BLOOD CHOLINESTERASE LEVEL AS BIOMARKER OF ORGANOPHOSPHATE AND CARBAMATE PESTICIDE EXPOSURE EFFECT AMONG RICE FARMERS IN TARNLALORD SUB-DISTRICT PHIMAI DISTRICT NAKHON RATCHASIMA PROVINCE THAILAND

Mr. Ekarat Sombatsawat

A Thesis Submitted in Partial Fulfillment of the Requirements
for the Degree of Master of Public Health Program in Public Health
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ระดับโคลีนเอสเตอเรสในเลือดตัวบ่งชี้ผลจากการรับสัมผัสสารกำจัดศัตรูพืชกลุ่มออร์กาโนฟอสเฟตและคาร์บาเมตในชาวนาตำบลธารละหลอด อำเภอพิมาย จังหวัดนครราชสีมา ประเทศไทย

นายเอกราช สมบัติสวัสดิ์

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาสาธารณสุขศาสตรมหาบัณฑิต
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Thesis Title	BLOOD CHOLINESTERASE LEVEL AS BIOMARKER OF ORGANOPHOSPHATE AND CARBAMATE PESTICIDE EXPOSURE EFFECT AMONG RICE FARMERS IN TARNLALORD SUB-DISTRICT PHIMAI DISTRICT NAKHON RATCHASIMA PROVINCE THAILAND
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เอกราช สมบัติสวัสดิ์ : ระดับโคลีนเอสเตอเรสในเลือดตัวบ่งชี้ผลจากการรับสัมผัสสารกำจัดศัตรูพืชกลุ่มออร์กาโนฟอสเฟตและคาร์บาเมตในชาวนาตำบลธารละหลอด อำเภอพิมาย จังหวัดนครราชสีมา ประเทศไทย. (BLOOD CHOLINESTERASE LEVEL AS BIOMARKER OF ORGANOPHOSPHATE AND CARBAMATE PESTICIDE EXPOSURE EFFECT AMONG RICE FARMERS IN TARNLALORD SUB-DISTRICT PHIMAI DISTRICT NAKHON RATCHASIMA PROVINCE THAILAND) อ.ที่ปรึกษาวิทยานิพนธ์หลัก: ผศ. ดร. วัฒน์สิทธิ์ ศิริวงศ์, 91 หน้า.

การศึกษาครั้งนี้ มีวัตถุประสงค์เพื่อหาระดับโคลีนเอสเตอเรสในเลือดจำนวน 3 ระยะของการปลูกข้าวฤดูแล้ง (นาปรัง) และประเมินผลกระทบทางสุขภาพจากการรับสัมผัสสารกำจัดศัตรูพืชกลุ่มออร์กาโนฟอสเฟตและคาร์บาเมตในชาวนา ซึ่งเป็นการศึกษาแบบภาคตัดขวางโดยใช้แบบสอบถามในการสัมภาษณ์แบบตัวต่อตัวและทดสอบระดับเอมไซม์โคลีนเอสเทอเรสในเม็ดเลือดแดง (AChE) และ ในพลาสม่า (PChE) โดยใช้เครื่องมือการทดสอบระดับโคลีนเอสเตอเรส Test-mate ChE (Model 400) โดยผู้เข้าร่วมการศึกษาครั้งนี้ คือ ชาวนาเพศชาย จำนวน 33 คน ที่ใช้สารกำจัดศัตรูพืชกลุ่มออร์แกนโนฟอสเฟตและคาบาร์เมตในการทำนา และวิเคราะห์ผลทางสถิติโดยใช้ไคสแควร์ โดยผู้เข้าเข้าร่วมการศึกษามีอายุเฉลี่ย (±ส่วนเบี่ยงเบนมาตรฐาน) 46 (± 9.38) ปี ผลการตรวจวัดระดับโคลีนเอสเตอเรสของชาวนา พบว่า ครั้งแรกภายใน 24 ชั่วโมงหลังจากใช้สารกำจัดศัตรูพืช ชาวนาร้อยละ 72.70 มีผลเลือดผิดปกติ ครั้งที่สอง 15 วันหลังจากใช้สารกำจัดศัตรูพืชครั้งแรก ชาวนาร้อยละ 48.50 มีผลเลือดผิดปกติ และครั้งที่สาม 30 วันหลังจากใช้สารเคมีครั้งแรก ชาวนาร้อยละ 42.40 มีผลเลือดผิดปกติ และยังพบว่าทั้ง AChE และ PChE จากผลการตรวจเลือดภายใน 24 ชั่วโมงและ 15 วันหลังจากการใช้สารกำจัดศัตรูพืช มีความสัมพันธ์อย่างมีนัยสำคัญทางสถิติ และ 30 วันหลังจากการใช้สารกำจัดศัตรูพืชกับการใช้สารกำจัดศัตรูพืชภายใน 24 ชั่วโมง และ 15 วัน หลังจากใช้สารกำจัดศัตรูพืช มีความสัมพันธ์อย่างมีนัยสำคัญทางสถิติ (ANOVA, $p<0.05$) และ ชาวนาเกิดผลกระทบต่อสุขภาพในระบบทางเดินอาหาร ระบบทางเดินปัสสาวะ การมองเห็น ผิวหนัง และระบบประสาทส่วนกลาง นอกจากนี้พบว่าระดับ AChE และ PChE ภายใน24 ชั่วโมงวันหลังจากการใช้สารเคมีความสัมพันธ์กับอาการมองภาพไม่ชัดอย่างมีนัยสำคัญทางสถิติ (Chi-square, $p<0.05$) ผลการวิจัยสรุปว่า หลังจากที่เกษตรกรฉีดพ่นสารกำจัดศัตรูพืชระดับ AChE และ PChE ในเลือดของเกษตรกรมีระดับที่ต่ำกว่าระดับปกติ อย่างไรก็ตามสามารถคืนกลับสู่ระดับปกติเมื่อเวลาผ่านไป ดังนั้นควรจะแนะนำชาวนาเกี่ยวกับการใช้อุปกรณ์ป้องกันส่วนบุคคลที่ถูกต้องและการปฏิบัติตัวเหมาะสมในการใช้สัมผัสสารกำจัดศัตรูพืช เพื่อลดผลกระทบต่อสุขภาพที่เกิดจากการสัมผัสต่อไป

สาขาวิชา สาธารณสุขศาสตร์	ลายมือชื่อนิสิต ....
ปีการศึกษา 2556	ลายมือชื่อ อ.ที่ปรึกษาวิทยานิพนธ์หลัก ....

5678816753 : MAJOR PUBLIC HEALTH
KEYWORDS: CHOLINESTERASE ORGANOPHOSPHATE (OPS) AND CARBAMATE MALE GENERAL HEALTH EFFECTS

EKARAT SOMBATSAWAT: BLOOD CHOLINESTERASE LEVEL AS BIOMARKER OF ORGANOPHOSPHATE AND CARBAMATE PESTICIDE EXPOSURE EFFECT AMONG RICE FARMERS IN TARNLALORD SUB-DISTRICT PHIMAI DISTRICT NAKHON RATCHASIMA PROVINCE THAILAND. ADVISOR: ASST. PROF. WATTASIT SIRIWONG, Ph.D., 91 pp.

This study aims to measure blood cholinesterase levels of acetyl cholinesterase (AChE) and plasma cholinesterase (PChE) during 3 times in dry-season crops and to assess health effects of organophosphate and carbamate pesticides exposure among rice farmers in Tarnlalord Sub-District, Phimai District, Nakhon Ratchasima Province, Thailand. The study design was a cross-sectional study using face to face questionnaire interview and blood cholinesterase level tested by Test-mate ChE (Model 400). The participants were 33 male farmers and average age (± SD) was 46 (± 9.38) years old. The results showed that the ChE levels in rice farmers in 3 times including (1) the first blood collection, 24 hours after application, 72.70% of the farmers were abnormal (2) the second blood collection, 15 day after the first collection, found 48.50% of the farmers were abnormal, and (3) the third blood collection, 30 day after the first collection, found 42.40% of them were abnormal. The activity of AChE and PChE level was assessed at 3 time differences showed a statistically significant association between within 24 hours after first application and 15 days after first application and 30 days after first application and previous (24 hours and 15 days after first application) was significant associated (ANOVA, $p<0.05$). The farmers reported their adverse health effects related to gastrointestinal system, urinary system, eye, skin, and central nervous system. Additionally, the AChE level within 24 hours after first application was significant association in eye symptoms (Chi-square, $p<0.05$). In conclusion, this study showed after the rice farmers applied pesticides at the beginning, both AChE and PChE level were abnormal and self-recovering to normal level by time. The appropriated self-practices and prevention from pesticides exposure should be recommended to rice farmer regarding proper use of personal protective equipment (PPE) and pesticides handling to reduce adverse health effects from pesticides exposure.

Field of Study: Public Health	Student's Signature ....
Academic Year: 2013	Advisor's Signature ....

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CHAPTER I

INTRODUCTION

1.1 Background and Rationale

Thailand covers about 513,115 km^2 and has a population of around 64 million citizens of these people (National statistical office Thailand, 2013), 64.1% of population live in rural areas. With the exception of Bangkok's highly concentrated industrial sector, Thailand's major source of income and occupation is agriculture. Agricultural contribution to the total GDP was approximately 11% in 2001. In 2012, the morbidity rate from toxic substance was 2.35 per 100,000 populations or 1,509 people (Siriphanich & Meesree, 2012). In recent years, concern has been growing that improper agrochemical use can create hazards for humans and the environment. Along with the green revolution policy of the Thai government, the use of pesticides has skyrocketed over the past 40 years. The extensive use of pesticide has resulted in the reduction of natural insect habitats, earthworms, micro-organisms and cover crops. Beside, pesticide residue in agricultural products can cause the rejection of the exported goods due to the residue has an effect on consumers' health, which can lead to economic damage to country.(Plianbangchang, Kanchalee, & Sakchai, 2009).

Pesticides are extensively used across the world. Thailand has been imported pesticides including herbicide, coinciding with the expansion of the country's agricultural system from domestic to industrial production and mono-cropping agriculture (Siriwong et al., 2009). Organophosphate (OPs) and Carbamate insecticides form are the groups of chemicals that are primarily used in agriculture. And pesticides have caused several harmful effects on the health of humans (Roshini, Peiris-John, & Rajitha, 2008).

The exposure of organophosphate and carbamate insecticides with the accompanying health risks can use biological monitoring an instrument for the measurement of pesticide exposure level that enters the body. The measurements of biological monitoring are used in blood, urine, saliva, or breast milk as biological media by the estimate of the amount of pesticide as its metabolite or its reaction product which is absorbed into the body (Pidgunpai, 2012).

Nowadays, the agricultural system in Thailand has changed from domestic to industrial production and mono-cropping agriculture. This result in an extensive use of pesticide through hour the country, especially OPs and carbamate which are the groups of chemicals primarily used in agriculture. Exposure to these pesticides can cause harmful effect on human health.

Most of organophosphate or carbamate pesticides was inhibited the blood enzymes that call “acetyl cholinesterase” (AChE) and “plasma cholinesterase” (PChE). AChE is basically chosen because of its lower biological variability and destitution of interferences than relative to PChE. After exposure to pesticide, recovery of AChE activity is always slower than PChE due to its longer half-life (1 month for AChE and 2 week for PChE). The quantitative determination of cholinesterase in whole blood can show the level of pesticide exposure. Organophosphate and carbamate insecticides have the highest morbidity rate of poisoning among the farmer. (Public Health, 2008) By the way they can avoid or decreasing the risk of exposure pesticide by using Personal Protective Equipment (PPE) (OSHA, 2003).

The regional environmental office 5 reported that highest number of patients and died of pesticides toxic used is Nakhon Ratchasima, Chaiyaphum, Surin, Buriram, and Mahasarakham respectively. (Laoprasert P, Seupsoue W, & Laoprasert K, 2008) Most of cause is the invalid knowledge in practices using the pesticides (Kumpon P, 2009).

Tarnlalord sub-district, Phimai district, Nakhon Ratchasima province has total population was 3,920 people including 1,983 male and 1,937 female. Number of villages is 14 villages and number of households is 987 households. The main occupation is farmer. Total of area is 30,005 Rai included agricultural area, residential areas, public areas, forest areas, public areas, forest areas and other areas (Tarnlalord Sub-district Administration Organization, 2008). Tarnlalord Sub-District has a Lalord river to flow through and there have the water to support farmers for agriculture. That's the reason of farmers when they do the paddy throughout the year they also have to use the pesticide throughout the year. Therefore this is a part to made Nakhon Ratchasima province had highest number of patients and died from pesticides toxic used.

Since rice farmers in Tarnlalord sub-district can do farming throughout the year. Thus, they have high chance to expose and receive effect to OPs and carbamate, the most frequency use pesticides in rice farm. Therefore, the researcher is interested in monitoring blood cholinesterase level and also analyzes the correlation among rice farmers, farming description and their practices in pesticides use. These results can be used to improve practices of rice farmers in the pesticides use to correctly and can prevent health problems.

1.2 Research Question

1. What are the demographic characteristics, farming characteristics and proper practices of pesticides application among rice farmer in Tarnlalord sub-district?

2. What is the level of cholinesterase in blood among rice farmer in Tarnlalord sub-district?

3. Are there relationships between demographic characteristics, farming characteristics, practices of pesticides application and blood cholinesterase levels among rice farmers in Tarnlalord Sub-District?

4. Is there relationship between blood cholinesterase levels and health effect of organophosphate and carbamate pesticide exposure among rice farmers in Tarnlalord Sub-District?

1.3 Hypothesis

Null Hypothesis (H_0)

- There are no relationships between demographic characteristics, farming characteristics, practices of pesticide application and blood cholinesterase levels among rice farmers.

- There is no relationship between blood cholinesterase level and health effect of organophosphate and carbamate pesticide exposure among rice farmers.

Alternative Hypothesis (H_1)

- There are relationships between demographic characteristics, farming characteristics, practices of pesticide application and blood cholinesterase levels among rice farmers.

- There is relationship between blood cholinesterase level and health effect of organophosphate and carbamate pesticide exposure among rice farmers.

1.4 Research Objective

General Objective

- To find relationship between demographic characteristics, farming characteristics, practices of pesticide application and blood cholinesterase levels among rice farmers.

- To find relationship blood cholinesterase levels related to health effect of organophosphate and carbamate pesticide exposure among rice farmers.

Specific Objective

- To describe demographic characteristic, farming characteristics and proper practices of pesticide application among rice farmers.

- To find relationship between demographic characteristics, farming characteristics, practices of pesticide application and blood cholinesterase levels among rice farmers.

- To identify blood cholinesterase levels related to health effect of organophosphate and carbamate pesticides exposure among rice farmers.

1.5 Benefits and expected outcome

1. The finding of this study can be used as base line to improve pesticide use practices of rice farmers.

2. To support health promotion and prevention related to pesticide usage.

3. Rice farmers will increase their awareness of pesticide use and protect themselves from pesticides exposure by using personal protective equipment.

1.6 Conceptual Framework

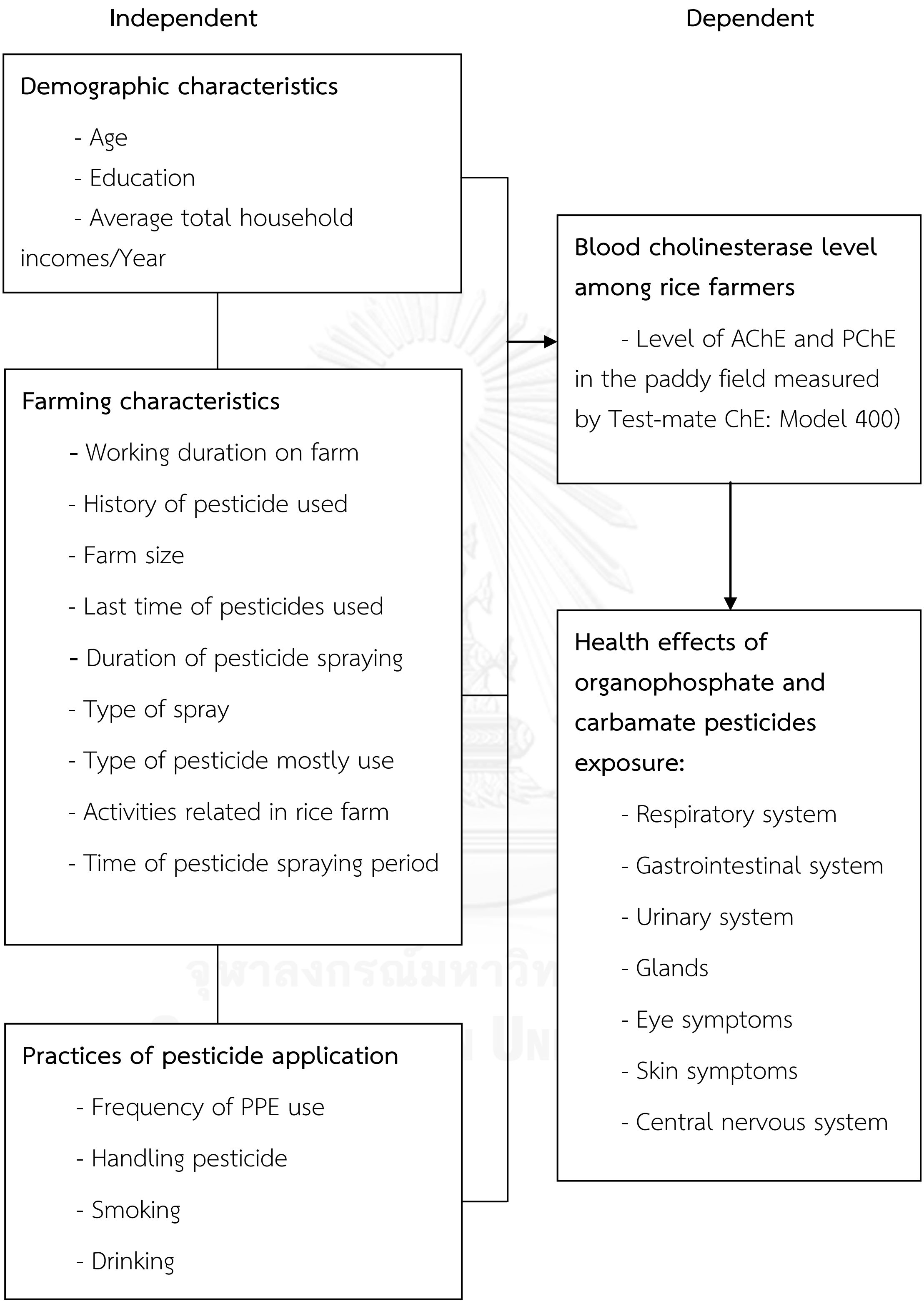

Figure 1: Conceptual framework

1.7 Operational Definition

Farmer means person who has work in paddy field more than 1 year and use chemical pesticides in Tarnlalord Sub-District, Phimai District, Nakhon Ratchasima Province, Thailand.

Pesticide means substance used for pest control. Pesticides are classified according to the type of pest they are active against such as insecticides, herbicide, fungicides, acaricides, rodenticides, nematocides and algaecides.

Dry-season farming refers to agricultural operations almost without irrigation in a climate with a moisture deficiency. Growing after finished wet-season rice harvest, around December and harvested around March.

Demographic characteristics

- Age: The length of time that a person has lived

- **Education level**: The process of receiving systematic instruction divided into never, primary school, secondary school, high secondary school, a diploma, bachelor's degree, master degree and other.

- **Average total household incomes/Year** means the total income of everyone in the household in that year.

Farming characteristics

- Working duration of farming: The number of year that farmers be a rice farmer.

- **History of pesticide used**: The number of year that farmers used the pesticide.

- **Farm size**: The number of paddy field that farmers growing rice.

