The Use of Organophosphate and Carbamate Pesticides on Paddy Fields and Cholinesterase Levels of Farmers in Sam Chuk District, Suphan Buri Province, Thailand

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Abstract
Several types of organophosphate and carbamate pesticides have been used extensively by the farmers in Thailand during the last few decades. This study examines the use of organophosphate and carbamate pesticides and cholinesterase levels in the blood (AChE) of farmers by using reactive paper on samples which were taken from 236 farmers in Sam Chuk district, Suphan Buri province. Moreover, information on the application, code of practices, duration, and frequency of the use of organophosphate and carbamate pesticides was obtained from questionnaires and analyzed by using descriptive statistics and correlation coefficients. The results showed that 37.3% had AChE levels lower than standard (< 87.5 units/ml) indicating a risk to health (28.8%) and an unsafe condition (8.5%). The use of chemical pesticides was related to the AChE levels of farmers as follows: methods of uses (r = 0.452, p < 0.001), practice (r = 0.396, p < 0.001), duration (r = 0.206, p < 0.004), chemical content (r = - 0.243, p < 0.001), frequency (r = - 0.210, p < 0.001) and chemical type (r = 0.171, p < 0.001). While 80.9% of farmers used the pesticides in both groups, most farmers (76.7%) used the labeled instruction incorrectly by overusing the chemical pesticides, followed by wrong practices (53%), application (51.3%), duration (39.4%), and frequency (39.4%). The results of the AChE levels of farmers were 62.7% within standard (≥ 87.5 units/ml) which were considered safe levels.

However, in order to be safe, farmers should strictly follow the instructions on the label/packaging of chemicals for application procedures and use personal protective equipment regularly. Especially, the farmers who had AChE levels lower than standard.

Keywords: Pesticides, Organophosphate, Carbamate, Cholinesterase, Farmers, Suphan Buri.

1. Introduction
The reliance on chemicals, especially pesticides which are toxic and highly dangerous, in agriculture is increasing in developing countries [1, 2]. Pesticides are used extensively in agricultural production to check or control pests, diseases, weeds and other plant pathogens in an effort to reduce or eliminate yield losses and preserve high product quality [3, 4].

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Moreover, chemical pesticides are used in varying amounts on rice crops to maintain yield and quality. Several types of organophosphate and carbamate pesticides have been used extensively by farmers in many countries such as Thailand during the last few decades. However, using pesticides incorrectly can affect the environment and human health [5-10]. Thus, the use of pesticides has caused health problems to the population that handles, or is exposed to, them [11-13], especially rice farming with the use of pesticides. Farmers are at risk and in danger of toxic chemicals increasingly used during their employment. In Thailand, this occupational health problem is one with acute exposure and effects. Organophosphates have become the most abundantly used insecticide group, e.g. chlorpyrifos, dichrotofos, parathion methyl, and profenofos [14-15] followed by carbamates e.g. methomyl and fenobucarb [16]. They exhibit their acute effects by inhibiting the function of the nervous system enzyme acetylcholinesterase [17-20].

In Thailand, Suphan Buri province has the largest low lands in the Tha Chin basin with large irrigation systems. The rice-paddy area is the largest in the country and is planted all year round. In 2011-2012, [21] Suphan Buri was reported to have the country's top rice production with 808,558 tons and the highest grain yield, in Sam Chuk district, at 90,600 tons. The continuous rice plantation in a large area causes diseases and insect pests which damage rice crops, for example, the outbreak of the brown planthopper (Nilapavata lugens) in paddy fields. As a result, farmers have to use dangerous chemicals more than is usually necessary. The use of pesticides in intensive farming, including the rice-paddy area like Suphan Buri, has been instrumental in reducing crop loss both before and after harvest. Currently, pesticide use is on the ascent. It was found that rice farming in Suphan Buri province, used chemical pesticides 1-15 times per rice cultivation crop. However, associated with the increased use of pesticides are health and environmental problems which have arisen due to indiscriminate use and inappropriate handling of the chemicals [22].

