RISK ASSESSMENT OF ORGANOPHOSPHATE PESTICIDES FOR CHILLI CONSUMPTION IN CHILLI FARM AREA, UBONRACHATHANI PROVINCE, THAILAND

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การประเมินความเสี่ยงจากการได้รับสารกำจัดศัตรูพืชกลุ่มออร์แกนโนฟอสเฟต จากการบริโภคพริก ในพื้นที่จังหวัดอุบลราชธานี ประเทศไทย

นางสาวสุทธิสาร์ อุไรกุล

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรมหาบัณฑิต
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Thesis Title	RISK ASSESSMENT OF ORGANOPHOSPHATE PESTICIDES FOR CHILLI CONSUMPTION IN CHILLI FARM AREA, UBONRACHATHANI PROVINCE, THAILAND
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สุทธิสาร์ อุไรกุล : การประเมินความเสี่ยงจากการได้รับสารกำจัดศัตรูพืชกลุ่มออร์แกนโนฟอสเฟต จากการบริโภคพริกในพื้นที่ จังหวัดอุบลราชธานี ประเทศไทย (RISK ASSESSMENT OF ORGANOPHOSPHATE PESTICIDES FOR CHILLI CONSUMPTION IN CHILLI FARM AREA, UBONRACHATHANI PROVINCE, THAILAND) อ. ที่ปรึกษาวิทยานิพนธ์หลัก : อ.ดร.วัฒน์สิทธิ์ ศิริวงศ์, อ. ที่ปรึกษาวิทยานิพนธ์ร่วม: อ.ดร.สุมนา สิริพัฒนากุล, 115 หน้า.

การประเมินความเสี่ยงจากการได้รับสารกำจัดศัตรูพืชกลุ่มออร์แกนโนฟอสเฟตได้ศึกษาในผู้บริโภคพริกในตำบลหัวเรือ จังหวัดอุบลราชธานี ช่วงระหว่างเดือนตุลาคม 2553 ถึงเดือนกุมภาพันธ์ 2554 แบบสอบถามข้อมูลพื้นฐานและการบริโภคได้ทำการสำรวจจากประชากรในพื้นที่ทั้งหมด 110 คน ด้วยวิธีการสัมภาษณ์โดยตรงกับผู้ตอบ (ชาย 45 คน และหญิง 65 คน) ผลการศึกษาพบว่าประชากรในกลุ่มตัวอย่างนี้มีช่วงอายุระหว่าง 15 ถึง 79 ปี และมีน้ำหนักเฉลี่ย (± ความเบี่ยงเบนมาตรฐาน) 57 ±10 กิโลกรัม อัตราการบริโภคพริกเฉลี่ยของประชากรในพื้นที่นี้คือ 0.018 กก./วัน ซึ่งมีค่าสูงกว่าค่าเฉลี่ยของคนไทยทั่วไป (0.005 กก./วัน) สำหรับการวิเคราะห์สารกำจัดศัตรูพืชตกค้างในพริก โดยนำตัวอย่างพริกจำนวน 33 ตัวอย่าง มาสกัดโดยใช้วิธี QuEChERS และวิเคราะห์โดย GC-FPD ผลการวิเคราะห์ตรวจพบคลอร์ไพรีฟอสและโพรฟีโนฟอสระหว่าง <0.010-1.380 มก./กก. และ 0.520-6.290 มก./กก. ตามลำดับ โดยจากตัวอย่างทั้งหมดพบการปนเปื้อนคลอร์ไพรีฟอสจำนวน 9 ตัวอย่าง (27%) และโพรฟีโนฟอสจำนวน 5 ตัวอย่าง (15%) ที่มีปริมาณปนเปื้อนสูงกว่าค่า MRLs สำหรับการประเมินความเสี่ยงด้านสุขภาพ พบว่าปริมาณการรับสัมผัสคลอร์ไพรีฟอสเฉลี่ยต่อวันจากการบริโภคพริกในพื้นที่นี้ เท่ากับ 1.07×10^{-4} มก./กก./วัน และปริมาณการรับสัมผัสโพรฟีโนฟอสเฉลี่ยต่อวันจากการบริโภคพริกในพื้นที่นี้เท่ากับ 8.00×10^{-4} มก./กก. ผลการศึกษาชี้ให้เห็นว่า ผู้บริโภคอาจจะได้รับความเสี่ยงจากการรับสัมผัสโพรฟีโนฟอสมากกว่าคลอร์ไพรีฟอส การระบุความเสี่ยงของสารไม่ก่อมะเร็งโดยอาศัยค่าดัชนีบ่งชี้อันตราย (HQ) พบว่า ค่าดัชนีบ่งชี้อันตรายของคลอร์ไพรีฟอสสำหรับประชาชนในพื้นที่ อยู่ในระดับความเสี่ยงที่ยอมรับได้ (HQ<1) ส่วนค่า HQ ของโพรฟีโนฟอสมีค่าสูงกว่าค่าที่ยอมรับได้ (HQ>1) เมื่อพิจารณาที่ค่ารับสัมผัสสูงสุดของผู้บริโภคที่ระดับ 95 เปอร์เซนไทล์ และที่ค่าสูงสุดของสารโพรฟีโนฟอส พบว่ามีค่าสูงกว่าค่าปริมาณอ้างอิงมาตรฐาน (RfD) ถึง 45 และ 110 เท่า การศึกษานี้ชี้ให้เห็นว่าควรมีการจัดการความเสี่ยงที่เหมาะสมควรและดำเนินการอย่างเร่งด่วนในพื้นที่หัวเรือ เพื่อป้องกันและลดความเสี่ยงจากการบริโภคพริกที่มีสารกำจัดศัตรูพืชปนเปื้อนต่อไป

