RISK ASSESSMENT OF CHLORPYRIFOS (ORGANOPHOSPHATE PESTICIDE) ASSOCIATED WITH DERMAL EXPOSURE IN CHILLI-GROWING FARMERS AT UBONRACHATANI PROVINCE, THAILAND

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การประเมินความเสี่ยงจากการได้รับสัมผัสคลอร์ไพรีฟอส (สารกำจัดศัตรูพืชกลุ่มออร์แกนโนฟอสเฟต) ผ่านทางผิวหนังของเกษตรกรผู้ปลูกพริก
จังหวัดอุบลราชธานี ประเทศไทย

นางสาวณัฏฐา ฐานีพานิชสกุล

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาสาธารณสุขศาสตรมหาบัณฑิต
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Thesis Title	RISK ASSESSMENT OF CHLORPYRIFOS (ORGANOPHOSPHATE PESTICIDE) ASSOCIATED WITH DERMAL EXPOSURE IN CHILLI-GROWING FARMERS AT UBONRACHATANI PROVINCE, THAILAND
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Accepted by College of Public Health Sciences, Chulalongkorn University in Partial Fullfillment of the Requirements for the Master Degree

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ณัฏฐา ฐานีพานิชสกุล: การประเมินความเสี่ยงจากการได้รับสัมผัสคลอร์ไพรีฟอส (สารกำจัดศัตรูพืชกลุ่มออร์แกนโนฟอสเฟต) ผ่านทางผิวหนังของเกษตรกรผู้ปลูกพริก จังหวัดอุบลราชธานี ประเทศไทย (RISK ASSESSMENT OF CHLORPYRIFOS (ORGANOPHOSPHATE PESTICIDE) ASSOCIATED WITH DERMAL EXPOSURE IN CHILLI-GROWING FARMERS AT UBONRACHATANI PROVINCE, THAILAND) อ.ที่ปรึกษาวิทยานิพนธ์หลัก: ดร.วัฒน์สิทธิ์ ศิริวงศ์ อ.ที่ปรึกษาวิทยานิพนธ์ร่วม: ศ.ดร.มาร์ค เกรกอรี รอบสัน, 62 หน้า

การประเมินความเสี่ยงจากการรับสัมผัสสารคลอร์ไพรีฟอส (กลุ่มออร์แกนโนฟอสเฟต) ผ่านทางการรับสัมผัสทางผิวหนังของเกษตรกรผู้ปลูกพริกในตำบลหัวเรือ อำเภอเมือง จังหวัดอุบลราชธานี ประเทศไทย ได้ทำการศึกษาในช่วงระหว่างเดือนธันวาคม 2552 ถึงเดือนมกราคม 2553 เพื่อตรวจวัดปริมาณสารคลอร์ไพรีฟอสตกค้างบนมือทั้งสองข้างของเกษตรกรผู้ปลูกพริกหลังจากการพ่นสารกำจัดศัตรูพืช โดยใช้เกษตรกรจำนวน 35 คน (ชาย 26 คน และหญิง 9 คน) โดยเทคนิคการสุ่มตัวอย่างแบบง่ายจากเกษตรกรผู้ปลูกพริกทั้งหมดในตำบลหัวเรือ ผลการศึกษาพบว่าเกษตรกรในกลุ่มนี้มีอายุระหว่าง 40 ถึง 50 ปี น้ำหนักเฉลี่ยเท่ากับ 56.34 (±11.11) กิโลกรัม พื้นผิวมือทั้งสองข้างของเกษตรกรผู้ปลูกพริกชายเท่ากับ 0.088 ตารางเมตร และหญิงเท่ากับ 0.075 ตารางเมตร ค่าเฉลี่ยความเข้มข้นของสารคลอร์ไพรีฟอสตกค้างบนผิวมือของเกษตรกรมีค่าเท่ากับ 6.95 ± 18.24 มก./กก. (0.01 – 98.59 มก./กก.) ในการศึกษาครั้งนี้ได้ทำการประเมินค่าการรับสัมผัสสูงสุดของเกษตรกรที่ระดับ 95 เปอร์เซนไทล์ เพื่อการป้องกันผลกระทบต่อสุขภาพของเกษตรกรจากการได้สัมผัสสารคลอร์ไพรีฟอสในปริมาณสูงสุดที่อาจจะเกิดขึ้นได้ จากการคำนวณค่าการรับสัมผัสสารต่อวันของเกษตรกรพบว่า ค่าการรับสัมผัสสารคลอร์ไพรีฟอสของเกษตรกรทั้งหมดเท่ากับ 2.51×10^{-9} มก./กก./วัน โดยกลุ่มเกษตรกรชายมีการรับสัมผัสต่อวันเท่ากับ 2.57×10^{-9} มก./กก./วัน มากกว่าเกษตรกรหญิง (2.41×10^{-9} มก./กก./วัน และทำการระบุความเสี่ยงโดยใช้ค่าดัชนีบ่งชี้อันตราย (Hazard Quotient, HQ) พบว่า กลุ่มเกษตรกรดังกล่าว อาจจะไม่ได้รับความเสี่ยงจากการรับสัมผัสสารคลอร์ไพรีฟอสทางการรับสัมผัสทางผิวหนัง เนื่องจากค่าดัชนีบ่งชี้อันตรายของเกษตรกรทั้งหมดเท่ากับ 1.67×10^{-6} (กลุ่มเกษตรกรชาย 1.71×10^{-6} และกลุ่มเกษตรกรหญิง 1.61×10^{-6}) ซึ่งมีค่าน้อยกว่าค่าที่ยอมรับได้ ($HQ < 1$) งานวิจัยนี้ได้เสนอแนะให้ทำการประเมินความเสี่ยงจากการได้รับสัมผัสสารคลอร์ไพรีฟอสผ่านการรับสัมผัสทางการสูดดมและการบริโภค เนื่องจากกลุ่มเกษตรกรได้ระบุว่ามีอาการเจ็บป่วยจากการรับสัมผัสสารในระยะเฉียบพลันและเรื้อรังหลังจากมีการฉีดพ่นคลอร์ไพรีฟอส

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NUTTA TANEEPANICHSKUL: RISK ASSESSMENT OF CHLORPYRIFOS (ORGANOPHOSPHATE PESTICIDE) ASSOCIATED WITH DERMAL EXPOSURE IN CHILLI-GROWING FARMERS AT UBONRACHATANI PROVINCE, THAILAND. THESIS ADVISOR: WATTASIT SIRIWONG, Ph.D., THESIS CO-ADVISOR: PROF. MARK G. ROBSON, Ph.D., 62 pp.

Risk assessment of Chlorpyrifos (Organophosphate Pesticide) associated with dermal exposure in chilli-growing farmers was studied during growing season from December 2009 to January 2010 at Hua-rau sub-district, Muang district, Ubonratchathani province, Thailand. Chlorpyrifos residue on chilli-growing farmers' hands after spraying were collected using hand-wiping technique from 35 farmers (26 men and 9 women) by using simple random sampling technique from all chilli-growing farmers in this area. The results showed that an age range of the participants was 40-50 years old. The average weight (mean±SD) was 56.3 ±11.1 Kg. Hand surface areas of male and female were 0.088 m^2 and 0.075 m^2, respectively. The mean concentration (±SD) of chlorpyrifos analyzed by using gas chromatograph with a selective detector, flame photometric detector (FPD) was 6.95 ±18.24 mg/kg/two hands (0.01 – 98.59 mg/kg/two hands). To evaluate health risk of the chilli-growing farmers in this community, an Average Daily Dose (ADD) was calculated using reasonable maximum exposure (RME) at 95th percentile of Chlorpyrifos concentration in order to health awareness and prevention. The ADD of farmers was 2.51×10^{-9} mg/kg/day and the ADD of male farmers (2.57×10^{-9} mg/kg/day) was higher than female farmers ($2.41 \text{ x } 10^{-9}$ mg/kg/day). Using hazard quotient (HQ) for risk characterization, it indicated that the HQ of farmers was lower than the acceptable level 1.0 (HQ = 1.67×10^{-6}). Both of the HQ for male and female farmers were lower than the acceptable level, 1.71×10^{-6} and 1.61×10^{-6}, respectively. In conclusion, the chilli-growing farmers were not at risk with non-carcinogenic effects from dermal exposure. This study suggests that other exposure routes e.g. inhalation and oral should be considered and evaluated because the farmers had mentioned on acute and repeated or prolonged effects of organophosphates after their application.

Field of Study : Public Health	Student's Signature: NUTTA TANEEPANICHSKUL
Academic Year: 2009	Advisor's Signature:
	Co-Advisor's Signature:

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For chilli-growing farmers, I would like to give my sincere thanks to their kindness and their friendly. My appreciation is offered to Mrs.Kranchana at Ubon Ratchathani university laboratory for her kindness and laboratory preparation. Moreover, I would like to thanks to my colleagues at Ubon Ratchathani, Mr.Karnchit and Mr.Sakchai, for their help in the data collection step. I also express my appreciation to Ms.Saowanee Narkaew for her great friendship and coordination in my field work.

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ABBREVIATIONS

ABS	= Absorption factor
ADD	= Average Daily Dose
BW	= Body Weight
C	= Concentration
CF	= Contact frequency
CR	= Contact rate
ED	= Exposure duration
EF	= Exposure frequency
HI	= Hazard Index
HQ	= Hazard Quotient
IRIS	= Integrated Risk Information System
LOAEL	= Lowest observed-adverse-effects level
NOAEL	= No- observed-adverse-effects level
RfD	= Reference dose
US EPA	= United State Environment Protection Agency

CHAPTER I
INTRODUCTION

1.1 Background and Significance of the Problem

Thailand is one of the most important countries in Southeast Asia to support agricultural products to the world. Thailand is the country's primary exports of agricultural goods. A half of population in the country is in agricultural sector and their mainly income to support their family also is earned from agricultural product (Agricultural Extension Department, 2007). Agricultural workers are the largest occupational group in developing countries (Hanshi, 2001).

Pesticides are a mainstay of pest control. Pesticides can be classified according to the types of pests which they destroy (Robson et al., 2005). A use of pesticides, such as insecticides, fungicides, insecticides, herbicides etc., is required to protect crop from pests and weeds (Sematong et al., 2008). There are over 5.6 billion pounds of pesticide were applied world-wide (Robson et al., 2005). Thousand tons of pesticides are imported to Thailand in order to keep high crop yields. Because of the pressure of yield, the heavy loads of pesticides are applied to the farms. Many drugs can be purchased without prescription and pesticides are widely used, especially in the home (Issaragrisil et al., 1997). Farmers commonly use pesticides in the organophosphate group, as they are highly effective (Jirachaiyabhas et al., 2004). Many pesticides have the potential to harm human health. The use of pesticides is steadily increasing. Pest resistance to pesticides in some developing countries, and aggressive marketing are among the causes for the growing use (Hanshi, 2001). Thus, some residues are contaminated the environment, such as soil, water, and air, and affect the health of humans in the crop area (Thapinta et al., 1998; Siriwong et al., 2007;2008;2009).

In Thailand, chilli is a famous agricultural product. Most of the products, include chilli, are grown in Northeast of Thailand. Most village households engage primarily in agriculture as their primary or secondary occupation (Coleman, 1999). Chilli is one of the crops that use a lot of load of pesticides. But chilli-growers are lack of knowledge to protect themselves from pesticide exposure. Chilli-growing farmers are getting risk because of lacking knowledge about pest and control. Most of them frequently use pesticide with overdose applications. Due to pricing of pesticides, short reentry intervals, and inefficient sprayer maintenance, not only farmers but also their family expose to these agro-chemicals and they are at risk in this situation (Rola et. al., 1993).

Hua rau sub-district, Muang district, Ubon Ratchahtani province, is a large area of agricultural. About 77.27% of family in this area is farmers. This research will try to use exposure assessment method to study about pesticide exposure (via dermal route) and estimate risk for chilli-growers in Ubon Ratchathani province

1.2 Research Question

1.2.1. What are the risk factors from Chlorpyrifos spraying among chilli-growing farmers?

1.2.2. Are chilli-growing farmers at risk from Chlorpyrifos spraying via dermal contact?

1.3 Hypothesis

1.3.1 Chilli-growing farmers in Hua rau sub-district are at risk of Chlorpyrifospesticide exposure from dermal pathway.

1.4 Purpose of the Study

The main objective of this study is to estimate organophosphate pesticides (OPPs) exposure through dermal contact. The specific objectives are:

1.4.1 To measure residue of Chlorpyrifos on chilli-growing farmers on the hands

1.4.2 To assess human risk associated with dermal exposure to Chlorpyrifos in chilli-growing farmers.

1.5 Benefit of the Study

1.5.1. The concentration of Chlorpyrifos, that chilli-growing farmers expose, will be estimated.

1.5.2. A risk of dermal exposure route in chilli-growing farmers will be assessed.

1.5.3. Chilli-growing farmers will aware and protect themselves from expose to Chlorpyrifos.

1.6 Brief Description of the Study Area

The study area is Hua-rua sub-district (a large area of chilli-growing), Muang district, Ubon Ratchathani province, Thailand (Figure 1.1).

