



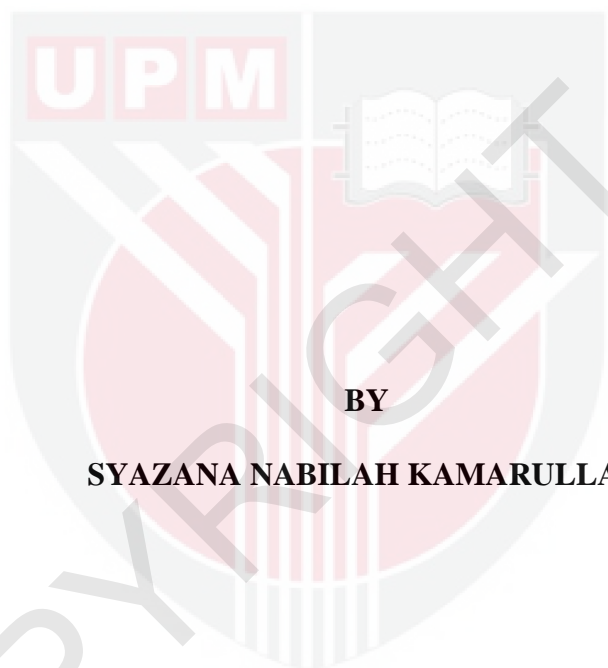
UNIVERSITI PUTRA MALAYSIA

***OCCURRENCE OF DIFENOCONAZOLE, TEBUCONAZOLE,
TRICYCLAZOLE AND PROPICONAZOLE IN BLOOD SERUM
SAMPLES AMONG PADDY FARMERS IN TANJUNG KARANG,
SELANGOR***

SYAZANA NABILAH BINTI KAMARULLAH

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AMONG PADDY FARMERS IN TANJUNG KARANG, SELANGOR**



BY

SYAZANA NABILAH KAMARULLAH

**This 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

THE OCCURRENCE OF DIFENOCONAZOLE, TEBUCONAZOLE, TRICYCLAZOLE AND PROPICONAZOLE IN BLOOD SERUM SAMPLES AMONG PADDY FARMERS IN TANJUNG KARANG, SELANGOR

SYAZANA NABILAH KAMARULLAH

Introduction: The high demand of rice consumption in Malaysia had urge a large production of crop and in order to meet this demand, and this leads to increase use of pesticide. Pesticides plays important roles in maintaining a good condition of crops in terms of quantity and also quality. However, increase use of pesticide in agricultural field gives concern towards farmers if they failed to wear appropriate personal protective equipment when they handling the pesticides. **Objectives:** The aim of the study is to quantify the concentration of difenoconazole, tebuconazole, propiconazole and tricyclazole in blood serum samples and its associate the reported health symptoms among farmers. **Methodology:** A total of 86 questionnaires on demographic and occupational were distributed to the farmers and their blood were extracted using QuEChERS method (quick, easy, cheap, effective, rugged and safe). The extracted were analysed using Ultra High Performance Liquid Chromatography Mass Spectrometry (UHPLC-MS/MS). **Results and Discussion:** The mean concentrations of difeconazole, tebuconazole, tricyclazole and propiconazole in blood serum samples among paddy farmers were 1063.18 ng/mL, 779.55 ng/mL, 638.52 ng/mL and 617.26 ng/mL, respectively. There is no significant association between concentration of pesticides in blood serum samples with the reported health symptoms(breathing difficulties and chest pain). There were other contributing factors such as work duration, smoking status and education level. **Conclusion:** As a conclusion, there is a significant value of concentration of pesticide in blood serum sample of farmers. The usage of personal protective equipment (PPE) according to Malaysian standard code of recommended practice (MS479:2012) were recommended. Further study should be conducted which may benefit relevant authorities. This study has a lot of challenges and limitation such as short period of time due to pandemic and the breakdown of UHPLC-MS/MS during the study period.

Keywords: Pesticide, Blood Serum, ultra-high performance liquid chromatography-mass spectrometry (UHPLC-MS/MS), QuEChERS (quick,easy, cheap, effective, rugged, and safe)

ABSTRAK

KEJADIAN DIFECONAZOLE, DIFECONAZOLE, PROPICONAZOLE, TRICYCLAZOLE DAN TEBUCONAZOLE DI DALAM SAMPEL SERUM DARAH DI KALANGAN PETANI DI TANJUNG KARANG, SELANGOR

SYAZANA NABILAH KAMARULLAH

Pengenalan: Permintaan tinggi penggunaan beras di Malaysia mendorong pengeluaran tanaman yang besar dan untuk memenuhi permintaan menyebabkan peningkatan penggunaan racun perosak. Racun perosak memainkan peranan penting dalam menjaga tanaman dari segi kuantiti dan juga kualiti. Walau bagaimanapun, peningkatan penggunaan racun perosak dalam bidang pertanian adalah merisaukan kepada petani jika mereka gagal memakai peralatan pelindung diri yang sesuai ketika mereka mengendalikan racun perosak. **Objektif:** Tujuan kajian ini adalah untuk mengukur kepekatan difeconazole, tebuconazole, propiconazole dan tricyclazole dalam sampel serum darah dan mengaitkan gejala kesihatan yang dilaporkan di kalangan petani. **Metodologi:** Sebanyak 86 borang soal selidik yang berdasarkan status demografik dan pendedahan pekerjaan diedarkan kepada para petani dan darah mereka diekstraksi menggunakan kaedah QuEChERS (cepat, mudah, murah, berkesan, kasar dan selamat). Sampel diekstrak dianalisis menggunakan Spektrometri Massa Kromatografi Cecair Berprestasi Tinggi (UHPLC-MS / MS). **Keputusan dan Perbincangan:** Kepekatan rata-rata difeconazole, tebuconazole, tricyclazole dan propiconazole dalam sampel serum darah di kalangan petani padi adalah masing-masing 1063.18 ng / mL, 779.55 ng / mL, 638.52 ng / mL dan 617.26 ng / mL. Tidak ada hubungan yang signifikan antara kepekatan racun perosak dalam sampel serum darah dengan gejala kesihatan yang dilaporkan (kesukaran bernafas dan sakit dada). Terdapat faktor penyumbang lain seperti tempoh kerja, status merokok dan tahap pendidikan. **Kesimpulan:** Sebagai kesimpulan, terdapat sejumlah besar racun perosak dalam sampel serum darah petani namun nilainya tidak signifikan. Penggunaan alat pelindung diri berdasarkan kod standard yang disyorkan Malaysia (MS479:2012) telah disarankan. Oleh itu, kajian lebih lanjut harus dilakukan yang dapat memberi manfaat kepada pihak berwajib. Kajian ini mempunyai banyak cabaran dan batasan seperti jangka masa pendek kerana pandemi dan kerosakan UHPLC-MS / MS selama tempoh kajian.

Kata kunci: Racun, Sampel Serum Darah, Petani, UHPLC-MS/MS, QuEChERS (cepat, mudah, murah, berkesan, kasar dan selamat)

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Figure 3.1 Location of the sampling at Kampung Sawah Sempadan,
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LIST OF ABBREVIATIONS

ESI	Electrospray ionization
IQL	Instrument quantification limit
IS	Internal standard
LOD	Limit of detection
LOQ	Limit of quantification
MeOH	Methanol
MgSO ₄	Magnesium sulphate
MRM	Multiple reaction monitoring
NHL	Non-hodgkin lymphoma
PPE	Personal protective equipment
QuEChERS	Quick, Easy, Cheap, Effective, Rugged, and Safe
R ²	Coefficient of determination
SPSS	Statistical Package for Social Science
UHPLC-MS/MS	Ultra high performance liquid chromatography tandem mass spectrometry

CHAPTER 1

INTRODUCTION

1.1 Background

Food is the basic need of human to survive. In Malaysia, the essential food is rice. The total population of Malaysia is 3.6 million on 2019 and this number is increasing year by year. This has created high demand of rice consumption in Malaysia which require agriculture sector especially paddy field to increase the production of rice in order to meet the food demand. To produce a large production of rice, farmers may face various problem such as rice disease and also pest. Traditional ways farming could not cope with these problems which in turns modern ways are being used. In modern ways, various pesticide and fertilizer are being used to keep the quality and quantity of the crop. Pesticide such as insecticide, fungicide, herbicide, rodenticide, molluscicide and nematocide are being used extensively (Anis et al., 2019).

Pesticides are categorized in to different classes depending on their function. Pesticides such as fungicide are important to fight with rice disease such as rice tungro disease (RTD) or well known with the name of 'penyakit merah' which caused by virus named tungro virus which potentially affect the quality and quantity of the rice. Fungicide such as difenoconazole, tebuconazole, tricyclazole and propiconazole are commonly used by the farmers in Malaysia to fight the disease. However, the increase use of pesticide in agricultural field gives concern towards human health and environment (Anis et al., 2019). Based on the material safety data sheet (MSDS) these fungicides are associated with respiratory problem and also reproductive toxicity.

Generally, pesticide can reside in environment such as in soil, water and air and it will also eventually received by human. Pesticide will reside in soil after spraying process and absorbed by the crop planted on it. The crop will eventually consumed by human and caused health effects. In a certain pesticide such as organophosphates, the main pathway of exposure were through soil ingestion and this can risk both children and adolescent (Bhandari et al., 2020). In water context, pesticide can cause water pollution as the water runoff from the agriculture area and consumed by human. In a study by Aligardashi and Jalali (2017) that was conducted in one of the river in Iran showing high risk of health effects towards people around and might risk from gastrointestinal cancer and mutagenic disorder. In air, most pesticide will vaporized in air and inhaled by the workers who involved directly during mixing, handling and spraying of pesticide. In a study carried out in cotton field in China, the result of study showing high risk of dermal and inhalation exposure among the operators while spraying forward walking (Kumar et al., 2013).

