J Occup Health 2017; 59: 165-176

Journal of Occupational Health

Original

Association of health symptoms with low-level exposure to organophosphates, DNA damage, AChE activity, and occupational knowledge and practice among rice, corn, and double-crop farmers

Surat Hongsibsong[1], Nalin Sittitoon[2] and Ratana Sapbamrer[3]

[1]Environment and Health Research Unit, Research Institute for Health Sciences, Chiang Mai University, 110 Intavaroros Road, Sriphum Sub-district, Muang District, 50200 Chiang Mai, Thailand, [2]School of Environmental Health, Institute of Medicine, Suranaree University of Technology, 111 University Avenue, Muang District, Nakhon Ratchasima, 30000 Thailand and [3]School of Medicine, University of Phayao, 19 Moo 2, Mae Ka Sub-district, Muang District, Phayao Province, 56000 Thailand

Abstract: Objectives: This study aims to determine (1) total dialkylphosphate (ΣDAP) levels, occupational knowledge and practice, DNA damage, AChE activity, and health symptoms in rice, corn, and double-crop farmers; (2) the association of health symptoms with ΣDAP levels, occupational knowledge and practice, DNA damage, and AChE activity in farmers; and (3) the prevalence of health symptoms between farmers and non-farmers. **Methods:** A cross-sectional study was conducted by interviewing as well as analyzing urine and blood samples during July to August 2014. **Results:** There were no differences in ΣDAP levels, AChE activity, and occupational knowledge and practice scores among all farmer groups. In terms of health symptoms related to ΣDAP, AChE activity, DNA damage, and occupational knowledge and practice, pesticide-related symptoms were determined, including breathlessness, chest pain, dry throat, numbness, muscle weakness, cramp, headache, dizziness, eye irritation, white/red rash, and white/red pimple, which were classified as respiratory, muscle, nervous, and epithelial symptoms. A remarkable finding was that farmers had a significantly higher prevalence of muscle weakness (odds ratio (OR)=3.79) and numbness (OR=3.45) as compared with non-farmers. **Conclusion:** Our findings, therefore, suggest that a long-term low-level exposure to organophosphates (OPs) may be associated with an increasing prevalence of muscle symptoms. However, a further cohort study incorporating sensitive health outcomes and measurement of multiple pesticides monitoring on a larger scale is warranted.

(J Occup Health 2017; 59: 165-176)
doi: 10.1539/joh.16-0107-OA

Key words: AChE activity, DNA damage, Farmer, Organophosphate, Pesticide, Symptom

Received April 20, 2016; Accepted November 30, 2016
Published online in J-STAGE January 11, 2017
Correspondence to: R. Sapbamrer, School of Medicine, University of Phayao, 19 Moo 2, Mae Ka Sub-district, Muang District, Phayao Province, 56000 Thailand (e-mail: lekratana56@yahoo.com and lekratana56@gmail.com)

Introduction

Rice and corn are two major economic crops cultivated in Thailand. Cultivation areas of rice rank fifth (11,705,920 hectares) and that of corn rank tenth (1,166,880 hectares) among cultivation areas in countries worldwide. In Northern Thailand, cultivation areas for rice and corn crops are approximately 3,067,688 and 793,333 hectares, respectively[1]. Farmers used to grow single rice crop in the past, but they have begun to change cultivation areas from rice crops to corn or double crops at present. Rice is planted in lowland during June and December, whereas corn is planted in upland during May and October[2]. Cocktail of pesticides were used in the cultivation of both crops due to multiple pests, especially insects. Organophosphates (OPs) were regularly used for killing insects on farms, resulting in environmental pollution and several adverse health effects. OPs have a potential to inhibit acetylcholinesterase, produce an effect of excess acetylcholine in the brain and neuromuscular junction, and consequently cause cholinergic acute and chronic poisoning effects[3].

It is well understood that acute effects of exposure to OPs produce a wide range of neurological symptoms and it can be monitored by clinical signs and inhibition of acetylcholinesterase activity[3,4]. However, a moderate or low level of exposure remains doubtful and begins to suspect their adverse health effects. Due to misdiagnosis or mild poisoning symptoms, there were no cases reported in hospitals. A long-term low level of exposure was associated with increased health neurological symptoms in some studies[5,6]. Besides, some studies suggested that a low level of exposure to OPs can induce oxidative stress and damage a strand break in DNA, resulting in an increased risk for chronic diseases, such as cancer and neurodegenerative diseases[7-10].

The study area is Mae Na Reur Sub-district, located in Muang District, Phayao province of Northern Thailand. The major crops cultivated here include rice and corn. Farmers planted these crops and used pesticides on farms for a long time. OPs, especially chlorpyrifos, are commonly used in surrounding areas and detected in harvested crops[11]. The parameters, including DAP levels, DNA damage, AChE activity, and occupational knowledge and practice, were used as biomarkers of acute effects of exposure to OPs. This study was, therefore, conducted to assess the following specific objectives: (1) comparison of ΣDAP levels, DNA damage, AChE activity, occupational knowledge and practice, and health symptoms among rice, corn, and double-crop farmers; (2) assessment of the association of health symptoms with ΣDAP levels, DNA damage, AChE activity, and occupational knowledge and practice among farmers; and (3) assessment of the prevalence of health symptoms between farmers and non-farmers.

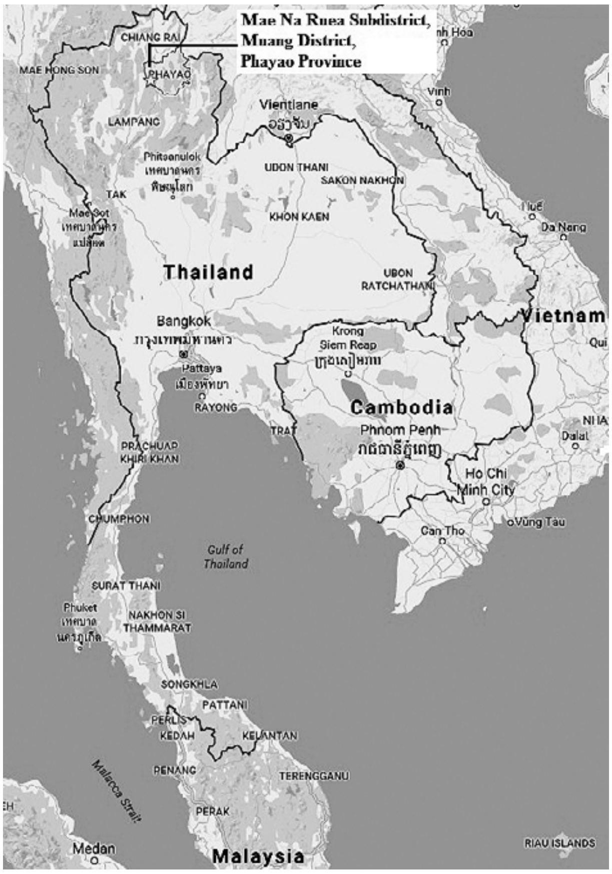

Fig. 1. Location of study site in Northern Thailand

Methods

Setting, participants, and interviews

A cross-sectional study was conducted by interviewing as well as analyzing urine and blood samples during July to August 2014. Farmers were categorized into the following 3 groups: Rice farmers, corn farmers, and double-crop farmers, whose urinary ΣDAP levels, DNA damage, AChE activity, occupational knowledge and practice, and health symptoms were investigated. Non-farmers were included for comparing their DNA damage, AChE activity, and health symptoms with that of farmers. The study collaborations included School of Medicine from University of Phayao, Research Institute for Health Sciences from Chiang Mai University, and School of Medicine from Suranaree University of Technology. The research project was approved by Human Ethical committee, University of Phayao (Certificate Ethical Clearance No. HE 5602040007, December 1, 2014).

