TOXICITY STUDY OF ISOPROTHIOLANE ON *Macrobrachium lanchesteri* IN SURFACE WATER OF PADDY FIELD ECOSYSTEM

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by

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LIST OF ABBREVIATIONS

BOD	Biochemical oxygen demand
COD	Chemical oxygens demand
TSS	Total suspended solid
ERA	Environmental Risk Assessment
EU	European Union
GC-MS	Gas Chromatography-Mass Spectrometry
IUPAC	International Union of Pure and Applied Chemistry
LOEC	Lowest-Observed Effect Concentration
LC	Lethal Concentration
NOEC	No-Observed Effect Concentration
PNEC	Predicted No-Observed Effect Concentration
SPE	Solid Phase Extraction
SPSS	Statistical Package for the Social Sciences
TSS	Total suspended solid
US EPA	United States Environmental Protection Agency
WHO	World Health Organization
DOA	Department of Agriculture
ANZECC	Australian an New Zealand Environment and Conservation Council
ARMCANZ	Agriculture and Resource Management Council of Australia and New Zewland

LIST OF SYMBOLS

°C	Degree Celsius
km	Kilometer
mg/l	Miligram per litre
mg/kg	Milligram per kilogram
ml	Mililitre
min	Minute
m/s	Meter per second
ppm	Part per million
%	Percentage

KAJIAN KETOKSIKAN ISOPROTHIOLANE TERHADAP *Macrobrachium lanchesteri* DALAM AIR PERMUKAAN SAWAH PADI

ABSTRAK

Penggunaan isoprothiolane dalam penanaman padi boleh memberi kesan kepada ekosistem akuatik. Kajian ini bertujuan untuk mencari paras sisa isoprothiolane di dalam permukaan air di sawah padi dan juga menentukan tahap ketoksikan akut dan kronik isoprothiolane kepada udang air tawar, *Macrobrachium lanchesteri*. LC_{50} atau kepekatan maut median untuk kematian 50 peratus dalam populasi digunakan sebagai titik akhir bagi paras ketoksikan akut manakala untuk paras ketoksikan kronik, paras tiada kesan tercerap (NOEC) telah dipilih. Ramalan tahap ketoksikan kronik ditentukan dengan menggunakan faktor penilaian dan kaedah regresi linear dua langkah (LRA). Paras tiada kesan tercerap yang diramal (PNEC) dibandingkan dengan nilai NOEC daripada ujian kronik yang dijalankan selama 21 hari bertujuan untuk menentukan tahap konservatif antara nilai ramalan dan nilai eksperimen. Sampel air dari 12 titik pensampelan dikumpul daripada kawasan ujikaji dan diekstrak menggunakan kaedah pengekstrakan fasa pepejal (SPE). Sampel kemudian dianalisa dengan menggunakan kromatografi gas- spektrometri jisim (GC-MS) untuk mengukur paras isoprothiolane yang dikesan di dalam sampel air. Residu isoprothiolane dikesan di kedua-dua peringkat pertumbuhan (maksima pecahan anak) dan peringkat masak padi. Kepekatan sisa isoprothiolane yang terdapat pada peringkat masak adalah jauh lebih tinggi daripada peringkat pertumbuhan. Kepekatan tertinggi residu isoprothiolane yang dikesan adalah pada tahap 9.37 mg/L daripada sampel air di titik pensampelan 5 (S5) pada peringkat masak. Tahap ketoksikan akut dianalisa dengan mencari nilai LC_{50} isoprothiolane pada *M. lanchesteri* ditentukan selepas 96 jam

pendedahan menggunakan analisis Probit daripada perisian SPSS. Hasil keputusan menunjukkan bahawa LC_{50} isoprothiolane berada pada tahap 18.21 mg/L. Hasil mortaliti untuk setiap ulangan untuk 21 hari pendedahan dianalisa dan keseluruhan LC_{50} isoprothiolane untuk kematian *M. lanchesteri* dianggarkan pada tahap 9.1 mg/L (pada batas keyakinan 95%). Bagi tahap ketoksikan kronik, kepekatan kesan terendah tercerap (LOEC) dan tiada kesan buruk tercerap (NOEC) untuk kematian *M. lanchesteri* telah dianalisa dan masing-masing pada kadar 6 mg/L dan 3 mg/L. Mortaliti kawalan diterima untuk semua julat kepekatan apabila semua kematian berada di bawah 20%. PNEC yang dihasilkan menggunakan ekstrapolasi data $LC_{50/5}$ dalam analisis LRA adalah lebih konservatif dengan nilai NOEC eksperimen. Berdasarkan kajian ini, dapat disimpulkan bahawa kaedah ekstrapolasi LRA $LC_{50/5}$ adalah yang paling menghampiri dengan nilai NOEC eksperimen. Penilaian darjah risiko (RQM) telah digunakan untuk mengkelaskan risiko ekologi yang dihasilkan oleh isoprothiolane. Hasilnya menunjukkan pencemaran isoprothiolane memberi risiko yang tinggi kepada *M. lanchesteri* yang hidup dikawasan sawah padi. Kesimpulanya, penggunaan isoprothiolane di sawah padi boleh memberi risiko yang tinggi kepada ekosistem sawah padi.