Pesticide residue can be found in human through biological monitoring by using biological sample such as urine, serum, hair and saliva. In a study conducted at Catalonia and Glacia by (Garí et al., 2018), they found the existence of organophosphate pesticide in urine sample among exposed and non-exposed group in his study. In a study located at Burkina Faso by Lehmann et al., (2018) found 17 pesticides through the hair samples of the volunteers in their study. However, blood and urine are classified as the benchmark for pesticide biomonitoring globally (Sweeney et al., 2019). Blood is the ideal biological samples for pesticide residue as most pesticide are lipophilic while urine are choice of determination of pesticide metabolites as well as parent compound (Sweeney et al., 2019).

The study took place in paddy cultivation area as this field use a lot amount of pesticide to grow the crops. Tanjung Karang is well known for its paddy cultivation activity. Tanjung Karang is the third largest area of paddy field in Peninsular Malaysia and also known as 'the rice bowl of Selangor' (Fuad et al., 2012). In paddy cultivation, rice disease is the most important factors to be taken care of as it can interrupt with quality of rice. Rice disease can cause an average of 10-15% of losses globally (M. K. P. Kumar et al., 2013). In order to keep crops free from this problem, fungicide such as difenoconazole, tebuconazole, tricyclazole and propiconazole are used widely. However, fungicide can bring adverse effects by interfering biosynthesis of amino acids and protein which in turns defect the biological function of target organism (Yang et al., 2011). Others side effects of fungicide includes interfering with signal transduction, give effects on respiration, mitosis, cell division and also nucleic acid synthesis (Yang et al., 2011).

1.2 Problem Statement

When massive production of rice is required, many sorts of method are being used to produce high quality of rice. This includes chemical control which is the usage of pesticide (Kumar et al., 2013). Fungicide is used to keep the crop free from disease (Kumar et al., 2013). However, pesticide can enter human body through three modes of action which are dermal, inhalation and ingestion (Damalas & Koutroubas, 2016). This can happen when a Personal Protection Equipment (PPE) are not worn or not sufficient to protect the farmers. Through dermal, the pesticide could enter through the penetration of skin directly in contact with the pesticide. A study was conducted by Pedon et al., (2020) to evaluate dermal absorption among farmers with in vivo and in vitro study. The result for in vitro showing that the farmers were potential exposed to dermal absorption between 0.87 mg/person/h until 2.85 mg/person/h and in vivo result showing amount of pesticide retained in the pig skin was $52.63 \text{ ng/cm}^2 \pm 10.73 \text{ ng/cm}^2$ and $135.15 \text{ ng/cm}^2 \pm 31.8 \text{ ng/cm}^2$ after 0.4 and 1.8 μ amount of pesticide were applied directly. Inhalation modes of exposure will risk the person who handle the pesticide directly during mixing, handling and spraying of the pesticide (Hamsan et al., 2017). As for ingestion, the possible ways to enter human body is mainly sourced from self-hygiene practice of oneself. Pesticide can be accidentally consumed when personal hygiene like washing hands before eating and after smoking are not being practised (Aylward, 2019). Taking shower after handling pesticide, washing hands and changing clothes that are used during handling pesticide activities are also one of good hygiene practise to prevent ingestion modes of entry in human body.

Unfortunately, most of fungicide are volatile compound and easily vaporized into air and inhaled by the farmers who are not wearing proper (PPE) while handling

the pesticide. Exposure of pesticide are highly associated with respiratory problems among farmer

Common disease that associates with pesticide includes cancer, parkinson's disease, reproductive disorders and also respiratory disorders might affect person who in contact with pesticide (Sabarwal et al., 2018). Pesticide can generate aerosols that can goes directly to human respiratory system and cause disease related to respiratory problem (Sabarwal et al., 2018). Pesticide can enter human body without sufficient protection from personal protective equipment (PPE). Most of the pesticide applicators in india did not wear proper PPE and this has bring them in contact with pesticide (Sabarwal et al., 2018). Pesticide sprayers are more prone to get symptoms for respiratory diseases such as dry cough, productive cough, wheezing, irritation of throat and blood stained (Fareed et al., 2013). This study also found that occupational exposure of OP pesticides can cause lung function to be decreased and caused haematological alterations among pesticide sprayers (Fareed et al., 2013).

In previous study by Hamsan et al., (2017) reported that there is existence of commonly use pesticide in personal air sample among farmers in Tanjung Karang. The occurrence of pesticide in the personal air sample is being associated with the health symptoms experienced by the farmers. In this study, the occurrence of commonly used pesticide among the paddy farmers includes tebuconazole, propioconazole, difenoconazole and tricyclazole are being analysed in the blood serum samples among farmers in Tanjung Karang.

1.3 Research Justification

Pesticide had bring adverse effect to the person who in contact with it through occupational exposure. The trace of pesticide in human body can be prove through biological monitoring with using biological sample such as blood. However, research study about concentration of pesticide in blood serum samples are not much to referred to. Pesticide exposure can be associated with several health symptoms. Most of the pesticide are lipophilic which makes it easier attached to the blood as blood contains lipoproteins. The pesticide can bind to blood and end up in the target organ such and cause illnesses. This study is important as it can proves the concentration of pesticides can be associated with the respiratory health symptoms experienced by the farmers. The outcomes of this study might be useful as an evidence for the authorities that involves in agricultural sector especially the management. A proper suggestion such as training on usage of sufficient PPE in order to reduce the exposure of farmers towards pesticide can be done (Hamsan et al., 2017). Most of the study involving animals such as mice or rats to mimic the response of pesticide towards human. These animals may showing similar response however their repair machinery are different from human and they only have fewer repair enzymes compared to human (Sabarwal et al., 2018b). For the sake of study, human cannot be asked to voluntarily consumed pesticide. Thus, more study of occupational exposure on pesticide in human especially in blood must be done. The past study from Hamsan et al., (2017) reported the presence of pesticide in air sampler and associate it with the health reported symptoms experienced by the farmers. This past study can be strengthened with proves of concentration of pesticides in blood serum samples with the health symptoms experienced. Many studies reported concentration of pesticide in human body through biological samples such as nail, hair and urine. However, study about concentration of pesticide in human blood serum still little. Most of the

study are done in animal such as rat and mice. These animals models may show similar response as human however they have few repair enzymes compared to human which affects the outcome of study directly (Sabarwal et al., 2018).



1.4 Conceptual Framework

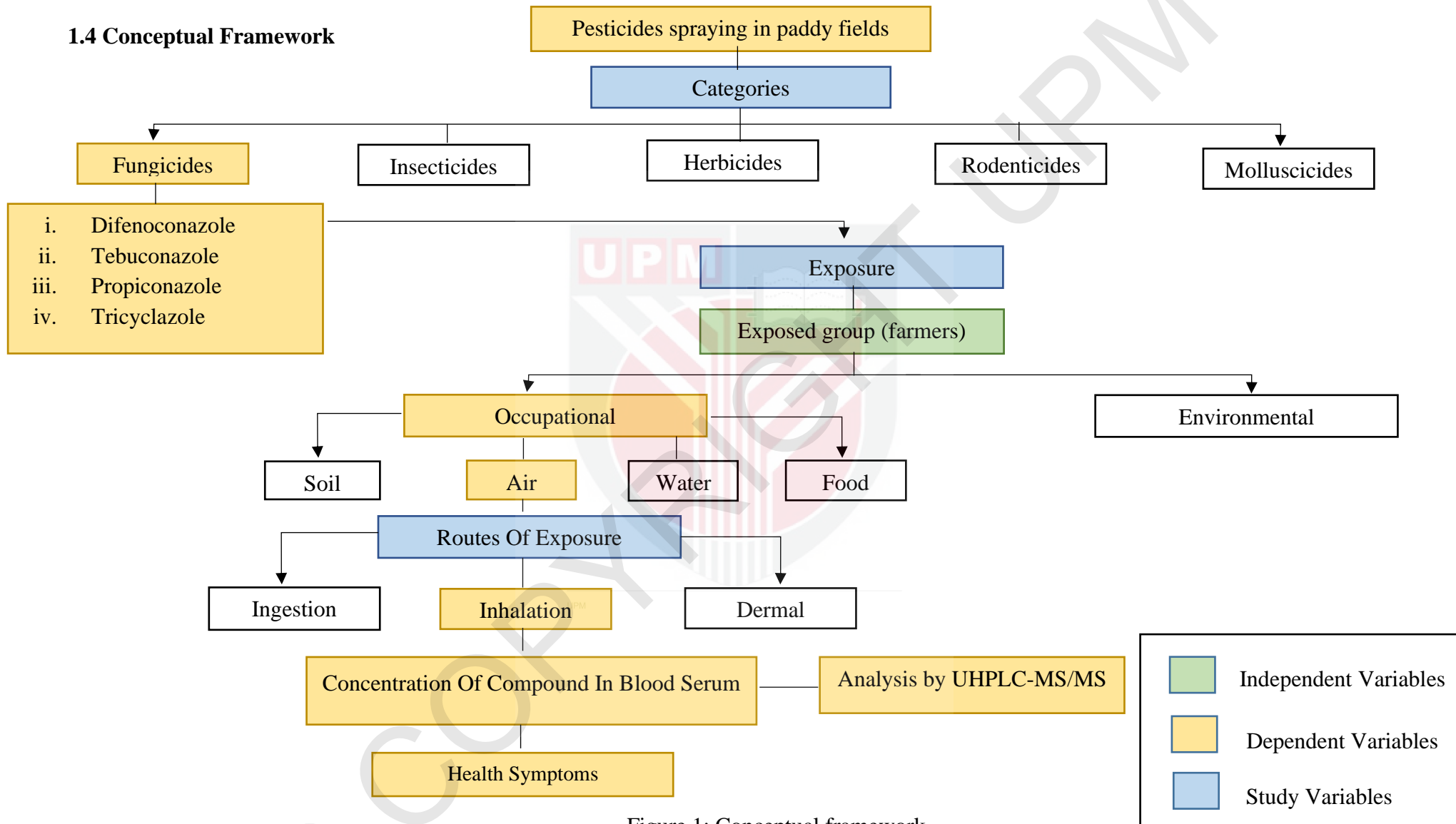


Figure 1: Conceptual framework

1.5 Research Question

- i. What are the concentration of difenoconazole, tebuconazole, propiconazole and tricyclazole in blood serum samples among paddy farmers in Tanjung Karang?
- ii. Is there any differences of self-reported respiratory health symptoms among paddy farmers?
- iii. Is there any association between the concentration of difenoconazole, tebuconazole, propiconazole and tricyclazole in blood serum samples and the reported health symptoms among paddy farmers?
- iv. Does the contributing factors affect self-reported respiratory health symptoms among paddy farmers?

