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

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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.

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

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. 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. 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. 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. 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 \(\Sigma\)DAP 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 \(\Sigma\)DAP 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.

**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 \(\Sigma\)DAP 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 mL urine containers and stored in ice cooler until they were transported to a laboratory. The samples were aliquoted to 15 mL ×3 tubes and frozen at -20°C until they were analyzed. Urinary concentrations of dimethylphosphate (DMP), dimethyldithiophosphate (DMTP), dimethylidithiophosphate (DMDTP), diethylphosphate (DEP), diethylthiophosphate (DETP), and diethylidithiophosphate (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 213.

Furthermore, 10 mL of blood samples were collected from farmers and non-farmers. Thereafter, 9 mL of blood samples were separated to a heparin tube to determine DNA damage and 1 mL 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 method15.

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 (P25-P75), 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 Kruksal-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 (χ²). 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.
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 $46 \pm 8$ 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,
harvesting products, scattering seed, packing products, and watering. They obtained the information about pesticide usage from neighbors, retailers, and government officers. Most farmers disposed unused 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 DETP (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 0.26 μg/g creatinine for double-crop farmers), followed by DEDTP (0.4 μg/g creatinine for rice farmers, 0.36 μg/g creatinine for corn farmers, and 0.26 μg/g creatinine for double-crop farmers), and then DETP (0.36 μg/g creatinine for rice farmers, 0.26 μg/g creatinine for corn farmers, and 0.15 μg/g creatinine for double-crop farmers), respectively.

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

| Parameters                                      | Median (P25th, P75th) | Rice farmers (n=21) | Corn farmers (n=33) | Double-crop farmers (n=49) | Non-farmers (n=47) | P value |
|------------------------------------------------|-----------------------|---------------------|---------------------|----------------------------|-------------------|---------|
| 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)       |                            |                   | 0.409   |
| DMTP                                          | 0.4 (0.23-0.56)       | 0.3 (0.18-0.45)     | 0.26 (0.18-0.45)    |                            |                   | 0.271   |
| DMDTP                                         | 0.36 (0.22-0.51)      | 0.33 (0.18-0.48)    | 0.26 (0.18-0.4)     |                            |                   | 0.378   |
| DEP                                           | 0.51 (0.34-1.2)       | 0.36 (0.26-0.87)    | 0.33 (0.19-0.81)    |                            |                   | 0.103   |
| DETP                                          | 0.26 (0.15-1.0)       | 0.16 (0.09-0.26)    | 0.15 (0.09-0.36)    |                            |                   | 0.151   |
| DEDTP                                         | 0.4 (0.27-0.96)       | 0.33 (0.18-0.48)    | 0.26 (0.18-0.48)    |                            |                   | 0.084   |
| ΣDMP**                                        | 6.4 (3.5-8.8)         | 6.6 (3.8-12.7)      | 4.0 (2.8-10.6)      |                            |                   | 0.407   |
| ΣDEP**                                        | 1.8 (0.75-3.7)        | 1.1 (0.65-1.9)      | 0.82 (0.47-2.1)     |                            |                   | 0.097   |
| ΣDAP**                                        | 9.4 (5.2-12.3)        | 7.7 (4.4-17.0)      | 6.5 (3.1-13.9)      |                            |                   | 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)          |                            |                   | 0.152   |
| During pesticide spraying                      | 12 (11-13)            | 12 (10-12)          | 11 (10-13)          |                            |                   | 0.371   |
| After pesticide spraying                       | 7 (6-7)               | 7 (6-7)             | 7 (6-7)             |                            |                   | 0.455   |
| Total knowledge score                          | 30 (28-30)            | 29 (25-30)          | 28 (27-31)          |                            |                   | 0.226   |
| Occupational practice score (62 full marks)    |                       |                     |                     |                            |                   |         |
| Before pesticide spraying                      | 21 (18-22)            | 19 (17-21)          | 20 (17-22)          |                            |                   | 0.481   |
| During pesticide spraying                      | 25 (20-26)            | 23 (20-24)          | 22 (19-24)          |                            |                   | 0.056   |
| After pesticide spraying                       | 13 (12-14)            | 12 (11-13)          | 13 (12-14)          |                            |                   | 0.118   |
| Total practice score                           | 59 (50-60)            | 53 (49-58)          | 55 (48-58)          |                            |                   | 0.183   |