- **Last time of pesticides used**: The last time that farmers used pesticides

- Duration of pesticide spraying: The number of hours of pesticide spraying in each time divided into 4 groups as; less than ½ hour, ½ - 1 hour, 1½ - 2 hours and more than 2 hours.

- Type of sprayer: The equipment that used in pesticides spraying divided into; spraying by tractor, spraying by hand, spraying by a backpack spray, spraying by a smoker spray, spraying by others.

- Type of pesticide use: The type of pesticide that farmers used are insecticides, herbicides, fungicides and other pesticides.

- Activities related in rice farm: The activities that you use before spraying pesticide divided into; spraying pesticide, mixing pesticide, loading pesticide, other.

- Pesticide spraying period: Time period that farmers spraying pesticide divided into; 9.00 am. - 12.00 pm., 1.00 pm. – 4.00 pm., after 4.00 pm., other.

Practices of pesticide application

Proper practices of pesticides application means self-practices of correct and appropriate pesticides usage for preventing the risk factors and including mixing, loading, and spraying that can be effect to health of the rice farmers in Tarnlalord Sub-District, Phimai District, Nakhon Ratchasima Province, Thailand.

- Frequency of PPE use: refer to designed to protect workers from serious workplace injuries or illnesses resulting from contact with chemical, radiological, physical, electrical, mechanical, or other workplace hazards (OSHA, 2006).

- **Handling pesticide:** The activities that farmers do for avoid and protect themselves from pesticides toxicities before you use pesticide.

- Smoking: Refers to the smoker or non-smoker rice farmers while mixing, loading, spraying pesticides.

- **Drinking**: Refers to the rice farm drinking or non-drinking while mixing, loading, spraying pesticides.

Level of cholinesterase in blood among rice farmers

Cholinesterase level or ChE refers to the level (U/mL) of blood enzymes acetyle cholinesterase (AChE) and plasma cholinesterase (PChE) related with OPs and Carbamate insecticides measure by Test-mate ChE Cholinesterase Test System; Model 400.

Acetyl cholinesterase or AChE refers to red blood cell cholinesterase. There is identical to the enzyme found in the nervous system, and it is thought to be a good indicator of actual neuronal activity. The turnover rate for red blood cells is slow (about 3 months), and AChE measurements reflect this slow replacement rate. Thus, AChE is typically used as a marker of chronic exposure.

Plasma cholinesterase or PChE refers to plasma. PChE turnover is much quicker. PChE is a better short-term indicator due to its more rapid response to exposure; it is used as an indicator of recent, acute exposure.

CHAPTER II

LITERATURE REVIEW

2.1 General information of Tarnlalord Sub-District, Phimai District, Nakhon Ratchasima Province, Thailand

Tarnlalord Sub-District, Phimai District, Nakhon Ratchasima Province has total population is 3,920 people, Male 1,983 people female 1,937 people. Number of villages is 14 villages, Number of households 987 households. The main occupation is the farmers; include plants, livestock, joint plantation, fishery and Food processing. Total of area is 30,005 Rai, include agricultural area (most is for paddy), residential areas, public areas, forest areas, and other areas. There is Lalord rivers flows through this sub-district. So the areas divided by water and land use, include irrigation areas and rained supported areas (Tarnlalord Sub-district Administration Organization, 2008). And there also have Nakhon Ratchasima Rice Research Center in Phimai district.

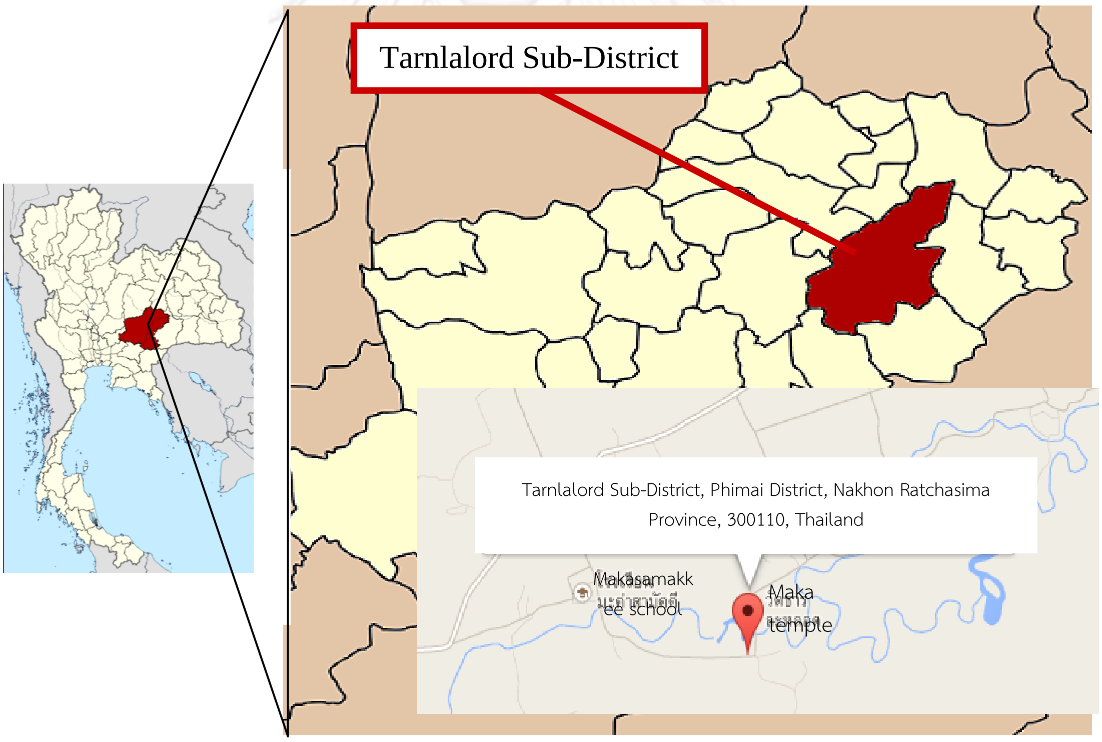

Figure 2: Map of study area

2.2 Process on rice farmer

Agriculture refers to the process of producing food, fiber, fuel and other products by the cultivation of crops. That have specific name is "Agriculturist" both husbandry terrestrial and aquatic animals in a systematic way, 42 percent of the world's labor in the agricultural sector. It is considered the most prevalent group (Gross World Product, 2013).

Farmers are a traditional occupation of Thailand. Thailand is one of the world's rice productions. People in the past inherit about how to do the farm to their descendants. However farm of rice is the main agricultural for people in Thailand. While farm of fruit and other vegetable for eaten in the household and share neighbors. If rest of their eaten, they has taken to sell. On the agricultural era adjusted to be like agriculture of western Thailand. Thailand was change the way to produce agricultural into the mixed farming and takes agricultural technology to replace traditional farming like a tractors, Sprayer, pumps, chemical fertilizers and any agricultural of chemicals etc. to make it into the agricultural economical for respond the demand of market and middleman.

2.2.1 Rice growing process

All farms are rain-fed and most of farms have soil including organic matter content which sandy soil type (Puntumetakul Rungthip, Wantana, Yodchai, Wichai, & Montien, 2011). Average rainfall all in year round Thailand is around 1,200-1,600 mm. (Reunglertpanyakul, 2002).

Paddy preparation process

(A) Paddy preparing (NETWORK, 2014)

(B) Paddy prepared (Photo by Ekarat Sombatsawat)

Figure 3: Paddy preparation process

The field, or paddy, is plough so that a sturdy root system will develop to support the plant and give them access to nutrients. The land must be ensure water is used efficiently and to help in controlling weeds. A drainage system that allows the fast removal of water is also made at this time. Fertilizer may also be used to prepare the soil (National Geographic, 2005).

Rice planting & transplanting process

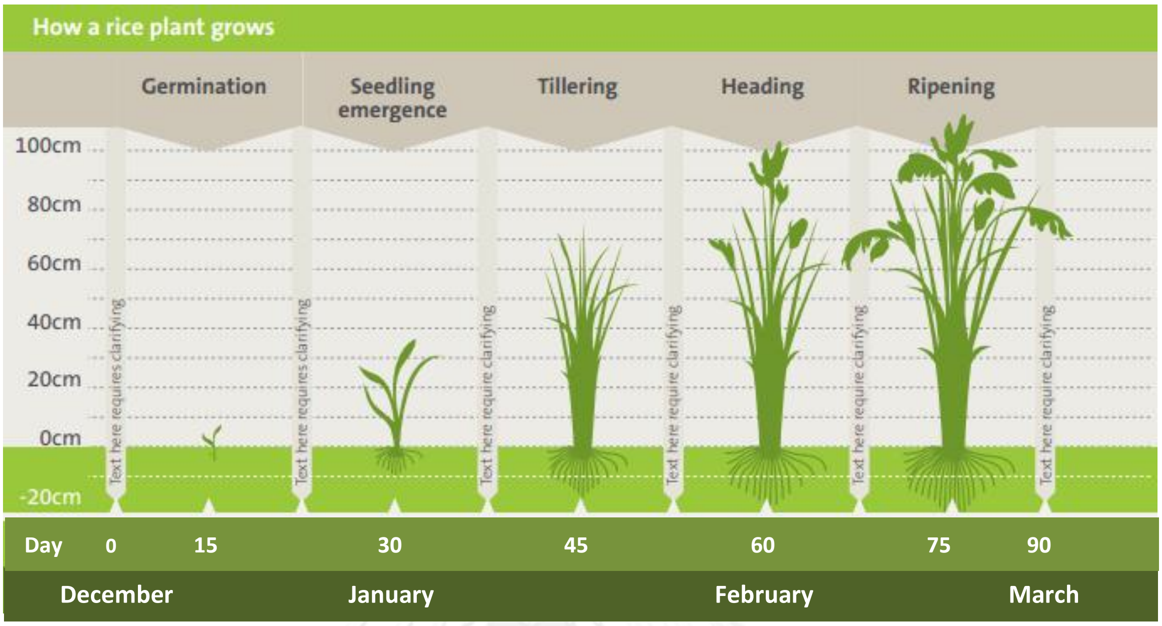

Figure 4: The growth of rice (About Rice FACTSHEET, 2009)

Rice cycle is generally around 90 days, with rice seed typically planted in spring. Seeds are often put into seed beds for germination and early growth. While seeds can be spread directly onto the land, saving time, this result in far lower crop yields. As the seeds germinate, the land is flooded in preparation for transplanting. When the seeds have germinated they are transplanted by hand to the wet rice paddies. This transplanting may occur from 20-80 days after planting (National Geographic, 2005).

In this process, farmers mostly use of pesticides to protect their crop. Normally farmers used OPs and carbamate pesticides to spraying their rice in the first time after rice germinating around 15 – 20 days. After that, farmers regularly to look after their rice, if having some of pest to threaten their rice around 15 days after first time of sprayed pesticides their will to spray OPs and carbamate pesticide and their will pray pesticide like this until their rice have no any pest to threaten. The farmers will spray pesticide and sow some of fertilizers to accelerate growth depend on the owner farm. In that time if the rice farms have no any pest, farmers will not spray any pesticide but their just only eradicate some weed and waiting time for their rice heading.

Harvesting process

When the rice is ready to be harvested, the paddies must be completely drained and the field allowed drying. Harvesting by hand is done with sickle or a scythe: the ears of rice are cut at about 20-30 cm. above the ground. After cutting, the ears of rice are left to dry on the stubble for two or three days (National Geographic, 2005).

(A) Man harvest (Khwanchanok, 2011)

(B) Car harvest (Narawong, 2012)

Figure 5: Harvesting rice process

2.3 Pesticide use in Paddy field

Pesticides are chemicals that are used to control pests, such as insects, rats, weeds and moulds. In most cases, these chemicals are poisons that kill the pest, but in some cases they act as a repellent (i.e. odors that make the pests stay away), or they stop the pest from growing and reproducing (IPM DANIDA, 2003). Pesticide was used in environment as though to be higher in developing countries more than used in developed countries. In developed countries, the aim of using the pesticides was make sure to be safe, hardly any harmful to human and also in the environment is certified by the suitable technique, infrastructure, regulation and economic support. Thailand also was used pesticides including herbicide, insecticide, and fungicide concomitant with the expansion of the agricultural country. And that's found many patients affected from pesticides and the poisoning for getting the diseases of chemicals.

The term pesticide is generic and applied to all chemical used pest control. Pesticide is classified according to type of pest they are active against: Insecticide (aphids, caterpillars, moths, and bugs), herbicides (weeds, grasses), fungicide (types of fungi), and ground squirrels), and nematocide or non-segmented soil worms. So pesticides can be classified in many different ways: according to the target peat, the chemical structure of the compound used or the degree or type of health hazard involved.(Markmee, 2005)

2.4 Application equipment use

In this each duration of rice cycle is the most of farmers using pesticide. And difference farmers they have difference equipment to spraying pesticide in their crops. So exposure of the farmers varies by different application methods; for example who use air-blast or hand spraying is more likely to be exposed to pesticides than those who use a boom sprayer (Ward, Prince, Stewart, & Zahm, 2001). The most common type of sprayers used in Thailand, are backpack sprayer and machine-operated sprayers. Backpacks sprayers are locally made and widely used the machine-operated sprays commonly used are mist blowers, booms on tractor and high pressure sprayers (Sriarunotai, C, W, T, & C, 1997). Farmers in Thailand, whose main crops are rice and maize, apply chemicals using backpack machine sprayers and by hands (Kunstadter et al., 2001). But mostly rice farming of rice farmers' use a backpack sprayer for spraying while fruit farmers used a tractor boom.

(A) Backpack sprayer (Photo by Ekarat Sombatsawat) (B) Boom on tractor (Hanchenlaksh, 2013).

Figure 6: Application equipment used in rice farm

2.5 Organophosphate (OP) and Carbamate use in rice farm

2.5.1 **Organophosphate**

Organophosphate insecticides have become the most widely used insecticides available today. More than forty of them are currently registered for use and all run the risk of acute and sub-acute toxicity. Organophosphates are used in agriculture, in the home, in gardens, and in veterinary practice. All apparently share a common mechanism of cholinesterase inhibition and can cause similar symptoms. Because they share this mechanism, exposure to the same organophosphate by multiple routes or to multiple organophosphates by multiple routes can lead to serious additive toxicity (USEPA, 2013). OPs are well absorbed after uptake by the oral, dermal or inhalation routes and are rapidly metabolized in the human body. 90% of the OPs are excreted within 6-24 hours (Heudorf, Butte, Schulz, & Angerer, 2006). It is important to understand, however, that there is a wide range of toxicity in these agents and wide variation in subcutaneous absorption, making specific identification and management quite important (United State Environmental Protection Agency, 2013).

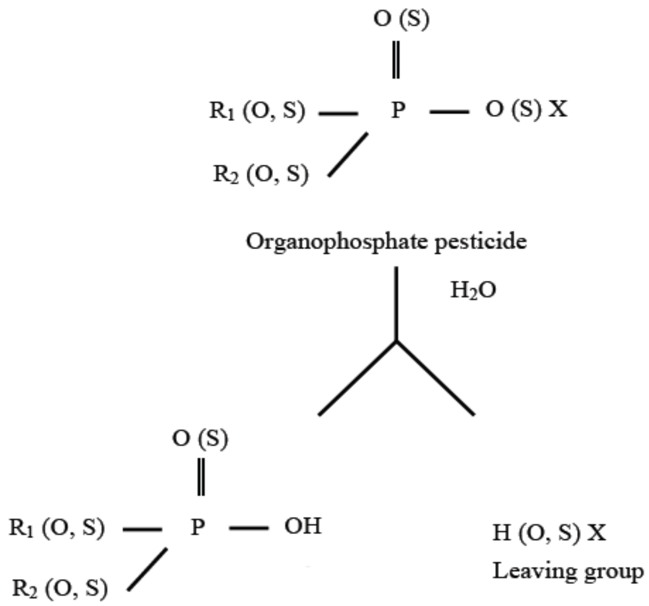

Figure 7: The common structure of organophosphate pesticide from hydrolysis pathway (Singh & Walker, 2006)

2.5.2 Carbamate

Carbamate insecticides are widely used in homes, gardens, and agriculture. They share with organophosphates the capacity to inhibit cholinesterase enzymes and therefore share similar symptom during acute and chronic exposures. However, exposure can occur by several routes in the same individual due to multiple uses, and there is likely to be additive toxicity with simultaneous exposure to organophosphates. However, due to the somewhat different affinity for cholinesterase, as compared to organophosphates, these poisonings are often somewhat easier to treat (EPA, 2012).

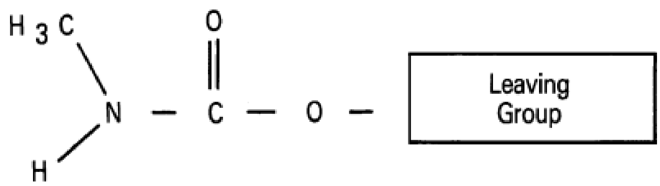

Figure 8: The common structure of Carbamate pesticide from hydrolysis pathway (EPA, 1989)

2.6 Effect of Organophosphate (OPs) and Carbamate

Organophosphate (OPs) and Carbamate insecticides form are the groups of chemicals that are primarily used in agriculture. The conditions of exposure to organophosphate and Carbamate insecticides at high degree along with the accompanying health risks in developing countries of the globe are famous. OPs pesticides, for example Malathion, Parathion, Phosalone, Fenitrothion, Dichlorvos, and Chlorpyrifos are rapidly decomposed and these components are somewhat non persistent in the environment. They are described as being highly acutely toxic. Organophosphate and Carbamate insecticides produce an effect on the nervous system by causing disorder in the enzyme which controls acetylcholine, a neurotransmitter. An unspecified number of them are very toxic. Organophosphate and Carbamate insecticides have the highest morbidity rate of poisoning among the farmer (EPA, 2012).

Despite their extensive and longstanding use for the public well, organophosphate (OPs) pesticides have direct to several adverse effects on human health. Environmental exposure to OPs and damaging on reproductive outcomes in men and women who are working on or are staying near farms are increasingly reported worldwide.

Low-level exposure effects are studied in occupationally or environmentally exposed groups, who are exposed to levels of OPs that basically do not plain in overt clinical features of acute OP poisoning as described in the medical literature. There are many reports on the “adverse health impacts of occupational exposure to OPs.” That was studies on health effects of low-level exposure to OPs have documented self-reported symptoms, effects on the nervous system and the respiratory system (Roshini et al., 2008)

Organophosphate and Carbamate insecticides produce an effect on the nervous system by causing disorder in the enzyme which controls acetylcholine, a neurotransmitter. An unspecified number of them are very toxic. They were grown gradually during the early 19th century. But 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 (EPA, 2012) with many system and organism. If exposed them, its cause many affect that showed by table 1 signs and symptoms depend on that systems or organisms.

Table 1: Signs and symptoms related to organophosphates and carbamate 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, dizziness
	Moderate	Nausea, vomiting, abdominal cramps
	Severe	Diarrhea, fecal incontinence
Respiratory system	Mild	Wheezing, dyspnea, running nose
	Moderate	Bronchorrhea, bronchospasm, shortness of breath
	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: (Wilaiwan, 2012).

