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ORIGINAL ARTICLE

ASSOCIATION BETWEEN BLOOD CHOLINESTERASE ACTIVITY, ORGANOPHOSPHATE PESTICIDE RESIDUES ON HANDS, AND HEALTH EFFECTS AMONG CHILI FARMERS IN UBON RATCHATHANI PROVINCE, NORTHEASTERN THAILAND

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ABSTRACT

Background. Use of pesticides has been documented to lead to several adverse health effects. Farmers are likely to be exposed to pesticides through dermal exposure as a result of mixing, loading, and spraying. Organophosphate pesticides (OPs) are widely used in most of the agricultural areas throughout Thailand. OPs are cholinesterase inhibitors and blood cholinesterase activity is used as a biomarker of OP effects.

Objective. This study aims to determine the association between blood cholinesterase activity and organophosphate pesticide residues on chili farmer's hands and their adverse health effects.

Materials and Methods. Ninety chili farmers directly involved with pesticide applications (e.g. mixing, loading, spraying) were recruited and were interviewed face to face. Both enzymes, erythrocyte acetylcholinesterase (AChE) and plasma cholinesterase (PChE), were tested with the EQM Test-mate Cholinesterase Test System (Model 400). Hand wipe samples were used for collecting residues on both hands and OP residues for chlorpyrifos and profenofos were quantified using gas chromatography equipped with a flame photometric detector (GC-FPD).

Results. The average activity (±SD) of AChE and PChE was 2.73 (±0.88) and 1.58 (±0.56) U/mL, respectively. About 80.0% of the participants had detectable OP residues on hands. The median residues of chlorpyrifos and profenofos were found to be 0.02 and 0.03 mg/kg/two hands, respectively. Half of participants reported having some acute health symptoms within 48 hours after applying pesticides. When adjusted for gender, number of years working in chili farming, and frequency of pesticide use, AChE activity (Adjusted OR = 0.03, 95%CI: 0.01-0.13) and detected OP residues on hands (Adjusted OR = 0.15, 95%CI: 0.02-0.95) were significantly associated with having health effects, but no significant association was found in PChE activity (Adjusted OR = 2.09, 95%CI: 0.63-6.99).

Conclusions. This study suggests that regular monitoring for blood cholinesterase and effective interventions to reduce pesticide exposure to prevent health effects should be provided to chili farmers.

Keywords: *cholinesterase activity, organophosphate pesticide residues, pesticide exposure, health effects*

INTRODUCTION

Thailand is one of the world's largest exporters of agricultural commodities. About 12.09 million Thai people work in the agricultural sector which leads to farming being the top occupation in Thailand [21]. Thailand has been promoting pesticide usage to increase yields and improve the quality of crops. In 2010-2015, around 147,746 tons of pesticides were annually imported to serve the agricultural sector, valued at 600 million USD per year [25]. However, the current use of pesticides among Thai farmers seems to be less effective due to their extensive and inappropriate use which causes environmental contamination and health problems [38]. In 2014, there were 7,954 Thai people afflicted with pesticide poisoning, or 12.25 per 100,000 population; 32.06% were farmers. The major cause of poisoning was organophosphates (OPs) and carbamates (CAs) [3].

Organophosphate pesticides (OPs) are widely used in agriculture throughout Thailand [3]. OPs are cholinesterase inhibitors and high dose exposure to OPs can cause acute effects such as gastrointestinal upset, sweating, tearing, urination problems, bronchial spasms, muscle twitching, muscle weakness, bradycardia, and coma [6, 13, 16, 29]. For chronic exposure at low to moderately high doses, poisoning symptoms include headache, dizziness, nausea, vomiting, abdominal pain, blurred vision, and chest tightness [3, 6, 13, 14, 15,16,

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29, 30, 31, 35, 41]. Moreover, there is evidence linking OP exposure to reproductive effects, non-Hodgkin's lymphoma, and cancer [13].

