UNIVERSITI PUTRA MALAYSIA

OCCURRENCE OF FIPRONIL, IMIDACLOPRID AND PRETILACHLOR IN BLOOD SERUM SAMPLES AMONG PADDY FARMERS IN TANJUNG KARANG, SELANGOR

NURSARAH BINTI MUHAMMAD ANWAR

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BY

NURSARAH BINTI MUHAMMAD ANWAR

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

OCCURRENCE OF FIPRONIL, IMIDACLOPRID AND PRETILACHLOR IN BLOOD SERUM SAMPLES AMONG PADDY FARMERS IN TANJUNG KARANG, SELANGOR

NURSARAH BINTI MUHAMMAD ANWAR

Introduction: Nowadays, the demand of rice has increased. To sustain the continual supply of rice, farmers has been using pesticides due to its effectiveness of generating higher yields and product quality. However, the occupational exposure to the farmers are a concern as it can result in acute or chronic health effects. **Objectives:** The aim of this study was to quantify the concentration of the commonly used pesticide (fipronil, imidacloprid and pretilachlor) in blood serum sample and associate the concentration of the pesticide with the reported respiratory health symptoms among paddy farmers. **Methodology:** A cross-sectional study was carried out in Tanjung Karang, Selangor. Twenty-nine questionnaires were given to the farmers to obtain the self-reported respiratory health symptoms while blood serum samples were taken to analyze the concentration of pesticides. The sample was extracted using QuEChERS method (quick, easy, cheap, effective, rugged, and safe) then analyzed using the Ultra High Performance Liquid chromatography-mass spectrometry (UHPLC-MS/MS). Logistic regression was also used to identify the association of the compounds with the self-reported respiratory health symptoms. **Results and Discussion:** The study shows that the concentrations of the commonly used pesticide (fipronil, imidacloprid and pretilachlor) were 309.63 $ngmL^{-1}$, 46.34 $ngmL^{-1}$ and 195.97 $ngmL^{-1}$, respectively. As for the reported health symptom, there was no significant association with the concentration of pesticides. There were other contributing factors such as work duration in years, weeks and hours, smoking and education that influenced the self-reported respiratory health symptoms. **Conclusion:** The preliminary findings of this study suggest that occupational exposure of pesticides may lead to occurrence of pesticides in blood serum samples of farmers. 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.

Keywords: pesticides, blood serum sample, ultra-high performance liquid chromatography-mass spectrometry (UHPLC-MS/MS), QuEChERS method (quick, easy, cheap, effective rugged, and safe)

ABSTRAK

KEJADIAN FIPRONIL, IMIDAKLOPRID AND PRETILACHLOR DALAM SAMPEL DARAH SERUM DALAM KALANGAN PESAWAH PADI DI TANJUNG KARANG

NURSARAH BINTI MUHAMMAD ANWAR

Pengenalan: Pada masa kini, permintaan beras telah meningkat. Untuk mengekalkan bekalan berterusan padi, petani telah menggunakan racun perosak kerana keberkesanannya menjana hasil yang lebih tinggi dan kualiti produk. Walau bagaimanapun, pendedahan pekerjaan kepada petani adalah satu kebimbangan kerana ia boleh menyebabkan kesan kesihatan yang akut atau kronik. **Objektif:** Tujuan kajian ini adalah untuk mengukur kepekatan racun perosak yang biasa digunakan (fipronil, imidacloprid dan pretilachlor) dalam sampel serum darah dalam kalangan petani dan juga mengaitkan kepekatan racun perosak tersebut dengan gejala kesihatan yang dilaporkan. **Metodologi:** Satu kajian keratan rentas telah dijalankan di Tanjung Karang, Selangor. Dua puluh sembilan borang soal selidik telah diberikan bagi melaporkan kesihatan melaporkan gejala manakala sampel serum darah diambil untuk menganalisis kepekatan sebatian racun perosak. Sampel diekstrak mengunakan kaedah 'QuEChERS' dan kemudiannya dianalisis dengan menggunakan ketinggian cecair kromatografi prestasi ultra-tinggi seiring spektrometri jisim (UHPLC-MS / MS). Analisis logistic regresi juga digunakan untuk mengenal pasti korelasi sebatian dengan gejala kesihatan yang dilaporkan. **Keputusan dan Perbincangan:** Kajian menunjukkan kepekatan racun perosak yang biasa digunakan (fipronil, imidacloprid dan pretilachlor) adalah 309.63 $ngmL^{-1}$, 46.34 $ngmL^{-1}$ dan 195.97 $ngmL^{-1}$. Bagi gejala kesihatan yang dilaporkan, tiada kaitan yang ketara terhadap kepekatan racun perosak. Terdapat juga factor penyumbang yang lain seperti tempoh pekerjaan dalam tahun, minggu dan jam, merokok dan tahap pembelajaran yang mempengaruhi gejala kesihatan yang dilaporkan. **Kesimpulan:** Dapatan awal mencadangkan bahawa pendedahan pekerjaan kepada racun perosak boleh menyebabkan kehadiran racun di dalam serum darah. Memandangkan racun perosak hadir dalam serum darah secara nyata, kajian yang lebih mendalam diperlukan untuk megukur kesan racun serangga terhadap kesihatan selain daripada gejala kesihatan yang dilaporkan.

Kata kunci: Racun serangga, sampel darah serum, ketinggian cecair kromatografi prestasi ultra-tinggi seiring spektrometri jisim (UHPLC-MS / MS), kaedah QuEChERS (laju, senang, murah, efektif, lasak, dan selamat)

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LIST OF ABBREVATIONS

DID	Drainage and Irrigation Malaysia
MADA	Muda Agricultural Development Authority
GDP	Gross Domestic Product
EPA	Environmental Protection Agency
AHS	Agriculture Health Study
PPE	Personal Protective Equipment
MREC	Medical Research and Ethics Committee
HCL	Hydrochloric Acid
US EPA	United State Environmental Protection Agency
LCMS	Hazard Quotient
ESI	Electrospray Ionization
SPSS	Statistical Package for Social Science
DCM	Dichloromethane
TDI	Tolerable daily intake
RfD	Reference Dose
IS	Internal Standard
RRF	Relative Response Factors
JKEUPM	University Research Ethics Committee of University Putra Malaysia, Selangor, Malaysia
UHPLC	Ultra-High performance or pressure liquid chromatography
WHO	World Health Organisation
HPLC	High performance or pressure liquid chromatography
C.E	Collision energy
MRM	Multiple reaction monitoring
LOD	Limit of detection

LOQ	Limit of quantification

CHAPTER 1

INTRODUCTION

1.1 Research background

Traditionally, Malaysia is a rice-growing country. Agriculture sector is one of the sectors that contribute to Malaysia's economy with 7.3% to the Gross Domestic Product (GDP) which showed an decrease from 2017 (Department of Statistics Malaysia, 2019). Rice is the staple food for almost half of the world's population and Asia is the biggest contributor to the production of rice. Furthermore, paddy or we call as rice, is the second largest planted crop in Malaysia after oil palm and also a staple food for Malaysia. In 2018, paddy had an increase in production of 2.7% from 2570.5 thousand tonnes to 2639.9 thousand tonnes compare to 2018 (Department of Statistics Malaysia, 2019).

