UNIVERSITI PUTRA MALAYSIA

RELATIONSHIP BETWEEN EXPOSURE OF AIRBORNE PESTICIDES WITH CONCENTRATION OF PESTICIDE RESIDUE IN BLOOD SERUM AMONG FARMERS IN TANJUNG KARANG, MALAYSIA

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FPSK(m) 2022 4

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By

SITI KHAIRUNNISAQ BINTI RUDZI

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Master of Science

June 2021

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Master of Science

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Pesticides are used to control pests and diseases, however not all sprayed pesticides will reach target area, hence causing exposure to farmers. To determine the concentration of pesticides in personal air samples, blood serum samples and health symptoms among paddy farmers and non-exposed group. This study was conducted in Tanjung Karang among 85 paddy farmers and 85 non-exposed individuals. Pesticides exposure was monitored using personal air sampler. Sorbents were extracted with acetone while blood serums were extracted with acetonitrile and analysed using ultra-high performance liquid chromatography tandem mass spectromtery (UHPLC-MS/MS). All target pesticides (azoxystrobin, buprofezin, chlorantraniliprole, difenoconazole, fipronil, imidacloprid, isoprothiolane, pretilachlor, propiconazole, pymetrozine, tebuconazole, tricyclazole, trifloxystrobin) were detected in personal air samples of paddy farmers with the highest mean concentration 32.00 ng m^{-3} (chlorantraniliprole); while azoxystrobin, buprofezin, difenoconazole, imidacloprid, pymetrozine, tebuconazole, and tricyclazole were detected among non-exposed group with the highest mean concentration 2.51 ng m^{-3} (imidacloprid). All target pesticides were detected in blood serum of paddy farmers with the highest mean concentration 23.42 ng mL^{-1} (chlorantraniliprole), while buprofezin, difenoconazole, fipronil, imidacloprid, tebuconazole, tricyclazole were detected among non-exposed group with the highest mean concentration 1.47 ng mL^{-1} (difenoconazole). The concentrations of pesticide in personal air (except azoxystrobin and imidacloprid) and blood serum (except tricyclazole) were significantly higher in paddy farmers. The concentrations of all pesticides in personal air were significantly correlated with blood serum of paddy farmers; while for non-exposed group, buprofezin, difenoconazole, imidacloprid, and tricyclazole were significanty correlated. The findings suggested that exposure to pesticides were significantly higher in paddy farmers than non-exposed group. No significant associations between proper use of personal protective equipment (PPE), lipid content, pesticides in personal air and blood serum with health symptoms. Notwithstanding, farmers should improve the use of PPE and maintain safe work practice to avoid long-term health effects.

Keywords: pesticides, inhalation exposure, pesticide sprayers, personal air monitoring, blood serum, UHPLC-MS/MS

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

HUBUNGAN DI ANTARA PENDEDAHAN RACUN PEROSAK DALAM UDARA DAN KANDUNGAN RACUN PEROSAK DALAM SERUM DARAH DALAM KALANGAN PESAWAH DI TANJUNG KARANG, MALAYSIA

