# ASSESSMENT OF FARMER AND NON-FARMER HEALTH EFFECTS RELATED TO ORGANOPHOSPHATE PESTICIDES EXPOSURE USING BLOOD CHOLINESTERASE ACTIVITY AS A BIOMARKER IN AGRICULTURAL AREA AT ONGKHARAK DISTRICT NAKHON NAYOK PROVINCE THAILAND

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for the Degree of Master of Public Health Program in Public Health
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# การประเมินผลกระทบสุขภาพของเกษตรกรและผู้ที่ไม่ใช่เกษตรกรจากการได้รับสารกำจัดศัตรูพืช กลุ่มออร์กาโนฟอสเฟต โดยใช้ตัวชี้วัดปฏิกิริยาโคลีนเอสเตอเรสในเลือด ในพื้นที่เกษตรกรรม อำเภอองครักษ์ จังหวัดนครนายก ประเทศไทย

นางสาววชิราภรณ์ วิไลวรรณ

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาสาธารณสุขศาสตรมหาบัณฑิต

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| Field of Study | Public Health |
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วชิราภรณ์ วิไลวรรณ: การประเมินผลกระทบสุขภาพของเกษตรกรและผู้ที่ไม่ใช่เกษตรกร จากการได้รับสารกำจัดศัตรูพืชกลุ่มออร์กาโนฟอสเฟต โดยใช้ตัวชี้วัดปฏิกิริยาโคลีนเอสเตอเรสในเลือด ในพื้นที่เกษตรกรรม อำเภอองครักษ์ จังหวัดนครนายก ประเทศไทย. (ASSESSMENT OF FARMER AND NON-FARMER HEALTH EFFECTS RELATED TO ORGANOPHOSPHATE PESTICIDES EXPOSURE USING BLOOD CHOLINESTERASE ACTIVITY AS A BIOMARKER IN AGRICULTURAL AREA AT ONGKHARAK DISTRICT NAKHON NAYOK PROVINCE THAILAND) อ.ที่ปรึกษาวิทยานิพนธ์หลัก: ผศ.ดร.วัฒน์สิทธิ์ ศิริวงศ์, 93 หน้า.

งานวิจัยนี้มีวัตถุประสงค์เพื่อประเมินผลกระทบสุขภาพจากการสัมผัสสารกำจัดศัตรูพืชกลุ่มออร์กาโนฟอสเฟต ในเกษตรกรและผู้ที่ไม่ใช่เกษตรกร ในตำบลศีรษะกระบือ อำเภอองครักษ์ จังหวัดนครนายก รูปแบบของงานวิจัยครั้งนี้เป็นการศึกษาภาคตัดขวางในช่วงเวลาที่มีการใช้สารกำจัดศัตรูพืช ในเดือนมกราคมถึงมีนาคม พ.ศ. 2556 เครื่องมือที่ใช้ประกอบด้วย แบบสอบถามและเทสต์เมท ซีเอชอี รุ่น 400 เพื่อตรวจวัดระดับโคลีนเอสเตอเรสในเลือด ทั้งในเม็ดเลือดแดง และในพลาสมา ผู้มีส่วนร่วมในงานวิจัยแบ่งออกเป็นผู้ชาย 25 คน และ 45 คนเป็นผู้หญิง อายุเฉลี่ย 42.63 (±10.41) ปี ผลการศึกษาพบว่า ระดับโคลีนเอสเตอเรสในเม็ดเลือดแดงของเกษตรกรต่ำกว่าในผู้ที่ไม่ใช่เกษตรกร และระดับโคลีนเอสเตอเรสในพลาสมาในกลุ่มเกษตรกรต่ำกว่าของกลุ่มผู้ที่ไม่ใช่เกษตรกรอย่างมีนัยสำคัญ ($p<0.001$) ระดับโคลีนเอสเตอเรสในเม็ดเลือดแดง มีความสัมพันธ์เป็นลบเล็กน้อยกับระดับโคลีนเอสเตอเรสในพลาสมา ระยะเวลาในการใช้สารกำจัดศัตรูพืชมีความสัมพันธ์กับระดับโคลีนเอสเตอเรสในพลาสมาอย่างมีนัยสำคัญ ($p<0.05$) การเป็นเกษตรกรมีความสัมพันธ์อย่างมีนัยสำคัญกับการเพิ่มขึ้นของอาการทางตา อาการทางระบบประสาท อาการทางระบบทางเดินหายใจ และ อาการทางอวัยวะคัดหลั่ง ($p<0.05$) ระดับโคลีนเอสเตอเรสในเม็ดเลือดแดงสัมพันธ์กับอาการทางระบบประสาทอย่างมีนัยสำคัญ ($p<0.05$) ระดับโคลีนเอสเตอเรสในพลาสมาสัมพันธ์กับอาการทางตา อาการทางระบบประสาท อาการทางระบบทางเดินหายใจและอาการทางอวัยวะคัดหลั่งอย่างมีนัยสำคัญ ($p<0.05$) สรุปได้ว่า เกษตรกรอาจจะได้รับความเสี่ยงมากกว่าผู้ที่ไม่ใช่เกษตรกรที่อาศัยอยู่ใกล้กับพื้นที่เกษตรกรรม จึงควรมีการแนะนำให้เกษตรกรและผู้ที่ไม่ใช่เกษตรกรป้องกันตัวเองอย่างเหมาะสมจากการรับสัมผัสสารกำจัดศัตรูพืช โดยเฉพาะอย่างยิ่งควรแนะนำให้เกษตรกรใช้อุปกรณ์ป้องกันส่วนบุคคลที่เหมาะสม

สาขาวิชา สาธารณสุขศาสตร์............ ลายมือชื่อนิสิต..............................

ปีการศึกษา 2555............................ ลายมือชื่อ อ.ที่ปรึกษาวิทยานิพนธ์หลัก..............................

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KEYWORDS : ORGANOPHOSPHATE/ CHOLINESTERASE/ HEALTH EFFECTS

WACHIRAPORN WILAIWAN: ASSESSMENT OF FARMER AND NON-FARMER HEALTH EFFECTS RELATED TO ORGANOPHOSPHATE PESTICIDES EXPOSURE USING BLOOD CHOLINESTERASE ACTIVITY AS A BIOMARKER IN AGRICULTURAL AREA AT ONGKHARAK DISTRICT NAKHON NAYOK PROVINCE THAILAND. ADVISOR: ASST. PROF. WATTASIT SIRIWONG, Ph.D**.,** 93 pp.

This study aims to assess health effects caused by organophosphate pesticides exposure among farmers (n=35) and non-farmers (n=35) in Sisa Krabue sub-district, Ongkharak district, Nakhon Nayok province. The research design of this study was a cross-sectional study during pesticide application period from January to March 2013. Questionnaire and Test-mate ChE (Model 400) for blood cholinesterase levels of both blood enzymes erythrocyte cholinesterase (AChE) and plasma cholinesterase (PChE) were used as measurement tools. Participants were consisted of 25 male and 45 female. Average age (±SD) was 42.63 (±10.41) years old. The results showed that AChE levels of farmers was likely lower than non-farmers and PChE levels in the farmer group was significantly lower than those non-farmer group ($p$<0.001). The association between AChE levels and PChE levels were likely low negative correlation. Years of using pesticides were significantly associated with PChE levels ($p$<0.05). The farmers were significantly associated with increase eye symptoms, central nervous system (CNS) symptoms, respiratory system symptoms, and glands ($p$<0.05). The AChE level was significantly associated with CNS symptoms ($p$<0.05). The PChE level was significantly associated with eye symptoms, CNS symptoms, respiratory system symptoms, and glands symptoms ($p$<0.05). In conclusion, farmers may be getting higher risk than non-farmers living nearby/around farmer area. It should be suggest that an appropriated self-prevention from pesticides exposure should be recommended to farmers and non-farmers, particularly, proper use of personal protective equipment (PPE) should be introduced to farmers.

Field of Study : Public Health ........ Student's Signature ........

Academic Year : 2012 ........ Advisor's Signature ........

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# CHAPTER I
# INTRODUCTION

## 1.1 Background and Rationale:

Thailand is an agricultural country and is also considered one of the main countries for rice farming. In 2009, the total area for rice farming in Thailand covered approximately 66 million rais across the country (Office of Agricultural Economics, 2009). Therefore, it is undeniably vital for the farmers to form a labor group that can promote and strengthen Thailand economy. However, the framework of the operations usually leads to the problems that undermine people's way of living throughout. One of those problems is health problems. In 2008, it was found that most patients with toxic chemicals from pesticides and from the poisoning used to get rid of animals represented 1,705 persons of all 2,141 patients across the country who suffers from diseases caused by chemical substances used in their profession. In this connection, the said chemicals used in pesticides are used in households for pest control and agricultural use. As a result, farmers as well as the general public may run the risk of exposure to such substances which come in various forms such as breath, oral cavity, and skin (Health Information System Development Office, 2009). In this regard, according to the report summarizing the import of agricultural hazardous materials, it has been revealed that in the year 2011, Thailand imported pesticides equivalent to the quantity of 34,672,233.30 kilograms with the value of 5,938,021,132.99 baht (Agricultural Extension Department, 2011).

Organophosphate (OP) pesticides contain chemicals which are primarily used in agriculture. The condition of exposure to OP pesticide at a high degree along with the accompanying health risks in developing countries of the globe is an alarming issue (Jaqa and Dharmani, 2003). OP pesticides, for example Malathion, Parathion, Phosalone, Fenitrothion, Dichlorvos, and Chlorpyrifos are used as biocides in the products of household from 1970s. Although these components can decompose rapidly and are somewhat nonpersistent in the environment, they are described as being highly acutely toxic (Zou et al., 2006). All organophosphates have a certain number of chemical properties in common. OP pesticides are composed of a central phosphorus atom with a dual bond to sulfur or oxygen, groups of R1 and R2, which are ethyl or methyl in terms of structure. Moreover, there is a leaving group that is specific to each OP pesticides (Kwong, 2002). Three routes of pesticides exposure are inhalation, dermal, oral exposure, typical sources of pesticides exposure from food, drinking water, residence, and worker who apply pesticides (US EPA, 2007). OP pesticides include a high toxicity on humans due to the fact that they act as acetyl cholinesterase inhibitor, resulting in the blockage of the nervous system. The inhibition of acetyl cholinesterase with severe toxicity is the cause of respiratory, myocardial as well as neuromuscular transmission debilitation (Deerasamee, 2009).

Nakhon Nayok is a province in the Central Region of Thailand. In 2010, the households of Nakhon Nayok province account for the number of 61,874 in total. Out of such number, 26,656 (43.1%) households are involved in agriculture and the majority of them earn their livelihood by being rice farmers. Currently, nearly 50% (612,504 rais) of the agricultural area totaling about 1.33 million rais serve as rice farming land (Nakhon Nayok Agricultural Extension Office, 2011). Additionally, the agricultural land is located in the irrigation area that gets water from different water projects. This enables rice cultivation throughout the year (Pathumthani Rice Research Center, 2008).

Ongkharak District, one district of Nakhon Nayok province, is divided into 11 sub-districts that are further subdivided into 116 villages. The district comprises a total of 17,890 households. In this regard, the number of 6,447 (36.0%) of them is concerned with agricultural households (Nakhon Nayok Agricultural Extension Office, 2011). Sisa Krabue sub-district is located in Ongkharak District with the highest number of agriculturists occupying the land. The sub-district also has the greatest number of fields in the district for agricultural occupation (Nakhon Nayok Agricultural Extension Office, 2011).

The activity of cholinesterase enzymes in the blood can be measured as a biomarker of effect for organophosphates (NPIC, 2012). A lot of methods can be used to test cholinesterase level. However normal method that was used is a screening test, which should be confirmed by Ellman methods. Therefore, this research aims to find the association between health effects and organophosphate pesticides exposure among rice farmers and non-farmers by using blood cholinesterase levels, both blood enzymes erythrocyte cholinesterase (AChE) and plasma cholinesterase (PChE) in Sisa Krabue sub-district, Ongkharak district, Nakhon Nayok Province. Cholinesterase level was tested by Ellman method, Test-mate ChE (Model 400) because this is can save time in the analysis and is also marked by the high degree of accuracy.

## 1.2 Research questions of the study:

1.2.1 Are there any differences in blood cholinesterase levels between farmers and non-farmers in Sisa Krabue sub-district?

1.2.2 Is there an association between pesticide use and the level of blood cholinesterase among farmers in Sisa Krabue sub-district?

1.2.3 Is there a relationship between blood cholinesterase levels and health effects in farmers and non-farmers in Sisa Krabue sub-district?

## 1.3 Research Hypothesis:

1.3.1 There are different blood cholinesterase levels that can be found from farmers and non-farmers in Sisa Krabue sub-district.

1.3.2 There is an association between pesticide use and the level of blood cholinesterase among farmers, Sisa Krabue sub-district.

1.3.3 There is a relationship between blood cholinesterase levels and health effects in farmers and non-farmers, Sisa Krabue sub-district.

## 1.4 Objectives of the study:

The main objective of this study is to assess health effects related to organophosphate pesticides exposure by using blood cholinesterase activity as a biomarker in farmers and non-farmers.

*Specific Objectives*

1.4.1 To estimate organophosphate pesticide exposure by using biological monitoring (cholinesterase level).

1.4.2 To determine the different blood cholinesterase levels among farmers and non-farmers in Sisa Krabue subdistrict.

1.4.3 To understand the general background, pesticide use, exposure factors, and health information in the community.

1.4.4 To explore an association between pesticide use and the level of blood cholinesterase in farmers, Sisa Krabue sub-district.

1.4.5 To identify health effects related to the blood cholinesterase level among farmers and non-farmers in Sisa Krabue sub-district.

**1.5 Variables in the study:**

1.5.1 Independent Variables

1.5.1.1 Socio-demographic data (age, gender, education, income, smoking behavior, and alcohol drinking behavior)

1.5.1.2 Pesticides use (type of work, work duration, farm size, amount of pesticide use, behavior of reading labels, contamination avoidance, and personal protective)

1.5.1.3 Related exposure factors (The number of farmers who used pesticides in a family, the distance between house area and paddy field, source of drinking water, behavior of washing fruits and vegetables)

1.5.2 Dependent Variables

1.5.2.1 The level of AChE and PChE in the paddy field measured by Test-mate ChE (Model 400)

1.5.2.2 Health effects (symptoms that related with OP pesticides exposure: respiratory system, gastrointestinal system, urinary system, glands, eye symptoms, skin symptoms, central nervous system)

## 1.6 Operational Definitions:

**Cholinesterase level** refers to the level of blood enzyme erythrocyte cholinesterase (AChE) and plasma cholinesterase (PChE) related with OP pesticides measured by Test-mate ChE Cholinesterase Test System (Model 400).

**Farmer** refers to a rice-growing farmer who is older than 18 years old and lives in Sisa Krabue sub-district, Ongkharak district, Nakhon Nayok Province. They have to load, mix and spray OP pesticides.

**Non-farmer** is a person older 18 years old, living in Sisa Krabue sub-district, Ongkharak district, Nakhon Nayok Province, who do not practice rice-growing or any activities in farms. They follow non-agricultural occupation.

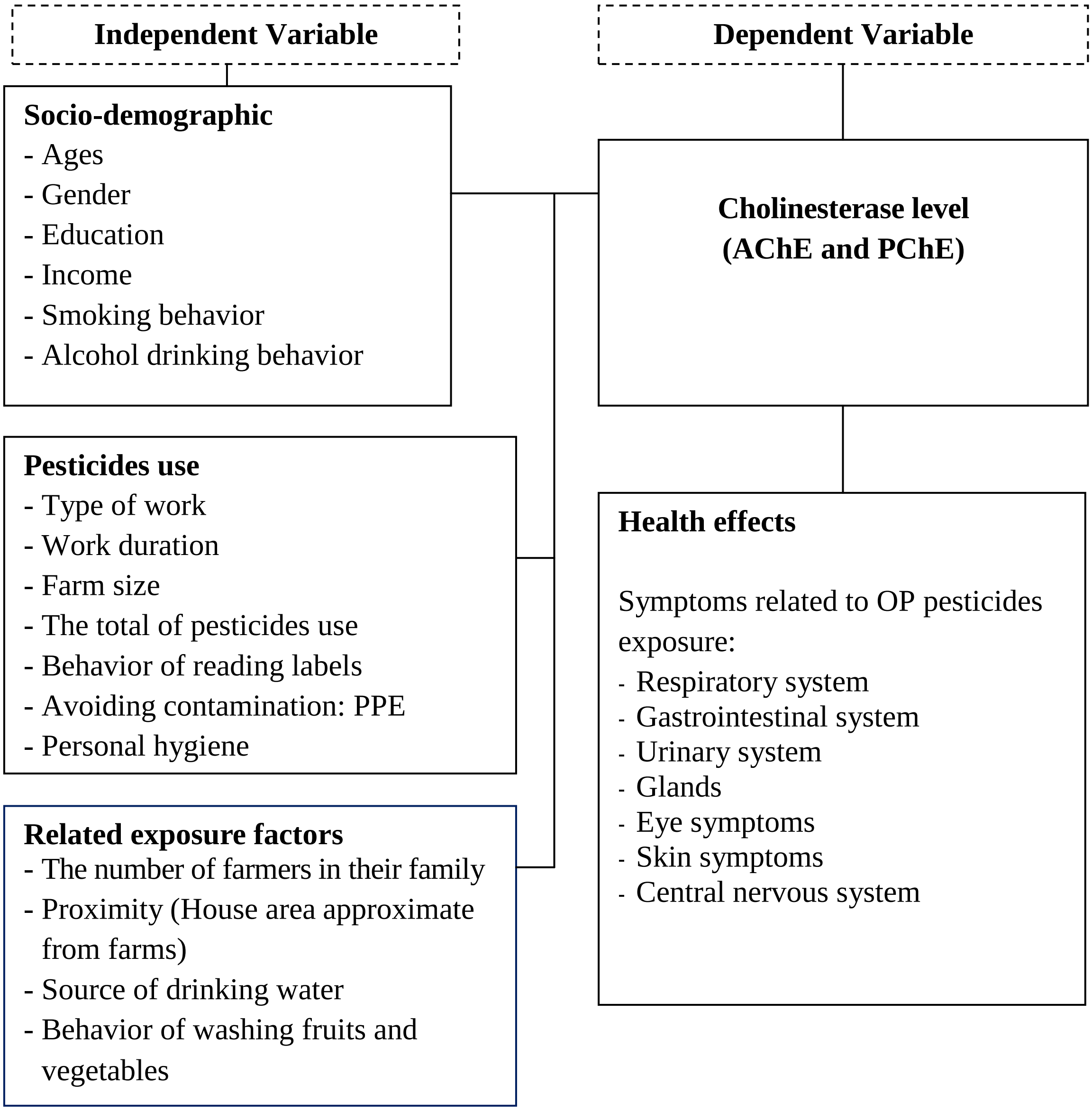
**Test-mate ChE (Model 400)** refers to the mobile instrument for the quantitative determination of cholinesterase in whole blood to monitor pesticide exposure (EQM Research, Inc., 2003).