Therefore, to sustain the production of paddy, the usage of pesticides is used. This is because although agriculture has begun to generate higher yields, it has been damaged by pest and diseases. 70% of agriculture products can be lost without the usage of agrochemicals due to around 67,000 species of organisms that can destroy agriculture crops (Archana Singh, 2014). On top of that, pesticides are used in agriculture production because of its ability to not only prevent but also control pests, diseases, weeds, and other plant pathogen as one of the initiative to minimize the losses

in agriculture as well as to produce high quality crops (Damalas & Eleftherohorinos, 2011).

However, the usage of agrochemicals can cause a lot of issues such as environmental damage, toxicity to humans as well as lower effectiveness of chemicals due to pest resistance. This worsen due to the poor knowledge of farmers on how to use the pesticides, the ignorance of using protective measure during agriculture activities and also the lack of awareness of the farmers regarding the health risk development (Arafa et al., 2013; Khan & Damalas, 2015). All these factors can lead to higher pesticides exposure (Arafa et al., 2013; Khan & Damalas, 2015).

Pesticides are divided into several types which are insecticides, fungicides, herbicides, rodenticides and molluscides. Insecticides function in terminating insects as well as other arthropods while fungicides kill fungi including blights, mildews, molds, and rusts. On top of that, herbicides are used to destroy weeds and other plants that grow in places where they should not while rodenticides have the ability to control the number of mice and other rodents such as cockroaches. Lastly, molluscicides inhibit or terminate mollusc's example snails that usually disturb growth of plants or crops (R. Kaur et al., 2019). In Malaysia, rice farmers are given subsidies for pesticides to increase productivity as well as to increase their life status. Tanjung Karang is one the location where paddy is being grew thus, they use a lot of synthetic pesticides including herbicides, fungicides and insecticides. The pesticides was selected based on a study by Hamsan et al. (2018) in which there were 13 compounds that are most applied in Tanjung Karang.

1.2 Problem Statement

Day by day, the food demand in Malaysia has increases. Thus, the traditional system in vegetable's plantation which do not use the chemicals input cannot fulfil the large demand (Mispan et al., 2015). Therefore, synthetic pesticides have been widely used due to its availability, simplicity in application, efficacy and economic returns (Bedi et al., 2015). However, a study has reported that only a small amount of pesticides which is less than 0.1% has been absorbed by the plant while the rest of it were dispersed into the air or environment. (Baharuddin et al., 2011).

People who works with pesticide are all at risk to the pesticide residues during mixing, handling and spraying of the pesticide. The exposure can happen through various ways which are inhalation, ingestion and skin contact. A study find out that, the usage of Personal Protective Equipment (PPE) during spraying activity is able to significantly reduce the level of inhalation exposure in farmers (Baharuddin et al., 2011). Furthermore, a study has shown in which the majority of the problem of farmers in Tanjung Karang is the improper usage of the PPE (Hamsan et al., 2018). Therefore, biological samples of farmers from the same study area was collected for analysis.

Thus, pesticide poses a big risk not only to its target but also to the environment including humans. Farmers that are directly involve with the usage of pesticides are exposed to various occupational disease brings a huge concern. In a study by Gangemi et al. (2016), in which he evaluated the effects pesticides on human health, mainly chronic disease. He found the relationship between pesticides with

some chronic diseases such as diabetes mellitus, neurological disease, reproductive disorder and also cancer. Some of the pesticides used as a basis for agricultural health studies in other countries cannot be used in Malaysia because of the differences in local management and also the paddy disease pattern. The target compounds under this study were selected according to their popularity among paddy farmers in Tanjung Karang. Thirteen commonly used pesticides were identified upon interviewing a number of farmers. The total number of target pesticides are 13 which are difenoconazole, fipronil, imidacloprid, trifloxystrobin, tebuconazole, azoxystrobin, chlorantraniliprole, pymetrozine, propiconazole, pretilachlor, isoprothiolane, buprofezin, tricyclazole and difenoconazole. On top of that, out of the 13 compounds, it is said by the study of Hamsan et al. (2018), these concentration were the factors that contribute to the self-reported health symptoms in that study.

1.3 Study Justification

Agriculture sector uses all sorts of pesticides to ensure its productivity as well to reduce loss during plantation. However, farmers face adverse health impacts arising from the usage of it such as respiratory problems as the main route of exposure is inhalation. There are plenty of studies related to pesticides in the environment but not personally to a human.

Most of the previous studies done involving farmers used hair or urine sample as the biological sample (Béranger et al., 2020; A. F. Hernández et al., 2019; Jouyban et al., 2019). However, a study by A. F. Hernández et al. (2019), he found out hair that is a better indicator than urine as the process of collecting, handling and

storing is much more easier as well as being able to measure the overall exposure. There is also a study regarding pesticide residue in food (Musarurwa et al., 2019). However, the process of analyzing pesticides in food is difficult due to the low concentrations of pesticides and the fact that certain food has complex matrix (Bordin et al., 2017). As for blood samples, it is preferred as biological sample because blood is a regulated fluid in which water, or any other substance can interfere with the volume. It is easier as no additional steps such as dilution needed to determine the concentration of the pesticide which are in contrast with urine and saliva (Barr et al., 2002). Therefore, in this study, blood is taken as an indicator to measure the concentration of pesticides among farmers.

Besides that, previous study by Hamsan et al. (2018), she found fipronil, pretilachlor and imidacloprid in personal air samplers among farmers in Tanjung Karang and only measured the health symptoms. In her study, she found out that concentration of fipronil and pretilachlor were contributing factors that lead to the reported health symptoms. Hence, this study was carried out in depth to determine the concentration of pesticides in blood serum samples of farmers with addition of studying the respiratory reported health symptoms by the farmers. Blood is taken from farmers as pesticides present in blood in the form of parent compound rather than its metabolite such us urine (Wessels et al., 2003).

Therefore, this study aims to determine the concentration of pesticides (fipronil, imidacloprid and pretilachlor) in blood serum and the reported health symptoms among paddy in Tanjung Karang.

1.4 Research Questions

1. What is the concentration of fipronil, imidacloprid and pretilachlor in blood serum samples among paddy farmers?

2. What are the self-reported health symptoms among paddy farmers?

3. Is there any association between the concentration of pesticides in blood serum samples with the self-reported health symptoms among paddy farmers?

4. What are the contributing factors that can affect the self-reported health symptoms?

1.5 Research Objectives

1.5.1 General objective

To determine the concentration of fipronil, imidacloprid and pretilachlor in blood serum samples among paddy farmers and the self-reported health symptoms in Tanjung Karang, Selangor.

1.5.2 Specific objectives:

1. To quantify the concentration of fipronil, imidacloprid and pretilachlor in blood serum samples among paddy farmers in Tanjung Karang, Selangor.