Inclusion criteria of farmers were people who lived in Mae Na Reur Sub-district, Phayao province of Thailand, aged between 25 and 65 years, cultivated rice and/or corn, and sprayed pesticides for at least 5 years. Inclusion criteria of non-farmers were people who were not farmers, aged between 25 and 65 years, and lived in Mae Na Reur Sub-district, Phayao province of Thailand for at least 5 years. Farmers and non-farmers who were diagnosed with cancer, diabetes, neurological disorder, pregnancy, and had a conflict with the study were excluded. The cluster sampling technique was used to randomize villages in Mae Na Reur Sub-district. The total 6 sampling villages included So Village, Rong Kham Luang Village, Rong Kham Noi Village, Rai Village, Rai San Jam Pa Village, and Rong Kham Sri Chum Village. Farmers and non-farmers who met the inclusion criteria were recruited to participate in this study and signed a written consent form. In total, there were 154 farmers and 60 non-farmers who met the inclusion criteria. Of these participants, there were 103 (66.9%) farmers and 47 (78.3%) non-farmers who signed a written consent form.

Participants were interviewed by native northern Thai-speaking interviewers for 15 to 20 minutes. The interview form of farmers consisted of the following 4 sections: (1) personal data; (2) pesticide usage and exposure; (3) occupational knowledge and practice before, during, and after spraying; and (4) recall health symptoms within one month after enrollment. The interview form of non-farmers consisted of the following 3 sections: (1) personal data; (2) pesticide exposure; and (3) recall health symptoms within one month after enrollment. The interview form was assessed for validity and reliability before collecting the data from participants.

The questions regarding pesticide usage were focused on agricultural experience, cultivation area, pesticide expenses, work tasks on farming, information and reason of pesticide usage, and disposal method of used pesticide containers. These 31 questions with regard to occupational knowledge and practice were presented within 3 sections focusing on (1) before pesticide spraying (11 items); (2) during pesticide spraying (13 items); and (3) after pesticide spraying (7 items). Farmers were asked about each knowledge item and answered either "yes" or "no." No mark for "no" and 1 mark for "yes" were assigned and the summation was calculated accordingly. For practice, farmers were asked about each item and answered either "never," or "sometimes," or "always." No mark for "never," 1 mark for "sometimes," and 2 marks for "always" were assigned and the summation was calculated accordingly. The knowledge score ranged from 0 to 31, whereas the practice score ranged from 0 to 62.

A total of 14 questions about recalled health symptoms due to pesticide exposure consisted of breathlessness, chest pain, cough, dry throat, numbness, muscle weakness, cramp, headache, dizziness, balance problem, eye irritation, white/red rash, white/red pimple, and diarrhea. For each item, farmers and non-farmers had to respond whether they "had symptom" or "had no symptom."

Collection of urine and blood samples

Pesticide exposure was assessed by determining urinary DAP levels. First morning urine samples of farmers were collected in 250 m*l* urine containers and stored in ice cooler until they were transported to a laboratory. The samples were aliquoted to 15 m*l* ×3 tubes and frozen at -20℃ until they were analyzed. Urinary concentrations of dimethylphosphate (DMP), dimethylthiophosphate (DMTP), dimethyldithiophosphate (DMDTP), diethylphosphate (DEP), diethylthiophosphate (DETP), and diethyldithiophosphate (DEDTP) were determined using a gas chromatograph-flame photometric detector (A Hewlett Packard 6890-FPD, Agilent Technology, CA, USA). The method for extraction and analysis were determined according to the method published in a study by Prapamontol et al. (2014)[12]. The limit of detection (LOD) ranged from 0.1 μg/*l* for DETP to 2.5 μg/*l* for DMP, while the limit of quantification (LOQ) ranged from 1 μg/*l* for DETP to 25 μg/*l* for DMP. Recovery ranged from 90.3% for DETP to 115.7% for DEP. Furthermore, %CV for intra-batch ranged from 7.6% for DEP to 18.7% for DEDTP and that for inter-batch ranged from 9.5% for DEP to 22% for DETP. Creatinine in urine samples was determined according to Jaffe's reaction. Concentrations of DAP metabolites were presented in microgram per gram of creatinine (μg/g creatinine). The sum of DMP, DMTP, and DMDTP was considered as total DMP metabolites (ΣDMP), that of DEP, DETP, and DEDTP was considered as total DEP metabolites (ΣDEP), and that of ΣDMP and ΣDEP was considered as total DAP (ΣDAP). Concentrations below LOD were replaced with LOD divided by the square root of 2[13].

Furthermore, 10 m*l* of blood samples were collected from farmers and non-farmers. Thereafter, 9 m*l* of blood samples were separated to a heparin tube to determine DNA damage and 1 m*l* of blood sample was separated to an EDTA tube to measure whole blood AChE activity. The comet assay was used to determine DNA damage and conducted under alkali conditions according to the method by Singh et al. (1988)[14]. Two major parameters included tail length and tail moment. Tail length was the distance of DNA migration and tail moment was the distance between the center of gravity of the head and the center of gravity of the tail. Whole blood AChE activity was measured according to the Ellman method[15].

Statistical analysis

Statistical Package for the Social Sciences (SPSS) version 16.0 was used for descriptive and inferential analysis. Descriptive statistics included frequency (n), percentage (%), arithmetic mean, median, percentile (P^{25th}-P^{75th}), and standard deviation (SD). Because of non-normal distribution of data, a non-parametric test was used. The differences in median urinary ΣDAP levels, tail length, tail moment, AChE activity, occupational knowledge and practice scores, and personal data among rice, corn, and double-crop farmers were tested using Kruskal-Wallis test variance, while the differences between farmers who had health symptoms and no symptoms were tested using the Mann-Whitney U test. The association of personal data, pesticide usage, occupational knowledge and practice, and health symptoms among 3 groups of farmers was tested using the Chi-square test (χ^2). Mantel-Haenszel statistics (OR) were used to investigate the prevalence of health symptoms in farmers and non-farmers.

Results

Demographic data and pesticide usage among rice, corn, and double-crop farmers

Demographic data and pesticide usage among rice, corn, and double-crop farmers are summarized in Table 1. The majority of farmers was males (n=20, 95.2% for rice farmers, n=29, 87.9% for corn farmers, and n=44, 89.8% for double-crop farmers,) and had received primary education (n=17, 81% for rice farmers, n=26, 78.8% for corn farmers, and n=37, 75.5% for double-crop farmers). It was found that 3 (14.3%) of the rice farmers, 15 (45.5%) of the corn farmers, and 12 (24.5%) of the double-crop farmers smoked cigarette. Moreover, 14 (66.7%) of the rice farmers, 22 (66.7%) of the corn farmers, and 39 (79.6%) of the double-crop farmers consumed alcohol.