**Type of work** refers to type of pesticides application (mixing, loading, and spraying)

**Avoiding contamination: PPE** refers to the use of personal protective equipment (gloves, long sleeved shirts, long legged, hat, mask, boots, and goggles)

**Personal hygiene** refers to practicing of pesticide use and personal hygiene (behavior of eating and drink during applying pesticides, washing hand before eating, taking shower, washing clothes, and removing of used pesticides bottles)

### 1.7 Conceptual Framework:



# CHAPTER II
# LITERATURE REVIEW

## 2.1 Organophosphate Pesticides:

Organophosphate Pesticides produce an effect on the nervous system by causing disorders in the enzyme which controls acetylcholine, a neurotransmitter. The majority of organophosphates are insecticides. The use of these substances gradually increased during the early $19^{th}$ century. However, the discovery of their effects on insects that resemble the ones on humans took place in 1932. An unspecified number of them are very toxic (they were used in times of World War II as nerve agents) (US EPA, 2012).

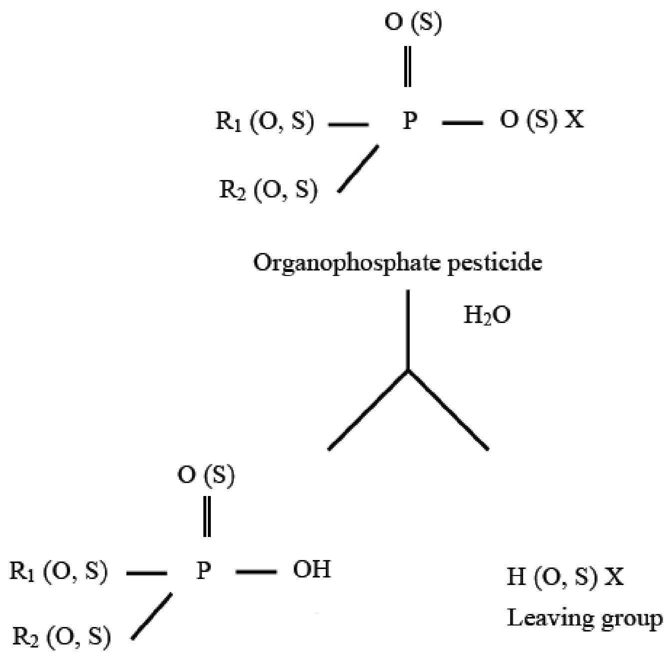
Presently, organophosphate (OP) pesticides form one category of pesticides that are extensively used as insecticides in the agricultural area for the purpose of pest control throughout the entire growth process of plants. The use of organophosphate (OP) pesticides is to provide a substitute for organochlorine pesticides due to their effectiveness at high level, comparatively low price and particularly low environmental persistence. Nevertheless, these pesticides comprise the toxicity of high level on humans because they act as acetyl cholinesterase inhibitor, leading to the obstruction of nervous system. The inhibition of acetyl cholinesterase with acute toxicity causes the emasculation of respiratory, myocardial and neuromuscular transmission (Deerasamee, 2009).

### 2.1.1 Organophosphate (OP) Compounds

The majority of organophosphorus compounds are ester or thiol derivatives of phosphoric, phosphonic or phosphoramidic acid. Their formula in general is shown in Figure 1. R1 and R2 are chiefly the aryl or alkyl group that can be adhered in a direct way to a phosphorus atom (phosphinates) or by the way of oxygen (phosphates) or a sulphur atom (phosphothioates). In an unspecified number of cases, R1 is directly joined with phosphorus and R2 with an oxygen or sulfur atom (phosphonates or thion phosphonates, in the order mentioned). Not less than one of these two groups is adhered with un-, mono- or di-substituted amino groups in hosphoramidates.

The X group can be varied and is likely to belong to an extensive range of aliphatic, aromatic or heterocyclic groups. Moreover, the X group is known as a leaving group now that on hydrolysis of the ester bond it is discharged from phosphorus (Sogorb and Vilanova, 2002).

**Figure 2.1** The common structure of organophosphate pesticide from hydrolysis pathway




Source: Singh and Walker (2006)

Total compounds may be put in four principal categories, being contingent on the character of the X constituent (Gallo and Lawlyk, 1991) as follows:

(1) Categories I; X contains quaternary nitrogen, for example, ecothiopate isodide.
(2) Categoiries II; X is F: Fluorophosphate groups have only a small number of compounds, for example, dimefox and diisopropyl fluorophosphates.
(3) Categories III; X is CN, OCN, SCN or Halogen not F, for instance, tabun, parathion.
(4) Categories IV; it may be divided into not less than eight subgroups based on their R1 and R2 constituents. Many of this group is different either qualitatively or qualitatively in toxicity. The foundation for the difference was also known in some instances.

### 2.1.2 Effect of organophosphate pesticides

Organophosphates poison insects as well as mammals mainly by phosphorylation of the acetylcholinesterase enzyme (AChE) at nerve endings. The consequence is a loss of existing AChE in order that the effect on organs becomes overmotivated by the incremental acetylcholine (ACh, the impulse-conveying substance) in the nerve ending. The enzyme is vital to the regular control of transmitting nerve impulses from nerve fibers to smooth and skeletal muscle cells, glandular cells, as well as autonomic ganglia within the central nervous system (CNS). Certain crucial proportion of the tissue enzyme mass needs to be inactivated by phosphorylation earlier than the time when symptoms and signs of poisoning become obvious. Loss of enzyme function at adequate dosage allows accumulation of Ach peripherally and centrally at cholinergic neuroeffector connections (muscarinic effects), skeletal nerve-muscle joints and automatic ganglia (nicotinic effects). ACh concentration at high level results in the respective contraction and secretion of muscle at cholinergic nerve junctions with smooth muscle and gland cells. At skeletal muscle connections, incremental ACh is likely to be arousing (causes muscle subtraction), but may debilitate or prevent the cell from acting normally by depolarizing the end-plate as well. In the CNS, ACh concentrations at high level result in sensory and behavioral perturbation, leads to an absence of coordination, makes motor function less active as well as causing respiratory depression. Increase in pulmonary secretions together with respiratory failure is the common causes of death resulting from organophosphate toxicity. Recovery is contingent eventually on the production of new enzyme in total crucial tissues.

Symptoms of severe organophosphate poisoning develop gradually throughout a period of or after exposure, within minutes to hours, dependent on the way of contact. Exposure through inhalation leads to the quickest appearance of toxic symptoms, followed by the gastrointestinal path and eventually the dermal way. Total signs and symptoms are inherently cholinergic and impinge on central nervous system receptors. The crucial symptoms in management are the respiratory symptoms. Adequate muscular fasciculation and weakness are frequently noticed because sudden respiratory arrest can happen to require respiratory support. Similarly, bronchorrhea as well as bronchospasm are frequently likely to hinder efforts of sufficient oxygenation of the patient. Bronchospasm and bronchorrhea can happen, generating tightness in the chest, wheezing, productive cough as well as pulmonary edema. The signification of life-threatening acuity of poisoning is related to loss of consciousness, lack of self-control, convulsions and respiratory depression. Respiratory failure is the primordial cause of death. Moreover, there is often an associate cardiovascular component. The typical cardiovascular sign is bradycardia that can grow gradually into sinus arrest. Nevertheless, this may be replaced by tachycardia and hypertension resulting from nicotinic (sympathetic ganglia) stimulation. Toxic myocardiopathy has been a conspicuous aspect of some acute organophosphate poisonings. An unspecified number of the most usually reported preliminary symptoms are nausea, dizziness, headache and hyper secretion. The latter symptoms are detected by sweating, salivation, lacrimation, as well as rhinorrhea. All signals indicating the aggravation of the poisoned state include vomiting, abdominal cramps, and diarrhea, muscle twitching, weakness, tremor, incardination. Miosis is frequently a diagnostic sign that is helpful. Furthermore, the patient may report dazzled and/or dark vision. The anxious state together with restlessness is conspicuous because there are a small number of reports of choreaform movements. Psychiatric symptoms involving depression, memory loss and confusion have been reported. Toxic psychosis shown as confusion or strange behaviors has been diagnosed erroneously as alcohol intoxication. Children are frequently presented with a little different clinical image by comparison with adults. Some of the classic cholinergic signs of lacrimation, sweating, bradycardia and muscular fasciculation were less common. Sudden attack of apoplexy (seizures) (22%-25%) coupled with mental status changes comprising lethargy and coma (54%-96%) was common. By comparison, merely 2-3% of adults show the symptom of seizures. Other common showing signs in children involve floppy muscle weakness, miosis and ample salvation (US EPA, 1999). Environmental exposure to OP pesticides along with unfavorable reproductive results in both men and women working on or living close to farms are more and more reported all over the world. No matter if it is exposure to OP below the level that leads to clinical manifestations of severe OP toxicity, this leads to a detrimental effect on fertility, growth as well as development. In this connection, the evidence regarding weakened fertility exists because of a decrease in semen quality as well as possible less testosterone levels in exposed men (John and Wickremasinghe, 2008).

**Table 2.1** Signs and symptoms related to organophosphate pesticides exposure

| **Manifestations** | **Exposure** | **Signs and symptoms** |
|---|---|---|
| Central nervous system | Mild | Headache, confusion, drowsiness, dizziness |
| | Moderate | Blurred vision, slurred speech, ataxia |
| | Severe | Convulsions, coma, heart block |
| Cardiovascular system | Moderate | Bradycardia |
| Gastrointestinal system | Mild | Anorexia |
| | Moderate | Nausea, vomiting, abdominal cramps |
| | Severe | Diarrhea, fecal incontinence |
| Respiratory system | Mild | Wheezing, dyspnea |
| | Moderate | Bronchorrhea, bronchospasm |
| | Severe | Cyanosis, pulmonary edema |
| Urinary system | Severe | Loss of urinary control |
| Glands | Mild | Hypersalivation, hyperlacrimation, sweating |
| Pupils | Mild | Miosis |
| | Severe | Pinpoint, unreactive to light |

Source: US EPA (1999)

### 2.1.3 Biomarkers of exposure to organophosphate pesticides

As the metabolites are afterwards removed from the body in the urine because the chemical structure of the leaving group is particular to the organophosphate, the detection and quantification of the leaving group is a moderately specific bio-marker of exposure to the parent compound. For instance, the leaving group 3, 5, 6-tricholoro-2-pyridinol (TCP) can be gauged in the urine as a quite specific biomarker of exposure to chlorpyrifos. A supplementary factor that may impinge on the interpretation of leaving groups as biomarkers of exposure is the observation that some organophosphates may experience hydrolysis reactions in the environment. For instance, Chlorpyrifos experience hydrolysis in the environment to yield the leaving group 3, 5, 6-tricholoro-2-pyridinol (TCP) as the main degradation product. Therefore, aside from expressing exposure to the parent compound (chlorpyrifos), the level of leaving group metabolites in the urine may be an indicator of exposure to TCP itself as well if it is existent in an individual's environment. This creates a challenge related to the interpretation of human health significance of metabolite levels as TCP does not restrain cholinesterase enzymes. Moreover, the interpretation of urinary dialkylphosphates is not simple because the hydrolysis of a particular organophosphate may produce more than one class of dialkylphosphates. This is obvious in the case of chlorpyrifos, where the products of hydrolysis can involve both diethylphosphate (DEP) and diethylthiophosphate, depending on whether the chlorpyrifos has undergone metabolic activation to chlorpyrifos-oxon in the body. Therefore, it is impossible to specify to which specific organophosphate a person was exposed on the basis of the detection or quantification of dialkylphosphate metabolites in the urine. The studies conducted not long ago have measured the quantity of biomarkers of exposure to organophosphates in vast samples of the United States population, by the use of urinary dialkylphosphate as well as other metabolites. Whereas this research offers significant information on exposure to organophosphate pesticides and their metabolites in the population generally accepted guidelines related to the interpretation of these biomarkers of exposure have not been created. The discovery of urinary alkylphosphates in the urine does not essentially show that they cause a harmful effect on health. There has been no study on correlation between urinary dialkylphosphates and acetylcholinesterase enzyme activity in the general population. Further research is required for the determination of links between these biomarkers of exposure and health effects and comparative role of dietary, residential, and occupational ways of exposure.

Other biomarker techniques are available to medical practitioners in the clinical evaluation of severe exposure. It is possible to measure the action of cholinesterase enzymes in the blood as a biomarker of effect for organophosphates. A person with severe symptomatic excessive exposure to organophosphates will most often have uncommonly low level of activity of cholinesterase enzymes gauged in the serum (as butyrylcholinesterase known as pseudocholinesterase as well) or in red blood cells (as RBC cholinesterase that is increasingly closely associated with acetycholinesterase activity in the nervous system). Blood cholinesterase measurements have restrictions in a manner that the time course for enzyme inhibition and recovery can be variable with exposure to different organophosphates. Additionally, cholinesterase activity can be affected by inter- and intra-individual tendency to vary. Nevertheless, in conjunction with a thorough exposure history is likely to provide important supplementary data for the health care provider assessing a person with severe excessive exposure to organophosphate insecticides. In an asymptomatic, healthy person with no history of recent excessive exposure to organophosphate insecticides (like the present case sequence), cholinesterase testing may not be of clinical value. There has been the development of guidelines for physicians concerned with the careful watch of workers with possible exposure to organophosphates by the use of monitoring in series of cholinesterase enzyme activity in the blood (NPIC, 2012).

## 2.2 Pesticides and Routes of Exposure

Three ways of pesticides exposure in people are getting pesticides in their mouth or digestive tract (oral exposure), inhaling pesticides (inhalation exposure), and absorbing pesticides through the skin (dermal exposure). Depending on the situation, pesticides could enter the body by one or all of these routes. Typical sources of pesticide exposure include:

(1) Food: Most of the foods we eat have been grown with the use of pesticides. Therefore, pesticide residues may be present inside or on the surfaces of these foods.
(2) Home and Personal Use Pesticides: You might use pesticides in and around your home to control insects, weeds, mold, mildew, bacteria, lawn and garden pests and to protect your pets from pests such as fleas. Pesticides may also be used as insect repellants which are directly applied to the skin or clothing.
(3) Pesticides in Drinking Water: Some pesticides that are applied to farmland or other land structures can make their way in small amounts to the ground water or surface water systems that feed drinking water supplies.
(4) Worker Exposure to Pesticides: Pesticide applicators, vegetable and fruit pickers and others who work around pesticides can be exposed to pesticide's chemical due to the nature of their jobs (US EPA, 2007).

## 2.3 Biological Monitoring

Biological monitoring (known as, biomonitoring) is an instrument for the measurement of pesticide exposure level that enters the body. It can evaluate human exposures in both environment and workplace. In case where exposure changes irregularly eventually or the skin is an important path of absorption, biological monitoring has proved to obtain the absorbed dose information. Generally, the measurements of biological monitoring are used with blood, urine, saliva, breast milk, or meconium as biological media by the estimate of the amount of pesticide as its metabolite or its reaction product which is absorbed into the body.

Biomarkers of exposure are significant in toxicology. This is because they are an indicator of inner dose, or the quantity of chemical exposure which has led to absorption into the body. Important improvements have been made in the development of analytical techniques that can discover and/or quantify the presence of several natural or synthetic toxins or their breakdown products (metabolites) in a biological matrix (for example blood or urine). The capability to correctly gauge biomarkers of exposure is dependent on a sufficient understanding of the chemistry and toxicology of the substance led by consideration.

### 2.3.1 Biomarker

The term "biomarker" is used in a wide sense to encompass practically any measurements expressing an interaction between a biological system and an environmental agent that is likely to be chemical, physical or biological. The biomarkers are arranged in three groups (IPCS, 2000).