2. To determine the self-reported health symptoms among paddy farmers in Tanjung Karang.

3. To determine the association between the concentration of pesticides in blood serum samples with the self-reported health symptoms among paddy farmers in Tanjung Karang.

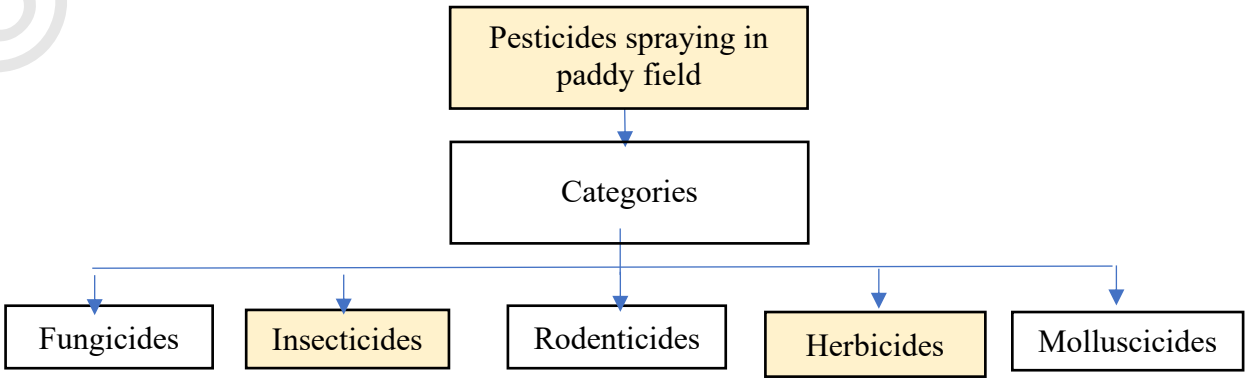
4. To determine the contributing factors that affect the self-reported health symptoms.

1.6 Research Hypothesis

1. The concentration of pesticides in blood serum samples are significantly associated with the self-reported health symptom among paddy in Tanjung Karang.

2. The contributing factors that affect the self-reported health symptoms were identified.

1.7 Conceptual Framework



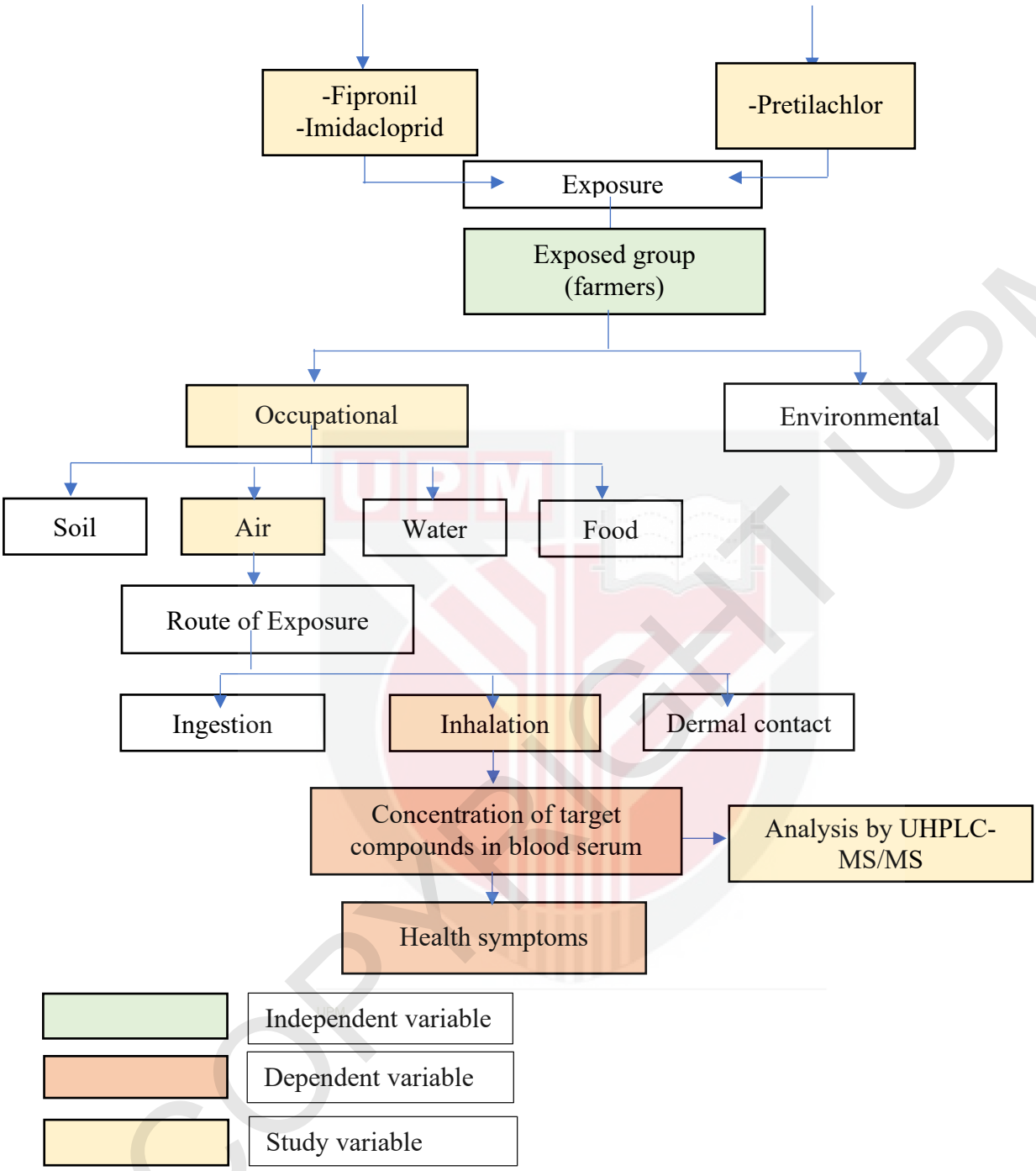


Figure 1.1: Conceptual Framework

CHAPTER 2

LITERATURE REVIEW

2.1 Pesticides used in Agriculture

Agriculture sector is one of the sectors that contribute to Malaysia's economy with 8.2% to the Gross Domestic Product (GDP) which showed an increase from 2016 (Department of Statistics Malaysia, 2019). Paddy field is the second contributor to Malaysia's agriculture after oil palm. In order to sustain the agriculture sector in Malaysia, the usage of pesticides, fertilizers are very common. Paddy fields require more chemical usage such as pesticides and fertilizers to grow healthier compared to other crops as stated by Mohamed et al., (2016).

The main concern of agriculture activities is the usage of pesticides. Without a doubt, pesticides have been widely used all around the world. Low and middle outcome countries tend to over produce crops which are aided with the usage of synthetic pesticides can later cause violation of the usage of it (Ecobichon, 2001; Schreinemachers & Tipraqsa, 2012). The increase of usage due high demand of food can cause negative impacts not only to the environment as well to human health. These exposure to pesticides can lead to either short or long term health impacts (Sutris et al., 2016).