2.7 Biomarkers of cholinesterase

Biological monitoring is an instrument for the measurement of pesticide exposure level that enters the body. It can evaluate human exposures to both environment and work place. In case where exposure changes irregularly eventually, and or the skin are a meaningful path of absorption, biological monitoring has proved to obtain the absorbed dose information. The measurements of biological monitoring are used in blood, urine, saliva, or breast milk as biological media by the estimate of the amount of pesticide as its metabolite or its reaction product which is absorbed into the body (IPCS, 2000)

Biomarkers used in environmental and occupational human health monitoring can be distinguished into three classes: biomarker of exposure, effect, and susceptibility (Knudsen & Hansen, 2007). Biomarkers of exposure involve measurement of parent compound, metabolites and reflect the dose of exposure. Biomarkers of effect are a measurable biochemical, physiological, and behavioral alteration within an organism that can be recognized as associated with an established or possible health impairment or disease. Biomarkers of susceptibility indicate an inherent or acquired ability of an organism to respond to specific exposure (Manno et al., 2010) Inhibition of plasma cholinesterase serves as a diagnostic tool for the risk assessment of exposure to toxic organophosphates (Amitai, Moorad, Adani, & Doctor, 1998). Inhibition of AChE activity has been widely used to assess OPs exposure. A study revealed a high prevalence of pesticides poisoning in agricultural farmers by OPs and carbamates exposure with the reduction of AChE (KARABAY, Berna, Ferah, & Günnehir, 2004).

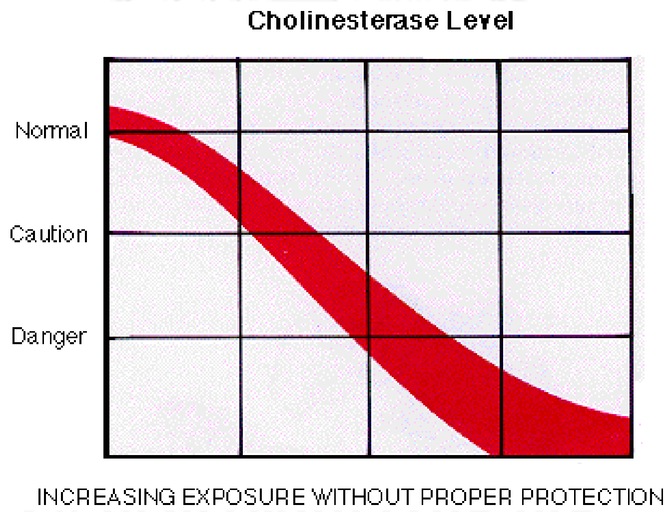

Figure 9: The cholinesterase level (Montana University, 2007)

Therefore, the studies 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 dialkyl phosphate as well as other metabolites. Whereas this research offers significant information on exposure to organophosphate insecticides and their metabolites in the population, in general accepted guidelines related to the interpretation of these biomarkers of exposure have not been created. The discovery of urinary alkyl phosphates in the urine does not essentially show that they cause a harmful effect on health. There has been no study on correlation between urinary dialkyl phosphates and acetyl cholinesterase 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 (Pidgunpai, 2012).

Therefore red blood cell AChE (acetylcholinesterase) and plasma PChE (plasma cholinesterase) can be used as sensitive biomarkers to detect exposure to OPs and carbamate nerve agents, pesticides, and cholinergic drugs in humans and animals. On 24 hours after the end of their exposure the AChE activities had lower (Mason, 2000).

2.8 Cholinesterase Measurements: EQM test-mate cholinesterase test system

Test Kit Cholinesterase Measurements

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

Figure 10: Test-mate ChE (Model 400)

The Test-mate ChE is useful for surveying occupational exposure to pesticides. There is the significant of the measurement on a ordinary basis of levels cholinesterase in blood 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 (Pidgunpai, 2012).

The Test-mate ChE is a test of cholinesterase testing system. The equipment and reagents in total that are required for performing 96 tests fit easily within the storage case. Only 10μL is required by system for each blood test that is likely to be obtained with ease from a finger stick sample. The whole assay may be finished in less than 4 minutes, making the quick evaluation of poisoning status easy. They are measuring quickly than the measurements performed in the clinic (EQM Research Inc., 2003).

2.9 Proper practices on Pesticide application

Personal Protective Equipment (PPE)

Personal Protective Equipment (PPE) is safety clothing and equipment for specified circumstances or areas, where the nature of the work involved or the conditions under which people are working, requires its wearing or use for their personal protective to minimize risk (UniSA, 2008). All end-use occupational use products must have the minimum baseline handler PPE of long-sleeved shirt, long pants and socks and shoes (OSHA, 2003).

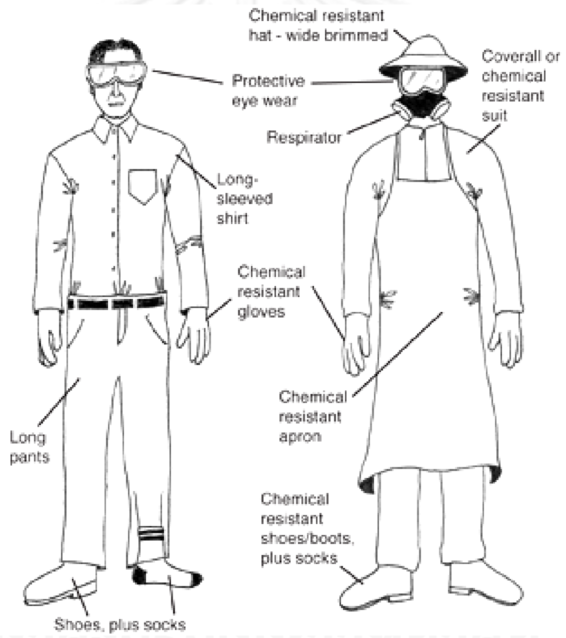

Figure 11: Proper of PPE (University of Hawaii, 2012)

PPE equipment of vapors, gases, and particulates from hazardous substance response activities place response personnel at risk. For this reason, response personnel must wear appropriate personal protective clothing and equipment whenever they are near the site. The more that is known about the hazards at a release site, the easier it becomes to select personal protective equipment (United State Environmental Protection Agency, 2012).

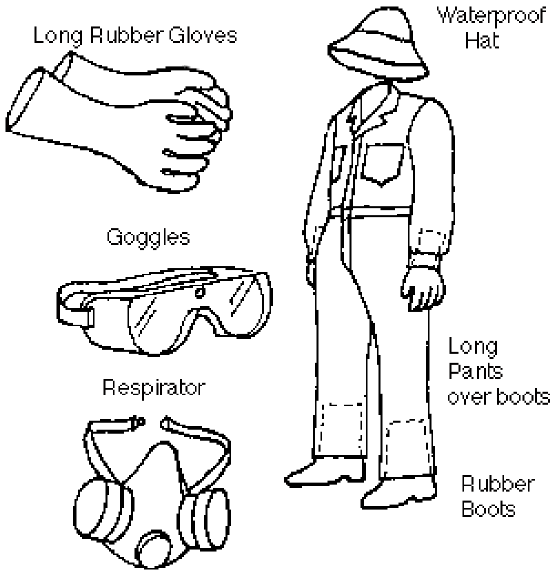

Figure 12: PPE (Montana University, 2007)

Majority of the pesticide absorption on the skin is absorbed in the first few minutes, so cause to health effect with both acute and chronic. The best way to avoid of contact with pesticides is to wear personal protective clothing. The pesticide will indicate the PPE required for mixing loading and sprayer pesticides.

- Chemical resistant hat:
- Respirator
- Waterproof long sleeve shirt
- Waterproof long pants
- Waterproof gloves
- Waterproof boots
- Goggles
- Waterproof apron

Identifying the Correct Product-Specific Handler Protective Clothing

Once the correct toxicity category has been established, the product-specific handler PPE can be identified. Reviewers may obtain the correct product-specific handler protective clothing from the Acute Toxicity Review. Table 2 below shows how the correct product-specific handler protective clothing is derived in the Acute Toxicity Review based on the toxicity category for a given product.

Table 2: Handler PPE for Worker Protection Standard (WPS) Products

Route of Exposure	Toxicity Category by Route of Exposure of End-Use Product			
Dermal Toxicity or Skin Irritation Potential1	**I DANGER**	**II DANGER**	**III WARNING**	**IV CAUTION**
	Coveralls worn over	long-sleeved shirt	and long pants	Coveralls worn over
	Socks	Socks	Socks	Socks
	chemical-resistant	footwear	Chemical-resistant	footwear
	Waterproof or Chemical resistant Gloves2	Waterproof or Chemical-resistant Gloves2	Waterproof or Chemical-resistant Gloves	No minimum4
Inhalation toxicity	Respiratory protection device3	Respiratory protection device3	No minimum4	No minimum4
Eye Irritation Potential	Protective eyewear5	Protective eyewear5	No minimum4	No minimum4

Source: (United State Environmental Protection Agency, 2007)

2.10 Related study

Leilanie J. Prado-Lu D., (Leilanie & Prado-Lu, 2007) aimed to determine associations between hematologic indices such as red blood cell cholinesterase (RBC) and mean corpuscular volume (MCV), with illnesses related to pesticide exposure among farmers in La Trinidad, Benguet. One hundred two (102) randomly selected farmers underwent comprehensive, personal physical health and laboratory examinations and answered a questionnaire on work practices and illness. Majority were males (52%) and most be longed to the 20–35 age group (45%). Majority of exposed farmers were symptomatic, with most common complaints being headache (48%), easy fatigability (46.1%) and cough (40. 2%). Analysis showed that RBC cholinesterase levels were positively associated with age ($p = 0.02$), and selling pesticide containers ($p = 0.008$). Number of years of using pesticides ($p = 0.022$), use of contaminated cloth (p = 0.033), incorrect mixing of pesticides (p = 0.041), sex (p = 0.002) and illness due to pesticides (p = 0.005) were correlated with abnormal MCV. Significant associations were also found for hemoglobin, hematocrit, RBC, white blood cell (WBC) and platelet count. Predictors of RBC cholinesterase were years of pesticide use (p = 0.037) and abnormalities on health (p = 0. 029). The findings of the study can be used for information dissemination and pesticide reduction programs for the farmers.

Ntow et al., (Ntow et al., 2009) did the survey to establish the extent of pesticide exposure in a farming community. Cholinesterase (ChE) activity in whole blood was used as a marker for assessing exposure to pesticides. Complete data were gathered for 63 farmers at Akumadan (exposed) and 58 control subjects at Tono, both prominent vegetable-farming communities in Ghana, by means of a questionnaire and blood cholinesterase analyses (acetylcholine assay). Although whole-blood ChE was significantly lower in the exposed than the control participants, it was not significantly correlated with either confounders of age, sex, body weight, and height or high-risks practices. The high-risks practices revealed during the survey included lack of use of personal protective clothing, short reentry intervals, and wrong direction of spraying of pesticides by hand or knapsack sprayer. About 97% of exposed participants had experienced symptoms attributable to pesticide exposure. The frequent symptoms were reported as weakness and headache. There is the need to review safety precautions in the use and application of pesticides in Ghana

Rendógn, (Rendógn, Rolando, MVM, & Lucia, 2010) surveyed agricultural production methods and pesticide use among subsistence farmers (campesinos) in 4 rural communities of Campeche, Mexico. Self-reports of symptoms of poisoning resulting from occupational pesticide exposure were elicited by questionnaire (N=121), and acetylcholinesterase (AChE) activity during insecticide use was evaluated from blood samples (N=127). In individuals from 2 of the 4 communities, AChE activity was significantly lower (p=0.05) than the mean of activity determined for individuals in a reference group. Results of this study show that erythrocyte AChE inhibition provides a good biomarker of exposure to organophosphate pesticides in field studies with human populations. Carbamates, particularly carbofuran, seem to be more associated with exuberant and diversified symptomatology of pesticide exposure than organophosphates. Studies found that in field communities where both carbamates and organophosphates are suspected to exist.

Blanco-Muñoz and Lacasaña, (Blanco-Munoz & Lacasana, 2011) found practices related to the safe handling of pesticides and uses of personal protective equipment (PPE) are largely unknown among agricultural workers in developing countries. The authors obtained information from 99 Mexican agricultural workers (35 women and 64 men) who answered questions on socio-demographic data, agricultural practices, use of pesticides, use of PPE, and risk perception. As expected, men handled pesticides more frequently than women (67% versus 20%). The workers carried out several agricultural tasks, as is customary in the case of field workers who (1) work in small agricultural enterprises; use a great number of pesticide products (59 commercial brands of pesticides, 33 active ingredients, and 20 chemical families); (2) use mostly manual application equipment; (3) have a low rate of correct usage of PPE (2%), which does not vary according to the education level, the time of year, or the risk perception; and (4) have insufficient hygienic practices. In addition, storage of pesticide products and application equipment at home is frequent among this group of workers (42%), and provides a significant source of Para-occupational exposure for the workers' families. These results show the need to develop prevention programs to reduce risks posed by pesticides to agricultural workers and their families.

Fareed, (Fareed et al., 2013) found that a fiber optic evanescent fluorosensor was developed for the rapid detection of anticholinesterases (AntiChEs) and was modified to measure cholinesterase (ChE) activities in whole blood. Quartz fibers coated with fluorescein isothiocyanate (FITC)-tagged acetylcholinesterase (AChE), detected AntiChEs by their reduction of quenching of fluorescence that was produced by protons generated during acetylcholine (ACh) hydrolysis. Blood ChE activity was detected by quenching the fluorescence of FITC bovine serum albumin immobilized on the quartz fiber. High ChE activity in blood samples produced strong fluorescence quenching and exposure to antiChEs reduced quenching. Fluorometric measurements were made in seconds to minutes by evanescent waveguide fluorometer on 200 µl blood samples. A 2-minute rinse in Krebs buffer was sufficient to prepare the fiber for another measurement.

CHAPTER III
RESEARCH METHODOLOGY

3.1 Study Design

The design of this study was a Cross-sectional study.

3.2 Population and Sample of study

Population of study

This study population was rice famers living in Tarnlalord sub-district, Phimai district, Nakhon Ratchasima province. There were 75 rice farmers growing dry-season rice.

Inclusion Criteria

- Rice famers live in Tarnlalord Sub-District, Phimai District, Nakhon Ratchasima
- Male who involve with pesticides handling and application
- Age between 18-59 years old.
- Rice farmers growing in dry-season crop.
- Willing to participates
- Commonly use organophosphate (OPs) and carbamate pesticide in paddy areas in their crops.
- Duration of being rice farmers more than 1 year

Exclusion Criteria

- Having history of liver failure, Myocardial infarction and malnutrition

3.3 Sample of study

Sample of study was specifies to the rice farmers who live in Tarnlalord Sub-District, Phimai District, Nakhon Ratchasima Province. The farmers growing rice in dry-season crop were 75 rice farmers. Then sample size was calculated were used the formula for the sample size for the mean (Israel, 1992). The equation calculates sample size for the mean, as shown below.

Formula

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

N_0 = sample size

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

σ^2 = variance of an attribute in the population = 0.02

e = desired level of precision = 0.05

Sanidcheu and Ausanawarong, (Sanidcheu & Ausanawarong, 2011) reported the level cholinesterase enzyme of 80 agriculturist in post-harvest period. This study calculates the S.D. and variance of ChE level from eleven farmers who has the lower AChE level such as 1.06, 1.07, 1.09, 1.10, 1.12, 1.14, 0.80, 0.81, 0.88, 0.91 and 0.98 respectively (U/ml).

Standard deviation sample = 0.126

Variance sample = 0.02

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

= 30.73

N_0 = 31 cases

Giving a 5% drop out rate, allowance was added to the sampling figures namely to add its sample size. 5% of 31 cases were 1.55 = 2 cases.

Therefore the sample size of this study were **33 cases**

3.4 Sampling Technique

Step 1: Sampling of districts

Nakhon Ratchasima province was divided into 32 districts, Phimai district was chosen by means of purposive sampling because rice can be grown throughout the year in this area due to the land and water can be supplied from 2 rivers, Moon and Jakkarat River.

Step 2: Sampling of sub-districts

Phimai District is divided into 12 sub-districts and used the simple random sampling for select sub-district in which Tarnlalord sub-district won chance.

Step 3: Sampling of subjects

The representatives of the farmers are growing rice in dry-season and the farmers are mixing, loading and spraying pesticide by themselves (1 subject per household). The 33 target objects by sampling criteria were random selected.

3.5 Validity and Reliability

The developed instrument was tested for validity and reliability

Validation: The questionnaire was consulted 3 experts of pesticide. Then experts considered and inspected on the questionnaire and gave index of item – objective congruence (IOC) score for each question, took the questions lead to calculated the IOC value. The IOC score was 0.90: After that researcher adjusts the questionnaire to obtain validity of content.

Reliability: The questionnaires were tried out with 30 farmers in Naimuang sub-district, Phimai district, Nakhon Ratchasima province. The cronbach's alpha coefficient was calculated using SPSS statistics computer licensed program Version 17. The cronbach's alpha coefficient value was 0.731.

3.6 Data Collection

This research study in blood cholinesterase level as biomarker of organophosphate and carbamate pesticide exposure among farmers in Tarnlalord sub-district, Phimai district, Nakhon Ratchasima province, Thailand

1. The objectives of this study, to find relationship between demographic characteristics, framing characteristics, practice of pesticide application and blood cholinesterase level and find blood cholinesterase level related with health effect. The population of this study was participated of farmers growing in dry-season rice.

2. The sample size were collected by pamphlet promotion and talked with farmers who interesting and willing to participate. Then researcher observed their house about pesticides used of organophosphate and carbamate group. The researcher surveyed and recorded trade name, chemical family and active ingredient of pesticides that rice farmer's used. Then the researcher observed and took photo of the farmer when they mixing, loading and spraying pesticides in their farm.

3. The Cholinesterase level in blood was measured by using EQM Test-mate Cholinesterase Test System, Model 400 for 3 times. First blood collected, within 24 hours after farmer exposed pesticides. Blood were collected 20μL of finger-prick blood sample from the farmers. Then, the researcher test cholinesterase in blood sampling by using EQM Test-mate Cholinesterase Test System, Model 400. After blood cholinesterase test, if who was abnormal level of blood cholinesterase, research need to recommend of them to get treatment at primary health care first and if blood cholinesterase in the severe level the researcher need to refer the participant to see the doctor at the hospital.

- Questionnaire: the researcher collected questionnaire by face to face interviewed at one time per 1 person approximately 20 min/1 person after they finish blood collected.

4. Researcher checks the accuracy and completeness of questionnaires and results of EQM Test-mate Cholinesterase Test System, Model 400 of each farmer. The participation in this study is voluntary, but the farmers can refuse to participate or withdraw from the study any time without the reasons. So the data, lab-result and photos used in the study, were kept confidential, and were destroyed after the end of the study.

5. Data analysis by SPSS statistics computer program licensed Version 17.

3.7 Instrument of study

3.7.1) Questionnaire

Questionnaires consist of closed and open-ended questions to collected data by face to face interviewed. This questionnaire was modified from a questionnaire previously used in the pesticide safe use in rice farmers (Pidgunpai, 2012).

- The questionnaires were divided into four parts as follow;

Part 1 Demographic characteristics
comprise of 3 open ended questions

Part 2 Farming characteristics

Comprise of 9 open ended questions

Part 3 Practices of pesticide use

Comprise of questions asking about frequency of PPE use, handling pesticide, smoking, and drinking alcohol while mixing, loading and spraying pesticides.

Part 4 Health effects of organophosphate and carbamate pesticides exposure

Comprise of questions asking about related symptoms including respiratory system, gastrointestinal system, urinary system, glands, eye symptoms, skin symptoms, central nervous system.

3.7.2) Blood collection and measurement

Blood collecting at 20μL per person of farmers, farmers have to clean and wash their hands with soap. Then the nurse use surgical cotton moistened with alcohol to clean the finger in order to take sample. After the alcohol dried off, a nurse uses a needle to puncture. Take the first drop of blood out and collect. The second drop then stores it with a capillary tube. Next, cholinesterase levels were test by EQM

Test-mate Cholinesterase Test System (Model 400). The results were reported to the participants directly. If the blood cholinesterase is abnormal at level, then researcher recommended the participant to meet the doctor (Wilaiwan, 2012).