Cholinesterase, an enzyme in the body has two types, type I or true cholinesterase or acetyl cholinesterase (AChE), an enzyme in the blood and nervous system that damages acetylcholine (ACh) is released from the nerve synapses and is an indicator of toxicity by organophosphate and carbamate in the body. Type II or plasma pseudocholinesterase or butyrylcholinesterase (BChE), an enzyme found in the body such as the brain, liver, skin, blood and plasma is responsible for the destruction of ACh hydrolysis esters and others such as benzoylcholine, succinylcholine, procaine, and propanidid [23-28]. We can use it to identify the amount of exposure to toxins or residues, or accumulation of certain chemicals in the body.

AChE is a biological marker for monitoring serum cholinesterase and cholinesterase enzymes in red blood cells which can assess actual exposure to pesticides, particularly organophosphates [29-31]. They inhibit the action of cholinesterase thus increasing the cholinergic effects of the neurotransmitter, acetylcholine in the body, and depolarization of the neural transmission [32-34]. AChE can be used to detect the level of cholinesterase, or the destruction of toxic chemicals and the remaining level in order to check the risk levels to health [35] by specific organophosphate and carbamate pesticides certain cholinesterase inhibitors, [6, 36] e.g. using reactive paper [37]. [5] The WHO/FAO organization estimates an annual worldwide total of 3 million cases of acute and severe pesticide poisoning, resulting in some 220,000 deaths. Occupational pesticide poisoning is a major
health problem among agricultural workers. It has been estimated that 1.3 billion workers are active in agricultural production worldwide [38-39]. Ecobichon [1] reported that the largest proportion of human acute toxicity of pesticides due to organophosphate. In Thailand, the Ministry of Public Health [40] showed the situation with pesticide poisoning in 1,452 patients, most of whom were farmers (48.76%) and the labor force (23.21%). In the classification of toxins that cause most illness was organophosphate (10.61%). In 2010, the number of patients increased to 2,158 with the majority of farmers in the central region including Suphan Buri with one of the highest illness rates, in the top five of the country, at rate of 12.8 cases per 100,000 persons [41].

Therefore, the use of organophosphate and carbamate pesticides by most rice farmers of Sam Chuk district, Suphan Buri province, in order to prevent and eliminate insects in rice paddy fields, is associated with AChE and health. The objectives of this study were to determine the use of organophosphate and carbamate pesticides, the AChE of farmers, and the relationship between AChE and the use of organophosphate and carbamate pesticides by farmers in Sam Chuk district, Suphan Buri province. Further, the farmers should be informed about the level of pesticide exposure by AChE and the pesticides that cause health problems, especially due to improper usage of pesticides on paddy fields.

2. Materials and methods

2.1 Samples

A total of 236 farmers in Sam Chuk district, Suphan Buri province from seven sub-districts were selected by simple random sampling.

2.2 Tools

Questionnaires containing personal characteristics data and the use of organophosphate and carbamate pesticides were completed about the farmers. A review of several reports and research works were used to relate behavioral factors to the use of pesticides. Common factors for farmers at risk of exposure to toxic chemicals include: chemical type, chemical content, frequency of use, duration, methods and practices or personal protection when using hazardous chemicals [9, 35, 42-45]. The test kit in this study uses reactive paper. It is the standard for detection of AChE in the body from exposure to pesticides, which was purchased from The Government Pharmaceutical Organization, Ministry of Public Health, Thailand. It is used to detect the cholinesterase (true cholinesterase or acetyl cholinesterase or AChE) in the blood of farmers indicating poisoning by organophosphate and carbamate in the body. Blood samples were taken by public health officials in primary health care in the area, Sam Chuk district. The comparison of color changes with the standard were divided into four levels: normal, safe, risk, and unsafe conditions of health [37]. Thus:

- A yellow colored test paper indicates (AChE ≥ 100 units/ml), a normal condition.