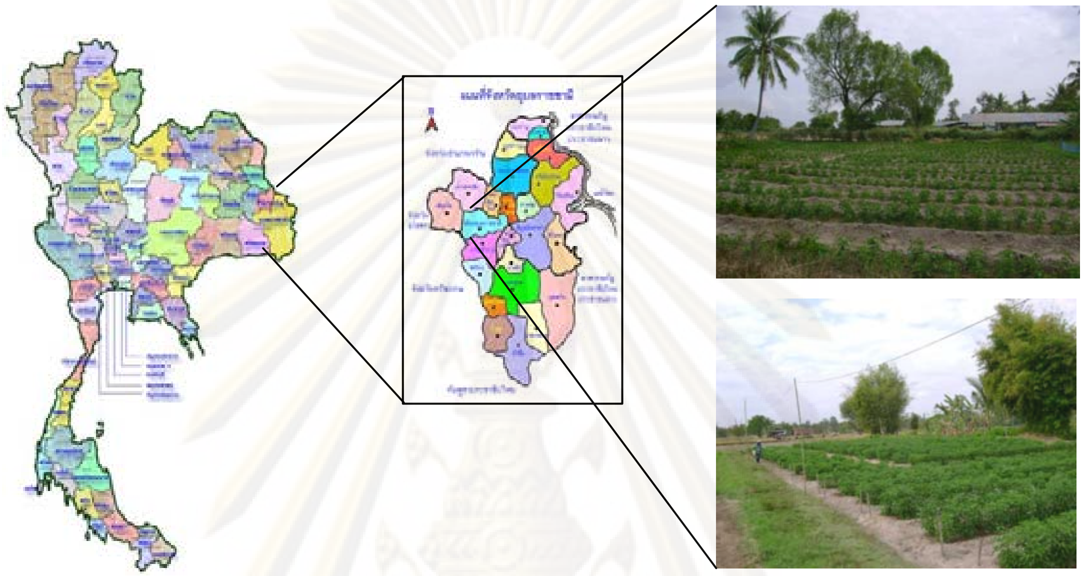


Figure1.1 The study area, Hua-rua sub-district, Muang district, Ubon Rachathani province, Thailand

1.7 Conceptual Framework

Dependent variables

Dermal contact
(Concentration of Chlorpyrifos)

Personal data

- Gender
- Body weight (kg)
- Age (years)
- Height (Centimeters)

Personal protect equipment

- Gloves
- Frequency of washing or cleaning Gloves

Field data

- Area cultivated (rai)
- Chlorpyrifos used (mL)
- Spray mix(CPF ml per L water)
- Duration of application (hr.)
- Tank Condition
- Frequency of spraying Chlorpyrifos

Independent variables

Exposure Assessment
Average Daily Dose (ADD)

Risk Characterization
Hazard Quotient (HQ)

Source : Aponso et.al.,2002

1.8 Operational Definitions

Dermal contact refers to the chilli-growing farmers who contact with Chlorpyrifos. The amount of exposure will depends on concentration of chemical and the dermal adherence, only hand (EPA, 1992).

Personal data refers to Chilli-growing farmers' body weight and age which are important factors for exposure assessment steps. These physiological factors are used fro calculating potential dermal exposures (EPA, 1997).

Personal protective equipment (Gloves) refers to Chilli-growing farmers use gloves to protect their hands form Chlopyrifos exposure.

Field data refers to all of details, such as spray mix and duration of application, which are investigate from chilli-growing farmers. The amount of exposure depends on concentration of chemical, the duration and frequency of contact to Chlorpyrifos (EPA, 1992).

Exposure Assessment means a process to determine of extension of humans, animals or other life exposure to hazardous agent. The concentration is a tool to measure the exposure. It depends on agent, duration, and presented frequency in environment (Robson et al., 2007)

CHAPTER II
LITERATURE REVIEW

2.1 Chlorpyrifos

Chlorpyrifos is an organophosphte compound. Chlorpyrifos has been used as pesticides or ingredient of pesticide products to spray on the farm to control crop pests. It may also be applied to crops in a microencapsulated form. According to EPA, tolerances of Chlorpyrifos defined as a raw agricultural commodities, foods, and animal feeds.

In the environment phase, Chlorpyrifos enters through volatilization, spills, and the disposal of chlorpyrifos waste. Volatilization is the major way when chlorpyrifos disperses into environment. Generally, Chlorpyrifos is broken down by sunlight, bacteria, or other chemical processes.

2.1.1 Chemical and Physical Properties

"O,O-diethyl- O-3,5,6-trichloro-2-pyridyl phosphorothioate" is a chemical name of Chlorpyrifos (Figure 2.1). The technical form is a white crystal-like solid with a strong odor (Table 2.1). It does not mix well with water, so it is usually mixed with oily liquids before it is applied to crops.

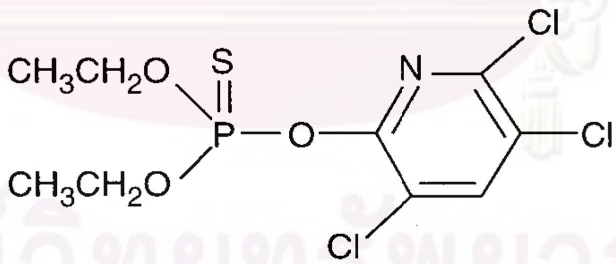


Figure 2.1 Structure of Chlorpyrifos

Table 2.1 Physical and Chemical properties of Chlorpyrifos

Characteristic	Information	Reference
Chemical name	O,O-diethyl O-(3,5,6-trichloro-2-pyridyl) phosphorothioate	Merck 1989
Synonym(s)	Phosphorothioic acid O,O-diethyl O-(3,5,6-trichloro-2-pyridinyl) ester; chlorpyrifos-ethyl; chlorpyriphos	Merck 1989
Registered trade name(s)	Dowco 179; ENT 27311; Dursban; Lorsban; Pyrinex; DMS-0971	Merck 1989
Chemical formula	$C_9H_{11}Cl_3NO_3PS$	Merck 1989

Property	Information	Reference
Molecular weight	350.57	Merck 1989
Color	White granular crystals White to tan Amber solid cake with amber oil Colorless crystals	Merck 1989 EPA 1988 Verschueren 1983 Worthing 1987
Physical state	Crystalline solid	EPA 1988
Melting point	41–42 °C	Merck 1989
Boiling point	Decomposes at approximately 160 °C	Verschueren 1983
Density at 43.5 °C	1.398 g/cm^3	Verschueren 1983
Odor	Mild mercaptan	EPA 1988
Odor threshold:		
Water	No data	
Air	No data	
Solubility:		
Water at 20 °C	0.7 mg/L	Bowman 1983
Water at 25 °C	2 mg/L	Merck 1989
Organic solvent(s)	79% w/w in isooctane 43% w/w in methanol Readily soluble in other organic solvents	Merck 1989
Partition coefficients:		
Log K_{ow}	4.82	McCall et al. 1980
Log K_{oc}	3.73	
Vapor pressure at 20 °C	1.87x10^{-5} mm Hg	Verschueren 1983
Vapor pressure at 25 °C	1.87x10^{-5} mm Hg	Merck 1989
Henry's law constant: at 25 °C	1.23x10^{-5} atm-m^3/mol	HSDB 1995
Autoignition temperature	No data	
Flashpoint	None	EPA 1988b
Flammability limits at 25 °C	No data	
Conversion factors (25 °C)	1 ppm=14.3 mg/m^3 1 mg/m^3=0.070 ppm	

Adopt from: Cattani, 2004

2.1.2 Mode of Action and Health Effects

Organophosphate pesticide exposure effected directly to inhibitor of the cholinesterase enzymes which from the basic of neurotransmission. Cholinesterase in human body has difference types which depend on the location in issue, substrate affinity, and physiological function.

Acetylcholinesterase (AChE), a kind of cholinesterase, presents beside nervous tissue and in red blood cell. AChE, normal condition, breakdown an acethylcholine, which is the chemical responsible for the physiological transmission of nerve impulses at different sites. The effects of organophosphate pesticide on human health are due to the inhibition of AChE. Especially Chlorpyrifos, the metabolism can be leading to rapid increasing in level of acetylcholine. It will be generated over stimulation of the nerve function.

The level of health effect depends on degree of exposure. Chronic or Acute effects are an association between cholinesterase inhibitor and exposure behavior. For instance, when inhibition occurs slowly and repeatedly, like chronic exposure, the correlation with illness may be low or nonexistent. On the other hand, if the correlation increases and the rate of inhibition is faster, it will be signs of acute intoxication. The association between AChE inhibition, level of poisoning and clinical symptoms are shown in Table2.2 (Cattani, 2004).

Table2.2 Severity and prognosis of acute organophosphate intoxication at different levels of AChE inhibition.

% AChE inhibition	Level of poisoning	Clinical symptoms	Progosis
50-60	Mild	Weakness, headache, dizziness, nausea, salivation, lacrimation, miosis, moderate bronchial spasm	Convalescense in 1-3 days
60-90	Moderate	Abrupt weakness, visual disturbances, excess salivation, sweating, vomiting, diahorrea, brachycardia, tremor of hands and head, disturbed gait, miosis, pain in the chest, cyanosis of the mucous membranes	Convalescense in 1-3 weeks
90-100	Severe	Abrupt tremor, generalized convulsions, psychic disturbance, intensive cyanosis, oedema of the lung, coma	Death from respiratory or cardiac failure

Source: Cattani, 2004

2.1.3 Metabolism

For toxicology study in the rat, 84 % of chlorpyrifos is excreted primarily in urine within 72 hours. The metabolism of chlorpyrifos in this case was extensive and changed parent compound was found in urine. The major urinary metabolites were 3,5,6-TCP, as well as glucuronide and sulfate conjugates of TCP.

In human (adult men), within 5 days following acute oral exposure approximately 70% of chlorpyrifos is excreted in the urine as TCP. The minimum dermal absorption is 1 to 3% of acute exposure. The mean pharmacokinetic half-life for 3,5,6-TCP in the urine was approximately 27 hours following both oral and dermal (EPA, 2000).

2.2 Environmental Health Risk Assessment

Risk is defined as a function of hazard and exposure. Basically, Risk Assessment is separated into four steps; Hazard identification, Dose-respond, Exposure assessment, and Risk characteristic. Each of steps will be described below (Robson et al., 2007):

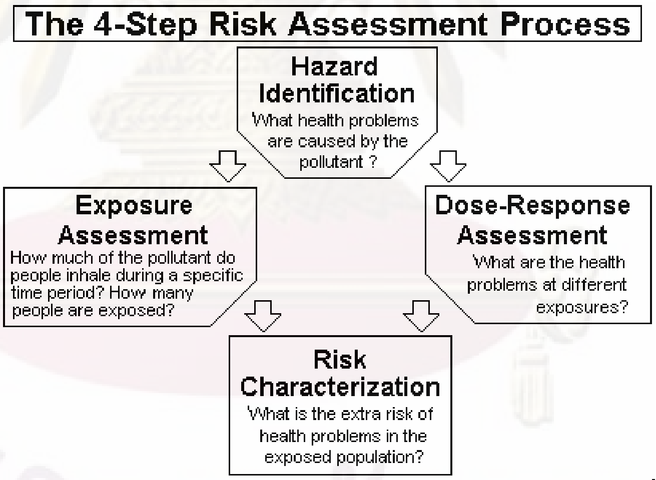


Figure 2.2 The 4-Step Risk Assessment Process

2.2.1 Hazard identification

The first step in the risk assessment process is to identify potential health effects that may occur from different types of pesticide exposure (US EPA, 2007a). Hazard identification is the process of determining when expose to chemical can cause of increasing in human health effects. This step always uses some data to support the health effect and chemical. For example, statistically controlled clinical studies on humans provide the best evidence that link between chemical and health effect. But, there is not a lot of available study in this method. Moreover, epidemiological studies involve a statistical evaluation of human populations to examine whether there is an association between exposure to a stressor and a human health effect. The advantage of these studies is that they involve humans who are weakness that result from expose to chemical. If the data of human is not available, the data from animal studies (rats, mice, rabbits, monkeys, dogs, etc) will be relied on to draw inference about the potential hazard to humans. But there are uncertainties associated with humans (IRIS, 2008).

2.2.2 **Dose-Response Assessment** (Cattani, 2004)

This step uses information that presented in the first step to estimate the amount of chemical that can be affected to human health. It attempt to combine qualitative measurements on the level of hazard in question. The association between level of exposure and health effect will be classified in this step (Robson et al., 2007). Dose-response assessment estimates potential risks to humans at exposure levels of interest. Dose-response assessments are useful in many applications: estimating risk at different exposure levels, estimating the risk reduction for different decision options, estimating the risk remaining after an action is taken, providing the risk information needed for benefit-cost analyses of different decision options, comparing risks across different agents or health effects, and setting research priorities. The purpose of the assessment should consider the quality of the data available, which will vary from case to case (US EPA, 2005).

The effect of Chlorpyrifos and its metabolites are classified as; acute cholinergic syndrome, intermediate syndrome and organophosphate-induced deployed polyneuropathy, and chronic.

Acute cholinergic syndrome

Acetylcholinesterase inhibition results in acetylcholine accumulation in cholinergic synapse. Hyperexcitation of postsynaptic neurons, that leads to autonomic and central nervous system symptoms, effect of this accumulation. There are some symptoms that are showed below (Cattani, 2004);

"headache, ocular pain, blurred version, miosis, conjunctival congestion, lachrymation, increased nasal secretion, increased salivation, chest tightness, bronchial secretions, laryngospasm, bracycardia, increased sweating, anorexia, nausea, vomiting, abdominal cramps, diarrhoea, fatigability, weakness, muscle twitching and fasciculations, confusions, confusion, slurred speed, are flexia, convulsions, coma, and respiratory paralysis"

The extent and combination of these symptoms varies in time of beginning, frequence and duration, depending on the dose and route of exposure.

Intermediate syndrome

Following absorption of high doses of organophosphates and treatment for acute symptoms a reversible muscle necrosis, distinct from delays polyneuropathy, and as such termed ‘intermediate syndrome’ has been reported (Cattani, 2004).

Organophosphate-induced deployed polyneuropathy (known as Delayed polyneuropathy)

Metabolism of chlorpyrifos in the river affects its active oxon, which causes the toxic effect of inhibition of target esterases in the peripheral and central nervous system. The clinical symptoms are distal degeneration of nerves together with ataxia or lower limb paralysis. After 2-4 weeks after a single exposure, the upper limbs are affected. In the period between exposure and development of symptoms the patient has usually recovered from acute symptoms, and is clinical normal (Cattani, 2004).

Chronic

Chlorpyrifos was evaluated for chronic toxicity in rats, mice and dogs. In all animal species, the most sensitive effect is inhibition of plasma, RBC and brain ChE that occurred at levels in the range of 0.03 to 3 mg/kg/day. Following chronic exposure dogs appear to be the most sensitive species for cholinesterase inhibition and systemic effects, as noted by increased liver weights in dogs exposed to 3 mg/kg/day that could be an adaptive response. Rats exposed to 7-10 mg/kg/day had decreased body weight and decreased body weight gain, ocular effects, adrenal gland effects and altered clinical chemistry and hematological parameters. Mice appear to be the least sensitive to chronic oral doses of chlorpyrifos, as exposure to 45-48 mg/kg/day resulted in decreased body weight and an increased incidence of non-neoplastic lesions (US EPA, 2000).