2.2 Effects of Pesticide to Human Health

Nowadays, people around the world are more aware about their health and quality of food. One of the major concerns are pesticide residues in food (Somasundram et al., 2016). In Tanjung Karang, pesticides are used by the paddy

farmers to control pests from invading the crops due to limited knowledge about the health effects from the usage of it (Hamsan et al., 2018). However too much usage of pesticides can give negative impacts not only to human health but also to the environment. According to (Hamsan et al., 2018), pretilachlor is one of the compounds that could affect most of the self-reported respiratory health symptoms.

Besides pesticides residue in food, studies reported the residues of pesticides in water and also soil (Abdullah, M. P.; Nabhan, K. J.; Al-Qaim, F. F.; Ishak, A.; Othman, M. R.; Afiq, W, 2017; Dharumarajan et al., 2011). Although these studies show the concentration of pesticides are not exceeding the limit, there are still concern regarding consumer's safety.

On top of that, biological monitoring has always been a useful tool to access exposure to pesticides such as human blood, urine or tissues. However, there are problems in using biomarkers because they are relatively low concentration and the complexity of some sample matrices such as urine, sample and whole blood. Hence, it is important that a reliable analytical method is followed to get accurate measurement (F. Hernández et al., 2002).

To determine pesticides in blood, serum is a better option than whole blood as whole blood has a major complexity of the matrix while serum is a better homogenous material (Gill et al., 1996). On top of that, the analysis of whole blood takes more procedures and need one or more clean up steps. Therefore, serum is a better biological marker than whole blood. On top of that, blood is a good indicator as

pesticides present in blood in the form of parent compound rather than its metabolite such us urine (Wessels et al., 2003).

2.3 Properties of Target Compounds

2.3.1 Fipronil

Fipronil is from the group of pyrazoles and is also a systemic insecticide. It has been widely used in vegetables, fruits and soil to not only destroy pests but also to increase yield production. Fipronil also serves as a reversible inhibitor for the y-aminobutyric acid receptor and the inotropic chloride channels that is activated by glutamate (Nicodemo et al., 2014). It is proven to be very effective in controlling pests. However, it's long persistence characteristics is a concern (Cravedi et al., 2013). Moreover, Fipronil also has the capability of binding to lipid rich matrices and later bioaccumulate through the food chain (Li et al., 2020). On top of that, it was found out that the metabolites of Fipronil can be more toxic that its parent. (Qu et al., 2016). Since then, Fipronil has been banned in China since 2009 to the risk it poses (Liang et al., 2019). Although most of the studies are been done on animals rather than human, Fipronil has potential hazard to human health and should be a concern (Duhan et al., 2015). Based on a study by Pes et al. (2010), the possible health effects of Fipronil are endocrine disruption, possible carcinogen, neurotoxic and reproductive effects. The other concern of fipronil is that when it is absorbed, it will be metabolized very fast and its' residues will be distributed to tissues especially the fatty tissues. A study has reported that Fipronil has changes the process of adipogenesis which causes the increment of lipids (Sun et al., 2016). In addition to that, the half-life of fipronil varies due to type of soil as well as the pH and other factors. Fipronil has a long half-

life which is 150-245 hours in the blood of a human being. The detailed information of fipronil is summarized in **Table 2.1**.

2.3.2 Imidacloprid

Imidacloprid is a representative of neonicotinoid compound which lives in the similar family to the tobacco chemical which is commonly used in agriculture. In animals, imidacloprid disturbs the transmission of the stimuli of the nervous system which later will cause permanent blockage in the receptors. This will later lead to overabundance of acetylcholine which later causes the stunt of the insect and death (Pes et al., 2014). In order to control sucking insects such as bees and termites, imidacloprid has been widely used (Quistad, 2004). On top of that, the degraded compound of imidacloprid poses a higher risk than the parent. However, as for now, imidacloprid has not been banned and fits in the moderately toxic category of insects by the Environmental Protection Agency (EPA). A study showed that the usage of imidacloprid has increased the loss of health and disease burden due to the consumption of residual pesticides (Qamar et al., 2017). Furthermore, based on a study by Pes et al. (2014), imidacloprid has been linked to several health effects such as reproductive, mutagenic and neurotoxic. A case study in India reported Imidacloprid poisoning in which the patient faced respiratory failure after consuming 200 ml of imidacloprid compound (Becker et al., 2018). Similar to the two other compounds in this study, imidacloprid has different half-life depending on what type of surface. Imidacloprid has a higher half-life in soil

rather than water. The half-life for imidacloprid in humans is still limited. The detailed information of imidacloprid is summarized in **Table 2.1**.

2.3.3 Pretilachlor

Pretilachlor is a commonly used herbicide that is used to control the nuisance weeds in rice fields (Wei et al., 2011). Based on Chemblink CAS data base 51218-49-6, pretilachlor is reported to be moderately toxic to human however to aquatic organism, it is very toxic as it can cause long term effects. Even though there are limited studies related to the toxic effects caused by pretilachlor, the usage of herbicides has a price as most of the herbicides accumulate in surface water and also contaminated diet which can later effect human population. There is a study in which pretilachlor is measure in a zebra fish embryo development. In that study, results show that pretilachlor can disturb the endocrine disruption, oxidative stress, apoptosis and immune pathways in a controlled condition (Zahan et al., 2018). Another study by Soni & Verma (2018) where the found out that pretilachlor can be toxic to fishes and should be reduced the usage of it. However, the effect of pretilachlor on humans are still limited until now. Pretilachlor's fate in the environment varies for soil and water. In a study by (Dharumarajan et al., 2011), he found out that the half-life for soil and water were different. The half-life for pretilachlor in soil is 4-10 days and 1-2 days for water. The detailed information of pretilachlor is summarized in **Table 2.1**.

Table 2.1: Information of the target compounds.

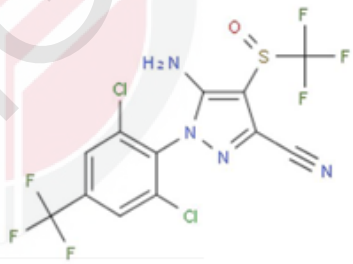
Common Name[a] Pesticide Type[a] Chemical Class[b] IUPAC Name[a] CAS Number[a]	Half-life in human body[e]	Molecular Formula[a]	Molecular Structure[b]	Molecular Weight ($g\ mol^{-1}$)[a]	Log Kow[a]	Cancer Classification[c]
Fipronil, Insecticide, Pyrazole, 5-amino-1-[2,6-dichloro- 4-(trifluoromethyl)phenyl]- 4-(trifluoromethylsulfinyl) pyrazole-3-carbonitrile, 120068-37-3	150-245 hours	$C_{12}H_4C_{l2}F_6N_4OS$		437.141 $gmol^{-1}$	4.0	Group C Possible Human Carcinogen

