 Malaysia legislation and regulation related to pesticides

Pesticides regulations differ from country to country. Malaysia has enforced Pesticides Act 1974 after the concerns were raised about the use of pesticides and its exposure to human. The Act covers the management of all pesticides and other chemicals utilized in agriculture activities including all the target pesticides (isoprothiolane, propiconazole and pretilachlor). Pesticides can be defined as any substance that contains an active ingredient or any preparation, mixture or material that contains any one or more of the active ingredients as one of its constituents but does not include contaminated food or any article listed in the Second Schedule of this act (Act 149 Laws of Malaysia, 2015).

Pesticides must be registered before being sold or used. According to Department of Agriculture (2018), all the target pesticides in this study are registered to be used in Malaysia. Registration is important to ensure that pesticides sold and used in the country are effective, good in quality and do not cause unacceptable effects to human and the environment. There are also other laws in Malaysia that related to pesticides which are Environmental Quality Act 1974 and Food Act 1983. The objective of Environmental Quality Act 1974 is to control chemical and industrial wastes including pesticides into the environment. Meanwhile, Food Act 1983 prescribes the maximum residue levels of pesticides in food.

CHAPTER 3

METHODOLOGY

3.1 Chemicals and standards

HPLC-grade methanol, HPLC-grade dichloromethane and HPLC-grade acetone and HPLC-grade acetonitrile were purchased from Fisher Scientific (UK). Analytical Reagent Grade dichloromethane, acetone and methanol were also purchased from Fisher Scientific (UK). Hydrochloric acid, formic acid and sodium thiosulphate 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). 1.0 μm glass fibre filter (GFF) was purchased from Sartorius (Germany), and 0.45 μm and 0.22 μm nylon membrane filter paper were purchased from Membrane Solutions (USA). Oasis HLB solid-phase extraction (SPE) cartridge (3 cc, 60 mg) was obtained from Waters (MA, USA). Ultra performance liquid chromatography tandem mass spectrometry (UPLC-MS/MS) was equipped with Zorbax Eclipse Plus C18 column (2.1 mm × 50 mm I.D., 1.8 μm) from Agilent Technologies (CA, USA). Reference standards of isoprothiolane (97.8%), propiconazole (99.0%) were purchased from Dr. Ehrenstorfer (Germany). Pretilachlor (98.7%) and internal standard imidacloprid -d4 (99.0%) were sourced from Sigma-Aldrich (MO, USA). Individual stock standard solutions (1000 mg/L) of target compounds were prepared in HPLC grade methanol. The stock solution was diluted (10 mg/L) in HPLC grade 25% methanol as working standard solutions and kept in flame-sealed amber-glass ampoules before storing at -20°C. Ultrapure water was purified in Milli-Q water system (Merck Millipore, USA).

3.2 Study design

This study used a cross sectional study design. It was a preliminary study and conducted within time frame of four months (a short period of time) with laboratory analysis for three targeted compounds which were isoprothiolane, propiconazole and pretilachlor. Dermal health risks of paddy farmers were assessed by using the pesticides concentrations found in this study and the self-reported pesticides exposure information of paddy farmers.

3.3 Study location

This study was conducted in the paddy field areas of Kampung Sawah Sempadan, Tanjung Karang, Selangor (Figure 3.1). The study area is well-known for its paddy cultivation activity as it is the main activity of residents there. It is also known as the third largest paddy field in Peninsular Malaysia. Kampung Sawah Sempadan comprised of residential areas where the paddy farmers who live in the same area were selected as respondents in this study. Generally, there are 24 blocks of paddy field namely as Block A until Block X with the total area about 2,300 hectares. There were a few considerations that has been taken into account before selecting this area as a study location. The points of interest were as following:

i. Agriculture areas where paddy cultivation is the main activity.

ii. The distance of study location is near to the laboratory which preservation and analysis are carried out.

iii. The residents who are volunteer to cooperate as respondents in this study.

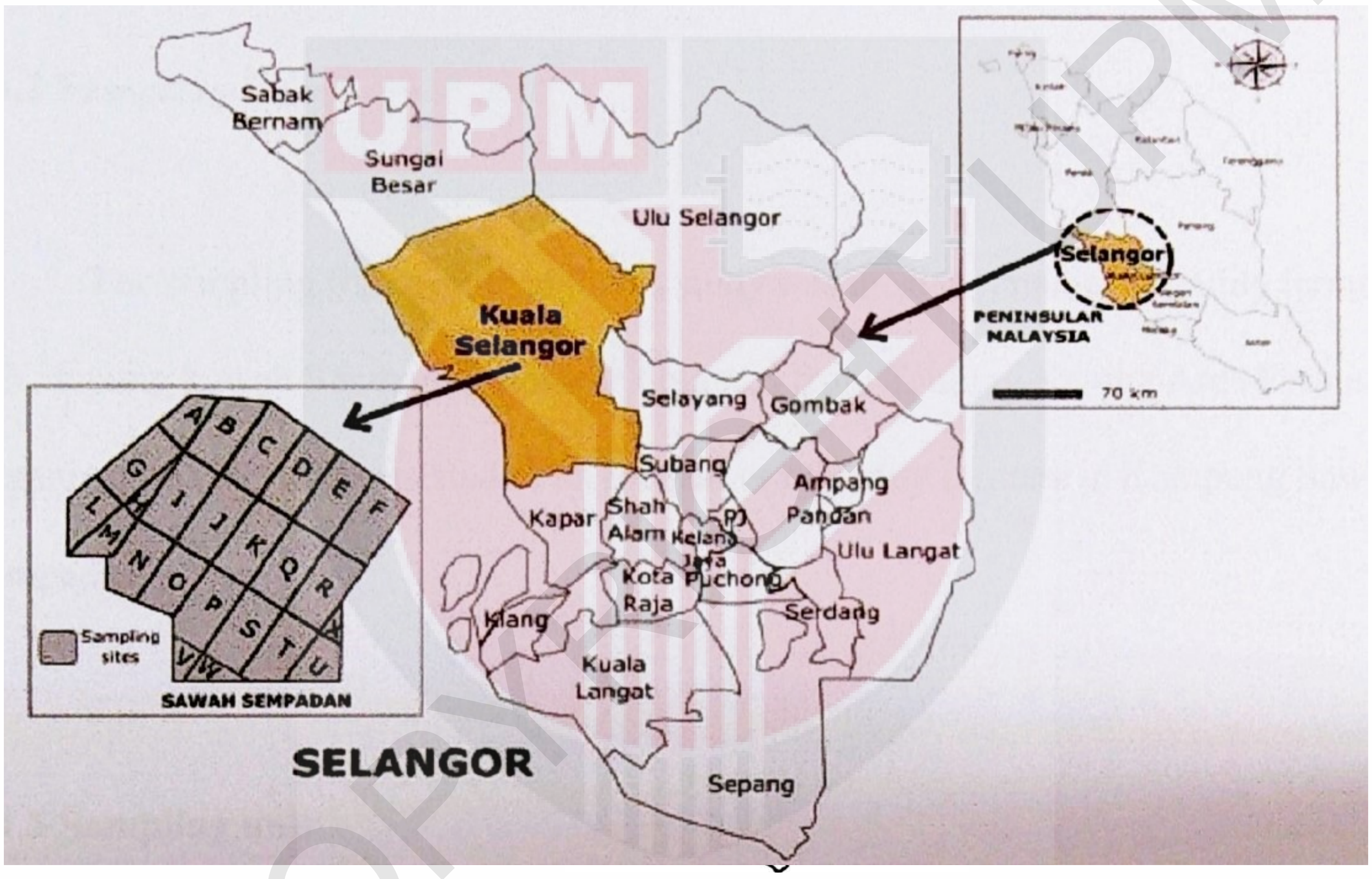


Figure 3.1: Map of Kampung Sawah Sempadan, Tanjung Karang, Kuala Selangor, Malaysia

3.4 Sampling

3.4.1 Sampling population

The target population for this study were paddy farmers in Kampung Sawah Sempadan, Tanjung Karang, Kuala Selangor.

3.4.2 Sampling frame

The sampling frame defined in this study was the list of names of paddy farmers in Kampung Sawah Sempadan that was obtained from Tanjung Karang Area Farmer's Organization. The list consisted of all the names of paddy farmers in Kampung Sawah Sempadan.

3.4.3 Sampling unit

Paddy farmers were selected as sampling unit. Below were the inclusion criteria for paddy farmers that being included throughout this study:

i. Aged between 18 and 60 years old.
ii. Worked directly in paddy field for more than six months.

The exclusion criteria was important to obtain reliable results. Therefore, exclusion criteria throughout this study was paddy farmers who hired workers or helper to manage their paddy field.

3.4.4 Sampling size

The total population of paddy farmers in Tanjung Karang, Kuala Selangor is approximately 7679. Meanwhile, the total population of paddy farmers in Kampung Sawah Sempadan, Tanjung Karang is around 1147. The sample size for present study was ascertained by using formula adapted from Lemeshow et al. (1990). Hence, the required sample size was calculated by using following formula:

$$n = \frac{Z^2\, 1 - \frac{a}{2}\, P\,(1 - P)}{d^2}$$

Equation 3.1

Where,

n	= Sample size
$Z^2\, 1 - \frac{a}{2}$	= Standard errors associated with confidence intervals, 95% (1.96)
P	= Highest prevalence, 0.1

d = Desired precision, 0.05

The calculation was as following:

$$n = \frac{(1.96)^2 \, x \, (0.1)(1-0.1)}{(0.05)^2}$$

$$\boldsymbol{n = 138}$$

The value if prevalence P was 0.1, from the study by Anh et al. (2007) which mentioned the overall prevalence of dermatitis among farmers due to exposure to water in which they engaged in agricultural activities in Hanoi, Vietnam.

The minimum sample size for this study was 138 respondents. In order to achieve appropriate response rate, 10% of minimum sample size were added to 138.

The calculation was as following:

10% of minimum sample size $= \frac{10}{100}$ x minimum sample size

$= \frac{10}{100}$ x 138

$= 13.8 \approx 14$

Total respondents = 138 + 14

= 152

Therefore, at least 152 questionnaires were disseminated among the target population.

3.5 Sampling technique

3.5.1 Water sampling

The water samples were collected from paddy field in Kampung Sawah Sempadan to determine concentrations of pesticides. There were a total of 24 blocks of paddy field (Block A to Block X) included in this study. Composite paddy water samples of each of the paddy block were taken in five different sampling points as illustrated in Figure 3.2. One liter of the paddy water was collected from each sampling point. Five water samples collected from each block were homogenised. Then, three replicates of composite water samples were collected by using one liter amber glass bottle and kept immediately in ice box at 0-4°C. A total of 72 water samples were collected from 24 blocks of paddy field in this study.