Reference Dose (RfD) of Chlorpyrifos (IRIS, 1998)

The Reference Dose (RfD) is depended on the assumption that thresholds of certain toxic effects. RfD is showed in units of mg/kg-day and derives for the noncarcinogenic health effects of substances that are also carcinogens. It is an estimate of a daily exposure to the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious effects during a lifetime. It can be derived from a NOAEL, LOAEL, or benchmark dose, with uncertainty factors generally applied to reflect limitations of the data used. So, the estimate value of Chlorpyrifos RfD is $3x10^{-3}$ mg/kg/day.

Experimental Doses

NOAEL (No observed adverse effect levels) where US EPA defines as the highest exposure level at which there are no biologically significant increases in the frequency or severity of adverse effect between the exposed population and its appropriate control; some effects may be produced at this level, but they are not considered adverse or precursors of adverse effects

NOAEL: 0.03 mg/kg/day

LOAEL (Lowest observed adverse effect levels) where US EPA the lowest exposure level at which there are biologically significant increases in frequency or severity of adverse effects between the exposed population and its appropriate control group.

LOAEL: 0.10 mg/kg/day

Uncertainty and Modifying Factors (UF)

The UF of 10 is the standard factor allowing for the range of human sensitivity for cholinesterase inhibition. It used in operationally deriving from experimental data. The factors are intended to account for;

1. Variation in susceptibility among the members of the human population (i.e., inter-individual or intraspecies variability)
2. Uncertainty in extrapolating animal data to humans (i.e., interspecies uncertainty)
3. Uncertainty in extrapolating from data obtained in a study with less-than-lifetime exposure (i.e., extrapolating from subchronic to chronic exposure)
4. Uncertainty in extrapolating from a LOAEL rather than from a NOAEL
5. Uncertainty associated with extrapolation when the database is incomplete.

2.2.3 Exposure Assessment (EPA, 1992a)

Exposure Assessment is a process to determine of extension of humans, animals or other life exposure to hazardous agent. The concentration is a tool to measure the exposure. It depends on agent, duration, and presented frequency in environment (Robson et.al., 2007). Exposure assessment is the determination (qualitative and quantitative) of the magnitude, frequency, and duration of exposure and internal dose. Exposure assessment generally consists of four major steps: defining the assessment questions, selecting or developing the conceptual and mathematical models, collecting data or selecting and evaluating available data, and exposure characterization (US EPA, 2005).

Human body has a hypothetical outer boundary separating inside and outside the body. The skin and the openings into the body such as the mouth the nostrils and punctures are called outer boundary. An exposure to chemical is defined as a contact of the chemical with outer boundary. “Exposure Assessment” is the evaluation of the contact. It concentrated on the intensity, frequency, and duration of contact, and often evaluates the rates at which the chemical crosses the boundary (chemical intake or uptake rates), the route by which it crosses the boundary (exposure route; e.g., dermal, oral, or respiratory), and the resulting amount of the chemical that actually crosses the boundary (a dose) and the amount absorbed (internal dose). In risk assessment, exposure assessment is a part that uses a dose-response relationship.

The chemical enter the body by two steps; contact (means exposure) and followed by cross the boundary. Due to crossing the boundary step, there are two processes; intake and uptake. Intake means that the chemical moves through an opening in outer boundary (mouth and nose). The chemical cross from outside to inside the body also called uptake. The chemical absorbs through the skin or other exposed tissue, such as eye.

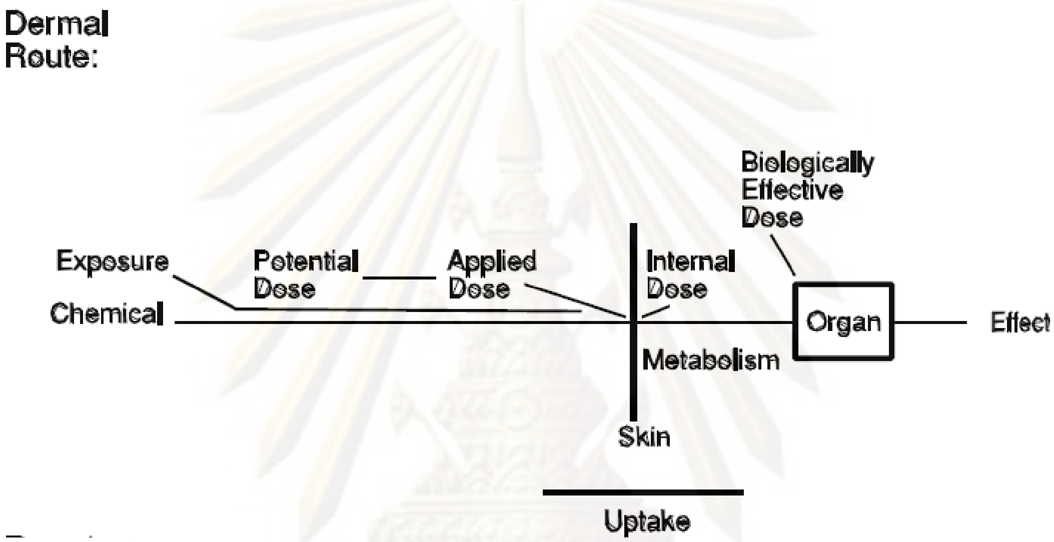


Figure 2.3 Dermal Schematic of dose and exposure
Source: EPA, 1992a

Figure 2.1 is Dermal Schematic of dose and exposure. The definition of each component describe below:

Exposure is the condition of a chemical contacting the outer boundary of a human. The chemical in the air is the exposure concentration.

Applied dose is the amount of a chemical at the skin that available for absorption. dose and internal dose sometimes has a relationship that can establish experimentally.

Potential dose is the amount of the chemical applied to the skin or the amount of chemical in the medium applied to skin.

Internal Dose is the amount of absorbed chemical and interacts with biologically significant receptors. After absorbed, the chemical can go through metabolism, storage, excretion, or transport within the body.

Occupational and residential exposures to chlorpyrifos can occur during handling, mixing, loading and applying activities. Occupational postapplication exposure can occur for agricultural workers during scouting, irrigation and harvesting activities. Residential postapplication exposure can occur following treatment of lawns, or residences for cockroaches, carpenter ants, termites, and other insects. In addition, there is a potential for inadvertent oral exposure to children from eating chlorpyrifos-treated turf and soil or hand to mouth activities following contact with treated surfaces or turf. Postapplication exposure to children can occur in locations other than the home, including schools, daycare centers, playgrounds, and parks. There is insufficient use information and exposure data to assess exposure resulting from use in vehicles and other current label uses such as treatment of indoor exposed wood surfaces, supermarkets, theaters, furniture, and draperies (US EPA, 2000).

Reasonable Maximum Exposure (RME)

The reasonable maximum exposure is defined as the highest exposure that is reasonably expected to occur at a site. It is likely to approximate the worst-case scenario and estimates for individual pathways. Exposure combination more than one pathway also represent as RME. The aim of the RME is to estimate a conservative exposure case that is still within the range of possible exposures. The RME excess risk estimates are representative of the most conservative exposure assumptions (Urban et al., 2009). The concentration term in the intake equation is the arithmetic average of concentration. It is contacted over the exposure period. However, this concentration does not indicate the maximum concentration that could be contacted at any one time. It is a reasonable estimate of the concentration likely to be contacted over time. In most situations, long-term contact with the maximum concentration is not assumed as reasonable. The uncertainty associated with any estimate of exposure concentration, the upper confidence limit (such as, the 95 percent upper confidence limit) on the arithmetic average will be used for this variable. If there is great variability in measured or modeled concentration values (such as too few samples), the upper confidence limit on the average concentration will be high, and possibly could be above the maximum detected or modeled value. In these cases, the maximum detected or modeled value should be used to estimate exposure concentrations. This could be regarded by some as too conservative an estimate, but given the uncertainty in the data in these situations, this approach is regarded as reasonable. For some sites, where a screening level analysis is regarded as sufficient to characterize potential exposures, calculation of the upper confidence limit on the arithmetic average is not required. In these cases, the maximum detected or modeled concentration should be used as the exposure concentration (US EPA, 1989; Siriwong, 2009a)

Average Daily Dose (ADD) Calculation

The average daily dose (ADD) is used for exposure to chemicals with non-carcinogenic non-chronic effects expressed as a daily dose on a per-unit-body-weight basis. ADD is a measurement that uses to estimate the exposure of non-carcinogenic effects. ADD is calculated by the route-specific mathematical algorithms. For dermal contact with chemicals in soil or water, dermal absorbed average daily dose can be estimated by the equation below (EPA, 1997):

$$ADD = \frac{DAevent \times EV \times ED \times EF \times SA}{BW \times AT} \qquad \text{eq. 1}$$

Where:

ADD	=	average daily dose (mg/kg-day)
DAevent	=	absorbed dose per event (mg/cm^2 -event)
EV	=	event frequency (events/day)
ED	=	exposure duration (years)
EF	=	exposure frequency (days/year)
SA	=	skin surface area available for contact (cm^2)
BW	=	body weight (kg)
AT	=	averaging time (days) for non-carcinogenic effects (AT = 45 years)

Application of Body Surface Area Data (Hands and Arms) (EPA, 1997)

The chemical can contact all of the body parts. It must be considered to estimate the total surface area of the body exposed. For exposure to both hands and arms, mean surface areas may be summed to estimate the total surface area exposed. The mean surface area of these body parts for men and women shows below:

Table2.3 Default values of surface area (m^2) recommended by US EPA

Surface Area (m^2)	Men	Women
Arms (includes upper arms and forearms)	0.228	0.210
Hands	0.084	0.075

Source: EPA, 1997

A formula to calculate body surface area is published in 1916. It can apply to calculate hand areas by using subjective height and weight. This model can be written below:

$$SA = a_0 H^{a1} W^{a2} \qquad \text{eq.2}$$

where:

SA	= surface area in square meters;
H	= height in centimeters; and
W	= weight in kg.
a_0, a_1, a_2	= constant values from US EPA (EPA, 1997)

2.2.4 Risk Characterization

Risk Characteristic is the important process that combines the result of analysis of effects and exposure assessment. It decrypts the nature and level of risk to human health, other life, or the environment. (Robson et.al., 2007) The risk characterization also brings together the assessments of hazard, dose response, and exposure to make risk estimates for the exposure scenarios of interest. This analysis that follows the summary is generally much more extensive. It typically will identify exposure scenarios of interest in decision making and present risk analyses associated with them. (US EPA, 2005).

Risk Characterization, the final step of risk assessment, summarizes both qualitative and quantitative findings. It is an instrument to communicate the findings of risk assessment to the risk manager or decision makers (Muller et al., 2005). The Chlorpyrifos is non-carcinogen pesticide. The criterion, that is used in non-carcinogen risk characterization, is reference dose (RfD). Individual Risk is a comparison between exposure and RfD. It indicates the degree of exposure; greater or less than RfD. The degree is called Hazard Quotient (HQ) (Siriwong, 2006; Jaipieam, 2008).

$$\text{Hazard Quatient(HQ)} = \text{Exposure/RfD} \qquad \text{eq. 3}$$

Where:

Exposure = total exposure to a single contaminant from source(mg/kg-d)

RfD = reference dose or other non-carcinogenic exposure limit

2.3 Related Article

Aponzo *et al.* (2002) designed to assess the internal dose experienced and the risk for nineteen farmers exposed to parent compound, chlorpyrifos using measurement of urinary levels of 3,5,6 trichloro-2-pyridinol(TCP), the major metabolite of chlorpyrifos. Moreover questionnaire was used to record personal application information. It found that cumulative TCP of creatinine and was equivalent to an internal dose off chlorpyrifos. Moreover, TCP levels were correlated with the amount of active ingredient used in spray session and condition of the tanks and self-protection. Calculated dermal dose ranged from 4.8 to 19.6 µg/cm^{2}on exposed skin.

Brenner *et al.* (2002) studied an organophosphate acute poisoning by survey in hospitals in Israel. Ninety-seven patients from 6 different hospitals were participated in this study. The group of study composed of 64 men and 33 women. The age range was 1-70 years (mean 19.8 years). Acute poisoning was the cause of intoxication of organophosphate in 51.5% of the patients, and suicide in 20.6% of exposures. Household insecticides and agricultural pesticides were the cause of intoxication in 64% and 36% respectively. The important route of intoxication was oral, inhalation and dermal respectively.

Coronado *et al.* (2006) studied on 218 farmworkers in 24 communities and labor camps in eastern Washington State, it examined the association between agricultural crop and OP pesticide metabolite concentrations in urine samples of adult farmworkers and their children and OP pesticide residues in house and vehicle dust samples. Farmworkers who worked in the pome fruits had significantly higher concentrations of dimethyl pesticide metabolites in their urine and elevated azinphos-methyl concentrations in their homes and vehicles than workers who did not work in these crops. Adult urinary concentrations showed significant correlations with both the vehicle and house-dust azinphos-methyl concentrations, and child urinary concentrations were correlated significantly with adult urinary concentrations and with the house-dust azinphos-methyl concentration.

Jaipieam et al. (2009) investigated inhalation exposure to organophosphate pesticides (OPPs) and evaluated the associated health risks to vegetable growers living in the Bang-Rieng agricultural community. Air samples were collected by using personal sampling pumps with sorbent tubes placed in the vegetable growers' breathing zone. Samples were collected during both wet and dry seasons. Residues of organophosphate pesticides, that is, chlorpyrifos, dicrotofos, and profenofos, were analyzed from 33 vegetable growers and 17 reference subjects. Results showed that median concentrations of OPPs in air in farm areas were in the range of 0.022–0.056 mg/m^3 and air in nonfarm areas in the range of <0.0016–<0.005 mg/m^3. The concentration of the three pesticides in the vegetable growers was significantly higher than that of the references during both seasons. The results also indicate that the vegetable growers may be at risk for acute adverse effects via the inhalation of chlorpyrifos and dicrotofos during pesticide application, mixing, loading, and spraying.

