Health Risk Assessment of Rice Consumption: Analysis of Imidacloprid Residue in Paddy and Rice from Tanjung Karang, Selangor

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Abstract

50 samples of paddy, brown rice and white rice were collected from paddy fields in Tanjung Karang, Selangor and were analysed for imidacloprid residues. This study objective was to evaluate the non-carcinogenic health risks on Tanjung Karang's population (n=552) through rice consumption. The population were divided into 3 age groups. The QuEChERS of extraction with (Ultra High Performance Liquid Chromatography- Ultra Violet) UHPLC-UV analysis was carried out to analyse the residues. Imidacloprid was detected in 20%, 14% and 10% of paddy samples, brown rice and white rice, respectively with 4% of the total samples exceeded the MRL. The mean imidacloprid concentration was 0.14 mg/kg in the paddy sample, 0.04 mg/kg in brown rice sample and 0.03 mg/kg in white rice sample. The EDI for adult, adolescent and children were 0.08 mg/kg, 0.03 mg/kg and 0.0002 mg/kg bw, respectively. HQ value of adult was 1.32 showed that this group was exposed to non-carcinogenic health risk through rice consumption.

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1.   Introduction

A large scale of paddy farming is found in Malaysia as rice is a staple food for the population. About one-third of the agricultural products are produced by using pesticides [1]. Without the use of pesticide, crops production decreased drastically due to pest attack [2]. Imidacloprid is frequently used by the farmers because it helps to combat the brown planthoppers. The brown planthoppers (BPH, *Nilaparvata lugens*) is considered as one of major pests of paddy cultivation in Southeast Asia [3].

Imidacloprid, [1-(6-chloro-3-pyridinyl) methyl-4, 5-dihydro-N-nitro-1H-imidazole-2-amine] is a member of neonicotinoid insecticides class which was first introduced by Bayer Agricultural Product [4] It was first registered for use in the United States in 1994 and is currently one of the best-selling insecticides globally [5]. Neonicotinoid is belonged to the chloronicotinyl insecticide and has a great affinity for nicotinergic acetylcholine receptors which is the receptor protein of insect nervous systems [6]. Due to this broad efficacy characteristic as a systemic insecticide, imidacloprid has been widely used by paddy farmers as early plant growth enhancer, foliar spray, soil treatment and seed dressing for paddy cultivation. Imidacloprid has the molecular formula $C_9H_{10}ClN_5O_2$ with the molecular weight of 255.7g/mol (Figure 1). Imidacloprid is very effective for the control of sucking insect pests such as aphids, whiteflies, leaf and plant hoppers and thrips [7] was categorized by the Environmental Protection Agency (EPA) as a Class II/III agent [8].
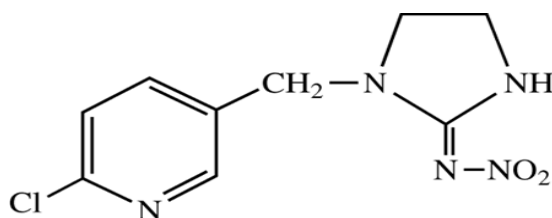
Fig. 1 Molecular structure of imidacloprid

Imidacloprid can enter human body through ingestion, inhalation and dermal contact. [5] described drowsiness, dizziness, vomiting, disorientation, and fever as the signs of toxicity of imidacloprid poisoning. Contamination of the imidacloprid residues through the dietary intake has been the significant issue in many areas of the world [1, 10]. Thus, it is essential to monitor pesticides residue in food for the evaluation of food safety in order to avoid any risk to human consumption [11]. It is important to ensure that pesticides levels found in foods remain safely within the limit such as maximum residue limit (MRL). For the present study, Health risk assessment (HRA) via dietary intake, known as Estimated Daily Intake (EDI) was applied in order to measure the safety of consuming rice containing imidacloprid residues from Tanjung Karang, Selangor while Hazard Quotient (HQ) for imidacloprid was calculated to measure non-carcinogenic risk posed by imidacloprid residue to Tanjung Karang population. There was non-carcinogenic risk evaluated as imidacloprid were only posed a non-carcinogenic risk to human population. The objectives of this study were (1) to determine imidacloprid residue