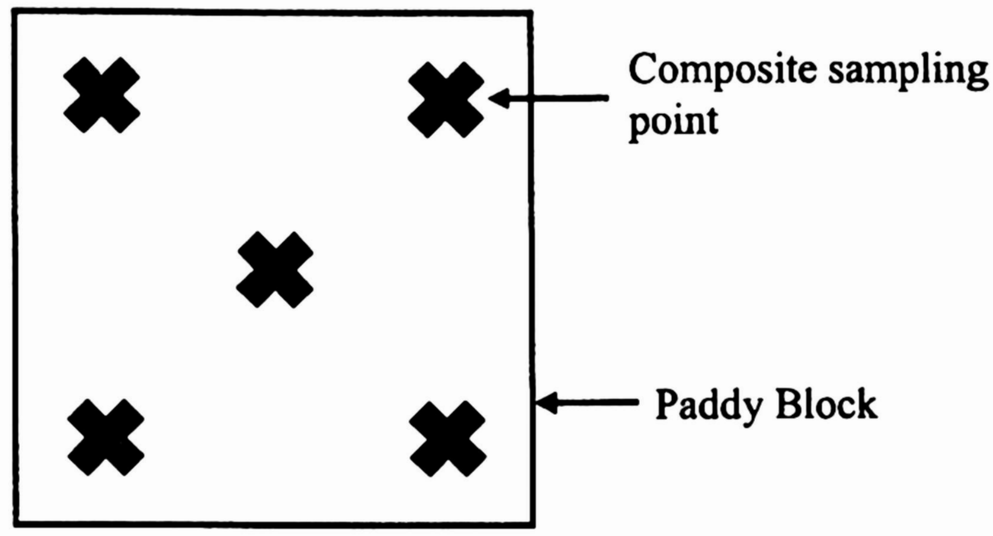


Figure 3.2: Five sampling points for water samples collection for each block

The collected water samples were labeled on-site using self-administered labeling stickers and transported back to the Environmental Health Laboratory at the Faculty of Medicine and Health Sciences, Universiti Putra Malaysia on the same day. Samples were kept in refrigerator with an average temperature of 4°C and analysed within one week after sampling by using UPLC-MS/MS.

3.5.1.1 Measurement of *in situ* water quality parameters of water

The *in situ* water quality parameters which includes pH of water, temperature, electrical conductivity, dissolved oxygen, turbidity, salinity and total dissolve solid (TDS) were measured in this study by using respective instruments. pH of water samples were measured by using Milwaukee Portable pH meter, USA. Temperature, electrical conductivity, salinity and TDS were measured by using Waterproof Portable Meter (CyberScan Series 600, USA). Dissolved oxygen was measured by using Eutech Instrument Cyberscan DO110 Dissolved Oxygen Portable meter (Thermo Fisher Scientific Inc, USA) meanwhile HACH 2100P Turbiditimeter, USA was used to measure turbidity. All parameters were measured and recorded at the same time on site to ensure that the reading or data taken were accurate. The readings of each of the parameters were recorded three times.

3.5.1.2 Water samples preservation

Based on Guidelines for Field-Measured Water-Quality Properties by Wilde (2008) with modifications, paddy water samples were collected from all paddy blocks in Kampung Sawah Sempadan by using silanized amber glass bottles and were kept in temperature in the range of 0-4°C. Silanized glass bottles were used for water samples collection to prevent pesticides compounds react with the bottle as no harmful chemicals can leach into its content. Furthermore, amber glass bottle can prevent change of the water samples compositions via photodegradation.

After the water collection, 80 mg of sodium thiosulphate was immediately added into each of the silanized amber glass bottles to neutralize the water samples. Before undergoing extraction and analysis processes, the water samples were preserved and undergone pre-treatment to ensure that significant changes in composition did not happen before the analysis were made.

3.5.2 Selection of target compounds

There were 13 compounds of regular used pesticides that have been distinguished upon interviewing paddy farmers and only three of these compounds were selected to be analysed throughout this study. Pesticides active ingredients with their frequency and percentage of usage is summarized in Table 1.1.

The three compounds chosen for this study were isoprothiolane, propiconazole and pretilachlor. Information of the individual targeted compounds which encompasses International Organization for Standardization (ISO) commmon name, pesticides type, IUPAC name, Chemical Abstracts Service (CAS) number, molecular formula, molecular structure and molecular weight, log K_{ow} and cancer classification are summarized in Table 2.1.

3.5.3 Extraction and preparation of samples

Two hundred and fifty millilitres of water samples were filtered with 1.0 μm GFF and further filtered with 0.45 μm and 0.22 μm nylon membrane to remove particles that may interfere during the analysis process. Prior SPE, 50 μg/L of internal standard (imidacloprid-d_4) were added to 250 mL of filtered water samples. The water samples were extracted by using SPE method that was modified according to Herrero-Hernández et al. (2013) before UPLC-MS/MS analysis. SPE and UPLC-MS/MS were used to quantify the pesticides concentrations in the water samples. The SPE cartridges, Oasis HLB (3 cc, 60 mg) (Waters, MA, USA) were conditioned with 5 mL of acetone followed by 5 mL of acetonitrile. Then, the cartridges were washed with 5 mL of ultrapure water and loaded with 250 mL of water samples. The cartridges were dried under vacuum pump for 20 minutes to remove residual water and analytes. After 20 minutes, the cartridges were eluted with 2 mL of acetonitrile followed by 2 mL of acetone. The 4 mL of solvents containing acetonitrile and acetone were then be evaporated to dryness at 40°C under a gentle stream of nitrogen. The extracts were reconstituted with 1 mL of methanol-water (50:50, v/v) solution. Analyses were performed by injecting 2 μL of the final extract into the UPLC-MS/MS system.

3.6 Study instrumentation

3.6.1 Questionnaire

A set of questionnaire was developed based on information gathered from various studies in order to assess the dermal health risks of paddy farmers against pesticides exposures.

The questionnaire consists of three sections which were Section A, Section B and Section C. Section A was regarding the personal information of respondent which included age, gender, races and weight. Section B was regarding of respondent's exposure to pesticides. This section was to obtain the frequency and duration of respondent exposed to pesticides. Meanwhile, Section C was related to PPE usage.

3.6.2 Ultra performance liquid chromatography tandem mass spectrometry (UPLC-MS/MS)

The analysis of pesticides using UPLC-MS/MS was performed on an Zorbax Eclipse Plus C18 column (2.1 mm × 50 mm I.D., 1.8 μm particle size) (Agilent Technologies, CA, USA). The mobile phase were composed of: (A) a gradient of 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. Then, the mobile phase were mixed as in Table 3.1.

Table 3.1: UPLC-MS/MS gradient program

Time (minutes)	A (%)	B (%)	Flow Rate (mL/min)
0.00	94	6	0.5
15.00	2	98	0.5
18.00	2	98	0.5
18.01	94	6	0.5
20.00	94	6	0.5

Mobile phase A (aqueous) : 0.1% formic acid and 5 mm ammonium formate in ultrapure water.

Mobile phase B (solvent) : 0.1% formic acid and 5 mm ammonium formate in methanol.

The flow rate used was at 0.5 mL/min and the total runtime was 20 minutes. The column temperature used was set at 40°C and the injection volume was 2 μL. All pesticides were detected using electrospray ionization (ESI) in positive ion mode. The optimised operating conditions were as follows: capillary voltage, 3500 V; gas temperature, 220 °C; gas flow, 11 L/min and nebulizer of 30 psi. The optimised ESI and MS/MS parameters, comprising of precursor ions, products ions, collision energy (C.E.) and fragmentation voltage are shown in Table 3.2. The optimised 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, *m/z***	**Product, *m/z* (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 Standard	**Precursor Ion, m/z**	**Product, m/z (Collision Energy, V)**	**Fragmentation, V**
Imidacloprid-d_4	260.10	212.90 (21)	380

3.7 Quality control

3.7.1 Cleaning of glassware

All the glasswares were washed using 5-10% hydrochloric acid wash before used to ensure they were free from any contaminants as glassware could contaminate its content, in light of EPA method 1699 (2007). All glasswares were soaked with 5-10% hydrochloric acid overnight followed by washed with a detergent solution, Decon 90. Next, all the glasswares were rinsed immediately by using methanol and followed with hot tap water. From that point forward, all the glasswares were rinsed with methanol again and followed by acetone and dichloromethane. After washing, all glasswares were dried at 60°C and capped with solvent rinsed aluminium foil to prevent any accumulation of dust or other contaminants.

3.7.2 Calibration of UPLC-MS/MS

UPLC-MS/MS was calibrated with each analytes at a five-point calibration curve. The labelled internal standards (imidacloprid-d_4) corresponding to the analytes was added to each calibration point at a concentration of 50 ng/mL to generate relative response factors. Calibration curve were obtained by injecting the native standards of isoprothiolane, propiconazole and pretilachor with the range from instrument quantitation limit (IQL) to 500 µg/L.

3.7.3 Extraction recovery

The extraction recovery was evaluated to validate the analytical procedure and this was done by spiking the samples with varied amounts of standard solutions of each compounds (Zhuang et al., 2009). The percent of recovery was calculated by comparing the concentrations of each compounds spiked before extraction (C_p) to its concentration spiked after extraction (C_a) in the similar sample matrix using Equation 3.2 (Ho et al., 2012) where C_{qc} is the concentration of analyte in blank sample.

$$\text{Recovery (\%)} = \frac{C_p - C_{qc}}{C_a - C_{qc}} \; x \; 100$$

Equation 3.2

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.

3.7.4 Preparation of blank materials

One liter of ultrapure water that stored in a silanized glass bottle was served as field blank to check for background contamination during sampling. For every 12 samples, a blank sample was analyzed same as the samples from the extraction until the instrumental analysis in order to check if there is any interference or contamination. Ultrapure water was utilized as blank sample throughout this study.