Kishi *et al.* (1995) studied on correlation between pesticide exposure and signs and symptoms of toxicity among Indonesian farmers. The study concentrated on acute illness and signs of poisoning by using interview at the time or within a few hours after spraying period. It also observed spray frequency and pesticide handling, dermal exposure, and the chemicals used. The study found that signs and symptoms occurred significantly more often during spraying than during nonspraying seasons. The number of spray operations per week, the use of hazardous pesticides, and skin and clothes being wetted with the spray solution were significantly and independently associated with the number of signs and symptoms. Moreover, the neurobehavioral signs and symptoms associated with the use of multiple organophosphates.

Kongtip et al. (2009) studied on assessment of health risk and cholinesterase levels due to chlorpyrifos exposure among rice farmers in Phatthalung Province. There was 31 study subjects used chlorpyrifos insecticides. Air samples were collected in the breathing zone of the rice farmers using versatile sampler tubes, containing a glass fiber filter and two sections of XAD-2 adsorbent. The accuracy, precision and detection limit of this method were also tested. Blood samples were collected and questionnaires were also administered by interviewers. The average occupational chlorpyifos exposure among rice farmers was 0.062 ± 0.092 mg/m3. Thirty subjects (96.8%) had been exposed to chlorpyrifos concentrations less than the TLV-TWA of 0.1 mg/m3 recommended by the American Conference of Governmental Industrial Hygienists (ACGIH). Many farmers had developed signs and symptoms, sweating (80.7%), chest tightness (32.3%), vomiting (25.8%) and blurred vision (35.5%). A high correlation coefficient was found between chlorpyrifos exposure and levels of cholinesterase in blood ($r=0.872$; $p=0.01$). The estimated daily intake of chlorpyrifos exposure through inhalation was 0.004 mg/ kg-day. The risk of exposure to chlorpyrifos was not acceptible ($HQ \geq 1$).

Lambert *et al.* (2005) studied on children of migrant farmworkers. They are at increased risk of exposure to organophosphate pesticides because of "carry-home" transport processes and residential location. Dialkyl phosphate (DAP) levels in serial samples of urine from 176 children, 2-6 years of age, in three Oregon communities are indicators in this study. Dimethylthiophosphate (DMTP), the most commonly detected metabolite, was significantly higher in urine samples from children in each of the three agricultural communities relative to a reference group of children who lived in an urban community and whose parents did not work in agriculture. The observed variability in urinary DAP levels, between communities and over time, could be attributed to the types and amounts of organophosphate pesticides used, the timing of applications and degradation of residues in the environment, work operations and hygiene practices.

Lee *et al.* (2007) studied an Chlorpyrifos exposure was associated with mortality in Iowa and North Carolina, United State among 55,071 pesticide applicators by using exposure data and other information were obtained from self-administered questionnaires. Poisson regression analysis was used to evaluate the exposure–response relationships between chlorpyrifos use and causes of death after adjustment for potential confounders. It found that the relative risk (RR) of death from all causes combined among applicators exposed to chlorpyrifos was slightly lower than that for non-exposed applicators. For most causes of death analyzed, there was no evidence of an exposure–response relationship. However, the findings may reflect a link between chlorpyrifos and depression or other neurobehavioral symptoms that deserves further evaluation.

L´ opez *et al.* (2009) studied an insecticides (Chlorpyrifos and/or Methamidophos) residue on hands of subsistence farmers in 12 communities of Nicaragua. Twenty-eight male farmers were participated in this study. The operation of the study combined mixing, filling and spraying a pesticide. A concentration of pesticide was collected by hand wiping samples with gauze (3 gauzes in each hand). Duration of applications was from 21 to 163 min (median 65 min). This study found that the total hand residues (n=30) were 791.0 (±1358.54) µg/observation period. The highest correlation was hand residue and application volume (r 0.43, p 0.02). Total hand residue correlated with not washing hands (r 0.41, p 0.04), spraying nozzle forward (r 0.26, p 0.17), manipulation of hose (r 0.32, p .09), and insecticide type (r 0.31, p 0.10).

CHAPTER III
RESEARCH METHODOLOGY

3.1 Study Design

This study was approved by The Ethical Review Committee for Research Involving Human Research Subjects, Health Science Group, Chulalongkorn University, Thailand with the certified code number No. 013/2010. All participants in this research had to agree with Participant Information sheet and signed in Informed Consent Form before they got involved in this study.

The research design of this study is “observational and experimental design”. The purpose of this study is to determine Chlorpyrifos exposure to farmers, who contact directly with this chemical, living in Hua-rua sub-district, Muang district, Ubon Ratchathani province. All samples were collected from December 200 to February 2010. Concentrations of Chlorpyrifos chilli-growing farmers’ hand were measured.

3.2 Study Population

All of participants in this study were chilli-growing farmers in Hua-rua sub-district, Muang district, Ubon Ratchathani Province. This research was concentrated on farmers who use Chlorpyrifos pesticide to control pest in chilli farm. Pre-survey and observation at pesticide selling store in the area of study found that Chlorpyrifos was a well known pesticide and widely used in this area. Moreover, most chilli-growing farmers always did not protect themselves from pesticide by using personal protection equipment (PPE) while they were spraying. A place of mixing and loading pesticide is around the farmers’ house or close to their kitchen. Some of them also lack of knowledge of using, mixing, or loading pesticides. Thus, this study measured the concentration of Chlorprifos that can be absorbed into farmers’ body by dermal route. The residue concentration were used for evaluate risk.

Approximately, thirty chilli-growing farmers found in randomly participate in this study. The samples were collected from after spraying pesticide immediately. Hand wipe method was applied for sample collection. Both hands of each subject were wiped and measured Chlorpyrifos concentration.

3.3 Sampling Method

Hua-rau subdistrict, Muang district, Ubon ratchathani Province was previously selected to be a study area because this area is one of the biggest chilli-growing area in

Thailand. Chilli-growing farmers in this area mainly grew a lot of ton of chilli in order to support both in and out country consumers.

Chilli-growing farmers were selected randomly by drawing technique from a group of chilli-growing farmers in this area; however, they were persons who apply Chlorpyrifos pesticide to the field directly. Most of them was the owner of farm and they mix and load volume of pesticide then apply to the field by themselves.

3.4 Data Collection

3.4.1 Questionnaire

Thirty chilli-growing farmers were interviewed. All of general information in this study, such as personal data, using PPE and field data, was collected by questionnaires. In this step, research assistances played a major role to interview chilli-growing farmers. Research assistance can well speak local language and familiar with the farmers in order to avoid communication bias.

All of questions in questionnaire are separated into 3 parts; General information, Personal protection equipment (PPE) data and field and pesticide using data (adopt from Aponso et al., 2002). Components of each part shows below. More information is in Appendix A.

General information

1. Gender
2. Body weight (kg)
3. Age (years)
4. Height (Centrimetes)

PPE data

1. Gloves
2. Frequency of washing or cleaning gloves

Field and pesticide using data

1. Area cultivated (rai)
2. Chlorpyrifos used (mL)
3. Spray mix(Chlorpyrifos ml per L water)
4. Duration of application (hr.)
5. Tank Condition
6. Frequency of spraying chlorpyrifos

3.4.2 Wipe Sample

Two moistened Gauze with 40% isopropanal was used to wipe pesticide on each hand of each farmer. Both hands were collected. Samples of each farmer were transferred to zip-lock bag and frozen until analysis step. After that, all of samples were kept in dry ice and sent to laboratory to analyze concentration of Chlorpyrifos by Gas Chromatography analysis.

3.5 Concentration Analysis

3.5.1 Extraction of Gauze Pads

The QuEChERs method is a multiresidue method for analysis of pesticide residue in low-fat products. QuEChERs method, which stands for quick, easy, cheap, effective, rugged, and safe, is a simple, fast, and inexpensive method for the determination of pesticide residues (Anastassiades, 2003). It entails extracting the pesticide residues from sample by vortex mixing with acetonitrile. Water is removed from the extrct by salting out with sodium chloride and magnesium sulfate and subsequent cleanup with a small quality of solid phase extraction (SPE) sorbent. QuEChERs method uses by Canadian and US Government regulatory agencies (Schenck, 2004).

After field collection, gauze pads were kept in the box at 4ºC until the GC-analysis process. Extraction and clean up samples in this research developed from QuEChERs method which showed the process in a flow chart (Figure 3.1). First, the gauze pad was weighted 1 gram approximately into a 50 mL centrifuge tube, which 5g NaCl, 10mL Acetonitrile(HPLC Grade), 2g $MgSO_4$ and 10 mL De-ionize water were added. Then, the tube was centrifuged for 10 minutes at 3,500 U/min, 5ºC. A supernatant was taken 5 mL and evaporated until the volume was less than 0.2 mL. After that, Acetonitrile HPLC grade was added to adjust volume to 1 mL, and 0.5g $MgSO_4$ and 0.5g PSA (Primary-Secondary Amine) were added. The tube was shaken by vortex mix machine for 1 min. Then, the supernatant was dispersive through Spring filter Nylon (0.2 µm diameter). Finally, it was injected to Gas Chromatography with Flame Photometric Detector (GC-FPD).

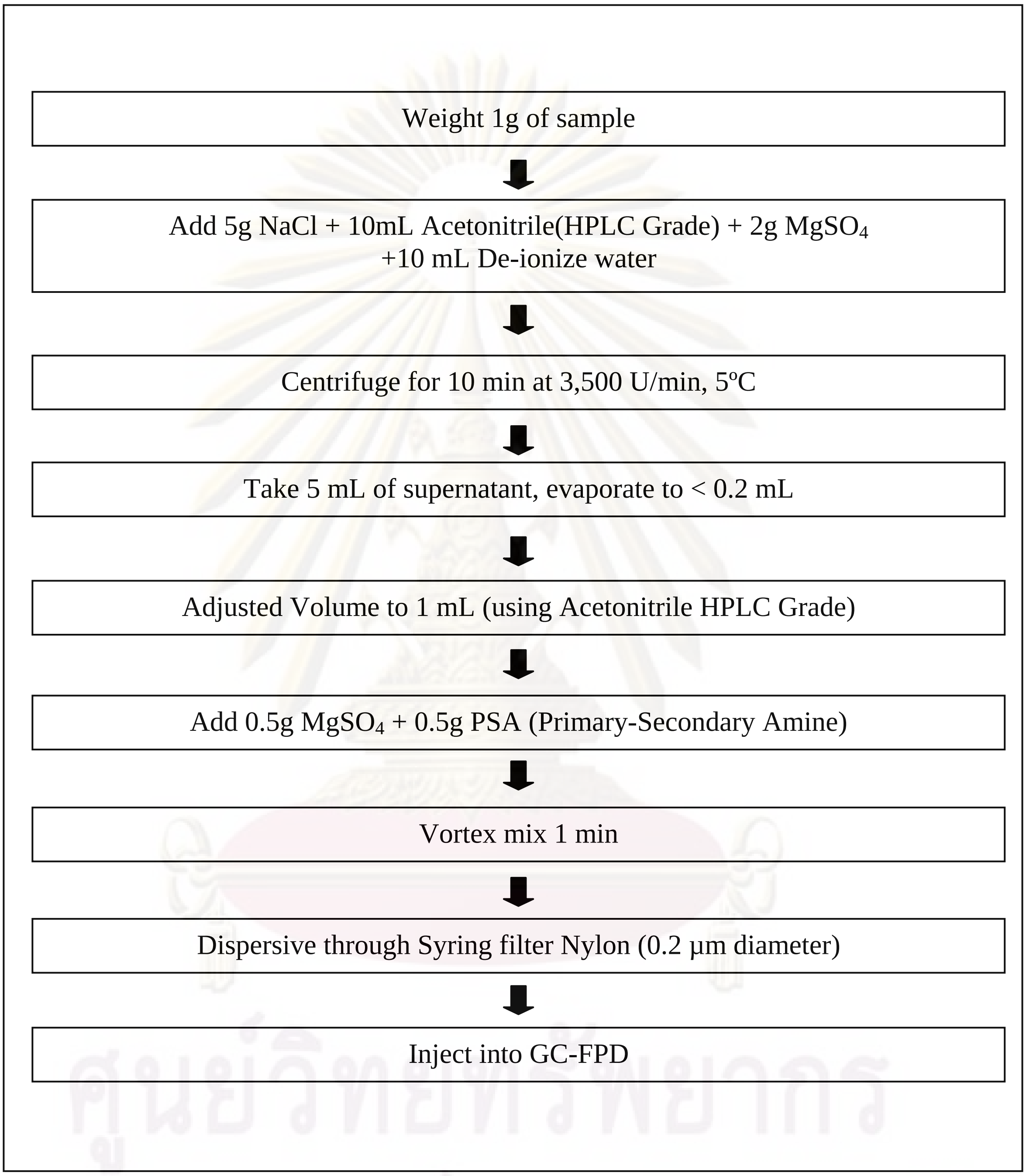


Figure 3.1 Flow Chart of the modified QuEChERs method

3.5.2 Gas Chromatography (GC) Analysis

In this study, an Agilent 6890N GC with Flame Photometric Detector was use to analyze concentration of wipe samples. The capillary column, which uses to separate compound, was DB-1701 (30.0 m length, 0.25 mm i.d., 0.25 µm film thickness) coated with 14% Cyanopropylphenyl and 86% methyl polysiloxane (J&W Scientific). External standards were used to perform sample quantification. A 2 µL of sample was injected into GC on splitless mode. The initial temperature of injection was 200ºC. The oven initial temperature was 80 ºC for 0 min, the programmed to increase at 12ºC/min to 195ºC. Then, it increased at 2ºC/min to 210ºC, held for 7 min. It increased to 225ºC at 15ºC/min, held 10min. The last temperature was 275ºC which increased at 35ºC/min and held for 7 min. Total run time was 24 min. The helium gas was used as a carrier gas with a flow rate at 1.5 mL/min.