TOXICITY STUDY OF ISOPROTHIOLANE ON Macrobrachium lanchesteri IN SURFACE WATER OF PADDY FIELD ECOSYSTEM

ABSTRACT

The application of isoprothiolane in rice cultivation may give risk to the aquatic ecosystem. The present study was undertaken to determine the level of isoprothiolane in surface water of paddy fields and consequently, to determine the acute and chronic toxicity level of isoprothiolane to freshwater prawn, *Macrobrachium lanchesteri*. LC_{50} or lethal concentration for 50 percent mortality in population was used as the endpoint for acute toxicity level while for chronic, no-observed effect concentration (NOEC) is preferable. Prediction on chronic toxicity level was determined using assessment factor and two-step linear regression (LRA) method. The predicted no-observed effect concentration (PNEC) was compared to the NOEC value from 21 days chronic test to determine the conservativeness between the prediction and the experimental value. Water samples from 12 sampling points were collected from sampling sites and extracted by solid phase extraction method. The samples were then analysed by gas chromatography-mass spectrometry (GC-MS) to quantify the concentration of isoprothiolane detected in the samples. Isoprothiolane residue was detected in both the vegetative stage (maximum tillering) and ripening stage of paddy growth. The concentration of isoprothiolane residue found in the ripening stage was higher than in the vegetative stage. The highest amount of isoprothiolane residue detected was at 9.37 mg/L from water sample collected at sampling point 5 (S5) at the ripening stage. LC_{50} of isoprothiolane on *M. lanchesteri* was determined after 96 hours of exposure, interpreted by Probit analysis from SPSS software. The results showed that LC_{50} of

isoprothiolane was 18.21 mg/L. Mortality results after 21 days exposure time were analysed and the overall LC_{50} of isoprothiolane on mortality of *M. lanchesteri* was estimated at 9.1 mg/L (at 95% confidence limit). The lowest effect concentration (LOEC) and NOEC were 6 mg/L and 3 mg/L respectively. Control mortalities were acceptable for all replicated concentration ranges when all mortality was below 20%. PNEC produced using $LC_{50/5}$ data extrapolation in LRA analysis were more conservative with the equivalent experimental NOECs. This study concluded that the LRA $LC_{50/5}$ extrapolation method is the most protective. Risk quotient assessment method was used to characterize ecological risk generated by isoprothiolane. The results indicate an high risk for *M. lanchesteri*. The application of isoprothiolane is therefore an activity generating risk for the aquatic in rice filed ecosystems.

CHAPTER 1

INTRODUCTION

1.1 Research background

Agricultural activities in Malaysia have led to the deterioration of biodiversity and sustainability of the ecosystem. Malaysia's agriculture activities include the extensive plantation of industrial commodities (palm oil, cocoa, and rubber) and food sub-sectors (paddy, vegetables, fruits, meats, and fish) (Fahmi et al., 2013). Various agrochemicals are used in this sector to prevent or control pests, diseases, weeds and other plant pathogens and enhance the production of crops (Fuad et al., 2012). Pesticides tremendously give a good impact in repelling or protecting the plants from pest and diseases, but they are one of the dangerous contaminants to human and ecosystem (Belmonte Vega et al., 2005).

Agrochemicals include pesticides, and fertilizer may affect the physical, chemical and biological activity of the ecosystem as they leach, deposit and accumulate into the surrounding environment (Kouzayha et al., 2012). Ismail et al., (2011) has found that the pesticides usage in Malaysia agriculture sector especially in rice cultivation have been rising over the years. These chemicals can be classified into insecticides, herbicides, fungicides, rodenticides, nematicides, molluscicides, plant growth regulator and others (Aktar et al., 2009) with over eight hundred active ingredients in over tens of thousands of formulations (Hernández et al., 2013).

Fungicides are extensively used in industry, agriculture, the home and garden (Mohamed & Marzouk, 2015) and isoprothiolane is one of the fungicides that is widely used by the farmers especially in rice growing activity. Idayu et al., (2014) have found that isoprothiolane (Fujoine 40 EC) is one of the two fungicides commonly used by rice farmers in Permatang Pauh. While in Korea, isoprothiolane is ranked in the twenty-third place out of fifty pesticides based on the quantity of use (Cha et al. 2014). Isoprothiolane (IPT) has widely been used to control the rice blast fungus *Pyricularia oryzae*, *Helminthosporium sigmoideum* and *Fusarium nivale* (Kaveeshwar & Gupta, 2014). This fungicide has been classified under class II (moderately hazardous) by the World Health Organization (WHO). Exposure to this compound has adverse effects on humans, causes severe eye irritation, respiratory irritation (respiratory tract irritation), may be fatal if swallowed and it is carcinogenic (ACM, 2015).

The application of isoprothiolane usually being made by spraying them to the whole plant or affected area of the plant. Isoprothiolane can reach various segments of the aquatic environment either via transfer of the chemicals from soil or by spraying against target organisms (Oruc et al., 2002). Aktar et al. (2009) stated that the sprays of isoprothiolane onto the affected terrestrial will affect the ecosystems such as air and soil. Also, Prusty et al. (2011) have reported that isoprothiolane residues in the aquatic ecosystem were found at a higher level than other ecosystems.

Figure 1.1 displays the distribution of pesticide in the environment. The residue of isoprothiolane in the environment is a crucial issue as their uptake may cause the accumulation of primary products (Yuichi et al., 1993). Besides, isoprothiolane likely flows into water bodies due to the high solubility of the fungicide (Fushiwaki & Urano, 2001). Realizing the effect of isoprothiolane on the aquatic ecosystem, it is essential to evaluate the potential toxicology effect of this pesticide on freshwater organisms. Extent research needs to be done about the application of pesticides in Malaysia due to the expansion of land use and rice production in the development of rice cultivation activity (Ahmad, 2016).