The method, it was interpreted by mean of the continuous scale of cholinesterase level (Pidgunpai, 2012).

<u>AChE</u>

More than 2.92 U/ mL*	=	Normal
Less than or equal 2.92 U/mL *	=	Abnormal

<u>PChE</u>

More than 1.56 U/mL*	=	Normal
Less than or equal 1.56 U/mL*	=	Abnormal

* (U/mL reference from Test-mate ChE (Model 400)

3.8 Data Analysis (Statistics)

3.8.1 Descriptive

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

3.8.2 Inferential Statistics

- Chi-square was used to find a relationship between demographic characteristics, farming characteristics, practices of pesticide application and blood cholinesterase levels

- Chi-square was used to identify blood cholinesterase levels related to health effect of organophosphate and carbamate pesticides exposure

- Repeated ANOVA used to find the difference within cholinesterase in blood in the different times.

3.9 Ethical 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 **COA No.055/2014.**

CHAPTER IV

RESULTS

The studies of blood cholinesterase level as biomarker of organophosphate and carbamate pesticide exposure effect among rice farmers in Tarnlalord sub-district, Phimai district, Nakhon Ratchasima, Thailand. This chapter presents the results obtained from analysis of interviews and laboratory findings. The total number of sample size was 33 male of rice farmers to participate in the study. Female farmers were excluded because of a known influence of gender on ChE activity and owing to the few women participating in the study (Jors et al., 2006). The study was collected data and description of the results obtained from the analysis of the survey. The results in each variables are described by percentages, mean, standard deviations, rang, information of questionnaire and the association of blood cholinesterase activity.

The 33 rice farmers were evaluated of participants during farm visitations and observations. The questionnaire divided into 4 parts follow as; Part 1: demographic characteristics including age, education level, and average total household income (baht/year). Part 2: farming characteristics including working duration of farm, history of pesticide used, farm size, last time of pesticides used, duration of pesticide spraying, application equipment of spraying pesticide, type of pesticide mostly use, activities related in rice farm and time of pesticide spraying. Part 3: proper practices of pesticide application including PPE use, handling pesticide, smoking, and drinking. Part 4: health effects of organophosphate and carbamate pesticides exposure including respiratory system, gastrointestinal system, urinary system, glands, eye symptoms, skin symptoms, and central nervous system.

Data from blood collection by EQM test-mate ChE test system, model 400

Blood cholinesterase level among rice farmers

Data of variables relationship

The relationship between demographic characteristics and blood cholinesterase levels

The relationship between farming characteristics and blood cholinesterase levels

The relationship between practices of pesticide application and blood cholinesterase levels

The relationship between blood cholinesterase levels and health effect of organophosphate and carbamate pesticides exposure

4.1 Demographic characteristics

The participants in this study were rice farmers consisting of 33 males. Table 3 showed the age range between 27 to 59 years old. The average age of participants was 46 (Mean) years with a standard deviation (S.D.) of 9.38. The majority of the participants age were in the range of 46-59 (n = 20, 60.60%), 32-45 (n = 9, 27.30%), and 18-31 (n = 4, 12.10%). In terms of education level, 26 people (78.80%) had graduated from primary school, 4 people (12.10%) had graduated from secondary school, and 3 people (9.10%) graduated from high secondary school respectively. The majority of the participants had an income per year more than 90,000 (n = 15, 45.50%) baht annually, while inferior had an average income of less than 30,001 (n = 8, 24.20%) bath and few participants had an income 30,001-60,000 (n = 3, 9.10%) bath per year.

Table 3 Demographic characteristics of rice farmers (n=33)

Demographic characteristics	Total (n)	Percentage (%)
Age		
18 – 31	4	12.10
32 – 45	9	27.30
46 - 59	20	60.60
Mean (±S.D.) = 46 (±9.38) Median = 47.00		
Education level		
Primary school	26	78.80
Secondary school	4	12.10
High secondary school	3	9.10

Table 3 Demographic characteristics of rice farmers (n=33) (Cont.)

Demographic characteristics	Total (n)	Percentage (%)
Average total household income (Baht/year) (1 USD ~ 30 THB)		
Less than 30,001	8	24.20
30,001 – 60,000	3	9.10
60,001 – 90,000	7	21.20
More than 90,000	15	45.50
Mean (S.D.) = 101666.67 (±70,107.80) Median = 85,000		

4.2 Farming characteristics

The farming characteristics of 33 subjects was summarized in table 4 The working duration of farm , most of the participants were found to be in the group of <12 and 12-23 (n = 11, 33.30%) years, inferior more than 35 (n = 6, 18.20%) years and least 24-35 (n = 5, 15.20%) years. 13 people (n = 39.40%) used pesticide for less than 10 years, 8 people (n = 24.20%) used pesticide within 10 – 19 years and another 8 people than 29 years. The average of farm size of the majority of the participants were less than 10 (n = 10.00, 30.30%) rais with the inferior group (n=8, 24.20%) having 10 – 19 rais and more than 29 rais (n = 8.00, 24.20%) and 7 people had farm land about 20-29 rais (21.20%). The lastest pesticide use was collected after one day after the activity (mixing, loading (n= 24.20%) 20 – 29 years. Only 4 people (n = 12.10%) used pesticide for more, and spraying).

The average duration of pesticide spraying was more than 2 hours/day (n = 11, 33.30%), followed by ½ - 1 hour/day (n = 10, 30.30%) and the least duration of pesticide spraying of less than ½ hour/day (n = 5, 15.20%). The methods used for spraying pesticides were spraying by backpack spray (n = 25, 78.50%) and spraying by hand (n = 8, 24.20%). Pesticides used were types herbicide (n = 20, 60.60%), insecticide (n =10, 30.30%) and other (rodenticides, larvicides, organic fertilizer) (n = 3, 9.10%). Rice farmers usually mix, load and spray pesticide by themselves. Lastly, the pesticide spraying period for most rice farmers is in the early morning 6.00 – 9.00 am. (n = 15, 45.50%), inferior the late morning (n = 9, 27.30%) and least in the afternoon (n = 3, 9.10%).

Table 4 Farming characteristics (n=33)

Farming characteristics	Total (n)	Percentage (%)	Mean (S.D.)
Working duration of farm (year)			19.64
Less than 12	11	33.30	(1.43)
12 – 23	11	33.30	
24 – 35	5	15.20	
More than 35	6	18.20	
History of pesticide used (year)			13.85
Less than 10	13	39.40	(1.04)
10 – 19	8	24.20	
20 – 29	8	24.20	
More than 29	4	12.10	
Farm size (Rais) (1 Rais ~ 1,600 Sqm.)			18.58
Less than 10	10	30.30	(1.41)
10 – 19	8	24.20	
20 – 29	7	21.20	
More than 29	8	24.20	
Duration of pesticide spraying			
Less than ½ hour/day	5	15.20	
½ - 1 hour/day	10	30.30	
1½ - 2 hours/day	7	21.20	
More than 2 hours/day	11	33.30	
Type of sprayer			
Hand sprayer	8	24.20	
Backpack sprayer	25	75.80	

Table 4 Farming characteristics (n=33) (Cont.)

Farming characteristics	Total (n)	Percentage (%)
Type of pesticide mostly use		
Insecticide	10	30.30
Herbicide	20	60.60
Others (rodenticides, larvicides, organic fertilizer)	3	9.10
Activities related in rice farm		
Spraying, Mixing, Loading pesticides	33	100.00
Pesticide spraying period		
Early morning 6:00 am. – 9:00 pm.	15	45.50
Late morning 9:01 – 12:00 am.	9	27.30
Afternoon 1:00 pm. – 4:00 pm.	3	9.10
Evening 4:00 – 6:00 pm.	6	18.10

4.3 Practices of pesticide application

Table 5 showed the frequency of personal protective equipment (PPE) that the rice farmers use while applying pesticide in the paddy fields. Majority of the farmers always wear long sleeve shirt (n = 29, 87.9%), Long pants (n = 26, 78.8%). Paper mask or handkerchief and boots were same (n = 24.00, 72.70%). However, the rice farmers use glove only at times (n = 15, 45.50%). Most of participants did not use aprons (n = 30, 90.90%), goggles (27, 81.80%) and hat (n = 20, 60.60%). Lastly, none of the farmers had used all PPE (n = 33, 100%).

Table 5 Frequency of Personal Protective Equipment (PPE) (n=33)

Personal Protective Equipment (PPE)	Frequency of PPE use		
	Always	Sometimes	Never
	n (%)	n (%)	n (%)
Hat	8 (24.20)	5 (15.20)	20 (60.60)
Paper mask or handkerchief	24 (72.70)	7 (21.20)	2 (6.10)
Long sleeve shirt	29 (87.90)	4 (12.10)	0 (0.00)
Long pants	26 (78.80)	7 (21.20)	0 (0.00)
Gloves	10 (30.30)	15 (45.50)	8 (24.20)
Boots	24 (72.70)	5 (15.2)	4 (12.10)
Goggles	1 (3.00)	5 (15.20)	27 (81.80)
Apron	0 (0.00)	3 (9.10)	30 (90.90)
Overall	0 (0.00)	0 (0.00)	33 (100.00)

4.4 Proper use of Personal Protective Equipment (PPE)

Table 6 had showed that 96.55% of the rice farmers were proper using boots (n = 28), 72.00% of them used gloves (n = 18), 50.00% of them used goggles (n = 3), 66.67% of them used aprons (n = 2) and only 3.23% of them used paper masks or handkerchiefs (n = 1), respectively. The usage of hat, long sleeve shirt, long pants, and overall of PPE were improperly used.

Table 6 Proper use of Personal Protective Equipment (PPE)

Proper of Personal Protective Equipment (PPE)	**Equipment's proper**	
	Yes	**No**
	n (%)	**n (%)**
Hat (n=13)	0 (0.00)	13 (100.00)
Paper mask or handkerchief (n=31)	1 (3.23)	30 (96.70)
Long sleeve shirt (n=33)	0 (0.00)	33 (100.00)
Long pants (n=33)	0 (0.00)	33 (100.00)
Gloves (n=25)	18 (72.00)	7 (28.00)
Boots (n=29)	28 (96.55)	1 (3.45)
Goggles (n=6)	3 (50.00)	3 (50.00)
Apron (n=3)	2 (66.67)	35 (33.33)
Overall (n=33)	0 (0.00)	33 (100.00)

* *Evaluated of PPE use base on USEPA standard (University of Hawaii, 2012)*

4.5 Handling Pesticides

Table 7 showed that the majority (93.90%) of rice farmers always mix pesticides by themselves (n = 31), 90.90% of them often keep pesticide products and application equipment at home (n = 30). 90.90% of them wash their clothes by separating work clothes and other clothes (n = 26), 75.80% take shower by soap or shower cream immediately after using pesticides (n = 25) and 51.50% of them read the label of pesticides products before use (n = 17). In the inferior group, the results showed that 72.70% of them wash the equipment after use (n = 24), 63.60% of them use the recommended amounts of pesticides (n = 21), of 54.50% washing hands before eating and drinking after using pesticides (n = 18) and of 45.50% wear gloves when mixing, loading, spraying pesticides (n = 15) respectively when farming. However, most rice farmers did not dig a hole to bury bottles of pesticide (54.50%, n = 18). Farmers, summary of drinking and smoking observed were reported by 12.20% of participants who smoke (n = 6) and 81.80% of them were non-smokers during used pesticides. While, 30.30% of them were drinking (n = 10) and 69.70% of them were not drinking (n = 23) while involved with pesticides..

Table 7 Handling Pesticides (n=33)

Handling Pesticides	**Frequency of Handling Pesticides**		
	Always	**Sometimes**	**Never**
	n (%)	**n (%)**	**n (%)**
Reading the label of pesticides products before use	17 (51.50)	14 (42.40)	2 (6.10)
Using the recommended amounts of pesticides	11 (33.30)	21 (63.60)	1 (3.10)
Wearing gloves when mixing, loading, spraying pesticides	10 (30.30)	15 (45.50)	8 (14.20)
Self-Mixing pesticides	31 (93.80)	1 (3.10)	1 (3.10)
Washing hands before eating and drinking after used pesticides	15 (45.50)	18 (54.50)	0 (0.00)
Taking shower by soap or shower cream of immediately after applying pesticides	25 (75.80)	7 (21.20)	1 (3.00)
Washing clothes by separated working clothes and normally clothes	26 (78.80)	6 (18.20)	1 (3.00)
Washing equipment after use	7 (21.20)	24 (72.70)	2 (6.10)
Keeping of pesticide products at home	30 (90.90)	2 (6.10)	1 (3.00)

Table 7 Handling Pesticides (n=33) (Cont.)

Handling Pesticides	Frequency of Handling Pesticides		
	Always	Sometimes	Never
	n (%)	n (%)	n (%)
Keeping of application equipment (sprayer) at home	30 (90.90)	2 (6.10)	1 (3.00)
Digging a hole to bury a bottle of pesticide used	3 (9.10)	12 (36.40)	18 (54.50)

4.6 Health effects of organophosphate and carbamate pesticides exposure

The health effects of organophosphate and carbamate pesticides exposure reported by the rice farmers were completed face to face via questionnaire. Furthermore, as demonstrated in Table 8, the farmers had reported that rice farmers had problem with the respiratory system. Most of them had dyspnea 63.60% (n = 21), a few of rice farmers had shortness of breath 42.40% (n = 14), followed by bronchorrhea 27.30% (n = 9) and the least is running nose 12.10% (n = 4). A few farmers also reported having problems with gastrointestinal system; 33.30% of them had dizziness/vomiting (n = 11), 18.20% of them had anorexia (n = 6) and 12.10% had Stomach ache (n = 4). Only few of them informed about loss of urinary control 12.10% (n = 4). In the same direction, rice farmers also reported the rice farmers had sweat in glands (n = 5, 15.20%) and hyper salivation (n= 4, 12.10%).

The eye symptoms of participants were irritation 54.50% (n = 18), particularly, blurred vision 51.50% (n = 17), and lacrimation 12.10% (n = 4), respectively. For the skin systems, 39.40% of them had skin rash, itchiness, burn and muscular twitching, and cramps (n = 13) and 33.30% of them had numbness of the hands (n = 11). Most farmers conveyed that they had symptoms related to central nervous system. Majority of the participants had headache 60.60% (n = 20), followed by dizziness 54.50% (n = 18) and memory problem 51.50% (n = 17). On the other hand, only few participants had symptoms of drowsiness 33.30% (n = 11), ataxia 12.10% (n = 4), slurred speech and trembling of the hands 9.10% (n = 3).

Table 8 Health effects related to OPs and carbamate pesticides exposure (n=33)

Health effects of OPs and carbamate pesticides exposure	Yes	No
	n (%)	n (%)
Respiratory system		
Dyspnea	21 (63.60)	12 (36.40)
Bronchorrhea	9 (27.30)	24 (72.70)
Running nose	4 (12.10)	29 (87.90)
Shortness of breath	14 (42.40)	19 (57.60)
Gastrointestinal system		
Anorexia	6 (18.20)	27 (81.80)
Dizziness/ Vomiting	11 (33.30)	22 (66.70)
Stomach ache	4 (12.10)	29 (87.90)
Urinary system		
Loss of urinary control	4 (12.10)	29 (87.90)
Glands		
Hyper salivation	4 (12.10)	29 (87.90)
Sweating	5 (15.20)	28 (84.80)
Eye symptoms		
Blurred vision	17 (51.50)	16 (48.50)

Table 8 Health effects related to OPs and carbamate pesticides exposure (n=33) (Cont.)

Health effects of OPs and carbamate pesticides exposure	Yes	No
	n (%)	n (%)
Lacrimation	4 (12.10)	29 (87.90)
Irritation	18 (54.50)	15 (45.50)
Skin symptoms		
Skin rash, itch and burn	13 (39.4)	20 (60.60)
Numbness of hands	11 (33.30)	22 (66.70)
Muscular twitching and cramps	13 (39.40)	20 (60.60)
Central nervous system		
Headache	20 (60.60)	13 (39.40)
Dizziness	18 (54.50)	15 (45.50)
Drowsiness	11 (33.30)	22 (66.70)
Slurred speech	3 (9.10)	30 (90.90)
Ataxia	4 (12.10)	29 (87.90)
Trembling of hands	3 (9.10)	30 (90.90)
Irritability	10 (30.30)	23 (69.70)
Memory problem	17 (51.50)	16 (48.50)

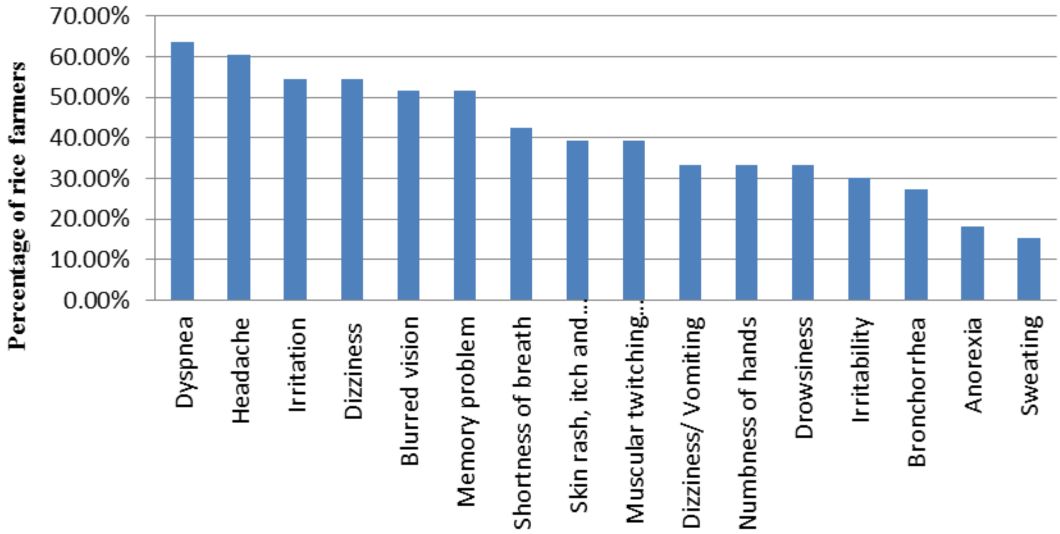

Figure 13: Symptoms of organophosphate and carbamate pesticides exposure report

4.7 Blood cholinesterase levels among rice farmers

In this study, there were a total of 33 rice farmers. They were registered in the study of blood cholinesterase collected in Tarnlalord sub-district, Phimai district, Nakhon Ratchasima province to find levels of blood enzymes acetyl cholinesterase (AChE) and plasma cholinesterase (PChE) for 3 times.

First, blood was collected from participants 24 hours after being exposed to OPs and carbamate via mixing, loading and spraying pesticides. AChE levels and PChE levels means (U/ml) were showed in Table 9, The first blood collection demonstrated that 27.30 % of participants, AChE levels were normal (n = 9) and 72.70% of the rice farmers had an abnormal AChE levels (n = 24). PChE levels were 42.40% indicating that participants were normal (n = 14) and 57.60% of participants had abnormal PChE level (n = 14). Second time of blood was collected from participants 15 days after the pesticide exposure. The results implied that 51.50 % of participants had normal AChE levels (n = 17) and 48.50% had abnormal AChE levels (n = 16). 57.60% of the participants had normal PChE levels (n = 19) and 42.40% of participants had PChE level abnormality (n = 14). Third time of blood collection was collected 30 days after being exposed to pesticides. 57.60 % of participants, AChE levels were normal (n = 19) and 42.40% of the participant had an abnormal AChE levels (n = 14). 69.70% of participants had normal PChE levels (n = 23) while 30.30% of participants were abnormal (n = 10).

The result of blood cholinesterase level was shows became graph (Figure 14) to comparison the normal of blood cholinesterase level.