- If the color of the test paper is yellow brown (AChE 87.5-99.9 units/ml), the health condition is safe.  

- If the color of the test paper is green there is a level of risk (AChE 75-87.4 units/ml), indicating toxicity from pesticides.  

- If the color of the test paper turns blue to blue-green the level is unsafe (AChE < 75 units/ml), indicating that toxicity from pesticides is very high.  

Additionally, the AChE on health effects could be divided into two groups. The first group includes AChE levels within the standard (AChE ≥ 87.5 units/ml) indicating normal and safe health
The other group includes AChE levels lower than the standard (AChE < 87.5 units/ml), indicating risk and unsafe conditions of health [37, 46].

2.3 Data Analysis

The statistics using the SPSS version 11.5 was used by employing descriptive statistics to analyze mean, percentage and standard deviation from personal data. The relationship between the use of organophosphate and carbamate pesticides and each of the factors was considered, including: chemical type, chemical content, method, duration, frequency, and practices. The AChE of farmers was the qualitative data used for correlation coefficients (r) and p-values.

3. Results and Discussion

3.1 Personal characteristics

The demographic characteristics of the sample of 236 farmers consisted of males (44.5%) and females (55.5%) of which 77.5% were married. The majority of participants were aged between 40-49 years (37.7%) and 50-59 years (37.3%) with an average age of 50 years. The majority of workers were family farmers in agricultural activities, including the Sam Chuk district. This constitutes a majority of the agricultural labor families which was the same as the previous study [16] because of the age of farmers that have been rice farming for a long time. Especially, in paddy fields with usage of organophosphate and carbamate pesticides, farmers are likely to be exposed to toxic chemicals that affect AChE in the body and pose a risk to their health.

Most of the farmers had a primary school education (68.6%) and secondary school education (26.7%). Their limited education be a cause for a lack of knowledge and understanding by farmers on practices for the use of pesticides in strictly following the instructions on the label / packaging, which was the same as the previous study, [16] and without regard to their health or the importance of using personal protective equipment (PPE) to prevent exposure to the use of pesticides. Moreover, low levels of care with regard to pesticide use, but high levels of awareness among the farmers with regard to the environmental impacts of pesticide use were found [47-48].

Nearly half of the farmers were average income earners at 2501-5000 baht / month (49.2%), which was derived from farming as the main occupation in Sam Chuk district. The majority of farmers based their incomes on no other sources. Thus, to increase income to the family, farmers focus on productivity with the use of chemical pesticides because they are generally easy to buy, convenient, and labor saving. In addition, a limited income affects the availability of PPE.

The majority of farmers did not have congenital diseases (86.4%) because some congenital diseases affect the reduction of AChE. Some patients may have diminished enzyme (pseudocholinesterase deficiency) which will cause a decreasing AChE in the body [49]. In this study, the diseases included: heart disease, liver disease, chronic kidney disease, tuberculosis, malnutrition, AIDS, and Alzheimer's disease. Therefore, for farmers who had these congenital diseases, care was taken in interpreting the results of AChE [26]. Almost all 92.8%, of the farmers had never been monitored for the use of pesticides and their effect on health.

3.2 The use of pesticides by the farmers in Sam Chuk district, Suphan Buri province

Most of the farmers used pesticides both organophosphate and carbamate (80.9%), chemical content followed the instructions of the label / packaging sometimes (32.2%), frequency 1-2 times /
month (39.4%), duration 1-2 hours / times (39.4%), methods of use had followed the instructions of the label / packaging every time (48.7%), and practice the use of personal protective equipment (PPE) every time (47.0%), as shown in Table 1. From Table 1, based on the use of organophosphate and carbamate pesticides on paddy fields and AChE of the farmers, it was found that 88 farmers (37.3%) had AChE lower than the standard (AChE < 87.5 units/ml). The majority of these farmers reported incorrect usage of pesticides, especially, 80.7% used PPE only sometimes, 48.9% of them followed the label / packaging only sometimes, 46.6% used the chemical content on the advice of neighbors more than by following the instructions of the label / packaging, 45.5% of these farmers have duration 2-4 hours / time, 30.7% frequency 1 or 2-3 times / week, and 87.5% used the pesticides in both groups. Thus, they are at risk on conditions of health from exposure to toxic chemicals and decreased AChE in the body more than farmers that had AChE within the standards.

