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PESTICIDE EXPOSURE OF RICE FARMERS AND HERBICIDE RESIDUE IN PADDY FIELD, SUPHAN BURI, THAILAND

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ABSTRACT

Suphanburi is rice cultivation area for two or three growing cycles. Agropesticide application is on intensively cultivated rice crop land when water conditions allow had occurred. This study was aimed to evaluate serum cholinesterase (SChE) and associated risk factors of pesticides exposure; and to determine paraquat and glyphosate in soil, water supply and harvesting rice samples as environmental residues. Gathered information concerning of long-term pesticide exposures from 50 rice farmers and 50 control respondents was conducted by questionnaire interviewing and SChE screening. Risky and unsafe rice farmers were confirmed for SChE level by automatic analyzer. The paraquat and glyphosate levels in environmental samples were analyzed by liquid chromatography- mass spectrometry/ mass spectrometry (LC-MS/MS). Rice farmers were long-term pesticide exposure over 10 years. Serum cholinesterase level was not significantly different between farmer and control group ($p = 0.033$). Pesticides relating symptoms were rarely observed and unexpected finding may due to unspecific symptoms, imprecisely explain and tolerance of frequently exposed farmers. Paraquat and glyphosate were detected from the soil (12/10), water (6/5) and rice (3/8) samples; however, the amount was lower than 0.05 mg/kg or mg/mL based on US EPA and FAO/WHO standard. Insignificant level of SChE in long-term exposure may lead to recommendation of biomarker measurements, such as, DAPs, 3-PBA and urinary glyphosate for evaluation of multiple pesticide use. Paraquat and glyphosate residues were within the acceptable range. Therefore, health and environmental effects of herbicide use were still concerned in rice farmers, passive exposures and consumers.

KEY WORDS : Environmental residues, Glyphosate, Herbicide, LC-MS/MS, Paraquat

INTRODUCTION

The agricultural system in Thailand has shifted from a traditional to a commercial agricultural system. Many previous studies were reviewed regarding pesticide use, poisoning, and knowledge and unsafe occupational practices in Thailand over the last decade (2006-2017); and Thai government is responsible in making policies and regulations and encouraging all agricultural activities to be sustainable (Sapbamrer, 2018). Herbicides are extensively used in agriculture to protect crops and increase yields.Paraquat and glyphosate are most common herbicide uses among Thai agriculture (Ofce of agricultural economics, 2017). Paraquat or gramoxone (1,1-dimethyl-4,4-bipyridnium dichloride) is a highly effective non-selective fast-acting contact herbicide and is widely used in more than 100 countries (Rashidipour *et al.*, 2019). Due to high environmental and human toxicity, it was banned in some countries (Bang *et al.*, 2017). Paraquat can cause neurological damage, and dysfunctional kidneys and liver in humans and animals (Shadnia *et al.*, 2018). Glyphosate is the most

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heavily used agricultural and residential herbicide in the world, and has been detected in soil, air, surface water, and groundwater, as well as in food (International Agency for Research on Cancer, 2015). Based on meta-analysis study, exposure of glyphosate-based-herbicides are at increased risk for risk of non-Hodgkin lymphoma (Zhang *et al.*, 2019).

The serum cholinesterase (SChE) is commonly useful clinical test for intoxication of neurotoxins, such as carbamate and organophosphate pesticides by monitoring of enzyme level in blood, which chemically interferes cholinesterase action. Decline of SChE is useful to indicate the rate or level of exposure including acute and chronic anticholinesterase exposures among field workers especially in sprayers (Giacobini, 2004; Sudjaroen, 2015). Thai Ministry of Public Health launched a nation-wide cholinesterase activity screening test, which had developed by using a reactive-paper finger blood test to determine and categorize risk of pesticide exposure (Bureau of Policy and Strategy, 2008). In a previous study, rice farmers in three Thai provinces were found to have the high prevalence of allergies, nasal congestion, wheezing, and acute symptoms after pesticide use (Kongtip *et al.*, 2018). Occupational health and safety problems among rice farmers may result from unsafe behaviors. These behaviors are related to agrochemical exposure, such as the use of faulty spraying equipment or lack of attention to safety precautions (Buranatrevedh and Sweatsriskul, 2005). Rice-growing farmers in NakhonNayok Province, the central part Thailand may be at risk for adverse health effects due to continuous dermal pesticide exposure from their improper use of personal protective equipment (PPE) (Lappharat *et al.*, 2014). Suphanburi province is located in central plain, which is a member of "rice bowl" of Thailand. Because of enough water supplies in this cultivation area, two or three rice growing cycles can be done rather than Northeast area. Agrochemicals for seeding and planting period are more use in Central area than in Northeast area, thus, farmers may expose to pesticides more frequenty rather than other area. Moreover, agropesticide application in Central of Thailand is on intensively cultivated rice crop land when water conditions improved (Grandstaff and Srisupun, 2004). This is a significant factor in allowing many farmers to continue growing rice, but it has also created health hazards (Monarca *et al.*, 2009). Objective of this study was to evaluate SChE level and associated risk factors of pesticides exposure, such as, degree of pesticide use, signs/symptoms after pesticide exposed practice and knowledge of personal protective equipment (PPE). Common herbicides used in Thailand, such as, paraquat and glyphosate, were also determined from soil, water supply and harvesting rice samples, which were collected from paddy field for environmental contaminant evaluation.