(1) Biomarker of exposure: an exogenous substance or its metabolite or the product of an interaction between a xenobiotic agent and certain target molecule or cell which is gauged in a compartment within an organism.
(2) Biomarker of effect: a biochemical, physiological, behavioral or other changing that can be measured within an organism which, dependent on the scale, can be accepted as correlated with an established or feasible health weakness or disease.
(3) Biomarker of susceptibility: this is concerned with an indicator of an inherent or acquired ability of an organism to react to the challenge of exposure to a particular xenobiotic substance.

## 2.4 Organophosphate Pesticides and Biomarker Monitoring

Though it is the inhibition of AChE in the nervous system which is the cause of toxicity, resembling kinds of cholinesterase are found in the blood with the possibility to be used as indicators of exposure to OP pesticides. OP pesticides stick to both AChE that is found in the synapses and joined to red blood cells and bind to butyryl cholinesterase called likewise plasma cholinesterase (PChE) and found in plasma. It is possible for pesticides to have various affinities for AChE and PChE. Therefore, power as an inhibitor changes according to the specific pesticide. Levels of inhibition of AChE as well as PChE give not much different information. Moreover, it is wise to do both tests for each patient. Red blood cell cholinesterase is similar to the enzyme existing in the nervous system. It is also believed to be a good indicator of real neuronal activity. The rate of turnover for red blood cells is not quick (approximately 3 months). AChE measurements also show the nature of this replacement rate that is not fast. Therefore, AChE is characteristically used as an indicator of chronic exposure. On the contrary, PChE turnover is much more rapid. PChE is a more efficient short-term indicator because of its faster response to exposure. It is used as an indicator of latest, severe exposure. When a person's exposure comes to an end, both enzymes return to their usual action levels as turnover happens (Brown et al., 2006).

The Test-mate ChE (Model 400) is beneficial for observing occupational exposure to pesticides. By means of the measurement on a habitual basis of blood cholinesterase levels of workers involved in handling pesticides, these workers are likely to be protected from excessive exposure to pesticides prior to the appearance of symptoms. Moreover, it is possible to evaluate pesticide handling safety programs for effectiveness as well as compliance, resulting in the improved protection of workers in the long term.

The Test-mate ChE Cholinesterase Test System is on the basis of Ellman method. Acetylthiocholine (AcTC) or butyrylthiocholine (BuTC) is hydrolyzed by AChE or PChE, respectively, producing carboxylic acid and thiocholine with reaction to the Ellman reagent (DTNB, dithionitrobenzoic acid) so as to create a yellow color that is gauged spectrophotometrically at 450 nm. The rate of color formation is in proportion to the amount of either AChE or PChE (EQM Research, Inc., 2003).

Cholinesterase

thiocholine ester (AcTC/BuTC) =============> thiocholine

thiocholine + DTNB =============> TNB-thiocholine + TNB (yellow)

**Figure 2.2** Test-mate ChE (Model 400)

## 2.5 Related Articles

Mekonnen and Ejigu's (2005) study on cholinesterase levels in farm workers with changing exposure to chemical pesticide, plasma cholinesterase (PChE) was gauged in workers in two Ethiopian farms. A standard questionnaire taken from the British Medical Research Council was used for the determination of health status of the subjects. The outcome indicated that 82 farm workers and 47 controls in total took part in the study. Whereas the mean values of plasma cholinesterase were in general less in workers, this difference was only meaningful in the sprayers at Birr farm. Four sprayers had cholinesterase activity less than 50% of normal. The sprayers in both farms were the groups that are affected at most, indicating that improved control on exposure to pesticide at workplace is necessary in these groups of workers.

A study by Sirivarasai et al. (2009) aimed at determining cholinesterase activity, pesticide exposure and health effects in the exposed people. Techniques 90 individuals in total exposed to OPs due to occupations and 30 controls were recruited. Erythrocyte acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE) activities were measured in two phases of exposure at low and high levels. The outcome indicated the correlation between occupational pesticide exposure and inhibition of cholinesterase. Therefore, medical monitoring of cholinesterase inhibition and intervention programs with regard to safety practices during weld work are key issues with the purpose of reducing harmful health effects of pesticide to the smallest degree.

Chakraborty et al. (2009) found that the upper and lower respiratory symptoms as well as considerable decrease in spirometric measurements are more prevalent among agricultural worker. On the whole, lung function reduction was recorded in 48.9% of agricultural workers by comparison with 22.7% of the controls and a restricting type of deficit was preponderant. Chronic obstructive pulmonary disease (COPD) was diagnosed in 10.9% of agricultural employees by comparison with 3.4% of the controls ($p$<0.05 in $\chi 2$ test). Additionally, the acuity of the disease was greater in agricultural employees. Red blood cell (RBC) AChE was lessened by

34.2% in agricultural employees. Moreover, the fall in AChE level was positively correlated with respiratory symptoms, lung function decrement as well as COPD following the control for education as well as income as potential confounders. In conclusion, exposure to cholinesterase-inhibiting pesticides used in agriculture in the long term which is presently in use in India is correlated with a decrease in lung function, COPD as well as an increase of respiratory symptoms.

In a study conducted by Kavalci et al. (2009) which illustrates the evaluation of a special kind of mass poisoning,particularly by putting emphasis on the way of poisoning, the demographic aspects and clinical results of patients were analyzed. The consequences of eating a wheat bagel were that 13 patients, 7 males and 6 females, were admitted to the department of emergency because of the organophosphate poisoning. 26 ± 13.9 was the mean age of the patients. The level of mean serum acetylcholinesterase was 2945.1±2648.9 U/L. 9 patients with supportive treatment who were given atropine and pralidoxime were hospitalized about 6.8±6.5 days. All patients recovered after the treatment with no occurrence of deaths. The failure to diagnose and treat organophosphate poisoning in a timely manner may be fatal. When cases of food poisoning are admitted to the hospital, a particular attention is needed to examine if they are concerned with mass poisoning.

The study done by Hofmann et al. (2010) indicates potential risk factors for serum cholinesterase (BuChE) inhibition among agricultural pesticide operators exposed to organophosphate (OP) and N-methyl-carbamate (CB) insecticides. Use longitudinal study was conducted among 154 agricultural pesticide operators who took part in the Washington State cholinesterase monitoring program in 2006 and 2007. The analysis of BuChE inhibition with relation to reported exposures was conducted before and following adjustment for potential confounders by the use of linear regression. Additionally, ORs estimating the risk of BuChE depression (>20% from baseline) were calculated for chosen exposures on the basis of unconditional logistic regression analyses. A whole reduction in mean BuChE activity was noticed among the participants in times of follow-up testing during the OP/CB spraying season in relation to pre-season baseline levels (mean reduction of 5.6%, $p<0.001$).

Score related to estimated cumulative exposure to OP/CB insecticides in the past 30 days was an important predictor of BuChE inhibition ($\beta=-1.74$, $p<0.001$). Many particular work practices as well as workplace conditions were related to greater BuChE inhibition, encompassing mixing/loading pesticides as well as cleaning spray equipment. Factors that protected against BuChE inhibition comprised full-face respirator use, wearing chemical-resistant boots as well as storing personal protective equipment in a locker at work.

Park et al. (2012) conducted a research on whether occupational exposures to pesticides were correlated with lessened nerve conduction studies among farmers. On two different occasions, the authors carried out a cross-sectional study of a group including 31 male farmers who used pesticides sporadically. Even though total median values remained within the laboratory normal limits, significant differences between the first and second tests were detected in sensory conduction velocities on the median and sural nerves, and motor conduction velocities on the posterior tibial nerve. Duration of life days of pesticide application was negatively correlated with nerve conduction velocities at the majority of nerves following the adjustment for potential confounders. These results are likely to reflect a connection between occupational pesticide exposure and peripheral neurophysiologic anomaly that is worth additional assessment.

Takayasu et al. (2012) state a case of lethal intoxication produced by ingestion of an organophosphate pesticide, methidathion (DMTP). It was found that a male aged 80 years was dead in his bed. No notable morphological change was revealed by the forensic autopsy. Nevertheless, in a test of toxicological screening, methidathion was detected in qualitative terms in extracts of stomach contents. Concentrations of methidathion (μg/g) in body fluids and organ tissues on the basis of the determination by gas chromatography-mass spectrometry are namely: 66.2 in heart blood, 8.33 in peripheral blood, 8.80 in urine, 2000 in the brain (frontal lobe), 4800 in the left lung, 810 in the liver, 150 in the left kidney, and 64,000 in the stomach contents (total 1.9 g). The said outcome strongly indicated that the victim orally took methidathion into the body. Besides, xylene was specified in body fluids as well as organ tissues. Based on toxicological information along with autopsy results, the diagnosis revealed that his death was caused by severe poisoning by an emulsion of methidathion.

Mwila et al. (2012) showed that, as a result, the effect of five various pesticides (carbaryl, carbofuran, parathion, demeton-S-methyl, and aldicarb) on AChE activity was examined to determine whether the relevant combinations had an additive, synergistic, or antagonistic inhibitory effect. The related findings suggested that the mixtures involved an additive inhibitory effect on AChE activity. The data obtained from the analysis of the mixtures were used for developing and training an artificial neural network (ANN) that was then utilized with success to identify pesticides as well as their concentrations in mixtures. This study is important now that it assessed mixtures of OPs and CPs whereas prior studies emphasized only either OPs or CPs. Former studies looked into only up to three pesticides whereas the present study assessed mixtures of five pesticides concurrently. OPs constitute a group of chemical compounds used across the globe. In the United Kingdom (UK), OPs have been used in agricultural and horticultural pesticides, certain veterinary medicines (especially, in human medicines (malathion only – as a treatment for head lice), as well as in different hygiene products for people in general, both for being used by professional operators (such as, for the control of cockroaches and other insect pests in public buildings like hospitals, schools etc.). Furthermore, OPs are used by the public members (insecticides used in household and garden). It is concerned with the use of OPs for these objectives on which this report focused. Moreover, certain OPs have been developed as nerve agents. It is proved that acute (in other words, happening within a few days) effects on health of humans can occur following exposure to enough high levels of OPs; such effects are relevant to the acute cholinergic syndrome. Despite being rare in the UK, there have been vast numbers of causes related to severely acute OP poisoning elsewhere in the world. It is accepted that (chronic) neurotoxic effects in the long term are likely to follow occasionally those short term effects.

More lately, there are more available data indicating that unfavorable effects on human health in the long run are likely to arise from being exposed to low levels of OPs, which, in themselves, does not cause symptoms of acute toxicity. If accurate, this would have significant implications for risk evaluation as well as the regulation of OP products. Nevertheless, up to now no scientific consensus on this likelihood has been found. The primary source of worry is concerned with possible neurological effects in the long term on farmers who have used OP sheep dips. Anyhow, it extends to cover people with exposure to OP products used for other objectives as well.

## CHAPTER III

## RESEARCH METHODOLOGY

### 3.1 Research Design:

This research was approved by Ethics Review Committee for Research Involving Human Research Subjects, Health Sciences Group, Chulalongkorn University with the certified code no.002/2013. The research design of this study is a cross-sectional study. The purpose of this study is to study the health problems of farmers and non-farmers exposure organophosphate (OP) pesticides in Sisa Krabue, Ongkharak, Nakhon Nayok, Thailand. All samples were collected in March 2013. Blood cholinesterase (ChE) levels were measured.

### 3.2 Study Area:

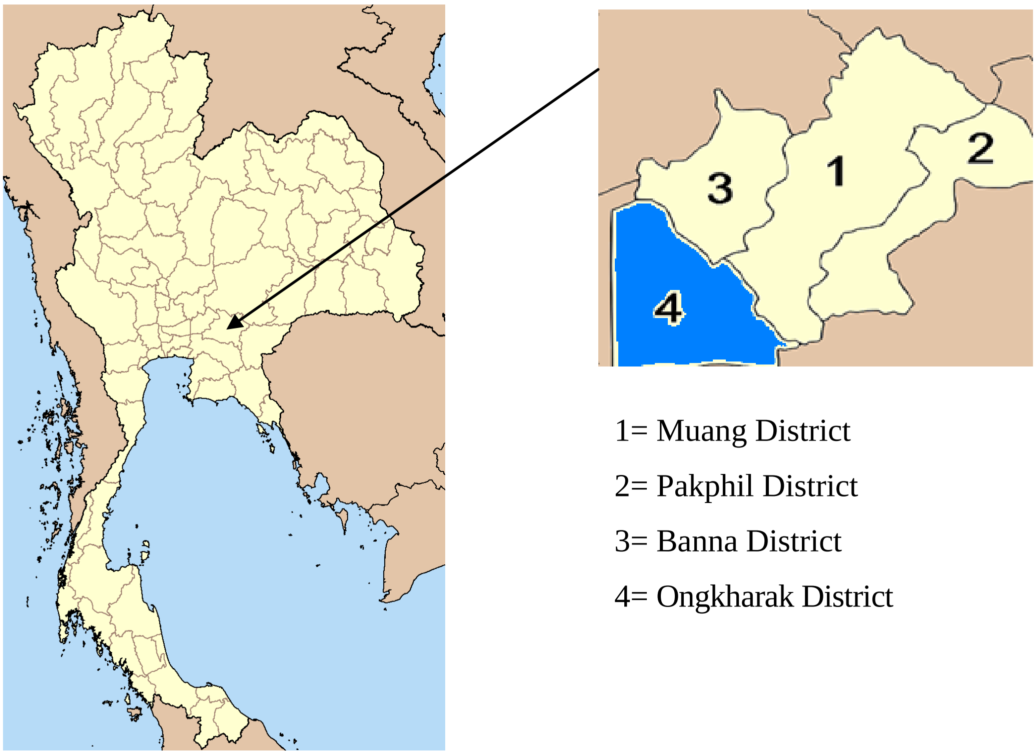



**Figure 3.1** The maps of the study Area, Sisa Krabue sub-district, Ongkharak district, Nakhon Nayok province Thailand

Sisa Krabue Sub-district in Ongkharak, Nakhon Nayok, Thailand, was purposively selected in this study due to a large number of farmers, as well as non-farmers, who were directly exposed to organophosphate (OP) pesticides since the area is occupied by the largest number of agriculturists and has the most rice paddies in the District (Nakhon Nayok, Agricultural Extension Office, 2011). Pre-survey and observation conducted at stores selling pesticides in the area of this study found that OP pesticide, such as Chlorpyrifos, was well-known and widely used in this area. Furthermore, according to a number of records related to injury and illness at work, there were several cases regarding this matter, 201 of them were among agriculturists in five years period (2007 – 2011). Additionally, it indicates an upward tendency of having an increasing number of patients at the hospital (North-Klong 23 Sub-district Health Promoting Hospital).

**3.3 Study Population:**

Accordingly, this study had divided the target populations into 2 groups which were farmers and non-farmer as follows:

3.3.1 Farmers:

Rice farmers, both male and female, in Sisa Krabue, Ongkharak, Nakhon Nayok, Thailand**,** who work frequently with OP pesticides.

*Inclusion criteria:*

- Age between 18-59 years old
- Growing rice
- Willing and being able to participate in this research.
- Applying organophosphate (OP) pesticides in paddy areas, loading, mixing, or/and spraying a day (24 hours) before blood collection.

*Exclusion criteria:*

- Having communication problems
- Having a history of liver failure, cardiovascular disease, taking anti-malarial drugs, malnutrition, and taking amphetamine.

### 3.3.2 Non-farmers:

In this study, non-farmers were participants living in the study area and may only occasionally exposed to pesticides though resides in area around house, landscape, treated crops or through residues on foods and drink.

*Inclusion criteria:*

- Age between 18-59 years old
- Non-daily farm workers
- Willing and being able to participate in this research.
- No pesticides application within 3 months in household or planting.

*Exclusion criteria:*

- Having communication problems
- Having a history of liver failure, cardiovascular disease, taking anti-malarial drugs, malnutrition, and taking amphetamine.

## 3.4 Sample Size:

The sample size will be calculated by using the formula for the sample size for the mean (Israle, 1992). The equation calculates sample size for the mean, as shown below.

$$N_0 = (Z^2 \sigma^2) / e^2$$

From Sanidcheu and Ausanawarong, 2011 study, a report of the level of cholinesterase Enzyme (ChE) in Post Harvest Farmers, was collected from 80 agriculturists in the study group and 40 people the control group. This study calculated the standard deviation and variance from the farmers who have the AChE lower level (n=11) (1.06, 1.07, 1.09, 1.10, 1.12, 1.14, 0.81, 0.88, 0.91, 0.98).

| | | |
|---|---|---|
| Standard deviation sample | = | 0.126 |
| Variance sample | = | 0.02 |

Where:

$N_0$ = sample size

Z = abscissa of the normal curve that cuts off an area α at the tails = 1.96

$\sigma^2$ = variance of an attribute in the population = 0.02

e = desired level of precision = 0.05

$$N_0 = (1.96)^2(0.02) / (0.05)^2$$
$$= 30.73$$

From the calculation above, the sample size was equal to 31 cases. Since there might be some losts to follow up, the sample size would be 10% increased covering for dropout rates. The 10% of 30.73 was 3.07 or 4 cases so the sample size was equal to 35 cases per group. Therefore, the total sample size for both groups was 70 cases. The sample sizes of previous studies were enough to find a significantly lower level of plasma cholinesterase between vegetable growers, the study group, (n=35) and the control group (n=35) (Soogarun et al., 2003). Rastogi, et al. (2008) also found that plasma butyrylcholinesterase (PBChE) decreased significantly in workers when compare the worker group (n=34) with the reference group (n=18).