Table 9 Blood cholinesterase levels among rice farmers (n=33)

Blood cholinesterase levels	Normal n (%)	Abnormal n (%)
First collection: within 24 hours after 1st application		
The AChE level	9 (27.30)	24 (72.70)
The PChE level	14 (42.40)	19 (57.60)
Second collection: 15 days after 1st application		
The AChE level	17 (51.50)	16 (48.50)
The PChE level	19 (57.60)	14 (42.40)
Third collection: 30 days after 1st application		
The AChE level	19 (57.60)	14 (42.40)
The PChE level	23 (69.70)	10 (30.30)

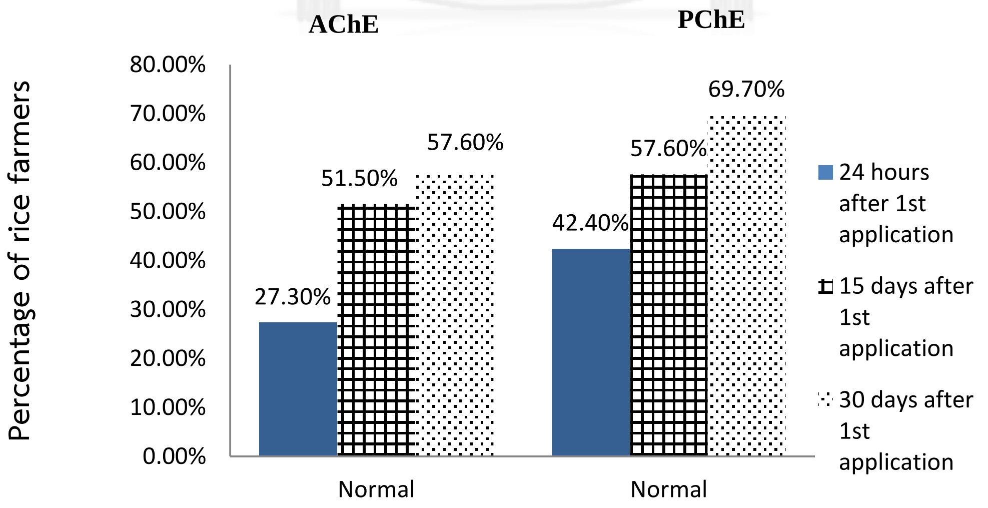

Figure 14: The comparison of ChE levels among rice farmers (n = 33)

Blood cholinesterase concentration in rice farmers

Table 10 that showed median of blood cholinesterase levels; first of blood collection found median of AChE equaled 2.75 (±0.73) were less than the standard of AChE (≤2.92 U/mL) that was abnormal and PChE equaled 1.50 (±0.539 is less than the standard (>1.56 U/mL) was abnormal. The second of blood collection, median of AChE equaled 3.10 (±0.98), it was more than the standard of AChE (>2.92 U/mL) that was normal and PChE equal 1.68 (±0.54) is more the standard of PChE (>1.56 U/mL) means normal. While the third of blood collection median of AChE equaled 3.20 (±0.90) and PChE equal 1.85 (±0.58) were over the standard (>2.92 U/mL) and (>1.56 U/mL), respectively that was normal.

Table 10 Median (S.D.) of blood cholinesterase concentration in rice farmers (n=33)

Cholinesterase levels	Concentration ChE in blood (U/mL)		
	First collection	Second collection	Third collection
	Median (±S.D.) (min - max)	Median (±S.D.) (min - max)	Median (±S.D.) (min - max)
AChE	2.75 (±0.73) (1.32 – 4.84)	3.01 (±0.98) (1.24 – 5.13)	3.13 (±0.90) (1.67 – 5.04)
PChE	1.50 (±0.53) (0.52 – 2.81)	1.59 (±0.54) (0.61 – 2.68)	1.81 (±0.58) (0.83 – 3.01)

Average of blood cholinesterase levels

The study reported activity of blood cholinesterase levels showed that the average of blood cholinesterase levels in the different time; 24 hours, 15 days and 30 days after 1st application found activity of blood cholinesterase levels within 24 hours after 1st application of participants had the average of AChE (2.75 U/mL) and PChE (1.50 U/mL) level less than the standard point are 2.92 and 1.56 U/mL, respectively. That showed the AChE and PChE level within 24 hours after 1st application was abnormal. Moreover, when time was passed 15 days after 1st application showed AChE (3.01 U/mL) and PChE (1.59 U/mL) level was increased became normal level. That's means AChE and PChE level more than the standard point were 2.92 and 1.56 U/mL. Likewise, AChE and PChE level at 30 days after 1st application were 1.13 and 1.81 U/mL, respectively also became normal level (figure 15).

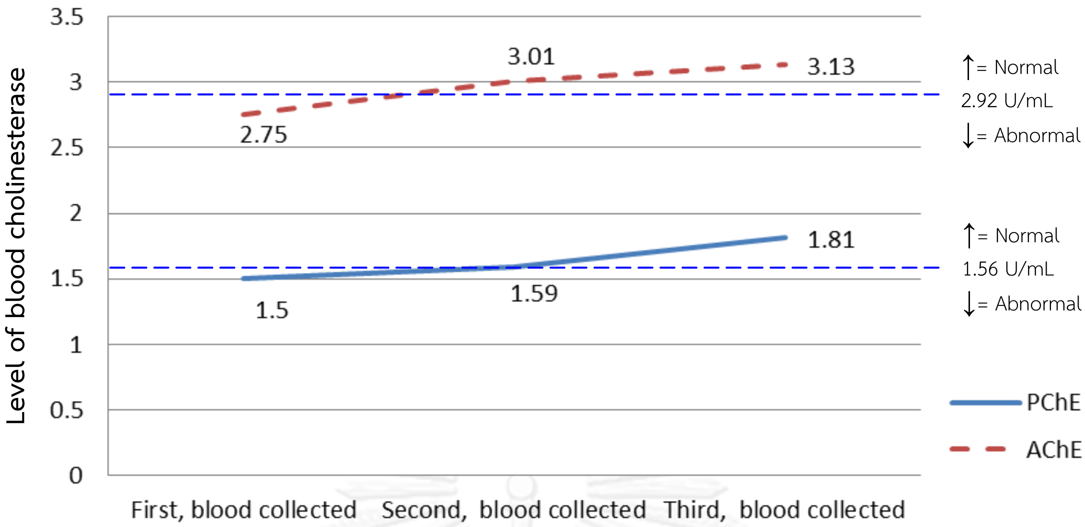

Figure 15: Blood cholinesterase levels (AChE and PChE) in rice farmers

The activity of cholinesterase level among rice farmers was assessed at different points of time. The activity of AChE level of them (Table 11) showed a statistically significant between within 24 hours after 1st application and 15 days after 1st application (p = 0.003, F =10.24), and 30 days after 1st application VS. within 24 hours and 15 days after 1st application (p = 0.002, F = 12.00). Likewise, the difference times of PChE levels observed showed the significant between 24 hours after 1st application VS. 15 days after 1st application (p = 0.023, F = 5.71), and 30 days after 1st application VS. within 24 hours and 15 days after 1st application (p = 0.007, F = 8.24).

Table 11 The different of blood cholinesterase levels among rice farmers (n=33)

Type of ChE	Blood cholinesterase levels	F	*P*-value
AChE	24 hours after 1st application VS. 15 days after 1st application	10.24	0.003*
	30 days after 1st application VS. 24 hours and 15 days after 1st application	12.00	0.002*
PChE	24 hours after 1st application VS. 15 days after 1st application	5.71	0.023*
	30 days after 1st application VS. within 24 hours and 15 days after 1st application	8.24	0.007*

** Significant at 0.05 probability level*

Repeated ANOVA-test

This study showed value of blood cholinesterase (AChE and PChE) level for 3 times (figure 16 and 17) that found after the rice farmers sprayed pesticides (within 24 hours) the AChE and PChE levels of them had lower standard point. When time was passed 15 days rice farmers had cholinesterase level increased to normal level while most of rice farmers had PChE level greater than AChE level because PChE were faster excrete from the body than AChE. However, both AChE and PChE level of rice farmers was continue increased to normal level. However, a few of rice farmers still had lower AChE and PChE level that may cause by their were probable more spray pesticides or expose pesticides from nearly farm that used pesticides.

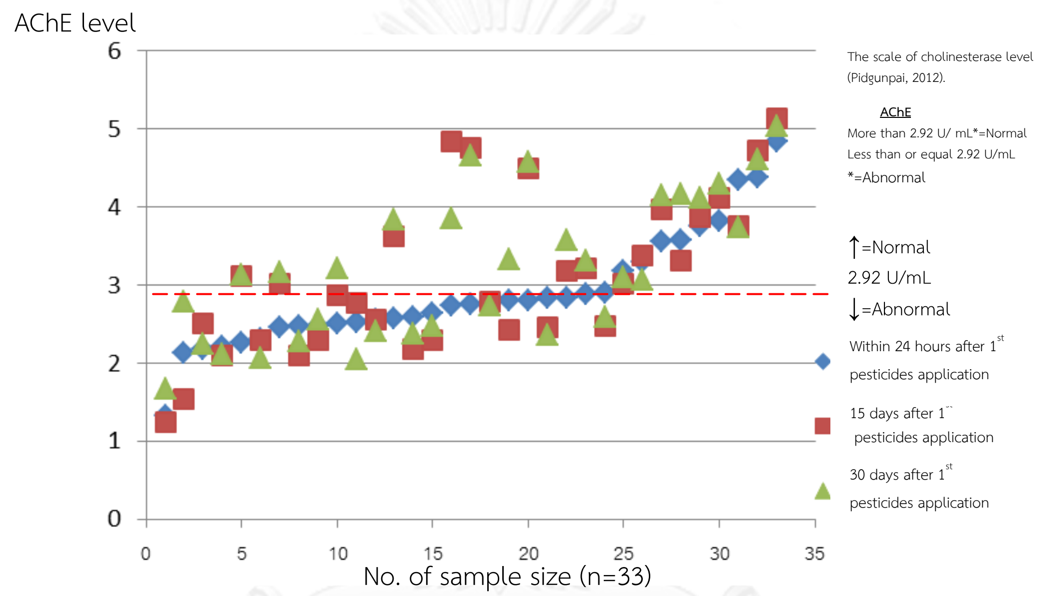

Figure 16: AChE level in farmers (30 days)

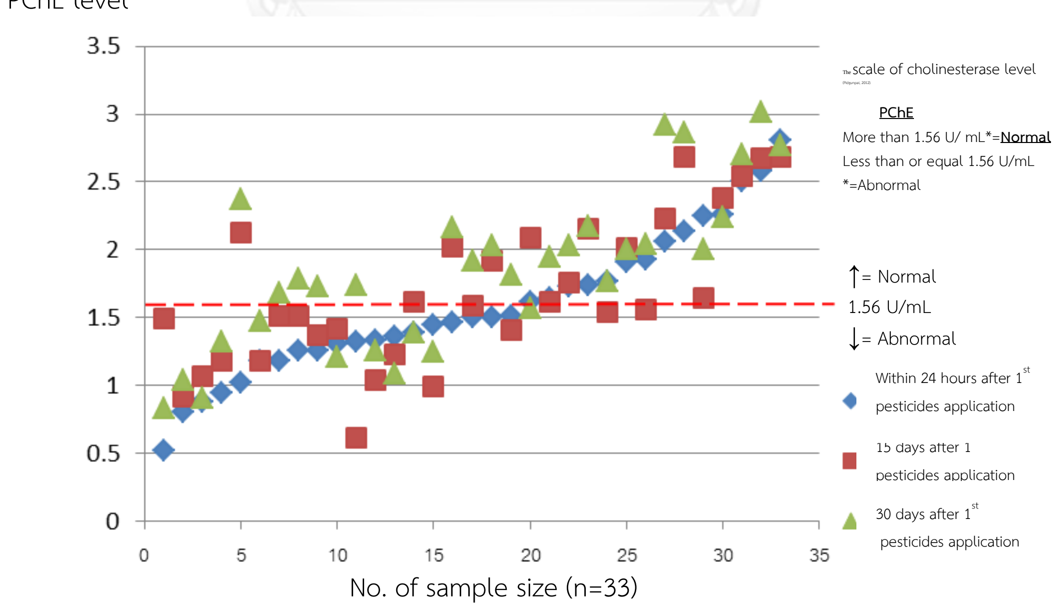

Figure 17: PChE level in farmers (30 days)

4.8 Relationship between demographic characteristics and blood cholinesterase levels of rice farmers

The relationship between demographic characteristics and blood cholinesterase levels of participants was statistically analyzed that showed in table 12. Age was significantly related with AChE in first application (24 hours after 1st application) (p = 0.007) but age were showed not significant with PChE. On the other hand, demographic characteristics showed that education level and average total household income (Baht/Year) were not significant relationship with both AChE and PChE.

Table 12 Relationship between demographic characteristics and blood cholinesterase levels of participants (24 hrs. after 1st application) (n = 33)

Demographic characteristics	**Blood cholinesterase levels**			
	AChE		**PChE**	
	χ^2	**(*P*-value)**	χ^2	**(*P*-value)**
Age	10.06	0.007*	1.18	0.554
Education level	2.588	0.274	0.983	0.612
Average total household income (Baht/Year) (1 USD ~ 30 THB)	1.219	0.748	0.838	0.840

** Significant at 0.05 probability level*
Chi-square test

4.9 Relationship between farming characteristics and blood cholinesterase levels of participants

The statistical analysis of relationship between farming descriptions and blood cholinesterase levels among rice farmers (table 13) found that AChE in first application (within 24 hours after 1st application) were significantly with duration of pesticide spraying (p = 0.018), type of sprayer (p = 0.047), type of pesticide mostly use (p = 0.012) and time of pesticide spraying period (p = 0.033). While, the other factors of farming characteristics including working duration of farm, history of pesticide used, and farm size were not significant related with AChE levels.

Tables 13 showed there were not significant associations with farming characteristics and PChE level.

Table 13 Relationship between farming characteristics and blood cholinesterase levels of participants (within 24 hours after 1st application) (n = 33)

Farming characteristics	Blood cholinesterase levels			
	AChE		PChE	
	χ^2	(*P*-value)	χ^2	(*P*-value)
Working duration of farm	1.665	0.645	1.042	0.791
History of pesticide used	2.023	0.568	0.445	0.931
Farm size	0.085	0.994	4.225	0.238
Duration of pesticide spraying	7.648	0.018*	1.306	0.728
Type of sprayer	3.960	0.047*	1.313	0.252
Type of pesticide mostly use	8.800	0.012*	0.794	0.672
Time of pesticide spraying period	6.615	0.033*	0.294	0.961

** Significant at 0.05 probability level*
Chi-square test

4.10 Relationship between personal protective equipment (PPE) and blood cholinesterase levels

The relationship between personal protective equipment (PPE) and blood cholinesterase levels was summarized in the table 14 that found although personal protective equipment (PPE) including hat, paper mask or handkerchief, long sleeve shirt, long pants, gloves, boots, goggles, and apron and AChE level in first application (within 24 hours after 1st application) were not significant association.

The relationship between personal protective equipment (PPE) and blood cholinesterase levels was statistically analyzed (Table 14) it showed all factor of personal protective equipment (PPE) and PChE levels were not significant association.

Table 14 Relationship between Personal Protective Equipment (PPE) and blood cholinesterase levels (within 24 hours after 1st application) in rice farmers (n = 33)

Personal Protective Equipment (PPE)	Blood cholinesterase levels			
	AChE		PChE	
	χ^2	(*P*-value)	χ^2	(*P*-value)
Hat	4.641	0.098	5.877	0.053
Paper mask or handkerchief	1.790	0.409	2.100	0.350
Long sleeve shirt	1.707	0.191	0.566	0.452
Long pants	1.088	0.297	0.001	0.979
Gloves	1.700	0.427	4.308	0.166
Boots	0.187	0.911	3.523	0.172
Goggles	3.422	0.181	1.401	0.496
Apron	2.582	0.108	0.794	0.373

** Significant at 0.05 probability level*
Chi-square test

4.11 Relationship between proper of personal protective equipment (PPE) and blood cholinesterase levels

The statistical analysis of relationship between proper of personal protective equipment (PPE) and blood cholinesterase levels (Table 15) showed the factors of proper of personal protective equipment (PPE); paper mask or handkerchief, gloves, boots, goggles, apron, and overall and AChE levels were not significant relationships.

However the statistical analysis of relationship between proper of personal protective equipment (PPE) and blood cholinesterase levels among rice farmers (table 15) found that the PChE level in first application (within 24 hours after 1st application) were significantly with gloves (p = 0.002) and boots (p = 0.037). Beyond, other proper of personal protective equipment (PPE) like paper mask or handkerchief, goggles, apron, and overall were not significant relationships with PChE level.

Table 15 Relationship between proper of personal protective equipment and blood cholinesterase levels (within 24 hours after 1st application) in rice farmers (n = 33)

Proper of personal protective equipment (PPE)	**Blood cholinesterase levels**			
	AChE		**PChE**	
	χ^2	(*P*-value)	χ^2	(*P*-value)
Paper mask or handkerchief	2.750	0.097	0.760	0.383
Gloves	0.005	0.943	9.528	0.002*
Boots	0.157	0.692	4.342	0.037*
Goggles	0.061	0.805	0.794	0.373
Apron	0.798	0.372	1.596	0.210
Overall	0.387	0.534	1.400	0.237

** Significant at 0.05 probability level*
Chi-square test

4.12 Relationship between handling pesticides and blood cholinesterase levels

The relationship between handling pesticides and blood cholinesterase levels showed the results from statistical analysis that found only washing hands before eating and drinking after used pesticides was significant related with AChE in first application (within 24 hours after 1st application) (*p* = 0.022). Therefore, the AChE levels and other factors of handling pesticides were not significant relationship (table 16).

The results from statistical analysis found the wearing gloves when the participants who mixed, loaded, sprayed pesticides and dig a hole to bury a bottle of pesticide used were significant related with PChE in first application (within 24 hours after 1st application) (*p* = 0.010 and 0.033), respectively. However the other factors of handling pesticides and PChE levels were not significantly relations.

Table 16 Relationship between handling pesticides and blood cholinesterase levels (within 24 hours after 1st application) in rice farmers (n = 33)

Handling pesticides	Blood cholinesterase level			
	AChE		PChE	
	χ^2	(*P*-value)	χ^2	(*P*-value)
Reading the label of pesticides products before use [a]	3.597	0.166	2.502	0.286
Using the recommended amounts of pesticides [a]	2.925	0.232	1.560	0.458
Wearing gloves when mixing, loading, spraying pesticides [a]	4.094	0.129	9.118	0.010*
Self-mixing pesticides [a]	0.798	0.671	1.569	0.456
Washing hands before eating and drinking after used pesticides [a]	5.215	0.022*	1.340	0.247
Taking shower by soap or shower cream of immediately after applying pesticides [a]	3.960	0.138	3.944	0.139
Washing clothes by separated working clothes and normally clothes [a]	3.332	0.189	2.977	0.226
Washing equipment after use [a]	0.799	0.671	2.588	0.274
Keeping of pesticide products at home [a]	3.422	0.181	2.841	0.242
Keeping of application equipment at home[a]	3.422	0.181	2.841	0.242
Dig a hole to bury a bottle of pesticide used [a]	2.330	0.312	6.796	0.033*
Smoking while using pesticide (mixing, loading and spraying) [b]	2.750	0.097	1.992	0.158
Drinking while using pesticide (mixing, loading and spraying) [b]	0.383	0.536	2.954	0.086

** Significant at 0.05 probability level*

a = 2x3 table, b = 2x2 table

Chi-square test

4.13 Relationship between blood cholinesterase levels and health effect of organophosphate and carbamate pesticides exposure

Table 17 showed only blurred vision was significantly related with AChE in first application (within 24 hours after first application) (p = 0.039, OR = 0.171; 95%CI = 0.029-1.012) in the eye symptoms. As well as the other symptoms of other body systems and AChE levels were not significantly association.