1.6 Research Objectives

1.6.1 General Objectives

To determine the concentration of difenoconazole, tebuconazole, propiconazole and tricyclazole in blood serum samples and associate with the self-reported respiratory health symptoms among paddy farmers in Tanjung Karang, Selangor

1.6.2 Specific Objectives

- i. To quantify the concentration of difenoconazole, tebuconazole, propiconazole and tricyclazole in blood serum samples among paddy farmers in Tanjung Karang.
- ii. To identify the self-reported respiratory health symptoms among paddy farmers in Tanjung Karang.
- iii. To determine the association of pesticides in blood serum samples and the reported health symptoms among paddy farmers in Tanjung Karang.
- iv. To determine the contributing factors that affect self-reported respiratory health symptoms among paddy farmers in Tanjung Karang.

1.7 Hypothesis

- i. The concentration of difenoconazole, tebuconazole, propioconazole and tricyclazole in blood serum samples are significantly associated with the self-reported respiratory health symptom among paddy farmers.
- ii. The contributing factors that affect self-reported respiratory health symptoms among paddy farmers were determined.



CHAPTER 2

LITERATURE REVIEW

2.1 Agriculture and Pesticide

Agriculture sector is one of the essential sector in our country which contribute high income for Malaysia. In agriculture, one of the most important sector is paddy as rice is the staple food for Malaysian. Many efforts have been given out by government to encourage farmers increase production in this field. This includes providing subsidize of pesticide and fertilizer towards farmer. This, have increased the uses of pesticide over a long period of time. The uses of pesticide is important to keep crop healthy. However, the use of pesticide can be harmful to human and environment if inappropriate measures are not taken seriously by the farmers.

As indicated by Food and Agriculture Organization of the United Countries (FAO) pesticide is synthetic, natural substance or can be combination or both that acts to control and prevent any pest. Pesticides can exist in various state such as solid, liquid or gas (Damalas & Koutroubas, 2016). According to the United States Environmental Protection Agency (US EPA), pesticides contain both 'active' and 'inert' ingredients. Active ingredients act to prevent, destroy, repels or mitigates a pest, or a plant regulator, defoliant, desiccant or nitrogen stabilizer. Other ingredients are called inert ingredients by the federal laws and they are important for product performance and usability. Pesticides can be divided into several groups depending on its function and also pest organism that it can kill which are insecticides, fungicides, herbicides, rodenticides and also molluscicides (Kaur et al., 2019).

2.2 Pesticide In Environment And Effects Of Human Health

Generally, Pesticide can reside in environment such as in soil, water and air and that will eventually receive by human. Fungicide can reside in soil

depending on its half-life. The residue of fungicide may end up in groundwater and surface water without proper drainage control measure as pesticide in paddy were applied at the surface water of paddy field (Latiff et al., 2010). This will also cause harm to the human who consumed water from the contaminated source of water. In a study conducted by Hamsan et al., (2017) detected 13 compounds of pesticide in personal air samples and associate it with health symptoms among paddy farmers in Tanjung Karang, Selangor. Many past studies have shown that pesticide can interrupt in the development of human with persistent use of pesticide. In a study carried out in Egypt, pesticide applicators working in agriculture having more ADHD symptoms compared to non-exposed group (Rohlman et al., 2019). Pesticides also associated with genotoxic damage in Bolivian farmers (Barrón et al., 2019). Pesticides also can be related to various cancer such as prostate cancer, breast cancer, colorectal cancer and also non-hodgkin lymphoma (NHL) (Sabarwal et al., 2018a). Addition to that, pesticides also can cause parkinson's disease, Alzheimer's disease, reproductive disorders, and also respiratory disorders (Sabarwal et al., 2018a).

2.4 Pesticide Residue in Blood Serum

Pesticides can give various effects to humans based on the modes of entry. These modes of entry include dermal (skin contact), mouth (ingestion) and also inhalation (Damalas & Koutroubas, 2016). After the pesticides enter the human body through any of these three channels, the residue will eventually end up in the blood stream. Blood consists of cells such as erythrocytes, leukocytes and platelets in an aqueous matrix called plasma which mainly consists of water with dissolved electrolytes (Aylward, 2019). Blood serum is the plasma without clotting factors (Aylward, 2019). The determination of pesticide residue can be assessed through blood serum which acts as a biomarker of exposure (Bedi et al., 2015).

Action modes of most pesticides are through lipids and sterols. Blood serum consists of triglycerides, cholesterol and lipoprotein which makes it the matrix of choice for most lipophilic chemicals like pesticides (Aylward, 2019). Many past studies have shown the use of blood serum to detect pesticide residues in humans. A study conducted by Farhan et al. (2017), blood serum samples were collected from the community of Vehari District in Pakistan to compare the OCP residues in blood between farm workers and people who live far from agricultural fields. Another similar study was conducted in Bathinda district of Punjab, India to detect the presence of pesticide residues among the users of pesticides in the area (Bedi et al., 2015).

2.5 Properties of Target Compound

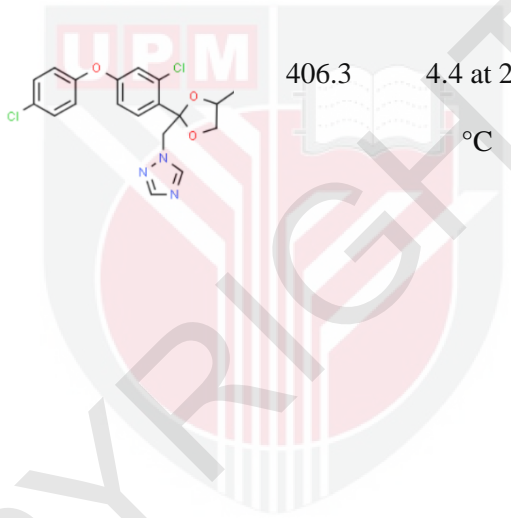
The target compounds for this study are difenoconazole, propiconazole, tebuconazole and tricyclazole. These four pesticides were categorized under fungicides and widely used in paddy farming. Fungicides generally help to kill fungi

that could bring disease to the crop which in turns reduced the quality and quantity of the crop (Kaur et al., 2019).

2.5.1 Difenoconazole

In one of the study conducted by Anis et al. (2019), among rice farmers in Northern Peninsular Malaysia showing that the difenoconazole is one of the frequently used pesticide in this field. Difenoconazole provide preventive and curative action towards crop under triazole systemic fungicide (Lucini & Magistrati, 2009). On the other side, fungicide can gives adverse effect to both human and environment. In human, fungicide can give acute or chronic health effects. A person may experienced several symptoms such as headache, dizziness, nausea and skin rashes under acute effect while chronic effect will lead to more serious condition such as birth defect, cancer endocrine disruption, and also gives reproduction effects (Kaur et al., 2019). Fungicide can also bring cytogenetic damage to infant (Ruiz-guzmán et al., 2017). A study by Lucini and Magistrati (2009) showing that difenoconazole giving the hazard rating of 4 ,showing that it can give adverse health effect to human without proper control. In a study conducted by wang et al. (2012) at the three field site in province of China reported that half –life of difenoconazole in water, rice plant and soil were vary where in water are in the range of 0.3 until 6.61 days, rice plant 1.39 until 2.59 days and soil 2.82 until 23.26 days. A study conducted in rice production area in Tajar Kedah, Malaysia highest value of 235.16 ppm of difeconazole were detected in paddy water (Latiff et al., 2010). The information of compound summarized in **Table 2.5.1**.