3.7.5 Instrumental detection limit (IDL), instrumental quantitation limit (IQL), method detection limit (MDL) and method quantitation limit (MQL)

In this study, instrumental detection limit (IDL) and instrumental quantitation limit (IQL) for chromatographic method were determined based on chromatographic response that produce signal-to-noise ratio (S/N) (Sobańska et al., 2015) by preparing different concentrations of pesticides mixtures standards from 0.0001 ng/mL to 0.1 ng/mL. Each concentration was directly injected into UPLC-MS/MS instrument and as the concentrations that produce peaks with S/N of 3 and 10, it determined the IDL and IQL respectively. On the other hand, method detection limit (MDL) and method quantitation limit (MQL) were estimated based on signal-to-noise method (Shrivastava & Gupta, 2011). This method performed by measuring the peak-to-peak noise produce by the target analyte from the test sample and therefore, the concentration of analyte would produce a certain value of S/N were estimated. A S/N of 3 and 10 was considered acceptable for determining the MDL and MQL respectively (International Conference on Harmonisation, 2005).

3.8 Health risk assessment

Characterization of risk was the final step in health risk assessment to estimate the type and magnitude of an adverse health effect of pesticides exposures could cause under particular circumstances. The risk for dermal exposure to pesticides in paddy water was assessed under the present environmental conditions at Kampung Sawah Sempadan, Tanjung Karang.

In this study, the quantitative dermal health risk assessment was focused only to non-carcinogenic health risk. The non-carcinogenic health risk was estimated using a standard EPA method by HQ. It is a division of dermal absorbed dose (DAD) to the compounds-specific reference dose (RfD). Based on USEPA (2004), the HQ calculation was calculated using Equation 3.3.

$$Dermal\ hazard\ quotient = \frac{DAD}{\mathrm{RfD}}$$

Equation 3.3

Where,

DAD = Dermal Absorbed Dose (mg/kg-day)

RfD = Absorbed reference dose (mg/kg-day)

According to USEPA (2005), if the value of HQ is less than one, it indicates that there is no significant risk of non-carcinogenic health risk whereas, if HQ value is more than one means there is a significant non-carcinogenic health risk. The risk may increase as HQ value is increasing. On top of that, determination of HQ value requires the RfD value. It is appropriate to use chronic reference dose (cRfD) values to determine the HQ for all recommended exposure pathways (USEPA, 2005). The cRfD for the three chosen analytes are shown in Table 3.3.

Table 3.3: The chronic reference dose

Analytes	cRfD (mg/kg-day)
Isoprothiolane	N/A
Propiconazole	0.1[a]
Pretilachlor	N/A

[a]USEPA (2015)

N/A – not available

The RfD value obtained in Table 3.3 was for oral reference dose (RfD_{oral}). Dermal reference dose (RfD_{dermal}) was required for this study to determine the non-carcinogenic dermal health risk. According to Kegley et al. (2014), the RfD_{oral} is used to estimate RfD_{dermal} by using Equation 3.4.

$$RfD_{dermal} = \frac{RfD_{oral}}{AF}$$

Equation 3.4

Absorption factor (AF) was calculated using the following formula (Kegley et al., 2014):

$$AF = 1 - e^{-Ka*ET}$$

Equation 3.5

Meanwhile, the dermal absorption coefficient (K_a) was calculated according to Equation 3.6.

$$\log K_a = 0.233 \times logK_{ow} - 0.00566 \times MW - 1.49$$

Equation 3.6

The following equation was used to calculate DAD (USEPA, 2004):

$$DAD = \frac{DA_{event} \times EV \times ED \times EF \times SA}{BW \times AT}$$

Equation 3.7

DA_{event} was calculated using the following formula (USEPA, 2004):

$$DA_{event} = 2\ FA \times K_p \times C_w \times \sqrt{\frac{6\ \tau_{event} \times \tau_{event}}{\pi}}$$

Equation 3.8

In order to determine DA_{event}, value of τ_{event} and K_p need to be calculated according to the following equations (USEPA, 2004):

$$\tau_{event} = 0.105 \times 10^{(0.0056\ MW)}$$

Equation 3.9

$$log K_p = -2.8 + 0.66\ log K_{ow} - 0.0056\ MW$$

Equation 3.10

The values of each parameters stated in Equation 3.4 to Equation 3.10 were tabulated in Table 3.4.

Table 3.4: Values of each parameters

	Parameters	Values	References
RfD_{dermal}	Dermal Reference Dose (mg/kg-day)	Based on calculation	Equation 3.4
DA_{event}	Absorbed dose per event (mg/cm^2-event)	Based on calculation	Equation 3.8
EV	Event frequency (events/day)	1	Question 12
ED	Exposure duration (years)	27	Question 6
EF	Exposure frequency (days/year)	220	Question 12
BW	Average body weight (kg)	70.161	Question 4
AT	Average time (days)	5940	Product of ED and EF

		Surface Area	**50th Percentile Surface Area of Male Adults (cm^2)**	
SA	Skin surface area available for contact	Forearms	1310	USEPA (2004)
		Hands	990	
		Lower legs	2560	
		Feet	1310	
K_p	Dermal permeability coefficient of compound in water (cm/hr)	Based on calculation		Equation 3.10

C_w	Chemical (pesticides) concentration in water (mg/cm^3)	Based on laboratory analysis	Analysis
t_{event}	Event duration (hr/event)	2	Interview
τ_{event}	Lag time per event (hr/event)	Based on calculation	Equation 3.9
K_a	First-order dermal absorption coefficient (h^{-1})	Based on calculation	Equation 3.6
K_{ow}	Octanol/water partition coefficient of the non-ionized species (dimensionless)	**Analytes** / **Log K_{ow}**	PubChem (2018)
		Isoprothiolane / 2.880	
		Propiconazole / 3.720	
		Pretilachlor / N/A	

AF	Absorption factor (dimensionless)	Based on calculation	Equation 3.5
FA	Fraction absorbed water (dimensionless)	1	Calandra et al. (2016)
ET	Total exposure time (hr)	24	Kegley et al. (2014)
MW	Molecular weight (g/mole)	**Analytes** / **Molecular weight (g/mole)** Isoprothiolane: 290.392 Propiconazole: 342.220 Pretilachlor: 311.850	PubChem (2018)

N/A – not available

3.9 Statistical analysis

All the data obtained in this study were analyzed by using Statistical Package for the Social Sciences (SPSS) Version 24. Descriptive statistical parameters such as mean and standard deviation (SD) were used to describe pesticides concentrations in paddy water samples. Spearman correlation was used to determine the correlation between concentrations of pesticides and *in situ* water quality parameters at a significant level of $p < 0.05$.

3.10 Ethical consideration

All respondents were briefed on the study and signed a written informed consent. This study was approved by the University Research Ethics Committee of Universiti Putra Malaysia, Selangor, Malaysia (JKEUPM) [Ref No.: JKEUPM-2017-187].

CHAPTER 4

RESULTS

4.1 Quality Control

Calibration curves were obtained for all target compounds to determine the concentration of pesticides in the water 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 µg/L and 500 µg/L with regression coefficients (R^2) ranging from 0.9995 to 0.9996. The IDL and IQL for all target compounds ranged from 0.001 µg/L to 0.050 µg/L. The MDL and MQL for all target compounds ranged from 0.005 µg/L to 0.500 µg/L respectively. The recovery for all target compounds ranged from 98% to 105%. Details information of IDL, IQL, MDL, MQL, linear range, R^2 and recovery are summarized in Table 4.1.

Table 4.1: IDL, IQL, MDL, MQL, linear range, R^2 and recovery values for all target compounds

Compounds	IDL (µg/L)	IQL (µg/L)	MDL (µg/L)	MQL (µg/L)	Linear Range (µg/L)	R^2	Recovery % Mean ± SD 100 µg/L, n=3
Isoprothiolane	0.001	0.010	0.040	0.500	0.01 – 500	0.9996	105 (11)
Propiconazole	0.001	0.010	0.050	0.100	0.01 – 500	0.9995	98 (12)
Pretilachlor	0.010	0.050	0.005	0.040	0.05 – 500	0.9996	101 (12)

4.2 Demographic background of paddy farmers

The questionnaires were distributed among 152 paddy farmers to obtain their demographic background. Table 4.2 summarized the demographic information of paddy farmers. From the table, it showed that 100% of respondents (n=152) were Malay farmers. All of the paddy farmers were male aged between 18 years and 60 years with an average of 51 years old. Meanwhile, the average weight of the paddy farmers was 70.16 kg which were ranged from 45 kg to 120 kg.

Table 4.2: Demographic background of paddy farmers (N=152) in Kampung Sawah Sempadan

Variable	Category	Frequency	Percentage
Gender	Male	152	100.0
Race	Malay	152	100.0
	Mean (SD)	**Min**	**Max**
Age (years)	51.29 (12.604)	18	60
Weight (kg)	70.16 (12.425)	45	120

4.3 Personal hygiene and use of PPE

One hundred and fifty (98.7%) of the paddy farmers used pesticides to control pests in their paddy field while the rest (1.3%) did not use pesticides. The paddy farmers were also asked in the questionnaire regarding their personal hygiene and the use of PPE during their working in paddy field, as outlined in Table 4.3.

Table 4.3: Personal hygiene and the use of PPE among paddy farmers (N=152)

Variable	Category	Frequency	Percentage
Taking a 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 of PPE during pesticides application	Yes	71	46.7
	No	81	53.3

The results from Table 4.3 showed that, 127 out of 152 (83.6%) paddy farmers were immediately taking shower after pesticides application and 25 (16.4%) of them were reported did not immediately shower up after application of pesticides. Additionally, changing clothes after pesticides application were reported by most of paddy farmers (95.4%) and only 7 out of 152 (4.6%) of them did not change their clothes. Other than personal hygiene, paddy farmers were also asked if they use PPE during their activities in paddy field. Most of the paddy farmers did not use any PPE during application of pesticides (53.3%) and it was only 46.7% of them use PPE while working with pesticides.

Following the use of PPE, paddy farmers were also asked regarding the type of PPE they worn during pesticides application. Table 4.4 summarized the type of PPE used by paddy farmers.

Table 4.4: 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 illustrated in Table 4.4, hat or hood (44.7%) was the most popular PPE among paddy farmers and this was followed by boots (41.4%), face shield (36.8%) and gloves (34.2%). Also, 37 out of 152 paddy farmers used goggles (24.3%) and 17 out of 152 paddy farmers used overalls (11.2%) during their activities in paddy field. Meanwhile, the least used PPE was apron (2.0%) where only 2 out of 152 of paddy farmers used apron.

4.4 Concentrations of isoprothiolane, propiconazole and pretilachlor in paddy water

The three target compounds were detected in 24 blocks of paddy field (Block A to Block X) in Kampung Sawah Sempadan which include isoprothiolane, propiconazole and pretilachlor. Table 4.5 illustrates the concentrations of target compounds in paddy water.