สาขาวิชา การจัดการสิ่งแวดล้อม ลายมือชื่อนิสิต……………………………………........................

ปีการศึกษา 2553 ลายมือชื่อ อ.ที่ปรึกษาวิทยานิพนธ์หลัก………………………...

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SUTISAR OORAIKUL : RISK ASSESSMENT OF ORGANOPHOSPHATE PESTICIDES FOR CHILLI CONSUMPTION IN CHILLI FARM AREA, UBONRACHATHANI PROVINCE, THAILAND. ADVISOR : WATTASIT SIRIWONG, Ph.D., CO-ADVISOR : SUMANA SIRIPATTANAKUL, Ph.D., 115 pp.

Risk assessment of organophosphate pesticides exposure was investigated in chilli consumption from October 2010 to February 2011 at Hua Rua sub-district, Ubonratchathani. Questionnaire-based, socio-demographic, and dietary surveys were completed by face-to-face interviewing among 110 local people (45 males and 65 females). The result showed that the age of participants ranged from 15 to 79 years. The average weight (±standard deviation) was 57±10 kg. The average chilli intake rate of people in this area was 0.018 kg/day which was higher than the average of general Thai people (0.005 kg/day). For determination of pesticide residue in chilli, thirty-three chilli samples were extracted followed QuEChERS method and analyzed by gas chromatography equipped with flame photometric detector. Chlorpyrifos and profenofos contaminated chilli with range of <0.010-1.380 mg/kg and 0.520-6.290 mg/kg were detected, respectively. Among all samples, 27% and 15% samples were found chlorpyrifos and profenofos contamination over MRLs, respectively. To evaluate potential health risk regarding chilli consumption, average daily dose (ADD) of chlorpyrifos and profenofos were 1.07×10^{-4} mg/kg-day and 8.00×10^{-4} mg/kg-day, respectively. The results indicated that the local chilli consumers exposure to profenofos was higher than that of chlorpyrifos. To characterize non-carcinogenic risk, hazard quotient (HQ) ratio was applied. The HQ for chlorpyrifos of the local people in this area was in the acceptable risk level (HQ<1.0) while the HQ for profenofos was over the acceptable risk level (HQ>1.0). It was found that the reasonable maximum exposure of profenofos (95^{th} percentile) and maximum levels were 45- and 110-times higher than the reference dose (RfD), respectively. It is suggested that appropriated risk management should be hastily implemented in Hua Rua area to reduce the risk due to the consumption of pesticide residues on chilli.

Field of Study : Environmental Management Student's Signature ....

Academic Year : 2010 Advisor's Signature ....

Co-advisor's Signature ....