3.3 The levels of AChE

Detection of the AChE of 236 farmers in Sam Chuk district, Suphan Buri province by reactive paper shown in Table 2. From Table 2, the standard of AChE levels on health effects were divided into two groups. The first with AChE within the standard (AChE ≥ 87.5 units/ml) and the other with AChE lower than the standard (AChE < 87.5 units/ml) [27]. It was found that 62.7% of the farmers’ levels were within the standard and 37.3% were lower than the standard. These results were the same as previous studies [25, 46, 50] stating that about 1 in 3 farmers tested for AChE by reactive paper had AChE levels lower than the standard.

3.4 Relationships between AChE of the farmers in Sam Chuk district, Suphan Buri province and the use of organophosphate and carbamate pesticides

The results from detection of the level of cholinesterase in the blood (AChE) by reactive paper of 236 farmers in Sam Chuk district, Suphan Buri province and the study of the relationship between the use of pesticides and the AChE, showed that AChE is associated with the use of pesticides at high significance (p < 0.001) Table 3. This study found that 68.3% of farmers had AChE within the standard. Most farmers used pesticides by following the instructions of the label / packaging strictly every time, whereas farmers had AChE lower than the standard with only 15.9% (r = 0.452, p < 0.001). This was the same as the previous studies [51-52]. The major risk factors were related to agrochemical exposure resulting from the misuse of pesticides, including erroneous beliefs of farmers regarding pesticide toxicity, the use of faulty spraying equipment, and the lack of proper maintenance of spraying equipment.

Considering chemical content, 33.8% of farmers had an AChE within the standard with strictly following the instructions of the label / packaging every time, while farmers had AChE lower than standard 5.7% (r = -0.243, p < 0.001) similar to Chanwijitra et. al.’s [53] finding that the farmers used an amount of chemical less than the label, 48.0% of farmers, with AChE within standard, used 1-2 times / month, while 25%, with AChE lower than standard (r = -0.210, p < 0.001), similar to [16] that 16.5% of farmers in Phitsanulok, Thailand used pesticides once or twice a month. For duration, 41.9% of farmers, AChE within standard, used for 1-2 hours/time, and 35.2%, AChE lower than standard (r = 0.206, p < 0.001), which was the same as the previous study [53]. For practice while using, 66.5% AChE within standard, used PPE every time while 15.9% of farmers, had AChE lower than standard (r = 0.396, p < 0.001) which was the same as
previous studies [29, 53-55]. Farmers who did not use PPE could have more experience with toxic chemicals. The previous study mentioned that the major risk factors related to agrochemical exposure resulted from the misuse of pesticides because of the lack of PPE (e.g. protective gear and appropriate clothing) [52, 56-57]. Additionally, this study found that most farmers used both types of pesticides, organophosphate and carbamate pesticides (80.9%) which was the same as previous studies [10, 31]. Organophosphate and carbamate pesticides are widely used in agriculture. And have AChE within standard and lower than standard ($r = 0.171, p < 0.004$). There was a relationship between decreasing AChE and more health risk. The previous study mentioned that farmers who spray more pesticides are more likely to have health problems. Besides these visible effects, exposure to pesticides has significant less visible impacts on farmers’ health (e.g. neurological, liver and kidney systems) [49].