Table 4.5: Mean and SD for concentrations (µg/L) of isoprothiolane, propiconazole and pretilachlor in paddy water samples of all paddy blocks (n=24)

Block	Isoprothiolane	Propiconazole	Pretilachlor
	Mean ± SD	Mean ± SD	Mean ± SD
A	3.699 ± 4.537	<MQL	<MQL
B	9.223 ± 0.330	<MQL	<MQL
C	2.635 ± 0.239	<MQL	<MQL
D	<MQL	<MQL	<MQL
E	<MQL	<MQL	<MQL
F	1.011 ± 0.167	0.290 ± 0.015	<MQL
G	51.542 ± 41.363	0.106 ± 0.135	<MQL
H	0.621 ± 0.051	<MQL	<MQL
I	0.632 ± 0.031	<MQL	<MQL
J	23.962 ± 12.774	12.434 ± 7.062	<MQL
K	17.871 ± 5.130	<MQL	<MQL
L	10.562 ± 7.954	0.179 ± 0.156	<MQL
M	7.295 ± 1.895	0.419 ± 0.060	<MQL
N	14.246 ± 4.817	<MQL	<MQL
O	3.674 ± 4.450	0.245 ± 0.013	<MQL
P	1.471 ± 1.032	<MQL	0.109 ± 0.116
Q	<MQL	<MQL	<MQL

Block	Isoprothiolane Mean ± SD	Propiconazole Mean ± SD	Pretilachlor Mean ± SD
R	1.162 ± 0.128	<MQL	<MQL
S	<MQL	<MQL	<MQL
T	<MQL	<MQL	<MQL
U	<MQL	0.059 ± 0.016	<MQL
V	0.853 ± 0.469	<MQL	<MQL
W	<MQL	<MQL	<MQL
X	<MQL	<MQL	<MQL

MQL – method quantitation limit

From the table above, it illustrates the highest mean concentration of isoprothiolane was at Block G (51.542 µg/L). The lowest mean concentration of isoprothiolane detected among 24 paddy blocks was at Block H (0.621 µg/L). Meanwhile, Block J was detected with the highest mean concentration of propiconazole (12.434 µg/L) and the lowest mean concentration of propiconazole was at Block U (0.059 µg/L). However, the mean concentration of pretilachlor was detected only at Block P among 24 paddy blocks with the mean concentration of 0.109 µg/L. Therefore, isoprothiolane had the highest mean concentration, while pretilachlor had the lowest mean concentration among all target compounds. Other than that, the concentrations of the target pesticides were below MQL in some of the paddy blocks.

4.5 *In situ* water quality parameters

In situ water quality parameters measured in this study include temperature, pH, electrical conductivity (EC), dissolved oxygen (DO), turbidity, salinity and total dissolved solid (TDS). The results for water temperature and pH were almost similar for each of the paddy blocks. The temperature readings were ranged from 27.03 °C to 31.33 °C, while pH readings were ranged from 5.35 to 7.26. The values of electrical conductivity, dissolved oxygen, turbidity, salinity and TDS were 1.19 µS/cm to 474.30 µS/cm, 2.30 mg/L to 7.53 mg/L, 14.13 NTU to 573.97 NTU, 1.21 ppm to 461.63 ppm, 1.17 mg/L to 462.87 mg/L, respectively. The mean and SD of *in situ* qualitative parameters of water are attached in Appendix E for reference.

4.6 Correlation between the concentrations of target compounds and *in situ* water quality parameters

Spearman correlation was used to determine the significant non-linear relationships between concentration of target compounds and *in situ* water quality parameters. The relationship between concentration of target compounds in the water samples and the *in situ* qualitative parameters of water were summarized in Table 4.6.

Table 4.6: Results of non-linear correlation analysis of pesticides concentrations and *in situ* water quality parameters

	Isoprothiolane	Propiconazole	Pretilachlor	Temperature	pH	EC	DO	Turbidity	Salinity	TDS
Isoprothiolane	1.000									
Propiconazole	-.174	1.000								
Pretilachlor	-.046	-.094	1.000							
Temperature	-.365	.004	-.316	1.000						
pH	-.005	-.095	.045	.281	1.000					
EC	.223	.056	.045	.010	.051	1.000				
DO	-.180	.268	-.105	.317	.011	.037	1.000			
Turbidity	.045	.312	-.256	.221	.005	-.094	.017	1.000		
Salinity	.219	.070	.045	.018	.053	**.999****	.044	-.097	1.000	
TDS	.223	.056	0.45	-.002	.046	**.999****	.045	-.105	**.998****	1.000

* Correlation is significant at the 0.05 level (2-tailed).

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

As shown in Table 4.6, there were no significant correlation between the concentrations of target compounds (isoprothiolane, propiconazole and pretilachlor) with the *in situ* water quality parameters. Nevertheless, there were a strong positive correlations between (i) electrical conductivity and salinity ($r = 0.999$, $p = 0.01$), (ii) electrical conductivity and TDS ($r = 0.999$, $p = 0.01$), and (iii) salinity and TDS ($r = 0.998$, $p = 0.01$).

4.7 Pesticides exposure

Paddy farmers were asked regarding their exposure to pesticides using questionnaires. This information was used to assess their dermal health risk due to pesticides exposure during work activities in paddy field. Table 4.7 summarized the informations of paddy farmers' exposure to pesticides.

Table 4.7: Pesticides exposure among the paddy farmers (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

Paddy farmers were interviewed regarding their exposure to pesticides based on their working hour per day and this is refer to t_{event}. Results showed that the paddy farmers work for 2 hours a day. Meanwhile, the paddy farmers were asked in the questionnaire regarding the event frequency (EV) which was based on their engagement in paddy cultivation activities for a daily basis. The result shown that the paddy farmers work once a day. As shown in the table, the questionnaire also comprises of paddy farmers' exposure duration (ED) to pesticides in years. Results showed that the paddy farmers involved in this study have been exposed to pesticides for approximately 27 years. The exposure frequency (EF) of paddy farmers within a year was 220 days. The averaging time (AT) of paddy farmers' exposed to pesticides was 5940 days, which was the result of the product of ED and EF.

4.8 Dermal health risk assessment

Among all the target compounds, only HQ for propiconazole was calculated. There were certain possible body surfaces considered in determining the HQ of paddy farmers which include forearms, hands, lower legs and feet. The HQ of paddy farmers exposure to propiconazole is illustrated in Table 4.8.

Table 4.8: HQ of paddy farmers exposed to propiconazole

Block	HQ				
	Forearms	Hands	Lower Legs	Feet	HQ (Total)
F	2.184×10^{-7}	1.650×10^{-7}	4.267×10^{-7}	2.184×10^{-7}	1.029×10^{-6}
G	7.985×10^{-8}	6.033×10^{-8}	1.560×10^{-7}	7.985×10^{-8}	3.760×10^{-7}
J	9.367×10^{-6}	7.076×10^{-6}	1.830×10^{-5}	9.367×10^{-6}	4.411×10^{-5}
L	1.347×10^{-7}	1.019×10^{-7}	2.633×10^{-7}	1.347×10^{-7}	6.346×10^{-7}
M	3.155×10^{-7}	2.385×10^{-7}	6.165×10^{-7}	3.155×10^{-7}	1.486×10^{-6}
O	1.846×10^{-7}	1.395×10^{-7}	3.607×10^{-7}	1.846×10^{-7}	8.694×10^{-7}
U	4.444×10^{-8}	3.358×10^{-8}	8.682×10^{-8}	4.444×10^{-8}	2.093×10^{-7}
Average	$\mathbf{1.478 \times 10^{-6}}$	$\mathbf{1.116 \times 10^{-6}}$	$\mathbf{2.887 \times 10^{-6}}$	$\mathbf{1.478 \times 10^{-6}}$	$\mathbf{6.959 \times 10^{-6}}$

The results showed that all HQ values did not exceed one. Lower legs exposure (2.887×10^{-6}) has the highest average value of HQ, followed by forearms and feet exposure which had the same value of HQ (1.478×10^{-6}). The lowest value for HQ was hands exposure (1.116×10^{-6}). On the other hand, the highest HQ for total exposures (forearms, hands, lower legs and feet) was found in Block J (4.411×10^{-5}) while the lowest HQ was found in Block U (2.093×10^{-7}). Lastly, the average value for all four body surfaces exposure which labelled as HQ total was 6.959×10^{-6}.

CHAPTER 5

DISCUSSION

5.1 Demographic background of paddy farmers, personal hygiene and use of PPE

In this study, we determined the demographic of paddy farmers. All of paddy farmers involved in this study were Malay as majority of residents in Kampung Sawah Sempadan were Malay. In terms of gender, the paddy farmers were all male which suggest that the female residents were not engaged in cultivation activities like male residents. The mean age of paddy farmers was 51 years old. This is an indication that rice cultivation activities are usually carried out by the middle-aged group. The average weight of paddy farmers was 70.16 kg. This finding is close to the body weight for adult which is 70 kg as suggested by USEPA (2004).

Most of paddy farmers used pesticides to control pests in their paddy field and only a small number of paddy farmers did not directly used pesticides. This finding is similar to a study in Kampung Sawah Sempadan done by Mazlan et al. (2016) where large percentage of paddy farmers (92.5%) been exposed to pesticides imazapic and the rest (7.5%) were never used of the pesticides. Some of paddy farmers in the current study did not directly used pesticides, but they hire others to spray pesticides in their paddy field. Eventually, the pesticides sprayed by others will enter the paddy water and the paddy farmers will be exposed to pesticides in the paddy water.

Due to the importance of personal hygiene and PPE, this study gave special attention to paddy farmers' personal hygiene and the usage of PPE among them. Most of the paddy farmers immediately taking a shower and change their clothes after working with pesticides. This shows that the paddy farmers practiced good personal hygiene after application. The use of PPE when working with pesticides is important to prevent paddy farmers from pesticides exposure. According to Nyatuame & Polytechnic (2015), the use of PPE help to prevent farmers from being exposed to hazardous chemicals used in agricultural areas during farming activities. Despite the advantages of PPE usage, in the current study, there was a great majority of paddy farmers did not use PPE during pesticides application. According to Sharifzadeh et al. (2017), the reasons that caused the paddy farmers not using PPE could be due to (i) high cost of PPE (ii) PPE items are not available locally (iii) none of colleagues use PPE during working with pesticides (iv) use of PPE is too time-consuming and (v) PPE reduces physical flexibility. Thus, this would increasing their exposure to the hazardous chemicals. The study by Okoffo et al. (2016) indicates that farmers' noncompliance to use PPE when working with pesticides lead to increase the potential of pesticides exposure, which cause serious health consequences. Wearing of PPE in this study was defined as wearing boots, apron, overalls, gloves, goggles, face shield and hat or hood at the time of working in paddy field. In this study, most of paddy farmers wore a hat or hood could be due to avoid being directly exposed to hot weather. Meanwhile, the least use of PPE was an apron. Only a small numbers of paddy farmers used apron as PPE as they think wearing a shirt is sufficient to avoid dermal contact with pesticides particularly at their abdomen regions.