concentration residue concentrations in paddy and rice (white and brown rice) samples from Tanjung Karang area, (2) to compare the imidacloprid residue concentration with Malaysian MRL. This study also (3) determined the estimated daily intake (EDI) of imidacloprid for different age group of Tanjung Karang population and finally (4) to assess and predict the non-carcinogenic risk for each age group.

2.   Materials & Methods

2.1 Paddy and rice sampling area

Kampung Sawah Sempadan was selected in this study as it is one of the largest of the seven areas forming the Tanjung Karang Rice Belt as the "rice bowl" of Selangor Kampong. The total paddy cultivation area of Kampung Sawah Sempadan was 5,908 acres, and neatly subdivided into 23 blocks (A-V) of about 250 acres each [12]. The 150 samples were collected randomly during the harvest season from May to June 2013. At each sampling point in the paddy field, 1 kg of paddy was collected (Figure 2.). The rice (white and brown) samples were randomly collected from the local rice milling factory. Organic rice which is free from pesticides was used as blank sample for calibration and validation purposes. The samples were collected in polythene zip lock bags, sealed with aluminum foil and transported to the laboratory and stored at 4°C until analysis.

2.2 Community survey

A community survey was conducted to determine the pattern of rice consumption among the Kampung Sawah Sempadan's population. Population in Kampung Sawah Sempadan, Tanjung Karang was selected because they lived around the paddy field and they consumed their own cultivated rice. The survey was carried from house to house until the number of respondent reached the targeted sample size (N = 552) with 184 respondents for each age group. The sample size was calculated by using formula from Neuman, (1997). The three age group included in this study were children (1 to 4 years), adolescent (5 to 18 years) and adult (19 to 64 years) old, respectively [13]. A standardized Food Frequency Questionnaire (FFQ) adapted from [14] was used for the survey.

2.3 Sample Analysis

2.3.1 Reagents and Chemicals

Imidacloprid analytical standard (purity >99%) was procured from Fluka Sigma-Aldrich. HPLC grade acetonitrile (ACN) was purchased from Sigma-Aldrich. The solvents were filtered through 0.45μm nylon Whatman filter paper before used. Water was purified using Elga /UK Pure Water System Model: Purelab S & BP MK1 (resistivity 18.2MΩ cm) water purification system. The salt such as anhydrous magnesium sulfate ($MgSO_4$), sodium chloride (NaCI) and primary secondary amine (PSA) included in roQ QuEChERS kit from Phenomenex were purchased for extraction and cleanup stages to save the analysis time. Ceramic homogenizer was used to break up salt agglomerates during the extraction which promoting more consistent sample extraction and thus increasing product recovery percent during the extraction process. Analysis of imidacloprid residue in paddy and rice sample was carried out by using Perkin Elmer Flexar FX-15 UHPLC equipped with