Table 1. Demographic data and pesticide usage among rice, corn, and double-crop farmers

		Rice farmers (n=21)	Corn farmers (n=33)	Double-crop farmers (n=49)	P value
Gender [a]	Male	20 (95.2)	29 (87.9)	44 (89.8)	0.629
	Female	1 (4.8)	4 (12.1)	5 (10.2)	
Education [a]	No education	0 (0)	2 (6.1)	0 (0)	0.175
	Primary education (Grade 1-6)	17 (81)	26 (78.8)	37 (75.5)	
	Secondary education (Grade 7-12)	4 (19)	5 (15.2)	11 (22.4)	
	Bachelor degree	0 (0)	0 (0)	1 (2)	
Smoking a cigarette [a]		3 (14.3)	15 (45.5)	12 (24.5)	0.128
Alcohol consumption [a]		14 (66.7)	22 (66.7)	39 (79.6)	0.585
Farm tasks [a]	Spraying pesticides	21 (100)	33 (100)	49 (100)	No statistics
	Mixing pesticides	15 (71.4)	24 (72.7)	29 (59.2)	0.375
	Harvesting crops	11 (52.4)	15 (45.5)	25 (51)	0.847
	Scattering seed	10 (47.6)	15 (45.5)	22 (44.9)	0.978
	Packing product	3 (14.3)	5 (15.2)	7 (14.3)	0.993
	Watering	4 (19)	3 (9.1)	6 (12.2)	0.574
Information of pesticide usage [a]	Neighbors	10 (47.6)	20 (60.6)	33 (67.3)	0.299
	Retailers	9 (42.9)	13 (39.4)	18 (36.7)	0.888
	Government officers	8 (38.1)	8 (24.2)	9 (18.4)	0.211
Reason of pesticide usage [a]	Protect the problem in advance	14 (66.7)	19 (57.6)	39 (79.6)	0.097
	Face a pest problem and plant diseases	12 (57.1)	17 (51.5)	27 (55.1)	0.912
	Save time, labor and cost	12 (57.1)	16 (48.5)	20 (40.8)	0.440
	Need good appearance products	8 (38.1)	12 (36.4)	23 (46.9)	0.591
Disposal methods of used pesticide containers [a]	Burying	16 (76.2)	11 (33.3)	18 (36.7)	0.003*
	Selling	4 (19)	11 (33.3)	19 (38.8)	0.274
	Throw in garbage	2 (9.5)	8 (24.2)	10 (20.4)	0.399
	Burning	0 (0)	6 (18.2)	4 (8.2)	0.078
	Reuse for other purposes	0 (0)	0 (0)	2 (4.1)	0.325
Age, years old [b]	Mean±SD.	55±8	52±8	52±7	0.271
	Median (P^{25th}-P^{75th})	56 (51-61)	53 (48-58)	52 (46-58)	
Agricultural experience, years [b]	Mean±SD.	31±11	31±10	28±11	0.473
	Median (P^{25th}-P^{75th})	31 (24-41)	32 (23-39)	28 (21-39)	
Area of cultivation, Acres [b]	Mean±SD.	3.1±1.5	3.6±2.6	8.1±9.4	<0.001**
	Median (P^{25th}-P^{75th})	3.2 (2-4.4)	2.4 (1.6-4.4)	5.5 (3.6-8.7)	
Pesticide expense per cultivation area, Bahts/acre/year [b]	Mean±SD.	261±306	462±397	235±271	0.005**
	Median (P^{25th}-P^{75th})	125 (53-360)	281 (198-777)	162.5 (65-313)	

[a] Presented in frequency and percentage; [b] Presented in Mean±SD. and Median (P^{25th}-P^{75th}); *$P<0.05$; **$P<0.01$

The range of the mean age and agricultural experience of individual groups of farmers was between 52 and 55 years and 28 and 31 years, respectively. The range of the mean area for cultivation and pesticide expenses was between 3.1 and 8.1 acres and 235 and 462 bahts/acre/year, respectively. The cultivation area for double-crop farmers was significantly larger than those for other farmers ($P<0.001$), while pesticide expenses for corn farmers were significantly higher than those for other farmers ($P=0.005$). The mean age of non-farmers was 46±8 years. The majority of non-farmers was males (n=41, 87.2%), had received primary education (n=28, 59.5%), did not smoke cigarette (n=39, 83%), and consumed alcohol (n=25, 53.2%) (data not shown).

With regard to pesticide usage, all farmers had a farm task of spraying pesticides followed by mixing pesticides,

Table 2. Urinary DAP metabolites, DNA damage, AChE activity, and occupational knowledge and practice scores

Parameters	Median (P25th-P75th)				P value
	Rice farmers (n=21)	Corn farmers (n=33)	Double-crop farmers (n=49)	Non-farmers (n=47)	
Urinary DAP metabolite concentrations, (μg/g creatinine)					
DMP	5.0 (2.8-7.6)	5.7 (2.8-11.8)	3.5 (2.4-8.9)	[d]	0.409
DMTP	0.4 (0.23-0.56)	0.3 (0.18-0.45)	0.26 (0.18-0.45)	[d]	0.271
DMDTP	0.36 (0.22-0.51)	0.33 (0.18-0.48)	0.26 (0.18-0.4)	[d]	0.378
DEP	0.51 (0.34-1.2)	0.36 (0.26-0.87)	0.33 (0.19-0.81)	[d]	0.103
DETP	0.26 (0.15-1.0)	0.16 (0.09-0.26)	0.15 (0.09-0.36)	[d]	0.151
DEDTP	0.4 (0.27-0.96)	0.33 (0.18-0.48)	0.26 (0.18-0.48)	[d]	0.084
ΣDMP [a]	6.4 (3.5-8.8)	6.6 (3.8-12.7)	4.0 (2.8-10.6)	[d]	0.407
ΣDEP [b]	1.8 (0.75-3.7)	1.1 (0.65-1.9)	0.82 (0.47-2.1)	[d]	0.097
ΣDAP [c]	9.4 (5.2-12.3)	7.7 (4.4-17.0)	6.5 (3.1-13.9)	[d]	0.420
DNA damage					
Tail length, μm	6.4 (5.8-6.8)	6.3 (5.7-6.5)	6.4 (6.1-6.7)	6.3 (5.6-6.5)	0.336
Tail moment, μm	3.3 (3.1-3.4)	3.2 (3.0-3.3)	3.3 (3.1-3.3)	3.2 (2.9-3.3)	0.393
AChE, U/*l*	10,764 (10,530-11,466)	10,296 (9,126-11,232)	10,530 (9,360-11,700)	10,530 (9,594-11,466)	0.662
Occupational knowledge score (31 full marks)					
Before pesticide spraying	11 (10-11)	10 (9-11)	11 (10-11)	[d]	0.152
During pesticide spraying	12 (11-13)	12 (10-12)	11 (10-13)	[d]	0.371
After pesticide spraying	7 (6-7)	7 (6-7)	7 (6-7)	[d]	0.455
Total knowledge score	30 (28-30)	29 (25-30)	28 (27-31)	[d]	0.226
Occupational practice score (62 full marks)					
Before pesticide spraying	21 (18-22)	19 (17-21)	20 (17-22)	[d]	0.481
During pesticide spraying	25 (20-26)	23 (20-24)	22 (19-24)	[d]	0.056
After pesticide spraying	13 (12-14)	12 (11-13)	13 (12-14)	[d]	0.118
Total practice score	59 (50-60)	53 (49-58)	55 (48-58)	[d]	0.183

DMP=dimethylphosphate; DMTP=dimethylthiophosphate; DMDTP=dimethyldithiophosphate; DEP=diethylphosphate, DETP=diethylthiophosphate; DEDTP=diethyldithiophosphate; [a] ΣDMP=DMP+DMTP+DMDTP; [b] ΣDEP=DEP+DETP+DEDTP; [c] ΣDAP=ΣDMP+ΣDEP; [d] data not collected in non-farmers

harvesting products, scattering seed, packing products, and watering. They obtained the information about pesticide usage from neighbors, retailers, and government officers. Most farmers disposed used pesticide containers by buying and selling. The major reasons of pesticide usage were that farmers wanted to ensure protection against pests in advance as they faced pest problems and plant diseases.