Oleh

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Racun perosak digunakan untuk mengawal perosak dan penyakit, namun tidak semua racun yang disembur menepati target, menyebabkan pendedahan kepada pesawah. Untuk menentukan kepekatan racun perosak dalam sampel udara peribadi, sampel serum darah dan gejala kesihatan dalam kalangan pesawah padi dan kumpulan tidak terdedah. Kajian ini dijalankan di Tanjung Karang, melibatkan 85 orang pesawah padi dan 85 orang tidak terdedah. Pendedahan kepada racun perosak dipantau menggunakan pam udara peribadi. Penapis gentian kaca diekstrak dengan aseton manakala serum darah diekstrak dengan asetonitril dan dianalisa menggunakan *ultra-high performance liquid chromatography tandem mass spectromtery* (UHPLC-MS/MS). Kesemua racun perosak (azoxystrobin, buprofezin, chlorantraniliprole, difenoconazole, fipronil, imidacloprid, isoprothiolane, pretilachlor, propiconazole, pymetrozine, tebuconazole, tricyclazole, trifloxystrobin) dikesan dalam sampel udara peribadi pesawah dengan purata kepekatan tertinggi 32.00 ng m^{-3} (chlorantraniliprole); manakala azoxystrobin, buprofezin, difenoconazole, imidacloprid, pymetrozine, tebuconazole, tricyclazole dikesan dalam sampel udara peribadi kumpulan tidak terdedah dengan purata kepekatan tertinggi 2.51 ng m^{-3} (imidacloprid). Semua racun perosak dikesan dalam serum darah pesawah dengan purata kepekatan tertinggi 23.42 ng mL^{-1} (chlorantraniliprole); manakala buprofezin, difenoconazole, fipronil, imidacloprid, tebuconazole, tricyclazole dengan difenoconazole dikesan dalam sampel udara peribadi kumpulan tidak terdedah dengan purata kepekatan tertinggi 1.47 ng mL^{-1} (difenoconazole). Kepekatan racun perosak dalam sampel udara peribadi (kecuali azoxystrobin dan imidacloprid) dan serum darah (kecuali tricyclazole) adalah lebih tinggi dalam pesawah berbanding kumpulan tidak terdedah. Kepekatan racun perosak dalam sampel udara peribadi mempunyai korelasi dengan kepekatan dalam

serum darah kumpulan pesawah; manakala bagi kumpulan tidak terdedah, buprofezin, difenoconazole, imidacloprid, dan tricyclazole mempunyai korelasi.
Hasil kajian mendapati pendedahan kepada racun perosak adalah lebih tinggi dalam kalangan pesawah berbanding kumpulan tidak terdedah. Tiada sebarang hubungan di antara penggunaan alat perlindungan diri, kandungan lipid, kandungan racun perosak dalam sampel udara peribadi dan serum darah dengan gejala kesihatan. Walaubagaimanapun, pesawah perlu meningkatkan penggunaan alat pelindung diri dan mengekalkan cara kerja selamat untuk mengelakkan kesan kesihatan jangka masa panjang.

Kata kunci: racun perosak, pendedahan pernafasan, penyembur racun perosak, pemantauan udara peribadi, serum darah, UHPLC-MS/MS

ACKNOWLEDGEMENT

Foremost, I would like to thank Allah S.W.T, the Almighty God for making it possible for me to go through this journey and face all the hurdles along the way. I believe it is impossible to get through this without His blessings. Besides, the completion of this thesis could not have been possible without the assistance and guidance from my supervisor, Associate Professor Dr. Ho Yu Bin. My sincere gratitude goes to my supervisor for the endless support, motivation, patience, and shared knowledge during years of my study. She has always been helpful and supportive throughout the journey and it is only possible that this research was able to meet the end because of her support.

To my parents, Rudzi Yamat and Wan Aida Rosma Wan Abdullah, I am forever thankful for their love, unconditional support and continuous encouragement throughout my study. My journey in this research was not easy however I am grateful for all great people I met that had helped me to complete this study. I appreciate all the respondents involved in this study for giving me their full co-operation and permission on performing the monitoring and interview.

I also thank University Putra Malaysia for granting me the scholarship (GRF) throughout my study which helps a lot to ease the financial burden.

Lastly, I sincerely thank everyone involved directly or indirectly in the completion of this research.

Thank you.

This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Master of Science. The members of the Supervisory Committee were as follows:

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Declaration by Members of Supervisory Committee

This is to confirm that:

- the research conducted and the writing of this thesis was under our supervision;
- supervision responsibilities as stated in the Universiti Putra Malaysia (Graduate Studies) Rules 2003 (Revision 2012-2013) are adhered to.