Table 2.1: Continued…

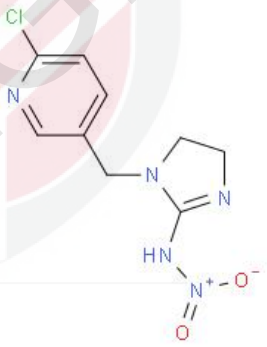
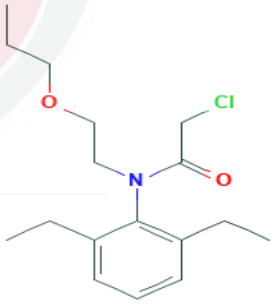
Common Name[a] Pesticide Type[a] Chemical Class[b] IUPAC Name[a] CAS Number[a]	Half-life in human body[e]	Molecular Formula[a]	Molecular Structure[b]	Molecular Weight (g mol^{-1})[a]	Log Kow[a]	Cancer Classification[c]
Imidacloprid, Insecticide, Imidazolidine, N-[1-[(6-chloropyridin- 3-yl)methyl]-4,5-dihydroimidazol-2-yl]nitramide, 138261-41-3	N/A	$C_9H_{10}ClN_5O_2$		255.662 $gmol^{-1}$	0.57 at 21°C	Group E Evidence of Non-Carcinogenicity for Humans

Table 2.1: Continued...

Common Name[a] Pesticide Type[a] Chemical Class[b] IUPAC Name[a] CAS Number[a]	Half-life in human body[e]	Molecular Formula[a]	Molecular Structure[b]	Molecular Weight (g mol^{-1})[a]	Log Kow[a]	Cancer Classification[c]
Pretilachlor, Herbicide, Amide, 2-Chloro-N-(2,6- diethylphenyl)-N-(2- propoxyethyl)acetamide, 51218-49-6	N/A	$C_{17}H_{26}ClNO_2$		311.85 $gmol^{-1}$	N/A	N/A

[a]Pubchem, 2017. National Center for Biotechnology Information (NCBI). https://pubchem.ncbi.nlm.nih.gov/ (accessed October 2018)

[b]Chemspider, 2017. Royal Society of Chemistry. http://www.chemspider.com/ (accessed October 2018)

[c]USEPA (2017). Chemicals Evaluated for Carcinogenic Potential (Annual Cancer Report 2017). http://npic.orst.edu/chemicals_evaluated.pdf (accessed October 2018)

[d]Data were not available.

[e]Pes, B., Pes, B., Pes, B., Pes, B., Sta, A., & Protec, E. (2010). *Chemical Watch Factsheet Glyphosate*. 6–9.

2.4 Socio-demographic Backgrounds, Lifestyle and Human Health

Sociodemographic backgrounds such as age and level of education as well as lifestyle (smoking status, consumption of vegetables and fruits, physical inactivity) are some of the factors that can affect human health.

According to J. Kaur et al., (2015), physical inactivity which a risk factor to mortality, increases with age as well level of education. In this study, with the increase of age as well as no education, have led to the decrease of physical activity. This shows that education as well as age plays a role in determining health.

Smoking has been a leading cause of lung cancer with 90% following chronic bronchitis and emphysema (75%) (Longstreth et al., 1998). This shows that smoking is an important factor leading to deterioration to human health. According to Geneva: World Healh Organization, (2018) there are about 30% of the male population that smokes globally and if this trend continues, it will cause the number of smokers to increase to one in six people by 2030. In conjunction to Sustainable Development Goal (SDG) number 3 which is to ensure healthy lives and promote well-being for all ages it is important that smoking is taken seriously by all.

On top of that, the recommended exercise for adults is 150 minutes in a week of moderate intensity exercise (Shahid & Shahid, 2016). Based on WHO (2018), physical inactivity is in fourth place of leading risk factor for mortality globally while number one in Malaysia.

Based on Malaysian Dietary Guidelines, the recommended serving for adolescents and children is at least 3 serving of vegetables and 2 servings of fruits. Previous studies has proven that the sufficient intake of fruits and vegetables can protect against cancer, cardiovascular diseases and diabetes. (Ministry of Health, 2013)

Therefore, it shows that all these factors such as socio-demographic background (age and level of education) and lifestyles (consumption of fruit, vegetables, physical inactivity and smoking status) may affect the results of the self-reported symptoms.

2.5 Legislation and regulation related to pesticide in Malaysia

Every county has its own law regarding the usage of pesticides to ensure that the usage of pesticide is under control. Food and Agriculture Organization (FAO) of the United Nations and also World Health Organization (WHO) together will some other organization has join forces to develop International Code of Conduct on Pesticide Management, Guidelines on Pesticide Legislation (FAO/WHO, 2015). This shows that the usage of pesticides is taken very seriously all around the world. In Malaysia, to control and manage the usage of pesticides, Pesticides Act 1974 has been used. this act comprises of 11 parts which include control of importation and manufacture by registration and permit, control of manufacture, sale and as well as storage, enforcement and many more (Act 149 Laws of Malaysia, 2015).

On top that, there are also other legislation that governs the issue of pesticide such as Poison Act 1952, Food Act 1983, Environmental and Quality Act 1974, Occupational Safety and Health Act 1994 and many others. Although there is a broad scope of laws and regulation that governs pesticide, there are still problems that arise from mismanagement of the pesticides (Ali & Shaari, 2015).

2.6 Personal Protective Equipment

Pesticide is a common usage in agriculture and almost avoidable. However, the usage of pesticides has impact to human as well as the environment. Therefore, to minimize the impact on human health, it is important that farmers use the proper personal protective equipment (PPE). This is to ensure, acute along with chronic poisoning from pesticides can be avoided. (Yarpuz-Bozdogan, 2018).

Pesticides enter the human body by various ways. Inhalation, dermal and oral are the main routes of exposure. In a study by Matthews (2006) it stated that the farmers during spraying the pesticides with either fog, aerosol will have to wear a mask as it will later accumulate in the lung as well as the breathing tube. On top of that, according to Malaysian Standard Code of Recommended Practice (MS479:2012) developed by the Department of Standards Malaysia and SIRIM Berhad (DOS), it stated that the process of preparation (mixing and loading) and also application (spraying) needs attention from farmers to wear appropriate protective clothing according to the label. Recent study by Baharuddin et al. (2011) find out that even though pesticide exposure by inhalation has a relationship with wind speed, usage of PPE during spraying activity can reduce significantly the level of pesticide.

CHAPTER 3

METHODOLOGY

3.1 Study Design

This study is a cross-sectional study in which to access pesticides exposure in blood serum among paddy farmers in Tanjung Karang, Selangor.

3.2 Chemicals and standards

Ammonium formate (10 M in H_2O) 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 ($MgSO_4$) 99% were purchased from R&M Chemicals (Malaysia).