The study of relationship between health effect of organophosphate and carbamate pesticides exposure and blood cholinesterase levels that showed the significant relationship between PChE in first application (within 24 hours after first application) and blurred vision in the eye symptoms (p = 0.024, OR = 0.185; 95%CI = 0.041-0.836). However the study was found the other symptoms of other body systems and PChE levels were not significant association.

Table 17 Relationship between health effect of organophosphate and carbamate pesticides exposure and blood cholinesterase levels (within 24 hours after 1st application) in rice farmers (n = 33)

Health effect of organophosphate and carbamate pesticides exposure	**Blood cholinesterase levels**							
	AChE				PChE			
	χ^2	(*P*-value)	OR	95%CI	**χ^2**	(*P*-value)	OR	95%CI
Respiratory system								
Dyspnea	0.049	0.825	1.200	0.239-6.025	0.443	0.506	0.615	0.147-2.582
Bronchorrhea	0.229	0.632	1.500	0.284-7.934	0.021	0.886	1.120	0.239-5.251
Running nose	0.012	0.913	0.875	0.079-9.696	0.107	0.744	1.417	0.174-11.507
Shortness of breath	0.874	0.350	2.083	0.441-9.844	0.571	0.450	1.714	0.422-6.968
Gastrointestinal system								
Anorexia	0.416	0.519	0.475	0.048-4.740	0.248	0.618	0.625	0.097-4.012
Dizziness/ Vomiting	0.688	0.407	1.943	0.399-9.453	0.248	0.618	0.686	0.155-3.036
Stomach ache	1.707	0.191	NC	NC	1.977	0.160	4.909	0.452-53.267

** Significant at 0.05 probability level*

Chi-square test

NC – Not calculated

Table 17 Relationship between health effect of organophosphate and carbamate pesticides exposure and blood cholinesterase levels (within 24 hours after 1st application) (n = 33) in rice farmers (Cont.)

Health effect of organophosphate and carbamate pesticides exposure	Blood cholinesterase levels							
	AChE				PChE			
	χ^2	(*P*-value)	OR	95%CI	χ^2	(*P*-value)	OR	95%CI
Urinary system								
Loss of urinary control	1.707	0.191	NC	NC	1.977	0.160	4.909	0.452-53.267
Glands								
Hyper salivation	1.185	0.276	3.143	0.371-26.621	0.107	0.744	1.417	0.174-11.507
Sweating	0.157	0.692	0.625	0.060-6.486	0.745	0.388	2.318	0.332-16.185
Eye symptoms								
Blurred vision	4.251	0.039*	2.171	1.129-14.012	5.125	0.024*	1.685	1.471-9.836
Lacrimation	1.707	0.191	NC	NC	0.107	0.744	1.417	0.174-11.508

** Significant at 0.05 probability level*

Chi-square test

NC – Not calculated

Table 17 Relationship between health effect of organophosphate and carbamate pesticides exposure and blood cholinesterase levels (within 24 hours after 1st application) (n = 33) (Cont.)

Health effect of organophosphate and carbamate pesticides exposure	Blood cholinesterase levels							
	AChE				PChE			
	χ^2	(*P*-value)	OR	95%CI	χ^2	(*P*-value)	OR	95%CI
Irritation	0.509	0.475	0.571	0.122-2.679	0.066	0.797	1.200	0.299-4.817
Skin symptoms								
Skin rash/ itching/ burning	1.528	0.216	0.338	0.058-1.972	0.138	0.710	0.764	0.184-3.169
Numbness of hands	0.688	0.407	0.476	0.081-2.811	0.248	0.618	0.686	0.155-3.036
Muscular twitching and cramps	0.132	0.716	1.333	0.282-6.300	0.138	0.710	0.764	0.184-3.169
Central nervous system								
Headache	1.354	0.245	0.400	0.084-1.913	1.146	0.284	0.462	0.111-1.921

** Significant at 0.05 probability level*

Chi-square test

NC – Not calculated

Table 17 Relationship between health effect of organophosphate and carbamate pesticides exposure and blood cholinesterase levels (within 24 hours after 1st application) (n = 33) in rice farmers (Cont.)

Health effect of organophosphate and carbamate pesticides exposure	Blood cholinesterase levels							
	AChE				PChE			
	χ^2	(*P*-value)	OR	95%CI	χ^2	(*P*-value)	OR	95%CI
Dizziness	0.733	0.392	2.000	0.404-9.909	1.340	0.247	0.438	0.107-1793
Drowsiness	0.000	1.000	1.000	0.197-5.000	0.248	0.618	0.686	0.155-3.036
Slurred speech	1.238	0.266	NC	NC	0.112	0.738	0.654	0.053-8.019
Ataxia	1.707	0.191	NC	NC	0.566	0.452	0.410	0.038-4.426
Trembling of hands	0.061	0.805	1.375	0.109-17.316	2.432	0.119	NC	NC
Irritability	0.383	0.536	0.571	0.096-3.409	0.907	0.341	0.468	0.096-2.271
Memory problem	1.638	0.201	0.357	0.072-1.780	1.588	0.208	2.475	0.597-10.269

** Significant at 0.05 probability level*

Chi-square test

NC – Not calculated

CHAPTER V

DISCUSSIONS

5.1 Demographic characteristics

This study describes some demographic characteristics among rice farmers in Tarnlalord sub-district, Phimai district, Nakhon Ratchasima, Thailand were 33 men of rice farmers to participate in the study. Female farmers were not included in this study because of a known influence of gender on ChE activity and owing to the few women participating in the study (Jors et al., 2006). The average age of farmers in this study was 46 years old, ranging from 27-59 years old, which is similar to other studies conducted in Thailand. A study found that rice growing farmers in Thailand had an average age 47.0 years old and ranging from 25 and 56 years old (Chaiklieng & Praengkrathok, 2011). However another study in different country presents the mean age of Ethiopian farm workers was 36.4 years old (Mekonnen & Ejigu, 2005). The results showed mostly of rice farmers graduated primary school, similar with questionnaires information was reported from rice growing farmers in Ongkharak, Nakhon Nayok, Thailand (Wilaiwan, 2012). Most of rice farmers had income less than 30,001 THB per year depending on water system and weather. The results were difference from other study showing that farmers gain approximately 30,000-60,000 TBH per year (Wilaiwan, 2012).

In the farming characteristics, this is the general characteristics found most of rice farmers had working duration of farm less than 12 years which is similar to a previous study (Un Mei Pan, 2009). The average history of pesticide used was 13.85 years. Other study reported years of using pesticides was 18.64 years (Wilaiwan, 2012). The average farm size was 18.58 rais, (1 rai = 1,600 Sqm.). Most of participants had the duration of pesticide spraying between ½-1 hour/day. On the other hand, the study of farmers were had duration pesticides spraying 1-2 hours/day in Sam Chuk district Suphan Buri Province, Thailand (Duangchinda, Anurugsa, & Hungspreug, 2014). Application equipment of spraying pesticide 78.50% of them were used spraying by a backpack sprayer. Farmers in Thailand, whose main crops are rice and maize, apply chemicals using backpack machine sprayers and by hands (Kunstadter et al., 2001). The results were supported by other study found 48.60% of application method that farm workers were mostly uses (Hanchenlaksh, 2013). While, type of pesticide mostly that the rice farmers use herbicide 60.60%. The activities related in rice farm found all of the rice farmers were spraying, mixing, and loading pesticides

-by themselves and 45% of participants were praying pesticides in the early morning (6:00 am.–9:00 pm.) which are similar to previous study.

The results showed a similarity to other in a way that the most frequency of personal protective equipment (PPE) use while applying pesticides were used paper mask or handkerchief, long sleeve shirt, long pants and boots. The results of the present study are also very similar (Kachaiyaphum, Howteerakul, Sujirarat, Siri, & Suwannapong, 2010). However a few of rice farmers used hat, gloves and goggles. In the other hand the rice farmers never used over all of personal protective equipment (PPE) during applying pesticides at that time which similar with previous study (Johnstone, 2006). All end-use occupational use products must have the minimum baseline handler PPE of long-sleeved shirt, long pants and socks and shoes (OSHA, 2003) to minimize risk (UniSA, 2008). Even through the rice farmers used personal protective equipment (PPE) but the important thing is proper of equipment that the rice farmers used. The study showed the rice farmers used proper of equipment is both gloves and boots, while a few of participants used equipment's proper are paper mask or handkerchief, goggles and apron. Results of the present study are also very similar to those reported by (Blanco-Munoz & Lacasana, 2011)). In addition, weather condition is an important thing that was determined PPE used of the rice farmers such as temperature and humidity may cause discomfort since most protective apparel has low heat dissipation. Moreover the rice farmers never used proper of equipment like hat, long sleeve shirt, long pants and overall. Although the evaluation was assessed by the researchers but hold to the principles of Personal Protective Equipment (PPE) by United States Environmental Protection Agency (University of Hawaii, 2012).

In this study showed that 51.50% of them read the label of pesticides products before use, 63.60% of them use the recommended amounts of pesticides, 78.80% of them wash their clothes by separating work clothes and other clothes and 72.70% of them wash the equipment after use, the results similar with the study of 35 farmers in Ongkharak, Nakhon Nayok, Thailand (Wilaiwan, 2012). While 45.50% of rice farmers wear gloves when mixing, loading, spraying pesticides, 93.90% of them always mix pesticides by themselves, 54.50% washing hands before eating and drinking after using pesticides and 75.80% take shower by soap or shower cream immediately after using pesticides. The result of this study was supported by (Jors et al., 2006). However 90.90% of them often keep pesticide products at home, application equipment at home and not dig a hole to bury the bottles of pesticide they used 54.50%. Moreover, 49 agricultural workers in Australian reported agricultural workers no smoked a cigarette (48%) and drank (35%) (Johnstone, 2006), similar with this study found 81.80% of them were non-smokers during used pesticides and 69.70% were not drink.

The finding showed that health effects related to pesticides exposure that farmers had top three problems with the respiratory system, eye system and central nervous system, very similar with the study 40 sprayers in North India found top three systems that the sprayers had were respiratory (27%), neurological (25%) and ocular (7%), respectively (Jors et al., 2006). Likewise the symptom of pesticides exposed reported that the respiratory system found most had dry cough, bronchrrhea, dyspnea and short of breath (Thiravirojana & Pusapukdeepob, 1999). Although the gastrointestinal system very similar with the study of 35 rice growing farmers in Ongkharak, Nakhon Nayok, Thailand showed 22.90% of them had abdominal cramp, followed by anorexia 17.10% and vomiting 11.40% (Wilaiwan, 2012) but the study of 29 rice-growing farmers found the different, most of them never have abdominal cramp, nausea/vomiting (Un Mei Pan, 2009). While urinary system, found the very few participants had loss of urinary control, also glands including hyper salivation and sweating which similar to those reported by (Wilaiwan, 2012). In this study reported eye symptom showed 54.50% of rice farmers had irritation symptom was partially consistent with those obtained by (Rendógn et al., 2010). Also 51.50% of them had blurred vision like the results of 128 farmers in Bolivia that 40.00% had blurred vision (Jors et al., 2006). The skin systems found 39.40% of participants had skin rash, itch and burn symptoms and 33.30% of participants had numbness of hands was supported by the studies of 136 farmers in northern, Thai reported 29.40% of farmers also had numbness (Sapbamrer, Arrak, & Prajak, 2013). However the most of systems that occurred to rice farmers who used pesticides, that is central nervous system, including headache 60.60%, followed by dizziness 54.50% and memory problem 51.50%. On the contrary, only few participants had symptoms of drowsiness 33.30%, ataxia 12.10%, slurred speech and trembling of the hands 9.10%. Even though the results of the presented in the study are also very similar to those reported by (Wilaiwan, 2012) but the results of (Wilaiwan, 2012) found very few of farmers had memory problem and non-slurred speech of them which is the differences thing between both results of central nervous system.

5.2 Blood cholinesterase level

The study reported activity of blood cholinesterase that showed average of blood cholinesterase levels for 3 time (Lotti, 1995); first of blood collection: within 24 hours after first application found activity of AChE was 2.89 (±0.73) U/mL is less than the standard of AChE less than 2.92 U/mL (abnormal level) and PChE equal 1.58 (±0.539) U/mL is more than the standard more than 1.56 U/mL (normal level). The AChE activity in this study was higher than the AChE activity in previous study which reported AChE activity of farm workers was 1.36 (±0.199) U/ml (Sanidcheu & Ausanawarong, 2011) and BuChE activity of farmers was 2.47 (±0.46) U/ml. While AChE activity in 15 days after first application showed AChE activity was 3.10 (±0.98) U/mL is more than the standard of AChE more than 2.92 U/mL) (normal level) and PChE was 1.68 (±0.54) U/mL was greater than the standard of PChE more than 1.56 U/mL (normal). This result was supported by human exposure studies/ five healthy men were given pirimiphos-methyl (purity, 97.8%) at a dose of 0.25 mg/kg per day orally for 28 days. Blood samples for measurement of plasma and erythrocyte cholinesterase activity were taken on days 14. Likewise changes in cholinesterase activity were within 12%. However, the group means for each time interval did not differ significantly and the variations noted were within the range of variations found by others for normal untreated subjects (WHO, 2012) and short-term studies of toxicity identified decreased cholinesterase activity, studies did not show any consistent time-related progression in the inhibition of plasma or erythrocyte cholinesterase activity but there was evidence of significant but not complete recovery of cholinesterase activities after 2 or more weeks (Marable, Maurissen, Mattsson, & Billington, 2007). However, the third of blood collection at 30 days after first application found activity of AChE was 3.20 (±0.90) U/mL and PChE equal 1.85 (±0.58) U/mL are greater than the standard (> 2.92 U/mL) and (> 1.56 U/mL), respectively that means normal. Therefore, the study similar with 64 spraymen which reported the cholinesterase (ChE) levels (U/mL) of exposure showed average of ChE levels that the spraymen exposed (5136.55 ± 19.057 U/mL) which it in normal level (Lal, 2004)

The activity of cholinesterase level among rice farmers was assessed at different points of time. The activity of AChE level among rice farmers showed a statistically significant between within 24 hours after first application and 15 days after first application (p = 0.003, F =10.24), and 30 days after first application and previous (p = 0.002, F = 12.00). Likewise, the difference of PChE levels observed showed significant between 24 hours after first application and 15 days after first application (p = 0.023, F = 5.71), and 30 days after first application VS. Previous (p = 0.007, F = 8.24). In 1993, Fakhei (Fakhei, 1993) found out the same results that AChE level was significant with the day of pesticides exposed (p = 0.001, 0.001 and 0.010, respectively) and the results of ChE level within 2 days and day 13 after spraying OPs, cholinesterase levels showed a significant drop.

5.2 Relationship between demographic characteristics and blood cholinesterase levels

In this study had only male, female farmers were excluded because of a known influence of gender on ChE activity and owing to the few women participating in the study (Jors et al., 2006). The results showed that age was significantly related with AChE (within 24 hours after 1st application) which the researcher expectation most of them had 46 – 59 years old. Therefore, when age was increased any metabolism activity in body adverse decreased the pesticides that rice farmers exposed make them had slowly eliminated of pesticides out of their body caused them had lower AChE. The result was different to previous study of (Chaiklieng & Praengkrathok, 2011). The presented study indicated both AChE and PChE levels were no significant with education level and average total household income (Baht/year).

5.3 Relationship between farming characteristics and blood cholinesterase levels

The study found AChE in first application were significantly with duration of pesticide spraying, type of sprayer, type of pesticide mostly use and time of pesticide spraying period. That why the rice farmers who sprayed pesticides more than 2 hours had lower AChE, In fact, If their sprayed pesticides more time that means they're have chance to get more pesticides exposure, similar with previous study (Duangchinda et al., 2014). In the study of Magauzi reported the result consistent by used multivariable analysis to confirm the positive relationship between ChE levels within 24 hours after first application and type of using pesticides (Magauzi et al., 2011). The rice farmers who used backpack sprayer have chance to get exposure from pesticides than hand sprayer because backpack was created very small drop or aerosol of pesticides that rice farmers can exposed pesticides more than who used hand sprayer. However, not only AChE activity in erythrocytes was associated with the duration of the workers exposure to pesticides (Singh Satyender et al., 2011) but also found relationship between AChE and method of use, practice, chemical content, frequency and chemical type of farmers in Sam Chuk, Suphan Buri, Thailand (Duangchinda et al., 2014). While, other factors of farming characteristics and AChE levels were not significant.

5.4 Relationship between proper practices of pesticide application and blood cholinesterase levels

The study of personal protective equipment (PPE) had the principle emphasis has been aims to avoid exposure of skin, mouth, nose and eye when handling pesticides product. In this study showed that relationship between personal protective equipment (PPE) and blood cholinesterase levels that the rice farmers used was showed, although personal protective equipment (PPE) including hat, paper mask or handkerchief, long sleeve shirt, long pants, gloves, boots, goggles, and apron and AChE within 24 hours after 1^{st} application were not significant related. While, PChE level found were significantly related between PChE within 24 hours after 1^{st} application and personal protective equipment (PPE).

In fact the workers normally used personal protective equipment (PPE) but not consider about proper of personal protective equipment (PPE) that they use. This is the important thing that all of rice farmers must be realizes, the PPE required for early entry to treated areas that is permitted under the worker protection standard and that involves contact with anything that has been treated, such as plants, soil, or water, is coveralls over chemical-resistant of long-sleeved shirt and long pants, chemical-resistant gloves, such as butyl rubber, nitrile rubber, or neoprene rubber, shoes and socks, protective eyewear, chemical-resistant headgear for overhead exposure (Fishel, 2007). The results of this study showed proper of personal protective equipment (PPE) and blood cholinesterase levels within 24 hours after 1^{st} application showed AChE levels were not significant relationships. Moreover the proper of personal protective equipment (PPE) and blood cholinesterase levels among rice farmers found PChE within 24 hours after 1^{st} application were significantly with Gloves and Boots. That’s why the rice farmers who wore proper gloves and boots had only lower PChE but AChE was normal, which rice farmers wearing or using for their personal protective equipment (PPE) can be minimize risk (UniSA, 2008) and rice farmers who used proper gloves and boots had less chance to expose pesticides than who used improper gloves and boots.

5.5 Relationship between handling pesticide application and blood cholinesterase levels

The results was presented that only washing hands before eating and drinking after used pesticides was significant related with AChE within 24 hours after pesticides application. Although this reported was supported by Sapbamrer (Sapbamrer et al., 2013) found the significant difference association between AChE and preventive practices during application but the study of wilaiwan found who washing hands before eating when used pesticides were not significant association with AChE (Wilaiwan, 2012). From the anticipant that may cause by rice farmers washed hand before ate and drank after used pesticides can protect them from pesticides exposure. Therefore, AChE level and other factors of handling pesticides including reading the label of pesticides products before use, using the recommended amounts of pesticides, self-mixing pesticides by themselves, taking shower by soap or shower cream of immediately after applying pesticides, washing clothes by separated working clothes and normally clothes, washing equipment after use keeping of pesticide products at home, keeping of application equipment at home, Dig a hole to bury a bottle of pesticide used, smoking while using pesticide (mixing, loading and spraying) and drinking while using pesticide (mixing, loading and spraying) were not significant association.

In this study showed results from statistical of PChE analysis found the wearing gloves when the participants who mixed loaded, sprayed pesticides and dig a hole to bury a bottle of pesticide used were significant relationship with PChE in first application, respectively. However the PChE levels and factors of handling pesticides were not significantly relations which the presented results were similar with previous study (Wilaiwan, 2012).