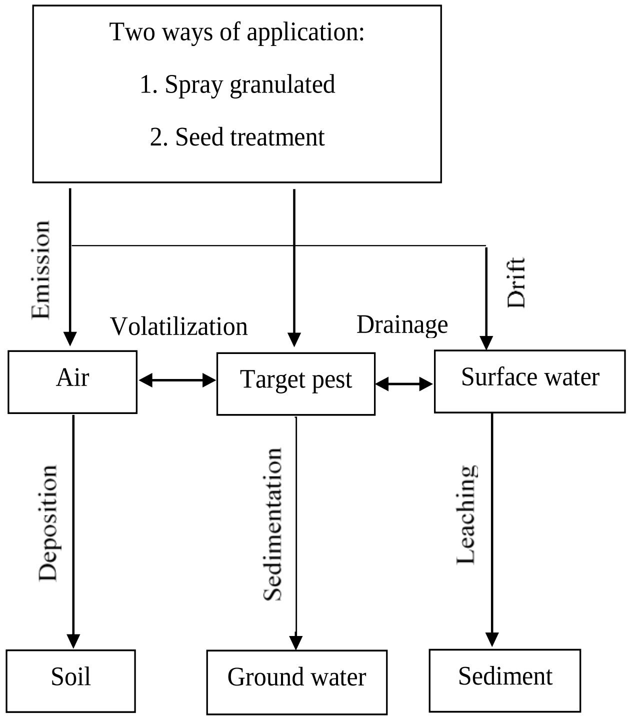

Figure 1.1 Distribution routes and “receptor” organisms for pesticides used in agriculture (WHO, 2009)

The methods for toxicity assessment can be classified into acute toxicity test used for the rapid evaluation of effluent discharge and chronic tests for the assessment of long-term effects on mortality, growth, and reproduction of aquatic animals (Vosylienë, 2007). The present study was undertaken to assess the toxicity effect of isoprothiolane towards freshwater prawn, *Macrobrachium lanchesteri*. Freshwater prawn is an important aquatic organism in toxicity testing because of its role as food to vertebrates like fish especially in food chain indicate the reliability of the species (Samuel et al., 1988). Besides, this aquatic organism is widely populated in a tropical region (De Grave et al., 2008), and it is an excellent animal representative for Malaysia ecosystem. In one of the study conducted by Shuhaimi-Othman et al. (2011), LC_{50}

values for metals for this *M. lanchesteri* is equally or more sensitive than most other tested crustaceans

In the present study, the acute and chronic toxicity assessment of isoprothiolane towards freshwater prawn, *M. lanchesteri* have been conducted. The chronic toxicity level was determined by performing the estimation method and experimental test. The chronic lethality was predicted based on the regression analyses of the acute toxicity data. The screening of isoprothiolane fungicide has been done to determine the residual of the chemical in rice field water bodies.

1.2 Problem statement

Isoprothiolane has high solubility in water and tends to flow easily in water. This fungicide transmitted in rivers and waterways can be very harmful to aquatic organisms. The implications of isoprothiolane to the environment have been studied across the globe, but limited studies were found in the literature for tropic climate including Malaysia. Besides, isoprothiolane is classified as toxic to aquatic life with long-lasting effects, and this may lead to the imbalance in the ecosystem and the data on chronic toxicity level of isoprothiolane towards any aquatic organism is still limited. The estimation of chronic toxicity is a decisive step in estimating the long-term effect of pesticide on organisms. Moreover, the information on isoprothiolane toxicity towards invertebrate was only tested on *Daphnia magna* or also called the water flea. This species is mostly absent from the tropics and commonly inhabit at the temperate region. This creates a bias towards the tropical ecosystem and may not represent the specific ecotoxicological risk.

1.3 Research objectives.

The primary purpose of this research is to determine the toxicity effects of isoprothiolane towards *M. lanchesteri* and to assess the residue contamination of the pesticide on paddy ecosystem. This research has the following objectives:

1. To determine the isoprothiolane residual concentration in paddy field ecosystem surface water.
2. To determine the acute and chronic toxicity level of isoprothiolane to freshwater prawn, *Macrobrachium lanchesteri.*
3. To predict the risk of isoprothiolane to *Macrobrachium lanchesteri*.

1.4 Scope of study

This research involved toxicity assessment and data analysis, sample collection and laboratory analysis. A 96 hours acute toxicity test of isoprothiolane towards freshwater prawn, *M. lanchesteri* were done in the laboratory. While the experiment and prediction method was conducted for chronic toxicity assessment. Experimentally, the chronic toxicity test was conducted for 21 days, and the chronic prediction was calculated using two different methods; assessment factor method and Two – Step Linear Regression Analysis (LRA).

Mukim 5, Penaga, Seberang Perai, Utara Pulau Pinang was chosen as the sampling site for this research. Water sampling was conducted at three different stages of rice cultivation period (seedling phase, vegetative phase and ripening phase) from April 2016 to August 2016. Water samples were collected from irrigation canal, river and estuarine. *In-situ* water quality assessment that includes pH, biochemical oxygen demand (BOD), chemical oxygen demand (COD), total suspended solid (TSS), total dissolved solid (TDS) and turbidity was conducted. All water samples were extracted by solid phase extraction (SPE) and analyzed using gas chromatography (GC).

1.5 Significance of study

A comprehensive set of chronic data for freshwater prawn, *M. lanchesteri* against which results of acute to chronic extrapolations can be benchmarked. This research finding will assist the national agricultural board in pest management control operation as a guideline to develop the maximum residue limit (MRL) for isoprothiolane in a freshwater ecosystem. Toxicology and bio-monitoring can be a strong demonstration and a good reference to identify pesticide exposure and its effect on the environment and guidance to fellow researchers to do extensive research regarding pesticides exposure to the environment.

The outcomes of the study will enhance the fundamental understanding of the effect of isoprothiolane contamination towards the ecosystem in paddy fields, river, and estuary particularly in tropic climate. Standard toxicity test procedure for freshwater prawns can be established and be used in toxicity assessment as the more representative invertebrate organism for a tropical country. Analysis of isoprothiolane residues can provide significant data on the level of contamination of isoprothiolane on the surface water of paddy field that may give inimical effects to the environment.

CHAPTER 2

LITERATURE REVIEW

2.1 Isoprothiolane usage in the agriculture industry

2.1.1 General background

Isoprothiolane is one of the compounds used to control fungus attack on plant. The IUPAC name of this fungicide is Diisopropyl 1,3-dithiolane-2-ylidenemalonate with a molecular weight of 290 g/moles. This active ingredient was introduced to overcome the famous rice plant fungi, *Pyricularia oryza* due to high demand in the late 1970s (Yasuhiko, 2001). This organo-sulfur compound containing two carboxylic acid groups combine with one dithiolane group as shown in Figure 2.1.