Urinary DAP metabolite concentrations among rice, corn farmers, and double-crop farmers

ΣDAP, ΣDMP, and ΣDEP levels among rice, corn, and double-crop farmers are provided in Table 2. The median ΣDAP levels of rice farmers was the highest among three farmer groups and ΣDMP levels were higher than ΣDEP levels in all farmer groups. However, it was noted that these differences among all groups were not statistically significant. Among DMP metabolites, the majority was DMP (5.0 μg/g creatinine for rice farmers, 5.7 μg/g creatinine for corn farmers, and 3.5 μg/g creatinine for double-crop farmers), followed by DMTP (0.4 μg/g creatinine for rice farmers, 0.3 μg/g creatinine for corn farmers, and 0.26 μg/g creatinine for double-crop farmers), and then DMDTP (0.36 μg/g creatinine for rice farmers, 0.33 μg/g creatinine for corn farmers, and 0.26 μg/g creatinine for double-crop farmers), respectively. Among DEP metabolites, the majority was DEP (0.51 μg/g creatinine for rice farmers, 0.36 μg/g creatinine for corn farmers, and 0.33 μg/g creatinine for double-crop farmers), followed by DEDTP (0.4 μg/g creatinine for rice farmers, 0.33 μg/g creatinine for corn farmers, and 0.26 μg/g creatinine for double-crop farmers), and then DETP (0.26 μg/g creatinine for rice farmers, 0.16 μg/g creatinine for corn farmers, and 0.15 μg/g creatinine for double-crop farmers), respectively.

Table 3. The association between occupational knowledge and practice among farmers (n=103)

Questions	Frequency (%) of farmers who answer "Yes"			χ^2	P value
	Never	Sometimes	Always		
Section 1: practice before pesticide spraying					
1 Buy pesticides having right labels, warning signs, chemical and manufacturing names	1 (1)	15 (15.5)	81 (83.5)	36.6	<0.001**
2 Survey types and amounts of pests before buying pesticides	2 (2.1)	16 (16.7)	78 (81.3)	13.4	0.001**
3 Study suitable types and formulation of pesticides	2 (2.1)	13 (13.3)	83 (84.7)	18.96	<0.001**
4 Use pesticides with suggestion of agriculture officers	2 (2.3)	13 (15.1)	71 (82.6)	23.68	<0.001**
5 Read suggestion of pesticide label before using	1 (1)	21 (21.4)	76 (77.6)	26.08	<0.001**
6 Mixing pesticides with specified formulation in label	2 (2.1)	17 (18.1)	75 (79.8)	17.33	<0.001**
7 Check spraying equipment	3 (3)	6 (6.1)	90 (90.9)	5.13	0.077
8 Mixing pesticide in outdoor	2 (2)	10 (10.1)	87 (87.9)	5.97	0.050
9 Stand upwind during mixing pesticides	4 (4.1)	20 (20.6)	73 (75.3)	10.07	0.007**
10 Do not use mouth for opening pesticide containers	14 (14)	4 (4)	82 (82)	6.18	0.046*
11 Do not use hands for mixing pesticides	8 (10.8)	6 (8.1)	60 (81.1)	49.97	<0.001**
Section 2: practice during pesticide spraying					
12 Wear gloves	4 (4.5)	6 (6.7)	79 (88.8)	42.04	<0.001**
13 Wear boots	2 (2)	2 (2)	95 (96)	14.8	0.001**
14 Wear long-sleeved shirt	2 (2)	1 (1)	97 (97)	16.02	<0.001**
15 Wear glasses or goggles	5 (7.6)	3 (4.5)	58 (87.9)	51.02	<0.001**
16 Wear long pants	2 (2)	1 (1)	97 (97)	16.02	<0.001**
17 Wear hat	2 (2)	1 (1)	96 (97)	23.72	<0.001**
18 Wear mask	4 (4.2)	7 (7.4)	84 (88.4)	22.43	<0.001**
19 Stand upwind	2 (2.1)	12 (12.4)	83 (85.6)	6.82	0.033*
20 Do not spray during windy	14 (16.1)	4 (4.6)	69 (79.3)	4.24	<0.001**
21 Do not eat, drink or smoke	3 (4.7)	23 (35.9)	38 (59.4)	31.69	<0.001**
22 Do not use mouth for blowing or sucking nozzle	15 (14.7)	3 (2.9)	84 (82.4)	24.99	<0.001**
23 Do not take a Break while wearing dirty work clothes	5 (7.6)	13 (19.7)	48 (72.7)	43.47	<0.001**
24 Do not rub eyes or scratch skin	9 (11)	8 (9.8)	65 (79.3)	47.01	<0.001**
Section 3: practice after pesticide spraying					
25 Wash body and hair immediately	4 (4.1)	6 (6.1)	88 (89.8)	10.86	0.004**
26 Wash dirty work clothes separately from family laundry	1 (1)	5 (5.1)	92 (93.9)	44.38	<0.001**
27 Wear new clothes	1 (1)	0 (0)	98 (99)	11.62	0.001**
28 Wash spraying equipment before keeping	1 (1.2)	4 (4.7)	80 (94.1)	84.08	<0.001**
29 Keep pesticides away from children and pets	2 (2)	1 (1)	96 (97)	23.72	<0.001**
30 Remove and dispose the used pesticide containers	5 (5.3)	22 (23.2)	68 (71.6)	19.27	<0.001**
31 Do not wash spraying equipment in river or canal	11 (11.7)	8 (8.5)	75 (79.8)	20.4	<0.001**

Presented in frequency and percentage; *P<0.05; **P<0.01

Occupational knowledge and practice among rice, corn, and double-crop farmers

Occupational knowledge and practice scores among rice, corn, and double-crop farmers are provided in Table 2. Rice farmers had the highest knowledge and practice scores (score=30 and 59) as compared with other farmers. Knowledge in all items was associated with practice, except mixing pesticides with specified formulation and checking spraying equipment (Table 3). Among individual items of occupational knowledge, no association was found between knowledge and farmer groups in all items. For individual items of occupational practice, there were 4 items associated with farmer groups, including (1) buying pesticides with correct labels, warning signs, and chemical and manufacturing names (χ^2=12.88, P=0.012); (2) using pesticides with the suggestion of agriculture officers (χ^2=13.16, P=0.011); (3) not eating, drinking, or smoking during spraying (χ^2=13.09, P=0.011); and (4) washing the spraying equipment before keeping them back (χ^2=13.77, P=0.008) (data not shown).