Thai farmers are exposed to pesticides *via* multiple routes i.e., inhalation, dermal absorption, and unintentional ingestion [36]. For most pesticide handling situations, dermal is most likely to be the given exposure [7]. OP residues on hands can be represented as indicators of exposure [4], and blood cholinesterase enzymes can be used as a biomarker of exposure effects particularly to OPs and CAs [27]. Both enzymes, erythrocyte acetylcholinesterase (AChE) and plasma cholinesterase (PChE) should be measured, as these results will have different value. The AChE activity measure is advantageous to evaluate chronic exposure of OPs and CAs, while the PChE measure is worthwhile in detecting early acute effects of OPs and CAs poisoning [13, 27, 29].

Few studies have investigated the association between cholinesterase (ChE) activity, pesticide exposure, and adverse health effects [5, 14]. Therefore, this study aims to determine the potential association between blood cholinesterase activity, organophosphate pesticide residues on hands, and adverse health effects in chili farmers.

MATERIALS AND METHODS

Study area and subjects

This study was a cross-sectional descriptive study located at Hua Ruea Subdistrict, Mueang District, Ubon Ratchathani Province, Thailand. This area was chosen because of the large number of farmers and agricultural area. It covers a total area of 7,978.9 acre. Over 84% of the total area is under cultivation and the main crops year round include rice, chili, and vegetables. With a population of 9,075 residing in 2,632 households, most of the population is farmers [12].

A total of 90 chili farmers living in Hua Ruea Subdistrict were enrolled. To be eligible for the study, the farmers had to be ≥18 years of age, settled in this area at least 1 year, directly involved with all steps of pesticide application (e.g., mixing, loading, spraying), and no communication problems. Those who had health problems e.g., alcoholism, liver failure, cardiovascular disease, malnutrition, drug addiction, and taking anti-malarial drugs, were excluded.

Data collection

Data collection was done in April 2015 (the high chili growing season). Face-to-face interviews were completed with all participants. Each participant was questioned about demographic characteristics, work characteristics, types and frequency of pesticide use, and acute symptoms related to pesticide exposure.

Cholinesterase measurement

Blood samples (20 µL) were taken from a cleaned fingertip of each participant in capillary tubes during the period of pesticide application by nurses. Blood enzymes erythrocyte acetylcholinesterase (AChE) and plasma cholinesterase (PChE) were tested with the Test-mate ChE Cholinesterase Test System (Model 400) [9, 23, 28, 31, 41], a field spectrophotometric analyzer based on the Ellman method [8]. The results were expressed as units per milliliter (U/mL).

Pesticide residue measurement

Hand wipe sampling method

The hand wipe sampling method was modified from *Geno* et al. [11] and *Taneepanichskul* et al. [36, 37]. Hand wipes were performed using sterilized and chemical free gauze pads (size: 4´4 inches, 8 ply) wetted with a 40% solution of isopropyl alcohol, 10 mL per pad. Two gauze pads were used for wiping pesticide residues on both hands of each participant. Then the wipes were wrapped in laboratory aluminum foil and placed in zip-lock plastic bags. All hand wipe samples were stored in cold boxes with ice packs, shipped to the laboratory, and refrigerated at -20°C until extraction within 7 days and analyzed afterward by gas chromatography.

Extractions of organophosphate pesticides in wipe samples

An extraction method of OP pesticides was adapted from *Farahat* et al. [10] and *Lapparat* et al. [17] to measure pesticide residues on farmer's hands. First, wipe samples were put into a 250-mL flask with 40 mL of ethyl acetate, then agitated *via* a mechanical shaker for 10 min at 150 rpm. Wipe samples were transferred into a second 250-mL flask with 40 mL of ethyl acetate and shaken with a mechanical shaker for 5 min at 150 rpm. The solvent from both flasks were combined and then evaporated by using air pumps until the volume was less than 1.0 mL. The residue was dissolved in 1.0 mL of acetone (pesticide grade). The solution was transferred to a 1.5-ml microcentrifuge tube. After centrifugation for 10 min at 10,000 rpm, only the liquid phase was transferred to a sample vial. Finally, the volume was adjusted with acetone (pesticide grade) to 1.0 mL.