Table 2.5.1: Information of Difeconazole

Common Name ^a Pesticide Type ^a Chemical Class ^b IUPAC Name ^a CAS Number ^a	Molecular Formula ^a	Molecular Structure ^b	Molecular Weight (g mol ⁻¹) ^a	Log Kow ^a	Cancer Classification ^c	Half-life of compound	
						Environment ^a	Biological ^a
Difeconazole						Water: 1-3 days	Liver, kidney and pancreas:
Fungicide	C ₁₉ H ₁₇ Cl ₂ N ₃		406.3	4.4 at 25	Suggestive	Aerobic soil: 175-1600 days	1-3 days
Dioxolanes	O ₃			°C	Evidence of		Fat: 9 days
1-((2-(2-Chloro-4-(4-chlorophenoxy)phenyl)-4-methyl-1,3-dioxolan-2-yl)methyl)-1H-1,2,4-triazole					Carcinogenic for Humans		
119446-68-3							

^aPubchem, 2017. National Center for Biotechnology Information (NCBI). <http://pubchem.ncbi.nlm.nih.gov/> (accessed March 2020)

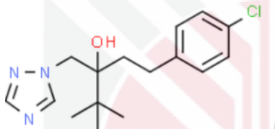
^bChemspider, 2017. Royal Society Of Chemistry. <http://www.chemspidder.com/> (accessed March 2020)

^cUSEPA (2017). Chemicals Evaluated for Carcinogenic Potential (Annual Cancer Report 2018). http://npic.orst.edu/chemicals_evaluated.pdf (accessed March 2020)

2.5.2 Tebuconazole

Tebuconazole are one of the fungicide that are being used widely to fight against mildew, brown rot blossom, twig blight, dry rot, leaf spots, and as growth regulator (Oerlemans et al., 2019). Tebuconazole used widely to control fungi in grain, vegetables and also fruit (Taxvig et al., 2008). Tebuconazole are harmful to human in context of genotoxic nor mutagenic with acceptable daily intake of 0.03mg/kg body weight according to Pesticide Residues (JMPR) (Oerlemans et al., 2019). In a another study by Taxvig et al., (2008), found that commonly fungicide like tebuconazole act as endocrine disruptor in vivo but the mode of action varies. Tebuconazole also inhibit enzymes that involves in conversion of progesterone to testosterone (Kjærstad et al., 2010). Tebuconazole also harmful to rat as it can bind to the steroid binding pocket which can potentially inhibited rat's neurosteroidogenic enzymes (shen et al., 2017) Human being can be exposed to tebuconazole by consumption of crops such as fruit, vegetables and grains that contain residue of pesticide (Oerlemans et al., 2019). In occupational setting, human can be exposed to tebuconazole through inhalation and dermal during spraying activity and the resident who living nearby might be affected too when visited or passing by the sprayed field (Oerlemans et al., 2019). The trace of tebuconazole can be monitored through human biological monitoring (HBM) such as urine, saliva, or exhaled air (Oerlemans et al., 2019). The information on tebuconazole were summarized in **Table 2.5.2**.

Table 2.5.2 : Information on tebuconazole

Common Name ^a Pesticide Type ^a Chemical Class ^a IUPAC Name ^a CAS Number ^a	Molecular Formula ^a	Molecular Structure ^b	Molecular Weight (g mol ⁻¹) ^a	Log Kow ^a	Cancer Classification ^c	Half-life of compound	
						Environment ^d	Biological ^e
Tebuconazole Fungicide A tertiary alcohol that is pentan-3-ol substituted by a 4-chlorophenyl, methyl, methyl, and a 1,2,4-triazol-1-ylmethyl at positions 1, 4, 4 and 3 respectively. 1-(4-chlorophenyl)-4,4-dimethyl-3-(1,2,4-triazol-1-ylmethyl)pentan-3-ol 80443-41-0	C ₁₆ H ₂₂ ClN ₃ O		307.82	3.7	Group C— Possible Human Carcinogen	Soil: 796 days	Oral: 7.8 hour Dermal: 16 hour

^aPubchem, 2017. National Center for Biotechnology Information (NCBI). <http://pubchem.ncbi.nlm.nih.gov/> (accessed March 2020)

^bChemspider, 2017. Royal Society Of Chemistry. <http://www.chemspider.com/> (accessed March 2020)

^cUSEPA (2017). Chemicals Evaluated for Carcinogenic Potential (Annual Cancer Report 2018). http://npic.orst.edu/chemicals_evaluated.pdf (accessed March 2020)

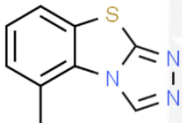
^dMontague, B., Al-mudallal, A., Fate, E., Division, E., Costello, K., Fite, E., Abel, S., Fate, E., & Division, E. (n.d.). *Environmental Fate and Effects Division*. 1–29.

^eOerlemans, A., Verscheijden, L. F. M., Mol, J. G. J., Vermeulen, R. C. H., Westerhout, J., Roeleveld, N., Russel, F. G. M., & Scheepers, P. T. J. (2019). Toxicokinetics of a urinary metabolite of tebuconazole following controlled oral and dermal administration in human volunteers. *Archives of Toxicology, Ec 2007*. <https://doi.org/10.1007/s00204-019-0>

2.5.3 Tricyclazole

Tricyclazole are one of the best treatment for disease in irrigated rice (Wandscheer et al., 2017). Tricyclazole used to control fungus *pyricularia grisea* that is well known as rice blast and one of the popular disease arise from rice crop (Wandscheer et al., 2017). The application of pesticide like tricyclazole have proven to cause an increase of genotoxic activity rice crop water through the abnormalities of chromosome (Wandscheer et al., 2017). Tricyclazole rapidly absorbed in human with approximately 90% and distribute through blood and liver (Food & Authority, 2015). Tricyclazole can be rapidly excreted through urine and bile (Food & Authority, 2015). Tricyclazole can persist and degrade in soil based on several factors such as moisture level, light and soil type (N. Kumar et al., 2017). According to study by N. Kumar et al., (2017), found that tricyclazole half-life in paddy field was 60.20 days. Propiconazole. The information on tricyclazole is summarized in **Table 2.5.3.**

Table 2.5.3: Information on tricyclazole

Common Name ^a Pesticide Type ^a Chemical Class ^a IUPAC Name ^a CAS Number ^a	Molecular Formula ^a	Molecular Structure ^b	Molecular Weight (g mol ⁻¹) ^a	Log Kow ^a	Cancer Classification ^c	Half-life of compound	
						Environment ^d	Biological ^e
Tricyclazole Fungicide Triazolobenzothiazole 8-methyl- [1,2,4]triazolo[3,4- b][1,3]benzothiazole 41814-78-2	C ₉ H ₇ N ₃ S		189.24	1.70	Not Likely to be Carcinogenic to Human	Water: 4.64- 4.85 days Soil: 3.57- 3.83days	2.5 hour

^aPubchem ,2017. National Center for Biotechnology Information (NCBI). <http://pubchem.ncbi.nlm.nih.gov/> (accessed March 2020)

^bChemspider,2017. Royal Society Of Chemistry. <http://www.chemspider.com/> (accessed March 2020)

^cUSEPA (2017). Chemicals Evaluated for Carcinogenic Potential (Annual Cancer Report 2018). http://npic.orst.edu/chemicals_evaluated.pdf (accessed March 2020)

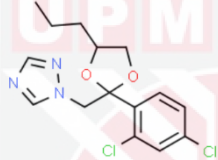
^d Meng, Z., Chen, X., & Fan, T. (2018). *Dissipation kinetics and risk assessments of tricyclazole during Oryza sativa L . growing , processing and storage.*

^e Science, M. O. F. (1984). *EFFECTS OF TRICYCLAZOLE ON SELECTED REPRODUCTIVE PARAMETERS IN ABSTRACT EFFECTS OF TRICYCLAZOLE ON SELECTED REPRODUCTIVE PARAMETERS IN MALLARDS AND BOBWHITE FOLLOWING CHRONIC DIETARY EXPOSURE.*

2.5.4 Propiconazole

Fungicide such as propiconazole gives best control for unwanted disease of rice such as *Oryza sativa* L that caused by *Ustilaginoidea virens* in China (Chen et al., 2013). Propiconazole can act as inhibitor for fungal infections by stopping spore germination which slows the disease progress of crop (N. Kumar et al., 2017). In a study by N. Kumar et al., (2017) reported 1.15 µg/L concentration of propiconazole in US stream. In a study by Knebel et al., (2019) reported that both propiconazole and tebuconazole caused expression of steatosis-related genes and cellular triglyceride accumulation in human. Propiconazole rate of absorption can reach up to 80% and having highest concentration in liver and kidney (Gupta, 2018). The excretion of propiconazole were more than 95% in urine and feces within 48 hour (Gupta, 2018). Propiconazole associated with liver toxicity and CNS effects such as general restlessness, alternating phases of increased and reduced motility, and also aggressive behaviour (Gupta, 2018). The information on propiconazole is summarized in **Table 2.5.4**.

Table 2.5.4: Information on propiconazole

Common Name ^a Pesticide Type ^a Chemical Class ^b IUPAC Name ^a CAS Number ^a	Molecular Formula ^a	Molecular Structure ^b	Molecular Weight (g mol ⁻¹) ^a	Log Kow ^a	Cancer Classification ^c	Half-life of compound (days)	
						Environment ^a	Biological ^a
Propiconazole Fungicide The cyclic ketal obtained by formal condensation of 1-(2,4-dichlorophenyl)-2-(1,2,4-triazol-1-yl)ethanone with pentane-1,2-diol. 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 ₂ N ₃ O ₂		342.2	3.72	Group C— Possible Human Carcinogen	Soil: 60-96 days Aerobic water: 25-85 days	Rats: 24-31 hours

^aPubchem, 2017. National Center for Biotechnology Information (NCBI). <http://pubchem.ncbi.nlm.nih.gov/> (accessed March 2020)

^bChemspider, 2017. Royal Society Of Chemistry. <http://www.chemspider.com/> (accessed March 2020)

^cUSEPA (2017). Chemicals Evaluated for Carcinogenic Potential (Annual Cancer Report 2018). http://npic.orst.edu/chemicals_evaluated.pdf (accessed March 2020)

2.6 Mechanism of Pesticide

Pesticide can modified many cellular and sub-cellular activities that can lead to epigenetic changes which cause disease or the dead of cell (Sabarwal et al., 2018b). when pesticide binds to the hormone receptor that available on cell membrane or nucleus, it will cause endocrine disruption because cellular signalling were disrupted on this state (Sabarwal et al., 2018b). Pesticide also potential to trigger inflammatory signals or worst, can generate reactive oxygen species (ROS) which leads to accumulation of unfolded protein aggregate deforming ubiquitin proteasome system and this will cause genetic and epigenetic alteration and causing various disease (Sabarwal et al., 2018b). The toxic properties of pesticide can destroy cellular function in a way by causing the failure of mitochondria and endoplasmic reticulum (Sabarwal et al., 2018b). When there is environmental signals such as communication pathway and transduction pathway, cell will adapt with various mechanism to cope and respond with it (Sabarwal et al., 2018b). Multiple sensors and receptors that presence on the cell will detect foreign substance and react to neutralize or eliminate the substance (Sabarwal et al., 2018b). In most case, the metabolism that too place gives out harmful metabolites such as ROS that dangerous to cell (Sabarwal et al., 2018b). The metabolism of compound give harmful effect to human as it disrupt essential process that took place in cell (Sabarwal et al., 2018b)

2.7 Legislation and regulation related to pesticide in Malaysia

Regulation of law is important to keep the usage of pesticide under control. Pesticide Regulation are being regulate by governments in order protect human and also environment from harm. In Malaysia, management of pesticides are being controlled under Pesticide Act 1974 which are being implement and enforced by Department of Agriculture under the Ministry of Agriculture and Agro-Based Industry. The scope under pesticide Act 1974 includes, regulate the labelling, importation, manufacturing, advertising, sale, storage and also reporting and investigation of incidents happened caused by pesticides.