Table 4. Association of health symptoms with urinary ΣDAP, AChE activity, tail length, and tail moment of farmers (n=103)

Symptoms	Median (P^{25th}-P^{75th})					
	ΣDAP, μg/g creatinine			AChE activity, U/*l*		
	Has symptom	No symptom	P value	Has symptom	No symptom	P value
Breathlessness	7.7 (4.4-15.7)	3.1 (2.0-5.3)	0.012*	8,658 (8,190-9,828)	10,764 (9,535-11,700)	0.015*
Chest pain	4.7 (2.4-6.3)	7.7 (4.4-15.4)	0.064	8,424 (8,073-10,413)	10,764 (9,535-11,700)	0.039*
Cough	7.7 (5.0-9.9)	7.4 (4.3-15.4)	0.709	10,062 (8,249-10,823)	10,764 (9,477-11,700)	0.101
Dry throat	7.7 (3.1-15.4)	7.5 (4.4-14.2)	0.510	10,062 (8,424-10,764)	10,764 (9,653-11,700)	0.029*
Numbness	7.7 (4.6-13.9)	7.5 (4.2-14.7)	0.920	10,530 (8,190-11,115)	10,647 (9,594-11,700)	0.039*
Muscle weakness	8.0 (4.4-15.4)	7.3 (4.3-13.5)	0.659	10,413 (8,658-11,173)	10,764 (9,828-11,700)	0.158
Cramp	6.6 (3.5-15.4)	7.7 (4.3-13.5)	0.726	10,647 (8,249-11,174)	10,530 (9,594-11,700)	0.207
Headache	10.1 (4.7-15.4)	6.9 (4.2-13.1)	0.246	9,243 (8,190-10,589)	10,764 (9,828-11,700)	0.001*
Dizziness	7.7 (3.7-16.6)	7.5 (4.4-12.9)	0.972	10,062 (8,424-10,764)	10,764 (9,535-11,700)	0.041*
Balance problem	11.3 (5.1-18.5)	7.4 (4.3-14.0)	0.464	10,998 (8,366-14,099)	10,530 (9,360-11,466)	0.578
Eye irritation	11.5 (4.4-15.5)	7.3 (4.3-12.3)	0.436	10,062 (8,424-10,764)	10,764 (9,653-11,700)	0.046*
White/red rash	9.7 (3.7-15.6)	7.4 (4.4-13.1)	0.942	10,764 (8,424-11,759)	10,530 (9,360-11,466)	0.956
White/red pimple	15.4 (4.7-17.7)	7.3 (4.3-12.6)	0.144	10,764 (8,658-11,934)	10,530 (9,360-11,466)	0.689
Diarrhea	7.8 (3.7-17.9)	7.7 (4.4-14.0)	0.882	9,828 (8,190-10,355)	10,764 (9,360-11,700)	0.082

Symptoms	Median (P^{25th}-P^{75th})					
	Tail length (μm)			Tail moment (μm)		
	Has symptom	No symptom	P value	Has symptom	No symptom	P value
Breathlessness	6.6 (6.6-7.3)	6.4 (5.9-6.6)	0.017*	3.3 (3.3-3.4)	3.2 (3.0-3.3)	0.027*
Chest pain	6.6 (6.5-7.3)	6.4 (5.9-6.6)	0.031*	3.3 (3.3-3.5)	3.2 (3.0-3.3)	0.030*
Cough	6.2 (5.3-6.4)	6.4 (6.0-6.7)	0.141	3.2 (2.8-3.4)	3.3 (3.1-3.4)	0.109
Dry throat	6.3 (5.4-6.6)	6.4 (6.0-6.7)	0.273	3.2 (2.9-3.3)	3.3 (3.1-3.4)	0.155
Numbness	6.3 (5.7-6.6)	6.4 (6.0-6.7)	0.491	3.2 (2.9-3.3)	3.3 (3.1-3.4)	0.479
Muscle weakness	6.4 (6.1-6.8)	6.3 (5.8-6.6)	0.110	3.3 (3.1-3.4)	3.2 (3.0-3.3)	0.198
Cramp	6.5 (6.2-6.8)	6.4 (5.7-6.6)	0.138	3.3 (3.1-3.4)	3.2 (3.0-3.3)	0.173
Headache	6.4 (5.9-6.6)	6.4 (6.0-6.7)	0.758	3.2 (3.0-3.3)	3.3 (3.1-3.4)	0.526
Dizziness	6.5 (6.3-6.7)	6.4 (5.7-6.7)	0.265	3.3 (3.1-3.4)	3.2 (3.0-3.3)	0.257
Balance problem	6.5 (6.2-7.0)	6.4 (6.0-6.7)	0.418	3.3 (3.1-3.3)	3.3 (3.2-3.4)	0.464
Eye irritation	6.4 (6.0-6.6)	6.4 (6.0-6.7)	0.936	3.3 (3.0-3.3)	3.3 (3.1-3.4)	0.808
White/red rash	6.3 (5.7-6.6)	6.4 (6.0-6.7)	0.640	3.2 (3.0-3.4)	3.3 (3.1-3.4)	0.612
White/red pimple	6.3 (6.2-6.5)	6.4 (6.0-6.7)	0.891	3.2 (3.0-3.3)	3.3 (3.1-3.4)	0.984
Diarrhea	6.4 (6.1-6.7)	6.4 (5.9-6.7)	0.673	3.2 (3.0-3.3)	3.3 (3.1-3.4)	0.871

*P<0.05

DNA damage and AChE activity among farmers and non-farmers

The median tail length was 6.4 μm for rice farmers, 6.3 μm for corn farmers, 6.4 μm for double-crop farmers, and 6.3 μm for non-farmers. The median tail moment was 3.3 μm for rice farmers, 3.2 μm for corn farmers, 3.3 μm for double-crop farmers, and 3.2 μm for non-farmers. The median AChE activity for rice farmers was the highest (10,764 U/*l*), followed by non-farmers (10,530 U/*l*), double-crop farmers (10,530 U/*l*), and then corn farmers (10,296 U/*l*), respectively. However, it was noted that these differences among all groups were not statistically significant (Table 2).

Health symptoms among farmers and non-farmers

All items of health symptoms among individual groups of farmers were not significantly different (data not shown). With regard to the association of health symptoms with ΣDAP levels, AChE activity, and DNA damage, farmers who had a breathlessness symptom had significantly higher ΣDAP levels (7.7 μg/g creatinine) than those farmers who had no symptom (3.1 μg/g creatinine). Breathlessness, chest pain, dry throat, numbness, headache, dizziness, and eye irritation were significantly associated with AChE activity. Farmers who had these symptoms had lower AChE activity than those who had no symptoms. Breathlessness and chest pain were signifi-

Table 5. Association of health symptoms with occupational knowledge and practice scores (n=103)