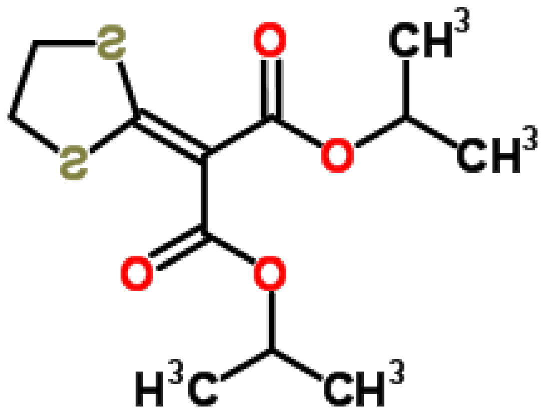

Figure 2.1 Chemical structure of isoprothiolane

Isoprothiolane can act as an insecticide in rice fields to reduce the population of brown planthoppers and leafhoppers (Ishizuka et al., 1998; Yasuhiko, 2001; Hu et al., 2014; Saravanan et al., 2015). According to the European Food Safety Authority (2012), isoprothiolane is a systematic plant growth with protective and curative action to help the rooting process of the plant. The application of isoprothiolane has not been approved in the EU, and no uses in Europe were registered.

2.1.2 Environmental fate and behaviour

This compound has high solubility in water with a concentration of 48 mg/L (Fushiwaki & Urano, 2001) with n-octanol/water partition coefficient of 3.16 (Tsuda et al., 1997). The half-life of isoprothiolane under sunlight depends on the pH value of soil or water. When the pH is 9.0, the half-life was 91 days while at pH 7.2 the half-life was 16 days and 13 days' half-life at pH 4 (Park et al., 1998). On the contrary, according to the Food and Drug Administration (2000), isoprothiolane was observed to be hydrolytically stable at all pH range in the environmental temperatures. Also, Ahmad et al. (2017) reported that isoprothiolane could remain at least a month after its application. The expected role of pesticides is to reach the target organisms and rapidly decomposed into less harmful by-products (Snelder et al., 2008). There are several degradation products of isoprothiolane such as isoprothiolane sulfoxide, diisopropyl chloromalonate, and diisopropyl dichloromalonate.

2.1.3 Mode of action

Generally, isoprothiolane fungicide act as an inhibitor for choline biosynthesis (Arul Selvi et al., 2013; Saravanan et al., 2015). There are two main pathways in Phosphatidylcholine biosynthesis, Greenberg's pathway and Kennedy's pathway (Hu et al., 2014). As shown in Figure 2.2, Greenberg's pathway transmethylation happened at the final step, while in Kennedy's pathway, it occurs at the preliminary step yielding choline before the formation of phosphatidylcholine (Yasuhiko, 2001). Isoprothiolane in Kennedy's pathway was claimed by Yoshida et al. (1984) as an inhibitor in choline synthesis by disrupting the transmethylation process. Isoprothiolane, iprobenfos and edifenphos were classified as choline biosynthesis inhibitors (CBIs).

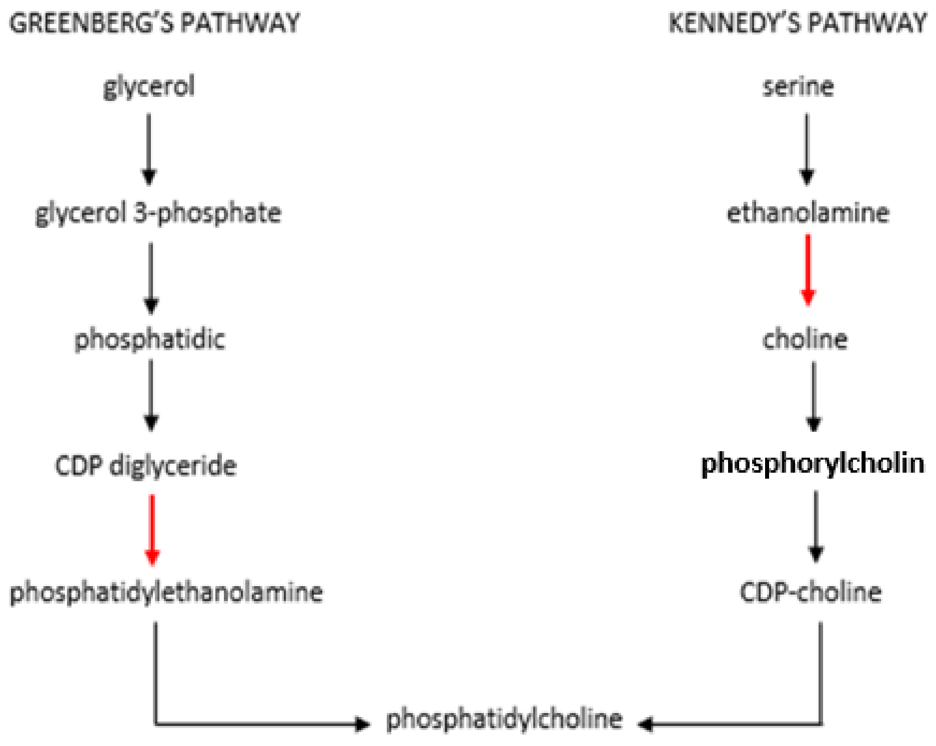

Figure 2.2 Two significant phosphatidylcholine pathways biosynthesis, the red arrow shows the transmethylation steps (Yasuhiko, 2001)

2.1.4 Ecotoxicology

The ability of isoprothiolane residue to accumulate into the natural raw material is one of the concerns (Yuichi et al., 1990). Several studies have been conducted to determine the toxicity effect of isoprothiolane on freshwater organisms. Tsuda et al., (1997) studied the 48-hour acute toxicity test of isoprothiolane and its degradation products on killifish (*Oryzias lattpes*) and the LC_{50} observed for isoprothiolane was 5.9 mg/L. The toxicities of the isoprothiolane degradation products were nearly equal to or lower than that of isoprothiolane. The adult killifish was reported to be low -toxicity in acute toxicity test compared to the larvae state (Hiromitsu et al., 2003). Table 2.1 shows the toxicity data on freshwater organisms that were collected from different authors.