reverse phase C_{18} column Brownlee HRes (30mm x 2.1 mm, 1.9 µm particle size) as stationary phase. UV detector was used because it very suitable for detection of imidacloprid due to their strong absorbance at 270nm [15, 16, 17].
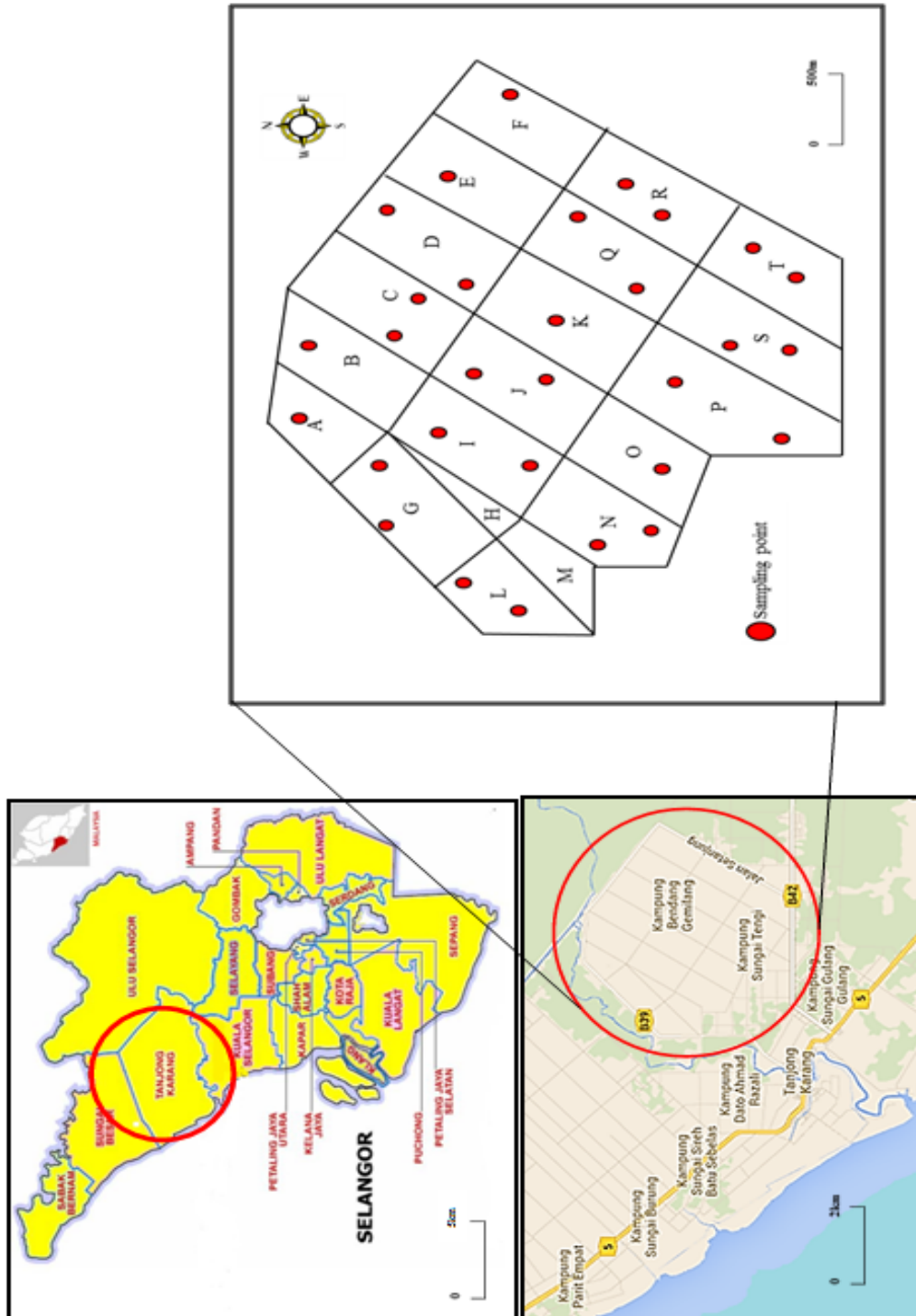