MATERIALS AND METHODS

Research area

The study was conducted inU-Thong district, Suphan Buri province, about 150 km from west of Bangkok, where rice is main harvesting product. Main of rice varieties in this area are Suphan Buri 1 (SPR1), Suphan Buri 2 (SPR2) and Khao Dawk Mali 105. Due to low river plain area of this province, rice growing cycles are two to three per year. Main of pesticide use is paraquat and glyphosate. Outsource pesticide sprayers were commonly found in rice field.

Sample collection

Soils and waters (n = 30) were collected from various location around rice cultivation area that is known as the intensive location on using of pesticides. The soil was collect using hand auger on the deep of 0-20 cm as a composite sample from 5 point in which each distance was 5 m using the diagonal method. Water was collected using grab water sampler that equipped with a clean plastic bottle. Preparation of these bottles included washing with detergent, rinsing with tap water and ultrapure water. Unrefined rice samples (n=15) were collected by random subsampling technique during end of harvesting season.

Environmental residue preparation and LC-MS/MS determination

Each sample was prepared and extracted by Quick Easy Cheap Effective Rugged and Safe (QuEChERS) technique (Villaverde *et al.*, 2018; Lozowicka *et al.*, 2017). Briefly, representative portion of soil (500 g) was air-dried at about 40 °C, sieved through a mesh with a grain size of 2 mm. Each water sample (500 mL) was centrifuged and filtered. Rice sample was air-dried as soil sample and grinded into powder form.

All sample types were stored at room temperature until analysis. An aliquot of 15 g of homogenized sample was placed in a 50 mL centrifuge, and 15 mL of acetonitrile was added. The mixture was vortexed for one minute, followed by adding 4 g of magnesium sulphate and 1 g of sodium chloride. The sample was centrifuged, and the supernatant was removed for clean-up. The clean-up was carried out by transferring the supernatant into another tube containing 50 mg of primary and secondary amine (PSA), 50 mg of graphite carbon black (GCB) and 150 mg of magnesium sulphate. After agitation and centrifugation, the aliquots of the extract were reconstituted to 3 mL with acetonitrile for liquid chromatography-mass spectrometry/ mass spectrometry (LC-MS/MS) analysis. Chromatographic separation was achieved using Agilent zorbax RRHD Eclipse plus C_{18}, 2.1x100 mm, 1.8 nm and conducted with Agilent 6460 triple quadrupole LC/MS with Agilent set Stream source. Mobile phase elution was included a) 5 mM ammonium formate and 0.01% formic acid in water; and b) 5mM ammonium formate and 0.01% formic acid in methanol. The flow rate was 0.3 mL/min. Mass spectrometric condition included gas temperature = 325 °C, gas flow = 9 L/min, nebulizer pressure = 40 psi, sheath gas temp = 400 °C and sheath gas flow = 11 L/min. Mass spectrometric analysis was performed under electrospray ionization in positive-ion mode (Jallow *et al.*, 2017). The precursor ions and the two transition ions (m/z) adopted for each of two analysts were paraquat (185; 169 and 115) and glyphosate (170; 88 and 60), respectively (Tsao *et al.*, 2016). The limit of detection (LoD) was >1 ppb (µg/L or µg/kg).