### 3.5 Sampling Technique:

This study used multi-stage sampling to select samples of both farmer and non-farmer groups as follows:

**Step 1: Sampling of districts**

Nakhon Nayok Province is divided into 4 districts and was chosen by means of purposive sampling because the area is occupied by a high number of agriculturists. The majority of them are rice farmers.

**Step 2: Sampling of sub-districts**

Ongkharak District is divided into 11 sub-districts. For this purposive sampling, Sisa Krabue Subdistrict was chosen as study groups owing to having the highest number of agriculturists and the possession of the largest fields for agricultural purposes.

**Step 3: Sampling of subjects**

The representatives of the households were recruited as subjects, (one subject per household). The simple random sampling was done by drawing in accordance with the criteria is used to get target number of sample (70 subjects) consisted of 35 farmers and 35 non-farmers.

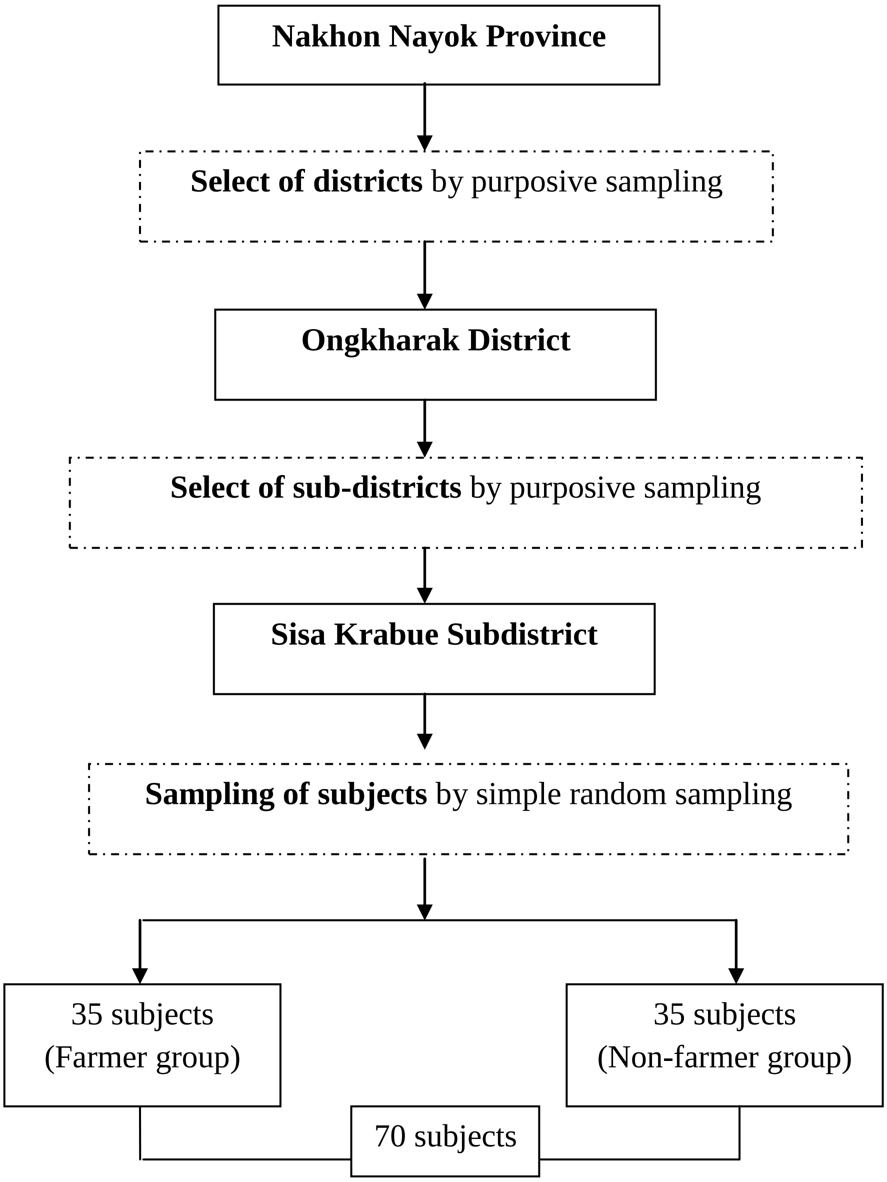



**Figure 3.2** Diagram of sampling technique

## 3.6 Measurement Tools:

### 3.6.1 Questionnaire

Information was obtained by questionnaires. Moreover, the principal researcher assessed the subjects by conducting site visits and observations. Questionnaire is divided into 4 parts. Part 1, 3 and 4 were both for farmers and non-farmers group while Part 2 was for farmer group only.

Part 1: Obtain general information and individual background, age, gender, education, income, smoking behavior and drinking behavior).

Part 2: Obtain pesticides use (type of work, work duration, farm size, the total of pesticides use, behavior of reading labels, avoiding contamination, and personal protective).

Part 3: Obtain related exposure factor (approximately house area far from paddy field, source of drinking water, eating behavior, the number of farmers in their family who apply pesticides).

Part 4: Obtain information about health–related problems to evaluate any health problems that are possibly connected with organophosphate pesticides exposure as well as symptoms (Symptoms that related with OP pesticides exposure: respiratory system, gastrointestinal system, urinary system, glands, eye symptoms, skin symptoms, central nervous system).

The structure of the questionnaire was modified by established agricultural heath studies (Taneepanichskul, 2012; Bureau of Occupational and Environmental Diseases, 2012; Thiravirojana and Pusapukdeepob, 1999) and was a guideline for personal protection when working with pesticides in tropical climates (FAO, 1990). The questionnaire is shown in Appendix.

3.6.2 Test-mate ChE (Model 400)

The Test-mate ChE is a full cholinesterase testing system. The equipment is used as reagents in total required for performing. There were 96 tests fit easily within the storage case. Only 10μL is required by the system for each blood test that is likely to be obtained with ease from a finger stick sample. The whole examination may be finished in less than 4 minutes, making it a quick evaluation of poisoning status easily (EQM Research, Inc., 2003). One of studies that study acute organophosphate poisoning in 14 patients measures AChE and plasma cholinesterase (PChE) by using Test-mate ChE (Model 400) compared with laboratory. The results showed that the Test-mate ChE field kit reliably provides rapid measurement of AChE in acute organophosphorus poisoning (Rajapakse et al., 2011).

## 3.7 Data Collection:

Data were collected in March 2013. The most appropriate time since farmers had grown rice for a whole year and pesticides were mostly use during this time. The place to collect the data is in the Ban Khlong 23 North Side Health Promoting Hospital (Appendix C).

3.7.1 Qualitative data

- Collected questionnaires and checked for data input. It took 10-15 minutes per person.

3.7.2 Quantitative data

- Identification of different blood cholinesterase levels by using Test-mate ChE (Model 400). A nurse took blood from farmers and non-farmers at 20μL per person. For the farmers, their bloods were collected for samples 1 day after the end of their exposure (loading, mixing, and/ or spraying) to OP pesticides (Mason, 2000). Farmers, whose blood was drawn from, need to wash hands with soap before participating in the process. Then used surgical cotton moistened with alcohol to clean the finger in order to take sample. After the alcohol dried off, use a needle to puncture. Take the first drop of blood out. Wait for the second drop to leak out then store it with a capillary tube until the tube is filled. A nurse was to not squeeze the finger. If the blood is not enough, puncture the finger again. Next, test the cholinesterase level by using Test-mate ChE Cholinesterase Test System (Model 400). The results were told to the participants directly. If the blood cholinesterase is not normal at level, then researcher recommended the participant to meet the doctor.

**3.8 Data Analysis:**

3.8.1 Interpret level of cholinesterase: depression of cholinesterase less than 50% normal indicates possible pesticide poisoning requiring removal from exposure and/or treatment with anticholinergics (Coye et al, 1986).

| | | |
|---|---|---|
| More than 50%Normal | = | normal |
| Less than or equal 50%Normal | = | abnormal |

3.8.2 This study was use statistical analysis: using the licensed SPSS version 17 for windows.

- The general characteristics and study variables of the study population were described by frequency, percentage, and mean.

- Independent T-Test was used to find differentiation between blood cholinesterase levels of farmer group and non-farmer group.

- Chi-square was used to find an association between pesticides use and blood cholinesterase level, a relationship between health effects and level of blood cholinesterase that information from interviews.

**3.9 Ethic Consideration:**

The experimental protocol was approved by the Ethics Review Committee for Research Involving Human Research Subjects, Health Sciences Group, Chulalongkorn University with the certified code no.002/2013. The objective of the research clearly informed to the study population. The data was used for the study purpose only. Inform consent was signed by subjects prior to the study.

# CHAPTER IV
# RESULTS

Data were collected in Sisa Krabue subdistrict, Ongkharak district, Nakhon Nayok province, Thailand. All participants were chosen by simple random sampling. Farmers and non-farmers were not accommodated in the same house. Researcher had explained to every participant about the research before they signed the assent form.

Chapter IV gives descriptive details of the results acquired from the field research which are questionnaire and blood cholinesterase activity. Variables are described by mean, standard deviations, range, percentages, and the association of blood cholinesterase activity and information gathered questionnaire.

## 4.1 Questionnaires Information:

There were 70 participants for the questionnaire part; 35 farmers and 35 non-farmers. Participants were evaluated during site visitations and observations. The questionnaire itself was divided into 4 parts. Part One contained general information asking every participant; the information included ages, gender, education, annul income, smoking and drinking behavior. Part Two was required to be filled only by farmers. This part contained questions regarding pesticides use such as activities related to pesticides exposure, area cultivated, type of work, working hours, label reading, personal protective equipment (PPE) use, and personal hygiene. Part Three was about factors related to pesticides exposure in both farmer and non-farmer participants. Part Four was about symptoms associated with organophosphate (OP) pesticides exposure including skin symptoms, eyes symptoms, central nervous system symptoms, respiratory system symptoms, gastrointestinal system symptoms, urinary system symptoms, and glands symptoms.

### 4.1.1 Characteristics of farmers and non-farmers

In this study, participants were divided into two groups; farmer and non-farmers which were consisted of 25 male and 45 female. Average age (±SD) was 42.63 (±10.41) years old. Most of them finished primary school (57.1%). About sixty three percent of respondents had an income of 30,000-60,000 baht per years. Most of them were not smokers (72.9%) but about half (47.1%) were drinkers.

*Farmers:*

Farmers who were participated in this study were male (40%) and female (60%). There were four age groups: 18 to 30 (11.4%), 31 to 40 (28.6%), 41 to 50 (37.1%), and 51-60 (22.9%). Average age (±SD) was 42.40 (±9.42) years old. Most of them finished primary school (62.9%). Sixty percent of them had an income of about 30,000-60,000 baht per year. More than half of them (60%) were not smokers but they were drinkers (62.9%) (Table 4.1).

*Non-farmers:*

In the non-farmer group, there were both men (31.4%) and women (68.6%). Age of participants was ranged from 20 to 59 years old. Age groups were 18 to 30 (14.3%), 31 to 40 (28.6%), 41 to 50 (28.6%), and 51-60 (28.6%). Average age (±SD) was 42.86 (±11.44) years old. Half of them graduated from primary school (51.4%). About sixty six percent of them had an income of about 30,000 – 60,000 baht annually while only 14.3% had an income more than 90,000 baht per year. Up to 85.7% of them were none smokers and 68.6% were none drinkers, which was in the higher rate compared to those of the farmers (Table 4.1).

**Table 4.1** General characteristics of the farmers and the non-farmers

| **General Information** | **Farmers** | **Non-Farmers** | **Total** |
|---|---|---|---|
| | (n = 35) | (n = 35) | (n = 70) |
| **Gender (n (%))** | | | |
| Men | 14 (40%) | 11 (31.4%) | 25 (35.7%) |
| Women | 21 (60%) | 24 (68.6%) | 45 (64.3%) |
| **Age Groups (n (%))** | | | |
| 18 - 30 | 4 (11.4%) | 5 (14.3%) | 9 (12.8%) |
| 31 - 40 | 10 (28.6%) | 10 (28.6%) | 20 (28.6%) |
| 41 - 50 | 13 (37.1%) | 10 (28.6%) | 23 (32.9%) |
| 51 - 60 | 8 (22.9%) | 10 (28.6%) | 18 (25.7%) |

| General Information | Farmers | Non-Farmers | Total |
|---|---|---|---|
| | (n = 35) | (n = 35) | (n = 70) |
| Mean ±SD | 42.40 (±9.42) | 42.86 (±11.44) | 42.63 (±10.41) |
| Range | 19 - 58 | 20 - 59 | 19-59 |
| **Education (n (%))** | | | |
| Uneducated | 1 (2.9%) | 1 (2.9%) | 2 (2.9%) |
| Primary School | 22 (62.9%) | 18 (51.3%) | 40 (57.1%) |
| Lower Secondary School | 6 (17.1%) | 7 (20.0%) | 13 (18.5%) |
| Upper Secondary School | 4 (11.4%) | 2 (5.7%) | 6 (8.6%) |
| Vocational Certificate | 1 (2.9%) | 1 (2.9%) | 2 (2.9%) |
| High Vocational Certificate | 1 (2.9%) | - | 1 (1.4%) |
| Bachelor or equal | - | 6 (17.2%) | 6 (8.6%) |
| **Annual Income (Baht)*** | | | |
| Less than 30,000 | 3 (8.6%) | 4 (11.4%) | 7 (10%) |
| 30,000-60,000 | 21 (60%) | 23 (65.7%) | 44 (62.9%) |
| 60,001-90,000 | 6 (17.1%) | 3 (8.6%) | 9 (12.8%) |
| More than 90,000 | 5 (14.3%) | 5 (14.3%) | 10 (14.3%) |
| **Smoking Status (n (%))** | | | |
| Smokers | 14 (40%) | 5 (14.3%) | 19 (27.1%) |
| Non-Smokers | 21 (60%) | 30 (85.7%) | 51 (72.9%) |
| **Drinking Status (n (%))** | | | |
| Drinkers | 22 (62.9%) | 11 (31.4%) | 33 (47.1%) |
| Non-Drinkers | 13 (37.1%) | 24 (68.6%) | 37 (52.9%) |

** 1 USD = 30 THB*

4.1.2 Information of agricultural works and pesticides use in the farmers

The average (±SD) year of using pesticides was 18.64 (±11.58) years. The average working hours was about 5.1 hours per day and the average of cultivation area was 30.74 (±19.81) rais. 37% of the farmers used the pesticides by mixing, loading, and spraying, some of them mixed and sprayed the pesticides (25.7%), only sprayed (25.7%), only mixed (8.6%), and only loaded (2.9%). The methods that they used to sprayed pesticides were mostly spraying by pump (60%) and by hand (40%). Most farmers used pesticides on their crops about 2 times a day; in the early morning and in the evening (65.7%) (Table 4.2).

**Table 4.2** Agricultural works and farming characteristics in farmers (n=35)

| | |
|---|---|
| **Years of using pesticides (n (%)) (mean ±SD)** | 18.64 (±11.58) |
| 0-10 | 14 (40%) |
| 10-20 | 7 (20%) |
| 21-30 | 9 (25.7%) |
| More than 30 | 5 (14.3%) |
| **Hours of working/day (Hrs) (mean ±SD)** | 5.1 (±1.8) |
| **Area cultivated (rai)* (n (%)) (mean ±SD)** | 30.74 (±19.81) |
| Less than 10 | 7 (20%) |
| 10-20 | 8 (22.9%) |
| 21-30 | 8 (22.9%) |
| More than 30 | 12 (34.2%) |
| **Type of pesticides application (n (%))** | |
| Mixing | 3 (8.6%) |
| Loading | 1 (2.9%) |
| Spraying | 9 (25.7%) |
| Mixing and Spraying | 9 (25.7%) |
| Mixing, Loading, and Spraying | 13 (37.1%) |
| **Spraying method (n (%))** | |
| Spraying by hand | 14 (40%) |
| Spraying by pump | 21 (60%) |
| **Spraying time (n (%))** | |
| Early morning | 5 (14.3%) |
| Evening | 1 (2.9%) |
| Convenient time | 2 (5.7%) |
| Early morning and Evening | 23 (65.7%) |
| Early morning, Middle day, and Evening | 4 (11.4%) |

**1 rai = 0.4 acre*

Table 4.3 shows that about 91.4 % of the farmers read the label on the pesticide products and up to 82.9% of them used the proper amount of the pesticides as stated on the label. While working in the fields, 88.6% of them did not eat and drink near the area and after using pesticides about 97.1% of them washed their hands before eating. About ninety four percent of them immediately take shower after finishing their work. They mostly washed their chemical stained clothes and normal clothes separately (94.3%). However, only 8.6% of the participants bury the pesticides bottle after finishing it while most of them did not (91.4%).