Fig. 2 Study location and sampling area

2.4 Extraction and Clean-up

The process of extraction and clean-up of imidacloprid residue in paddy and rice in this study were done by using QuEChERS method and followed EN 15662 original non- buffered method which was introduced by [18]. The extraction process were started with homogenization of sample and grounded by using Waring blender through cryogenic milling technique. Then, 5 g of the comminuted homogenous and frozen sample were weighed using the calibrated balancing scale. The homogenized sample was then mixed with 10ml of ultrapure water and 10 mL of acetonitrile in a 50 mL screw cap centrifuge tube. After that, 4 g anhydrous magnesium sulphate ($MgSO_4$) and 1 g of sodium chloride (NaCI) from roQ QuEChERS extraction kit were added into the centrifuge tube. Then the tube is closed and shaken vigorously by hand for 1 minute. The tube was then centrifuged for 5 minutes at 4000rpm. A further 1ml supernatant or the top liquid layer from the extraction process was transferred by using pipette into 2ml screw cap tube contained 150mg $MgSO_4$ and 25mg primary secondary amine (PSA) to precede the cleanup process. The tube was closed and shaken vigorously by hand for 30 seconds. Then the tube was centrifuged for 5 minutes at 4000rpm to separate solid materials from the liquid layer. The supernatant was then filtered through filter 0.22µm Thermo-filter and transferred into 1.5 mL amber vials before injected to UHPLC-UV for analysis.

2.5 Recovery studies and quality control

Imidacloprid were identified by matching the retention time of the sample with its external calibration standard curve. All the cleaning and washing procedure glass wares used during the analysis were followed the USEPA Method 1694 to avoid any contamination. Recovery study was conducted in 3 replicates. The replicates samples were fortified at 1 mg/kg which indicates overall recovery ranged from 92.02 to 95.61% and relative standard deviation (RSD %) from 2.47% to 4.54% for each type of samples. The 6 points of imidacloprid calibration curve was generated, and the linear relationship was evaluated across the range of expected sample concentrations. Linearity was obtained by a linear regression plot of known concentration versus response using a minimum of six different concentrations of imidacloprid. The regression equation was y = 356314.0725x + 39983.2015, with r^2 = 0.9996. The limit of detection and quantification for imidacloprid in the present of study were obtained in the range of 0.001mg/kg and 0.003 mg/kg, respectively.

2.6Estimated Daily Intake (EDI) and Hazard Quotient (HQ) Analysis

EDI is a realistic estimation of pesticides residues exposure, calculated in the agreement with the international guidelines [19]. The [20] was used as a main guide to calculate EDI which was expressed as milligram pesticides per kilogram body weight (mg/kg BW) as shown in Equation 1. The value of F and W were obtained from the community survey Tanjung Karang, Selangor. In this present study, EDI of imidacloprid residue were calculated for the three age groups. The assessment were calculated using the pattern of white rice intake only as the studied population did not consumed brown rice in their daily meal. The result of EDI was then compared to Acceptable Daily Intake (ADI) to determine if the imidacloprid exposure through the rice intake among the Tanjung Karang population was within the safe limit. It is expressed in milligrams of the chemical per kilogram of body weight (mg/kg/day). According to [21] USEPA, (2011), HQ is the ratio of the potential exposure to the

substances and the level at which no adverse effects are expected. If the HQ is calculated to be equal to or less than 1, then no adverse health effects are expected as a result of exposure as shown in Equation 2.

Estimated Daily Intake (EDI) (mg/kg bw/day) = Σ C x F/D x W

Equation 1

Where,

C= mean of imidacloprid concentration in samples (mg/kg)

F=mean of annual intake of white rice (kg)

D=number of days in a year (365 days)

W=mean body weight (kg)

Hazard Quotient (HQ) = EDI / ADI

Equation 2

3.   Results

### *3.1   Analysis of imidacloprid in different type of samples*

Table 1 shows the result of mean values of Imidacloprid concentrations for paddy and rice samples in Tanjung Karang, Selangor. The highest imidacloprid concentration was observed in paddy sample while the lowest imidacloprid concentration was found in white rice. The reduction of imidacloprid residue concentration was observed from paddy to white rice samples.

Table 1: Mean concentrations of imidacloprid in paddy, brown rice and white rice samples.