There are limited studies about the effect of isoprothiolane towards freshwater aquatics. Marcial et al. (2005) has found the twenty-four-hour medial lethal concentrations of isoprothiolane to the rotifer *Brachionus plicatilis* or so-called marine rotifer at 25 ^{o}C was 64.1 mg/L. Saravanan et al. (2015), suggested that the isoprothiolane can cause harmful effect on aquatic organisms even at very low concentrations in the aquatic environments. The study has found that the hemoglobin, hematocrit, red blood cell, serum glucose, cholesterol, triglycerides, aspartate aminotransferase, and gill Na^+/K^+-ATPase in *Cyprinus carpio* levels were significantly ($p>0.05$) decreased when isoprothiolane was treated. While on mammal, Mohamed and Marzouk (2015) have studied the the acute oral toxicities of three pesticides (fenitrothion, thiobencarb, and isoprothiolane) to Albina rats and they have found that the LD_{50} values for isoprothiolane (Fuji-one 40% EC) on male and female rats were 966.445 mg/kg and 895.698 mg/kg at 24 hrs which indicated that males generally were more sensitive than females.

Table 2.1 Toxicity data of isoprothiolane on different freshwater aquatics

Aquatic organism	Toxicity test	Endpoint value	Authors
Fish			
- Killifish (*Oryzilas latipes*)	Acute 48-hour LC_{50}	5.9 mg/L	Tsuda et al. (1997)
- Killifish (*Oryzilas latipes*)	Acute 96-hour LC_{50}	10.0 mg/L	Hiromitsu et al. (2003)
- Common carp (*Cyprinus carpio*)	Acute 96-hour LC_{50}	4.14 mg/L	Liangan et al. (1990)
- Silver carp (*Hypophthalmichthys molitrix*)	Acute 96-hour LC_{50}	3.18 mg/L	Liangan et al. (1990)
Invertebrate			
- Water flea (*Daphnia magna*)	Acute 48-hour LC_{50}	1.80 mg/L	Liangan et al. (1990)
	Acute 48-hour LC_{50}	<10 mg/L	Matsumoto et al. (2009)
Algae			
- *Microcystis aeruginosa*		1.72 µg/L	
- *Scenedesmus quadricauda*	ErC_{50} (48h)	0.96 µg/L	(Jr & Nakahara, 2002)
- *Aulacoseira granulata*		2.55 µg/L	

2.1.5 Isoprothiolane pollution of freshwater around the world.

Most of the isoprothiolane contamination was recorded in Asia region because this fungicide is usually used in paddy plantation. In Mekong Delta, 46.2 % of farmers using isoprothiolane based fungicides in their agricultural activity (Toan et al., 2013). Compared to another region, the level of the isoprothiolane residue was the highest at Yun San river in South Korea with 26.3 µg/L respectively. While in Japan, Isoprothiolane was the most frequently detected fungicide in the effluent river from Lake Biwa (Sudo et al., 2002). In Japan, water pesticide residual monitoring was extensively being conducted. From Table 2.2, we can see the comparison of isoprothiolane residual level in surface water from different regions. Until now, the monitoring of isoprothiolane in Malaysia is still limited. In one of the studies from (Hamsan et al., 2017), they have determined the contamination level of isoprothiolane in personal air sample at Tanjung Karang Selangor, and the mean concentration was 57.42 ng/m^3. Ahmad (2016), has found that isoprothiolane was the second most used fungicide by Permatang Pauh's rice farmers beside difenoconazole, but the contamination level was not being analyzed. Other than that, no studies have been found regarding the level of isoprothiolane in the water system in Malaysia.

Table 2.2 Level of isoprothiolane from different countries.

Study area	Mean residual concentration	Sources
Mekong Delta, Vietnam	3.34 µg/L	Toan et al. (2013)
Northeast China watershed	0.876 ± 0.365 µg/L	Ouyang et al. (2017)
South Korea	Namhan River: 3.2 µg/L Yung San River: 26.3 µg/L	Park et al. (2007) (National Institute of Environmental Research , 2009)
Japan	Seta river: 0.331 µg/L	(Tanabe & Kawata, 2009)
	Shin river: 1.2 µg/L	
	Shinano river: 50 ng/L	Narushima et al. (2014)
	Agano river: 40 ng/L	

2.2 Malaysia paddy plantation

Oryza sativa L. or commonly known as rice is the most important source of food in the most of the world (Sabere et al., 2013). In Malaysia, rice is one of staple dish, and it is planted in the low regions of north Peninsular Malaysia which was contributed by ten granary areas as shown in Figure 2.3 and Sabah in Borneo (Ibrahim, 2007). Rice is typically cultivated either as lowland or upland. The production of rice from the lowland area is higher than the upland. The primary production system for lowland is a flooded system or paddy. George *et al*., (2001) states that the production of upland rice was limited due to infertile of the soils the leads to a low harvest index of traditional cultivars.