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LIST OF ABBREVIATIONS

AUDPC	Areas Under Disease Progress Curve
BMI	Body Mass Index
CAS-No	Chemical Abstract Service Number
CUPs	Currently used pesticides
DCM	Dichloromethane
ESI	Electrospray Ionization
GC	Gas Chromatography
GC-MS	Gas Chromatography-Mass Spectrometry
GHS	Globally Harmonized System
HCl	Hydrochloric Acid
HPLC	High Performance Liquid Chromatography
IADA	Integrated Argricultural Development Area
IDL	Instrumental Detection Limit
IQL	Instrumental Quantification Limit
IS	Internal Standard
JKEUPM	University Research Ethics Committee of University Putra Malaysia
KADA	Kemubu Argricultural Development Authority
LOQ	Limit of Quantification
LC	Liquid chromatography
LC-MS/MS	Liquid chromatography-tandem mass spectrometry
MADA	Muda Argricultural Development Authority
MDL	Method Detection Limit
$MgSO_4$	Magnesium Sulphate

MLR	Multiple Logistic Regression
MQL	Method Quantification Limit
NaCl	Sodium Chloride
OC	Organochlorine
OP	Organophosphate
OR	Odd Ratio
PEL	Permissible Exposure Limit
PPE	Personal Protective Equipment
PUF	Polyurethane Foam
QuEChERS	quick, easy, cheap, effective, rugged, and safe
RSD	Relative Standard Deviation
SD	Standard Deviation
SDS	Safety Data Sheet
SPSS	Statistical Package for the Social Sciences
TG	Triglycerides
TWA	Time-weighted Average
UHPLC-MS/MS	Ultra-high Performance Liquid Chromatography Tandem Mass Spectrometry
USEPA	United State Environmental Protection Agency

CHAPTER 1

INTRODUCTION

1.1 Background of study

Agriculture is one of the vibrant sectors of Malaysia's economy that contribute up to 7.1% to national Gross Domestic Product in 2019 (Department of Statistics Malaysia, 2020). Malaysia's economy has been depending on agriculture over decade as Malaysia's products are exported worldwide. Agriculture, which is often labelled as the poor man's sector has slowly changing its pace towards a large-scale farming. Paddy is the top three widely planted crops in Malaysia following oil palm and rubber. The production of paddy was reported at 2,348.9 thousand tonnes in 2019 (Department of Statistics Malaysia, 2020).

The growing of population in Malaysia has increased the demand for rice consumption. Rice is staple food for Malaysians as they consume it as a cooked rice or rice-based food from rice flour. The traditional system in agriculture which do not use chemicals input cannot fulfil the high demand (Mispan et al., 2015). Therefore, to satisfy this demand, commercial pesticides have been widely used in agriculture because they are easily available, simple to apply, and effective. In addition, pesticides are used to control pests and diseases which pose serious problems in crop production.

Intensive paddy cultivation activities are responsible for the use of synthetic pesticides in Tanjung Karang. Not all sprayed pesticides will reach to the target area and it was estimated that more than 95% of applied pesticides had the potential to disperse in the environment and affect non-target organisms (Farcas et al., 2013). Due to the chemical properties of its compounds and ingredients, pesticides can penetrate into air, soil, water, and eventually contaminating yields.

Pesticides can be acutely and chronically toxic to human health. Some effects can be seen immediately or few hours after exposure such as eye and skin allergy, dizziness, nausea, fatigue, vomiting, abdominal pain and poisoning (Singh et al., 2018); while some adverse effects can only be seen after an extended period or few years after exposure. These include neurological diseases and cancers (Bonner et al., 2017; Brouwer et al., 2017).

1.2 Problem statement

While chemical pesticides are used as pests control, it was reported that not all pesticides reached intended target while another portions were deposited in environment (Garcerá et al., 2017). Agriculture activities are mainly responsible for the pesticides contamination in environment (Souza et al., 2017). Numerous pesticides had been detected in air indicating pesticides dispersion and contamination (Coscollà et al., 2017; Fuhrimann et al., 2020; Hamsan et al., 2017). In addition, pesticides can enter human body and absorbed into blood stream (Damalas & Koutroubas, 2016). As pesticides handlers are directly in contact with pesticides, evaluating pesticides exposure in blood provide evidence of exposure and signs of residues in body (Saeed et al., 2017).

Agriculture workers often neglect the significant of personal protective equipment (PPE) and safe application of pesticides (Gesesew et al., 2016; Okoffo et al., 2016; Oyekale, 2018). Upon observation during site visit and interview done among paddy farmers in Tanjung Karang, majority of them did not wear appropriate PPE especially for inhalation protection, reflecting the negligence of PPE usage. Without appropriate safety consideration and PPE, extended exposure to pesticides may cause adverse health effects.