3.5.3 Quality Control

All samples in this study were used DB-1701 (30.0 m length, 0.25 mm i.d., 0.25 µm film thickness). A calibration curve used external mixed standard. In appendix F showed quantitative recovery was 93%. The average precision of the matrices was 6.7% Relative standard deviation (RSD). Limit of detection (LOD) and limit of quantification (LOQ) were calculated from mixed external standard which are responded from 3 and 10 times the signal/ noise, respectively. The average LOD in this study was 0.050 ng/mL. The average LOQ was 0.100 ng/mL. The Method limit detection (MDL) was determined by multiplying the approximate (i.e. n-1 degree of freedom) one-sided 95th percent Student’s t-statistic ($t_{0.95}$) by the standard deviation (SD) which was from the replicate analyses of spiked matrices. MDL in this study was 30.95 ppb. All values were in the standard that AOAC Peer Verified Methods Program (1993) recommended (appendix F).

3.6 Data Analysis

3.6.1 Statistical Analysis

Data was analyzed by using SPSS for Window.

Mean, median and percentage, was described the general information of all population in the study. Moreover, this study concentrated on 95th percentile (upper bound) in order to over protection of all chilli-growing farmers.

3.6.2 Average Daily Dose(ADD) Calculation

ADD is a measurement that uses to estimate the exposure of non-carcinogenic effects. ADD is calculated by the route-specific mathematical algorithms that based on the equation below.

$$\text{ADD (mg/kg-day)} = \frac{\text{DAevent x EV x ED x EF x SA}}{\text{BW x AT}}$$

Where:

ADD	=	average daily dose (mg/kg-day)
DAevent	=	absorbed dose per event (mg/cm -event)
EV	=	event frequency (events/day)
ED	=	exposure duration (years)
EF	=	exposure frequency (days/year)
SA	=	skin surface area available for contact (cm)
BW	=	body weight (kg)

3.6.3 Non-Carcinogen Risk Estimation

Hazard Quotient (HQ) expresses the risk estimation in this condition. The non-carcinogenic effects are calculated by the relationship below:

Hazard Quotient (HQ)= Exposure / RfD

Exposure = chemical exposure level, or intake (mg/kg/day)
RfD = reference dose (mg/kg/day)

Where: $HQ > 1$ adverse non-carcinogenic effect concern
$HQ \leq 1$ acceptable level (no concern)

3.7 Ethic consideration

This study was approved by the Ethic Review Committee for Research Involving Human Research Subjects, Health Science Group, Chulalongkorn University (certified code number No. 013/2010). All participants signed a consent form prior to participation in this study

CHAPTER IV
RESULTS

4.1 General Information

In this study, there are 35 participants (26 men and 9 women) who get involved in this study. All of them are chilli-growing farmers who lived in the area of study. Both male and female were interviewed face to face by researcher. The questionnaires included both general information, such as gender, age, height, weight and pesticide using data, such as wearing gloves, spray mix (ml per 20 L water) and average times of spraying Chlorpyrifos. The data were illustrated in table 4.1.

The proportion between men and women who got involved in this study were 74.2% and 25.7% respectively. The results showed that age of male and female in the study was in the range of 41 – 50 years old and body weight was 51-60 kilograms. The average age and weight of this population were 56 (±11) years and 44.29 (±11.08) kilograms. The group of height was difference between men and women and average height for the subjects in this study was 161.31 (±7.89) centimeters, rank between 161 and 170 centimeters. The average height of men was 163.42 centimeters and women were 155.22 centimeters. About 60% of participants did not use glove as protective equipment during spraying period. However, few of chilli-growing farmers, who used gloves during farm period, did not reuse their gloves again. The result showed that most of them had never washed their glove. It also included farmers who did not use glove during farm period, so it was showed high percentage (79.4%). In this study, most chilli-growing farmers (65.7%) mixed Chlorpyrifos in an appropriate tank condition; however there was some leaking pesticide from the tank. The farmers mixed Chlorpyrifos with ratio of Chlorpyrifos (formulated product) 21-30 ml per 20L of water. The in average volume was 30.49 mL. Chilli-growing farmers (85.7% of participants) sprayed pesticide once per week, while some of them sprayed pesticide one time per two weeks. They also spent their time in the field to work approximately one hour including spraying pesticide and other agricultural activities. The duration of crop for chilli in this area was 5 month approximately. However, some farm in this area can grow either longer (about 6 months) or shorter (about 3 months) because the crop is depended on a kind of chilli, such as red, green, or black chilli.

TABLE 4.1 General information of chilli-growing farmers Hua-rau sub-district, Muang district, Ubon Ratchathani province, Thailand

General Information	Chilli-growing Farmers				Total	
	Male		Female			
	N	%	N	%	N	%
Gender	26	74.3	9	25.7	35	100
Age (years)						
≤ 30	4	11.4	-	-	4	11.4
31 – 40	5	14.3	2	5.7	7	20.0
41 – 50	10	28.6	5	14.3	15	42.9
51 – 60	5	14.3	2	5.7	7	20.0
≥ 61	2	5.7	-	-	2	5.7
Body Weight (kilograms)						
≤ 50	7	20.0	4	11.4	11	31.4
51 – 60	9	25.7	4	11.4	13	37.7
61 – 70	6	17.7	1	2.9	7	20.0
≥ 71	4	11.4	-	-	4	11.4
Height (centimeters)						
≤ 150	1	2.9	4	11.4	5	14.3
151 – 160	10	28.6	3	8.6	13	37.1
161 – 170	14	40.0	2	5.7	16	45.7
≥ 171	1	2.9	-	-	1	2.9
Use of PPE (Gloves)						
Use	6	17.1	1	2.9	7	20.0
Use but damage	3	8.6	4	11.4	7	20.0
Not use	17	48.6	4	11.4	21	60.0
Wash or clean gloves						
Not reuse	4	11.8	-	-	4	11.8
Once a week	3	32.2	1	2.9	4	35.1
Once a month	-	-	-	-	-	-
Never	19	55.9	8	23.5	27	79.4

General Information	Chilli-growing Farmers				Total	
	Male		Female			
	N	%	N	%	N	%
Formulated product						
(ml per 20 L water)	4	11.4	-	-	4	11.4
≤ 20	22	62.9	6	17.1	28	80.0
21 – 30	-	-	-	-	-	-
31 – 40	-	-	3	8.6	3	8.6
≥ 41						
Tank Condition						
Good	17	48.6	6	17.1	23	65.7
Average	9	25.7	3	8.6	12	34.3
Leaking	-	-	-	-	-	-
Frequency of Chlorpyrifos Spraying						
For a week						
One time / two week	2	5.7	2	5.7	4	11.4
One time / week	23	65.7	7	20.0	30	85.7
Two time / week	1	2.9	-	-	1	2.9
For a year						
3 months / year	3	8.6	-	-	3	8.6
4 months / year	-	-	-	-	-	-
5 months / year	22	62.9	7	20.0	29	82.8
6 months / year	1	2.9	2	5.7	3	8.6

4.2 Personal Monitoring (Hand Wiping Samples)

In the area of study, Hua-rau sub-district, Muang district, Ubon Ratchathani province, Thailand, chilli-growing farmers usually did not wear personal protective equipment, especially gloves. Mixing, loading and spraying pesticide on the farm became an important factor to expose to chemical. Wiping samples in this study was used to estimate residue pesticide. Chilli-growing farmers' both hands were wiped and collected. After samples collection, samples were kept in dry ice and sent to a laboratory for extraction and analysis of Chlopyrifos concentration.

There are 31 samples of chlopyrifos (figure 4.3) were collected in the field work. All concentrations which analyze by gas chromatography equipment were shown in Appendix D. The retention time of Chlorpyrifos in this analysis was 18 – 19 min approximately (figure 4.1). The mean, maximum and minimum of chlopyrifos concentrations (mg/kg) include dose estimates, values were shown in table4.4. The mean of residue Chlorpyrifos concentration on chilli-growing farmers' hands is 6.95 (±18.24) mg/kg. The rank between minimum and maximum of chlorpyrifos concentration were 0.10 and 98.59 mg/kg, respectively. The reasonable maximum exposure (RME) at 95th percentile was estimated for protection and prevention of high dermal exposure farmers. Other difference percentiles (25th, 50th and 75th) were also calculated and showed as bar chart in the figure 4.2.

TABLE 4.2 Dose estimate of Chlopyrifos concentration on chilli-growing farmers' hands (mg/kg) in Hua-rau sub-district, Muang district, Ubon Ratchathani province, Thailand

	Mean	SD	Min	25th	50th	75th	95th	Max
Chlorpyrifos (mg/kg)	6.95	18.24	0.10	0.10	1.47	4.32	55.57	98.59

Figure 4.1 The chromatogram of Chlopyrifos residues in wipe sample on chilli-growing farmer both hands using DB-1702 (30.0 m length, 0.25 µm film thickness)

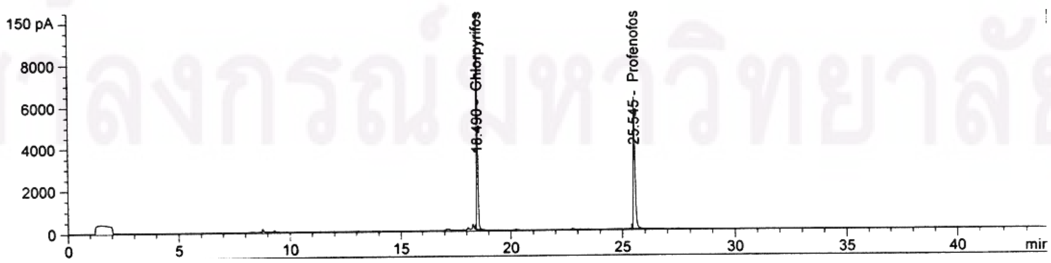


Figure 4.2 Chlorpyrifos concentration (mg/kg) by chilli-growing farmer in Hua-rua sub-district, Muang district, Ubon Ratchathani province, Thailand

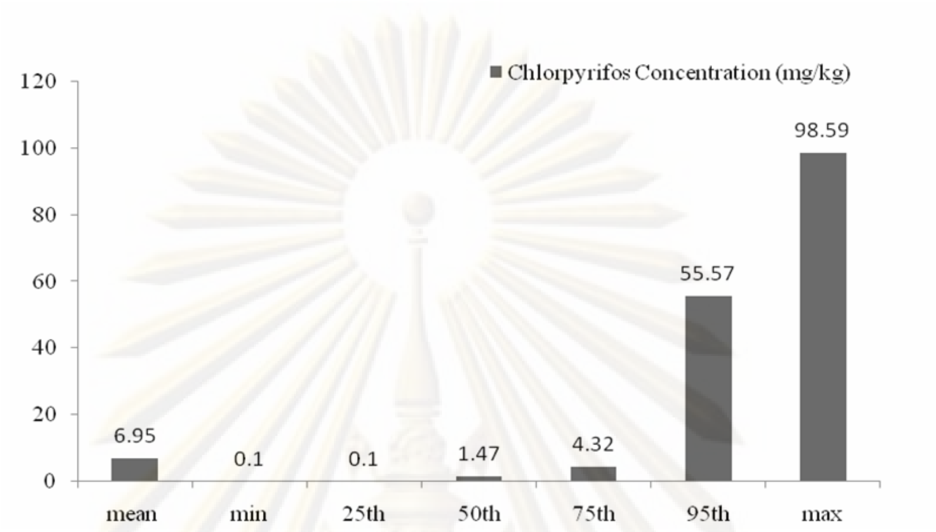
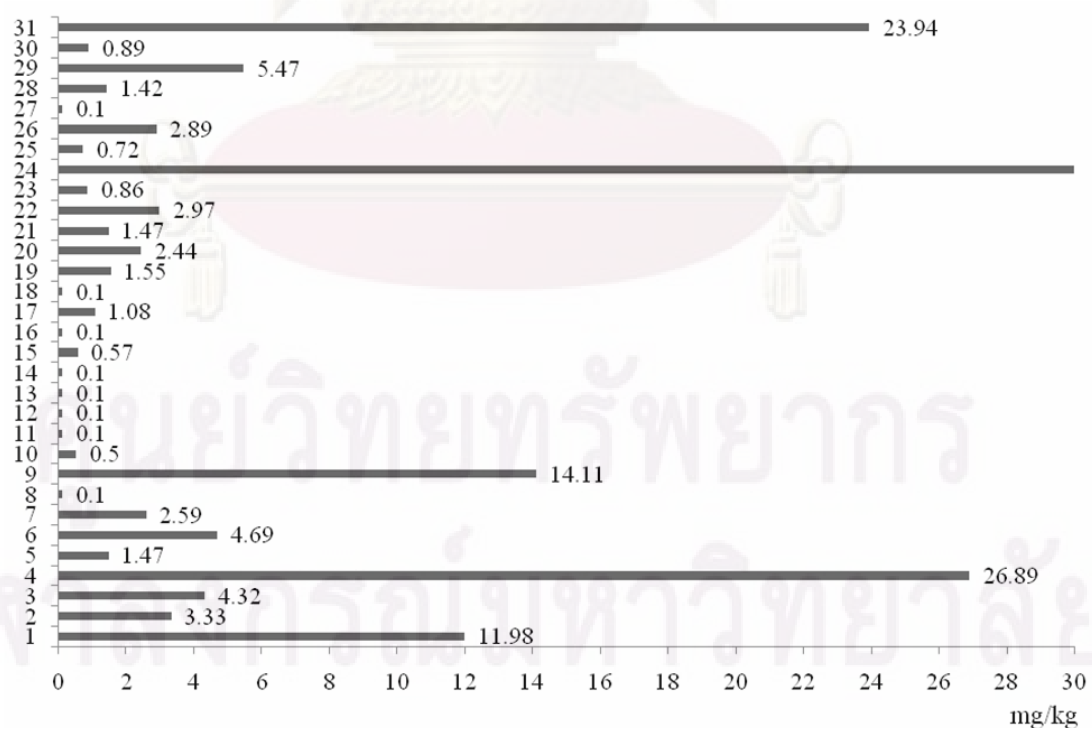


Figure 4.3 The 31 Chlorpyrifos Concentrations (mg/kg) by chilli-growing farmers in Hua-rua sub-district, Muang district, Ubon Ratchathani province, Thailand



4.3 Hand Surface Area

Due to Average daily dose calculation in Exposure Assessment step, hand surface area is one factor in the ADD equation (eq.1 in the chapter II). Hand Surface area of subjective in this study was calculated by the following equation.

$$SA = a_0 H^{a1} W^{a2}$$

Where:

SA	=	surface area (m^2)
H	=	height (cm)
W	=	weight (kg)
a_0, a_1, a_2	=	constant values from US EPA, 1997

The a_0, a_1 and a_2 in the equation are based on the US EPA's defaults values which are shown in the table in Appendix D.