Gas chromatography analysis

Wipe samples were analyzed for chlorpyrifos and profenofos, which were extensively used in this area [24, 36, 37], using an Agilent 7890A gas chromatography (GC) equipped with a flame photometric detector (FPD). The GC run conditions were [17]: HP-5 capillary column (HP-5, 30 m ´ 0.32 mm id, 0.25-µm film thickness) coated with 5% phenyl methyl siloxane. Nitrogen used as carrier gas was set to a flow rate at 2 mL/min, while makeup gas was at 45 mL/min. Air and hydrogen used as detector gas was regulated at 100 and 75 ml/min, respectively. Initially, 1.0 µL of sample was injected into the GC on splitless mode.

The initial temperature of injector and detector was 230 °C and 250 °C, respectively. The initial condition of the oven was set at 100 °C for 2 min, and then it was programmed to increase at 10 °C/min to 220 °C. The total run time was 24 min. The chromatogram in Figure 1 demonstrates the retention time of chlorpyrifos and profenofos at 9.903 and 11.540 mins, respectively.

Quality control

A calibration curve for quantification was performed using a series of standard solutions at nine concentration levels ranging from 0.001-10.000 µg/mL. The correlation coefficient (r^2) of chlorpyrifos and profenofos was 0.99951 and 0.99931, respectively. For analytical control, the standard solutions were confirmed in every 10 sample measurements presented in the range of linearity. The limit of detection (LOD) was 0.01 mg/kg for chlorpyrifos and 0.02 mg/kg for profenofos. The limit of quantitation (LOQ) for chlorpyrifos and profenofos was 0.02 and 0.05 mg/kg, respectively. The mean recovery of extractions for profenofos was 94.8%, which was in an acceptable range of 80-120% following the Association of Official Agricultural Chemists (AOAC) recommendations [2]. The mean recovery for chlorpyrifos was 64.9%, which was lower than the acceptable range and was a limitation of this study.

Data analysis

Descriptive statistics were used to describe information regarding demographic characteristics, types and frequency of pesticide use, and prevalence of symptoms related to pesticide exposure. *Kolmogorov-Smirnov* tests were used to test distributions for continuous variables. The associations between ChE activity and symptoms related to pesticide exposure were investigated by using point biserial correlation. The relationship between detected OP residues and symptoms were evaluated by using a *Chi*-square test and *Fisher's* exact test. Binary logistic regression analysis was performed to determine potential associations between ChE activity, detected OP residues on hands, and health effects related to pesticide exposure. In our logistic regression analyses, the dependent variable was having health effects (0 = no, 1 = yes), that was defined as "no" when participants reported having none of acute symptom related to pesticide exposure; it was defined as "yes" when participants reported having at least 1 symptom. The independent variables were AChE and PChE activity (continuous) as well as detected OP residues on hands (0 = no, 1 = yes). Odds ratios (OR) and 95% confidence intervals (95%CI) were derived from the logistic regression models. All analyses were conducted with the SPSS statistical software package version 16.0. The significance level was set at 0.05 and 0.01.

For statistical analysis, if the results of OP residues were reported as zero or below the LOD, they were substituted with the LOD [22]. The detected OP residues on hands were defined as "yes" if wipe samples found chlorpyrifos or profenofos or both residues higher than the LOD, otherwise if the residues were lower than the LOD they were considered as "no".

For interpretation of ChE results, the ChE values were classified by using mean values for cut-off points into 2 levels such as abnormal and normal level [23, 28, 31]. If the value was equal to or less than 2.73 U/mL for AChE, and 1.58 U/mL for PChE, it was considered "abnormal level". It was assumed that participants could possibly have pesticide poisoning. If the value of AChE and PChE was more than 2.73 and 1.58 U/mL respectively, it indicated "normal level".

Ethical consideration

This study was approved by the Ethic Review Committee for Research Involving Human Research Subjects, Health Science Group, Chulalongkorn University (Certified code no. 078/2558). All participants signed the written consent before participated in the study.

RESULTS

Demographic characteristics

Demographic characteristics of the participants are summarized in Table 1. Over half of the participants were males (53.3%). The participant's age was in the range of 29 to 83 years. The mean (±SD) age was 49.6 (±10.4) years. The majority of participants (73.6%) had graduated primary school. About 23.3% of participants reported having some chronic diseases, e.g., peptic ulcer, and hypertension. Only 36.7% of participants were drinkers and 20.0% were smokers. All participants worked on chili farms for an average (±SD) of 18.2 (±9.6) years and most of them (74.4%) had owned chili farms of approximately 0.4 to 0.6 acres in size. Also, 62.2% of participants grew other crops during the chili growing season, e.g., spring onions, corianders, and long beans. Over 70.0% of participants had another family members working in chili farming. All participants joined in pesticide application i.e., mixing or loading (83.3%), and spraying (93.3%). Approximately 47.8% of participants applied pesticides twice monthly.