Due to its presence in ecosystem, human is inevitable to pesticides exposure. Approximately, 70,000 cases of pesticides poisoning (yearly) among agriculture workers lead to fatalitiy and a higher number of cases involving diseases were estimated by International Labor Organization (Caldas, 2016). Among reported health symptoms associated with pesticides were excessive fatigue, vomiting, difficulty breathing, dizziness, nausea, blurred vision, and cough (Joko et al., 2020). The exposure of farmers to pesticides are significantly greater compared to general population (Özkara et al., 2016).

Physicochemical characteristics such as temperature and weather conditions may be related to pesticides contamination in air (Souza et al., 2017). According to Baharuddin et al. (2011), wind speed had the strongest impact on pesticide exposure via inhalation, while temperature and humidity also contribute to the exposure of pesticides in air. Thus, it is important to consider these climatological factors in determining pesticides concentrations in air samples.

Persistent pesticides such as organochlorine (OC) are slowly phased out and alternatively replaced with currently-used-pesticides (CUPs) (Climent et al., 2019). They were designed to be more friendly to environment than banned pesticides due to their higher effectivity on pests, lower persistency and lower non-target toxicity (Hvězdová et al., 2018). However, they have been detected outside their application area (Degrendele et al., 2016). Thus, there is a possibility for these pesticides to be exposed to human and enter the bodydue to their long-term negative health effects (Climent et al., 2019).

1.3 Research justification

Assessment of health effects among farmers is a potential concern due to their occupational exposure, yet only few studies have focused on health risks to this group in Malaysia (Ahmad et al., 2020; Baharuddin et al., 2011; Hamsan et al., 2017). To determine whether the massive use of pesticides pose health impact among farmers in Tanjung Karang, pesticides pollution needs to be determined in personal air (external dose) and blood serum (absorbed dose).

Very limited study integrated both quantifications of pesticides in air and blood samples. Whyatt et al. (2003) was one of the studies that investigate the pesticides concentration in personal air and blood plasma samples. However, the study was conducted among mother-infant pair for residential insecticides use on 1998 to 2001, which had left a huge time gap until our current study. In our study, 13 compounds were analysed simultaneously in both personal air and blood serum samples of farmers to evaluate the exposure due to occupational factor. Assessing farmers' risk during the application of pesticides and estimating the dose absorbed by the body is challenging but it is a necessity (Mandic-Rajcevic et al., 2019). Inhalation exposure causes the individual to breathe in pesticides, which is absorbed through the surface of lung and enter blood stream to be distributed in body (Damalas & Koutroubas, 2016).

Besides, there were limited studies on personal air sampling of pesticides in agriculture setting. Most of the previous research focused on ambient air (Coscollà et al., 2017; Désert et al., 2018; Zivan et al., 2017), thus there are limited number of studies that focus on personal exposure especially in Malaysia agriculture. Among few studies that cover personal air sampling are Baharuddin et al., (2011); Bakri, Hariri, Ismail, Abdullah, & Kassim, (2018); and Nurul, Shamsul, & Noor Hassim, (2016). Bakri et al., (2018) & Nurul et al. (2016) determine the concentration of heavy metals among welders and steel factory workers respectively, while Baharuddin et al. (2011) determine pesticides exposure among farmers. Therefore, the highlight of this study is we compare the personal exposure of 13 CUPs among farmers and control group which prove occupational exposure and provide information on current pesticides use in paddy field in Tanjung Karang specifically and Malaysia generally.

There were also lacking number of studies in CUPs which are commonly used in Tanjung Karang. Most of the pesticides studies in this country are mainly focusing on persistent pesticides such as OC group (Farina et al., 2018; Sutris et al., 2016; Zainuddin et al., 2020). Nowadays, many agriculture activities have shifted to CUPs which are less persistent. To overcome the gap, this study determines 13 CUPs in personal air and blood serum samples. The target compounds were selected according to their popularity among paddy farmers in Tanjung Karang which comprised of azoxystrobin, buprofezin, chlorantraniliprole, difenoconazole, fipronil, imidacloprid, isoprothiolane, pretilachlor, propiconazole, pymetrozine, tebuconazole, tricyclazole, and trifloxystrobin.