Data analysis

Herbicide residues were represented as frequency of detectable samples, which were higher than LoDand mean ± SD of each residue concentration. Statistical analysis was performed using the SPSS computer program version 20.0 (SPSS, Chicago, IL).

RESULTS AND DISCUSSION

Chronic pesticide exposure in rice farmers

No statistically significant difference between rice farmers and controls for gender and risked behaviors, such as, cigarette smoking and alcoholic consumption. All of rice farmers were long-term pesticide exposure and 60% of rice farmers used pesticide over 10 years; and rate of pesticide exposure was mainly for 1-2 time/week. The related

Table 1. Demographic data, pesticide-used history and practice in rice farmers and control

Demographic data		Frequency (%)		*p*-value
		Rice farmer (n = 50)	Control (n = 50)	
Gender :	Male	34 (68)	30 (60)	0.405
	Female	16 (32)	20 (40)	
Age :	< 40 years	22 (44)	40 (80)	0.0001*
	≥ 40 years	28 (56)	10 (20)	
Alcohol intake:	none	36 (72)	33 (66)	0.517
	drinking	14 (28)	17 (34)	
Duration of pesticide use				
	< 4 years	-	-	
	4-9 years	18 (36)	-	
	> 10 years	32 (64)	-	
Rate of pesticide exposure				
	1-2 days/week	30 (60)	-	
	3-4 days/week	4 (8)	-	
	5-6 days/week	16 (32)	-	
Clinical symptoms				
	None	36 (72)	-	
	Headache/vertigo	11 (22)	-	
	Abdominal cramp	3 (6)	-	
	Breathless	-	-	
	Muscle convulsion	-	-	
Health education:	none	13 (26)	-	
	educated	37 (74)	-	

pesticide used symptoms rarely occurred and almost of them had health education for awareness of pesticide uses. However, unexpected finding may due to unspecific symptoms, imprecisely explained by personal interviewing and tolerance of frequently exposed farmers.

Most of rice farmers were known about pesticide health effects (80%), however, some of them were ill informed on practice, such as, the reading of pesticide label, first aids for toxicity and appropriated skill on spraying (Table 1). The serum cholinesterase screening of rice farmers by paper test were interpreted as normal, safe, risky and unsafe, for 5, 19, 15 and 11, respectively. Therefore, serum cholinesterase level was within reference value when analyzed with automatic analyzer. Some of unsafe farmers had serum cholinesterase level at lower borderline of reference value (Table 2).

In this study, rice farmers had long-term pesticide exposure and used pesticide frequently. However, occurrence of related symptoms was rare, which may be due to non-specific symptoms and difficult to define. Most of the rice farmers are educated about health awareness and health protection on pesticide use. In contrast, poor practices were common among them, because of public health staffs from government are providing only education program, however, the on-site occupational practice and providing of PPE was done only occasionally. Risky and unsafe rice farmers were screened by paper test, which was useful by high sensitivity. However, unsafe rice farmers had serum cholinesterase level within reference range, which was analyzed by quantitative methods. It may imply that serum cholinesterase may not be a good marker for quantifying exposure to pesticide among sprayers, especially during spraying season. As agriculturists can be exposed to pesticides divided into sprayers, agriculturists and other professions, however, the serum cholinesterase levels among them were not significantly difference and level was still within reference range (Mathew *et al.,* 2015). Most of studies on Thai pesticide exposure had reported exposure of single type of pesticide; however, mixed pesticide uses are more common for multi-crop cultivation. Thus, screening of other biomarkers rather than serum cholinesterase such as, alkyl phosphate metabolites (DAPs), urinary 3-phenoxybenzoic acid (3-PBA) and urinaryglyphosate, is still necessary for public provider.

Environmental residues

The chromatogram and transition ion (m/z) of paraquat and glyphosate were represented in Fig. 1. Paraquat was detected from 12, 6 and 3 of soil, water and rice samples, respectively. Glyphosate was also detected from 10, 5 and 8 of soil, water and rice samples (Table 1). All detectable samples were lower than national standard of pesticide residues in soil, water and food (<0.05 mg/kg or mg/mL), which were based on United States Environmental