5.6 Relationship between blood cholinesterase levels and health effect of organophosphate and carbamate pesticides exposure

The results of this study showed that only blurred vision was significantly related with AChE within 24 hours after pesticides application in eye symptoms. These rice farmers' sprayed pesticides with an eye have no protective equipment (goggle); eventually eyes of these pesticides sprayer get exposed. Wilaiwan study found effects of pesticide are illnesses or injuries that do not appear immediately (within 24 hours) after exposure to a pesticide, may cause of a few rice farmers wore goggles to protect their eyes from the pesticides which harmoniously with the study

35 farmers found 32 farmers did not used goggles and showed significant between cholinesterase and eyes symptoms (Wilaiwan, 2012). Adverse effects can be delayed for weeks, months or even years after the first exposure to a pesticide. Depending upon the toxicity of the compound, dosage and exposure time, the adverse effects of pesticides poisoning ranges from headaches, vomiting, skin irritation, respiratory problems to other neurological disorders (Jors et al., 2006). The AChE measurements reflect this slow replacement rate. Thus, AChE is typically used as a marker of chronic exposure (Brown et al., 2006).

Rice farmers who sprayed pesticide, represent in the study were working at a very high risk of eye toxicity as they get exposed to agricultural pesticides regularly. The eyes of these pesticide sprayers get direct exposure of pesticides while spraying during pesticides application. These rice farmers never follow any hygienic work practices due to their unawareness of ocular toxicity due to pesticides. Sometimes, during spraying they touch or rub their eyes from fingers of their unwashed hands when they feel itching in their eyes. The effect of pesticide toxicity in the eyes may be worse than imagined, starting with mild visual problems; conditions could progress to more severe visual impairment, or possibly a total loss of vision.

The reported of OPs and carbamate poisoning showed the symptom that separation by the time occur; acute: the symptom began to emerge within 24 hours after expose OPs and carbamate pesticides including, dyspnea, dizziness, vomit, stomach ache, loss urinary control, burr vision. While sub-acute; 1-2 weeks showed the symptoms that mostly occur with respiratory system such as shortness and dyspnea. However chronic will show the symptom around 2-4 weeks or more, distal weakness, muscle weakness and numbness of hand/leg (Ramathibodi Poison center, 2013). The study reported PChE levels showed that the significant related between PChE in first application and blurred vision in the eye symptom. Therefore the study was found PChE levels were not significant related with respiratory systems, gastrointestinal systems, urinary systems, glands, skin symptoms and central nervous systems. In contrast, PChE turnover is much quicker. PChE is a better short-term indicator due to its more rapid response to exposure; it is used as an indicator of recent, acute exposure (Brown et. al., 2006). However some study cholinesterase which pesticide-related symptom was not significantly with AChE and PChE levels [(Thiravirojana & Pusapukdeepob, 1999), (Jors et al., 2006) and (Johnstone, 2006)].

CHAPTER V
CONCLUSION LIMITATION and RECOMMENDATION

6.1 Conclusion

The present investigation was designed to evaluate the cholinesterase levels in an exposed rice farmers at different times of observation. In this study was cross-sectional study and conducted in Tarnlalord sub-district, Phimai district, Nakhon Ratchasima, Thailand. The aims of this study were; to assess demographic characteristic, farming characteristics and proper practices of pesticide application and cholinesterase level in blood among rice farmers, to find relationship between demographic characteristics, farming characteristics, proper practices of pesticide application, blood cholinesterase levels and identify blood cholinesterase levels related to health effect of organophosphate and carbamate pesticides exposure among rice farmers. There was conducted only 33 male of the participants. We used questionnaire face to face for complete the information and used the EQM Test-mate cholinesterase Test System, Model 400 for complete the cholinesterase level.

The results shown the majority of participant age was in the range of 46-59 years old. While the education level showed that 78.80% of them had graduated from primary school, 45.50% of them had an income per year in ranging from more than 90,000. The farming descriptions of the subjects are summarized, the working duration of farm, most of the participants were found to be in the group of <12 and 12-23 (33.30%), 39.40% of them used pesticide for less than 10 years. The average of farm size of the majority of the participants were less than 10 rais (30.30%) while, duration of pesticide spraying was more than 2 hours/day (33.30%), methods that they used for spraying pesticides were spraying by backpack spray (78.50%). However, the majority of pesticide that the rice farmers used type-herbicide (60.60%) and they usually mix, load and spray pesticide by themselves, meanwhile 45.50% of them was sprayed pesticides period in early morning 6.00 – 9.00 pm. In the activity related with rice farming found the personal protective equipment (PPE) which rice farmers used, although the rice farmers always used long sleeve shirt (87.9%), long pants (78.8%), paper mask or handkerchief and boots were-same (72.70%), glove (30.30%), hat (24.20%) and goggles (3.00%) but most of these equipment were proper only boots (84.80%) and gloves (54.50%).

The presented of this study showed that the majority (93.90%) of rice farmers always mix pesticides by themselves, 90.90% of them keep pesticide products at home and application equipment at home, 78.80% of them wash their clothes by separating work clothes and other clothes, 75.80% take shower by soap or shower cream immediately after using pesticides and 51.50% of them read the label of pesticides products before use. Moreover the inferior group, the results are that 72.70% of them sometimes wash the equipment after use, 63.60% of them use the recommended amounts of pesticides, 54.50% washing hands before eating and drinking after using pesticides and 45.50% wear gloves when mixing, loading, spraying pesticides, respectively. However, most rice farmers do not dig a hole to bury the bottles of pesticide they used 54.50%. While, 12.20% of participants were smoke and 81.80% were non-smokers during used pesticides. The health effects of organophosphate and carbamate pesticides exposure reported most of participants had health effected with eyes symptoms; irritation (54.50%) and blurred (51.50%), followed by central nervous system; headache (60.60%), dizziness (54.50%) and memory problem (51.50%), and respiratory system had only Dyspnea symptom (63.60%).

The study of relationship between blood cholinesterase levels in difference tines and health effect of organophosphate and carbamate pesticides exposure showed only blurred vision was significantly related with AChE in first application (p = 0.039) in the eye symptoms. For PChE level showed that the significant relationship between PChE in first application and blurred vision in the eye symptoms (p = 0.024).

6.2 Benefit from the study

1. Were assessed demographic characteristic, farming characteristics and proper practices of pesticide application which related to cholinesterase level in blood among rice farmer.

2. Were completely analyzed relationship between demographic characteristic, farming characteristics and proper practices of pesticide application which related to cholinesterase level in blood among rice farmers.

3. Proper practices to reduce the farmers risk from pesticides exposure and increasing their awareness should be recommended the finding.

6.3 Limitation of the study

1. The amount of sample size was considered as small size.

2. The health related symptoms were reported by the participants that may not answers as diagnose by doctors.

3. In this study, the cut point of blood cholinesterase level (AChE and PChE) were used as recommendation from the test-mate cholinesterase base on general population in which it is not farmer population in Thailand.

6.4 Recommendation of the study

1. Farmers should increase of personal protective equipment (PPE) usage. Although the rice farmers used PPE but all of them must give precedence with the proper of equipment which rice farmers used.

2. This study should be introduced intervention regarding proper use of personal protective equipment (PPE) were increase farmers awareness on pesticides handling.

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APPENDIX

Appendix A

AF 02-12

The Ethics Review Committee for Research Involving Human Research Subjects, Health Science Group, Chulalongkorn University
Institute Building 2, 4 Floor, Soi Chulalongkorn 62, Phyat hai Rd., Bangkok 10330, Thailand,
Tel: 0-2218-8147 Fax: 0-2218-8147 E-mail: eccu@chula.ac.th

COA No. 055/2014

Certificate of Approval

Study Title No.006.1/57 : **BLOOD CHOLINESTERASE LEVEL AS BIOMARKER OF ORGANOPHOSPHATE AND CARBAMATE PESTICIDE EXPOSURE EFFECT AMONG RICE FARMERS IN TARNLALORD SUB-DISTRICT, PHIMAI DISTRICT, NAKHON RATCHASIMA PROVINCE, THAILAND**

Principal Investigator : MR. EKARAT SOMBATSAWAT

Place of Proposed Study/Institution : College of Public Health Sciences, Chulalongkorn University

The Ethics Review Committee for Research Involving Human Research Subjects, Health Science Group, Chulalongkorn University, Thailand, has approved constituted in accordance with the International Conference on Harmonization – Good Clinical Practice (ICH-GCP) and/or Code of Conduct in Animal Use of NRCT version 2000.

Signature: Prida Tasanapradit	Signature: Nuntaree Chaichanawongsaroj
(Associate Professor Prida Tasanapradit, M.D.)	(Assistant Professor Dr. Nuntaree Chaichanawongsaroj)
Chairman	Secretary

Date of Approval : 4 April 2014 Approval Expire date : 3 April 2015

The approval documents including

1) Research proposal
2) Patient/Participant Information Sheet and Informed Consent Form
3) Researcher
4) Questionnaire

Protocol No. 006.1/57
Date of Approval -4 APR 2014
Approval Expire Date -3 APR 2015

The approved investigator must comply with the following conditions:

1. *The research/project activities must end on the approval expired date of the Ethics Review Committee for Research Involving Human Research Subjects, Health Science Group, Chulalongkorn University (ECCU). In case the research/project is unable to complete within that date, the project extension can be applied one month prior to the ECCU approval expired date.*
2. *Strictly conduct the research/project activities as written in the proposal.*
3. *Using only the documents that bearing the ECCU's seal of approval with the subjects/volunteers (including subject information sheet, consent form, invitation letter for project/research participation (if available).*
4. *Report to the ECCU for any serious adverse events within 5 working days*
5. *Report to the ECCU for any change of the research/project activities prior to conduct the activities.*
6. *Final report (AF 03-12) and abstract is required for a one year (or less) research/project and report within 30 days after the completion of the research/project. For thesis, abstract is required and report within 30 days after the completion of the research/project.*
7. *Annual progress report is needed for a two- year (or more) research/project and submit the progress report before the expire date of certificate. After the completion of the research/project processes as No. 6.*

Appendix B

AF 01-12

คณะกรรมการพิจารณาจริยธรรมการวิจัยในคน กลุ่มสหสถาบัน ชุดที่ 1 จุฬาลงกรณ์มหาวิทยาลัย
อาคารสถาบัน 2 ชั้น 4 ซอยจุฬาลงกรณ์ 62 ถนนพญาไท เขตปทุมวัน กรุงเทพฯ 10330
โทรศัพท์: 0-2218-8147 โทรสาร: 0-2218-8147 E-mail: eccu@chula.ac.th

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COA No. 055/2557

ใบรับรองโครงการวิจัย

โครงการวิจัยที่ 006.1/57 : ระดับโคลีนเอสเตอเรสในเลือดตัวบ่งชี้ผลจากการรับสัมผัสสารกำจัดศัตรูพืชกลุ่มออร์กาโนฟอสเฟตและคาร์บาเมตในชาวนา ตำบลธารละหลอด อำเภอพิมาย จังหวัดนครราชสีมา ประเทศไทย

ผู้วิจัยหลัก : นายเอกราช สมบัติสวัสดิ์

หน่วยงาน : วิทยาลัยวิทยาศาสตร์สาธารณสุข จุฬาลงกรณ์มหาวิทยาลัย

คณะกรรมการพิจารณาจริยธรรมการวิจัยในคน กลุ่มสหสถาบัน ชุดที่ 1 จุฬาลงกรณ์มหาวิทยาลัย ได้พิจารณา โดยใช้หลัก ของ The International Conference on Harmonization – Good Clinical Practice (ICH-GCP) อนุมัติให้ดำเนินการศึกษาวิจัยเรื่องดังกล่าวได้

ลงนาม..
(รองศาสตราจารย์ นายแพทย์ปรีดา ทัศนประดิษฐ)
ประธาน

ลงนาม..
(ผู้ช่วยศาสตราจารย์ ดร.นันทรี ชัยชนะวงศาโรจน์)
กรรมการและเลขานุการ

วันที่รับรอง : 4 เมษายน 2557 **วันหมดอายุ** : 3 เมษายน 2558

เอกสารที่คณะกรรมการรับรอง

1) โครงการวิจัย
2) ข้อมูลสำหรับกลุ่มประชากรหรือผู้มีส่วนร่วมในการวิจัยและใบยินยอมของกลุ่มประชากรหรือผู้มีส่วนร่วมในการวิจัย
3) ผู้วิจัย
4) แบบสอบถาม

เลขที่โครงการวิจัย 006.1/57
วันที่รับรอง - 4 เม.ย. 2557
วันหมดอายุ - 3 เม.ย. 2558

<u>เงื่อนไข</u>

1. ข้าพเจ้ารับทราบว่าเป็นการผิดจริยธรรม หากดำเนินการเก็บข้อมูลการวิจัยก่อนได้รับการอนุมัติจากคณะกรรมการพิจารณาจริยธรรมการวิจัยฯ
2. หากใบรับรองโครงการวิจัยหมดอายุ การดำเนินการวิจัยต้องยุติ เมื่อต้องการต่ออายุต้องขออนุมัติใหม่ล่วงหน้าไม่ต่ำกว่า 1 เดือน พร้อมส่งรายงานความก้าวหน้าการวิจัย
3. ต้องดำเนินการวิจัยตามที่ระบุไว้ในโครงการวิจัยอย่างเคร่งครัด
4. ใช้เอกสารข้อมูลสำหรับกลุ่มประชากรหรือผู้มีส่วนร่วมในการวิจัย ใบยินยอมของกลุ่มประชากรหรือผู้มีส่วนร่วมในการวิจัย และเอกสารเชิญเข้าร่วมวิจัย (ถ้ามี) ***เฉพาะที่ประทับตราคณะกรรมการเท่านั้น***
5. หากเกิดเหตุการณ์ไม่พึงประสงค์ร้ายแรงในสถานที่เก็บข้อมูลที่ขออนุมัติจากคณะกรรมการ ต้องรายงานคณะกรรมการภายใน 5 วันทำการ
6. หากมีการเปลี่ยนแปลงการดำเนินการวิจัย ให้ส่งคณะกรรมการพิจารณารับรองก่อนดำเนินการ
7. โครงการวิจัยไม่เกิน 1 ปี ส่งแบบรายงานสิ้นสุดโครงการวิจัย (AF 03-12) และบทคัดย่อผลการวิจัยภายใน 30 วัน เมื่อโครงการวิจัยเสร็จสิ้น สำหรับโครงการวิจัยที่เป็นวิทยานิพนธ์ให้ส่งบทคัดย่อผลการวิจัย ภายใน 30 วัน เมื่อโครงการวิจัยเสร็จสิ้น

Appendix C

Questionnaire (English version)

Code................

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

Questionnaire
"Blood cholinesterase level as biomarker of organophosphate and Carbamate pesticide exposure effect among rice farmers in Tarnlalord sub-district, Phimai district, Nakhon Ratchasima province, Thailand"

Explanation Questionnaire is separates onto 4 parts

Part 1 Demographic characteristics
Part 2 Farming Descriptions
Part 3 Practices of pesticide use
Part 4 Health effects of organophosphate pesticides exposure

Part 1: Demographic characteristics

1.1 Age .... Years

1.2 Education level

( ) Never ( ) Primary school

( ) secondary school ( ) High secondary school

( ) a diploma ( ) Bachelor's degree

( ) Master' degree ( ) other

1.3 Average total household incomes/Year.......................................Bath

Part 2: Farming characteristics

2.1 How long have you been a farmer? ....year(s)

2.2 How long do you used of OPs and carbamate pesticides? ....year(s)

2.3 How many rais of your growing rice in this crop? ....Rai(s)

2.4 How long at last time that you used pesticides.....................day(s)

2.5 How long do you spraying of pesticide per day?

( ) Less than ½ hour/day ( ) ½ - 1 hour/day

( ) 1½ - 2 hours/day ( ) More than 2 hours/day

2.6 What did you use type of sprayer?

( ) hand sprayer

( ) backpack sprayer

( ) smoker sprayer

( ) others................................

2.7 Which type of pesticide do you mostly used?

( ) Insecticide

( ) Herbicide

( ) Fungicide

( ) Other...............................

2.8 What did your activities that related in rice's farming? (Can choose more than 1 choice)

( ) Spraying pesticide ( ) Mixing pesticide

( ) Loading pesticide ( ) other ....

2.9 When did you use the pesticide?

( ) Early morning 6:00 a.m. – 9:00 a.m. ( ) Late morning 9:01 a.m.–12:00 p.m.

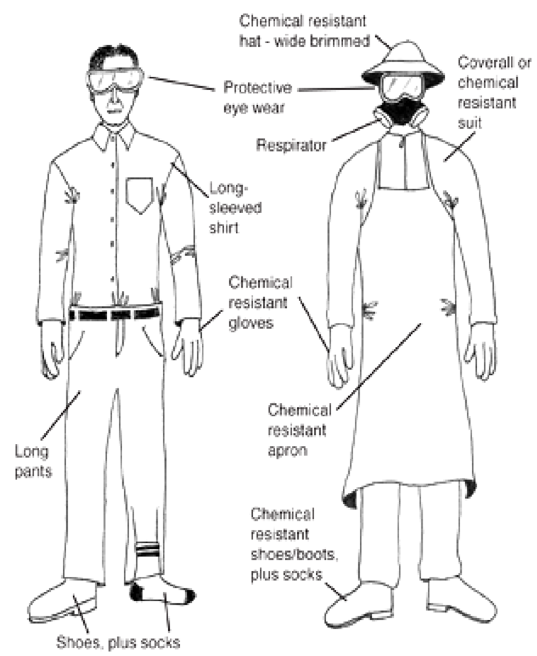
( ) Afternoon 1:00 pm. – 4:00 pm. ( ) Evening 4:00 – 6:00 pm.

<u>Part 3</u>: Proper practices of pesticide application

3.1 Do you use Personal Protective Equipment (PPE) or not? How? (Within 6 months)

Equipment	Frequency of PPE use		
	Always	Sometimes	Never
1. Hat			
2. Paper mask or handkerchief			
3. Long sleeve shirt			
4. Long pants			
5. Gloves			
6. Boots			
7. Goggles			
8. Apron			
9. Overall			

When you use Personal Protective Equipment (PPE), correct or not?



CTAHR Good Agricultural Practices Coaching

Equipment	Equipment's Correct	
	Yes	No
1. Chemical resistant hat		
2. Respirator		
3. Waterproof long sleeve shirt		
4. Waterproof long pants		
5. Waterproof gloves		
6. Waterproof boots		
7. Goggles		
8. Waterproof apron		
9. Waterproof overall		

3.2 Do you handling pesticides or not? How? (Within 6 months)

Handling Pesticides	Always	Sometimes	Never
Reading the label of pesticides products before use.			
Using the recommended amounts of pesticides.			
Wearing gloves when mixing, loading, spraying pesticides			
Self-mixing pesticides			
Washing hands before eating and drinking after applying pesticides.			
Taking shower by soap or shower cream of immediately after using pesticides.			
Washing clothes by separated working clothes and normally clothes.			
Washing equipment after use.			
Keeping of pesticide products at home			
Keeping of application equipment (sprayer) at home			
Dig a hole to bury a bottle of pesticide used.			
Smoking while you using pesticide (mixing, loading and spraying)			
Drinking while you using pesticide (mixing, loading and spraying)			

<u>Part 4</u>: **General health effects of organophosphate and carbamate pesticides exposure (Within 6 months)**

Symptoms Related	Yes	No
Respiratory system		
Dyspnea		
Bronchorrhea		
Running nose		
Shortness of breath		
Gastrointestinal system		
Anorexia		
Dizziness/ Vomiting		
Stomachache		
Urinary system		
Loss of urinary control		
Glands		
Hyper salivation		
Sweating		
Eye symptoms		
Blurred vision		
Lacrimation		
Irritation		
Skin symptoms		
Skin rash/ itching/ burning		
Numbness of hands		
Muscular twitching and cramps		

Symptoms Related	Yes	No
Central nervous system		
Headache		
Dizziness		
Sleep fragmentation		
Slurred speech		
Ataxia		
Trembling of hands		
Irritability		
Memory problem		

Appendix D

Questionnaires Thai version

แบบสัมภาษณ์ชุดที่..............