Pesticides used in chili farming

A variety of pesticides were used in chili farming. All of the participants used insecticides, 91.1% used herbicides, and 61.1% used fungicides. The most common insecticides used were avermectins (90.0%), followed by organophosphates, i.e., chlorpyrifos (35.6%), profenofos (33.3%), dimethoate (1.1%), as well as carbamates i.e., methomyl (20.0%), and are detailed in Table 2. Out of 82 participants using herbicides, paraquat (91.5%) was the most often used. Among 55 participants using fungicides, propineb (94.5%) was the most common used. However some participants could not remember the name of pesticides used, so the percent of pesticide use in Table 2 may be underestimated.

Table 1. Demographic characteristics of 90 chili farmers

Characteristics	No. of chili farmers	Percent (%)
Gender		
Male	48	53.3
Female	42	46.7
Age (year)		
Mean ± SD (Min-Max)	49.56 ± 10.36 (29.00-83.00)	
Education level		
Primary education	68	75.6
Secondary education	12	13.3
High school education	7	7.8
Bachelor's degree or higher	3	3.3
Had any chronic disease		
No	69	76.7
Yes	21	23.3
Alcohol consumption		
No	57	63.3
Yes	33	36.7
Smoking habit		
No	72	80.0
Yes	18	20.0
Number of years working in chili farming		
Mean ± SD (Min-Max)	18.16 ± 9.56 (1.00-42.00)	
Chili farm size (acres)		
0.4 - 0.8	67	74.4
0.9 - 1.6	13	14.4
>1.6	10	11.1
Growing other crops during chili growing season		
No	34	37.8
Yes	56	62.2
Frequency of pesticide use per month		
1	22	24.4
2	43	47.8
3	8	8.9
4	17	18.9

Table 2. List of insecticides used in chili farming reported by 90 chili farmers

Chemical class	Common name (Active ingredients)	Trade name	No. of response	%
Botanical, Macrocyclic Lactone	Avermectins	Abamectin, Avermectins	81	90.0
Organophosphate	Chlorpyrifos	Podium, Chlorpyrifos	32	35.6
	Profenofos	Selecron	30	33.3
	Dimethoate	Bazooka	1	1.1
Carbamate	Methomyl	Lannate	18	20.0
Neonicotinoids	Imidacloprid	Provado	31	34.4
Pyrethroid	Cypermethrin	Cypermethrin	8	8.9
	Chlorpyrifos+Cypermethrin	Lampard	3	3.3

Table 3. Cholinesterase activity of the 90 chili farmers

Biomarker	Mean ± SD (U/mL)	Range (U/mL)	Abnormal*		Normal**	
			n	%	n	%
AChE	2.73 ± 0.88	1.20 - 7.17	45	50.0	45	50.0
PChE	1.58 ± 0.56	0.47 - 3.11	46	51.1	44	48.9

* Abnormal level was considered if the value was ≤ 2.73 U/mL for AChE and ≤ 1.58 U/mL for PChE

** Normal level was considered if the value was > 2.73 U/mL for AChE and > 1.58 U/mL for PChE

Table 4. Percentage of positive wipe samples and OP residues (n=90)

Pesticides	Detection frequency [a] (%)	Residues (mg/ kg/ two hands)				
		Range	Median	Mean	SE	SD
OPs	72 (80.0%)	<LOD - 3.41	0.05	0.13	0.04	0.38
Chlorpyrifos	61 (67.8%)	<LOD - 0.96	0.02	0.04	0.01	0.11
Profenofos	58 (64.4%)	<LOD - 3.34	0.03	0.09	0.04	0.36

[a] Detection frequency = Number of wipe samples with detected OP residues higher than the limit of detection (LOD)
LOD = 0.01 mg/kg for chlorpyrifos, and 0.02 mg/kg for profenofos
SE = Standard error of mean, SD = Standard deviation

Cholinesterase activity

The average activity was 2.73 U/mL (±0.88 U/mL) for AChE and 1.58 U/mL (±0.56 U/mL) for PChE. The prevalence of abnormal AChE levels in farmers in this study was 50.0% equal to that of normal levels. For PChE, the prevalence of abnormal levels was 51.1% which was slightly greater than the normal level of 48.9% (Table 3).