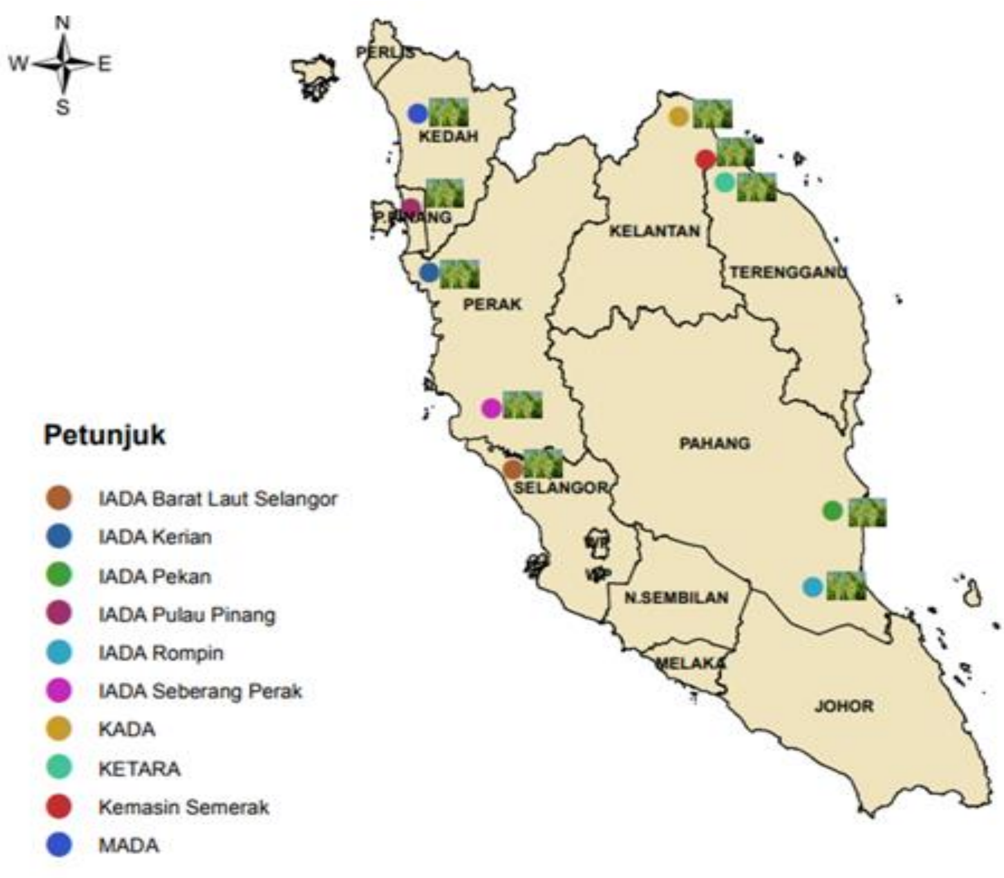

Figure 2.3 Map of paddy area. (Department of Agriculture, 2014)

Malaysia's rice was cultivated year-round due to the rainfall distribution and climate that is categorized as being hot and humid throughout the years. Rice approximately plant and harvest at the same cycle. Main Season is defined as the commencement date of paddy planting between 1st of August to 28/29th February of the following year and usually, know the as wet season. Off Season is defined as the commencement date of paddy planting between 1st March to 31st July of the year and also known as the dry season (Department of Agriculture, 2014).

Ministry of Agriculture has stated that in 2016, the total national rice production was approximately 2.74 million metric tonnes, covering an area of about 286, 579 hectares (see Table 2.3). Regarding per capita consumption, people in low-income Asian countries consume about 150-200 kg of rice per person per year. Muda in Kedah is the most massive granary (98 860 ha), followed by Kemubu in Kelantan (32 400 ha), Kerian in Perak (24 010 ha), Projek Barat Laut Selangor (19 920 ha), Seberang Perak in Perak (9510 ha), Sungai Manik in Perak (6510 ha), Besut in Terengganu 5100 ha) and Seberang Perai in Penang (1300 ha) (Karim et al., 2004).

Table 2.3 Annual Change of Paddy Plantation, 2012-2016 (Ministry of Agriculture and Agro-based Industry, 2016).

Commodities	2012	2013	2014	2015	2016
Paddy Parcel Area^2 (Ha)	292,500	289,882	289,882	291,0886	286, 579
Production ('000 Tonnes)	2599	2604	2849	2741	2740
Average Yield (Kg/Ha)	3797	3876	4194	4,022	3,978
Imports of Rice Quantity ('000 Tonnes)	983	853	863	961	748

2.2.1 Pesticide in Malaysia's agriculture

Malaysia is an agricultural-based country, located in the tropical rainforest province. Decades after independence, agricultural activity was the major contributor to economic growth in Malaysia (Fahmi et al., 2013). The Malaysian agriculture sector is still dependent on agrochemicals including chemical fertilizers and pesticides (Jamal & Yaghoob, 2014). After the Second World War, the application of pesticide for the agriculture and vector control was extensive (Ibrahim, 2007). Since 1955, pesticides have been used in Malaysia to control pests in rubber, cocoa, and oil palm plantation (Conway, 1973). DDT was used in 1955 to 1963 to control malaria in Malaysia as it was extraordinarily effective and later became the standard model to be applied throughout the world and other pesticides such as endrin, technical grade Hexachlorocyclohexane, lindane, lead arsenate, and trichlorphon were also used in the cocoa plantations (Ibrahim, 2007).

From 1956 to 1964, the use of DDT, dieldrin, and endrin were extensive to fight the attack of cockchafers, bagworm, and rhinoceros beetles in oil palm plantations (Conway, 1973). The number of registered pesticides keeps increasing throughout the years due to the new development and invention of pesticides formula. The amount of pesticides registered with the Ministry of Agriculture and Agro-based Industry in August 2018 is 3057 formulations of compounds. The market for pesticide in Malaysia is enormous due to the weather and the use of pesticide to protect the crops (Ali & Shaari, 2015). Table 2.4 shows the market of agrochemical in 2011, 2012 and 2017. Even though the market value for all pesticides shows a substantial decrement from 2012 to 2017, but the value in 2017 still indicates a high amount of pesticide (48235.5 tons) being traded and herbicide contributes 87% of total value.