**Table 4.3** Practicing of pesticide use and personal hygiene among farmers (n=35)

| | **Farmers: n (%)** | |
|---|---|---|
| | **Yes** | **No** |
| Read the label of pesticides products | 32 (91.4%) | 3 (8.6%) |
| Use the recommended amounts of pesticides | 29 (82.9%) | 6 (17.1%) |
| Eat and drink away from pesticides exposure area | 31 (88.6%) | 4 (11.4%) |
| After using pesticides, wash hands before eating | 34 (97.1%) | 1 (2.9%) |
| Take shower immediately after using pesticides | 33 (94.3%) | 2 (5.7%) |
| Wash chemical stained clothes and normal clothes separately | 33 (94.3%) | 2 (5.7%) |
| Bury used pesticide bottles after finishing them | 3 (8.6%) | 32 (91.4%) |

Table 4.4 shows the use of personal protective equipment (PPE) while applying pesticides in the paddy fields. Every farmer indicated that they wore was long sleeved shirts and long legged pants. Up to 97.1% of all participants wore hat and mask, only one participant had never worn hat and mask. Some farmers reported that they used gloves (37.1%). At the same time only 5.7% and 8.6% of them used boots and goggles respectively.

**Table 4.4** Personal protective equipment (PPE) use among farmers (n=35)

| | **Farmers: n (%)** | |
|---|---|---|
| **PPE** | **Use** | **Not Use** |
| Gloves | 13 (37.1%) | 22 (62.9%) |
| Long sleeved shirts and Long legged | 35 (100%) | - |
| Hat and Mask | 34 (97.1%) | 1 (2.9%) |
| Boots | 2 (5.7%) | 33 (94.3%) |
| Goggles | 3 (8.6%) | 32 (91.4%) |

4.1.3 The information of factors that related to pesticides exposure

Table 4.5 shows the information of farmers and non-farmers exposure to pesticides including community related factors. Thirty one percent of participants had one farmer among their family members, 30.1% had 2 farmers in their household. About half of them (51.4%) reported that they live near the paddy area, less than 51 meters to their house. The majority of participants reported that rain water was the main source of drinking water in family (74.3%), from bottled water (21.4%) and from tap water (4.3%). Every participant washed fruits and vegetables before eating.

*Farmers:*

In family of farmers, most of the participants had 2 farmers in their household (37.1%). Half of them (51.4%) live near the paddy area, less than 51 meters to the field. Most of them drink rain water (85.7%) and few of them drink from bottled water (14.3%). About eighty three percent of them always washed fruits and vegetables before eating and 17.1% sometimes washed fruits and vegetables before eating.

*Non-farmers:*

In non-farmers family, most family had 1 farmer in the household (45.7%). About half of them (51.4%) live near paddy area, less than 51 meters from the field. They reported that their sources of drinking water were rain water (62.9%), bottled water (28.6%), and tap water (8.6%). One hundred percent of them washed fruits and vegetables before eating, 82.9% always did it and 17.1% did it sometimes.

**Table 4.5** Related exposure factors in the respondents (n=70)

| **Related exposure factors** | **Farmers** (n=35) | **Non-Farmers** (n=35) | **Total** (n=70) |
|---|---|---|---|
| **Numbers of farmers in the family (n(%))** | | | |
| 1 | 6 (17.1%) | 16 (45.7%) | 22 (31.4%) |
| 2 | 13 (37.1%) | 8 (22.9%) | 21 (30.1%) |
| 3 | 5 (14.3%) | 6 (17.1%) | 11 (15.7%) |
| 4 | 6 (17.1%) | 4 (11.4%) | 10 (14.3%) |
| 5 | 4 (11.4%) | 1 (2.9%) | 5 (7.1%) |
| 6 | 1 (2.9%) | - | 1 (1.4%) |
| **Residential location (From paddy area: m) (n(%))** | | | |
| Less than 51 | 18 (51.4%) | 18 (51.4%) | 36 (51.4%) |
| 51-100 | 6 (17.1%) | 6 (17.1%) | 12 (17.2%) |
| 101-500 | 3 (8.6%) | 8 (22.9%) | 11 (15.7%) |
| More than 500 | 8 (22.9%) | 3 (%8.6) | 11 (15.7%) |
| **Source of drinking water (n(%))** | | | |
| Rain water | 30 (85.7%) | 22 (62.9%) | 52 (74.3%) |
| Tap water | - | 3 (8.6%) | 3 (4.3%) |
| Plastic bottled water | 5 (14.3%) | 10 (28.6%) | 15 (21.4%) |
| **Washing fruits and vegetables before eating (n(%))** | | | |
| Always | 29 (82.9%) | 29 (82.9%) | 58 (82.9%) |
| Sometimes | 6 (17.1%) | 6 (17.1%) | 12 (17.1%) |

### 4.1.4 Health effects related to OP pesticides exposure

The self-reported health symptoms related to OP pesticides exposure from both farmer and non-farmers group were completed by face to face technique during questionnaire (Table 4.6). In farmer group, they reported to have skin symptoms; about half of them suffered from skin rash, itch and burn (48.6%). Thirty four percent of them had hands numbness and thirty one percent had muscular twitching and cramps. The main eye symptoms were irritation (68.6%) and blurred vision (62.9%), respectively. In accordance with respiratory system, they mentioned on shortness of breath (42.9%), wheezing (37.1%), and dyspnea (28.6%). For gastrointestinal system, some of them reported the abdominal cramps (22.9%). They reported that about half of them had excessive sweating (40%). For central nervous system, up to 82.9% of them had headache and 80% had dizziness. Some of them had drowsiness (37.1%) and irritability (34.3%). However, only few of them got trembling hands (8.6%), ataxia (2.9%), and memory problem (2.9%).

The self-reported heath symptoms from non-farmers showed that some of them (28.6%) had skin rash, itch and burn and 34.3% were hands tingling and numbness. For eye symptoms were blurred vision (34.3%) and irritation (40%). Central nervous system symptoms were found 45.7% headache, 28.6% dizziness and 25.7% drowsiness. Shortness of breath was found 28.6%. Anorexia and abdominal cramps were found 11.4% and 14.3%, respectively. Few of non-farmers had excessive sweating (5.7%).

**Table 4.6** Subjective symptoms related to organophosphate pesticides exposure

| **Symptoms** | **Farmers** (n=35) **n(%)** | **Non-Farmers** (n=35) **n(%)** | **Total** (n=70) **n(%)** |
|---|---|---|---|
| **Skin symptoms** | | | |
| Skin rash/ itching/ burning | 17 (48.6%) | 10 (28.6%) | 27 (38.6%) |
| Tingling/Numbness of hands | 12 (34.3%) | 12 (34.3%) | 24 (34.3%) |
| Muscular twitching and cramps | 11 (31.4%) | 9 (25.7%) | 20 (28.6%) |
| **Eye symptoms** | | | |
| Blurred vision | 22 (62.9%) | 12 (34.3%) | 34 (48.6%) |
| Lacrimation | 16 (45.7%) | 3 (8.6%) | 19 (27.1%) |
| Irritation | 24 (68.6%) | 14 (40%) | 38 (54.3%) |

| Symptoms | Farmers (n=35) n(%) | Non-Farmers (n=35) n(%) | Total (n=70) n(%) |
|---|---|---|---|
| **Central nervous system** | | | |
| Headache | 29 (82.9%) | 16 (45.7%) | 45 (64.3%) |
| Dizziness | 28 (80.0%) | 10 (28.6%) | 38 (54.3%) |
| Drowsiness | 13 (37.1%) | 9 (25.7%) | 22 (31.4%) |
| Slurred speech | - | 2 (5.7%) | 2 (2.9%) |
| Ataxia | 1 (2.9%) | - | 1 (1.4%) |
| Trembling of hands | 3 (8.6%) | 2 (5.7%) | 5 (7.1%) |
| Irritability | 12 (34.3%) | 4 (11.4%) | 16 (22.9%) |
| Memory problem | 1 (2.9%) | 4 (11.4%) | 5 (7.1%) |
| **Respiratory system** | | | |
| Wheezing | 13 (37.1%) | 5 (14.3%) | 18 (25.7%) |
| Dyaspnea | 10 (28.6%) | 5 (14.3%) | 15 (21.4%) |
| Bronchorrhea | 1 (2.9%) | - | 1 (1.4%) |
| Running nose | - | 1 (2.9%) | 1 (1.4%) |
| Shortness of breath | 15 (42.9%) | 10 (28.6%) | 25 (35.7%) |
| **Gastrointestinal system** | | | |
| Anorexia | 6 (17.1%) | 4 (11.4%) | 10 (14.3%) |
| Vomiting | 4 (11.4%) | 2 (5.7%) | 6 (8.6%) |
| Abdominal cramps | 8 (22.9%) | 5 (14.3%) | 13 (18.6%) |
| Fecal incontinence | 1 (2.9%) | - | 1 (1.4%) |
| **Urinary system** | | | |
| Loss of urinary control | 1 (2.9%) | - | 1 (1.4%) |
| **Glands** | | | |
| Hyper salivation | 4 (11.4%) | - | 4 (5.7%) |
| Sweating | 14 (40%) | 2 (5.7%) | 16 (22.9%) |

Table 4.7 presents results for farmer and non-farmer group with report of health effects. Farmers who were directly exposed to pesticides were significantly associated with increase eye symptoms: blurred vision (OR=3.244, 95%CI 1.219-8.629),

lacrimation (OR=8.982, 95%CI 2.311-34.910), and irritation (OR=3.273, 95%CI 1.224-8.748). The table indicates that these positive associations of farmers are significantly related to central nervous system symptoms: headache (OR=5.740, 95%CI 1.906-17.282), dizziness (OR=10.000, 95%CI 3.308-30.230), and irritability (OR=4.043, 95%CI 1.154-14.164). Moreover, the study found farmers were significantly associated with an increase wheezing (OR=3.545, 95%CI 1.102-11.411) and sweating (OR=11.000, 95%CI 2.267-53.372). Farmer group were not associated with skin symptoms, gastrointestinal system symptoms and urinary system symptom.

**Table 4.7** The association between participant groups (n=70) and health effects

| **Symptoms** | **Farmers and Non-Farmer** | |
|---|---|---|
| | **P-value** | **OR (95% CI)** |
| **Skin symptoms** | | |
| Skin rash/ itching/ burning | 0.086 | 2.361 (0.879-6.345) |
| Tingling/Numbness of hands | 1.000 | 1.000 (0.373-2.683) |
| Muscular twitching and cramps | 0.597 | 1.324 (0.467-3.750) |
| **Eye symptoms** | | |
| Blurred vision | 0.017* | 3.244 (1.219-8.629) |
| Lacrimation | 0.000* | 8.982 (2.311-34.910) |
| Irritation | 0.016* | 3.273 (1.224-8.748) |
| **Central nervous system** | | |
| Headache | 0.001* | 5.740 (1.906-17.282) |
| Dizziness | 0.000* | 10.000 (3.308-30.230) |
| Drowsiness | 0.303 | 1.707 (0.614-4.744) |
| Slurred speech | 0.483 | NC |
| Ataxia | 1.000 | NC |
| Trembling of hands | 1.000 | 1.547 (0.242-9.878) |
| Irritability | 0.023* | 4.043 (1.154-14.164) |
| Memory problem | 0.356 | 0.228 (0.024-2.151) |

| Symptoms | Farmers and Non-Farmer | |
|---|---|---|
| | P-value | OR (95% CI) |
| **Respiratory system** | | |
| Wheezing | 0.029* | 3.545 (1.102-11.411) |
| Dyaspnea | 0.145 | 2.400 (0.725-7.949) |
| Bronchorrhea | 1.000 | NC |
| Running nose | 1.000 | NC |
| Shortness of breath | 0.212 | 1.875 (0.695-5.061) |
| **Gastrointestinal system** | | |
| Anorexia | 0.495 | 1.603 (0.410-6.264) |
| Vomiting | 0.673 | 2.129 (0.364-2.459) |
| Abdominal cramps | 0.356 | 1.778 (0.518-6.097) |
| Fecal incontinence | 1.000 | NC |
| **Urinary system** | | |
| Loss of urinary control | 1.000 | NC |
| **Glands** | | |
| Hyper salivation | 0.114 | NC |
| Sweating | 0.001* | 11.000 (2.267-53.372) |

**Significant at 0.05 probability level*
*NC- not calculated*

## 4.2 Blood Cholinesterase Levels:

A total of participants (35 farmers and 35 non-farmers) were registered in a study exploration of pesticides exposure. Blood samples were collected from Ban Khlong 23 North Side Health Promoting Hospital to find levels of both blood enzymes erythrocyte cholinesterase (AChE) and plasma cholinesterase (PChE) by using standard method. The averages temperature was 27.4 ºC.

For farmers, bloods were collected from each participant after 24 hours that they had finished using pesticides. AChE levels and PChE levels means (U/ml) and range were showed in Table 4.7. In farmer group, range of AChE level was 1.60-3.95 U/ml while in non-farmer group was 1.39-4.11 U/ml. The results found that average AChE of farmers (2.63 ±0.55 U/ml) was lower than non-farmers (2.80 ±0.53 U/ml)

PChE levels in farmer group showed that the minimum PChE was 0.08 U/ml while it was 1.36 U/ml in the non-farmer group. The maximum PChE level in non-farmers was 2.58 U/ml but in farmer group only 1.75 U/ml. The results showed that the average PChE levels in non-farmer group (1.81±0.30 U/ml) was significantly (t-test, $p < 0.001$) higher than in farmer group (1.01±0.44 U/ml) (Table 4.8).

**Table 4.8** Cholinesterase levels (U/ml) of farmers and non-farmers in Sisa Krabue Subdistrict, Ongkharak District, Nakhon Nayok Province

| | Farmers (n=35) | | Non-Farmers (n=35) | | **P-value** |
|---|---|---|---|---|---|
| | Mean ±SD | Range | Mean ±SD | Range | |
| AChE (U/ml) | 2.63±0.55 | 1.60-3.95 | 2.80±0.53 | 1.39-4.11 | 0.197 |
| PChE (U/ml) | 1.01±0.44 | 0.08-1.75 | 1.36-2.58 | 1.36-2.58 | <0.001** |

*** Significant at 0.001 probability level, (t-test*)

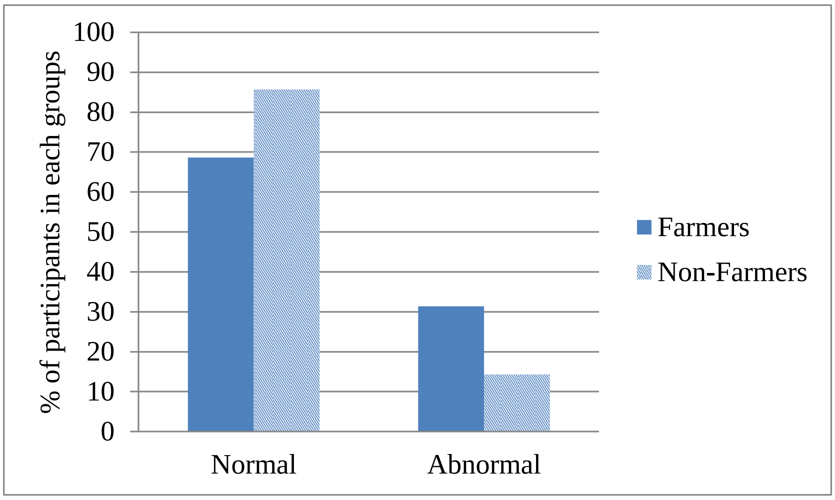
All participants (35 farmers and 35 non-farmers) were divided into 2 groups; normal ChE level and abnormal ChE level. For farmers, the prevalence of abnormal AChE levels was 31.4% and 68.6% of them had normal AChE levels. In non-farmer group, the prevalence of abnormal AChE levels was 14.3% and 85.7% of them had normal AChE levels. The result showed the prevalence of abnormal AChE levels in farmer group was more than in non-farmer group but not significant (t-test, $p$=0.184).

According to PChE levels, 25.7 % of the farmers had normal PChE levels and 74.3% of them had abnormal PChE levels while all of non-farmers had normal PChE levels (Table 4.8). The result showed the prevalence of normal PChE levels in non-farmer group was more than in farmer group (t-test, $p$<0.001). The association between AChE levels and PChE levels were likely low negative correlation (Pearson Correlation coefficient -0.121 at $p$=0.488 in farmer group) (Table 4.9).