ผู้สัมภาษณ์...

แบบสัมภาษณ์

เรื่อง "ระดับโคลีนเอสเตอเรสในเลือดตัวบ่งชี้ผลจากการรับสัมผัสสารกำจัดศัตรูพืชกลุ่มออร์กาโนฟอตสเฟตและคาร์บาเมต ในชาวนา ต.ธารละหลอด อ.พิมาย จ.นครราชสีมา ประเทศไทย"

เลขที่โครงการวิจัย 006.1/57
วันที่รับรอง - 4 เม.ย. 2557
วันหมดอายุ - 3 เม.ย. 2558

คำชี้แจง แบบสัมภาษณ์ประกอบด้วย 4 ส่วน

ส่วนที่ 1 ข้อมูลทั่วไป

ส่วนที่ 2 อธิบายการทำการเกษตร

ส่วนที่ 3 การปฏิบัติของการใช้สารกำจัดศัตรูพืช

ส่วนที่ 4 ผลกระทบทางสุขภาพของการสัมผัสสากำจัดศัตรูพืชกลุ่มออร์กาโนฟอสเฟต

<u>ส่วนที่ 1:</u> ข้อมูลทั่วไป

1.1 อายุ .... ปี

1.2 ระดับการศึกษา

( ) ไม่ได้เรียน　　( ) จบประถมศึกษา (ป.1 – ป.6)

( ) จบมัธยมต้น/เทียบเท่า　　( ) จบมัธยมปลาย/ปวช/เทียบเท่า

( ) จบอนุปริญญา/ปวส　　( ) จบปริญญาตรี/เทียบเท่า

( ) สูงกว่าปริญญาตรี　　( ) อื่นๆ (ระบุ).......................

1.3 รายได้เฉลี่ยของครอบครัว ....บาท ต่อปี

<u>ส่วนที่ 2</u>: อธิบายการทำการเกษตร

2.1 ท่านทำนาข้าวมาแล้วกี่ปี..............................ปี

2.2 ท่านใช้สารกำจัดศัตรูพืชกลุ่มออร์กาโนฟอสเฟตและคาร์บาเมตมาทั้งสิ้นกี่ปี....................ปี

2.3 ท่านกำลังปลูกข้าวในฤดูกาลปัจจุบันจำนวนกี่ไร่.........................ไร่

2.4 ท่านใช้สารกำจัดศัตรูพืชครั้งสุดท้ายมาแล้วกี่วัน...................วัน

2.5 ท่านฉีดพ่นสารกำจัดศัตรูพืช ท่านใช้ระยะเวลานานกี่ชั่วโมงต่อวัน

( ) น้อยกว่า ½ ชั่วโมงต่อวัน ( ) ½ - 1 ชั่วโมงต่อวัน

( ) 1½ - 2 ชั่วโมงต่อวัน ( ) มากกว่า 2 ชั่วโมงต่อวัน

2.6 ท่านใช้อุปกรณ์ใดในการฉีดพ่นสารกำจัดศัตรูพืช

( ) ฉีดพ่นด้วยเครื่องฉีดพ่นแบบมือ

( ) ฉีดพ่นด้วยเครื่องฉีดพ่นแบบสะพายหลัง

( ) ฉีดพ่นด้วยเครื่องฉีดพ่นแบบควัน

( ) ฉีดพ่นด้วยเครื่องมือชนิดอื่น.......................................

2.7 สารกำจัดศัตรูพืชชนิดไหนที่ท่านใช้บ่อยที่สุด

( ) สารกำจัดวัชพืช

( ) สารกำจัดแมลง

( ) สารกำจัดเชื้อรา

( ) อื่นๆ ....

2.8 ขั้นตอนการใช้สารกำจัดศัตรูพืชของคุณ (เลือกได้มากกว่า 1 ข้อ)

( ) ฉีดพ่นสาร ( ) ผสมสาร

( ) เทสารใส่เครื่องฉีด ( ) อื่นๆ......................

2.9 เวลาใดที่ท่านทำการฉีดพ่นสารกำจัดศัตรูพืช

( ) ช่วงเช้า 6.00 - 9.00 น. ( ) ช่วงสาย 9.01 – 12.00 น.

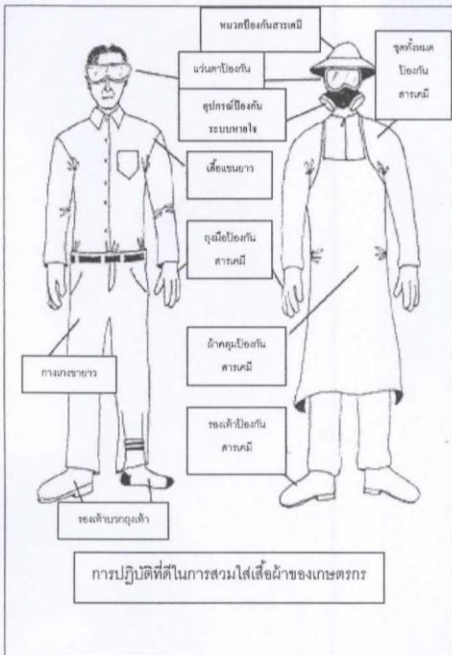
( ) ช่วงบ่าย 1.00 – 4.00 โมงเย็น ( ) ช่วงเย็น หลังจาก 4.00 โมงเย็น

<u>ส่วนที่ 3</u>: **การปฏิบัติของการใช้สารกำจัดศัตรูพืช**

3.1 ท่านสวมใส่อุปกรณ์ป้องกันตัวส่วนบุคคลหรือไม่ อย่างไร *(ภายใน 6 เดือนที่ผ่านมา)*

อุปกรณ์	ทุกครั้ง	บางครั้ง	ไม่เคย
1. หมวก (Hat)			
2. หน้ากากกระดาษหรือผ้าเช็ดหน้า (Paper mask or handkerchief)			
3. เสื้อเชิ้ตแขนยาว (Long sleeve shirt)			
4. กางเกงขายาว (Long pants)			
5. ถุงมือ (Gloves)			
6. รองเท้าบูท (Boots)			
7. แว่นตากันลม/ฝุ่น (Goggles)			
8. ผ้าคลุม (Apron)			
9. ทั้งหมด (Overall)			

เวลาที่ท่านสวมใส่อุปกรณ์ป้องกันตัวส่วนบุคคล มีการสวมใส่ที่ถูกต้อง หรือไม่?



อุปกรณ์	อุปกรณ์ที่ใส่ถูกต้อง	
	ใช่	ไม่ใช่
1. หมวกป้องกันสารเคมี		
2. หน้ากากกรอง		
3. เสื้อเชิ้ตแขนยาว (พลาสติกหรือผ้า) กันน้ำ		
4. กางเกงขายาว (พลาสติกหรือผ้า) กันน้ำ		
5. ถุงมือ (พลาสติกหรือยางพารา) กันน้ำ		
6. รองเท้าบูทกันน้ำ		
7. แว่นตากันลม/ฝุ่น		
8. ผ้า (หรือพลาสติก) คลุมกันน้ำ		
9. ทั้งหมดป้องกันน้ำ		

3.2 แนวทางการปฏิบัติของท่านเกี่ยวกับสารกำจัดศัตรูพืชหรือไม่ อย่างไร ***(ภายใน 6 เดือนที่ผ่านมา)***

การจัดการเกี่ยวกับสารกำจัดศัตรู	ทุกครั้ง	บางครั้ง	ไม่เคย
1. อ่านฉลากผลิตภัณฑ์ก่อนใช้			
2. ใช้สารกำจัดศัตรูพืชตามที่ฉลากกำหนด			
3. สวมถุงมือขณะที่ผสม, เทสารใส่เครื่องฉีด, ฉีดพ่นสารกำจัดศัตรูพืช			
4. ผสมสารกำจัดศัตรูพืชด้วยตนเอง			
5. ล้างมือก่อนรับประทานอาหารและดื่มน้ำหลังจากใช้สารกำจัดศัตรูพืช			
6. อาบน้ำทันทีด้วยสบู่หรือครีมอาบน้ำหลังการใช้สารกำจัดศัตรูพืช			

การจัดการเกี่ยวกับสารกำจัดศัตรู (ต่อ)	ทุกครั้ง	บางครั้ง	ไม่เคย
7. แยกเสื้อผ้าที่สวมตอนใช้สารกำจัดศัตรูพืชออกจากเสื้อผ้าปกติในการซักผ้า			
8. ทำความสะอาดอุปกรณ์ลังจากใช้สารกำจัดศัตรูพืช			
9. เก็บผลิตภัณฑ์สารกำจัดศัตรูพืชไว้ที่บ้าน			
10. เก็บอุปกรณ์ที่ใช้กับสารกำจัดศัตรูพืชไว้ที่บ้าน			
11. ขุดหลุมเพื่อฝังขวดสารกำจัดศัตรูพืชที่ใช้แล้ว			
12. ในขณะที่ท่านใช้สารกำจัดศัตรูพืช (ผสม, เทสารใส่เครื่องฉีด, ฉีดพ่นสาร) ท่านสูบบุหรี่หรือไม่			
13. ในขณะที่ท่านใช้สารกำจัดศัตรูพืช (ผสม, เทสารใส่เครื่องฉีด, ฉีดพ่นสาร) ท่านดื่มเครื่องดื่มหรือไม่			

ส่วนที่ 4: ผลกระทบทางสุขภาพทั่วไปที่สังเกตได้หลังจากการสัมผัสสารกำจัดศัตรูพืชกลุ่มออร์กาโนฟอสเฟตแลคาร์บาเมต *(ภายใน 6 เดือนที่ผ่านมา)*

ผลกระทบทางสุขภาพ	ใช่	ไม่ใช่
ระบบทางเดินหายใจ		
- หายใจลำบาก		
- มีเสมหะปริมาณมาก		
- น้ำมูกไหล		
- หายใจเป็นจังหวะสั้นๆ		
ระบบทางเดินอาหาร		
- เบื่ออาหาร		
- คลื่นไส้/อาเจียน		
- ปวดท้อง		
ระบบทางเดินปัสสาวะ		
- กลั้นปัสสาวะไม่อยู่		
อวัยวะคัดหลั่ง		
- มีน้ำลายมากกว่าปกติ		
- เหงื่อออกมากกว่าปกติ		

ผลกระทบทางสุขภาพ (ต่อ)	ใช่	ไม่ใช่
อาการทางตา		
– มองภาพไม่ชัดเจน		
– น้ำตาไหล		
– ระคายเคือง		
อาการทางผิวหนัง		
– คัน/ผื่นขึ้น/มีรอยไหม้		
– มือชา		
– กล้ามเนื้อกระตุกหรือเป็นตะคริว		
ระบบประสาท		
– ปวดหัว		
– เวียนศีรษะ		
– นอนหลับไม่สนิท		
– พูดไม่ชัด		
– เดินโซเซ		
– มือสั่น		
– หงุดหงิดง่าย		
– มีปัญหาเกี่ยวกับความจำ		

รหัสโครงการวิจัย 006-1/57

วันที่รับรอง - 4 เม.ย. 2557

วันหมดอายุ - 3 เม.ย. 2558

Appendix E

แบบบันทึก การใช้สารกำจัดศัตรูพืช

รหัส............................ อายุ..................ปี ครั้งที่................

วัน/เดือน/ปี	สารที่ใช้	ลักษณะการใช้
	....	ระยะเวลาในการใช้.......................ชม ☐ ผสมสาร ☐ เทสารลงเครื่อง ☐ ฉีดพ่นสาร
	....	ระยะเวลาในการใช้.......................ชม ☐ ผสมสาร ☐ เทสารลงเครื่อง ☐ ฉีดพ่นสาร
	....	ระยะเวลาในการใช้.......................ชม ☐ ผสมสาร ☐ เทสารลงเครื่อง ☐ ฉีดพ่นสาร
	....	ระยะเวลาในการใช้.......................ชม ☐ ผสมสาร ☐ เทสารลงเครื่อง ☐ ฉีดพ่นสาร
	....	ระยะเวลาในการใช้.......................ชม ☐ ผสมสาร ☐ เทสารลงเครื่อง ☐ ฉีดพ่นสาร

Appendix F

How to use the EQM Test-mate Cholinesterase Test System, Model 400 (English version)

1. Open the machine by slide the switch on right hand

2. Choose "Mode" (AChE or PChE)
3. Press "Test" the display will show message "Insert new tube" Put new tube into machine (Don't touch the tube in the water area) and press "Test" again
4. Wait about 10 second. The display will show message "To remove tube" then remove the tube
5. Open tube and add the capillary tube which has blood
6. Close tube and shake it about 15 second
7. Put the tube in assay tube (in machine). And the capillary tube must straight the black point
8. Press "Test" and wait about 10 second
9. Open tube. Add the reagent (we must to add liquor about 3 drip in the reagent and mix together)
10. Close tube and shake it about 5 second after that put it in the assay tube.
11. Press "Test". The display will show message "shake assay tube"
12. Press "Test" again. The display will show message "incubation"
13. Wait about 1 min. after that the display will show message "reading". Wait about 50 second.
14. When the display show message "remove". Remove the tube.
15. Read and record data
16. Press "Done" and test the new sample

** AChE mode has 6 variables PChE mode has 4 variables

Appendix G

How to use the EQM Test-mate Cholinesterase Test System, Model 400 (Thai version)

วิธีการใช้การทดสอบ EQM เพื่อนร่วมทดสอบระบบ Cholinesterase รุ่น 400

1. วางอุปกรณ์ตามรูป

2. เปิดเครื่อง (slide ด้านขวาขึ้น)
เลือก Mode AChE หรือ PChE

3. กด Test ในหน้าจอจะขึ้น “Insert a new tube”
จับบนฝาเกลียวขาวค่อยๆใส่ในช่อง analyzer (ห้าม! จับขวดแก้วครึ่งล่างเพราะจะรบกวนทางผ่านของแสง)

4. กด Test หน้าจอจะขึ้น “blank” ใช้เวลาอ่านค่า 10 วินาที
ให้เอาขวดออกเมื่อขึ้น “To remove tube”

5. หมุนฝาเกลียวออก (ระวัง! อย่าสัมผัสขวดแก้วด้านล่าง) วางขวดใน tube rack วางฝาข้างๆ
กด Test จะแสดงหน้าจอ “add blood”

6. ใส่ถุงมือ powder free เพื่อเจาะเลือด ผู้ถูกเจาะเลือดควรล้างมือด้วยสบู่ก่อน

7. ใช้สำลีชุบแอลกอฮอล์ทำความสะอาดนิ้วที่จะเจาะ ปล่อยให้แห้ง 30 วินาที ใช้เข็มเจาะลงไป เอาเลือดหยดแรกออก รอให้เลือดหยดที่สองซึมออกมาแล้วเก็บด้วย capillary tube จนเต็มหลอด (ห้ามบีบนิ้ว ถ้าเลือดไม่พอให้เจาะใหม่)

8. เช็ดเลือดด้วยการกลิ้งปลายหลอดบนกระดาษกรอง แล้วใส่ลงไปใน assay tube หมุนปิดฝาให้แน่น จับที่ส่วนบนและล่างของหลอดเขย่าให้เลือดออกมากระจายอยู่ในน้ำยา 15 วินาที

9. ตะแคงขวดลงให้หลอด capillary tube มาอยู่ที่ข้างขวด ค่อยๆตั้งขวดแล้วใส่ลงไปในช่อง analyzer โดยให้ด้านที่มีหลอด capillary tube อยู่ตรงกับจุดสีดำ

10. กด Test เพื่ออ่านค่า 10 วินาที แล้วเอาหลอดออก เปิดฝาและวางไว้บน tube rack กด Test หน้าจอจะขึ้น “add reagent”

11. ใช้คีมตัดและดึงฝา reagent plate ทิ้งไป หยดน้ำกลั่นลงไป 3 หยด ใช้ pipette คนจนละลาย (ผงอาจเปลี่ยนจากสีขาวเป็นสีเหลืองตามอายุของสารเคมี แต่ไม่มีผลกับการวิเคราะห์)

12. เอียงถาดและใช้ pipette ดูดสาร reagent ออกมาให้หมด ใส่ลงใน assay tube กด Test ทันที หน้าจอจะขึ้น “shake assay tube”

13. ปิดฝาให้แน่นเขย่าเบาๆ 5 วินาทีเพื่อผสมสารให้เข้ากัน ตะแคงหลอดให้ capillary tube อยู่ที่ด้านใดด้านหนึ่ง ใส่ลงไปในช่อง analyzer ให้หลอดเล็กตรงกับจุดดำ

14. กด Test หน้าจอจะขึ้น “incubation” ใช้เวลาประมาณ 1 นาที (แต่ไม่เกิน 80 วินาที) จากนั้นหน้าจอจะขึ้น “reading” ประมาณ 50 วินาที

15. เมื่อหน้าจอโชว์ “removes” ให้นำขวดออกและทิ้งไป

16. กด Test เพื่อแสดงผลการทดสอบ บันทึกผลแต่ละค่าลงในสมุดบันทึก กด Test เพื่อดูการแสดงผลไปจนกระทั่งครบทุกค่าที่ต้องการ

17. AChE mode จะแสดง 6 ค่า
PChE mode จะแสดง 4 ค่า

18. กด done เพื่อเตรียมวิเคราะห์ตัวอย่างต่อไป

Appendix H

Chemical of Organophosphate group 65 kinds

1. Accphate
2. Azinphos ethyl
3. Azinphos methyl
4. Bromophos
5. Carbophenothion
6. Chlorpyriphos
7. Chlorpyriphos-ethyl
8. Chlorpyriphos-mthyl
9. Coumaphos
10. Cyanofenphos
11. Demeton
12. Dialifor
13. Diaminon
14. Dichlorienthion
15. Dichlorvos
16. Dicrotoplus
17. Dimethoate
18. Dioxathion
19. Disulfoton
20. EPN
21. Ethion
22. Ethoprop
23. Etrimphos
24. Fenchlorphos
25. Fenitrothion
26. Fenthion
27. Fensultfonthion
28. Fonophos
29. Formothion
30. Iodofenphos
31. Isofenphos
32. Isazophos

33. I.cptophos
34. Malathion
35. Mephospholan
36. Methamidophos
37. Methyl parathion
39. Mevinphos
40. Monocrotophos
41. Naled
42. Omethoate
43. Oxydemetom-methyl
44. Parathion
45. Phenthoate
46. Phorate
47. Phosalon
48. Phosmet
49. Phosphamidon
50. Phoxim
51. Pirimiphos-methyl
52. Profenophos
53. Propetamphos
54.Prothiophos
55. Prothoate
56. Quinalphos
57. Sulprophos
58.Temophos
59. Tetrochorvinphos
60. Thiometon
61.Triazophos
62.Trichlorfon
63.Trithion
64. Vamidothion
65. Isoxathion

Appendix I

Chemical of Carbamate group 27 kinds

1. Aldicarb
2. Amitraz
3. Bendiocarb
4. Benfuracarb
5. Binapacryl
6. BPMC
7. Carbarryl
8. Carbofuran
9. Carbosulfan
10. Cartap
11. Diflubenzuron
12. Formetanate
13. Isoprocarb
14. Methiocarb
15. Methomyl
16. Mexacarbate
17. MIPC
18. MTMC
19. Oxamyl
20. Pirimicarb
21. Promccarb
22. Propoxur
23. Teflubenzuron
24. Thiocyclam hydrogenoxalate
25. Thiodicarb
26. Thiofanox
27. Tsumacide

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