Hand surface area of subjective in this study, include average height and average weight, were shown in Table 4.5.

TABLE 4.3 Average hand surface area of chilli-growing farmers in Hua-rua sub-district, Muang district, Ubon Ratchathani province, Thailand

Sex	**Average height (cm)**	**Average weight (kg)**	**a_0**	**a_1**	**a_2**	**Hand surface area (m^2)**
Male (n= 26)	163.42	59.27	0.0257	0.573	-0.218	0.088
Female (n=9)	155.22	51.00	0.013	0.412	0.0274	0.075

From table 4.5, average height and average weight of this population were calculated from general information data. The average height of male and female were 163.42 and 155.22 centimeters. Average weights were 59.27 kg for men and 51.00 kg for women. After calculation, the results showed that the average hand surface area of both male and female of this study were 0.088 m^2 and 0.075 m^2, respectively. From these values, they used as values to calculate in the ADD equation.

4.4 Exposure Estimation

4.4.1 Dermal (hands) Contact Exposure

The average daily dose of hands contact to pesticide was calculated by the ADD equation which recommended by US EPA. It was shown by the following equation (Jaipieam, 2008; US EPA, 1997).

$$ADD = (Cs \times SA \times DAevent \times EV \times ED \times EF) / (BW \times AT)$$

Where

ADD	=	Average daily dose (mg/kg/day)
Cs	=	Concentration of pesticide on both hands (mg/kg)
SA	=	Surface area (cm^2)
DAevent	=	absorbed dose per event (mg/cm^2-event)
EV	=	Event frequency (event/day)
ED	=	Exposure duration (years)
EF	=	Exposure frequency (day/year)
BW	=	Body weight (kg)
AT	=	Averaging time (days) for non-carcinogenic effects (ED x 365 days)

Adopt from: US EPA, 1997

The values of each factor in the equation were shown in the Table 4.6.

TABLE 4.4 Value of each factors in ADD equation for both male and female population in Hua-rua sub-district, Muang district, Ubon Ratchathani province

	Cs (mean) (mg/Kg)	**Cs (95th percentile)** (mg/Kg)	**SA**[a] (cm^2)	**DAevent**[b] ($mg/cm^2/h$)	**EV** (hour/day)	**ED** (years)	**EF** (days/year)	**BW** (kg)	**AT** (days)
Male	6.95	55.57	8.8×10^2	456×10^{-6}	1.00	44.38	19.54	58.19	16,198.70
Female	6.95	55.57	7.5×10^2	456×10^{-6}	1.00	44.00	18.89	51.00	16,060.00
Male & Female	6.95	55.57	8.2×10^2	456×10^{-6}	1.00	44.19	19.22	54.60	16,129.35

[a] SA values from direct calculation (table 4.5)

[b] DAevent value from Griffin et. al.,1999

The average daily dose (ADD) of Chlorpyrifos dermal (both hands) contact for female and male were estimated separately (showed in table 4.7). The ADD at 95th percentile was calculated in order to protect chilli-growing farmers who expose higher dose than others. The concentrations of Chlorpyrifos, which the farmers expose, are higher range between minimum and maximum (table 4.4)

The calculations of ADD at mean and 95th percentile for both in male, female and both of them were shown;

Male

$$ADD_{mean} = \frac{6.95 \text{ mg/kg} \times 10^{-6} \text{ kg/mg} \times 456 \times 10^{-6} \text{ mg/cm}^2\text{/h} \times 1 \text{ h/day} \times 44.38 \text{ years} \times 19.54 \text{ days/year} \times 8.8 \times 10^2 \text{ cm}^2}{58.19 \text{ kg} \times 44.38 \text{year} \times 365 \text{ days/year}}$$

$$= 2.57 \times 10^{-9} \text{ mg/kg-day}$$

$$ADD_{95th} = \frac{55.57 \text{ mg/kg} \times 10^{-6} \text{ kg/mg} \times 456 \times 10^{-6} \text{ mg/cm}^2\text{/h} \times 1 \text{ h/day} \times 44.38 \text{ years} \times 19.54 \text{ days/year} \times 8.8 \times 10^2 \text{ cm}^2}{58.19 \text{ kg} \times 44.38 \text{year} \times 365 \text{ days/year}}$$

$$= 2.05 \times 10^{-8} \text{ mg/kg-day}$$

Female

$$ADD_{mean} = \frac{6.95 \text{ mg/kg} \times 10^{-6} \text{ kg/mg} \times 456 \times 10^{-6} \text{ mg/cm}^2\text{/h} \times 1 \text{ h/day} \times 44.00 \text{ years} \times 18.89 \text{ days/year} \times 7.5 \times 10^2 \text{ cm}^2}{51.00 \text{ kg} \times 44.00 \text{ year} \times 365 \text{ days/year}}$$

$$= 2.41 \times 10^{-9} \text{ mg/kg-day}$$

$$ADD_{95th} = \frac{55.57 \text{ mg/kg} \times 10^{-6} \text{ kg/mg} \times 456 \times 10^{-6} \text{ mg/cm}^2\text{/h} \times 1 \text{ h/day} \times 44.00 \text{ years} \times 18.89 \text{ days/year} \times 7.5 \times 10^2 \text{ cm}^2}{51.00 \text{ kg} \times 44.00 \text{ year} \times 365 \text{ days/year}}$$

$$= 1.93 \times 10^{-8} \text{ mg/kg-day}$$

Male & Female

$$ADD_{mean} = \frac{6.95 \text{ mg/kg} \times 10^{-6} \text{ kg/mg} \times 456 \times 10^{-6} \text{ mg/cm}^2\text{/h.} \times 1 \text{ h/day} \times 44.19 \text{ years} \times 19.22 \text{ days/year} \times 8.2 \times 10^2 \text{ cm}^2}{54.6 \text{ kg} \times 44.19 \text{year} \times 365 \text{ days/year}}$$

$$= 2.51 \times 10^{-9} \text{ mg/kg-day}$$

$$ADD_{95th} = \frac{55.57 \text{ mg/kg} \times 10^{-6} \text{ kg/mg} \times 456 \times 10^{-6} \text{ mg/cm}^2\text{/h.} \times 1 \text{ h/day} \times 44.19 \text{ years} \times 19.22 \text{ days/year} \times 8.2 \times 10^2 \text{ cm}^2}{54.6 \text{ kg} \times 44.19 \text{year} \times 365 \text{ days/year}}$$

$$= 2.00 \times 10^{-8} \text{ mg/kg-day}$$

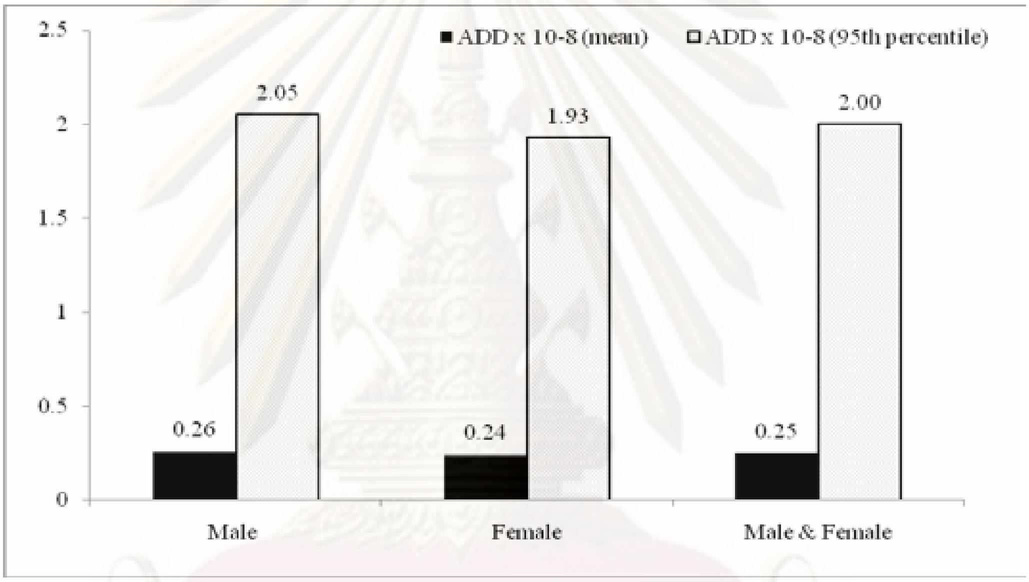
TABLE 4.5 Average daily dose of study population at Hua-rua sub-district, Muang district, Ubon Ratchathani province

ADD (mg/kg-day)	Male	Female	Male & Female
$ADD_{\text{at mean concentration}}$	2.57×10^{-9}	2.41×10^{-9}	2.51×10^{-9}
$ADD_{\text{at 95th percentile}}$	2.05×10^{-8}	1.93×10^{-8}	2.00×10^{-8}

From table 4.7, men's average daily dose (ADD) in this area was 2.57×10^{-9} mg/kg/day which is higher than women ADD (2.41×10^{-9} mg/kg/day) (figure 4.3).

However, the ADD for all participants in this study was 2.51 x 10^{-9} mg/kg/day. The reasonable maximum exposure (RME) at 95th percentile also estimated in this calculation. ADD for men and women were 2.05 x 10^{-8} and 1.93 x 10^{-8}, respectively. The results also showed that men in this area also expose to Chlorpyrifos more than women (figure 4.4). The ADD for both of them at RME level was 2.00 x 10^{-8} mg/kg/day.

Figure 4.4 Average daily dose (mg/kg/day) at mean and 95th percentile level by chilli-growing farmer in Hua-rua sub-district, Muang district, Ubon Ratchathani province, Thailand



4.5 Non-Carcinogenic Hazard Quotient

Hazard quotient (HQ) in this study was calculated by the following equation which recommended by US EPA (1997).

$$\text{Hazard Quotient (HQ)} \quad = \quad \text{Exposure / RfD}$$

Where:

Exposure = chemical exposure level (mg/kg/day)

RfD = reference dose (mg/kg/day)

HQ > 1 adverse non-carcinogenic effect concern

HQ ≤ 1 acceptable level (no concern)

In this study, ADDs in previous step were used in term of “exposure”. Chlorpyrifos dermal RfD equals to 0.0015 mg/kg/day (Jaipieam, 2008). Hazard

Quotients of the study population were calculated and showed in table 4.8. HQ at Reasonable maximum exposure (RME) 95th percentile was estimated in order to cover all chilli-growing farmer population.

TABLE 4.6 Hazard Quotient (HQ) of study population

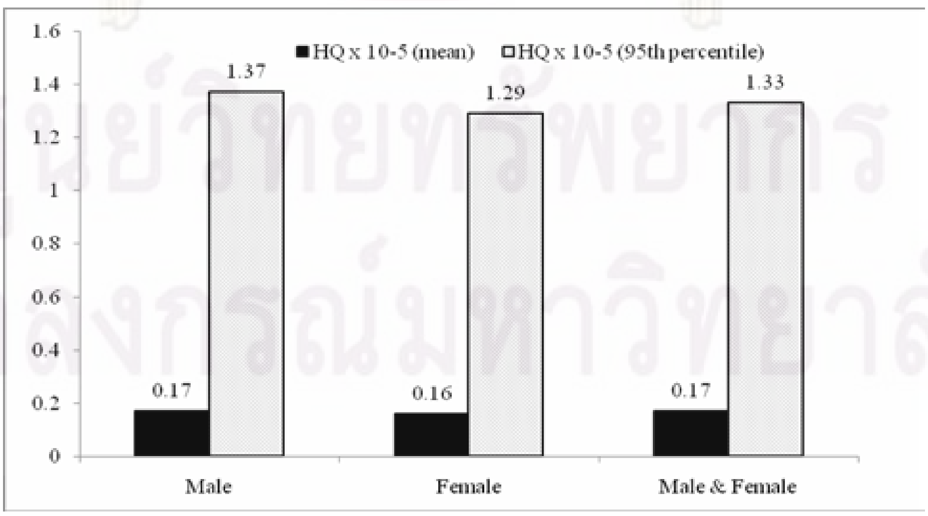
HQ	Dermal RfD* (mg/kg/day)	Male	Female	Male & Female
HQ_{mean}	0.0015	1.71 x 10^{-6}	1.61 x 10^{-6}	1.67 x 10^{-6}
$HQ_{95th\ percentile}$	0.0015	1.37 x 10^{-5}	1.29 x 10^{-5}	1.33 x 10^{-5}

*Dermal RfD value from Jaipieam (2008)

Hazard Quotients at mean level were 1.71 x 10^{-6} for male and 1.61 x 10^{-6} for women. HQ has no unit because "exposure term" and "RfD" have the same unit and they are divided. For male & female, HQ at mean was 1.67 x 10^{-6}. However, the results of HQ showed that chilli-growing farmers in this area were not getting from dermal exposure through their hands because HQ values were lower than 1, which is an acceptable level.

The RME at 95th percentile also showed the low HQ (Male = 1.37 x 10^{-5}, Female = 1.29 x 10^{-5} and Male & Female = 1.33 x 10^{-5}). HQ at RME level was higher that mean level (figure 4.5). However, the HQ values also lower than acceptable level (equal to 1). It indicated that chilli-growing farmers in this area of study did not get risk from dermal exposure (by hands) to Chlorpyrifos, although we considered at RME level. However, there is major route of exposure which the farmers might be getting risk that is inhalation.