Samples	No samples		Mean ± S.D	Range (mg/kg)	[a] No. samples above MRL	[b] No. samples above MRL
	Analyzed	Detected (%)	(mg/kg)		(> 0.05 mg/kg)	(> 0.1 mg/kg)
Paddy	50	10(20)	0.14 ± 0.09	0.006-0.016	6	5
Brown rice	50	7(14)	0.04 ± 0.05	0.005-0.012	2	1
White rice	50	5(10)	0.003 ± 0.02	0.022- 0.555	1	0

[a] Codex Alimentarius Commissions, CODEX
[b] Malaysia Food Act 1983; Food Regulation 1985 in Sixteenth Schedule
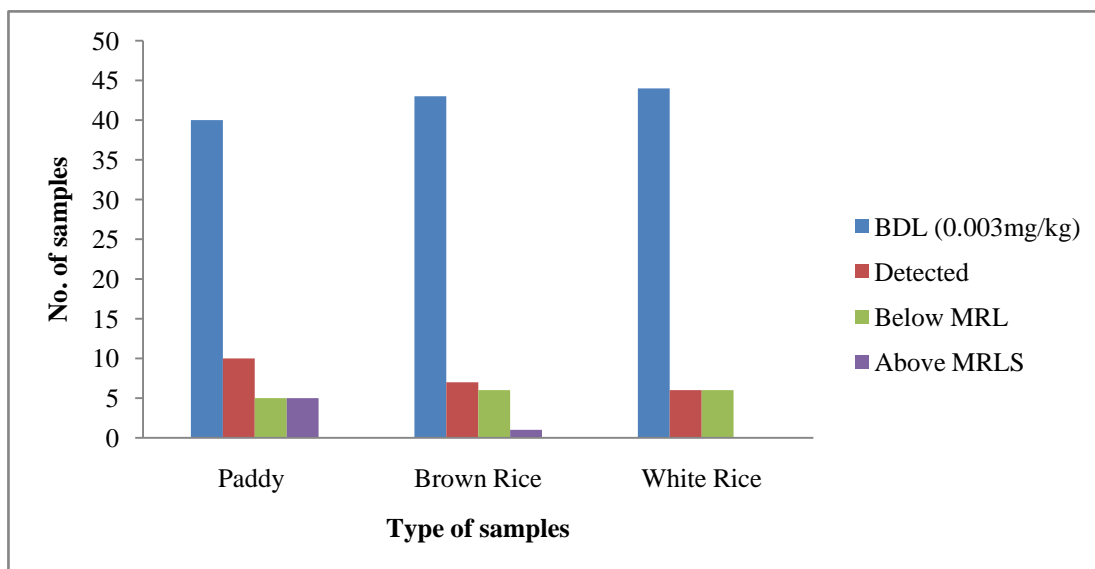
Fig. 3 Occurrences of imidacloprid residue in paddy and rice

Figure 3 shows the number of samples contained imidacloprid residue. The pesticides residue analysis result shows imidacloprid residue was found in all types of samples.

3.2 Health Risk Assessment

The health risk of imidacloprid on human health through the rice consumption was assessed through the Estimated Daily Intake (EDI) and Hazard Quotient (HQ). The EDI, ADI and HQ of imidacloprid residue among the age groups are shown in Table 2. The EDI was then compared to ADI set by the Joint FAO/WHO Meeting on Pesticide Residues (0.06kg) on the safe levels of pesticide residue in food.