Symptoms	Median (P^{25th}-P^{75th}) of knowledge score								
	Before pesticide spraying			During pesticide spraying			After pesticide spraying		
	Has symptom	No symptom	P value	Has symptom	No symptom	P value	Has symptom	No symptom	P value
Breathlessness	10 (6-11)	11 (10-11)	0.363	8 (5.5-11)	12 (10-13)	0.011*	7 (3.5-7)	7 (6-7)	0.542
Chest pain	10 (6-11)	11 (10-11)	0.363	9 (6.5-10.5)	12 (10-13)	0.008**	6 (3-6.5)	7 (6-7)	0.007**
Cough	10 (9.8-11)	11 (10-11)	0.093	10.5 (8.8-13)	12 (10-13)	0.373	7 (6.8-7)	7 (6-7)	0.348
Dry throat	10 (10-11)	11 (10-11)	0.141	10 (9-12)	12 (10.3-13)	0.008**	7 (6-7)	7 (6-7)	0.934
Numbness	10 (10-11)	11 (10-11)	0.400	11 (10-11.5)	12 (10-13)	0.017*	7 (6-7)	7 (6-7)	0.064
Muscle weakness	10 (10-11)	11 (10-11)	0.062	10 (8-12)	12 (10-13)	0.014*	7 (7-7)	7 (6-7)	0.130
Cramp	10.5 (10-11)	11 (10-11)	0.579	10 (8-11)	12 (10-13)	0.001**	7 (6-7)	7 (6-7)	0.241
Headache	11 (10-11)	11 (10-11)	0.646	10 (9.8-12.3)	12 (10-13)	0.080	7 (6-7)	7 (6-7)	0.442
Dizziness	11 (9.5-11)	11 (10-11)	0.649	10 (8.5-12)	12 (10-13)	0.043*	7 (5-7)	7 (6-7)	0.342
Balance problem	11 (9.8-11)	11 (10-11)	0.724	11 (8.8-12)	12 (10-13)	0.214	7 (6-7)	7 (6-7)	0.439
Eye irritation	10 (9-11)	11 (10-11)	0.010*	10 (9-11)	12 (10-13)	0.003**	6.5 (6-7)	7 (6-7)	0.353
White/red rash	10 (9.8-11)	11 (10-11)	0.095	10 (8-11)	12 (10-13)	0.003**	7 (6-7)	7 (6-7)	0.962
White/red pimple	10 (9-11)	11 (10-11)	0.317	10 (9-11)	12 (10-13)	0.044*	7 (6-7)	7 (6-7)	0.974
Diarrhea	10.5 (9.3-11)	11 (10-11)	0.671	10.5 (8.8-11.3)	12 (10-13)	0.082	7 (6.8-7)	7 (6-7)	0.380

Symptoms	Median (P^{25th}-P^{75th}) of practice score								
	Before pesticide spraying			During pesticide spraying			After pesticide spraying		
	Has symptom	No symptom	P value	Has symptom	No symptom	P value	Has symptom	No symptom	P value
Breathlessness	18 (14-19)	20 (17.8-22)	0.056	16 (11.5-22)	23 (19.8-25)	0.027*	12 (11-13)	13 (12-14)	0.225
Chest pain	18 (13.5-19.5)	20 (17.8-22)	0.078	16 (11.5-20.5)	23 (19.8-25)	0.007*	11 (9.5-12)	13 (12-14)	0.009*
Cough	20 (18.3-21.25)	20 (17-22)	0.986	22 (16.8-25.3)	23 (19-24.5)	0.836	13.5 (12-14)	13 (12-14)	0.180
Dry throat	20 (18-22)	20 (17-22)	0.704	21 (17-24)	23 (20-25)	0.196	13 (12-14)	13 (12-14)	0.445
Numbness	20 (17-22)	20 (17-22)	0.664	20 (16.5-24.5)	23 (20-25)	0.111	12 (12-14)	13 (12-14)	0.406
Muscle weakness	20 (16-21.75)	20 (18-22)	0.462	20 (17-23.8)	24 (21-25)	0.009*	12 (11.3-14)	13 (12-14)	0.524
Cramp	20 (15-22)	20 (17-22)	0.974	19.5 (16.3-22)	23 (20-25)	0.009*	12 (11-14)	13 (12-14)	0.247
Headache	20.5 (18.8-22)	20 (17-22)	0.321	21.5 (19.8-25.3)	23 (19-24)	0.941	13 (11.8-14)	13 (12-14)	0.961
Dizziness	20 (15-21.5)	20 (17.8-22)	0.635	20 (14.5-23.5)	23 (17.8-22)	0.026*	13 (11-14)	13 (12-14)	0.755
Balance problem	20 (18.8-22)	20 (17-22)	0.557	21 (17-25)	23 (19.5-24.5)	0.640	12 (11.8-13.3)	13 (12-14)	0.454
Eye irritation	20 (18-22)	20 (17-22)	0.749	21 (17-24)	23 (20-25)	0.051	13 (12-14)	13 (12-14)	0.254
White/red rash	20 (17.5-20.5)	20 (17-22)	0.602	17.5 (15.5-22.5)	23 (20-25)	0.013*	13.5 (11-14)	13 (12-14)	0.581
White/red pimple	20 (18-22)	20 (17-22)	0.931	19 (17-24)	23 (20-25)	0.136	14 (12-14)	13 (12-14)	0.279
Diarrhea	19.5 (17.3-20.5)	20 (17-22)	0.657	23 (17.8-24.3)	23 (19-25)	0.832	12.5 (11.8-14)	13 (12-14)	0.965

*P<0.05; **P<0.01

cantly associated with tail length and tail moment. Farmers who had breathlessness and chest pain (6.6 μm tail length and 3.3 μm tail moment) had higher tail length and tail moment than those who had no symptoms (6.4 μm tail length and 3.2 μm tail moment) (Table 4).With regard to health symptoms with occupational knowledge and practice, all symptoms, except cough, headache, balance problem, and diarrhea, were significantly associated with

Table 6. Odds ratio of health symptoms between farmers and non-farmers

Symptoms	Farmers (n=103)	Non-farmers (n=47)	Odds ratio (OR)	95%CI
Breathlessness	5 (4.9)	2 (4.3)	1.15	0.215, 6.143
Chest pain	5 (4.9)	6 (12.8)	0.35	0.101, 1.207
Cough	10 (9.7)	3 (6.4)	1.58	0.413, 6.018
Dry throat	19 (18.4)	7 (14.9)	1.29	0.502, 3.325
Numbness	25 (24.3)	4 (8.5)	3.45*	1.125, 10.552
Muscle weakness	32 (31.1)	5 (10.6)	3.79**	1.370, 10.466
Cramp	16 (15.5)	14 (29.8)	0.43	0.191, 0.986
Headache	18 (17.5)	14 (29.8)	0.5	0.223, 1.117
Dizziness	17 (16.5)	10 (21.3)	0.73	0.306, 1.747
Balance problem	6 (5.8)	3 (6.4)	0.91	0.217, 3.795
Eye irritation	19 (18.4)	11 (23.4)	0.74	0.320, 1.713
White/red rash	10 (9.7)	8 (17)	0.52	0.192, 1.428
White/red pimple	7 (6.8)	3 (6.4)	1.07	0.264, 4.332
Diarrhea	6 (5.8)	6 (12.8)	0.42	0.129, 1.388

Presented in frequency and percentage; *P<0.05; **P<0.01

knowledge and practice scores (Table 5). With regard to the prevalence of health symptoms between farmers and non-farmers, the prevalence of numbness and muscle weakness was significantly higher in farmers than in non-farmers (OR=3.45, P<0.05 and OR=3.79, P<0.01, respectively) (Table 6).