Pesticide can bring adverse effect to both human and environment without proper control measures, however this can be prevented if precautionary action were taken and implementation of laws and regulation involved were enforced. Among Pesticide Regulation that involves in Malaysia includes, Pesticide Act 1974, Environmental Quality Act 1974(Act 127) ,Pesticide (Registration) (Amendment) Rules 2008, P.U. (A) 353/2008, Pesticides (Labeling) Regulation 1984, P.U.(A) 251/1984, Pesticides (Importation for Educational or Research Purposes) Rules 1987, Pesticides (Licensing For Sale And Storage For Sale) Rules 2007, Pesticides (Licensing for Manufacturing) Rules, 2011, Food (Amendment) Regulations 2004, PU(A) 160/2004 and also Occupational Safety and Health Act 1994 (Act 514).

In order to protect environment, Environmental Quality Act 1974 were regulated to controls the pesticide effluent from the workplace. These will helps to reduce the pollution that caused by the pesticide residue itself. As for protecting human health, Food (Amendment) Regulations 2004, PU(A) 160/2004, were implemented to cater the problems on pesticide residue in food, gives education on pesticides users and act to improve pesticide applications. As for Occupational

Safety and Health Act 1994 (Act 514), it was implemented to provide guidelines to achieve a standards of safety and health of workers involved with pesticide handling.



CHAPTER 3

METHODOLOGY

3.1 Chemicals and Standard

Ammonium formate (10 M in H₂O) and reference standards of fipronil (99.0%) and imidacloprid (99.0%) were purchased from Dr. Ehrenstorfer (Germany). Pretilachlor (98.7%) was purchased from Sigma-Aldrich (Germany). The internal standard (IS) imidacloprid-d4 (99.9%) was purchased from Sigma-Aldrich (Germany). Stock standard solutions (1000 mg/L) were prepared monthly by dissolving the standards in methanol. Working standard solutions (10 mg/L) were prepared from the stock standard solutions weekly. HPLC-grade methanol, HPLC-grade acetonitrile and HPLC-grade acetone were purchased from Fisher Scientific (UK). Formic acid 98%, and magnesium sulphate (MgSO₄) 99% were purchased from R&M Chemicals (Malaysia).

3.2 Study Design

This is a cross-sectional study as the occupational exposure of pesticide were assessed in blood serum samples and associate their self-reported respiratory health symptoms among paddy farmers. The samples were collected on January 2019 until March 2019. Fungicides are frequently applied as it controls disease. At Tanjung Karang, the Cultivation periods for all plots are different from each other. Hence, fungicide are used frequently based on the paddy field needs to control the disease.

3.3 Study Location

The study were conducted at Tanjung Karang, Karang as the place is well known for its paddy cultivation activity. The sampling location were included in figure 3.1. The study location was chose based on some criteria which were:

- i) Agriculture area with paddy cultivation as the main activity. Tanjung Karang is the third largest area of paddy field in Peninsular Malaysia and also known as 'the rice bowl of Selangor' (Fuad et al., 2012).
- ii) The residents which will be the respondents are willing to give co-operation throughout the study
- iii) Short distance from the laboratory which preservation and analysis will be conducted.

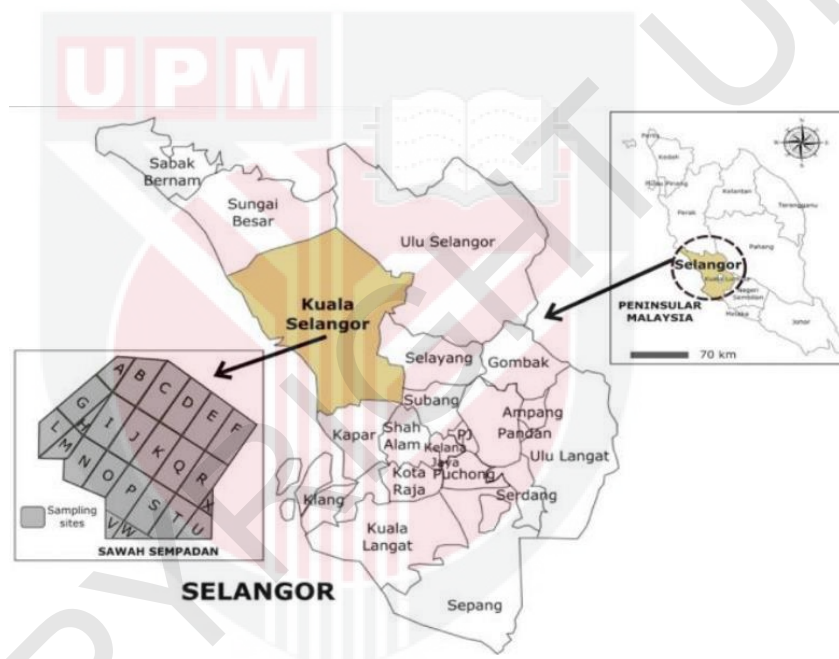


Figure 3.1: Location of the sampling at Kampung Sawah Sempadan, Tanjung Karang

3.4 Sampling Population

The sample frame of this study were paddy farmers in Kampung Sawah Sempadan, Tanjung Karang. The study populations were male adult farmers in Kampung Sawah Sempadan, Tanjung Karang. The respondents were selected based on the exclusion and inclusion criteria as follows:

Inclusion criteria

- i) Farmers who work in the paddy field and directly involved in preparation and application of pesticides
- ii) Age of 18-59 years old
- iii) Male

Exclusion criteria

- i) Individual that are not willing to participate in the study

3.5 Sample Size Calculation

The sample size calculation was determined using Lemeshow et al. (1990). The formula for calculating sample size is shown below:

$$n = \frac{(Z_{1-\alpha/2}\sqrt{2PQ} + Z_{1-\beta}\sqrt{P_1Q_1 + P_2Q_2})^2}{(P_1 - P_2)^2}$$

Where,

$Z_{1-\alpha/2}$ = Standard error associated with confidential interval, 95%

CI=1.96

$Z_{1-\beta}$ = Standard error associated with power, 84.2% of power = 0.842

P_1 = Estimated proportion which is (56.4%) (Abdul Hamid et al., 2016)

P_2 = Estimated proportion which is (33.3%) (Abdul Hamid et al., 2016)

$$P = \frac{P_1 + P_2}{2}, \quad Q = 1 - P$$

$$Q_1 = 1 - P_1 \text{ and } Q_2 = 1 - P_2$$

$$n = \frac{(1.96\sqrt{2(0.4485)(0.5515)} + 0.842\sqrt{0.564(0.436) + 0.333(0.667)})^2}{(0.564 - 0.333)^2}$$

$$n = 71.5 \sim 72$$

$$72 + 14 (20\%) = 86$$

Based on the sample size calculation for group, total number of study population in group was 72. **The sample size was optimized by 20% from the total considering lost data** making the total sample size 86. This population size shows the strength of study for this research. However, due to study limitation such as pandemic and the breakdown of UHPLC-MS/MS machine the samples analysed only achieved by 29 samples.



3.6 Sampling Method

The farmers who involves in activity of spraying pesticides were recruited. The recruitment were done randomly based on the farmers who fits the inclusion criteria as stated. The farmers required to answer the questionnaire and after the spraying activity were done they traveled to Klinik Kesihatan Sungai Tinggi Kanan for blood sampling. Each respondent from exposed and non-exposed group involved were paid RM50 as a token of appreciation in participating in this study

3.7 Study Procedure

3.7.1 Questionnaire

Demographic information and occupational exposure information were determined for all participants using questionnaire. The questionnaire was developed based on questionnaire used in (i) Agriculture Health Study (AHS) by Alavanja, (1996); Hou et al. (2013); Andreotti et al. (2015), and (ii) Vietnam: Pesticide Use Survey (Berg, 2001). The questionnaire comprised of five sections namely Section A (personal information of the respondents), section B (working background and experience), section C (lifestyle), Section D (exposure to pesticides) and Section E (use of personal protective equipment (PPE)). A sample of questionnaire is attached in the submission.

This questionnaire were pre-tested among farmers at Kampung Sungai Burung to assess the clarity of the questionnaire and words used. By doing this, any wording issue or difficult questions can be identified and corrected. Besides, pre-testing can help to estimate the time taken for each respondent to answer the questions. The number of respondents that will be involved in the pre-testing is 10%

from the total sample size of this study. Socio-demographic of the respondents in the pre-testing will also be similar to the characteristics of the respondents of the study.