However, this study found that the farmers who did not use PPE had AChE within standard of AChE (8.8%) more than farmers with AChE lower than standard (3.4%) because they may use pesticides correctly and strictly, especially, the chemical content and method that followed the instructions of the label / packaging every time. In addition to frequency and duration of the use of pesticides were less than farmers with AChE lower than standard which was the same as previous studies [55, 58]. The previous study mentioned that Thai farmers are at great risk of pesticide poisoning largely because they use inappropriate pesticide handling, incorrect use of PPE and inadequate understanding of the toxicity of the chemicals with which they work [44, 59-60]. Finally, unsafe practices can lead to measureable health effects in workers exposed to pesticides [61-62] and using a higher than recommended concentration of pesticides can lead to decreased AChE activity, a marker of organophosphate and carbamate pesticides exposure [43, 63].

<table>
<thead>
<tr>
<th>The use of pesticides</th>
<th>standard of AChE (units / ml)</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AChE ≥ 87.5 (%)</td>
<td>AChE &lt; 87.5 (%)</td>
</tr>
<tr>
<td>1. Chemical type</td>
<td>n</td>
<td>(%)</td>
</tr>
<tr>
<td>(1.1) Organophosphate</td>
<td>26</td>
<td>(17.6)</td>
</tr>
<tr>
<td>(1.2) Carbamate</td>
<td>8</td>
<td>(5.4)</td>
</tr>
<tr>
<td>(1.3) Organophosphate and carbamate</td>
<td>114</td>
<td>(77.0)</td>
</tr>
<tr>
<td>Total</td>
<td>148</td>
<td>(100)</td>
</tr>
<tr>
<td>2. Chemical content</td>
<td>n</td>
<td>(%)</td>
</tr>
<tr>
<td>(2.1) Follow the instructions of the label / packaging every time</td>
<td>50</td>
<td>(33.8)</td>
</tr>
<tr>
<td>(2.2) Follow the instructions of the label / packaging for sometimes</td>
<td>53</td>
<td>(35.8)</td>
</tr>
<tr>
<td>(2.3) By understanding themselves</td>
<td>16</td>
<td>(10.8)</td>
</tr>
<tr>
<td>(2.4) On the advice of neighbors</td>
<td>29</td>
<td>(19.6)</td>
</tr>
<tr>
<td>Total</td>
<td>148</td>
<td>(100)</td>
</tr>
</tbody>
</table>
3. Method of use
(3.1) Follow the instructions of the label / packaging strictly every time
101 (68.3) 14 (15.9) 115 (48.7)
(3.2) Follow the instructions of the label / packaging for sometimes
28 (18.9) 43 (48.9) 71 (30.1)
(3.3) On the advice of the shop / store fertilizer / pesticides
12 (8.1) 16 (18.2) 28 (11.9)
(3.4) By understanding / familiarity of their own or on the advice of neighbors
7 (4.7) 15 (17.0) 22 (9.3)
Total 148 (100) 88 (100) 236 (100)

4. Frequency
(4.1) 1-2 times / month
71 (48.0) 22 (25.0) 93 (39.4)
(4.2) > 2 times / month
23 (15.5) 12 (13.6) 35 (14.8)
(4.3) 1 time / week
18 (12.2) 27 (30.7) 45 (19.1)
(4.4) 2-3 times / week
36 (24.3) 27 (30.7) 63 (26.7)
Total 148 (100) 88 (100) 236 (100)

5. Duration
(5.1) < 1 hour / time
34 (23.0) 5 (5.7) 39 (16.5)
(5.2) 1-2 hours / time
62 (41.9) 31 (35.2) 93 (39.4)
(5.3) 2-4 hours / time
32 (21.6) 40 (45.5) 72 (30.5)
(5.4) > 4 hours / time
20 (13.5) 12 (13.6) 32 (13.6)
Total 148 (100) 88 (100) 236 (100)

6. Practice
(6.1) Use PPE every time
97 (65.5) 14 (15.9) 111 (47.0)
(6.2) Use PPE sometimes
38 (25.7) 71 (80.7) 109 (46.2)
(6.3) No use PPE
13 (8.8) 3 (3.4) 16 (6.8)
Total 148 (100) 88 (100) 236 (100)

Table 2. Number and percentage of farmers with AChE results by reactive paper in Sam Chuk district, Suphan Buri province.