Figure 4.5 Hazard Quotient and Hazard Quotient at 95th percentile by chilli-growing farmer in Hua-rua sub-district, Muang district, Ubon Ratchathani province, Thailand



CHAPTER V

DISCUSSIONS

5.1 Questionnaire Information

Chilli-growing farmers in Hua-rau sub-district, Muang district, Ubon Ratchathani province, Thailand who were participated in this study had average age 44 years old and the rank of age between 41 and 50 years old. The average age of rice farmers in Thailand were 44.0 years ranging from 23 to 63 years (Kongtip et al., 2009) and the average age of tangerine growers in the northern of Thailand also in the same range of age, between 41 and 60 years old (Chalermphol et al., 2009). The results show that the average age of farmers in Thailand were in the middle age group. The majority of chilli-growing farmers were male as same as other farmers (Kongtip et al.,2009; Jaipieam et al.,2009). Women applicators contributed 1% of the overall application of pesticide (Dosemeci et al., 2002). Chilli-growing farmers mixed loaded and sprayed chlorpyrifos by themselves. Dosemeci et al. (2002) also reported that 4% of pesticide applicators did not personally mix pesticides, 26% personally mixed the pesticide less than half of the time, and 70% personally mixed pesticides.

Most chilli-growing farmers in this area used good tank condition, hand spraying, which observed by the researcher, although the tank condition was good, but it had a little spill and leak from the containers. The use of hand spraying was the most common crop pesticide application technique (Dosemeci et al., 2002). Aponso et al. (2002) showed that there were some farmers used leaking spray tanks and they exposed through wet clothes. Most of them did not use gloves as personal protective equipment; therefore they might be getting risk through dermal contact to pesticide through their hands. The most common used PPE were rubber/chemically resistant gloves (Dosemeci et al.,2002). As well as, Blanco et al. (2005) found that none of the workers wore gloves, work practices such as blocking a leakage with bare hands, repairing the nozzles or inserting hands into the tank resulted in obvious contamination of the hands. Glove use was associated with the hand and total dermal exposure levels (Stewart et al., 1999). On the other hand, Nicol et al. (2008) demonstrated that individual equipment was variety use. Gloves were mostly worn, followed by a spraying suit and breathing protection. The half-day shift worked by the large majority of the sprayers indicates that pesticide spraying was usually only performed over certain hours of the day and it did not exposure for the whole day (Mekonnen et al., 2002). The concentration of the pesticide in the spray mixture, physical and chemical properties of the pesticide such as evaporation and skin penetration, and the time of residence of the pesticide from its deposition to the moment of sampling may play a role (Arago´n et al., 2006). Farmers are also exposed to pesticides while mixing, loading pesticide as well as while cleaning the equipment and disposing of empty containers. Other activities associated with exposure are sowing pesticide-preserved seeds, weeding and harvesting previously sprayed crops (Slhzdn, 2001). The applicator observed not to wear gloves during both the application and mixing operations, had the highest estimated exposure. Ninety percent of his exposure was on his hands (Karr et al., 1992).

5.2 Personal Monitoring (Hand Wiping Samples)

A hand wipe sampling and analysis procedure was developed for the measurement of dermal contact to pesticides (Geno et al., 1995). The 31 wiping samples were collected in this study. Both hands of chilli-growing farmers were individually wiped after spraying. The residue concentration of Chlorpyrifos on chilli-growing farmers' hands was 6.95 (± 18.24) mg/kg. Curwin et al. (2005) studied on the Chlorpyrifos concentration of hand wipe sample among farmers and non-farmers, it found that the concentration was between 0.36 and 19.00 ng/cm^2. In chilli-growing farmers study found wide range of concentration (0.1 – 98.59 mg/kg) greater than previous study of Jaipieam (2008). It studied on vegetable growers in Southern Thailand and found that residue of chlorpyrifos on hands was 0.070 mg/both hands. Karr et al. (1992) investigated six individual orchard organophosphate pesticide applicators. He found that the total estimated dermal exposure for ranged from 19 to 1235 µg. Another study suggested that mean chlorpyrifos surface concentrations varied across the body surface which was rank between 0.5 ng/cm^2 and 143.0 ng/cm^2 (Jagt et al., 2004). Another major route of expose to Chlorpyrifos was studies by many researchers. The average chlorpyrifos inhalation exposures of rice farmers were 0.062 ± 0.092 mg/m^3 ranging from 0.022 to 0.550 mg/m^3(Kongtip et al., 2009). The chlorpyrifos inhalation exposure concentrations did not exceed Recommended Exposure Limit (REL) of chlorpyrifos (0.6 mg/m^3) (Jaipieam et al., 2009).

About the quality constant, samples in this study analyses by the standard laboratory. Limit of detection (LOD) in this study was 0.050 ng/mL. Curwin et al. (2005) also studied on wipe sample and found that LOD was 0.12 ng/cm^2. A limit of quantitation (LOQ) was 0.10 ng/mL. The other study of analyze chlorpyrifos concentration showed that LOQ was 8 µg/L (Fenske et al., 2002). Method Detection Limit (MDL) was 30.95 ppb. The relative standard deviation and recovery of this analysis was 6.7% and 93% respectively. According to the Scientific Association Dedicated to Excellence in Analytical Methods (AOAC), all QC values showed the quality of this study was in the recommended standard level (Appendix E) (AOAC Peer Verified Methods Program, 1993).

5.3 Hand Surface Area

Hand surface areas of population in this study were calculated directly by the equation 1 which was recommended by US EPA (1997) (chapter II). Male and female hand surface areas were calculated separately. The study found that hand surface area for male and female were 0.088 m^2 and 0.075 m^2, respectively.

The direct calculation of hand surface areas was similar to the EPA default values for both male and female (table 5.1). There were some different of values substituted in the equation 2 (chapter II), such as weight and height, thus the values of area did not the same the default values.

Table5.1 Defaults values of surface area (m^2) recommended by US EPA

Surface Area (m^2)	Men	Women
Arms (includes upper arms and forearms)	0.228	0.210
Hands	0.084	0.075

Sources: US EPA,1997

In this study, both hands of farmers used as a part of body where usually contacted with pesticide during the spaying and mix period. During pouring and loading by hand, pesticides may contact to hands or other body parts of the sprayers (Mekonnen et al., 2002). Skin is the most exposed organ while spraying the pesticide on fields (Slhzdn, 2001). The frequent skin contact with pesticides was on the hands and face. About 30% of farmers had hand dermatitis, and more than two thirds had pigmentation and thickening on the hands (Guo et al., 1996). The applicators of organophosphate pesticide, the majority of observed exposure was to either the head or hands (Karr et al., 1992). Hands were the most frequently contaminated, and the back had the highest body segment scores which criteria by weighting the size of exposed body parts according to total body surface (Aregon et al., 2006) Stokes et al. (1995) found a significant increase in mean vibration threshold sensitivity for the dominant and non-dominant hand suggests previous organophosphate exposure among pesticide applicators was associated with a loss of peripheral nerve functions. Moreover, exposure pathway to residue chlorpyrifos was mainly found on the hands of the agricultural children (Fenske et al.,2002). The highest concentrations of Chlorpyrifos were found at the wrist and hands (Jagt et al., 2004).

5.4 Exposure Estimation

In this study, the average daily dose (ADD) of chilli-growing farmers via dermal exposure in Hua-rau sub-district, Muang district, Ubon Ratchathani province was 2.51 x 10^{-9} mg/kg/day. Jaipieam (2009) found that ADD of dermal exposure to chlorpyrifos in vegetable growers in Thailand was 3.23 x 10^{-5} mg/kg/day. Rigas et al.

(2001) studied on children exposure to chlorpyrifos using urinary biomarker measurements and found that the average dose of chlorpyrifos predicted by the model was 1.61 mg/kg per event. Average background dose rate for these children that reported exposure events was 0.0062 mg/kg/h, or 0.15 mg/kg/day. In the other study showed the dermal absorption of Chlorpyrifos was 3.61 x 10-4 nmol chlorpyrifos/cm^2/sec (Zartarian et al., 2000). Absorbed doses for the dermal exposure route were 10 ng/kg/day for Non-Occupational Exposure to Pesticides by all relevant pathways (Figueroa et al., 2008). Aggregate daily exposures for chlorpyrifos (inhalation, dietary and dermal) ranged from 13.5 ng/day to 12,821.0 ng/day, with a mean daily aggregate exposure of 1,390.0 ng/day (Weinhold, 2002). Albers et al. (2007) found that 3,5,6 trichloro-2-pyridinol, a chlorpyrifos metabolite, excretion among exposed workers suggested an estimated daily chlorpyrifos exposure averaging about 576 to 627 µg/day. Regression analyses revealed significant relations between dermal chlorpyrifos contamination and TCP in urine for 7 out of 10 investigated body regions. Exposure measurements by means of pads at the wrist, hands, and ankles were not significantly related to internal dose (Jagt et al., 2004).

Men, working on farm in this area of study, had higher ADD than women. In Thailand, most of farmers are men who have a responsible for earn income to their family. Therefore, they always exposed to agro-chemical in the field than women. Krieger et al. (2001) showed that farther whose family living in the spraying insecticide area exposed to the chemical more than mother. In contrast, the study showed that male children exposed to chemical lower than female children (Krieger et al., 2001).

5.5 Non-Carcinogenic Hazard Quotient (HQ)

The Hazard Quotients (HQ) of chilli-growing farmers in this study was not greater than 1.0 (HQ = 1.71 x 10^{-6}). Moreover, The reasonable maximum exposure (RME) at 95^{th} percentile also showed the low HQ than acceptable level (male = 1.37 x 10^{-5}, female = 1.29 x 10^{-5} and male & female = 1.33 x 10^{-5}). It is indicated that chilli-growing farmers in this area did not get risk from dermal exposure through their both hands. The weight of experimental evidence indicates that the risk of adults or children experiencing an adverse health effect from exposure to chlorpyrifos through both nondietary and dietary sources is insignificant (Gibson et al., 1998). The study of golfer exposure to chlorpyrifos via dermal and incidental ingestion pathways also found that the HQ for chlorpyrifos was not exceeded 1.0. The small RfD value determined for other chemical at similar levels to that of chlorpyrifos is estimated to be a great deal more hazardous. Therefore, choosing a pesticide that is less toxic can reduce the hazards associated with pesticide exposure (Cisar et al., 2001).

On the other hand, exposure to chlorpyrifos via oral route was found that hazard quotients were higher than acceptable level (HQ > 1). For example, Essumang et al. (2008) found that the risk assessment showed cancer risk for adults and children due to the presence of endosulfan and chlopyrifos through oral route. The hazard index for chlorpyrifos was greater than 1.0, which is a sign of contamination by chlorpyrifos.

Inhalation exposure was major possible route be assessed which should to access risk for chilli-growing farmers in this area. During the chlorpyrifos spray season measurable values were found in the air over a 28 day periods (Ramaprasad et al., 2009). The HQs for the median of children's acute exposures to chlorpyrifos via inhalation were 4.0 and 0.8, respectively (Harnly et al., 2005). Inhalation of indoor air contaminated with Chlorpyrifos accounted for 76.1% of the aggregate exposure to the population. However, chlorpyrifos is not very volatile (Weinhold, 2002).

5.6 Human Health Risk Management

Risk management is the process of identifying, evaluating, selecting, and implementing actions to reduce risk to human health and to ecosystems. The overall goal of risk management is to reduce or to prevent risks which related to social, cultural, ethical, political, and legal considerations in order to improve community's health (Charnley, 1998; US EPA, 1997). In this study, the result shows that chilli-growing farmers in this area may be not at risk from chlorpyrifos exposure. However, there are some evidences base showed that population in this area of study still had some effect from pesticide exposure, such as disability and mortality. Therefore, the other routes of exposure, such as inhalation and oral, should be consider as an important routes. To assess risk for other routes (inhalation and oral) is recommended for the future study. Moreover, other kind of pesticide should be concerned.

As a risk manager in this study, it can showed that personal care and personal manner of work, including the manner in which gloves are used, are other factors that were identified by some researches to affect dermal exposure. It is highly probably that the correct use of gloves leads to lower dermal exposure than insufficient use of gloves (Marquart et al., 2003).

Inhalation exposure is the main route of exposure to pesticide which should have a great concern (Weinhold, 2002). Using appropriated personal protective equipment (PPE) is an alternative to protect farmers' health. Marin et al. (2004) also found the need to wear personal protective equipment, including a respirator for the application of pesticides (Marin et al., 2004). Exposure to pesticides can be reduced by wearing PPE (Nicol et al., 2008). The prevention includes the knowledge of exposed workers with regard to the safe handling of chemicals should be suggested to farmers (Hanshi, 2001). However, farmers may recognize and concern about risk that pesticides may pose to their health and that of their families, but their decision to use exposure control practices may need to be negotiated through the cultural and practice norms of their community as well as the particular constraints of their own farming operation (Nicol et al., 2008).

Moreover, risk communication which is the process of informing people about potential hazards to their person, property, or community should apply to the community. The purpose of risk communication is to help residents of affected communities understand the processes of risk assessment and management, to form scientifically valid perceptions of the likely hazards, and to participate in making decisions about how risk should be managed. Risk communication tools are written, verbal, or visual statements containing information about risk. They should put a risks, include advice about risk reduction behavior, and encourage a dialogue between the sender and receiver of the message. The best risk communication occurs in contexts where the participants are informed, the process is fair, and the participants are free and able to solve whatever communication difficulties arise (US EPA, 2007b). The risk manager should educate the public about risks, risk analysis, and risk management , inform the public about specific risks and actions taken to alleviate them, encourage personal risk reduction measures, improve understanding of public values and concerns, increase mutual trust and credibility between the authorities and the public and resolve conflicts and controversies (US EPA, 2007c).

CHAPTER VI
CONCLUSIONS AND RECOMMRNDATIONS

6.1 Conclusions

In this study, it investigated the organophosphate pesticide (Chlorpyrifos) residue through dermal exposure (both hands) of chilli-growing farmers in Hua-rau sub-district, Muang district, Ubon Ratchathani province, Thailand. The results can be concluded as following.

1. The participants in this study were both male and female. Most of them were men; their age was between 41 and 50 years old. The weight and height of participants were in the range of 51-60 kilograms and 161-170 centimeters.

2. Most chilli-growing farmers in the area of study did not use gloves as protective equipment during spaying period. Spray mix condition of their pesticide was 21-30 mL per 20 L of water with good tank condition. The crop duration in this area was around 5 months in each year. The farmers also sprayed Chlorpyifos once a week in crop period.

3. Due to hand surfaces areas calculation, the value of hand areas in this population were close to US EPA default values. Male and female hand surface areas of this population were 0.088 and 0.075 respectively by direct calculation.