3.7.2 Blood Samples

This study was approved by University Research Ethics Committee of University Putra Malaysia, Selangor, Malaysia (JKEUPM) with its' reference number JKEUPM-2018-357. All of the respondents were briefed and been given their consent to participate in this study. Respondents travelled to Klinik Sungai Tengi Kanan for blood sampling. Blood samples were obtained by venepuncture procedure which was carried out by an authorized and certified medical personnel according to standard procedure. As a token of appreciation, all respondents were given RM50.00. For the usage of this study, 10 mL of blood was drawn from their veins of the inner forearms of each subject. Blood samples was then collected in residue free heparinised glass vials by using a sterilized syringe. Lastly, the blood samples are transported to the laboratory with dry ice and stored at -80 °C until analysis.

3.8 Sample Extraction and Analysis

3.8.1 Extraction of blood serum

The extraction method was based on Shin et al. (2018) in which QuEChERS (Quick, Easy, Cheap, Effective, Rugged, and Safe) method was used. Two milliliters of blood sample was centrifuged at 40 x 100 rpm for 10 minutes to obtain serum. 100µL of blood serum into a 2-mL microcentrifuge tube was extracted. Four hundred microliter of acetonitrile was then poured into the microcentrifuge tube. Then using MS-100 Thermoshaker Incubator (LABGENE Scientific SA ZI, Switzerland) the sample was shake for 1 minute at 1200 rpm. Next, 40 mg of Magnesium Sulphate

(MgSO₄) and 10 mg of sodium chloride (NaCl) were added into the sample under ice bath condition to avoid heating from the MgSO₄. After that, the sample was centrifuged for 5 mins at 13000 rpm using Sartorius Sigma 1–14 Microcentrifuge (Sartorius Stedim, Epsom, UK). The supernatant was then blow dried under a gentle stream of nitrogen gas. The remaining residue after evaporated with nitrogen gas was reconstituted with 400µL of reconstitute solution (H₂O:MeOH, 3:1). The sample was then filtered with 0.22µm nylon syringe filter (Membrane Solutions, USA) and transferred into 2-mL amber glass vial. The sample was then injected into the Ultra High Performance Liquid Chromatography tandem mass spectrometry (UHPLC-MS/MS) for analysis. The blood collected in this study is only used for the purpose of this study and the leftovers of unused blood are destroyed according to standard procedure after the study is completed.

3.8.2 Instrumentation (UHPLC-MS/MS analysis)

The extracts were analysed according to Zaidon et al., (2019). UHPLC-MS/MS (Agilent, USA) was used to analyse the concentrations of pesticides and the methods conditions are as follows: Chromatographic separation was conducted on an Eclipse Plus C18 column (2.1 mm × 50 mm I.D., 1.8 µm particle size) (Agilent, USA). The mobile phase was a gradient of, A: ultrapure water with 0.1% formic acid and 5mM ammonium formate, B: methanol with 0.1% formic acid and 5mM ammonium formate. The mobile phase was mixed as follows: 0 min 94% A, 6% B; 15 min 2% A, 98% B; 18 min 2% A, 98% B; 18.01 min 94% A, 6% B; and 20 min 94% A, 6% B. The flow rate and column temperature were set for 0.5 mL min⁻¹ and 40 °C, respectively. Other than that, injection volume and the total run time were 2 µL and 20 min, respectively. Electrospray ionization (ESI) in positive ion mode was used for detecting all the pesticides. The optimized operating conditions were as

follows: capillary voltage is 3500 V; gas temperature is 220 °C; gas flow is 11 L min⁻¹; and nebulizer is 30 psi. The optimized electrospray ionization (ESI) and tandem mass spectrometry (MS/MS) parameters, comprising of precursor ions, product ions and collision energy (C.E.) were shown in **Table 3.1**. The optimized ESI and MS/MS parameters were subsequently use for multiple reaction monitoring (MRM) in UHPLC-MS/MS analysis.

Table 3.1: Gradient Condition

	Time (minutes)	A%	B%	Flow rate (mL/min)
1	0	94.00	6.00	0.5
2	15.00	2.00	98.00	0.5
3	18.00	2.00	98.00	0.5
4	18.01	94.00	6.00	0.5
5	20.00	94.00	6.00	0.5

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

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

Table 3.2: Precursor ions, product ions and collision energy for the 4 compounds and 1 internal standard

Target compounds	Precursor ion, m ^z ⁻¹	Product, m ^z ⁻¹ (collision energy, V)
Difeconazole	406.10	337.00 (10)
		251.00 (20)
Tebuconazole	308.10	124.90 (47)
		70.00 (40)

Tricyclazole	190.00	163.10 (24) 136.20 (28)
Propiconazole	342.10	159.00(32) 69.10(16)
Internal standards	Precursor ion, mz^{-1}	Product, mz^{-1} (collision energy, V)
Imidacloprid-d ₄	260.10	212.90 (21)

3.9 Quality Control

3.9.1 Questionnaire

Pre-test were conducted among paddy farmers at Kampung Sungai Burung, Tanjung Karang for reliability and validity testing for this study. The time taken for each respondent to answer the questionnaire also recorded during the pre-test. The reason for choosing respondent from Kampung Sungai Burung is because of the same socio-demographic background of respondent in the study. The number of respondent involved in the pre-test were 10% from the total sample size in the study. The value of Cronbach alpha for the pre-test was 0.728.

3.9.2 Cleaning of Glassware

The method of cleaning glassware were according to US EPA (2007) method 1699. All glassware were soaked in the Decon 90 solution overnight. The glassware then rinsed with methanol and continued by hot tap water. The glassware were rinsed again by using methanol, followed by acetone and lastly dichloromethane. After all washing process finished, all the glassware were dried in the oven at 60°C and glassware were capped with aluminium foil.

3.9.4 Method Validation

The mixture of pesticide compound together with IS were spiked in the blank serum samples in order to determine the limit of detection (LOD) and limit of quantification (LOQ). Blood serum sample were extracted and analysed by using UHPLC-MS/MS. LOD and LOQ were determined with a signal-to-noise ratio of >3 and >10 (Choi et al., 2013). The LOD, LOQ, linear range, coefficient of determination (R^2) and mean total lipid were summarized in **Table 3.3**.



Table 3.3: LOD, LOQ, Linear range, coefficient of determination (R²) and Mean Total Lipid

Components	LOD (ngmL ⁻¹)	LOQ (ngmL ⁻¹)	Linear Range (ngmL ⁻¹)	R ²	Mean Total Lipid (mmolL ⁻¹)
Difenoconazole	0.005	0.05	0.05-500	0.9995	
Tebuconazole	0.001	0.01	0.01-500	0.9997	13.88
Tricyclazole	0.001	0.01	0.01-500	0.9995	
Propiconazole	0.001	0.01	0.01-500	0.9995	

Lipid analysis were done to find out the total lipid content as most pesticide compound are lipophilic. Lipophilic compound can build a non-specific bond with hydrophobic sites on blood proteins (Phillips et al., 1989). The total serum lipids were calculated based on Han et al. (2020).

$$\text{Total lipid (TLs)}: 2.27 \times \text{total cholesterol} + \text{triglycerides} + 0.623$$

3.9.5 Calibration of UHPLC-MS/MS

The instrument were calibrated for each compound by using five-point calibration limit of quantification (LOQ) curve ranging from 0.01 ng/mL to 500 ng/mL.

3.9.6 Method Blank

To monitor overall performance for quality control, all samples were spiked with internal standard (IS) mixture during extraction. Mixture of IS were spike into one

blank blood serum sample and run through entire sample preparation and extraction procedure for every batch in order to observe any possible background contamination in the blood serum samples. Imidacloprid-d₄ were used as internal standards to quantify the concentration of target compounds in the study.

2.9.4 Extraction recovery

The extraction recovery were done by spiking the serum samples with IS and mixture of 13 compound of pesticides. The percentage of recovery was calculated based on method 1694 by EPA (2007).

$$\text{Recovery (\%)} = \frac{\text{concentration found } \left(\frac{\text{ng}}{\text{mL}}\right)}{\text{concentration spiked } \left(\frac{\text{ng}}{\text{mL}}\right)} \times 100$$

2.9.5 Method Blank

All samples were spiked with internal standard (IS) mixture during extraction. Mixture of IS were spike into one blank blood serum sample and run through entire sample preparation and extraction procedure for every batch in order to observe any possible background contamination in the blood serum samples. Imidacloprid-d₄ were used as internal standards to quantify the concentration of target compounds in the study.

3.10 Statistical analysis

Questionnaire response tabulation and graphical summarizes will complete with Microsoft Excel 2010, while statistical analysis was analyzed by using Statistical Package for Social Science (SPSS) Version 22.0. The descriptive test was used to calculate mean, median, mode and standard deviation. Meanwhile, Kolmogrov Smirnov test was used to determine the normality of the data. Descriptive analysis were used to quantify the concentration of difenoconazole, tebuconazole, propiconazole and tricyclazole in blood serum samples among paddy

farmers. In this study, the association between the concentration of pesticides in blood serum samples and the reported health symptoms among paddy farmers was determine using logistic regression. Simple logistic regression and multiple logistic regression was done to determine the contributing factors that can affect the self-reported symptoms.



CHAPTER 4

RESULTS

4.1 Quality control

The total lipid was calculated based on Equation 1 and the result is tabulated as in Table 3.3. The mean concentration for lipid analysis was summarized in **Table 3.3**. Based in **Table 3.3**, the mean for total lipid was 13.88 mmolL^{-1} .

The recovery was calculated based on Equation 2 and summarized in **Table 4.1**.