5.2 Concentrations of isoprothiolane, propiconazole and pretilachlor in paddy water

In this study, the highest mean concentration of pesticides in paddy water was isoprothiolane. Correspondingly, a previous study done by Ouyang et al. (2017) found that the concentration of isoprothiolane (1.357 μg/L) in river water from agricultural area in northern China was the highest compared to other targeted pesticides in their study. Another study by Derbalah et al. (2003) on the pesticides contamination in Japan, also found that isoprothiolane (37 ng/L) was detected with the highest concentration compared to the other nineteen pesticides in their study. The maximum mean concentration of isoprothiolane detected in this study was 51.542 μg/L which was higher compared to previous studies. This could be due to the water sample was directly collected from paddy field, while previous studies were in the river water where agricultural water flows.

The results showed that propiconazole was the second highest mean concentration after isoprothiolane. The water samples from 7 out of 24 paddy blocks were contaminated with propiconazole while the rest of the water samples were below MQL (Table 4.5). Based on the previous study by Battaglin et al. (2011) in United States on 12 different fungicides, it was found that propiconazole concentration in the water sample was 1.15 μg/L. The concentration of their study is lower as compared to this study.

With the exception of water sampled from Block P which recorded a mean pretilachlor concentrations of 0.109 μg/L, all the other paddy blocks were found to be below MQL (Table 4.5). Arora et al. (2014) previously reported the pretilachlor residues were not detected in water samples of rice fields in Sonepat and Dehradun, India. However, pretilachlor has been detected in the water samples from Haryana, India ranging between 0.21 μg/mL to 0.81 μg/mL (Duhan & Punia, 2015). The concentration of pretilachlor that found in this study was lower compared to Duhan and Punia (2015).

Isoprothiolane and propiconazole are fungicides used to kill the presence of fungi in paddy field while pretilachlor is categorised as herbicides used to control the weedy rice. The results found in this study revealed that pretilachlor was not detected in 23 out of 24 paddy blocks may be due to the duration of sampling of paddy water that was two weeks before harvesting. During this time, herbicide was not as actively applied as fungicides since there were very minimal presence of weed. Other than that, previous study reported that pretilachlor readily dissipates in rice fields by photodecomposition, microbial degradation and volatilization (Kumar et al., 2018) and consequently, the data obtained from this study showed that most of the concentration of pretilachlor were below MQL. On the contrary, isoprothiolane and propiconazole concentrations were detected in most of the paddy blocks. The detection of fungicides (isoprothiolane and propiconazole) in most of the water samples could be due to duration of sampling was near to the harvesting period. Pesticides application at that time was more focussed on fungi that could infested the crops. Fungicides were widely used during the vegetative, reproductive and ripening phases of paddy to minimize crop damage and protect the quantity of agricultural products (Hamsan et al., 2017). Therefore, the paddy farmers tend to use fungicides to protect the production of rice.

5.3 Correlation between the concentrations of target compounds and *in situ* water quality parameters

The *in situ* water quality parameters are physical parameters while the concentrations of pesticides are chemical parameters. The results obtained from this study found that there were no significant correlation in pesticides concentrations collected from paddy water to the *in situ* water quality parameters. Electrical conductivity is one of the water parameters that is imperative and related to the amount of soluble salts concentrations (The Center for Agriculture, Food and the Environment, 2018). Salinity is a measure of salts in the water while solids can be obtained in nature in a dissolved form (Clean Water Team, 2004). Salts that dissolve in water break into positively and negatively charged ions (Clean Water Team, 2004). Since dissolved ions increase salinity and additionally conductivity, these explains why there is a significant positive correlation between (i) EC and salinity, (ii) EC and TDS, and (iii) salinity and TDS.

5.4 Dermal health risk assessment

In terms of estimating the risk posed by paddy farmers, this study focused on chronic exposures to the target compounds in the paddy water through dermal contact. Based on the available information in the risk assessment database, only propiconazole was calculated for the HQ. The RfD for isoprothiolane and pretilachlor was not available from literature search. HQ for paddy farmers exposed to propiconazole was calculated for certain possible exposures of body surfaces such as forearms, hands, lower legs and feet. After the calculation, all the HQ values for propiconazole were found to be less than value of one. These indicate that there were no significant dermal health risks for the exposure of propiconazole among the paddy farmers when working in paddy water. Based on the results, it showed that the average HQ for lower legs was much higher compared to other body parts. The findings in this study were in accordance with the findings of Mazlan et al. (2016) where the HQ values for legs was found to be the highest compared to other body surfaces for exposure of pesticides imazapic through dermal in the paddy water. Also, the average value for all four body surfaces exposure which labelled as HQ total was 6.959×10^{-6}, indicating that there was no significant dermal health risk as it did not exceed the value of one. Therefore, there were no adverse non-carcinogenic dermal health risks were likely to occur when the target population exposed to propiconazole in paddy water. In addition, to the best of our knowledge, no previous study has assessed the non-carcinogenic dermal health risk by using HQ for propiconazole. However, there was a study reported the HQ for propiconazole in personal air samples in Tanjung Karang, Selangor by Hamsan et al. (2017). The HQ value for propiconazole as found by Hamsan et al. (2017) was 7.17 ×

10^{-5}. The results reported by Hamsan et al. (2017) were comparable to this study where the value of HQ was less than one. On the other hand, HQ values for isoprothiolane and pretilachlor were not calculated due to unavailable data in any database for the reference dose value of both of the compounds.

CHAPTER 6

CONCLUSION AND RECOMMENDATIONS FOR FUTURE RESEARCH

6.1 Conclusions

The present study carried out in paddy fields at Kampung Sawah Sempadan, Kuala Selangor, revealed the concentrations of pesticides in paddy water. Isoprothiolane was detected with the highest mean concentrations at 51.542 µg/L and were followed by propiconazole (12.434 µg/L) and pretilachlor (0.109 µg/L).

The correlation analysis output exhibit that there were no significant correlation between concentrations of targeted compounds in paddy water and the *in situ* water quality parameters. However, there were significant correlation between electrical conductivity and salinity, electrical conductivity and total dissolved solids and salinity and total dissolved solids.

The non-carcinogenic dermal health risk assessment was calculated in this study. All of HQ values obtained were not exceed one, indicating that there was no significant chronic non-carcinogenic health risks via dermal exposure to propiconazole in paddy water among paddy farmers.

6.2 Limitations of the study

There were some limitations encountered while conducting this study. The dermal health risk assessment was calculated only for propiconazole compound. Since information on isoprothiolane and pretilachlor were unavailable in any existing database, their HQ values cannot be calculated. Furthermore, this present study only studied three types of targeted compounds. There are some other types of pesticides that are commonly used by paddy farmers in this study area which are thirteen all of them. Besides that, only dermal exposure of pesticides among the paddy farmers were assessed in this study. Other route of exposures of pesticides such as ingestion and inhalation were not assessed in this study.

6.3 Recommendations

6.3.1 Recommendation for future research

For future studies, we suggest to take into consideration for all of the thirteen compounds. By doing so, the actual dermal risk to human health associated to pesticides exposure in paddy water might be much more accurate. Since dermal health risk to pesticides were reported in this study, it is suggested to assess the other main exposure pathways in future studies. For instance, exposure through ingestion and inhalation. Paddy farmers may be exposed to pesticides through ingestion when they are not wearing gloves during their activities in paddy field as well as not maintaining good personal hygiene such as eating when working without washing hands. The present study reported that majority of paddy farmers did not wearing gloves while working with pesticides. Therefore, they may be exposed to pesticides through ingestion. On the other hand, there are paddy farmers using spraying methods for pesticides application in their paddy field. This causes paddy farmers exposed to pesticides through inhalation when they breathing the pesticides. Therefore, further studies are important for a better understanding pesticides exposures in the paddy field.

6.3.2 Recommendation for paddy farmers

Although the results obtained through this study showed that there were no significant non-carcinogenic health risks to paddy farmers, it is encouraged for paddy farmers to wear PPE during working in paddy field to reduce occupational pesticides exposures because there is still a minimal risk levels.

The results obtained from this study could help those agricultural community particularly, paddy farmers, as they will be more aware in relation to pesticides ingredients used by them. There are various types of pesticides used only for the needs of plants at the moment. Thus, the use of appropriate pesticides is important to prevent paddy farmers exposed with excessive pesticides residue in paddy water.

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APPENDIX A:

Questionnaire

JABATAN KESIHATAN PERSEKITARAN & PEKERJAAN

FAKULTI PERUBATAN DAN SAINS KESIHATAN

UNIVERSITI PUTRA MALAYSIA

PENDEDAHAN KEPADA ISOPROTHIOLANE, PROPICONAZOLE DAN PRETILACHLOR DI DALAM SAMPEL AIR PADI DAN RISIKO KESIHATAN DALAM KALANGAN PESAWAH DI TANJUNG KARANG, KUALA SELANGOR.

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 ISOPROTHIOLANE, PROPICONAZOLE DAN PRETILACHLOR DI DALAM SAMPEL AIR PADI DAN RISIKO KESIHATAN DALAM KALANGAN PESAWAH DI TANJUNG KARANG, KUALA SELANGOR.