3.3 Study Location

This study was conducted at Tanjung Karang, Selangor which is known for its agriculture in paddy fields. Figure 3.1 shows the location of the study. The study location was selected based on some few criteria which are as below:

i) Agriculture activity is rice cultivation. Tanjung Karang is the number one largest area of paddy field in Peninsular Malaysia which is also known as 'the rice bowl of Selangor'

ii) Respondent were self-willing to give information and cooperation throughout the study

iii) The distance of location from the laboratory is near in which preservation and analysis was conducted

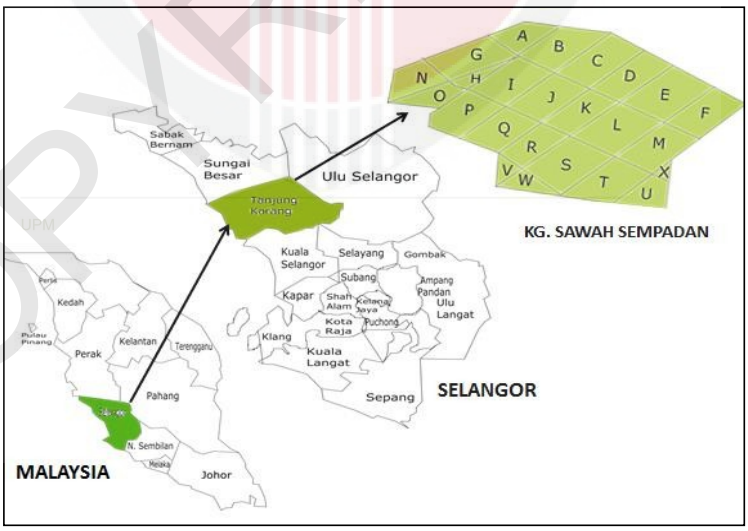


Figure 3.1:Location of Sampling Site, Kampung Sawah Sempadan, Tanjung Karang, Selangor

3.4 Sampling Population

The sample frame of this study consists of paddy farmer in Kampung Sawah Sempadan, Tanjung Karang. The study population were among adult male farmers of Kampung Sawah Sempadan, Tanjung Karang. Respondent were chosen based on the inclusion and exclusion criteria. The inclusion and exclusion criteria of the study were included below.

Inclusion criteria

i) Farmers who worked 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

ii) Farmers that have been diagnosed with cancer

3.5 Confounder

3.5.1 Lifestyle

I. People who are active smokers

II. People who rarely exercise

III. People who rarely consume vegetables and fruits

3.5.2 Socio-demographic background

I. Education level

II. People who are older

3.6 Sample Size Calculation

The sample size calculation was determined by Ogston et al., (1991), for group comparison between two groups. The formula for calculating sample size is shown below:

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

(Eq 1)

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 = $\frac{P1+P2}{2}$, Q = 1-P

Q_1 = 1-P_1 and Q_2 = 1-P_2

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

$$n = 71.5 \sim 71$$

Based on the sample size calculation, the total number of study population is 71. The sample size was then increased by 20% taking account of non-responsive respondents, missing data and errors. The final total number of sample size is 86. This population size shows the strength of study for this research. However, due to the recent pandemic and also machine breakdown, only 29 samples from exposed group was able to be analyzed.

3.7 Sampling Method

The farmers who fit the inclusion criteria were randomly recruited before the activity of spraying pesticide is done. They were required to answer the questionnaire and travel to Klinik Kesihatan Sungai Tengi Kanan for blood sampling.

3.8 Study Procedure

3.8.1 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 et al., (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). The questionnaire used is attached in the appendix.

This questionnaire was 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 helped to estimate the time taken for each respondent to answer the questions. The number of respondents that were involved in the pre-testing is 10% from the total sample size of this study. Socio-demographic of the respondents chosen in the pre-testing are similar to the characteristics of the respondents of the study.

3.8.2 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 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 venipuncture 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 heparinized glass vials by using sterilized syringe. Later, the blood samples were transported to the laboratory with dry ice and stored at -80 ºC until analysis.

3.9 Sample Extraction and Analysis

3.9.1 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 ($MgSO_4$) and 10 mg of sodium chloride (NACL) were added into the sample under ice bath condition to avoid heating from the $MgSO_4$. After that, the sample was centrifuged for 5 mins at 13000 rpm using Sartorius Sigma 1–14 Microcentrifuge (Sartorius Stedim, Epson, 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 (H_2O: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.

3.9.2UHPLC-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 gradient conditions as in **Table 3.1.** 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 3.2**. 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 3 compounds and 1 internal standard

Target compounds	Precursor ion, mz^{-1}	Product, mz^{-1} (collision energy, V)
Fipronil	435.00	330.00 (12)
		250.00 (28)
Imidacloprid	256.10	209.00 (13)
		175.10 (13)
Pretilachlor	312.17	252.10 (17)
		176.10 (29)
Internal standards	Precursor ion, mz^{-1}	Product, mz^{-1} (collision energy, V)
Imidacloprid-d_4	260.10	212.90 (21)

3.9.3 Statistical analysis

Questionnaire response tabulation and graphical summarizes was completed with Microsoft Excel 2010, while statistical analysis was analyzed by using Statistical Package for Social Science (SPSS) Version 25.0. The descriptive test is used to calculate mean, median, mode and standard deviation. Meanwhile, to ensure the normality of the data, Kolmogrov Smirnov test was used. The tests used to analyse the objectives in the study are stated as below.

Objective 1: To quantify the concentration fipronil, imidacloprid and pretilachlor in blood serum samples among paddy farmers in Tanjung Karang, Selangor.

Statistical analysis: Descriptive analysis

Objective 2: To determine the self-reported health symptoms among paddy farmers in Tanjung Karang, Selangor.

Statistical analysis: Descriptive analysis

Objective 3: To determine the association between the concentration of pesticides in blood serum samples and the self-reported health symptoms among paddy farmers in Tanjung Karang, Selangor.

Statistical analysis: Simple logistic regression

Objective 4: To determine the contributing factors that affect the self-reported health symptoms.

Statistical analysis: Linear Regression

3.10 Quality control

3.10.1 Cleaning of glassware

Cleaning of glassware was conducted according to US EPA method 1699. All glassware were soaked overnight with Decon 90. After that the glassware was rinsed with methanol continued with hot tap water. The glassware was rinse again with methanol, followed by acetone and then dichloromethane (DCM). After all washing process finished, all the glassware were dried at 60^0C and capped with solvent rinsed aluminium foil.

3.10.2 Lipid Analysis

Lipid analysis was also done due to the characteristic of the compounds which are distributed in fat and fatty tissue. The total serum lipids were calculated based on Han et al. (2020).

$$Total\ lipid\ (TLs): 2.27 \times total\ cholesterol + triglyerides + 0.623$$

(Eq 2)

3.10.3 Calibration of UHPLC-MS/MS

UHPLC-MS/MS was calibrated to ensure the validity and reliability of pesticides analysed. The instrument was calibrated with each analytes at a five-point calibration curve at a range of 0.1 to 500 ng mL^{-1}. At a concentration of 50 ng mL^{-1} of each calibration point, labelled internal standard (ISs), imidacloprid-d_4 corresponding to the analyte was added to generate relative response factors (RRF).

3.10.4 Method blank

To ensure the overall method performance for the quality control, the samples were spiked with internal standard (IS) mixture before extraction. The mixture of IS was spiked into one blank sample and continued with the whole sample preparation as well the extraction procedure to identify any possible background contamination in the samples. Imidacloprid-d_4 was used as internal standard to quantify the concentration of the target compounds in the study.