Organophosphate pesticide residues on chili farmers' hands

About 80.0% of 90 wipe samples were found to have OP residues, in which 52.2% were found to have both chlorpyrifos and profenofos, 27.8% were found to have either chlorpyrifos or profenofos, and the remaining 20.0% had no residues. As shown in Table 4, 67.8% of the samples were detected with chlorpyrifos, while 64.4% were found to have profenofos. The median residues of chlorpyrifos and profenofos were found to be 0.02 and 0.03 mg/kg/two hands, respectively.

Acute health symptoms related to pesticide exposure

Half of the participants reported having some health symptoms during 48 hours after applying pesticides, in which 27.8% of participants reported 1-3 symptoms and 22.2% of participants reported more than 3 symptoms. The prevalence of acute health symptoms related to pesticide exposure is shown in Table 5. The top three health symptoms were reported to be headache (31.1%), dizziness (27.8%), and fatigue or weakness (22.2%). The main gastrointestinal symptom commonly reported was nausea or vomiting (15.6%). The respiratory symptom most often reported was cough (14.4%). Itching or burning (13.3%) was the most often skin symptom reported.

Table 5. Prevalence of acute symptoms related to pesticide exposure and its associations with ChE activity and detected OP residues on hands

Symptoms	No. of response	AChE[*]		PChE[†]		Chlorpyrifos[**]		Profenofos[**]	
		r_{pb}	p	r_{pb}	p	c^2	p	c^2	p
Respiratory									
Cough	13 (14.4%)	-0.22	0.04[*]	0.05	0.68	0.58[a]	0.54	1.03[a]	0.37
Sore throat, dry throat	10 (11.1%)	-0.17	0.12	0.04	0.70	2.54[a]	0.16	0.15[a]	0.75
Difficulty in breathing	6 (6.7%)	-0.19	0.08	0.01	0.95	0.00[a]	1.00	1.00[a]	0.42
Chest pain	5 (5.6%)	-0.13	0.22	0.11	0.29	0.15[a]	1.00	4.56[a]	0.05
Skin									
Itching, burning	12 (13.3%)	-0.28	<0.01[**]	0.09	0.39	0.33[a]	0.75	3.14[a]	0.11
Rash	8 (8.9%)	-0.23	0.03[*]	0.18	0.09	0.11[a]	1.00	2.78[a]	0.13
Muscle									
Numbness	4 (4.4%)	-0.04	0.72	0.06	0.58	0.61[a]	0.59	2.84[a]	0.13
Cramp, pain	4 (4.4%)	-0.09	0.41	-0.10	0.36	0.10[a]	1.00	0.38[a]	0.61
Muscle weakness	3 (3.3%)	-0.12	0.25	-0.01	0.90	0.00[a]	1.00	0.01[a]	1.00
Central nervous system									
Headache	28 (31.1%)	-0.46	<0.01[**]	0.14	0.19	0.93	0.47	0.95	0.33
Dizziness	25 (27.8%)	-0.48	<0.01[**]	-0.04	0.73	0.23[a]	0.80	2.34	0.15
Fatigue, weakness	20 (22.2%)	-0.36	<0.01[**]	0.24	0.02[*]	0.06	1.00	0.22	0.64
Blurred vision	9 (10.0%)	-0.12	0.25	0.14	0.20	0.01[a]	1.00	1.75[a]	0.27
Gastrointestinal system									
Nausea, vomiting	14 (15.6%)	-0.29	<0.01[**]	-0.01	0.91	0.86[a]	0.54	5.97[a]	0.02[*]
Abdominal pain	9 (10.0%)	-0.12	0.26	-0.09	0.41	0.68[a]	0.46	7.78[a]	0.01[*]
Diarrhea	2 (2.2%)	-0.04	0.71	0.16	0.15	0.97[a]	0.56	1.13[a]	0.54
Others									
Excessive sweating	7 (7.8%)	-0.18	0.10	0.15	0.15	0.05[a]	1.00	0.18[a]	0.70
Excessive salivation	3 (3.3%)	-0.14	0.19	0.12	0.27	0.00[a]	1.00	0.01[a]	1.00
Lacrimation	2 (2.2%)	0.00	0.99	-0.02	0.84	0.30[a]	1.00	3.71[a]	0.12
Brittle nails, nail loss	2 (2.2%)	-0.02	0.82	-0.05	0.62	4.30[a]	0.10	3.71[a]	0.12