Table 2.4 Market values of pesticide in Malaysia for the year of 2011, 2012 and 2017 (Raja Abdul, 2017)

Pesticides	2011		2012		2017	
	Values (million USD)	Active ingredient (tons)	Values (million USD)	Active ingredient (tons)	Values (million USD)	Active ingredient (tons)
Insecticide	121.10	10,671.1	121.36	4,065.47	70.19	3,021.76
Herbicide	358.74	67607.73	352.93	60,231.52	255.87	42,360.30
Fungicide	185.56	6,980.25	115.19	4,420.97	78.95	2,828.59
Rodenticide	3.81	0.27	4.82	0.01	N/A	N/A
Mollucside	4.17	75.25	2.00	201.62	0.20	24.85
Total	673.38	85334.6	596.3	68919.59	405.21	48235.5

2.2.2 Pesticide used in rice cultivation.

Insects, diseases, and weeds in rice fields in tropical Asia are responsible for the lost of 120 and 200 million tons of grain annually (Willocquet et al., 2004). The humid and warm atmosphere in the tropical region facilitates the invasion of insect and diseases (Pathak & Khan, 1994). To increase rice production, the extensive use of pesticides has become routine to control the damage of the crop and to protect the quantity and quality of agricultural products (Hamsan et al., 2017). Dieldrin, lindane, trichlorfon, and diazinon were used since 1955 to control rice borer pests (Ibrahim, 2007). Extended period needed in paddy growth, gives a chance for insects, fungi, and weed to disrupt the production for example small brown planthopper, brown planthopper, and white backed plant hopper are the primary pest insects in paddy plantation (Ahmad, 2016).

Generally, 10- 15% of rice production in Asia was affected by various rice diseases (Savary et al., 2000). Sheath blight and blast are the vital rice disease that is responsible for losses of 5% of rice production (Gianessi, 2014). Rice yield losses caused by weeds reach 5-85% in Malaysia and depend on the planting method, season, location, predominant weed flora, weed density, management practices, and infestation duration (Dilipkumar et al., 2012). In general, weedy rice *(Oryza sativa L.)*, *Echinochloa* spp., *Leptochloa chinensis* (L.) Nees., *Ischaemum rugosum* Salisb., *Paspalum* spp., *Digitaria ciliaris* (Retz.) Koeler., *and Cynodon dactylon* (L.) Pers. were the predominant grass weeds in direct-seeded rice (Dilipkumar et al., 2017). According to International Rice Research Institute (2018), 5 to 10% loss of rice production comes from rats invasion every year.

In the 80s, pesticide residues such as aldrin/dieldrin, chlordane, Hexachlorocyclohexane, and Dichlorodiphenyltrichloroethane were found in fish samples in paddy-farming areas (Mohamed et al., 2016). Fuad et al. (2012) have studied the consumption of pesticide in Tanjung Karang rice field and found that 93% of the farmers used Paraquat that has been subsidized by the government. This highly toxic compound for human and animal is a quaternary nitrogen herbicide that targets on weeds and other vegetations before plant cultivation (Tan et al., 2013). Idayu et al. (2014) found that the significant pesticides in Permatang Keriang rice field were cypermethrin, chlorpyrifos, lambda-cyhalothrin, difenoconazole, bispyribac-sodium, isoprothiolane, and cartap hydrochloride. Table 2.5 shows the symptoms of a various type of common pest invasion and its pesticides recommendation.

Table 2.5 Type of pest attack in rice plantation (Department of Agriculture, 2012)

Pest	Pesticides recommendations
Insects pest	
1. Brown planthopper (*Nilaparvata lugens*)	Dimethoate, etofenprox, and malathion at the recommended rate on the label
2. Whitebacked planthopper (*Sogatella furcifera*)	Phenobucarb, etofenprox, and buprofezin according to the recommended rate on the label.
Rice disease	**Pesticides recommendations**
3. Rice blast (caused by *Pyricularia oryzae*)	Treat seeds with benomyl fungicide (4 gm / kg of seeds) or captan (2 gm / kg of seeds). Use recommended fungicides such as benomyl, isoprothiolane and carbendazim when the blast attacks are detected.
4. Sheath blight (*Rhizoctonia solani*)	Use fungicides such as edifenphos (0.1% b.a) or isoprothiolane (2 kg b.a./ha) Treat seeds with fungicides such as thiram (3 g / 100 g seeds), benomyl (4g / kg seeds) or captan (3g / 100 g seeds
Weed	**Pesticides recommendations**
5. Weedy rice (*Oryza sativa* L.)	The application of glyphosate before land preparation or seeding reported being effective
6. Jungle rice (*Echinochloa colona* (L.) Link)	Preemergence application of oxadiazon or pendimethalin or postemergence application of cyhalofop, butachlor, and fenoxaprop can be effective.
7. Red sprangletop (*Leptochloa chinensis* (L.) Nees)	Quinclorac, propanil, pendimethalin, fenoxaprop, pretilachlor, or benthiocarb
8. Wrinkle duck beak, saromacca grass (*Ischaemum rugosum* Salisb.)	Butachlor, thiobencarb, pendimethalin, or mixtures of thiobencarb or butachlor and propanil, cyhalofop, and fenoxaprop can give effective control.

2.3 *Macrobrachium lanchesteri*

Macrobrachium lanchesteri is a freshwater prawn that can be found in almost every inland waterbody (Uraiwa & Sodsuk, 2004) such as rice fields, ponds, reservoirs, streams, and rivers (De Grave et al., 2008). This prawn is widely found in Malaysia, Indonesia, Thailand, Cambodia, Laos, India, Philippines, and Vietnam. *M. lanchesteri* was initially described from southern Thailand by Lanchester in 1901 and has subsequently been reported from central Thailand. In Peninsular Malaysia and Sabah *M. lanchesteri* were found in 1961 and 1995 respectively. In Thailand, the people like to eat the prawn even though its size is petite. The International Union for Conservation of Nature (IUCN) classified the species as less threatened in their population. Taxonomically, this invertebrate/animal is classified as the following scheme:

Kingdom	: Animalia
Phylum	: Arthropoda
Class	: Malacostraca
Order	: Decapoda
Family	: Palaemonidae
Synonyms	: *Palaemon (Eupalaemon) lanchesteri, Cryphiops lanchesteri, Palaemon paucidens*
Common names	: Glass prawn, Ghost prawn

Table 2.6 The morphological characteristics of *M. lanchesteri*. (Uraiwa & Sodsuk, 2004)

Morphological characteristics	*M. lanchesteri*
Rostral teeth	1 (7-10)/ 3-4
Five pairs of walking legs	Clear, without red-brown spot
Rostrum	Slender and same length as antennular peduncle
The position of the hepatic spine	Behind the first one of the upper rostral teeth
The second pair of walking legs	With little short hair appearing only on the fingers of mature males

Plate 2.1 *Macrobrachium lanchesteri*

2.3.1 Lifecycle of freshwater prawn

There are two major reproductive strategies found in many *Macrobrachium* species, first is the classical r-strategists and the second is called K- strategists. The species that follow the classical r-strategists (also referred as prolonged larval development will produce a large number (up to 150,000) of small eggs and those with small eggs are amphidromous which means the adults are found in freshwater, but they migrate to marine environments for spawning (Wowor et al., 2009). The K-strategists is also known as abbreviated larval development (ALD). This second major reproductive strategy is when the life cycle of the species is entirely completed in freshwater.

As opposed to r- strategist's reproduction, the species lay relatively fewer eggs that are larger, and the development of the larvae is highly abbreviated and consists of only 1 or 2 zoeas, and also has a significant reduction in the timing of larval stages. *M. lanchesteri* has reportedly had a somewhat intermediate strategy in that they have ‘‘semi-abbreviated development.” due to the medium size of the eggs (0.027 mm). They spend their entire life cycle in freshwater, and the first two zoeal stages are free-swimming, and they need another three to four stages of larval development as opposed to 1 or 2 zoeal stages in ALD species (Sabar, 1979; Chong and Khoo, 1987). The species with intermediate-sized eggs inhabit exclusively slow-flowing rivers, canals, streams, lakes, ponds, and pools along the mid stretches of major rivers (Wowor et al., 2004).

2.4 Bioavailability, bioconcentration and biomagnification.

The contamination of pesticides in environment is happened depends on the amount of pesticides residue in the substrate and the sensitivity of the surrounding biota. Bioaccumulation in water is common process. Bioaccumulation is a process when the compound is accumulated in the organism because the pesticide cannot be broken down to be used by organism. Bioaccumulation is the total accumulation of contaminants in the tissue of an organism through any route, such as food items as well as from the dissolved phase in water (EPA, 2000). A persistent compound is accumulated in the organism tissue if the degradation and elimination processes are slower than the uptake rate, and the concentration in the tissue thus becomes higher than in the surrounding media

Some pesticides cross gills and intestine more easily than others. The state of being potentially available for biological uptake by an aquatic organism when that organism is processing or encountering a given chemical is called as bioavailability (EPA, 2000). Photodegradation alters the chemical structure, physical properties or concentration of a contaminant might change its bioavailability and usually photodegradation will help in lowering the residue of pesticide in water system. Contrary, according to Hamelink et al. (1994), some photo-degradation products may be even more persistent and toxic than the original compound.

The movement of the pesticides from surrounding into the organisms are in various ways. The mobility of pesticides or free- dissolved compound through nondietary passage defined the term of bioconcentration (Bacci, 1994). Some pesticides, such as DDT, are "lipophilic", meaning that they are soluble in, and accumulate in, fatty tissue such as edible fish tissue and human fatty tissue. Other pesticides such as glyphosate are metabolized and excreted.

Biomagnification is the process by which bioaccumulation causes an increase in tissue concentrations from one trophic level to the next from food to consumer. (EPA, 2000). This process indicates the increase in concentration of a substance along the food chain (Widenfalk, 2002). Higher tissue level in predators will concentrate the substance in the body when they consumed they affected preys. However, according to Suedel (1994) the validated biomagnification data on aquatic ecosystem are still lacking, especially for those compounds that are not hypothesized to readily biomagnify. Therefore, conclusions regarding their potential to biomagnify cannot be drawn until such data are available.

2.5 Toxicity studies in environmental application.

Nuffield Council on BioEthics (2005) identify toxicity study as to assess the degree of harmful chemical or toxin to humans, animals, and environment. Apart from that, toxicity studies also include the determination of the potential risks of substances, and the characterization of a compound action. Most of the toxicity testing is carried out on animals (Hodgson, 2004). The purpose of this study are to demonstrate the mode of action for a substance and also to determine the right dose for the substances before they are approved to be used as new chemical products such as drugs, pesticides or detergents (Arome & Chinedu, 2014).

In addition to dose, these few factors may also influence the toxicity of the compound such as the route of entry, duration, and frequency of exposure, variations between different species (interspecies) and variations among members of the same species (intraspecies) (UNL Environmental Health and Safety, 2002). The recommended methods can be divided into short-term, acute toxicity bioassays used for the rapid assessment of effluent discharge and chronic tests which are applied for the evaluation of long-term effects on growth, development, and reproduction of aquatic organisms.

2.5.1 Acute toxicity test

Acute tests were defined as tests where exposure time was greater than or equal to 24 hours and less than or equal to 96 hours (Slaughter, 2005). The purpose of this assessment is to provide the data for the design and selection of dose levels for more prolonged studies. Acute toxicity tests (such as the "classical" LD_{50} test) are designed to determine the mean lethal dose of the test substance.

The median lethal dose (or LD_{50}) is defined as the dose of a test substance that is lethal for 50% of the animals in a dose group (Food and Drug Administration, 2000). Acute toxicity studies are the most popular among the researches due to the animal welfare aspect as the number of animals used is fewer than for chronic experimental studies. This test will provide the primary data for the other extensive testing assessment, for example the selection of dose for prolonged toxicity studies (Arome & Chinedu, 2014).