3.10.5 Extraction recovery

The extraction recovery was determined in order to validate the analytical procedure by spiking the samples with varied amount of standard solution of each compound (Zhuang et al., 2009). The percentage for recovery was then calculated based on EPA (2007).

$$\text{Recovery (\%)} = \frac{Concentration\ found\ (ng/mL)}{Concentration\ spiiked\ (ng/mL)} \text{ x } 100$$

(Eq 3)

3.10.6 Method validation

The mixture of pesticide as well with internal standard were spiked into the blank sample to determine the limit of detection (LOD) and limit of quantification (LOQ). Extraction and analysis of sample was then followed as described in 3.9.1. Determination of LOD and LOQ uses with signal-to-noise ratio of >3 and >10 (Choi et al., 2013).

3.11 Ethical consideration

University Research Ethics Committee of University Putra Malaysia, Selangor, Malaysia (JKEUPM) has approved this study with its' reference number JKEUPM-2018-357. Respondents that agreed to take part in this study was given information regarding the study as well as a written consent was obtained from each of them before the study took place. All information obtained from the respondents were confidential and used for study purposes only.

CHAPTER 4

RESULTS

4.1 Quality Control

The LOD, LOQ, linear range, coefficient of determination (R^2), mean total lipid and recovery are summarized in **Table 4.1.**

Table 4.1 LOD, LOQ, Linear range, coefficient of determination (R^2), Mean Total Lipid and recovery

Components	LOD ($ngmL^{-1}$)	LOQ ($ngmL^{-1}$)	Linear Range ($ngmL^{-1}$)	R^2	Mean Total Lipid ($mmolL^{-1}$)	Recovery (%)
Fipronil	0.001	0.01	0.01-500	0.9992		90.42
Imidacloprid	0.001	0.01	0.01-500	0.9999	13.88	63.89
Pretilachlor	0.01	0.05	0.01-500	0.9997		69.12

Five-point calibration curves for all target compounds were derived in the range of 0.1-500 $ngmL^{-1}$. The selectivity was excellent as no other interfering peaks was observed during the retention time of all target compounds. All calibration cruves of the target compounds showed good linearity with regression coefficient (R^2) from 0.9992 to 0.9999. The LOD and LOQ for all compounds ranged from 0.01 to 0.001 and 0.01 to 0.05, respectively. The extraction recovery ranged from 64% to 90%.

4.2 Socio-demographic background of respondents

A total of twenty-nine farmers were involved in this study. Their socio-demographic background such as age, gender, race and level of education were obtained from the questionnaire and are summarized in **Table 4.2**

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

Variables	Mean ± SD	Minimum	Maximum
Age (years)	43.97±10.15	27	59
Variables	Category	Frequency	Percentage (%)
Gender	Male	29	100
Race	Malay	29	100
Education level	Primary	5	17.2
	Secondary	24	82.8
Smoking	Yes	15	48.3
	No	14	51.7

All of the respondents that are involved in this study were Malay and male. From **Table 4.2**, the average age for paddy famers were 44 years old with the range of 27 years old to 59 years old. As for the educational level, majority of the paddy farmers have secondary education with (82.8%) and primary education with (17.2%). As for smoking status, the farmers, have almost equal percentage which yes (48.3%) and no (51.7%).

4.3 Concentration of pesticides (fipronil, imidacloprid, pretilachlor) collected for paddy farmers

The concentration of fipronil, imidacloprid and pretilachlor collected in paddy farmers in blood serum samples are summarized in **Table 4.3**.

Table 4.3 Concentrations of target compounds in blood serum sample among paddy farmers ($ngmL^{-1}$) (n=29)

Target compounds	Mean	Standard deviation	Minimum	Maximum	Frequency of detection in blood serum
Fipronil	309.63	362.82	<LOQ	1430.40	28
Imidacloprid	46.34	249.52	<LOQ	1343.73	1
Pretilachlor	195.97	323.38	<LOQ	1449.42	20

LOQ – Limit of quantification

Based on the results summarized in **Table 4.3**, the mean concentration of fipronil in blood serum sample among paddy farmers was 309.63 $ngmL^{-1}$ with the minimum concentration was below LOQ and the maximum concentration was 1430.40 $ngmL^{-1}$. As for the frequency, fipronil was detected in majority of the blood serum samples in farmers which were 28 out of 29 paddy farmers. Next, the mean concentration of imidacloprid was 46.34 $ngmL^{-1}$ with the minimum concentration below LOQ and the maximum concentration was 249.52 $ngmL^{-1}$. Out of 29 farmers, only one of the farmer's serums has detected imidacloprid. Lastly, the mean concentration of pretilachlor was 195.97 $ngmL^{-1}$ with the minimum concentration was below LOQ and the maximum concentration was 1449.42 $ngmL^{-1}$. There were 20 samples that were detected with pretilachlor among 29 samples.

4.4 The Self-Reported Health Symptoms among paddy farmers

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

Table 4.4 The self-reported health symptoms among paddy farmer (n=29)

Health Symptoms Reported	Yes n (%)	No n (%)
Breathing difficulties	8 (27.6)	21 (72.4)
Chest pain	10 (34.5)	19 (65.5)
Cough	10 (34.5)	19 (65.6)
Wheezing	2 (6.9)	27 (93.1)
Sore throat	7 (24.1)	22 (75.9)
Nausea	11 (37.9)	18 (62.1)
Vomiting	8 (27.6)	21 (72.4)
Dizziness	14 (48.3)	15 (51.7)
Runny nose	4 (13.8)	25 (86.2)

Based on the **Table 4.4**, it was found out that dizziness was the most self-reported health symptoms among paddy farmers which is 14 farmers (48.3%) out of 29 farmers followed by nausea (37.9%) and evenly between chest pain and cough (34.5%).

4.5 Association between the concentration of pesticides in blood serum samples and the self-reported health symptoms among paddy farmers

The association between concentrations of pesticides (fipronil, imidacloprid and pretilachlor) and self-reported health symptoms among paddy farmers were summarized in **Table 4.5.**

Table 4.5: Association between concentrations of pesticides (fipronil, imidacloprid and pretilachlor) and self-reported health symptoms

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]
Fipronil	0.065	0.119	0.601	0.127	0.223	0.278	0.805	0.567	0.180
Imidacloprid	0.641	0.662	0.549	0.870	0.762	0.649	0.998	0.998	0.997
Pretilachlor	0.096	0.086	0.527	0.072	0.250	0.212	0.778	0.610	0.291

[a]Breathing difficulty, [b]Chest pain, [c]Cough, [d]Wheezing, [e]Sore throat, [f]Nausea, [g]Vomitting, [h]Dizziness, [i]Runny nose

Based on the result in **Table 4.5**, there was no significant association between the concentrations of pesticides (fipronil, imidacloprid and pretilachlor) and self-reported health symptoms.