The aim of this study is to investigate whether the occupational exposure to the mixtures of pesticides in personal air and blood serum are associated with health symptoms among farmers. Due to the volatility of pesticides, surface deposition happens immediately after application and may enter the body via respiratory tract (Mamane et al., 2015). Upon entering the body, it is often absorbed into the blood (Damalas & Koutroubas, 2016) as blood is rapidly exposed to absorbed chemicals (Lushchak et al., 2018). When inhaled, they can irritate and damage the alveolar and subsequently causing symptoms (Fareed et al., 2013).

1.4 Conceptual framework

In this study, 13 CUPs were selected as target compounds which comprised of three categories of pesticides namely fungicides (azoxystrobin, difenoconazole, isoprothiolane, propiconazole, tebuconazole, tricyclazole, trifloxystrobin), insecticides (buprofezin, chlorantraniliprole, fipronil, imidacloprid, pymetrozine), and herbicides (pretilachlor). Human may exposed to pesticides from environmental and occupational exposure. Farmers and pesticides sprayers are the common groups exposed to pesticides due to occupational exposure (Damalas & Koutroubas, 2016). Therefore, we determine the occupational exposure of pesticides among farmers. Pesticides can contaminate soil, air and water (Singh et al., 2018) and enter human body through ingestion, inhalation and dermal contact (Nicolopoulou-Stamati et al., 2016). Due to the volatile characteristic of pesticides, inhalation exposure is a great concern (Amaral, 2014). We study inhalation exposure by sampling personal air of farmers while spraying the pesticides and collecting their blood serum samples to determine the absorbed concentration in the body. The climatological conditions (wind speed, temperature, and realative humidity) were measured and the correlations with pesticides concentration in personal air samples were determined. The association of concentration of pesticides in personal air samples and blood serum samples as well as the use of PPE with reported health symptoms (breathing difficulty, chest pain, cough, phlegm, wheezing, sore throat, nausea, vomiting and dizziness) were determined to study the effects of pesticides exposure to farmer's health. The conceptual framework was illustrated as Figure 1.1.