4. The Average daily dose (ADD) for chilli-growing farmer in the area of study was equal to 2.51 x 10^{-9} mg/kg/day. Male chilli-growing farmers' average daily dose (2.57 x 10^{-9}mg/kg/day) was higher than female (2.41 x 10^{-9}mg/kg/day). It indicated that men may expose to Chlorpyrifos more than women.

5. Hazard Quotient (HQ) of study population was not greater than 1.0 at both mean and RME 95th percentile level. Therefore, the value shows that chilli-growing farmers in this area of study were not at risk due to exposure to Chlorpyrifos through dermal (hands).

6. Risk management step was suggested to the area of study to concern other routes of exposure. Inhalation is another possible route which is recommended to evaluate risk because there are some studies showed that farmer could expose to pesticide during spraying period.

6.2 Contribution of this study

This research showed the organophosphate pesticide (Chlorpyrifos) exposure assessment of chilli-growing farmers in Hua-rau sub-district, Muang district, Ubon Ratchathani province, Thailand. This is the prior study of dermal exposure assessment which conducts in this area of study. Some of specific parameters of chilli-growing farmers, such as duration of spraying period, tank condition and duration of a crop, found in this study can be used to evaluate risk in other routes. Risk characterization was estimated to realize the situation in this area. Risk management and risk communication should provide for this community. Moreover, in this research also gave some suggestions of risk management to conduct for this area in the future.

5.3 Recommendations for future study

1. This study was took place in Hua rau sub-district, Muang district, Ubon Ratchathani province. Some chilli-growing farmers in this area are at risk, so intervention is needed to apply in this area in order to reduce risk and provide knowledge to the farmers.

2. This study determined only Chlorpyrifos dermal exposure after spraying period of day. The further study should determine other routes of exposure and other Organophosphate pesticide, such as Profenofos and Herbicide (such as Paraquot).

3. This study was concentrated on only spraying pesticide period. The chilli-growing farmers might be at risk from other agricultural activities, such as mixing and loading pesticide step. The further study should be concern about other activities.

4. Children and older people who are a susceptible in the area of study should be included for further study. They can be indirect exposure group form pesticides and agrochemicals.

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APPENDICES

APPENDIX A
(Questionnaire: English version)

Chlorpyrifos Exposure Questionnaire

Part I: General Information

1. Name____

2. Address_________________________________Moo_______

3. Gender O Male

O Female

4. Body weight ______________kg

5. Age_____________________ years

6. Height ___________________cm

Part II: Personal Protection Equipment data

6. Gloves O Use

O Use but Damage

O Not Use

7. Wash or clean gloves _________________times/week

Part III: Field and pesticide using data

8. Area cultivated___________________________ rai

9. Chlorpyrifos used_________________________ml

10. Spray mix(CPF ml per L water)__________________

11. Duration of application______________________hr.

12. Tank Condition O Good

O Average

O Leaking

13. Frequency of spraying Chlorpyrifos

____________________ times/day

____________________ times/week

____________________ times/month

APPENDIX B
(Questionnaire: Thai version)

แบบสอบถาม: การรับสัมผัสสารคลอไพริฟอส

ข้อมูลทั่วไป

1. ชื่อ____

2. ที่อยู่_________________________________หมู่ที่_________

3. เพศ O ชาย

O หญิง

4. น้ำหนัก______________กิโลกรัม

5.อายุ__________________ปี

6.ส่วนสูง_______________ซม.

ข้อมูล Personal Protection Equipment

6.ถุงมือ O ใช้และสภาพสมบูรณ์

O ใช้และสภาพมีรอยขาด

O ไม่ใช้

7.การซักล้างถุงมือ_______________ครั้ง/สัปดาห์

ข้อมูลพื้นที่และการใช้คลอไพริฟอส

8. พื้นที่____________________________ ไร่

9.ปริมาณคลอไพริฟอสที่ใช้_________________________มิลลิลิตร

10.อัตราส่วนการผสมสาร__________มิลลิลิตร ต่อน้ำ____________ลิตร

11.ระยะเวลาในการฉีดพ่น____________________ชั่วโมงต่อครั้ง

12.ลักษณะของถังผสมสาร O ดี O ปานกลาง O มีรอยรั่ว

13. ใน 1 วันมีการฉีดพ่น______________________ครั้ง

14. ใน 1 เดือนมีการฉีดพ่น____________________ครั้ง

15. ใน 1 สัปดาห์มีการฉีดพ่น__________________ครั้ง

APPENDIX C

TABLE 1-C Default values of parameters for calculating Hand Surface Areas (m^2)

Table 6-1. Summary of Equation Parameters for Calculating Adult Body Surface Area

Body Part	N	Equation for surface areas (m^2) a_0	W^{a1}	H^{a2}	P	R^2	S.E.
Head							
Female	57	0.0256	0.124	0.189	0.01	0.302	0.00678
Male	32	0.0492	0.339	-0.0950	0.01	0.222	0.0202
Trunk							
Female	57	0.188	0.647	-0.304	0.001	0.877	0.00567
Male	32	0.0240	0.808	-0.0131	0.001	0.894	0.0118
Upper Extremities							
Female	57	0.0288	0.341	0.175	0.001	0.526	0.00833
Male	48	0.00329	0.466	0.524	0.001	0.821	0.0101
Arms							
Female	13	0.00223	0.201	0.748	0.01	0.731	0.00996
Male	32	0.00111	0.616	0.561	0.001	0.892	0.0177
Upper Arms							
Male	6	8.70	0.741	-1.40	0.25	0.576	0.0387
Forearms							
Male	6	0.326	0.858	-0.895	0.05	0.897	0.0207
Hands							
Female	12[b]	0.0131	0.412	0.0274	0.1	0.447	0.0172
Male	32	0.0257	0.573	-0.218	0.001	0.575	0.0187
Lower Extremities[c]	105	0.00286	0.458	0.696	0.001	0.802	0.00633
Legs	45	0.00240	0.542	0.626	0.001	0.780	0.0130
Thighs	45	0.00352	0.629	0.379	0.001	0.739	0.0149
Lower legs	45	0.000276	0.416	0.973	0.001	0.727	0.0149
Feet	45	0.000618	0.372	0.725	0.001	0.651	0.0147

[a] $SA = a_0 \; W^{a1} \; H^{a2}$
W = Weight in kilograms; H = Height in centimeters; P = Level of significance; R^2 = Coefficient of determination;
SA = Surface Area; S.E. = Standard error; N = Number of observations
[b] One observation for a female whose body weight exceeded the 95 percentile was not used.
[c] Although two separate regressions were marginally indicated by the F test, pooling was done for consistency with individual components of lower extremities.
Source: U.S. EPA, 1985.

Source : US EPA, 1997

APPENDIX D

TABLE 1-D Chlorpyrifos concentrations on dermal contact (mg/ both hands) of chilli-growing farmers in Hua-rau sub-district, Muang district, Ubon Ratchathani province**,** Thailand

Samples	**Chlorpyrifos Concentration (mg/ both hands)**
1	11.98
2	3.33
3	4.32
4	26.89
5	1.47
6	4.69
7	2.59
8	0.10
9	14.11
10	0.50
11	< 0.01
12	< 0.01
13	< 0.10
14	< 0.01
15	0.57
17	1.08
18	< 0.01
19	1.55
20	2.44
21	1.47
22	2.97
23	0.86
24	98.59
25	0.72
26	2.89
27	< 0.01
28	1.42
29	5.47
30	0.89
31	23.94
Mean(±SD)	6.95 (±18.24)

*LOQ = 0.1

APPENDIX E

THE CHROMATOGRAM OF CHLORPYRIFOS RESIDUES

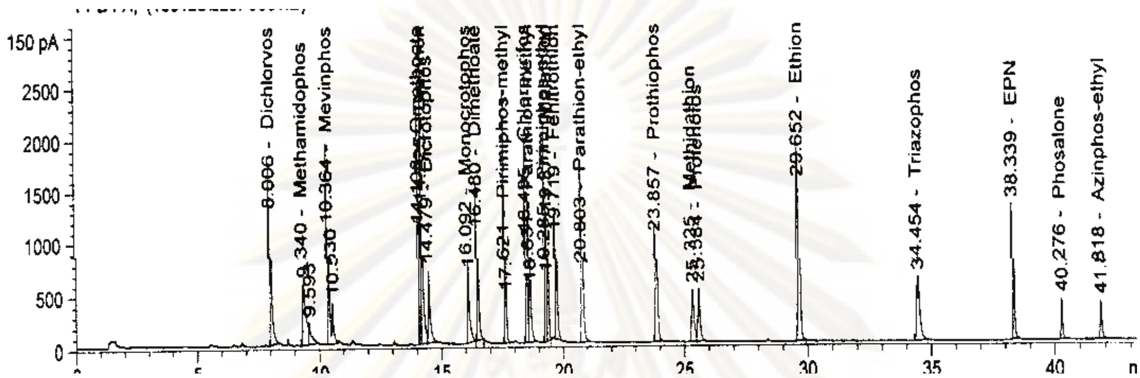

Figure 1-E The Chromatogram of organophosphate pesticide standard 2 µL using DB-1702 (30.0 m length, 0.25 µm film thickness)

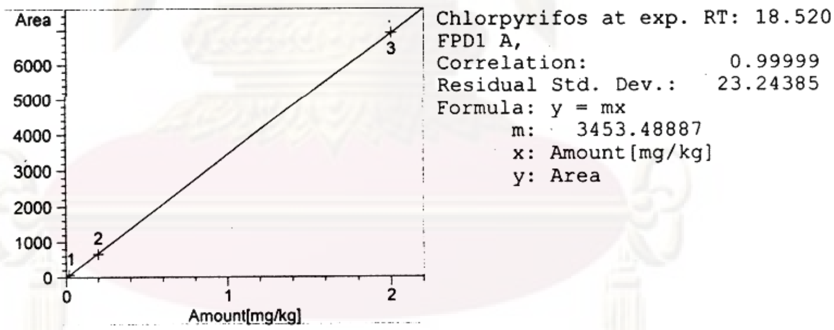

Figure 2-E The calibration curve of Chlorpyrifos

APPENDIX F
QUALITY CONTROL

TABLE 1-F Limit of detection (LOD), Limit of quantitation (LOQ), and method detection limit (MDL) of Organophosphate pesticide (Chlorpyrifos)

	LOD (ng/mL)	LOQ (ng/mL)	MDL (ppb)	RSD %	Recovery %
Chlorpyrifos	0.050	0.100	30.95	6.7	93

Limit of Detection (LOD) and Limit of Quantitation (LOQ)

The limit detection (LOD) is the lowest concentration level that can be determined to be statistically different from a blank (99% confidence). The LOD is determined to be in the region where the signal to noise ratio is greater than 5. Limits of detection are matrix, method, and analyze specific. In chromatography, results in peak with height at least twice or three times as the base line noise level is the detection limit. The limit of quantitation (LOQ) is the level above which quantitative results may be obtained with a specified degree of confidence. The LOQ is mathematically defined as equal to 10 times the standard deviation of the results for a series of replicates used to determine a justifiable limit of detection. It is the minimum injected amount that gives exact measurement. The LOD and LOQ can be calculated by the equation below (Siriwong, 2006).

$$LOD = 3\,\frac{Signal}{Noise} \qquad \text{(Equation F1)}$$

$$LOQ = 10\,\frac{Signal}{Noise} \qquad \text{(Equation F2)}$$

Method Detection Limit (MDL)

The method detection limit is the minimum concentration of a substance that can be measured and reported with 95% confidence that the analyze concentration is greater than zero. The MDL is determined by multiplying the appropriate (i.e., n-1 degree of freedom) one-sided 95 percent Student's t-statistic ($t_{0.95}$) by the standard deviation (SD) from a minimum of seven replicate analyses of spiked matrix sample containing analyze of interest at a concentration three to five times the estimated MDL.

$$MDL = t_{0.95(n-1)} \times SD \qquad \text{(Equation F3)}$$

Assessment of method precision

Relative Standard Deviation (RSD) or coefficient of variation (CV) used to estimate the precision for multiple samples. The % RSD was calculated from the equation below.

$$\%RSD = 100 \frac{SD}{Mean} \qquad \text{(Equation F4)}$$

The precision acceptance criterion depends on the type of analysis. The precision in environmental analysis depends on the sample matrix, the concentration of analyte and the analysis technique. It can vary between 2% and more than 20% (Siriwong, 2006).

TABLE 2-F Analyte concentration versus precision (relative standard deviation, RSD) recommended by AOAC

%Analyte	Analyte Ratio	Unit	%RSD
100	1	100%	1.3
10	10^{-1}	10%	2.8
1	10^{-2}	1%	2.7
0.1	10^{-3}	0.1%	3.7
0.01	10^{-4}	100 ppm	5.3
0.001	10^{-5}	10 ppm	7.3
0.0001	10^{-6}	1 ppm	11
0.00001	10^{-7}	100 ppb	15
0.000001	10^{-8}	10ppb	21
0.0000001	10^{-9}	1 ppb	30

Assessment of method accuracy

To access the method of accuracy is calculate by percent of recovery from analysis of reference materials, or laboratory control samples. The percent recovery is calculated by equation below.

$$\% \text{ Recovery} = [(M_s - M_u) / T_s] \times 100 \qquad \text{(Equation F5)}$$

Where;

M_s = Measured concentration of target analyte in the spiked sample

M_u = Measured concentration of target analyte in the unspiked sample

T_s = True concentration of target analyte added to the spiked sample

The concentration should cover the range of concern and should include one concentration close to the quantitation limit. The table3-F shows the estimate recovery data, recommended by AOAC.

TABLE 3-F Analyte recovery at different concentrations by AOAC

%Active Ingredient	Analyte Ratio	Unit	% Mean Recovery
100	1	100%	98-102
10	10^{-1}	10%	98-102
1	10^{-2}	1%	97-103
0.1	10^{-3}	0.1%	95-105
0.01	10^{-4}	100 ppm	90-107
0.001	10^{-5}	10 ppm	80-110
0.0001	10^{-6}	1 ppm	80-110
0.00001	10^{-7}	100 ppb	80-110
0.000001	10^{-8}	10ppb	60-115
0.0000001	10^{-9}	1 ppb	40-120

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