4.6 Contributing factors that affect the self-reported health symptoms

The contributing factors that may influence the self-reported health symptoms can be work duration (years), work duration (weeks), work duration (hours), smoking and education is summarized in **Table 4.6.**

Table 4.6: Contributing factors that affect the self-reported health symptoms

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 (-0.018, -0.152, 0.145, 0.425) respectively has a negative association with the self-reported health symptoms while for smoking (0.238), it is the only factor that has a positive association with the self-reported healthsymptoms.

CHAPTER 5

DISCUSSION

5.1 Socio-demographic background of respondents

Based on the results, all the paddy farmers involved in this study were male and Malay. In a study by Hamsan et al. (2018), she mentioned that the farmers in Tanjung Karang were all male and no female farmers are present. On top of that, a study stated that 90% of the people in Kampung Sawah Sempadan are Malay (Mohd. Fuad et al., 2012). As for the education level, majority of the paddy farmers have secondary education and only a few with primary education. The average age for the paddy farmers is 42 years old which is considered old. This is because older paddy farmers are more experienced while youngsters take on other jobs in the city as it is perceived to give a more payment.

5.2 Concentration of pesticides (fipronil, imidacloprid, pretilachlor) collected for paddy farmers

Among the three target compounds (fipronil, imidacloprid and pretilachlor), the highest mean concentration among the paddy farmers was fipronil (309.63 $ngmL^{-1}$), followed by pretilachlor (195.97 $ngmL^{-1}$) and lastly imidacloprid (46.34 $ngmL^{-1}$). Since this is a novel study, there is no exact standard to verify the concentration of fipronil, imidacloprid and pretilachlor in blood serum. However, there was a study conducted by Chang et al. (2017) in which the pesticides can be detected

in the blood serum with a range from 0.02 to 1423.62 $ngmL^{-1}$. This shows that there is significant amount of fipronil, imidacloprid and pretilachlor in the blood serum sample. On top of that, recently, a study was conducted by Tao et al. (2019) in which the first absorbed daily dose (ADD) for imidacloprid was estimated.

5.3 The Self-Reported Health Symptoms among paddy farmers

The reported health symptoms by paddy farmers were breathing difficulties, chest pain, cough, wheezing, sore throat, nausea, vomiting, dizziness and runny nose. Dizziness (48.3%) was the most reported health symptoms among paddy farmers followed by nausea (37.9%) and evenly between chest pain and cough (34.5%). Damalas & Koutroubas (2016) stated that dizziness is one of the severe symptoms to toxic exposure. The findings were also aligned with a study by Lamichhane et al. (2019), in which dizziness was also the most common reported health problems faced by farmers. Nausea and vomiting were also reported in that study. In a study by da Silva et al. (2016), skin irritation, eye irritation and headache were the most common reported symptoms among the rice farmers which were not aligned with the results of this study. This could be due to the different usage of pesticides such as thiobencarb mixed with propanil (73%) and 2,4-D (13%), followed by the insecticides dimethoate (3%), and endosulfan (1%)

5.4 Association between the concentration of pesticides in blood serum samples and the self-reported health symptoms 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. According to Hamsan et al. (2018), the concentration of fipronil, imidacloprid

and pretilachlor in air was contributing factors that affected the self-reported health symptoms. A study done by Bais (2019) mentioned that pretilachlor are known for its ability to cause oxidative stress on animals which later causes breathing difficulties. This is also similar to imidacloprid in which a study found out that acute exposure to imidacloprid can cause inflammation as well as oxidative stress to carps in Turkey (Özdemir et al., 2018). However, studies related to fipronil, imidacloprid and pretilachlor in humans are still limited.

5.5 Contributing factors that affect the self-reported health symptoms

There are several factors that affect the self-reported symptom such as work duration (years), work duration (weeks), work duration (hours), smoking and education. However, all of the factors are insignificant. Although all of the contributing factors are insignificant, the factors show an association. There is positive association for smoking and negative association such as work duration (years), work duration (weeks), work duration (hours) and education.

For smoking, based on the results, it is said that the more the person smokes, the higher the possibility the person will report the symptoms. This is because, a study by Jayes et al. (2016) states that smoking increases the risk of respiratory disease. Since the symptoms are self-reported, it is possible that the farmers are confused with the symptoms due to pesticide with symptoms due to smoking. The signs and symptoms of smokers such as breathing difficulty can be mistaken. When a person stops to smoke, breathing difficulties is one of the sign that a person faces (West, 2017).

As for the other contributing factors such as the work duration in years, hours and weeks it showed a negative association to the self-reported health symptoms. This may be caused due to the farmers that are used to the work after years and years. In other words, the longer the farmers work and the less possibility that the workers will report the health symptom. On top of that, since the farmers self-report the health symptoms and was not diagnosed by medical practitioner, the information might not be accurate. In a study by Andrianasolo et al. (2018), they reported that adults with a higher education level were prone to have a healthy lifestyle thus reports less asthmatic symptoms.

CHAPTER 6

CONCLUSION AND RECOMMENDATION

6.1 Conclusion

In conclusion, this study showed that the targeted compound of pesticides which are imidacloprid, pretilachlor and fipronil presented in the blood serum sample among the paddy farmers in Kampung Sawah Sempadan. Among the targeted compound, the highest mean concentration was fipronil (309.63 $ngmL^{-1}$), followed by pretilachlor (195.97 $ngmL^{-1}$) and lastly imidacloprid (46.34 $ngmL^{-1}$). Thus, there is significant amount of pesticides in the blood serum sample. Moreover, based on the results, there were no significant association for the reported health symptom. This is because there are several contributing factors such as work duration in years, weeks and hours, smoking and education that influenced the reported health symptoms. The preliminary findings of this study suggest that occupational exposure of pesticides may lead to occurrence of pesticides in blood serum samples of farmers. 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 the study

There are several limitations of this study that should be considered in future research. One of it is recall bias. This is because the reported health symptoms

answered are based on 12 months experiences. On top of that, the reported health symptoms are only accessed based on inhalation exposure only. However, other route of exposure such as dermal and ingestion was not considered during this study. Furthermore, climatological conditions should be considered as it can interfere with the effectiveness of spraying pesticides. For the blood serum sample, it was kept for a long time due to issues of machine. For the improvement of future research, the blood serum samples should be analyzed as soon as possible to avoid any degradation or loss of pesticides in the serum.

6.3 Recommendation

6.3.1 Authorities

Authorities should ensure that farmers that involve directly with the usage of pesticides, should be given training on how to use PPE properly. On top of that, authorities should also take initiative in providing PPE for the farmers and make sure they use the PPE properly.

6.3.2 Farmers

Farmers that handles directly with pesticide should take precaution steps and wear their PPE to protect themselves from the danger of pesticides.

6.3.3 Future Research

In the future, it would be better if the different route of exposure such as dermal and ingestion be considered as there are other possible route of exposure other than inhalation.

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APPENDIX I

APPROVAL LETTER (ETHICS COMMITTEE)

APPENDIX II

QUESTIONNAIRE

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

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 menegah)
☐ 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

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

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

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

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	**Keptusan**
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:
……...