**Table 4.9** The percentage of normal and abnormal ChE levels

| | AChE (%N) | | PChE (%N) | |
|---|---|---|---|---|
| | Normal (n(%)) | Abnormal (n(%)) | Normal (n(%)) | Abnormal (n(%)) |
| All (n=70) | 54 (77.1%) | 16 (22.9%) | 44 (62.9%) | 26 (37.1%) |
| Farmers (n=35) | 24 (68.6%) | 11 (31.4%) | 9 (25.7%) | 26 (74.3%) |
| Non-Farmers (n=35) | 30 (85.7%) | 5 (14.3%) | 35 (100%) | - |

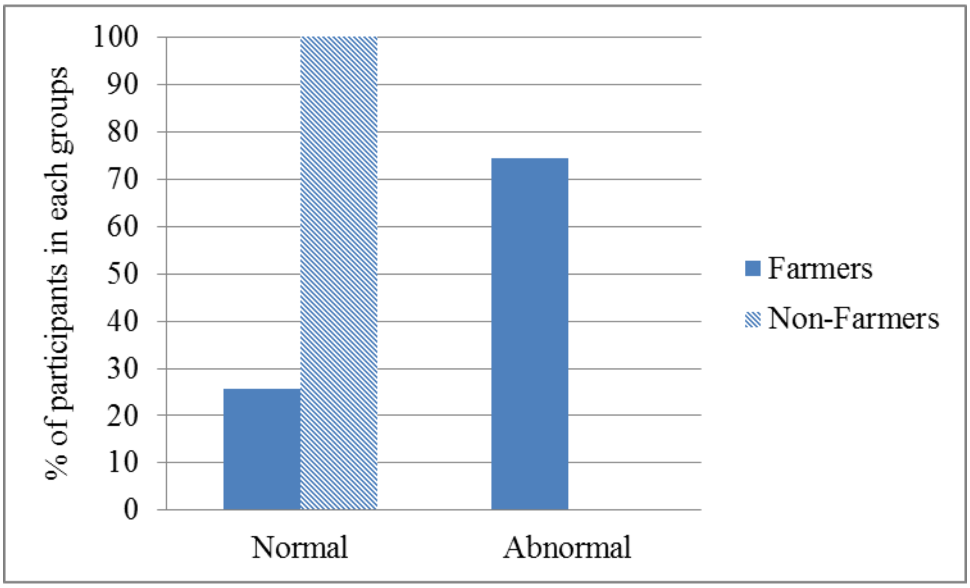
**Figure 4.1** The comparison of AChE levels (normal and abnormal) among farmers (n=35) and non-farmers (n=35)




*Normal = More than 50%Normal

*Abnormal = Less than or equal 50%Normal

**Figure 4.2** The comparison of PChE levels (normal and abnormal) among farmers (n=35) and non-farmers (n=35)




*Normal = More than 50%Normal

*Abnormal = Less than or equal 50%Normal

## 4.3 Association between ChE and Factors in participants:

### 4.3.1 Association between ChE levels and characteristics of participants

Association between ChE levels and general characteristics was statistically analyzed (Table 4.10). The results showed that gender was significantly associated with AChE levels (*p* = *0*.027), male had normal AChE levels more than female. On the other hand, AChE levels and characteristics: age, education, income, smoking status and drinking status were no significant association**.**

**Table 4.10** Association between AChE levels and the characteristics of participants (n=70)

| **Characteristics** | **AChE (%N)** | |
|---|---|---|
| | $\chi^2$ | **P-value** |
| Gender | 4.868 | 0.027* |
| Age | 1.180 | 0.758 |
| Education | 2.421 | 0.933 |
| Income | 3.100 | 0.376 |
| Smoking Status | 0.739 | 0.529 |
| Drinking Status | 2.102 | 0.147 |

**Significant at 0.05 probability level*

Table 4.11 shows the smoking and drinking behavior were significantly associated with PChE levels (*p*=0.001 and *p*=0.001, respectively). The characteristics of participants: gender, age, education, and income were no significant association with PChE levels.

**Table 4.11** Association between PChE levels and the characteristics ofparticipants (n=70)

| Characteristics | PChE (%N) | |
|---|---|---|
| | $\chi^2$ | **P-value** |
| Gender | 1.963 | 0.161 |
| Age | 3.227 | 0.358 |
| Education | 6.276 | 0.508 |
| Income | 2.920 | 0.404 |
| Smoking Status | 10.928 | 0.001* |
| Drinking Status | 11.165 | 0.001* |

**Significant at 0.05 probability level*

4.3.2 Association between ChE and pesticides use behaviors in the farmers

The statistical analysis of association between AChE levels and agricultural works and farming characteristics in farmers showed years of using pesticides, hours of working per day, size of cultivation area, and there was not significant association between spraying method and AChE levels (Table 4.12).

**Table 4.12** Association between AChE levels and agricultural works and farming characteristics in farmers (n=35)

| | AChE (%N) | |
|---|---|---|
| | $\chi^2$ | **P-value** |
| Years of using pesticides | 7.093 | 0.069 |
| Hours of working/day | 1.673 | 0.892 |
| Area cultivated | 5.889 | 0.117 |
| Spraying method | 0.199 | 0.721 |

The results from statistical analysis found that reading the label of pesticide products, recommendation of the amount pesticides use, eating and drinking behavior in paddy field, washing hands, taking shower after using pesticides, washing chemical stained clothes method, and the method to remove bottle of pesticide used were not significantly associated with both normal and abnormal AChE levels (table 4.13).

**Table 4.13** Association between AChE levels and practicing of pesticide use and personal hygiene among farmers (n=35)

| **Practicing of pesticide use and personal hygiene** | **AChE (%N)** | |
|---|---|---|
| | $\chi^2$ | **P-value** |
| Reading the label of pesticides products | 1.891 | 0.227 |
| Using the recommended amounts of pesticides | 0.012 | 1.000 |
| Eating and drinking away from pesticides exposure area | 0.723 | 0.575 |
| Washing hands before eating when used pesticides | 0.472 | 1.000 |
| Immediately take shower after using pesticides | 0.339 | 0.536 |
| Washing working clothes without normally clothes | 0.339 | 0.536 |
| Bury used pesticide bottles underground | 1.504 | 0.536 |

The use of PPE and AChE levels was statistically analyzed. Table 4.14 shows that the use of gloves, hat and mask, boots, and goggles when farmers applying pesticides were not significantly associated with AChE levels.

**Table 4.14** Association between AChE levels and personal protective equipment (PPE) use among farmers (n=35)

| **PPE** | **AChE (%N)** | |
|---|---|---|
| | $\chi^2$ | **P-value** |
| Gloves | 0.669 | 0.478 |
| Hat and Mask | 2.246 | 0.314 |
| Boots | 0.006 | 1.000 |
| Goggles | 0.339 | 0.536 |

The association between years of using pesticides was significantly associated with PChE levels ($p$ = 0.010). However, hours of working per day, size of cultivating area, and spraying method were not significantly associated with both normal and abnormal PChE levels (Table 4.15).

**Table 4.15** Association between PChE levels and agricultural works and farming characteristics in farmers (n=35)

| | **PChE (%N)** | |
|---|---|---|
| | $\chi^2$ | **P-value** |
| Years of using pesticides | 11.368 | 0.010* |
| Hours of working/day | 4.040 | 0.544 |
| Area cultivated | 2.811 | 0.422 |
| Spraying method | 3.590 | 0.112 |

**Significant at 0.05 probability level*

The statistical analysis was found normal and abnormal PChE levels were no significant association with reading the label of pesticides products, use of the recommended amounts of pesticides, behavior of eating and drinking around farm, taking shower after pesticides application, and washing working clothes method (table 4.16).

**Table 4.16** Association between PChE levels and practicing of pesticide use and personal hygiene among farmers (n=35)

| **Practicing of pesticide use and personal hygiene** | **PChE (%N)** | |
|---|---|---|
| | $\chi^2$ | **P-value** |
| Reading the label of pesticides products | 0.100 | 1.000 |
| Using the recommended amounts of pesticides | 0.310 | 1.000 |
| Eating and drinking away from pesticides exposure area | 0.001 | 1.000 |
| Washing hands before eating when used pesticides | 0.356 | 1.000 |
| Immediately take shower after using pesticides | 0.655 | 0.454 |
| Washing working clothes without normally clothes | 0.655 | 0.454 |
| Bury used pesticide bottles underground | 0.100 | 1.000 |

The table 4.17 shows association between PChE levels and use of PPE in famers. The statistical analysis was found normal and abnormal PChE levels were not significantly associated with use of gloves, hat and mask, boots, and goggles.

**Table 4.17** Association between PChE levels and personal protective equipment (PPE) use among farmers (n=35)

| **PPE** | **PChE (%N)** | |
|---|---|---|
| | $\chi^2$ | **P-value** |
| Gloves | 1.759 | 0.243 |
| Hat and Mask | 2.974 | 0.257 |
| Boots | 0.100 | 1.000 |
| Goggles | 0.734 | 1.000 |

4.3.3 Association between ChE levels and health effects

Table 4.18 shows the AChE level was significantly associated with dizziness (*p*=0.014) in central nervous system. However the AChE level was not significantly associated with other symptoms in central nervous system: headache, drowsiness, slurred speech, ataxia, trembling of hands, irritability and memory problem. The study was found AChE levels were not significantly associated with skin symptoms, eye symptoms, respiratory system symptoms, gastrointestinal system symptoms, urinary system symptoms, and glands symptoms.

The study showed that PChE level was not associated with skin symptoms. While The PChE level was found significant association with lacrimation and irritation (*p*=0.001 and *p*=0.003, respectively). For central nervous system, the study found the significant association between PChE level and headache, dizziness, and irritability (*p*=0.027, *p*=0.001, and *p*=0.003, respectively). In respiratory system, the PChE level was significantly associated with wheezing, dyaspnea, and shortness of breath (*p*=0.015, *p*=0.039, and *p*=0.015, respectively). But PChE level was not associated with symptoms in gastrointestinal system and urinary system.

The association between PChE level and hyper salivation was found ($p$=0.016). Also The association between PChE level and the sweating ($p$=0.000).

**Table 4.18** Association between ChE levels (AChE and PChE) and reported health symptoms in participants (n=70)

| Health symptoms | AChE (%N) | | PChE (%N) | |
|---|---|---|---|---|
| | $\chi^2$ | P-value | $\chi^2$ | P-value |
| **Skin symptoms** | | | | |
| Skin rash/ itching/ burning | 1.143 | .285 | 2.280 | 0.131 |
| Tingling/Numbness of hands | 0.794 | .373 | 0.320 | 0.572 |
| Muscular twitching and cramps | 0.810 | .366 | 0.055 | 0.814 |
| **Eye symptoms** | | | | |
| Blurred vision | 0.490 | .484 | 2.784 | 0.095 |
| Lacrimation | 0.177 | .752 | 10.928 | 0.001* |
| Irritation | 0.154 | .695 | 8.541 | 0.003* |
| **Central nervous system** | | | | |
| Headache | 2.600 | .107 | 4.895 | 0.027* |
| Dizziness | 6.077 | .014* | 11.690 | 0.001* |
| Drowsiness | 0.000 | .986 | 0.195 | 0.659 |
| Slurred speech | 0.610 | 1.000 | 1.217 | 0.526 |
| Ataxia | 3.424 | .229 | 1.717 | 0.371 |
| Trembling of hands | 0.025 | 1.000 | 1.205 | 0.353 |
| Irritability | 0.829 | .498 | 8.875 | 0.003* |
| Memory problem | 1.595 | .582 | 3.182 | 0.074 |
| **Respiratory system** | | | | |
| Wheezing | 0.333 | .536 | 5.962 | 0.015* |
| Dyaspnea | 0.157 | .734 | 4.272 | 0.039* |
| Bronchorrhea | 0.301 | 1.000 | 1.717 | 0.371 |
| Running nose | 0.301 | 1.000 | 0.599 | 1.000 |
| Shortness of breath | 0.583 | .445 | 5.923 | 0.015* |

| Health symptoms | AChE (%N) | | PChE (%N) | |
|---|---|---|---|---|
| | $\chi^2$ | P-value | $\chi^2$ | P-value |
| **Gastrointestinal system** | | | | |
| Anorexia | 1.944 | .221 | 0.041 | 1.000 |
| Abdominal cramps | 0.567 | .476 | 0.555 | 0.531 |
| Fecal incontinence | 3.424 | .229 | 1.717 | 0.371 |
| **Urinary system** | | | | |
| Loss of urinary control | 3.424 | .229 | 1.717 | 0.371 |
| **Glands** | | | | |
| Hyper salivation | 0.011 | 1.000 | 7.179 | 0.016* |
| Sweating | 0.198 | .748 | 12.732 | 0.000* |

**Significant at 0.05 probability level*

# CHAPTER V
# DISCUSSION

## 5.1 Questionnaires Information:

The majority of farmers in Sisa Krabue sub-district, Ongkharak district, Nakhon Nayok Province, Thailand were female like farmers in other areas (Sapbamrer et al., 2011). The average age of farmers in this study was 42.4 years old, ranging from 19-58 years old, which is similar to other studies conducted in Thailand. A study found that rice growing farmers in Thailand had an average age 44.0 years old and ranging from 23 and 63 years old (Kongtip et al., 2009). Moreover, another study in different country presents the mean age of Ethiopian farm workers was 36.4 years old (Mekonnen and Ejigu., 2005). The results showed that the average age of farmers were in the middle aged group. Farmers mostly finished primary school, the same information was reported from rice growing farmers in Rangsit area (Pan, 2009). Most famers gain approximately 30,000 – 60,000 THB (1 USD = 30 THB) per year depending on their cultivation area and whether they own the land or not. However, some famers were hired as pesticide sprayer and were hourly paid. Present study shows that 40% of farmers were smoker and 62.9 % were drinkers. The results were supported by other study showing that about 34 % of farm workers were smokers (Yassin et al., 2002) and 43.4% of them were drinker (Thiravirojana and Pusapukdeepob, 1999).

Most farmers in this study had been using pesticides for more than 10 years which is similar to a previous study (Catano, et al., 2008). Average year of pesticides exposure was 18.64 years. Other study found that the average working years with pesticide exposure was 19.2 years. The working hour in this study was higher than previous study but the mean of cultivated area was less than previous study (Pan, 2009). The number of years of using pesticide was higher because rice-growing is a major work in the area. Finishing primary school, they started working in rice growing industry right away. Most of the farmers who mixed, loaded, and sprayed pesticides by themselves which is similar to another study reported that 70% of the farm workers mixed pesticides on their own. From observations by researcher, most of farmers in this study area used pump spraying method because it is the most efficient way since up to 4-5 sprayers can spray pesticides at the same time. However, farmers who cultivated on their own land preferred to spray the pesticides by hand which in a common practice (Dosemeci et al., 2002). Most of the farmers applied pesticides to their fields twice a day in early morning and in the evening (65.7%) which means that they worked about 5 hours per day in the paddy fields. However, the non-farmer group did not work in the paddy fields thus they were not exposed to the pesticides in general. This information indicated that the farmers who work in paddy fields could be exposed to pesticides more than the non-farmer group.

The study showed that famers in the study area followed the pesticides instruction (82.9%) more than farmers in the Gaza Strip (56.1%) which was shown in another study. Moreover, the percentage of the farmers who mentioned of not drinking and eating during application of pesticides and took a shower after applying pesticides are higher than another farm worker group (Yassin et al., 2002; Kachaiyaphum et al., 2010). 94.3% of respondents washed their chemical stained clothes separately. Only 8.6% of respondents bury the used pesticide bottles while most of them did not (91.4%).

The results showed a similarity to other study in a way that the most frequently used personal protective equipment (PPE) by farmers while applying pesticides were long sleeved shirts and long legged, hat and facial shield, and gloves (Kachaiyaphum et al., 2010; Sapbamrer et al., 2011). Only few of the farmers used boots and goggles to protect themselves. No matter where and when pesticides are being used, there is a need to make sure that agriculturalists protect themselves well enough from contamination. However, weather condition such as heat and humidity may cause discomfort since most protective apparel has low heat dissipation. Moreover, farmers can not wear boots, which is an appropriate PPE, because it may damage the crops. Thus, the problem of wearing additional protective equipment in tropical countries is well recognized and has been commented upon over the years (FAO, 1990).

Another result which is similar to other’s study is the number of farmer in a family. The study showed that there were 2 members, including the respondent, in each farmer family who were farmers (Pan, 2009) while there were only one farmer in a non-farmer family. The study area of this research was in an agricultural area so that about half of the participant houses were near from field. Due to the house location, it could be said that participants were exposed to pesticides even if they were not directly contacted. This was because most participants drank from rain water and exposed the chemicals carried by the winds.

The present study found that common pesticides related symptoms that farmers suffered were headache and dizziness; this result was similar to other study (Kachaiyaphum et al., 2010). Moreover, the study is consistent with the previous study on pesticide usage in 136 farmers from Phayao, Thailand; it reported that the impacts on physical health were fatigue headache (40.4%), dizziness (36.8%),and numbness (29.4%), respectively (Sapbamrer et al., 2011). In a study of 190 rice farmers in the Mekong Delta, Vietnam, reported similar incidence rates for skin irritation (66%), headache (61%), dizziness (49%), eye andirritation (56%) and shortness of breath (44%) (Dasgupta et al., 2007). Moreover, 211 farm workers in Eastern Washington reported health symptoms such as headaches (50%), burning eyes (39%), pain in muscles, joints, or bones (35%), a rash or itchy skin (25%), and blurred vision (23%) (Strong, 2004).

From the statistically analysis presents the farmers were significantly associated with an increase eye symptoms. The results were similar with previous study which showed that the predominance of eye symptoms were found to be 40% among pesticide sprayers which was significantly higher ($p$<0.01) as compared to the control group. The symptoms were found to be blurred vision, lacrimation, pain in eyes, red swollen eyes, andirritation of eyes. The ratio showed a regular increase pattern related to the increase of the period of exposure time. In this study, chi-square value for linear trial was 34.5 and p-value was 0.001 which shows statistical significance trend that the increase of eye symptoms is related to increasing pesticide exposure. (Fareed, 2012). Eyes are exposed to external environment and thereby exposed to environmental contaminants. During agricultural operations, farm workers' eyes could be exposed to the pesticides while spraying if lacked proper preventive steps. As a result, these chemicals are being absorbed through the eye tissue and enter the blood circulation. Exposure of unprotected eyes to pesticides results in the absorption in ocular tissue and potential ocular toxicity (Jaga and Dharmai 2003). The positive associations of farmers are significantly related to central nervous system symptoms were found in the present study. It is reasonable to show a health report regarding the symptoms caused by organophosphates poison to insects and mammals mainly by phosphorylation of the acetylcholinesterase enzyme (AChE) at nerve endings. The consequence is a loss of existing AChE which makes organ becomes over motivated by the incremental acetylcholine (ACh, the impulse-conveying substance) at the nerve ending. The enzyme is vital to regular control of the transmission of impulse from nerve fibers to smooth and skeletal muscle cells, glandular cells, as well as autonomic ganglia and within the central nervous system (CNS) (US EPA, 1999). Moreover, the study found the farmers were significantly associated with an increase of wheezing. In addition to specific cases, an inhalation hazard, that is to say intake into the lungs through nose or mouth, is not often an obvious aspect of pesticide exposure. Exposure studies have indicated that during customary application of pesticides, the quantity of contamination from inhalation is a very small part from skin exposure. This is because the majority of pesticides are not enough volatile or the particle sizes produced during traditional application of sprays or dusts are excessively large for being inhaled into the lungs. When the method of application really generates a considerable number of particles with capacity of being inhaled into the lungs, for example mist blowing, or when the pesticide formulation is volatile, therefore respiratory protection would be necessary (FAO, 1990). Moreover, the farmers were significantly associated with an increase of sweating that is one of the classic chronic signs (US EPA, 1999).