Table 4.1: Percentage of recovery (n=4)

Target compounds	Mean \pm SD (%)
Difenoconazole	70.89 ± 11.04
Tebuconazole	90.42 ± 1.01
Tricyclazole	69.12 ± 9.51
Propiconazole	64.76 ± 8.10

Based on the **Table 4.1**, the highest mean recovery for four compound were tebuconazole (90.42), followed by difenoconazole (70.89%), tricyclazole (69.12%), propiconazole (64.76%).

4.2 Socio-demographic background of respondents

A total of seventy one respondents from exposed group which is farmers were involved in this study. However, due to pandemic and the breakdown of UHPLC-MS/MS, we were only able to analyse twenty nine samples. The socio-demographic background such as age, gender, race, level of education and smoking status were obtained from the questionnaire summarized in **Table 4.2**.

A total of eighty-six of farmers were involved in this study. However, due to pandemic and the breakdown of UHPLC-MS/MS, we only be able to analyse twenty nine samples only. The socio-demographic background such as age, gender, race, level of education and smoking status were obtained from the questionnaire. As for smoking status, the farmers, have almost equal percentage which yes (48.3%) and no (51.7%). All of the respondents involved were male farmers and Malay. The average age of paddy farmers involved was 43.97 years old with range from 27 years old to 59 years old. The majority level of education of paddy farmers was secondary level (82.8).

Table 4.2 Socio-demographic background of paddy farmers (n=29)

Variables	Mean \pm SD	Minimum	Maximum
Age (years)	43.97 \pm 10.15	27	59

Variables	Category	Frequency	Percentage (%)
Gender	Male	29	100
Race	Malay	29	100
Education level	Primary	5	82.8
	Secondary	24	17.2

smoking	Yes	15	48.3
	No	14	51.7

4.3 Concentrations of pesticides (difenoconazole, tebuconazole, tricyclazole and propiconazole) collected.

The concentration of difenoconazole, tebuconazole, tricyclazole and propiconazole in blood serum samples among paddy farmers were summarized in

Table 4.3.

Table 4.3 Concentration of target compounds in blood serum samples among paddy farmers (n=29).

Target compounds	Mean	Standard deviation	Minimum	Maximum	Frequency of detection in blood serum samples.
Difenoconazole	1063.18	1267.77	12.02	4898.20	29
Tebuconazole	779.55	806.55	16.80	3100.80	29
Tricyclazole	638.52	825.36	00.00	3121.22	24
Propiconazole	617.26	622.16	44.24	2281.28	29

Based on Table 4, the mean concentration of difeconazole in blood serum sample among paddy farmers was 1063.18 ng/mL with the minimum value of 12.02 ng/mL and maximum was 4898.20 ng/mL. All blood serum samples detect the presence of difeconazole. For second compound which was tebuconazole, the mean concentration in blood serum sample was 779.55 ng/mL with the minimum value of 16.80 ng/mL and maximum concentration was 3100.80 ng/mL. All blood serum samples detect the presence of tebuconazole. Next, the mean concentration for tricyclazole in blood serum sample among paddy farmers was 638.52 ng/mL with

maximum concentration was 3121.22 ng/mL. The frequency of tricyclazole detected in blood serum samples was 24 out of 29 paddy farmers. Lastly, the mean concentration for propiconazole was 617.26 ng/mL with minimum concentration of 44.24 ng/mL and maximum concentration of 2281.28 ng/mL.

4.4 The Self-Reported Respiratory Health Symptoms experienced by the paddy farmers.

The self-reported respiratory health symptoms by paddy farmers were breathing difficulties, chest pain, cough, phlegm, wheezing, sore throat, nausea, vomiting, dizziness and runny nose. The self-reported health symptoms were summarized in **Table 4.4**.

Table 4.4: Self-reported respiratory health symptoms experienced by the paddy farmers (n=29)

Health Symptoms	n (%)	
	Yes	No
Reported		
Cough	10 (34.5)	19 (65.5)
Phlegm	0	29(100)
Wheezing	2 (6.9)	27 (93.1)
Sorethroat	7 (24.1)	22 (75.9)
Nausea	11 (37.9)	18 (62.1)
Chestpain	10 (34.5)	19 (65.5)
Breathing	8 (27.6)	21 (72.4)
Vomiting	8 (27.6)	21 (72.4)
Dizziness	14 (48.3)	15 (51.7)
Runnynose	4(13.8)	25 (86.2)

Based on **Table 4.4**, dizziness was the most self-reported health symptoms recorded among paddy farmers which out of 14 of respondents (48.3%) from 29 respondents experiencing it, followed by nausea (37.9%) and evenly between chest pain and cough (34.5%). On the other hand, phlegm did not recorded any number as the most reported health symptoms among paddy farmers

4.5 Association between reported health symptoms and concentration of pesticide (difenoconazole, tebuconazole, tricyclazole and propiconazole) in blood serum samples and the self-reported respiratory health symptoms among paddy farmers.

The association between concentrations of pesticides (difenoconazole, tebuconazole, tricyclazole and propiconazole) and self-reported respiratory health symptoms among paddy farmers were summarized in **Table 4.5**.

Table 4.5: Association between reported health symptoms and concentration of pesticide (difenoconazole, tebuconazole ,tricyclazole and propiconazole) in blood serum samples and self-reported health symptoms (n=29)

Concentration of pesticides	p-value ^a	p-value ^b	p-value ^c	p-value ^d	p-value ^e	p-value ^f	p-value ^g	p-value ^h	p-value ⁱ
Difeconazole	0.277	0.379	0.725	0.483	0.779	0.599	0.392	0.744	0.217
Tebuconazole	0.057	0.631	0.550	0.733	0.614	0.949	0.628	0.644	0.205
Tricyclazole	0.138	0.195	0.183	0.642	0.317	0.896	0.438	0.977	0.485
Propiconazole	0.132	0.198	0.914	0.242	0.357	0.128	0.336	0.959	0.167

^aBreathing difficulties ^bChestpain ^cCough ^dWheezing ^eSorethroat ^fNausea ^gVomitting

^hDizziness ⁱRunny nose

*p value is significant at 0.05 levels

Based on the result in **Table 4.5**, there was no significant association between the concentrations of pesticides (difenoconazole, tebuconazole, tricyclazole and propiconazole) and self-reported health symptoms.

4.6 Contributing factors that affects the self reported health symptoms by paddy farmers

Contributing factor such as work duration in years ,weeks, and hours were recorded. Other factors such as smoking and education level were also recorded. The information were summarized on **Table 4.6**.

Table 4.6 : Contributing factors that affects the self-reported respiratory health symptoms by paddy farmers

Contributing factors	Simple linear regression			Multiple Linear regression		
	Unstandardized B	t	p	Unstandardized B	t	p
Work duration (years)	-0.018	-0.649	0.522	-0.004	-0.124	0.903
Work duration (weeks)	-0.152	-1.074	0.292	-0.060	-0.315	0.756
Work duration (hours)	-0.145	-1.009	0.322	-0.115	-0.687	0.499
Smoking	0.238	0.390	0.699	0.305	0.446	0.660
Education	-0.425	-0.528	0.602	-0.181	-0.189	0.852

Based on **Table 4.6**, work duration (years), work duration (weeks), work duration (hours) and education (b= -0.018, -0.152, 0.145, 0.425) respectively has a negative association with the total self-reported symptoms while for smoking (0.238), it is the only factor that has a positive association with the self-reported health symptom.

CHAPTER 5

DISCUSSION

5.2 Socio-demographic background of respondents

Based on the results, 86 paddy farmers involved in this study which took place in Tanjung Karang, Selangor. All of the information were collected from the questionnaires provided for this study. The farmers who involved in this study were malay, as malay are the majority race that lives there. This have been mentioned by a study by Fuad et al., (2012), where the two study areas involved in the study (Sungai Burung and Sawah sempadan) were dominantly lived by malay race. Male farmers were choosen as majority of farmers were male and male can carry out heavy work that required to be done in farming activities. This characteristics have been supported by a study conducted by Galiè, Jiggins and Struik (2013) ,which the study found that farming activities were majority participated by men as men are stronger to do heavy works. The average age of farmers was 43.97 years old. Youngster tend to move to city for a better job offer and left old folks to maintain the farm field (Fan et al., 2013).

5.3 Concentrations of pesticides (difenoconazole, tebuconazole, tricyclazole and propiconazole) collected.

Based on the results, the mean concentrations of difenoconazole, tebuconazole, tricyclazole and propiconazole in blood serum samples among paddy farmers were 1063.18 ng/mL, 779.55 ng/mL, 638.52 ng/mL and 617.26 ng/mL, respectively. There is no past studies that relating concentration of pesticides (difeconazole, tebuconazole, tricyclazole and propiconazole) in blood serum samples to be compared of. However, in study by Chang et al. (2017) , 76 pesticides that were detected in the blood serum of adult population in Jiangsu Province, China with a

range from 0.02 to 1423.62 ng/mL. Based on this study, shows that there is significant amount of difenoconazole, tebuconazole, tricyclazole and propiconazole in blood serum sample.

5.4 The Reported Health Symptoms experienced by the paddy farmers.

According to the results, the most reported health symptoms were dizziness (48.3%), followed by nausea (37.9%) and chest pain (34.5%). These were the common symptoms experienced by the farmers during application of pesticide. Dizziness is one of the severe symptoms of toxic exposure (Damalas & Koutroubas, 2016). A study conducted by Akter et al. (2018) reported that vomiting and headaches are the most symptoms experienced by the farmers during pesticide application. Farmers that works directly with pesticide tends to have common respiratory disease symptoms such as shortness of breathing and asthma attack (Dhananjayan & Ravichandran, 2018). In another study reported 73.8% of headache health symptoms experienced by the farmers during pesticide use (Bhandari et al., 2018).