Discussion

Although no statistical significance was found in the differences of ΣDAP levels, AChE activity, and occupational knowledge and practice scores among all farmers, the results can be roughly seen as that rice farmers had the highest ΣDAP levels as compared with other farmers, whereas corn farmers had the lowest AChE activity and occupational practice scores. Our results also determined that pesticide expenses per cultivation area for corn farmers were significantly higher than that for other farmers. Therefore, it is possible that corn farmers used pesticides, including OPs, in higher amounts than that used by other farmers, but it might be not high enough to produce significant differences. Another possibility is that the protective behavior to avoid pesticide exposure among corn farmers was rather poor as compared with other farmers. In our study, we determined that approximately 12.1 to 30.3% of corn farmers ate, drank, or smoked during spraying, did not buy pesticides with correct labels, did not use pesticides with the suggestion of agricultural officers, and did not wash the spraying equipment before keeping them back.

OPs play an important role to inhibit acetylchloinesterase and induce oxidative stress and damage DNA[10,16]. Therefore, determination of AChE activity and DNA damage can be useful for monitoring OPs exposure[8,9]. The comet assay is a common technique and a convenient tool for measuring DNA damage in individual cells[17]. Previous studies concluded that mean values of tail length and tail moment for lymphocytes from applicators and farmers were significantly greater than those from controls[7,18]. However, DNA damage and AChE activity between farmers and non-farmers in our results were not different. It is likely that occupational exposure to pesticides among farmers were not high enough to damage DNA or inhibit acetylcholiesterase. Another possibility is that non-farmers might be exposed pesticides from the environment. All non-farmers in this study living in an agricultural community and being a member of farmers' family may be exposed to pesticides that may have been inadvertently transported to their homes through farmers' skin and clothing[19].

Occupational knowledge among farmer groups was not significantly different, whereas some items of practice among farmer groups were different, and the results determined that the protective behavior in corn farmers was poor than that in other farmers. Besides, our results determined a positive association between knowledge and practice in most items. However, no association was found in 2 items—checking spraying equipment and mixing pesticides outdoor—possibly due to difficulties in practical usage in some working conditions or unawareness of safety in these practices. These results are in agreement with those by Mohanty et al. (2013) who reported that knowledge of agricultural workers in South India was associated with their practice related to pesticides[20].

With regard to the period of occupational practice, the period during pesticide spraying had more chances of exposure to pesticides than during the period before and after spraying. Exposure to OPs mainly occurs during pesticide spraying via dermal, inhalation, and ingestion. Although most farmers were concerned about protecting themselves by wearing protective measures, they used improper and simple protective measures. These findings were in conformity with that by Tamrin and Jamiluddin (2014) who reported that the knowledge score of pesticide management was high; however, the practice score, especially the use of personal protective equipment (PPE), was very poor[21]. In addition, the study by Yuantari et al. (2015) suggested that almost none of the farmers used standardized PPE and used PPE completely[22]. It may be due to the high cost of PPE, hot tropical climatic conditions, poverty, or lack of training programs by the government[21,23-25]. Some farmers had an inappropriate protective behavior, such as eating, drinking, smoking, taking a break, blowing or sucking a spray nozzle with their mouth, and spraying when it was windy. Furthermore, the belief among farmers that crops should be sprayed with pesticides before pest infestation. They also obtained more information about pesticides from neighbors than from government officers and retailers, possibly due to a lack of trust in government and pesticide retailers[26]. It can, therefore, be concluded that farmers had good knowledge and practice regarding pesticide safety, but they lacked awareness regarding pesticide usage and exposure risks in some issues.

In terms of health symptoms related to ΣDAP, AChE activity, DNA damage, and occupational knowledge and practice, this study determined that pesticide-related symptoms, including breathlessness, chest pain, dry throat, numbness, muscle weakness, cramp, dizziness, eye irritation, white/red rash, and white/red pimple, were classified as respiratory, muscle, nervous, and epithelial symptoms. These findings were in agreement with that by Sapbamrer et al. (2016) who reported that the type of crop cultivation was associated with an increasing prevalence of respiratory tract, muscle system, and skin irritation[27]. Besides, the study conducted with rice farmers from Northern Thailand also revealed that occupational exposure and agricultural tasks were associated with an increasing prevalence of breathlessness, chest pain, dry throat, cramp, numbness, and diarrhea[6]. A similar finding was previously reported with farmers in other countries. The study among Egyptian farm workers determined that the five toxicity symptoms associated with pesticide exposure included eye irritation (64.3%), dizziness (32.4%), breathlessness or chest pain (28.1%), skin irritation (27%), and headache (26.5%)[28]. The study with Indian farm workers determined that self-reported symptoms associated with pesticide use were skin rash (40.5%), headache (48%), excessive sweating (22.5%), and diarrhea (21.3%)[29]. The study among Thai chilli-farm workers from North-East Thailand determined that the most common pesticide-related symptoms included dizziness (38%), headache (30.9%), nausea/vomiting (26.9%), and fever (26.9%)[30]. Interestingly, our results determined that farmers had a significantly higher prevalence of muscle weakness (OR=3.79) and numbness (OR=3.45) as compared with non-farmers. It is possible that the major organ system affected by pesticide exposure was the muscle system. Acetylcholine is a neurotransmitter that motors neurons released for activating muscles in the body. Exposure to OPs causes acetylcholinesterase inhibition, leading to an excess of acetylcholine at the neuromuscular junction. It results in muscle overstimulation and a consequent nervous dysfunction[3]. The nervous system is not a homogenous single function; therefore, pesticides could produce various symptoms depending on the extent and level of effects, which range from mild to obvious neurological symptoms[31]. Besides, experimental animal and human evidences have shown the association between long-term low-level exposure to OPs and chronic toxicity and neurobehavioral symptoms[16,32]. However, muscle weakness and numbness may not be entirely attributed to pesticide exposure. Thus, another possibility is that these symptoms determined in our study may be caused due to ergonomic problems from agricultural work[33].

There are limitations in this study. First, this a cross-sectional study with a small sample size. Second, ΣDAP levels in this study were rather low, possibly due to an inappropriate time of urine collection. Urinary DAP metabolites can be useful for biological monitoring of human population exposed to OPs. If possible, urine samples should be collected within a 24-hour period after exposure to OPs. It is, therefore, usually not practical under a cross-sectional survey condition[34]. However, urine samples were collected from farmers who were reportedly exposed to OPs on the farm less than 7 days ago. Third, urinary DAP metabolites were not taken from non-farmers because of the limitation of time and research budget. Fourth, the data of health symptoms were obtained from interviews without physical examination, and some symptoms might not be specific only for exposure to OPs. At last, females and smokers constituted a small number of participants; thus, they may be a confounding factor in the study.

Conclusions

There were no differences in ΣDAP levels, AChE activity, and occupational knowledge and practice scores among all farmers. A remarkable finding was that farmers had a significantly higher prevalence of muscle weakness (OR=3.79) and numbness (OR=3.45) as compared with non-farmers. Our findings, therefore, suggest that a long-term low-level exposure to OPs may be associated with an increasing prevalence of muscle symptoms. However, a cohort study incorporating sensitive health outcomes and measurement of multiple pesticides monitoring on a larger scale should be conducted to warrant whether health symptoms were caused by a long-term low-level exposure to OPs.

Acknowledgments: The study was funded by University of Phayao Grant 2015. We are grateful to Research Institute for Health Sciences from Chiang Mai University and School of Medicine from Suranaree University of Technology for their cooperation. We would like to thank Ms. BenchamasPanya for her support. We thank all the scientists from School of Medicine, University of Phayao, as well as the officers in Health promotion Hospital of Rong Kham Sub-district for their assistance.