BAHAGIAN A: MAKLUMAT DIRI

1. Umur: .... tahun

2. Jantina:
   - [ ] Lelaki
   - [ ] Perempuan

3. Bangsa:
   - [ ] Melayu
   - [ ] Cina
   - [ ] India
   - [ ] Lain-lain (sila nyatakan): ....

4. Berat: .... kg

BAHAGIAN B: PENDEDAHAN KEPADA RACUN PEROSAK

5. Pekerjaan sekarang: ....

6. Tempoh bekerja di tempat sekarang: .... bulan/ tahun

7. Pekerjaan terdahulu: ....

8. Tempoh bekerja di tempat dahulu: .... bulan/ tahun

9. Adakah anda mandi selepas ke tempat kerja?
   - [ ] Ya
   - [ ] Tidak

10. Adakah anda menukar pakaian kerja setiap hari?

☐ Ya
☐ Tidak

11. Adakah anda menggunakan racun perosak pada tanaman anda?

☐ Ya
☐ Tidak

12. Nyatakan kekerapan anda menggunakan racun perosak:

.... kali/ hari
atau
.... kali/ minggu
atau
.... kali/ bulan
atau
.... kali/ tahun

BAHAGIAN C: PENGGUNAAN KELENGKAPAN PELINDUNG DIRI

13. Adakah anda menggunakan kelengkapan pelindung diri semasa bekerja?

☐ Ya
☐ Tidak

14. Jika "Ya", apakah jenis kelengkapan pelindung diri yang digunakan semasa bekerja?

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

15. Jika "Ya", adakah anda menukar kelengkapan pelindung diri setiap hari?

☐ Ya
☐ Tidak

~Tamat soal selidik~

APPENDIX B:

Consent Form

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

Kuantifikasi Isoprothiolane, Propiconazole dan Pretilachlor di Dalam Sampel Air Padi dan Risiko Kesihatan Dalam Kalangan Pesawah di Tanjung Karang, Kuala Selangor.

2. PENGENALAN

Isoprothiolane, Propiconzole dan Pretilachlor merupakan racun perosak. Pesawah merupakan individu yang berpotensi tinggi untuk terdedah kepada racun perosak tersebut kerana mereka menggunakan racun perosak di dalam aktiviti penanaman padi. Terdapat kajian sebelum ini mendapati bahawa racun perosak menyebabkan masalah kesihatan terhadap kesihatan seseorang individu tersebut. Penilaian risiko kesihatan dijalankan untuk menganggar kebarangkalian terjadinya penyakit kulit kronik jika terdedah kepada bahan kimia. Kajian ini dijalankan untuk mengenalpasti risiko penyakit kulit kronik yang boleh berlaku dalam kalangan pesawah disebabkan oleh penggunaan sisa racun perosak dalam aktiviti penanaman padi. Sekiranya ada, pesawah akan dinasihati untuk mengambil langkah berhati-hati semasa musim meracun.

3. APAKAH YANG PERLU ANDA LAKUKAN

Responden dikehendaki untuk menjawab semua soalan yang terdapat di dalam borang kaji selidik yang akan diedarkan oleh penyelidik bagi mendapatkan informasi berkaitan berat badan, tempoh dan kekerapan terdedah kepada racun perosak.

4. SIAPA YANG TIDAK BOLEH MENYERTAI KAJIAN INI?

Penduduk Kampung Sawah Sempadan yang tidak terlibat dalam aktiviti penanaman padi, pesawah yang tidak menggunakan racun perosak dalam tanaman padi dan kanak-kanak yang di bawah umur 18 tahun. Namun, penyertaan adalah secara sukarela. Keengganan untuk menyertai tidak akan melibatkan penalti atau apa-apa tindakan.

5. APAKAH FAEDAH MENYERTAI KAJIAN INI?

a) KEPADA ANDA SEBAGAI PESERTA?

Kajian ini akan menilai jika terdapatnya risiko kesihatan kulit atau tidak apabila anda terdedah kepada racun perosak. Selepas selesai kajian, anda akan diberitahu sekiranya penggunaan bahan racun perosak tersebut berisiko atau tidak berisiko untuk mendapat penyakit kulit. Di samping itu, anda akan menerima sejumlah RM10.00 sebagai tanda terima kasih atas penyertaan anda dalam kajian ini. Selepas menerima borang persetujuan anda yang telah dimaklumkan dan borang kaji selidik anda yang lengkap, penyelidik akan memberikan pampasan kepada anda.

b) KEPADA PENYELIDIK?

Melalui kajian ini, penyelidik akan dapat mengumpul maklumat berkaitan kehadiran racun perosak di dalam air padi di kawasan sawah padi di Kampung Sawah Sempadan dan risiko pesawah untuk mendapat penyakit kulit juga dapat ditentukan. Data yang didapati boleh digunakan lebih lanjut untuk kajian yang lebih mendalam dan spesifik mengenai penyakit yang dikenalpasti. Selain itu, hubungkait antara risiko, kekerapan penggunaan racun perosak dan penggunaan kelengkapan pelindung diri juga dapat dikenalpasti.

6. ADAKAH IA BERISIKO?

Terdapat risiko minimum. Proses kajian yang akan dijalankan 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 serta hanya akan digunakan untuk tujuan kajian sahaja.

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

Responden boleh menghubungi penyelidik kajian ini:

014-8860206
ASNA SYAHIRAH BINTI MOHAMAD SALIHIN
JABATAN KESIHATAN PERSEKITARAN DAN PEKERJAAN,
FAKULTI PERUBATAN DAN SAINS KESIHATAN,
43400 UPM SERDANG,
SELANGOR, MALAYSIA.

012-6140221/03-89472396
HO YU BIN, Ph.D
JABATAN KESIHATAN PERSEKITARAN DAN PEKERJAAN,
FAKULTI PERUBATAN DAN SAINS KESIHATAN,
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 Helaian 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 .... (*Responden*)

Tandatangan .... (*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 C:

Ethical Approval

APPENDIX D:

Original Data

Table 1: Concentration of compound replication in each paddy block

Block	Isoprothiolane (µg/L)	Propiconazole (µg/L)	Pretilachlor (µg/L)
A1	0.3746	0.0000	0.0044
A2	0.1705	0.0000	0.0027
A3	2.2292	0.0046	0.0074
B1	2.2686	0.0000	0.0018
B2	2.4001	0.0001	0.0030
B3	2.2482	0.0001	0.0022
C1	0.7023	0.0016	0.0021
C2	0.6832	0.0013	0.0019
C3	0.5907	0.0016	0.0026
D1	0.1594	0.0029	0.0094
D2	0.1064	0.0003	0.0042
D3	0.0664	0.0004	0.0073
E1	0.0888	0.0010	0.0028
E2	0.0961	0.0111	0.0020
E3	0.1037	0.0013	0.0024
F1	0.2537	0.0767	0.0028
F2	0.2106	0.0697	0.0017
F3	0.2942	0.0713	0.0024
G1	21.1470	0.0655	0.0192
G2	1.2887	0.0089	0.0051
G3	16.2210	0.0054	0.0024
H1	0.1488	0.0040	0.0092
H2	0.1699	0.0022	0.0133
H3	0.1470	0.0052	0.0046
I1	0.1670	0.0000	0.0054
I2	0.1525	0.0035	0.0063
I3	0.1547	0.0000	0.0038
J1	5.9970	3.5510	0.0165
J2	9.1810	4.6108	0.0132
J3	2.7938	1.1637	0.0167
K1	3.6397	0.0024	0.0059
K2	3.8183	0.0020	0.0078
K3	5.9449	0.0041	0.0087
L1	1.8866	0.0447	0.0101
L2	1.1393	0.0059	0.0142
L3	4.8959	0.0840	0.0000
M1	2.3681	0.1220	0.0089
M2	1.5035	0.0944	0.0065
M3	1.6000	0.0977	0.0055
N1	4.7508	0.0000	0.0134
N2	3.5907	0.0000	0.0123
N3	2.3431	0.0000	0.0112
O1	2.1886	0.0000	0.0195
O2	0.4516	0.0611	0.0120
O3	0.1156	0.0000	0.0098

P1	0.6577	0.0000	0.0607
P2	0.1633	0.0044	0.0110
P3	2.2824	0.0066	0.0098
Q1	0.0000	0.0000	0.0023
Q2	0.0206	0.0000	0.0020
Q3	0.0259	0.0000	0.0000
R1	0.2690	0.0000	0.0129
R2	0.2751	0.0000	0.0000
R3	0.3273	0.0000	0.0000
S1	0.0000	0.0000	0.0000
S2	0.0553	0.0000	0.0000
S3	0.0000	0.0000	0.0000
T1	0.0000	0.0000	0.0000
T2	0.0380	0.0000	0.0024
T3	0.0142	0.0000	0.0022
U1	0.1116	0.0184	0.0013
U2	0.0667	0.0107	0.0009
U3	0.0739	0.0152	0.0013
V1	0.1544	0.0041	0.0019
V2	0.1369	0.0000	0.0009
V3	0.3484	0.0003	0.0021
W1	0.0239	0.0026	0.0026
W2	0.0200	0.0000	0.0012
W3	0.0133	0.0004	0.0016
X1	0.0000	0.0000	0.0000
X2	0.0000	0.0000	0.0056
X3	0.0000	0.0000	0.0000

APPENDIX E:

Tables of Results

Table 2: Mean and standard deviation of *in situ* water quality parameters of all paddy blocks (n=24)

Block	Mean ± SD						
	Temperature (ºC)	pH	Electrical Conductivity (µS/cm)	Dissolved Oxygen (mg/L)	Turbidity (NTU)	Salinity (ppm)	Total Dissolved Solid (mg/L)
A	28.600 ± 0.600	6.407 ± 0.240	201.020 ± 215.869	5.670 ± 0.087	109.333 ± 1.155	194.420 ± 206.331	196.387 ± 210.951
B	28.833 ± 0.321	6.850 ± 0.128	176.333 ± 45.059	5.473 ± 0.107	14.127 ± 4.845	168.933 ± 43.553	172.100 ± 44.012
C	28.133 ± 0.379	6.167 ± 0.131	149.667 ± 11.070	6.630 ± 0.087	61.900 ± 0.361	142.800 ± 10.437	146.300 ± 11.122
D	27.033 ± 0.379	6.177 ± 0.292	217.167 ± 88.371	3.650 ± 0.204	55.067 ± 0.306	207.533 ± 85.652	212.000 ± 85.893
E	29.467	5.923	70.500	6.827	51.367	70.153	68.760

	±	±	±	±	±	±	±
	0.493	0.060	2.720	0.155	11.854	1.971	2.534
F	27.967	5.740	90.080	5.183	89.200	87.243	87.853
	±	±	±	±	±	±	±
	1.026	0.036	0.796	0.177	0.000	0.794	0.885
G	28.000	6.673	176.767	2.297	52.533	168.833	172.367
	±	±	±	±	±	±	±
	0.557	0.065	51.550	0.155	11.226	48.788	50.316
H	28.433	7.150	60.037	6.150	191.300	60.797	58.640
	±	±	±	±	±	±	±
	0.058	0.185	24.661	0.100	1.044	22.081	24.085
I	28.667	6.620	141.600	5.217	68.267	135.233	138.400
	±	±	±	±	±	±	±
	0.351	0.044	8.628	0.035	1.677	8.501	8.445
J	27.667	5.350	229.567	6.997	289.867	219.233	224.167
	±	±	±	±	±	±	±
	0.252	0.460	23.767	0.031	2.419	24.274	23.213