### 5.2 Blood Cholinesterase Levels:

The study showed that an average AChE activity of the farmers was 2.63 ±0.55 U/ml and 2.80 ±0.53 U/ml in non-farmers. The AChE activity in this study was higher than the the AChE activity in previous study which showed AChE activity of farm workers was 1.36 ± 0.199 U/ml in male and 1.35 ± 0.19 U/ml in female. In control group, AChE were 1.35±0.15 in male and 1.33 ±0.16 in female (Sanidcheu and Ausanawarong, 2011). The possible reasons were 1) it is likely that different crops are associated with different AChE activity. 2) Other studies were study in high risk areas. The study found that the farmers are likely to have lower AChE activity than the non-farmers as stated in Simoniello's et al. (2010) study that compared AChE between in the directly and indirectly exposed groups. Famers have directly exposure to pesticides in many ways. This is because pesticide products can be splashed or spilled to exposed skin during the pouring and mixing process of making concentrated pesticide and during application when spray or dust can contaminate to exposed skin or clothing. Inhalation hazard since most pesticides are not sufficiently volatile, or the particle sizes generated during conventional application of sprays or dusts. The results showed a significant decrease ($p$ <0.01) with an 25% AChE activity inhibition in the directly exposed group and 15% AChE activity inhibition in the indirectly exposed group. The prevalence of abnormal AChE due to pesticides poisoning among the farmers in present study was 31.4%. The result from another study showed 24.1% of the farm workers had abnormal AChE activity (Magauzi, 2011). Also one study in Vietnam was conducted, by blood testing for AChE among 190 rice farmers. The results found that over 35% of test participants experienced acute pesticide poisoning caused by AChE activity reducing (Dasgupta et al., 2007).

The present study revealed that the prevalence of abnormal PChE levels of the farmers was 74.3% but all of non-farmers had normal PChE levels. This is the same with the study result in 1999 by Thiravirojana and Pusapukdeepob, a study in Chonburi province, Thailand, which found that 41.1% of farm workers had abnormal PChE levels. This study shows that PChE levels in non-farmer group was significantly (t-test, $p$ <0.001) different from farmer group. The results are similar to other studies. One of previous study mentioned that PChE activity of sprayers, mechanics and operators were lower than the controls, but only in the sprayers was significantly lower than the control group. Ntow et al. (2009) studied 63 farmers exposed group and 58 control subject. That study was found PChE was significantly lower in the exposed than the control group. However, the study of London et al. in 1998 concluded that mean plasma cholinesterase levels for sprayers and non-sprayers were not significantly different.

The farmers who use OP pesticides in the present study were neurotoxic in nature, thus the AChE activity was likely found to be significantly depleted in the pesticide exposed group than that of non-exposure group and the PChE activity were found to be significantly depleted in the pesticide exposed group than that of non-exposure group. It is widely accepted that AChE and PChE are biomarkers for OP pesticide exposure which can be understood that the obstruction activities of AChE and PChE are due to OP pesticide exposure among the farmers.

## 5.3 Association between ChE and Factors in participants:

The results showed that female had abnormal AChE levels more than male. This result was similar to the study of Sanidcheu and Ausanawarong (2011); which found that there was a different of AChE level between male and female participants, male had a higher AChE levels than female. The possible reasons were most of the farmers are female and the female workers have other activity in paddy fields more than male farmers as they are mostly pesticides employee so female could get more pesticides exposure. Drinkers and smokers are associated with PChE activity. Drinking may contaminated with pesticides that store in their home or drinking behavior beside the farm may the factor exposure pesticides by oral pathway. Smokers get more exposure of pesticides from inhalation when they are smoking. The present study indicated both AChE and PChE levels were not significant with age, education, income in both farmer and non-farmer group**.** In 2009, Ntow et al. found out the same results that PChE was no significant correlation with age, sex, body weight, and height.

The study found PChE levels related with years of pesticides exposure. The result consistent with the study in 2008, Catano et al. used multivariate analysis to confirm the positive correlation between PChE activity and years of pesticides exposure. This finding may reflect an adaptive response to long-term challenge from OPs; in other words, chronic exposure to these compounds might lead to a higher enzyme activity (PChE induction) that would reduce OP binding to biological targets (Kashyap et al. 1986). However, it was not found that both AChE and PChE levels associated with agricultural works, farming characteristics of pesticide use, and personal hygiene among farmers. The possible reason may cause the farmers were reading the label but they do not always read the label or just listen from the salesman then they assume that they have read the label. Moreover, they reported that they always wash their hand after using pesticides but maybe it does not correct way so pesticides still in their skin and could exposure to them. So the study was not found ChE levels associated with pesticide use and wrong way of personal hygiene among farmers. From researcher observation found that the farmer used improperly PPE

(Appendix C), improperly handing protective increasing the level of risk because the exposure level increases. Improper use of PPE will make it useless. Therefore, the AChE and PChE levels were not found association with PPE use information from questionnaire.

Type of using pesticides is related to ChE levels, supported by Magauzi et al. (2011), field workers who had the most abnormal cholinesterase activity were sprayers (50%), followed by those who worked in previously sprayed areas 49%, loaders (31%), mixers (29%), repairers (22%), waste disposers (9%) and lastly stores managers (7%). AChE activity in erythrocytes was associated with the duration of the workers exposure to pesticides (Singh et al., 2011). Moreover, mixing and high concentrated using of pesticides was related with the prevalence of self-reported toxicity symptoms (Yassin, 2002). Nevertheless, PPE usage was significantly associated with higher PChE levels and with a lower risk of pesticide-related symptoms, which mentioned the advantage from using appropriate protective measures (Catano et al., 2008).

FAO, 1990 formulated guidelines giving a short general description of the measures for personal protection of pesticide for operators against exposure in all forms. The principal emphasis has been laid on avoiding skin contamination because it is the most probable way of exposure and inhalation as well. The first basic rule is to always read and act according to the label recommendations on the pesticide container. If it is possible to avoid or minimize direct exposure of the skin, nose, mouth or eyes when handling pesticide products, hence this decreases considerably the likelihood of personal contamination. It is suggested to try as much as possible to avoid splashing or spilling onto skin or clothing when pouring and mixing the concentrated product. The probability of contamination can be considerably diminished by the use of appropriate equipment to measure out and transfer the product. Work clothing is required to be comfortable but provides adequate protection to perform the job safely as well. The minimum requirement for all categories of pesticide operations is lightweight clothing that covers most of the body. In practice, this comprises long-sleeved upper apparel, clothing covering the lower part of the body including the legs, footwear (boots or shoes). In case of spraying high crops, a hat is required. Work clothing, as depicted above, will help protect the operator when working with pesticides. Personal protective equipments are required in some occasions. The most usual requirement is gloves and eye protection at the times of pouring, mixing and loading pesticide formulations. In other conditions, other protective tools may be necessary for protection from inhalation of vapor, fine dust or spray, protection against particularly hazardous products, specialized application conditions or applications in tall dense crops. Protective tools for these objectives may encompass boots, face masks, aprons, protective apparel or hats. Another fundamental principle of personal protection is good hygiene. When working with pesticides, the operators are not recommended to eat, drink or smoke during performing job and refrain from touching their face or other parts of bare skin with soiled hands or gloves. They are suggested to always wash their hands and face after dealing with pesticides and prior to eating, drinking, smoking or going to the toilet. After finishing work for the day, the operators are advised to wash themselves completely. Moreover, their working clothes should be washed after job separately from other apparel and dried after that.

The results from present study are similar with those of Midtling et al. (1985) who found that many patients continued to report health effect after erythrocyte cholinesterase had been recovered from a previous depression. The study found that PChE level was not associated with skin symptoms which the cause of health effects to the skin can be reduce by many way. When the farmers were pouring and mixing the concentrated product, they avoided splashing or spilling onto skin or clothing. Or they used suitable equipment for measuring out and transferring the product (FAO, 1990). The PChE level was found significant association with eye symptoms, central nervous system symptoms, and respiratory system symptoms that support by US EPA, 1990, the enzyme is regular control of transmitting nerve impulses from nerve fibers to smooth and skeletal muscle cells, glandular cells, as well as autonomic ganglia within the central nervous system (CNS). It is also believed to be a good indicator of real neuronal activity. The rate of turnover for red blood cells is not quick (approximately 3 months). Therefore, AChE is used as an indicator of chronic exposure. For PChE, turnover is much quicker. PChE is a more effective short-term indicator because of its faster response to exposure. It is used as an indicator of recent, acute exposure (Brown et al., 2006). However some study indicated that pesticide-related symptom was not significantly associated with PChE levels (Catano et al., 2008; Richter et al.,1992; Ngowi et al., 2001; Jors et al., 2006).

## CHAPTER VI
## CONCLUSIONS

### 6.1 Conclusion:

To evaluate organophosphate pesticides exposure by using cholinesterase activity, Test mate ChE was used in this study. This study found the farmers who were directly exposed OP pesticides had more likely lower AchE levels than non-farmers. All of non-farmers participants had normal PChE levels. Farmers had abnormal PChE levels significantly more than non-farmers. The results showed that farmers had health effects from pesticides exposure more than non-farmers. The association between AChE levels and PChE levels were likely low negative correlation (Pearson Correlation coefficient -0.121 at $p$=0.488 in farmer group).

Most of them had experienced years of using pesticides, more than 10 years to be exact, and worked in the field about 5 hours a day. Average area was approximately 30.74 rais. Most of the sample in the study population were mixing, loading, and spraying by themselves in the morning and evening. They usually wore PPE such as long sleeved shirts and long legged plants. They mostly had good practicing of pesticide use and personal hygiene. The farmers were significantly associated with increase eye symptoms, central nervous system (CNS) symptoms, respiratory system symptoms, and glands ($p$<0.05).

The study found that male participants had normal AChE levels more than female. Moreover, alcohol drinker, smoker, and years of using pesticides were significantly associated with PChE levels. For the practicing of pesticides use, the study found that the association between years of using pesticides was significantly associated with PChE levels ($p$ = 0.01). However, ChE activity was not associated with other agricultural works (hours of working per day, type of pesticides application, spraying method, and spraying time) and farming characteristics, hours of working per day, size of cultivating areas, and spraying method**.** Practicing of pesticide use and personal protective factors were not associated with ChE activity. Use of PPE in wrong practice and improper use were not associated with ChE levels.

To identify health effects which were related to the blood cholinesterase level among farmers and non-farmers, it was showed that the AChE level was significantly associated with central nervous system symptoms. The PChE level was significantly associated with eye symptoms, CNS symptoms, respiratory system symptoms, and glands symptoms. Thus, AChE is used as an indicator of chronic exposure and PChE is used as an indicator of acute exposure.

The farmers in the study area are at risk more than non-farmers. One way to reduce health effects on them is the appropriate prevention to reduce pesticides exposure. Thus there should be an intervention to reduce the risk by provide knowledge for the farmers to increase correct pesticides use, proper PPE use during working with pesticides.

**6.2 Benefit from the study:**

1. To give elaborate details of the different cholinesterase levels of farmers and non-farmers.

2. To access the general information and study variables that related to cholinesterase levels.

3. To understand the risk of OP pesticides exposure among farmers and non-farmers.

4. To increase awareness of pesticides use in the study area.

5. Researcher can help suggest appropriate ways to farmers and non-farmers participants to protect themselves from pesticides exposure such as reducing concentration of pesticides usage and wearing personal protective equipment.

### 6.3 Limitation of the study:

1. Subjective symptoms may be caused by other pesticides.

2. Evidence of pesticide-related symptoms was relied on self-report without physical examinations or clinical interview.

3. Budget and time limitation so the sample size was small.

4. The cross-sectional study design was limited to determining the causal associations of significant predictors and blood cholinesterase levels.

5. The standard normal ChE level from American people was use in this study. It would be better if ChE level with the standard normal from Thai people was used.

6. This study was focused only on OP pesticides. Other carbamates and some herbicide maybe used in this area but not included in the study. Therefore, the report of symptoms might be influenced by other pesticides.

### 6.4 Recommendation for future Studies:

1. This study was selected an area in Sisa Krabue subdistrict, Ongkharak district, Nakhon Nayok province as a place to study. Some farmers in the area are already at health risk, thus there should be an intervention to reduce the risk and provide knowledge to the farmers.

2. Proper use and appropriate use of PPE should be recommended to the farmers.

3. Pesticides exposure could also be investigated by contamination via Multiple-pathways and urinary metabolite level.

4. This study was only interested in farmers who used OP pesticides. Further study should focus on other pesticides such as carbamates which also affect blood ChE activity.

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# APPENDICES

# Appendix A

## Questionnaire (English version)

**Code.**...............

**Questionnaire of the Research**

**"ASSESSMENT OF FARMER AND NON-FARMER HEALTH EFFECTS RELATED TO ORGANOPHOSPHATE PESTICIDES EXPOSURE USING BLOOD CHOLINESTERASE ACTIVITY AS A BIOMARKER IN AGRICULTURAL AREA AT ONGKHARAK DISTRICT, NAKHON NAYOK. PROVINCE, THAILAND."**

**Explanation**

Questionnaire is separates onto 4 parts. Part 1,3 and 4 for both farmer and non-farmer group. Part 2 for farmer group only.

**Part 1** General Information

**Part 2** Pesticides Use

**Part 3** Related exposure factors

**Part 4** Health effects of organophosphate pesticides exposure

Interviewer's name...............................................................

**"ASSESSMENT OF FARMER AND NON-FARMER HEALTH EFFECTS RELATED TO ORGANOPHOSPHATE PESTICIDES EXPOSURE USING BLOOD CHOLINESTERASE ACTIVITY AS A BIOMARKER IN AGRICULTURAL AREA AT ONGKHARAK DISTRICT NAKHON NAYOK PROVINCE THAILAND."**

**Explanation** Please write down in the provides blank space or tick✓ in the parentheses**.**

**Part 1** General Information

1. Age...........................Years
2. Gender ( ) Male ( ) Female
3. Education Level

   ( ) Uneducated ( ) Primary School

   ( ) Lower Secondary School ( ) Upper Secondary School

   ( ) Vocation ( ) HighVocation

   ( ) Bachelor or equal ( ) Others.........................
4. Average total household incomes/Year.......................................Bath
5. Smoking ( ) Yes ( ) No
6. Drinking alcohol ( ) Yes ( ) No

**Part 2** Pesticides Use

1. How long have you been a farmer?...............Years………..Months
2. How many the hours you working per day?.............Hours/Day
3. How many rais you have to growing rice?................Rais
4. Progress of pesticides use

   ( ) Mixing ( ) Loading ( ) Spraying

5. How to spraying pesticides?

( ) Not spraying pesticides
( ) Spraying by tractor
( ) Spraying by hand
( ) Spraying by a backpack spray
( ) Spraying by a smoker spray
Or spraying by others ______________________________

When you spraying ( ) sprayed in the groove ( ) line sprayed

6. What time you spraying pesticides?

( ) Early morning………………………
( ) Mid-day……………………………..
( ) Evening…………………………….
( ) Convenient time……………………

| **Practice and self-protection** | **Always** | **Sometimes** | **Never** |
|---|---|---|---|
| 7. Reading the label of pesticides products. | | | |
| 8. Using the recommended amounts of pesticides. | | | |
| 9. Wearing gloves when spraying | | | |
| 10. Wearing long-sleeved shirt and trousers | | | |
| 11. Wearing hat | | | |
| 12. Wearing goggles | | | |
| 13. Wearing special boots | | | |
| 14. Eating and drinking far from pesticides exposure area. | | | |
| 15. Washing hands before eating when used pesticides. | | | |
| 16. Immediately take shower after using pesticides. | | | |
| 17. Washing clothes by separated working clothes and normally clothes. | | | |
| 18. Dig a hole to bury a bottle of pesticide used. | | | |

**Part 3** Related exposure factors

1. How many farmers in your family?.....................Person

2. How far between your house and paddy area? ...............................Metre

3. Source of drinking water

( ) Rain water ( ) Tap water

( ) Plastic bottled water ( ) Others…………………..