Conflicts of interest: We hereby declare that we do not have any competing interest.

References

1) Office of Agricultural Economics. Agricultural statistics of Thailand 2014. [Online]. 2014[cited 2015 Feb. 1]; Available from: URL: http://www.oae.go.th
2) Hauser S. Corn or maize, essential crop for millions of Thai farmers. [Online]. 2014[cited 2015 Feb. 1]; Available from: URL: http://www.sjonhauser.nl
3) Ray DE, Richards PG. The potential for toxic effects of chronic, low-dose exposure to organophosphates. Toxicol Lett 2001; 120(1-3): 343-351.
4) Ecobichon DJ. Toxic effects of pesticides. In: Klaassen CD, editor. Casarett & Doull's Toxicology: The basic science of poisons. USA. 5th edn. McGraw-Hill; 1996. p. 643-698.
5) Kamel F, Engel LS, Gladen BC, Hoppin JA, Alavanja MC, Sandler DP. Neurologic symptoms in licensed private pesticide applicators in the agricultural health study. Environ Health Perspect 2005; 13(7): 877-882.
6) Sapbamrer R, Nata S. Health symptoms related to pesticide exposure and agricultural tasks among rice farmers from Northern Thailand. Environ Health Prev Med 2014; 19(11): 12-20.
7) Muniz JF, McCauley L, Scherer J, et al. Biomarkers of oxidative stress and DNA damage in agricultural workers: A pilot study. Toxicol Appl Pharmacol 2008; 227(1): 97-107.
8) Lukaszewicz-Hussain A. Role of Oxidative stress in organophosphate insecticide toxicology-short review. Pestic Biochem Physiol 2010; 98(2): 145-150.
9) Singh S, Kumar V, Thakur S, et al. DNA damage and cholinesterase activity in occupational workers exposed to pesticides. Environ Toxicol Pharmacol 2011; 31(2): 278-285.
10) Koureas M, Tsezou A, Tsakalof A, Orfanidou T, Hadjichristodoulou C. Increased levels of oxidative DNA damage in pesticide sprayers in Thessaly Region (Greece). Implications of pesticide exposure. Sci Total Environ 2014; 496: 358-364.
11) Sapbamrer R, Hongsibsong S. Organophosphorus pesticide residues in vegetables from farms, markets, and a supermarket around Kwan Phayao Lake of Northern Thailand. Arch Environ Contam Toxicol 2014; 67(1): 60-67.
12) Prapamontol T, Sutan K, Laoyang S, et al. Cross validation of gas chromatography-flame photometric detection and gas chromatography-mass spectrometry methods for measuring dialkylphosphate metabolites of organophosphate pesticides in human urine. Int J Hyg Environ Health 2014; 217(4-5): 554-566.
13) Hornung RW, Reed LD. Estimation of average concentration in the presence of nondetectable values. Appl Occup Environ Hyg 1990; 5(1): 46-51.
14) Singh NP, McCoy MT, Tice RR, Schneider EL. A simple technique for quantitation of low levels of DNA damage in individual cells. Exp Cell Res 1988; 175(1): 184-191.
15) Ellman GL, Courtney KD, Andres V Jr, Feather-Stone RM. A new and rapid colorimetric determination of acetylcholinesterase activity. Biochem Pharmacol 1961; 7: 88-95.
16) Ray DE, Richards PG. The potential for toxic effects of chronic, low-dose exposure to organophosphates. Toxicol Lett 2001; 120(1-3): 343-351.
17) Kumaravel TS, Vilhar B. Comet assay measurement: a perspective. Cell Biol Toxicol 2009; 25(1): 53-64.
18) Kisby GE, Muniz JF, Scherer J, et al. Oxidative stress and DNA damage in agricultural workers. J Agromed 2009; 14(2): 206-214.
19) Salvatore AL, Bradman A, Castorina R, et al. Occupational behaviors and farm workers' pesticide exposure: findings from a study in Monterey County, California. Am J Ind Med 2008; 51(10): 782-794.
20) Mohanty MK, Behara BK, Jena SK, et al. Knowledge attitude and practice of pesticide use among agricultural workers in Puducherry, South India. J Forensic Leg Med 2013; 20: 1028-1031.
21) Shamsul BMT, Nurul ABJ. Knowledge, attitude, and practice of pesticide application at selected agricultural industries. In: Khairunnisa S, TShamsul BMT, editors. Occupational safety and health in commodity agriculture: case studies from Malaysian agricultural perspective. 1st edn. Tamrin SBM; 2014. p. 149-171.
22) Yuantari MG, Van Gestel CA, Van Straalen NM, Widianarko B, Sunoko HR, Shobib MN. Knowledge, attitude, and practice of Indonesian farmers regarding the use of personal protective equipment against pesticide exposure. Environ Monit Assess 2015; 187(3): 142-148.
23) Peiris-John RJ, Ruberu DK, Wickremasinghe AR, van-der-Hoek W. Low-level exposure to organophosphate pesticides leads to restrictive lung dysfunction. Respir Med 2005; 99 (10): 1319-1324.
24) Andrade-Rivas F, Rother H. Chemical exposure reduction: factors impacting on South African herbicide sprayers' personal protective equipment compliance and high risk work practices. Environ Res 2015; 142: 34-45.
25) Weng CY, Black C. Taiwanese farm workers' pesticide knowledge, attitudes, behaviors and clothing practices. Int J Environ Health Res 2015; 25(6): 685-696.

26) Fan L, Niu H, Yang X, et al. Factors affecting farmers' behaviour in pesticide use: insights from a field study in northern China. Sci Total Environ 2015; 537: 360-368.
27) Sapbamrer R, Hongsibsong S, Kerdnoi T. Urinary dialkylphosphate metabolites and health symptoms among farmers in Thailand. Arch Environ Occup Health 2016 (Epub ahead of print).
28) Yassin MM, Abu Mourad TA, Safi JM. Knowledge, attitude, practice, and toxicity symptoms associated with pesticide use among farm workers in the Gaza Strip. Occup Environ Med 2002; 59(6): 387-393.
29) Kumari P, Reddy KG. Knowledge and practices of safety use of pesticides among farm workers. IOSR-JAVS 2013; 6(2): 1-8.
30) Kachaiyaphum P, Howteerakul N, Sujirarat D, Siri S, Suwannapong N. Serum cholinesterase levels of Thai chilli-farm workers exposed to chemical pesticides: prevalence estimates and associated factors. J Occup Health 2010; 52(1): 89-98.
31) Brain PG. Adverse health effects after low-level exposure to organophosphates. Occup Environ Med 2001; 58(11): 689-690.
32) Jamal GA, Hansen S, Julu PO. Low level exposure to organophosphorus esters may cause neurotoxicity. Toxicol 2002; 181-182: 23-33.
33) Naeini HS, Karuppiah K, Tamrin SB, Dalal K. Ergonomics in agriculture: an approach in prevention of work-related musculoskeletal disorders (WMSDs). J Agric Environ Sci 2014; 3 (2): 33-51.
34) Richter ED, Kaspi L, Gordon M, Levy S, Israeli R, Gruener N. Monitoring for neurotoxic effects from low level exposures to organophosphate pesticides. Sci Total Environ 1984; 32(3): 335-344.