Table 2: EDI and HQ of imidacloprid residue in white rice samples

Age group	Mean concentration of imidacloprid in rice (mg/kg) (C)	Mean annual intake of white rice per person (kg) (F)	No days in year (D)	Mean weight of person (kg) (W)	ADI (mg/kg bw daily)	EDI (mg/kg bw daily)	Hazard Quotient (HQ)
Children	0.003	2.16	365	10.78	0.06	0.0002	0.0035
Adolescent	0.003	87.6	365	42.68	0.06	0.03	0.56
Adult	0.003	135.05	365	66.75	0.06	0.08	1.35

The HQ values for children, adolescent and children were outlined in Figure 4. The adult population were exposed to a non-carcinogenic health risk via rice consumption as the HQ value was 1.35.
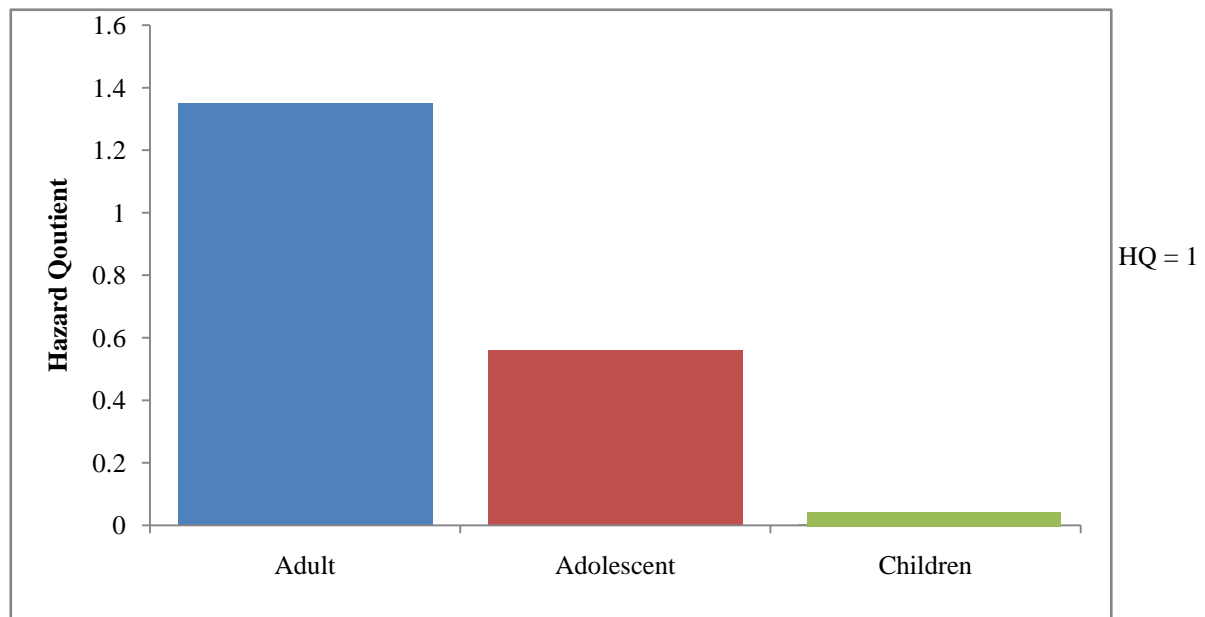
Fig. 4 Non cancer risk (HQ) of Imidacloprid in population adolescent and children

4. Discussion

4.1 Analysis of imidacloprid in different type of samples

Table 1 shows the result of mean values of Imidacloprid concentrations for paddy and rice samples in Tanjung Karang, Selangor. The highest imidacloprid concentration was observed in paddy sample while the lowest imidacloprid concentration was found in white rice. This supported by a study done by [22] which stated that the pesticides usage in Tanjung Karang is high in order maintain high level of rice output for local consumption. Imidacloprid was detected in 20% of the paddy sample, 14% of the brown rice sample and 10% of the white rice sample. The reduction of imidacloprid residue concentration was observed from paddy to white rice samples. The result was supported by a few of studies of which reported that this dissipation was due to the processing from paddy to rice [23, 24, 25, 26, 27].

In addition, [28] explained that pesticides residue was mostly dissipated during the rice processing. After harvesting and drying, the paddy was subjected to the primary milling operation which includes de-husking (hulling) as well as the removal of bran layers (polishing) before consumption. At the processing plant, the rice is cleaned and hulled to obtain unpolished rice also known as brown rice. The bran (the brown outer layer) and aleurone layer (the layer right under the bran) removal during polishing process attributed to the reduction in residue of imidacloprid as these layers generally bind the surface deposited pesticides residue [27].