DMP=dimethylphosphate; DMTP=dimethylthiophosphate; DMDTP=dimethylthiodiphosphate; DEP=diethylphosphate, DETP=diethylthiophosphate; DEDTP=diethylthiodiphosphate; a ΣDMP=DMP+DMTP+DMDTP; b ΣDEP=DEP+DET+DEDTP; c ΣDAP=ΣDMP+ΣDEP; d data not collected in non-farmers
Table 3. The association between occupational knowledge and practice among farmers (n=103)

| Questions                                                                 | Frequency (% of farmers who answer “Yes” | χ²       | P value |
|---------------------------------------------------------------------------|------------------------------------------|----------|---------|
| **Section 1: practice before pesticide spraying**                         |                                           |          |         |
| 1. Buy pesticides having right labels, warning signs, chemical and         | Never (1) | Sometimes (15.5) | Always (83.5) | 36.6 | <0.001** |
| manufacturing names                                                       |             |                       |              |     |         |
| 2. Survey types and amounts of pests before buying pesticides             | 1 (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) | 15 (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 (3.0) | 6 (6.1) | 90 (90.9) | 5.13 | 0.077   |
| 8. Mixing pesticide in outdoor                                            | 2 (2.1) | 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 (4.1) | 82 (82) | 6.18 | 0.046*  |
| 11. Do not use hands for mixing pesticides                                | 8 (8.8) | 6 (6.1) | 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 (15.7) | 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) | 75 (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 (χ²=12.88, P=0.012); (2) using pesticides with the suggestion of agriculture officers (χ²=13.16, P=0.011); (3) not eating, drinking, or smoking during spraying (χ²=13.09, P=0.011); and (4) washing the spraying equipment before keeping them back (χ²=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               | ΣDAP, μg/g creatinine | Median (P²50, P⁷⁵) | AChE activity, U/l | Median (P²50, P⁷⁵) | P value |
|------------------------|-----------------------|--------------------|--------------------|--------------------|---------|
| Has symptom            | No symptom            |                    | Has symptom        | No symptom         |         |
| Breathlessness         | 7.7 (4.4-15.7)        | 3.1 (2.0-5.3)      | 0.012*             | 8.656 (8.190-9.828) | 0.015*  |
| Chest pain             | 4.7 (2.4-6.3)         | 7.7 (4.4-15.4)     | 0.064              | 8.424 (8.073-10.413)| 0.039*  |
| Cough                  | 7.7 (5.0-9.9)         | 7.4 (4.3-15.4)     | 0.709              | 10.062 (8.249-10.823)| 0.101   |
| Dry throat             | 7.7 (3.1-15.4)        | 7.5 (4.4-14.2)     | 0.510              | 10.062 (8.424-10.764)| 0.029*  |
| Numbness               | 7.7 (4.6-13.9)        | 7.5 (4.2-14.7)     | 0.920              | 10.530 (8.190-11.115)| 0.039*  |
| Muscle weakness        | 8.0 (4.4-15.4)        | 7.3 (4.3-13.5)     | 0.659              | 10.413 (8.658-11.173)| 0.158   |
| Cramp                  | 6.6 (3.5-15.4)        | 7.7 (4.3-13.5)     | 0.726              | 10.647 (8.249-11.174)| 0.207   |
| Headache               | 10.1 (4.7-15.4)       | 6.9 (4.2-13.1)     | 0.246              | 9.243 (8.190-10.589)| 0.001*  |
| Dizziness              | 7.7 (3.7-16.6)        | 7.5 (4.4-12.9)     | 0.972              | 10.062 (8.424-10.764)| 0.041*  |
| Balance problem        | 11.3 (5.1-18.5)       | 7.4 (4.3-14.0)     | 0.464              | 10.998 (8.366-14.099)| 0.578   |
| Eye irritation          | 11.5 (4.4-15.5)       | 7.3 (4.3-12.3)     | 0.436              | 10.062 (8.424-10.764)| 0.046*  |
| White/red rash         | 9.7 (3.7-15.6)        | 7.4 (4.3-13.1)     | 0.942              | 10.764 (8.424-11.759)| 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)| 0.689   |
| Diarrhea               | 7.8 (3.7-17.9)        | 7.7 (4.4-14.0)     | 0.882              | 9.828 (8.190-10.355)| 0.082   |