[*] Point biserial correlation analysis, r_{pb} = point biserial correlation coefficient
[**] *Chi*-square test, [a] Fisher's exact test, [*] Significant at $p < 0.05$, [**] Significant at $p < 0.01$

Table 6. Associations between ChE activity, detected OP residues and having health effects related to pesticide exposure by binary logistic regression analysis

Variables	Crude			Adjusted†		
	OR	95%CI	*p-value*	OR	95%CI	*p-value*
AChE	0.13	0.05-0.32	<0.01**	0.03	0.01-0.13	<0.01**
PChE	1.13	0.54-2.39	074	2.09	0.63-6.99	0.23
Detected OP residues‡	0.57	0.20-1.64	0.30	0.15	0.02-0.95	0.04*

* Adjusted for gender, number of years working in chili farming, frequency of pesticide use

** Detected OP residues on hands (0= no, 1 = yes)

OR = odds ratios, 95% CI = 95% confidence interval

* Significant at P <0.05, ** P <0.01

Associations of ChE activity and detected OP residues to health symptoms related to pesticide exposure

The associations of ChE activity and detected OPs on hands to symptoms related to pesticide exposure are shown in Table 5. AChE activity had moderately inverse associations with dizziness (point biserial correlation coefficient, r_{pb} = -0.48, p <0.01) and headache (r_{pb} = -0.46, p <0.01). It also showed weakly inverse associations with fatigue or weakness (r_{pb} = -0.36, p <0.01), cough (r_{pb} = -0.22, p=0.04), skin itching or burning (r_{pb} = -0.28, p <0.01), skin rashes (r_{pb} = -0.23, p=0.03), and nausea or vomiting (r_{pb} = -0.29, p <0.01). PChE activity had a weakly positive association with only fatigue or weakness (r_{pb} = 0.24, p=0.02). Furthermore, detected profenofos residues were significantly related to nausea or vomiting and abdominal pain (Fisher's exact test, p=0.02 and 0.01, respectively), while detected chlorpyrifos residues did not show significant association with any symptoms.

Associations between ChE activity, detected OP residues on hands, and having health effects related to pesticide exposure

The results of binary logistic regression analysis are presented in Table 6. Increased activity of AChE was significantly associated with decreased odds of having health effects related to pesticide exposure (Crude OR = 0.13, 95%CI: 0.05-0.32), and its association was still in the same direction after adjusting for gender, number of years working in chili farming, and frequency of pesticide use (Adjusted OR = 0.03, 95%CI: 0.01-0.13). No statistically significant relationship was observed between PChE activity and having health effects whether adjusted for the confounding factors or not. Moreover, detected OP residues on hands were statistically significantly associated with having health effects (Adjusted OR = 0.15, 95%CI: 0.02-0.95) when adjusted for gender, number of years working in chili farming, and frequency of pesticide use.