Figure 3 shows the number of samples contained imidacloprid residue. The pesticides residue analysis result shows imidacloprid residue were found in all types of samples, however there are only paddy and brown rice sample had exceed the Malaysian MRL and Codex standard which were set by [28] (0.1mg/kg) and CODEX Alimentarius Commission (0.05mg/kg), respectively. More than 80% of the samples were observed below detection limit (BDL) (0.003mg/kg). From the 50 samples for each type of samples there were only 10, 7 and 5 of paddy, brown rice and white rice samples have been detected for imidacloprid residue, respectively. Apart

from that, 50% of paddy and 14% brown rice from the samples had exceeded the Malaysian MRL (0.1mg/kg). [30] Also stated that the pesticide distribution in food commodities is depending on many factors, such as matrix chemical composition and pesticide lipophilicity.

3.2 Health Risk Assessment

The health risk of imidacloprid on human health through the rice consumption was assessed through the Estimated Daily Intake (EDI) and Hazard Quotient (HQ). In this present study, no health risk assessment was carried out on the brown rice consumption as majority of the studied population did not consume brown rice. Thus the daily intake of imidacloprid residue was only determined on the consumption of white rice obtained from the community survey on the three age groups.

Generally, imidacloprid is a neonicotinoid insecticide which produces neurotoxicity through binding to specific areas of the nicotinic acetylcholine receptor [31]. According to the toxicological profiles from [32], Imidacloprid can give a non-carcinogenic health risk in which the health effects due to chronic exposure to Imidacloprid were observed on the liver, thyroid and reduction of body weight [31].

The EDI, ADI and HQ of imidacloprid residue among the age groups are shown in Table 2. The EDI was then compared to ADI set by the Joint FAO/WHO Meeting on Pesticide Residues (0.06kg) on the safe levels of pesticide residue in food. The average of rice consumption pattern (kg) and body weight (kg) for each age group were obtained from the community survey and were used in the HRA calculation. The EDI for children, adolescent and adult were 0.002, 0.03 and 0.08, respectively. The EDI of adult population exceeded the ADI value which indicated that the intake of imidacloprid residue through their rice consumption has exceeded the safe level.

The HQ values for children, adolescent and children were outlined in Figure 4. The adult population were exposed to a non-carcinogenic health risk via rice consumption as the HQ value was 1.35. [31] also stated that for general public, the highest HQ for ingestion exposures is 1.5 and this HQ is associated with the upper bound of plausible exposures for the longer-term consumption of contaminated food with Imidacloprid. However, Imidacloprid contamination in Tanjung Karang area did not pose non-carcinogenic health risks to adolescent and children via rice consumption because the HQ value was below than 1. Thus, this indicated that those adults are more vulnerable to health effects of Imidacloprid compared to the adolescent and children as adult consumed more rice than the adolescent and children, supported by study done by [33] also reported that adult had higher carbohydrate intake which is 240 g per day.

There a limitation found in this study. The limitation is the use of UHPLC- UV. This is because UHPLC-UV gave lower sensitivity compared to Liquid Chromatography Mass Spectrometry (LCMS). However, HPLC with conventional UV detection is still commonly used in most research laboratories as a routine method. This is because LC-MS is an expensive instrument as compared to the conventional HPLC-UV and DAD method [34, 35].

5.   Conclusions

The results underline that imidacloprid residues are present in paddy and rice samples originates from Tanjung Karang paddy cultivation area. This indicates that routine monitoring of this pollutant in food items is required to prevent, control and reduce the pollution and to minimize health risks. This research has provided important information on imidacloprid residues contamination on local paddy and rice for the first time

Acknowledgments

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