4. Washing fruits and vegetables before eating?

( ) Always ( ) Sometimes ( ) Never

**Part 4** Health effects of organophosphate pesticides exposure during the last 3 months.

| **Health effects** | **Yes** | **No** |
|---|---|---|
| **Skin symptoms** | | |
| − Skin rash/ itching/ burning | | |
| − Numbness of hands | | |
| − Muscular twitching and cramps | | |
| **Eye symptoms** | | |
| − Blurred vision | | |
| − Lacrimation | | |
| − Irritation | | |
| **Central nervous system** | | |
| − Headache | | |
| − Dizziness | | |
| − Drowsiness | | |
| − Slurred speech | | |
| − Ataxia | | |
| − Trembling of hands | | |
| − Irritability | | |
| − Memory problem | | |
| **Respiratory system** | | |
| − Wheezing | | |
| − Dyaspnea | | |
| − Bronchorrhea | | |
| − Running nose | | |
| − Shortness of breath | | |
| **Gastrointestinal system** | | |
| − Anorexia | | |

| **Health effects** | **Yes** | **No** |
|---|---|---|
| − Dizziness/ Vomiting | | |
| − Abdominal cramps | | |
| − Fecal incontinence | | |
| **Urinary system** | | |
| − Loss of urinary control | | |
| **Glands** | | |
| − Hyper salivation | | |
| − Sweating | | |

**Appendix B**

**Questionnaire (Thai version)**

แบบสัมภาษณ์ชุดที่..............

**แบบสัมภาษณ์ของโครงการวิจัย**

**เรื่อง "การประเมินผลกระทบสุขภาพของเกษตรกรและผู้ที่ไม่ใช่เกษตรกรจากการได้รับสารกำจัดศัตรูพืชกลุ่มออร์กาโนฟอสเฟตโดยใช้ตัวชี้วัดปฏิกิริยาโคลีนเอสเตอเรสในเลือด ในพื้นที่เกษตรกรรม อำเภอองครักษ์ จังหวัดนครนายก ประเทศไทย"**

**คำชี้แจง**

แบบสัมภาษณ์ประกอบด้วย 4 ส่วน ในส่วนที่ 1 3 และ 4 เป็นคำถามสำหรับทั้งกลุ่มเกษตรกรและกลุ่มผู้ที่ไม่ใช่เกษตรกร ในส่วนที่ 2 เฉพาะกลุ่มเกษตรกรเท่านั้น

**ส่วนที่ 1** ลักษณะของข้อมูลทั่วไป

**ส่วนที่ 2** ลักษณะการใช้สารกำจัดศัตรูพืช

**ส่วนที่ 3** ปัจจัยที่เกี่ยวข้องกับการรับสัมผัส

**ส่วนที่ 4** ผลกระทบทางสุขภาพของการสัมผัสสารกำจัดศัตรูพืชกลุ่มออร์กาโนฟอสเฟต

ผู้สัมภาษณ์.........................................................................

**แบบสัมภาษณ์ของโครงการวิจัย**

**เรื่อง “การประเมินผลกระทบสุขภาพของเกษตรกรและผู้ที่ไม่ใช่เกษตรกรจากการได้รับสารกำจัดศัตรูพืชกลุ่มออร์กาโนฟอสเฟตโดยใช้ตัวชี้วัดปฏิกิริยาโคลีนเอสเตอเรสในเลือด ในพื้นที่เกษตรกรรม อำเภอองครักษ์ จังหวัดนครนายก ประเทศไทย”**

**คำชี้แจง** จงเติมคำลงในช่องว่างหรือทำเครื่องหมาย ✓ ในวงเล็บ

**ส่วนที่ 1** ลักษณะของข้อมูลทั่วไป

1. อายุ...........................ปี
2. เพศ ( ) ชาย ( ) หญิง
3. ระดับการศึกษา

| | |
|---|---|
| ( ) ไม่ได้เรียนหนังสือ | ( ) ประถมศึกษา |
| ( ) มัธยมต้น | ( ) มัธยมปลาย |
| ( ) ปวช. | ( ) ปวส. |
| ( ) ปริญญาตรีหรือเทียบเท่า | ( ) อื่นๆ............................ |

4. รายได้เฉลี่ยในครอบครัวต่อปี....................................................บาท
5. ปัจจุบันคุณสูบบุหรี่หรือไม่ ( ) สูบ ( ) ไม่สูบ
6. ปัจจุบันคุณดื่มเครื่องดื่มมีแอลกอฮอล์หรือไม่ ( ) ดื่ม ( ) ไม่ดื่ม

**ส่วนที่ 2** ลักษณะการใช้สารกำจัดศัตรูพืช

1. คุณประกอบอาชีพเกษตรกรชาวนาระยะเวลา ................... ปี ..................... เดือน
2. ทำงานกี่ชั่วโมงต่อวัน ................................ชั่วโมงต่อวัน

3. ปัจจุบันคุณมีพื้นที่ในการปลูกข้าว ......................... ไร่

4. ขั้นตอนการใช้สารกำจัดศัตรูพืชของคุณ (เลือกได้มากกว่า 1 ข้อ)

( ) ผสมสาร ( ) เทสารใส่เครื่องฉีด

( ) ฉีดพ่นสาร ( ) อื่นๆ.......................

5. คุณใช้วิธีใดในการฉีดพ่น

( ) ไม่ฉีดพ่นสารกำจัดศัตรูพืช

( ) ฉีดพ่นด้วยรถแทรกเตอร์

( ) ฉีดพ่นด้วยเครื่องฉีดพ่นแบบมือ

( ) ฉีดพ่นด้วยเครื่องฉีดพ่นแบบสะพายหลัง

( ) ฉีดพ่นด้วยเครื่องฉีดพ่นแบบควัน

หรือ ฉีดพ่นด้วยเครื่องมือชนิดอื่น ______________________________

เมื่อท่านฉีดพ่น ( ) ท่านฉีดพ่นลงในร่องนา ( ) ฉีดพ่นเป็นแนว

6. เวลาใดที่ท่านทำการฉีดพ่นสารกำจัดศัตรูพืช

( ) เช้าตรู่...............................น.

( ) เที่ยงวัน............................น.

( ) ตอนเย็น...........................น.

( ) ไม่แน่นอนตามความสะดวก คือ ___________________________________

| **การปฏิบัติตนและการป้องกันตนเอง** | **ทุกครั้ง** | **บางครั้ง** | **ไม่เคย** |
|---|---|---|---|
| 7. อ่านฉลากผลิตภัณฑ์ก่อนใช้ | | | |
| 8. ใช้สารกำจัดศัตรูพืชตามที่ฉลากกำหนด | | | |
| 9. สวมถุงมือขณะที่ผสมสารกำจัดศัตรูพืช | | | |
| 10. ใส่เสื้อแขนยาวและกางเกงขายาวขณะใช้สารกำจัดศัตรูพืช | | | |

| การปฏิบัติตนและการป้องกันตนเอง | ทุกครั้ง | บางครั้ง | ไม่เคย |
|---|---|---|---|
| 11. สวมหมวกขณะฉีดพ่นหรือสัมผัสสารกำจัดศัตรูพืช | | | |
| 12. ใส่แว่นขณะฉีดพ่นหรือสัมผัสสารกำจัดศัตรูพืช | | | |
| 13. สวมรองเท้าบูธขณะใช้สารกำจัดศัตรูพืช | | | |
| 14. รับประทานอาหารและดื่มน้ำห่างไกลจากแหล่งที่มีการใช้สารกำจัดศัตรูพืช | | | |
| 15. ล้างมือก่อนรับประทานอาหารหลังจากใช้สารกำจัดศัตรูพืช | | | |
| 16. อาบน้ำทันทีด้วยสบู่หรือครีมอาบน้ำหลังการใช้สารกำจัดศัตรูพืช | | | |
| 17. แยกเสื้อผ้าที่สวมตอนใช้สารกำจัดศัตรูพืชออกจากเสื้อผ้าปกติในการซักผ้า | | | |
| 18. ขุดหลุมเพื่อฝังขวดสารกำจัดศัตรูพืชที่ใช้แล้ว | | | |

**ส่วนที่ 3** ปัจจัยที่เกี่ยวข้องกับการรับสัมผัส

1. จำนวนของสมาชิกในครอบครัวที่ประกอบอาชีพเกษตรกรรมหรือมีการใช้สารกำจัดศัตรูพืช......................คน (รวมคุณด้วย)

2. ระยะห่างจากบ้านกับนาข้าว..............................เมตร

3. แหล่งที่มาของน้ำดื่ม

( ) น้ำฝน ( ) น้ำประปา

( ) น้ำขวดพลาสติก ( ) อื่นๆ...............................

4. ล้างผักและผลไม้ก่อนรับประทาน

( ) ทุกครั้ง ( ) บางครั้ง ( ) ไม่เคย

**ส่วนที่ 4** ผลกระทบทางสุขภาพของการสัมผัสสารกำจัดศัตรูพืชกลุ่มออร์กาโนฟอสเฟต ภายในช่วง 3 เดือนที่ผ่านมา

| **ผลกระทบทางสุขภาพ** | **ใช่** | **ไม่ใช่** |
|---|---|---|
| **อาการทางผิวหนัง** | | |
| – คัน/ผื่นขึ้น/มีรอยไหม้ | | |
| – มือชา | | |
| – กล้ามเนื้อกระตุกหรือเป็นตะคริว | | |
| **อาการทางตา** | | |
| – มองภาพไม่ชัดเจน | | |
| – น้ำตาไหล | | |
| – ระคายเคืองตา | | |
| **ระบบประสาท** | | |
| – ปวดหัว | | |
| – เวียนศีรษะ | | |
| – นอนหลับไม่สนิท | | |
| – พูดไม่ชัด | | |
| – เดินโซเซ | | |
| – มือสั่น | | |
| – หงุดหงิดง่าย | | |
| – มีปัญหาเกี่ยวกับความจำ | | |
| **ระบบทางเดินหายใจ** | | |
| – หายใจมีเสียงวี๊ด | | |
| – หายใจลำบาก | | |
| – มีเสมหะปริมาณมาก | | |
| – น้ำมูกไหล | | |
| – หายใจเป็นจังหวะสั้นๆ | | |
| **ระบบทางเดินอาหาร** | | |

| ผลกระทบทางสุขภาพ | ใช่ | ไม่ใช่ |
|---|---|---|
| – เบื่ออาหาร | | |
| – คลื่นไส้/อาเจียน | | |
| – ปวดเกร็งท้อง | | |
| – กลั้นอุจจาระไม่อยู่ | | |
| **ระบบทางเดินปัสสาวะ** | | |
| – กลั้นปัสสาวะไม่อยู่ | | |
| **อวัยวะคัดหลั่ง** | | |
| – มีน้ำลายมากกว่าปกติ | | |
| – เหงื่อออกมากกว่าปกติ | | |

# Appendix C

**Figure C1** Pesticide application and exposure of farmer in Ongkharak district

**Figure C2** Participants and blood collection for AChE and PChE

**Appendix D**

**Assay Procedure of Test-mate ChE (Model 400)**

1. Turn on and select mode AChE or PChE
2. Press “Test”. “Insert a new tube” will appear on the screen. Hold onto the white screw lid. Put gradually in the box “analyzer”. Do not hold the bottom half of glass bottle due to the interference of light.
3. Press “Test”. “Blank” will appear on the screen. Take 10 seconds for reading. Take the bottle out when “To remove tube” appears.
4. Rotate the screw lid out. Place the bottle in tube rack. Place the lid aside.
5. Press “Test”. “Add blood” will show on the screen.
6. Wipe blood by rolling the tip of tube on the filter paper. Then put into the assay tube. Rotate to close the lid tightly. Hold the top and bottom of the tube, shake to allow blood to be dispersed in medicinal liquid for 15 seconds.
7. Lean the bottle, set the capillary tube to the side of the bottle. Gradually set the bottle up and put it in the box analyzer. The side with capillary tube corresponds with black spot.
8. Press “Test” to read for 10 seconds. Then, remove the tube. Open the lid and place it on tube rack. Press “Test”. The screen will show “add reagent”
9. Use pliers to cut and pull the lid reagent plate off. Drop three droplets of distilled water. Use pipette to stir until melted (powder may change from white to yellow with age of the chemical. But this does not affect the analysis)
10. Lean tray and use pipette to suck all reagents out. Put into assay tube. Press “Test” immediately. “Shake assay tube” will appear on the screen.

11. Close the lid tightly and shake gently for 5 seconds to mix substances together to be homogeneous. Lean tube in such a way that the capillary tube is at either side. Put into the box analyzer. Put a smaller tube corresponding with black spot.

12. Press "Test". The screen will show "incubation", taking around 1 minute (but not more than 80 seconds). Then the screen will show "reading" about 50 seconds.

13. When "Remove" appears on the screen, bring the bottle away and discard it.

14. Press "Test" to display the results of the test. Record each value in the notebook. Press "Test" to see the display of next results until getting all the desired values.

    AChE mode will display 6 values

    PChE mode will display 4 values

15. Press "Done" to prepare for the next analysis of samples.

# Appendix E

**Table 1-E** The descriptive statistic of farmers data

| | N | Minimum | Maximum | Mean | Std. Deviation |
|---|---|---|---|---|---|
| Temp (ıC) | 35 | 26.3 | 28.8 | 27.449 | .8521 |
| AChE (U/ml) | 35 | 1.60 | 3.95 | 2.6277 | .54743 |
| AChE (%N) | 35 | 34 | 84 | 55.74 | 11.688 |
| A_Hgb (g/dL) | 35 | 7.3 | 15.9 | 10.300 | 1.4287 |
| A_Hgb (%N) | 35 | 48 | 106 | 68.63 | 9.619 |
| Q (U/g) | 35 | 18.6 | 38.4 | 25.637 | 4.7890 |
| Q (%N) | 35 | 59 | 122 | 81.34 | 14.848 |
| PChE (U/ml) | 35 | .08 | 1.75 | 1.0086 | .43908 |
| PChE (%N) | 35 | 3 | 68 | 39.54 | 17.183 |
| P_Hgb (g/dL) | 35 | 6.7 | 12.5 | 10.211 | 1.1282 |
| P_Hgb (%N) | 35 | 45 | 95 | 68.37 | 8.468 |
| Valid N (listwise) | 35 | | | | |

**Table 2-E** The descriptive statistic of non-farmers data

| | N | Minimum | Maximum | Mean | Std. Deviation |
|---|---|---|---|---|---|
| Temp (บC) | 35 | 24.6 | 28.9 | 27.309 | 1.1536 |
| AChE (U/ml) | 35 | 1.39 | 4.11 | 2.7951 | .52858 |
| AChE (%N) | 35 | 30 | 87 | 59.40 | 11.091 |
| A_Hgb (g/dL) | 35 | 8.1 | 13.4 | 10.389 | 1.2117 |
| A_Hgb (%N) | 35 | 56 | 89 | 69.20 | 8.051 |
| Q (U/g) | 35 | 16.5 | 35.7 | 26.966 | 3.9215 |
| Q (%N) | 35 | 35 | 114 | 84.09 | 15.203 |
| PChE (U/ml) | 35 | 1.36 | 2.58 | 1.8054 | .29697 |
| PChE (%N) | 35 | 53 | 101 | 70.77 | 11.667 |
| P_Hgb (g/dL) | 35 | 8.0 | 13.7 | 10.257 | 1.1268 |
| P_Hgb (%N) | 35 | 53 | 91 | 68.17 | 7.306 |
| Valid N (listwise) | 35 | | | | |

# Appendix F

## Cholinesterase-inhibiting organophosphate pesticides

Cholinesterase-inhibiting pesticides are listed by common name, with trade names in parentheses. Check the active ingredient statement on the label of the pesticide to see if it contains one of the common names listed since not all trade names may be included. Newly registered active ingredients or those not commonly used may not be listed here.

acephate
azinphos-methyl
bensulide
carbophenothion
chlorethoxyfos
chlorfenvinphos
chlorpyrifos
chlorpyrifos-methyl
coumaphos
demeton diazinon
dichlorvos
dicrotophos
dimethoate
dioxathion disulfoton
EPN
ethion ethoprop
ethyl parathion
famphur
fenamiphos
fenitrothion
fensulfothion
fenthion
fonofos
isofenphos
malathion
methamidophos
methidathion
methyl parathion
mevinphos
monocrotophos
naled
omethoate
oxydemeton-methyl
parathion
phorate
phosmet
phosphamidon
phostebupirim
pirimiphos-ethyl
pirimiphos-methyl
profenofos
propetamphos)
sulfotepp
sulprofos
tebupirimiphos
temephos
terbufos
tetrachlorvinphos
tribufos
trichlorfon

Source: Brown (2006)

# Appendix G

## Administration and Time Schedule

| **Plan/Date** | Sep 2012 | Oct 2012 | Nov 2012 | Dec 2012 | Jan 2013 | Feb 2013 | March 2013 | April 2013 | May 2013 |
|---|---|---|---|---|---|---|---|---|---|
| Literature Review | → | → | → | → | → | → | | | |
| Writing Proposal | | → | → | | | | | | |
| Proposal Exam | | | | → | | | | | |
| Revise Proposal | | | | → | → | | | | |
| Sending Ethics Committee's Approval | | | | | → | | | | |
| Preparation for Data Collection | | | | → | → | | | | |
| Data Collection | | | | | | | → | | |
| Data Analysis | | | | | | | → | | |
| Report Writing | | | | | | | → | → | |
| Submit for Final Exam | | | | | | | | → | |
| Thesis Defense | | | | | | | | | → |
| Revise Thesis | | | | | | | | | → |
| Submit Final Thesis | | | | | | | | | → |

# VITAE


| | | |
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