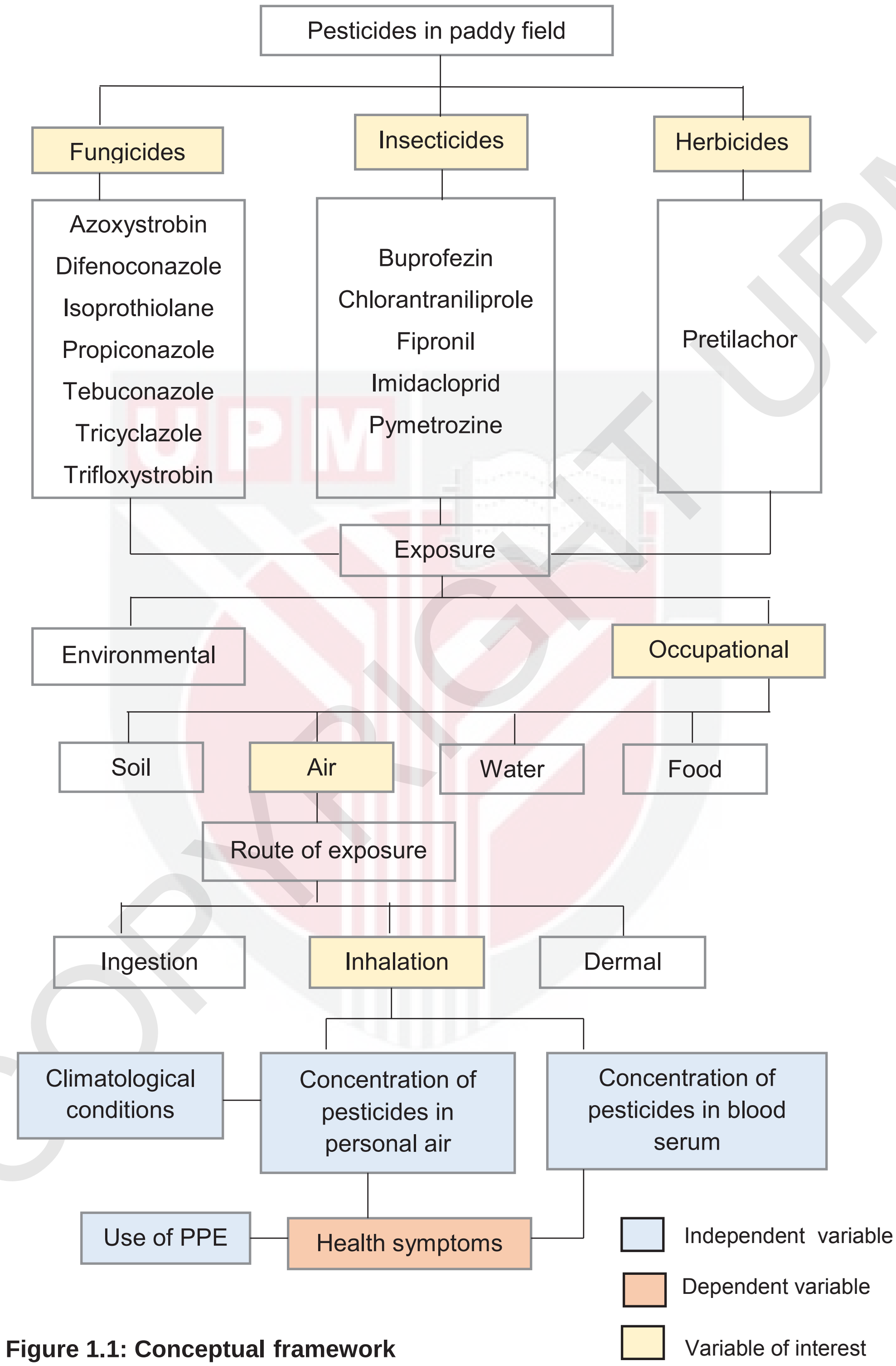

Figure 1.1: Conceptual framework

1.5 Research questions

Questions arise from this research is what is the relationship between pesticides concentration in personal air samples, blood serum samples and their association with reported health symptoms among paddy farmers in Tanjung Karang?

1.6 Objectives

General objective

To determine the relationship between pesticides concentration in personal air samples, blood serum samples and their association with reported health symptoms among paddy farmers in Tanjung Karang.

Specific objectives:

1. To quantify the concentration of pesticides in personal air and blood serum samples of farmers and non-exposed group.

2. To determine the awareness of using PPE among farmers.

3. To determine the reported health symptoms of farmers and non-exposed group.

4. To compare the concentration of pesticides in personal air and blood serum samples of farmers and non-exposed group.

5. To correlate the concentration of pesticides in personal air samples and blood serum samples of farmers and non-exposed group.

6. To correlate the concentration of pesticides in personal air samples and climatological conditions (wind speed, temperature, relative humidity) of farmers.

7. To associate the concentration of pesticides in personal air samples, blood serum samples, and use of PPE with reported health symptoms of farmers.

1.7 Hypothesis

1. The concentration of pesticides in personal air and blood serum samples of farmers is significantly higher than in non-exposed group.

2. The concentration of pesticides in personal air samples is significantly correlated with the concentration of pesticides in blood serums samples of farmers and non-exposed group.

3. The concentration of pesticides in personal air samples is significantly associated with climatological conditions of farmers.

4. The concentration of pesticides in personal air samples, blood serum samples, and use of PPE are significantly associated with reported health symptoms among farmers.

1.8 Ethical consideration

All participants' participation is voluntarily and they were briefed on the study before taking part in this project. All participants had signed a written consent form and the study was approved by the University Research Ethics Committee of University Putra Malaysia, Selangor, Malaysia (JKEUPM (FPSK-P161) 2017).

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