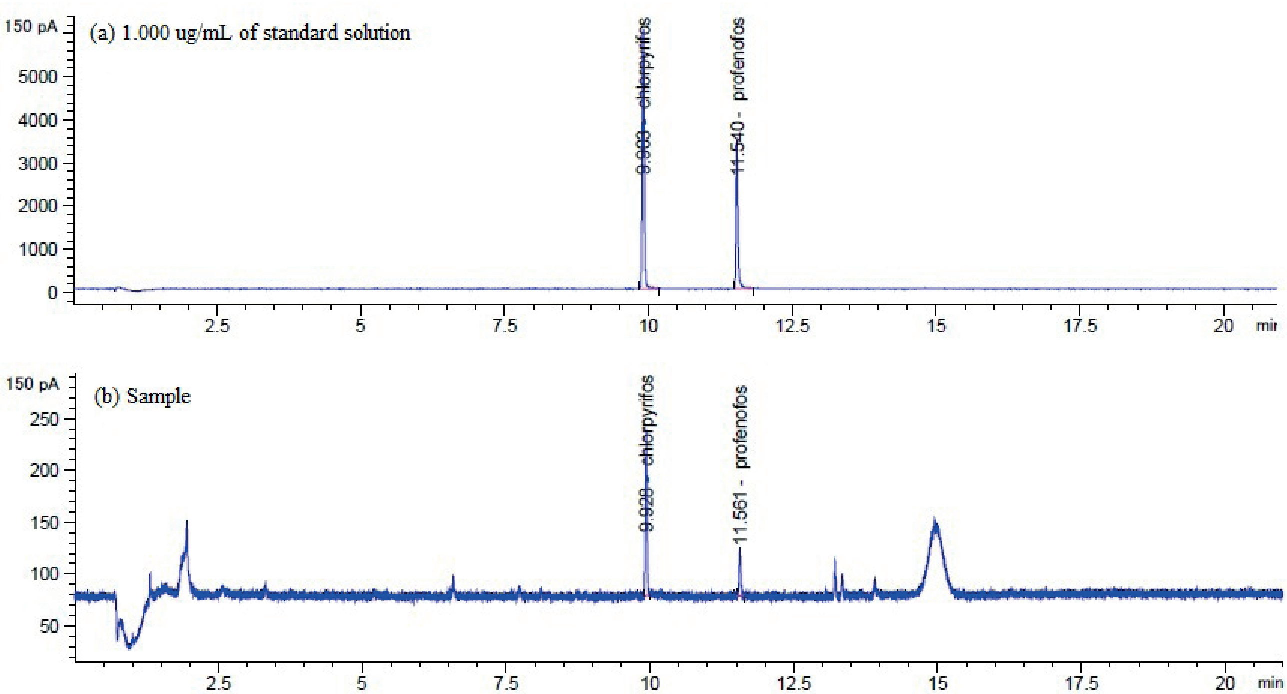

Figure 1. Chromatograms and retention time of chlorpyrifos and profenofos

DISCUSSION

Chili farmers in this study were directly involved in pesticide applications such as mixing or loading (83.3%), and spraying (93.3%). Three quarters of them applied pesticides twice a month or more. Various kinds of pesticides were used in chili farming such as insecticides (100.0%), herbicides (91.1%), and fungicides (61.1%). OPs and CAs, which were ChE inhibiting insecticides, were most commonly used. This study revealed that the average AChE activity of chili farmers (2.73 ±0.88 U/mL) was lower than previous studies, which reported that AChE activity was 3.31 ±0.56 U/mL for elderly people living in the agricultural area in Ubon Ratchathani province [23]; 2.92 ±0.60 U/mL for rice farmers in Chinart province, Central Thailand [28]; and 4.17 ±0.82 U/mL for Kenya agricultural workers [26]. Conversely, the average AChE activity of chili farmers was higher than that of rice farmers in Nakhon Nayok province, Central Thailand (2.63 ±0.55 U/mL) [41] and cacao farmers in Southwestern Nigeria (2.63 ± SE: 0.08 U/mL) [32]. This could be the result of the differences in crop types, agricultural tasks, types of pesticides used, pesticide exposure levels, and personal characteristics such as age, gender, genetic and therapeutic agents [16]. These factors could cause the variation of AChE activity. Furthermore, this study found that around half of chili farmers had abnormal AChE and abnormal PChE. It could be assumed that chili farmers were more likely to get pesticide poisoning and they should be removed from exposure and/or receive the medical treatment. Depression in ChE activity can cause constant firing of electrical signals across synapses in the nervous systems resulting in poisoning symptoms e.g., muscular twitching, trembling, paralyzed breathing, and convulsions [27].