<table>
<thead>
<tr>
<th>Results</th>
<th>the levels of AChE by reactive paper (units / ml)</th>
<th>n</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>≥ 100</td>
<td>23</td>
<td>9.7</td>
</tr>
<tr>
<td>Safe</td>
<td>87.5 – 99.9</td>
<td>125</td>
<td>53.0</td>
</tr>
<tr>
<td>Risk</td>
<td>75.0 – 87.4</td>
<td>68</td>
<td>28.8</td>
</tr>
<tr>
<td>Unsafe</td>
<td>&lt; 75.0</td>
<td>20</td>
<td>8.5</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>236</td>
<td>100</td>
</tr>
</tbody>
</table>

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Table 3. Correlation coefficients ($r$) and p-value of the relationship between AChE and the use of organophosphate and carbamate pesticides of farmers in Sam Chuk district, Suphan Buri province.

<table>
<thead>
<tr>
<th>the use of pesticides</th>
<th>$r$</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Method of use</td>
<td>0.452</td>
<td>0.000*</td>
</tr>
<tr>
<td>Practice</td>
<td>0.396</td>
<td>0.000*</td>
</tr>
<tr>
<td>Chemical content</td>
<td>-0.243</td>
<td>0.000*</td>
</tr>
<tr>
<td>Frequency</td>
<td>-0.210</td>
<td>0.000*</td>
</tr>
<tr>
<td>Duration</td>
<td>0.206</td>
<td>0.004</td>
</tr>
<tr>
<td>Chemical type</td>
<td>0.171</td>
<td>0.000*</td>
</tr>
</tbody>
</table>

*p-value < 0.001

4. Conclusions

This study on the use of organophosphate and carbamate pesticides and cholinesterase levels in the blood (AChE) of 236 farmers in Sam Chuk district, Suphan Buri province, focused on chemical type, method of use, chemical content, frequency, duration, and practice in the use of pesticides. Most farmers use chemical pesticides of both types, organophosphate and carbamate, (80.9%), method of use followed the instruction of the label / packaging strictly every time (48.7%), chemical content used followed the instruction of the label / packaging sometimes (32.2%), frequency of the most commonly used 1-2 times / month (39.4%), duration 1-2 hours / time (39.4%) and 2-4 hours / time (30.5%), while in practice use of pesticides with PPE every time (47%), sometimes (46.2%), and was not used (6.8%). The results of the AChE of farmers by reactive paper found 62.7% had levels higher than the standard ($\geq$ 87.5 units / ml); which were safe (53%) and normal (9.7%), and 37.3% of farmers had AChE levels lower than the standard (< 87.5 units/ml); which were at risk (28.8 %) and unsafe to health (8.5%). The use of pesticides was related to the AChE levels with high significance (p-value < 0.001) followed by: method of use ($r = 0.452, p < 0.001$), practice ($r = 0.396, p < 0.001$), chemical content ($r = -0.243, p < 0.001$), frequency ($r = -0.210, p < 0.001$), duration ($r = 0.206, p < 0.004$) and chemical type ($r = 0.171, p < 0.001$), respectively.

Therefore, to safeguard health and reduce the risk of exposure to toxic pesticides, farmers should strictly follow the instructions on the label / packaging and use PPE every time. It is especially important for farmers who have been incorrectly using pesticides and had AChE levels lower than the standard.

5. Acknowledgments

We would like to thank Suphan Buri Agricultural Office, and the Health Officer of primary health care in Sam Chuk district for their help, and the farmers for their cooperation in this study.

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