Table 2. Knowledge, attitude and practice of rice farmers for pesticide use

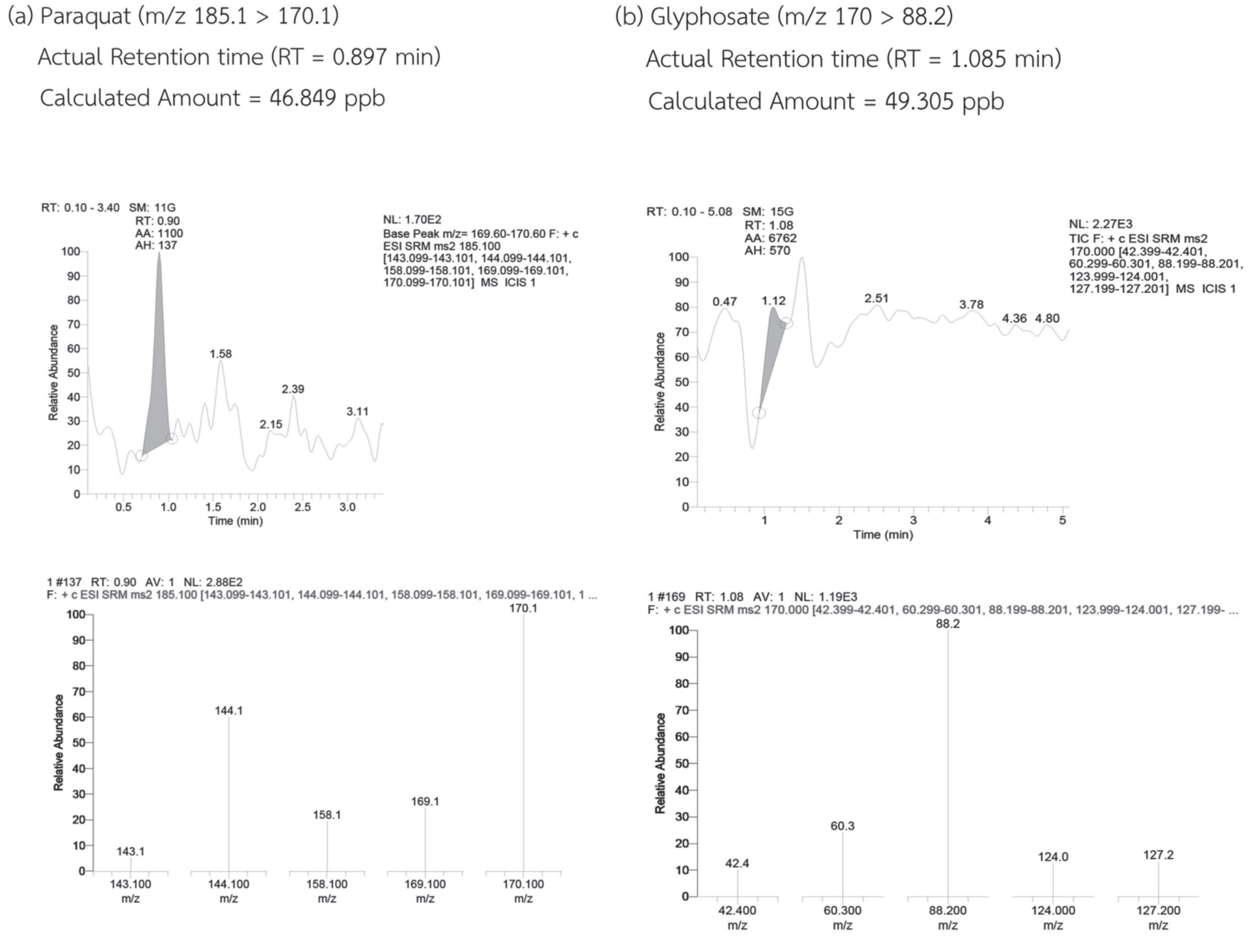
Topic of Pesticide use	Frequency (%)	
	Uneducated	Educated
Health effects of pesticide	10 (20)	40 (80)
First-aids for acute intoxication	23 (46)	27 (54)
Personal protective equipment	17 (34)	33 (66)
Reading and understanding on information of pesticide label	24 (48)	26 (52)
Cleaning of pesticide spraying clothes	15 (30)	35 (70)
Pesticide container management	17 (34)	33 (66)
Appropriate in pesticide spraying	21 (42)	29 (58)
Personal hygiene after pesticide spraying	15 (30)	35 (70)

Table 3. Comparison of serum cholinesterase between rice farmers and controls

Group	Serum cholinesterase (U/L) [a]	*p*-value
Control (n = 50)	7775.98 ± 1152.80	0.033
Rice farmer (n = 50)	7247.26 ± 1293.30[b]	
Reference value	**5,320 – 12,920**	**-**

[a] Calculated from automatic analysis and represented as mean ±SD

[b] Serum cholinesterase was screened by paper test before tested with automatic analyzer.