Four-fifths of the chili farmers had detectable OP residues on their hands, in which over half of them had both chlorpyrifos and profenofos. This is evidence that chili farmers were frequently exposed to pesticides through the dermal route. Additionally, 67.8% of wipe samples had detectable chlorpyrifos residues in the range of 0.01-0.96 mg/kg/two hands and 64.4% of wipe samples had profenofos residues in the detectable range of 0.02-3.34 mg/kg/two hands. The percentage of detectable wipe samples and the detectable range of both residues in our study were higher than those reported in the study of *Taneepanichskul* et al. in 2014 [36], although our study had a limitation on extraction recoveries for chlorpyrifos residues. In contrast, their previous study in 2010 reported chlorpyrifos residues on chili farmer's hands greater than those found in our study [37]. The exposure level of OPs varied by the measure of exposure.

In this study, half of the chili farmers experienced some health symptoms after applying pesticides and the most reported symptoms were headache and dizziness. Our findings were consistent with earlier studies [14, 15, 40, 41]. This study exhibited significantly inverse associations of AChE activity with respiratory, skin, central nervous system, and gastrointestinal symptoms, while PChE activity showed a significantly positive association with only fatigue or weakness. Our findings are consistent with the study of *Von Osten* et.al. [40] that demonstrated a significant relationship of AChE inhibition to respiratory and central nervous system symptoms. On the contrary, *Wilaiwan* et al. [41] showed a significant association between AChE level and dizziness, whereas PChE levels were found to have significant associations with respiratory, central nervous system, eye and gland symptoms. Associations between both biomarkers of exposure effects and health symptoms were altered by the measurement of exposure and symptoms.

In addition, this study examined the association between detected OP residues on hands and health symptoms. There was a significant association between detected profenofos residues and gastrointestinal symptoms (e.g. nausea or vomiting, abdominal pain), and no significant association was found for detected chlopyrifos residues. Both chlorpyrifos and profenofos are moderately hazardous pesticides (Class II) by the WHO recommended classification [42]. They can cause ChE inhibition in humans which is linked to overstimulation in the nervous system which causes health effects [1, 18, 19, 20, 39, 42]. Chlorpyrifos, at low levels can cause headache, dizziness, weakness, and runny nose, at moderate exposure, increased lacrimation, salivation and sweating, nausea, vomiting, abdominal cramps, muscle pain, weakness, or cramps, and at high exposure, unconsciousness, convulsion, respiratory depression and paralysis, as well as possible death [1, 19, 20]. Although profenofos is less likely toxic than chlorpyrifos [42], it can cause similar health symptoms [18, 39]. This study demonstrated a significant association between health symptoms and detected profenofos residues on hands which was used as an indicator of OP exposure; however it seems difficult to explain due to a lack of a comparable study. Only one study suggested no significant association was found between reported health symptoms and the proportion of detectable urinary pesticide metabolites used as an indicator of pesticide exposure [34].

Overall, the binary logistic regression results indicated that increased activity of AChE was significantly associated with decreased odds of having health effects. Raised activity of PChE might be related to increased odds of having health effects, but it failed to achieve statistical significance. Association of AChE and PChE with having health effects are not likely relative. Also the measurement of AChE and PChE had a weak negative association [33]. However, both AChE and PChE should be measured.

Surprisingly, chili farmers with detected OP residues on hands were significantly less likely to have health effects than those without OP residues when adjusted for gender, number of years working in chili farming, and frequency of pesticide use. Possible explanations could be the variety of pesticides were used in chili farming as mentioned previously, so chili farmers were potentially exposed to multiple pesticides through multiple routes other than the OP residues on hands which was focused on in this study. *Von Osten* et al. [40] mentioned that CAs were more likely related to adverse health effects than OPs. Therefore, further study would be required to assess OP and CA exposure through multiple routes such as inhalation and dermal routes and also to determine the potential association between pesticide exposure, ChE activity, and health effects. Several limitations were considered for this study. The prevalence of health symptoms were from subjective evaluation, so it might have recall bias. The health symptoms examined here have multiple causes and may not be caused solely by pesticide exposure.

CONCLUSIONS

Understanding the associations between ChE activity, OP residues on hands, and health effects related to pesticide exposure may be an advantage to prevent health effects related to pesticide exposure in chili farmers. Regular monitoring of AChE and PChE in addition to effective interventions in regards to reducing pesticide exposure to prevent health effects should be provided to chili farmers.

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Conflict of interest
The authors declare no conflict of interest.

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