K	27.533	6.213	438.400	6.390	112.000	427.367	428.233
	±	±	±	±	±	±	±
	0.289	0.790	79.841	0.040	4.359	82.908	78.215
L	28.467	6.617	223.767	3.653	115.667	215.333	218.733
	±	±	±	±	±	±	±
	0.252	0.146	170.429	0.059	2.517	164.063	166.595
M	28.567	6.493	106.583	5.963	115.867	102.693	103.727
	±	±	±	±	±	±	±
	2.309	0.021	12.837	0.064	17.200	11.275	11.848
N	28.800	7.257	1.189	5.747	185.333	1.206	1.169
	±	±	±	±	±	±	±
	0.608	0.117	0.085	0.085	1.155	0.106	0.078
O	29.733	6.673	209.700	6.750	143.000	201.367	204.767
	±	±	±	±	±	±	±
	0.153	0.795	9.619	0.053	1.732	9.642	9.471
P	31.200	6.297	134.000	6.420	261.667	128.533	131.200
	±	±	±	±	±	±	±

	0.000	0.050	6.762	0.079	16.197	6.380	6.669
Q	27.900	6.003	85.917	6.250	106.333	83.937	83.933
	±	±	±	±	±	±	±
	0.100	0.031	0.540	0.056	4.041	1.156	0.393
R	28.633	5.837	112.833	3.493	126.667	108.667	110.000
	±	±	±	±	±	±	±
	0.404	0.076	9.819	0.025	7.371	8.631	9.590
S	29.700	6.317	85.950	4.027	573.967	84.467	83.897
	±	±	±	±	±	±	±
	0.100	0.060	1.807	0.021	6.108	1.741	1.796
T	30.200	6.373	80.090	7.160	150.333	79.307	78.370
	±	±	±	±	±	±	±
	0.557	0.160	1.156	0.122	1.528	0.697	0.917
U	29.600	5.883	109.300	6.680	33.833	105.767	106.667
	±	±	±	±	±	±	±
	0.100	0.058	1.300	0.036	4.576	0.945	1.185
V	31.167	7.133	474.300	7.533	94.600	461.633	462.867

	±	±	±	±	±	±	±
	0.306	0.321	34.947	0.120	5.495	38.894	35.345
W	30.233	6.683	369.133	6.807	80.000	355.600	360.500
	±	±	±	±	±	±	±
	0.473	0.127	7.851	0.105	1.000	4.677	7.002
X	31.333	6.143	265.833	4.980	357.333	252.667	259.367
	±	±	±	±	±	±	±
	0.643	0.090	17.079	0.062	21.779	16.093	16.874
BL	25.000	7.540	0.180	7.603	0.263	10.430	0.170
	±	±	±	±	±	±	±
	0.000	0.000	0.000	0.025	0.015	0.000	0.000

APPENDIX F:

Calibration Curve

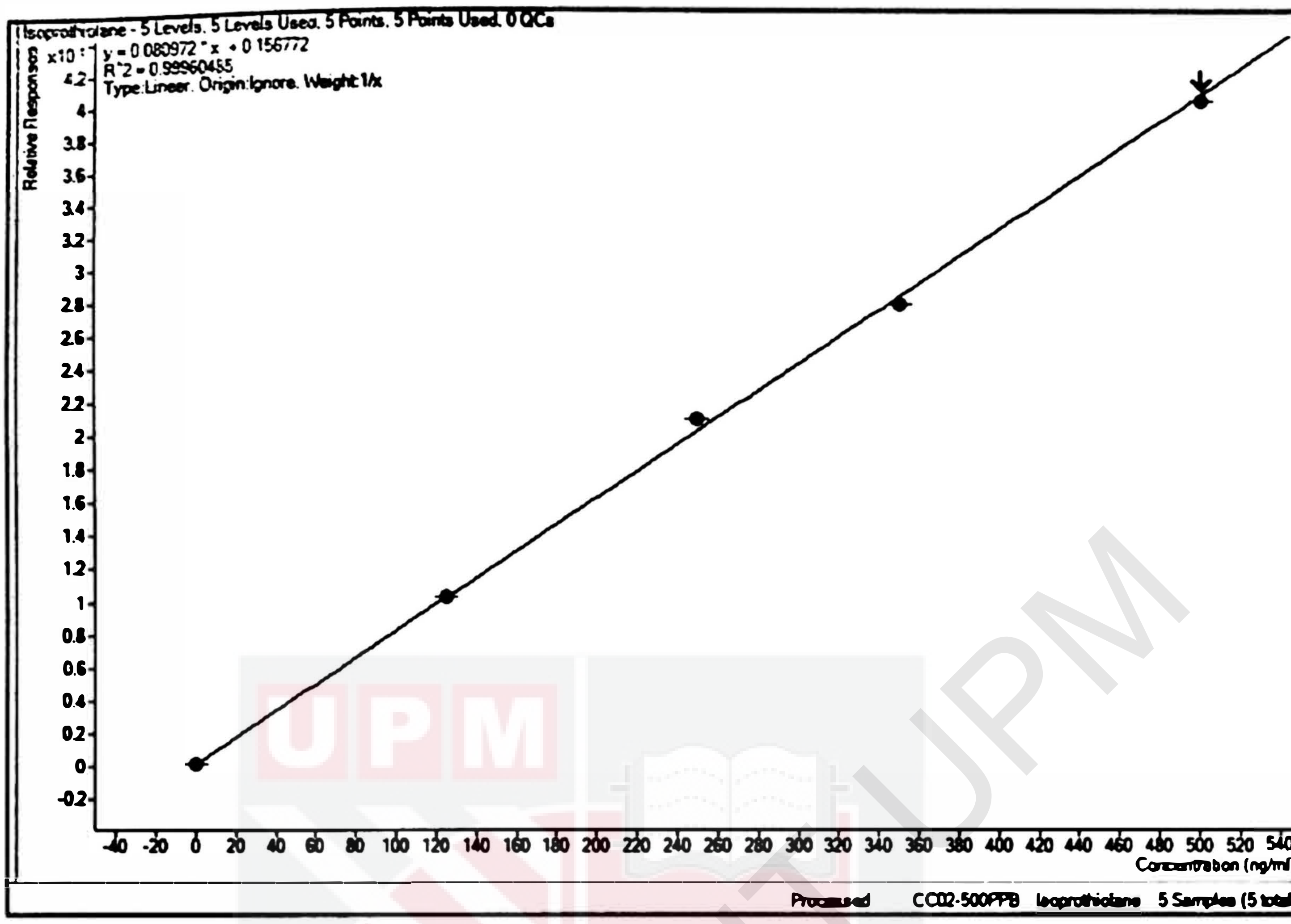

Figure 1: Calibration curve for isoprothiolane

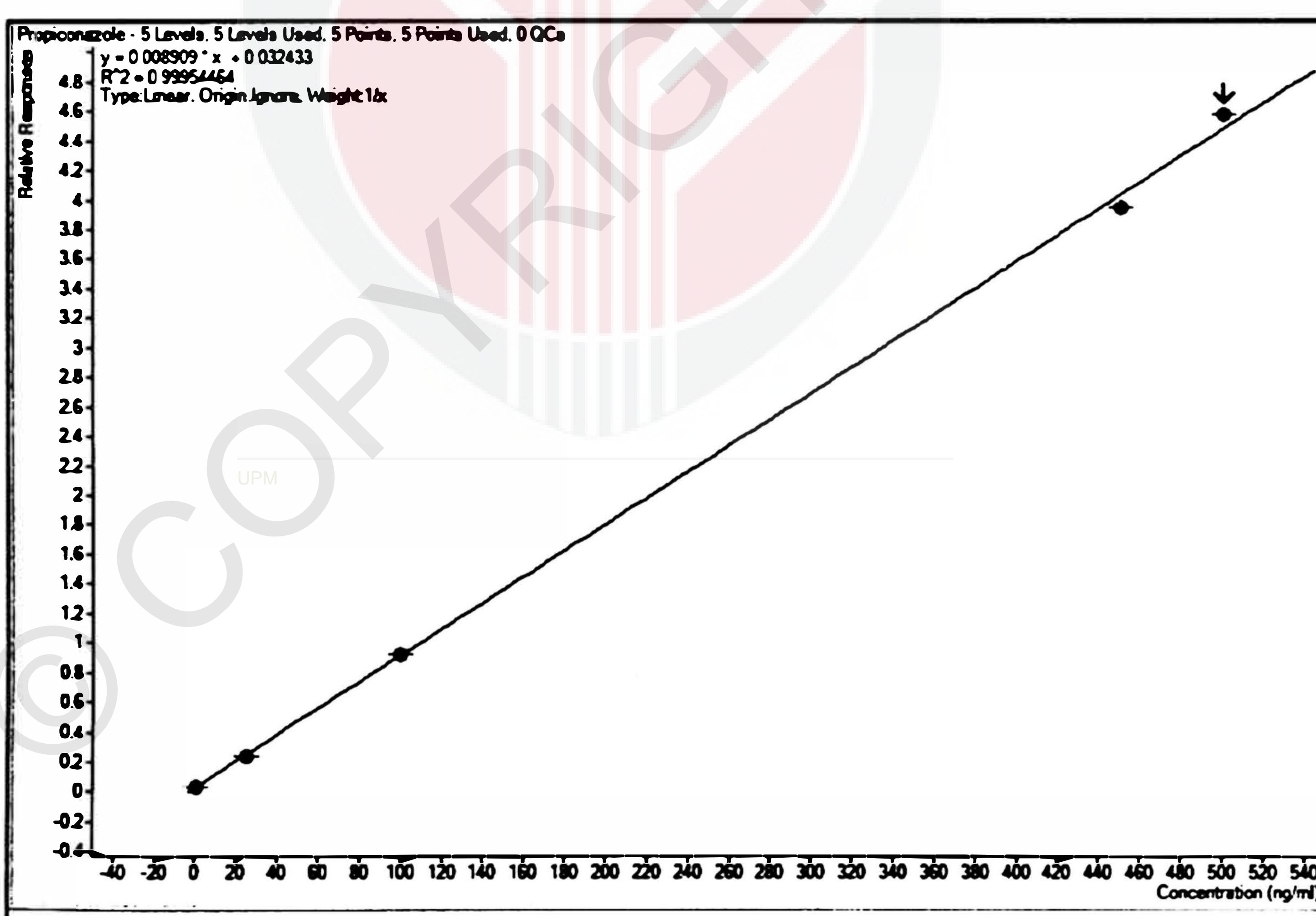

Figure 2: Calibration curve for propiconazole

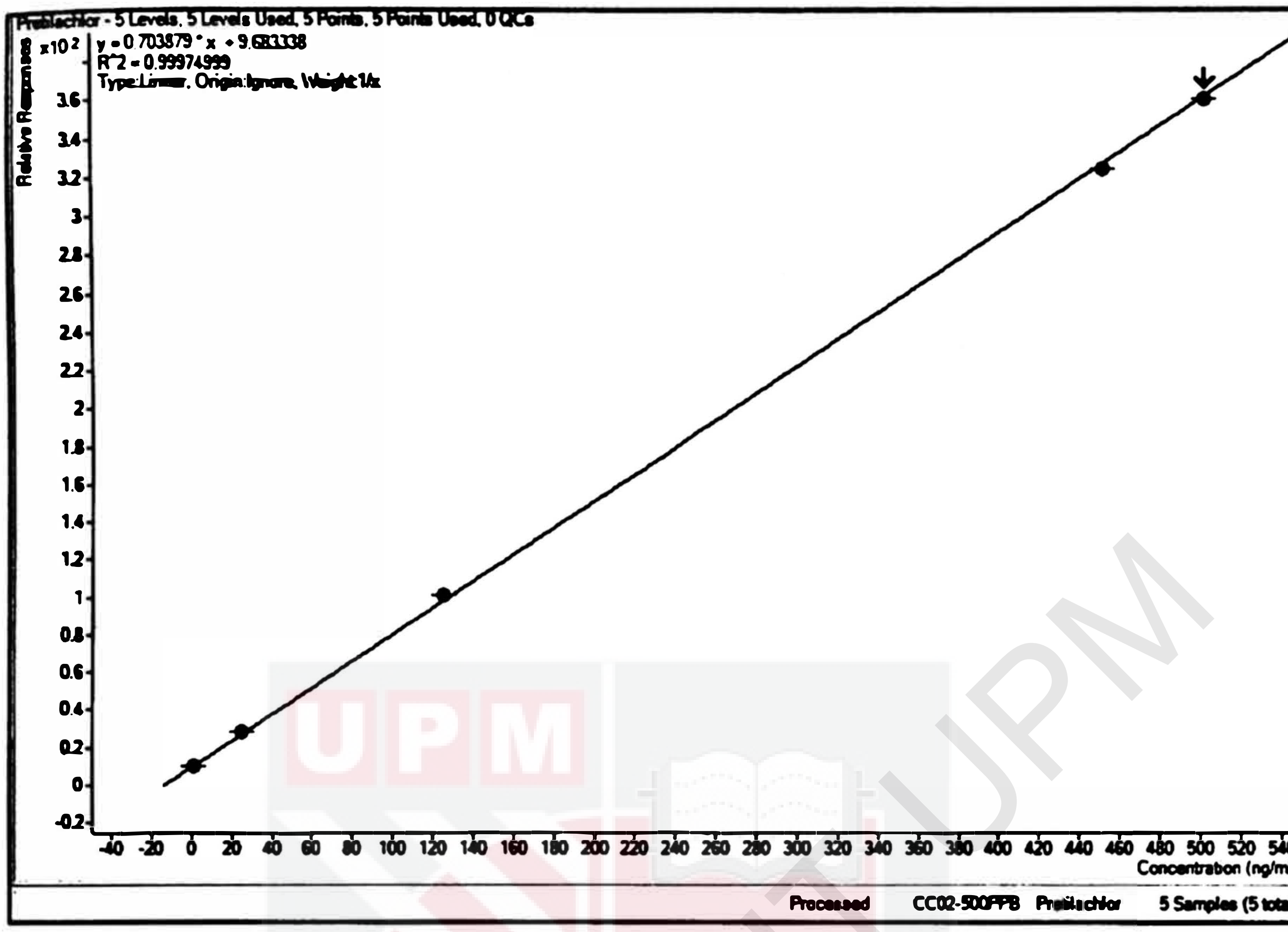

Figure 3: Calibration curve for pretilachlor

APPENDIX G:

Chromatogram Graph

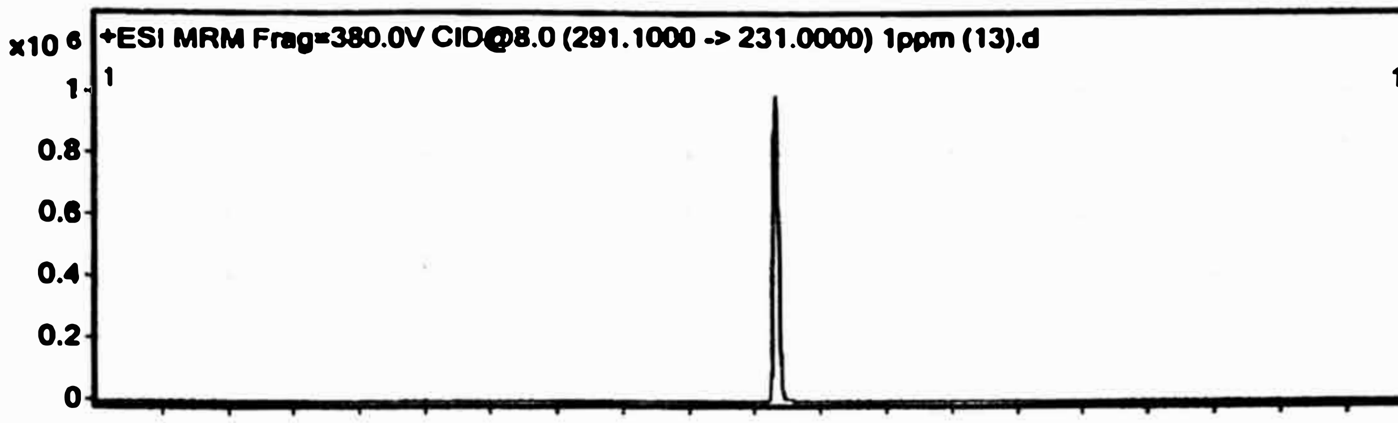

Figure 4: Chromatogram for isoprothiolane

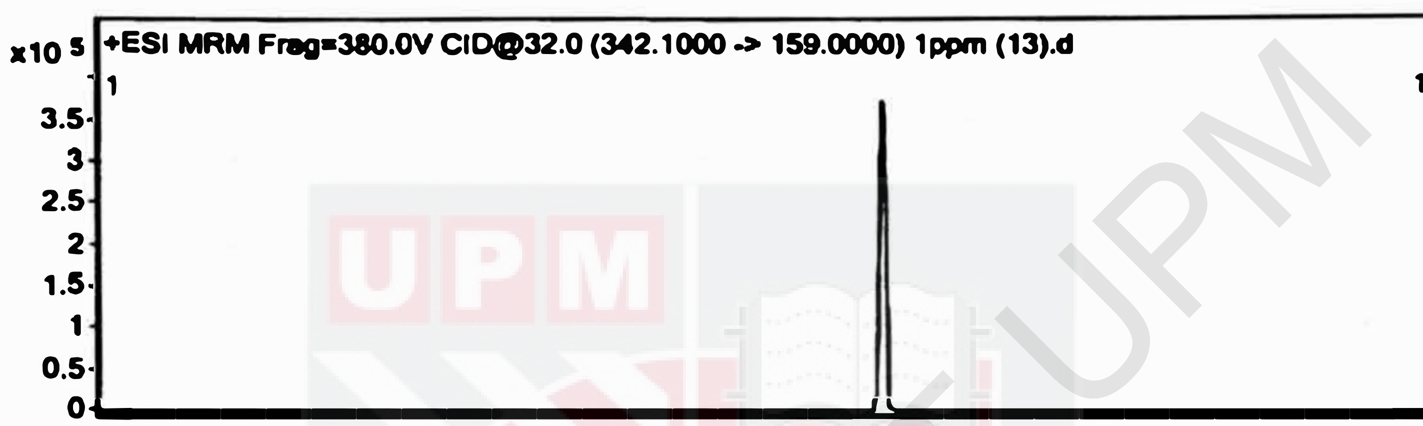

Figure 5: Chromatogram for propiconazole

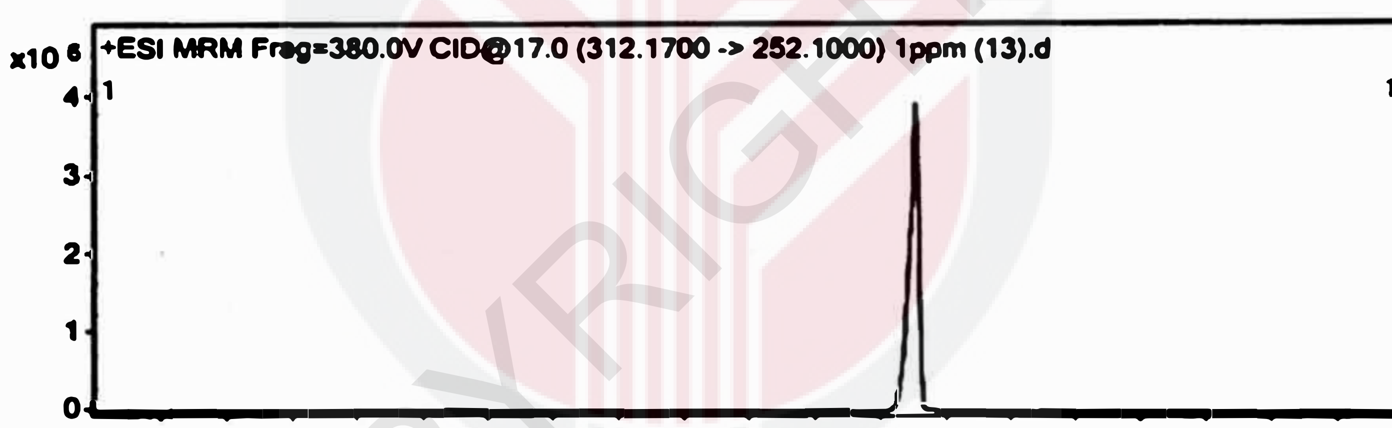

Figure 6: Chromatogram for pretilachlor