(a) Paraquat (m/z 185.1 > 170.1)
Actual Retention time (RT = 0.897 min)
Calculated Amount = 46.849 ppb

(b) Glyphosate (m/z 170 > 88.2)
Actual Retention time (RT = 1.085 min)
Calculated Amount = 49.305 ppb

Fig. 1. Chromatographic separation and transition ions (m/z) of (a) paraquat and (b) glyphosate in soil, water supplies and rice collected from rice paddy field

Protection Agency (U.S. EPA)and Food and Agricultural Organization (FAO) (U.S. EPA, 2017; FAO, 1998).

Long-term exposure of paraquat leads to harmful biomagnification in human and mammals (Frimpong *et al.,* 2018); and extensive paraquat use leads to widespread residues in soil and water resources, which are involved in food chain (Pateiro-Moure *et al.,* 2009). Paraquat is strongly adsorbed to soil and very existent up to 20 years, which is dependent on soil texture. Paraquat is also bound to aquatic suspension or sediment in long period depending on sunlight and water depth (Watts, 2011) and finding in surface water (Poranee *et al.*, 2012). Dietary exposure assessment of paraquat in Thai rice is 3.8% of Acceptable Dietary Intake (ADI), which will satisfy within 2.2-4.7% of ADI and lower than maximum residue limit (MRL) according by FAO/WHO(Joint FAO/WHO Meeting on Pesticide Residues, 2011). We were detected paraquat in soil, water supplies and harvesting rice, which were collected from the study area. The amount of paraquat was not of critical levels (<0.05 mg/kg or mg/mL) as environmental and food residue aspects.

In contrast to paraquat, individuals may be exposed to glyphosate through various routes such as food anddrinking water, both in the occupational and environmental settings, however, carcinogenic potential of glyphosate has been under review and debated by multiple authoritative and regulatory bodies. IARC classified glyphosate as a "probable human carcinogen"; EFSA declared that "glyphosate is unlikely to pose a carcinogenic hazard to humans"; and US EPA reviewed and concluded that it is "not likelyto be carcinogenic to humans". The controversy over glyphosate's carcinogenic classification is based on various aspects, including differences in the weight placed on the results of human epidemiological studies (Gillezeau *et al.*, 2019). Therefore, extensive glyphosate use may

Table 4. Concentration of paraquat and glyphosate in soil, water supplied and rice (n = 45)

Herbicide	*n*	Residue detected (LoD> 1 ppb)			Concentration (ppb)*		
		S	W	R	S	W	R
Paraquat	21	12	6	3	47.8 ± 0.03	23.5 ± 0.16	15.1 ± 0.78
Glyphosate	23	10	5	8	41.7 ± 0.28	18.5 ± 0.36	12.5 ± 0.76

n = numbers of detectable sample; S = soil, W = water supplies, R = harvesting unrefined rice
*Concentration is representing as mean ± SD

associate to various of human diseases, including cancer, kidney damage and mental conditions, suchas attention-deficit hyperactivity disorder (ADHD), autism, Alzheimer's and Parkinson's disease. Glyphosate is degrading to glyphosate derivative, aminomethylphosphonic acid (AMPA) by microorganisms. The half-life of AMPA is 60–240 days and can bedecomposed to inorganic phosphate, ammonium ions and carbon dioxide (Van Bruggen *et al*., 2018). The accumulation of glyphosate in soil by excessive amount was reduced earthworms and may affect to terrestrial environments (Battaglin *et al*., 2014). In this study, glyphosate levels from all sources were lower than ADI (0.3 mg/kg body weight) and drinking water standards (<0:9 mg/L), which were based on the Joint FAO/WHO meeting on pesticide residues (JMPR) (JMPR, 1994).

Paraquat and glyphosate residues were represented in soil, water supplies and harvesting rice, which were collected in nearby cultivation area and the detectable levels of both herbicides were within the acceptable range. Therefore, health and environmental effects of herbicide use were still concerned in rice farmers, passive exposures and consumers; organic cultivation is trend to solve this problem.

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