| Symptoms               | Tail length (μm) | Median (P²50, P⁷⁵) | P value |
|------------------------|-----------------|--------------------|---------|
| Has symptom            | No symptom      |                    |         |
| Breathlessness         | 6.6 (6.6-7.3)   | 6.4 (5.9-6.6)      | 0.017*  |
| Chest pain             | 6.6 (6.5-7.3)   | 6.4 (5.9-6.6)      | 0.031*  |
| Cough                  | 6.2 (5.3-6.4)   | 6.4 (6.0-6.7)      | 0.141   |
| Dry throat             | 6.3 (5.4-6.6)   | 6.4 (6.0-6.7)      | 0.273   |
| Numbness               | 6.3 (5.7-6.6)   | 6.4 (6.0-6.7)      | 0.491   |
| Muscle weakness        | 6.4 (6.1-6.8)   | 6.3 (5.8-6.6)      | 0.110   |
| Cramp                  | 6.5 (6.2-6.8)   | 6.4 (5.7-6.6)      | 0.138   |
| Headache               | 6.4 (5.9-6.6)   | 6.4 (6.0-6.7)      | 0.758   |
| Dizziness              | 6.5 (6.3-6.7)   | 6.4 (5.7-6.7)      | 0.265   |
| Balance problem        | 6.5 (6.2-7.0)   | 6.4 (6.0-6.7)      | 0.418   |
| Eye irritation          | 6.4 (6.0-6.6)   | 6.4 (6.0-6.7)      | 0.936   |
| White/red rash         | 6.3 (5.7-6.6)   | 6.4 (6.0-6.7)      | 0.640   |
| White/red pimple       | 6.3 (6.2-6.5)   | 6.4 (6.0-6.7)      | 0.891   |
| Diarrhea               | 6.4 (6.1-6.7)   | 6.4 (5.9-6.7)      | 0.673   |

| Symptoms               | Tail moment (μm) | Median (P²50, P⁷⁵) | P value |
|------------------------|-----------------|--------------------|---------|
| Has symptom            | No symptom      |                    |         |
| Breathlessness         | 3.3 (3.3-3.4)   | 3.2 (3.0-3.3)      | 0.027*  |
| Chest pain             | 3.3 (3.3-3.5)   | 3.2 (3.0-3.3)      | 0.030*  |
| Cough                  | 3.2 (2.8-3.4)   | 3.3 (3.1-3.4)      | 0.109   |
| Dry throat             | 3.2 (2.9-3.3)   | 3.3 (3.1-3.4)      | 0.155   |
| Numbness               | 3.2 (2.9-3.3)   | 3.3 (3.1-3.4)      | 0.479   |
| Muscle weakness        | 3.3 (3.1-3.4)   | 3.2 (3.0-3.3)      | 0.198   |
| Cramp                  | 3.3 (3.1-3.4)   | 3.2 (3.0-3.3)      | 0.173   |
| Headache               | 3.2 (3.0-3.3)   | 3.3 (3.1-3.4)      | 0.526   |
| Dizziness              | 3.3 (3.1-3.4)   | 3.2 (3.0-3.3)      | 0.257   |
| Balance problem        | 3.3 (3.1-3.3)   | 3.3 (3.2-3.4)      | 0.464   |
| Eye irritation          | 3.3 (3.0-3.3)   | 3.3 (3.1-3.4)      | 0.808   |
| White/red rash         | 3.2 (3.0-3.4)   | 3.3 (3.1-3.4)      | 0.612   |
| White/red pimple       | 3.2 (3.0-3.3)   | 3.3 (3.1-3.4)      | 0.984   |
| Diarrhea               | 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 (P25th-P75th) of knowledge score | Median (P25th-P75th) of practice score |
|-------------------|----------------------------------------|----------------------------------------|
|                   | Before pesticide spraying              | During pesticide spraying              | After pesticide spraying               |
|                   | 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                                  |

**P<0.01;
*P<0.05;
**P<0.001

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
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 not be 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 acetylcholinesterase 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 (7). 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 acetylcholinesterase. Another possibility is that non-farmers might be exposed to 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

### 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
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\(^6\). In addition, the study by Yuantari et al. (2015) suggested that almost none of the farmers used standardized PPE and used PPE completely\(^6\). It may be due to the high cost of PPE, hot tropical climatic conditions, poverty, or lack of training programs by the government\(^21,22,23\). 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\(^30\). 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\(^31\). Besides, the study conducted with rice farmers from Northern Thailand also revealed that occupational exposure included eye irritation (64.3%), dizziness (32.4%), breathlessness or chest pain (28.1%), skin irritation (27%), and headache (26.5%)\(^32\). 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%)\(^33\). 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%)\(^34\). 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\(^35\). 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\(^36\). Besides, experimental animal and human evidences have shown the association between long-term low-level exposure to OPs and chronic toxicity and neurobehavioral symptoms\(^37-39\). 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\(^31\).

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\(^31\). 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.

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

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