5.5 Association between reported health symptoms and concentration of pesticide (difenoconazole, tebuconazole ,tricyclazole and propiconazole) in blood serum samples among paddy farmers.

There were no significant association between the concentration of pesticides in blood serum samples and the self-reported health symptoms among paddy farmers. A study regarding concentration of pesticide (difenoconazole, tebuconazole, tricyclazole and propiconazole) in human being are still limited. However, in a study by Hamsan et al., (2017) , found that concentration of fipronil, imidacloprid and pretilachor in air was contributing to health symptoms experienced by farmers. The value might be not significant due to smaller sample size. The symptoms reported

also recorded based on questionnaire only and not being verified by medical personnel, the symptoms might be falsely reported.

5.6 Contributing factors that affects the self-reported respiratory health symptoms by paddy farmers

The results showing that there is a negative association between the work duration of paddy farmers with the self-reported health symptom. The level of education also showing negative association with the self-reported health symptoms. However, there is a positive association between smoking and the self-reported health symptoms. Smoking increase symptoms of disease related to respiratory system (Jayes et al., 2016). The sign of chest pain might be mistakenly thought comes from the use of pesticide instead of cause from smoking.

As for contributing factors such as work duration in years, hours and weeks it showing negative association with the self-reported health symptoms. The symptoms may be falsely recorded as the symptoms were not diagnosed by medical practitioner and being recorded only by questionnaire. The farmers who works longer might be used to the symptoms experienced and did not recorded the symptoms experienced.

CHAPTER 6

CONCLUSION AND RECOMMENDATION

6.1 Conclusion

As a conclusion, there is concentration of compound (difeconazole, tebuconazole, tricyclazole and propiconazole) in blood serum samples among paddy farmers but the value is not significant. Among targeted compound the highest concentration detected was difeconazole (1063.18 ng/mL), followed by tebuconazole (779.55 ng/mL), tricyclazole (638.52 ng/mL) and propiconazole (617.26 ng/mL). Based on the result, there were no significant association between reported health symptoms for all compound as the results may be affected by other contributing factors such as work duration, smoking status and education level. The preliminary findings of this study suggest that occupational exposure of pesticides may lead to occurrence of pesticides in blood serum samples of farmers. Hence, a proper and adequate must be worn by the farmers in order to control the risk. A proper guidelines such as Malaysian standard code of recommended practice (MS479:2012), developed by the Department of Standards Malaysia and SIRIM Berhad on adequacy of dermal and inhalation protection were recommended for the suitable control measure (Hamsan et al., 2017). Based on the guidelines, farmers who works directly during preparation (mixing/loading) and application (spraying) of pesticide should wear: (i) regular work clothes (long pants and sleeves; heavyweight and tightly woven fabrics of cotton or polyester/cotton blends); (ii) gloves (PVC, rubber, nitrile or neoprene gloves should be used in handling concentrates but are unsuitable for continuous wear); (iii) boots (rubber or PVC boots); (iv) face shield (face shields are intended for

face protection); (v) wide-brimmed hat or hood (may be a hat with a broad brim or a full hood that drapes to cover the shoulders); (vi) face masks (masks of gauze or similar material are capable of filtering the particles from a wettable powder spray and may be worn to reduce respiratory exposure if such protection is considered desirable for compounds of moderate toxicity); and (vii) respirators (designed to protect from toxic vapors, gases or droplets of very toxic materials; they must be specifically chosen for each compound being used) (DOS, 2012).

Since the pesticides are present in blood serum samples of farmers in a significant amount, further in depth studies are needed to study the health effects of the pesticides besides the self-reported respiratory health symptoms

6.2 Limitation of study

There are several limitations in this study that should be considered to improve on further research. The limitation includes the long period of time in keeping the blood serum samples from the date collected until analysed stage. This might cause some of the pesticide content in the blood serum degraded. This problem arises due to the breakdown of Ultra High Performance Mass Tandem Spectrometry (UHPLC-MS/MS) which holds the process of analysing of sample for quite a long time. The analysing process of data also stopped due to pandemic that happened throughout the study period which limits the sample that can be analysed. Recall bias also one of the limitation as the respondents have to recall all the health symptoms for the past 12 months and confusion might happened during that stage. The paddy farmers also did not wear a proper or sufficient PPE which may exposed them to spillage and caused a higher concentration in blood serum samples. The health reported symptoms also solely based on what reported by the farmers itself without confirmation from medical personnel.

6.3 Recommendation

Authorities involved should have a more effective initiative in order to make sure all paddy farmers have a better access knowledge of proper use of personal protective equipment (PPE) and also pesticide. A proper training on handling pesticide can be done in order to reduce the exposure of pesticide towards farmers. Farmers also must plays an important role in order to protect themselves from pesticide by practising a good hygiene and good use of proper PPE.

6.4 Ethical Consideration

All respondents which were the farmers from Tanjung Karang , Selangor were briefed on the study and signed a written informed consent. The study were approved by the University Research Ethics Committee of University Putra Malaysia Selangor, Malaysia (JKEUPM).

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APPENDIX II
QUESTIONNAIRE



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ID Responden:

Tarikh:



JABATAN KESIHATAN PERSEKITARAN & PEKERJAAN

FAKULTI PERUBATAN DAN SAINS KESIHATAN

UNIVERSITI PUTRA MALAYSIA

**KEJADIAN RACUN PEROSAK DI DALAM SAMPEL UDARA PERIBADI
DAN GEJALA KESIHATAN DALAM KALANGAN PESAWAH PADI DAN
KUMPULAN YANG TIDAK TERDEDAH DI TANJUNG KARANG,
SELANGOR**

ARAHAN SOALAN:

1. Borang soal selidik ini mengandungi Empat (4) bahagian iaitu: Soalan Saringan

Bahagian A : Maklumat Diri

Bahagian B : Latar Belakang Pekerjaan

Bahagian C : Gaya Hidup

Bahagian D : Maklumat Gejala/Tanda Kesihatan

2. Anda diminta menjawab semua soalan di dalam buku soalan ini
3. Buku soalan ini hendaklah dikembalikan kepada pengkaji setelah selesai menjawab.

KEJADIAN RACUN PEROSAK DI DALAM SAMPEL UDARA PERIBADI DAN GEJALA KESIHATAN DALAM KALANGAN PESAWAH PADI DAN KUMPULAN YANG TIDAK TERDEDAH DI TANJUNG KARANG, SELANGOR

KEGUNAAN
PENYELIDIK

BAHAGIAN A: MAKLUMAT DIRI

1. Jantina:

Lelaki

Perempuan

2. Umur : tahun

3. Bangsa:

Melayu

Cina

India

Lain-lain (sila nyatakan):

4. Tahap pendidikan:

Tiada pendidikan formal

Pendidikan Primer (Sekolah rendah)

Pendidikan Sekunder (Sekolah menengah)

Pendidikan Tertinggi (Peringkat Universiti/Kolej)

BAHAGIAN B: LATAR BELAKANG PEKERJAAN

5. Pekerjaan sekarang :

6. Tempoh bekerja di tempat sekarang: bulan/ tahun

7. Jumlah hari bekerja dalam seminggu: hari/seminggu

8. Jumlah masa bekerja dalam sehari: jam/sehari

9. Pekerjaan terdahulu:

10. Tempoh bekerja di tempat dahulu: bulan/ tahun

SULIT

KEJADIAN RACUN PEROSAK DI DALAM SAMPEL UDARA PERIBADI DAN GEJALA KESIHATAN DALAM KALANGAN PESAWAH PADI DAN KUMPULAN YANG TIDAK TERDEDAH DI TANJUNG KARANG, SELANGOR

KEGUNAAN PENYELIDIK

BAHAGIAN C: GAYA HIDUP

11. Adakah anda merokok sekarang?

Ya

Tidak

12. Adakah anda makan sayur-sayuran?

Ya

Tidak

13. Adakah anda makan buah-buahan?

Ya

Tidak

14. Adakah anda melakukan aktiviti senaman?

Ya

Tidak

15. Adakah anda mengambil sebarang makanan tambahan?

Ya

Tidak

SULIT

KEJADIAN RACUN PEROSAK DI DALAM SAMPEL UDARA PERIBADI DAN GEJALA KESIHATAN DALAM KALANGAN PESAWAH PADI DAN KUMPULAN YANG TIDAK TERDEDAH DI TANJUNG KARANG, SELANGOR

KEGUNAAN PENYELIDIK

BAHAGIAN D: GEJALA/TANDA KESIHATAN

16. Apakah jenis-jenis gejala/tanda yang anda alami dalam tempoh masa 12 bulan yang lepas? (Sila tandakan (/) pada mana yang berkaitan)

No	Gejala/Tanda Kesihatan	Pernah mengalami gejala/tanda		Berjumpa doktor apabila mengalaminya	
		Ya	Tidak	Ya	Tidak
1	Loya				
2	Muntah				
3	Pening				
4	Berkahak				
5	Sukar bernafas				
6	Batuk				
7	Sakit tekak				
8	Hidung Berair				
9	Sakit Dada				
10	Berdehit				

Parameter Komposisi Badan	Keputusan
Tinggi	m
Berat	kg
Indeks Jisim Badan	kg/m ²
Catatan:	

~ Borang soal selidik tamat ~

Terima kasih atas kerjasama yang telah anda berikan.

Adakah anda ingin mengetahui keputusan kajian ini. Jika ya, sila isikan maklumat berikut untuk makluman tentang keputusan kajian.

Ya

Tidak

Nama:

Alamat surat menyurat:

No. Telefon:



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