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“Thank you, I love you so much” :)

CONTENTS

Page

LIST OF TABLES

Page

LIST OF FIGURES

ABBREVIATIONS

AchE	Acetylcholinesterase Enzyme
ADD	Average Daily Dose
ADI	Acceptable Daily Intake
AOAC	The Scientific Association Dedicated to Analytical Excellence®
AT	Average Time
BW	Body Weight
C	Pesticide Concentration
D-SPE	Dispersive Solid Phase Extraction
ED	Exposure Duration
EF	Exposure Frequency
FDA	US Food and Drug Administration
GCB	Graphitized Carbon Black
GC-FPD	Gas Chromatography- Flame Photometric Detector
HQ	Hazard Quotient
IR	Intake Rate
IRIS	US EPA’s Integrated Risk Information System
LOD	Limit of Detections
LOQ	Limit of Quantifications
MRL	Maximum Residue Limit
NOAEL	No Observed Adverse Effect Level
OPPs	Organophosphate Pesticides
OPPRs	Organophosphate Pesticide Residues
ppb	Part per billion
ppm	Part per million
PSA	Primary-Secondary Amine
QuEChERS	Quick, Easy, Cheap, Effective, Rugged and Safe method
RfD	Reference Dose
RME	Reasonable Maximum Exposure
US EPA	United State Environmental Protection Agency

CHAPTER I
INTRODUCTION

1.1 Theoretical Background

The Earth's population is increasing rapidly, which in turn is increasing the global demand for food, a basic factor for life. In order to meet the rising demands, there are efforts to hasten the production process of agricultural products for the world market. Therefore, to meet the high consumer demands for food products pesticides are essential in agriculture during a crop's growth, storage, and transport to control plant pests and diseases. Thailand is known as an agricultural country with more than 54.2% of its total area dedicated to agricultural production. It is reported that over half of the agricultural area (54.4%) is cultivated using pesticides, of which 45.9% are chemical. The majority of the agriculture areas using pesticides are in the central and northeastern regions, 76.5% and 44.9%, respectively (Agricultural Census, 2003).

Prohibition of organochlorine pesticides in Thailand in 1981 (Thirakhupt, 2006a) had made organophosphate pesticides the most widely used pesticide available today due to their effectiveness, short half-life, and low price. However, organophosphate pesticides have higher acute toxicities than chlorinated pesticides. Organophosphate pesticides' toxicological effects are mostly due to the inhibition of the acetylcholinesterase enzyme (AChE) in the nervous system, resulting in respiratory, myocardial, and neuromuscular transmission impairments (Goh, 1990; Reigart and Roberts, 2010)

The WHO/FAO (1990) estimated an annual worldwide total of 3 million cases of acute and severe pesticide poisoning, resulting in some 220,000 deaths. Recently, Ecobichon (2001) reported the largest proportion of human acute toxicity of pesticides due to organophosphates. The general population is mainly exposed to organophosphate pesticides through the ingestion of contaminated foods (such as vegetables and fruits), which are directly treated with organophosphate pesticides or are grown in contaminated fields. Therefore, contamination of the environment and food by pesticide residues is a dramatically topical tissue in many area of the world, including Thailand.

In Thailand, chilli is one of important product for Thai people's daily life because Thai people, especially Northeasterners, like chilli's spiciness and prefer using the chilli as an ingredient in their daily cooking. The Ministry of Public Health reported Thai's chilli consumption of approximately 5 grams per day or 1 teaspoon. Chilli is a profitable crop in Thailand. In 2007, income from chilli production in Thailand was about 2,161 million baht. Hua Rua sub-district, Ubonratchathani province is indicated as one of the largest areas of chilli production in Thailand. Hua Rua produces about 4,000 tons of chilli and had an annual income of 50 million baht from chilli in 2004-2005 (Cluster, 2009).

In planting, the chilli-growing farmers have to use many loads of pesticides both pre- and post-harvest to control chilli pests, protect the crops from disease, and meet high production targets. At the beginning, chemicals for preventing chilli' plumule from aphides would be used when their sprouts were planted for 7 days. Pesticides would be sprayed increasingly every 7 days when chilli initially cropped and every 15 days when their crops matured. Later, chemicals for preventing them from disease and insects would be used after keeping the first generation of seeds and before gathering crops. However, chilli-growing farmers lack proper knowledge and awareness of pesticide usage. Namely, when chilli was growing in the period of high price, farmers would increase spraying chemicals for accelerating the chilli production and therefore collecting the chilli before chemical breakdown. In addition, most of them frequently overdose their crops with pesticides (Aksornsri, 2005; Norkaew, 2009; Taneepanichskul, 2009). Furthermore, Hua Rua Primary Healthcare Unit (2011) had surveyed the cholinesterase in blood for 624 of Hua Rua people in 2009. It was reported that 61.3% were at risk level, 36.7% were over the acceptable of cholinesterase in blood. The result indicated that Hua Rua people had pesticides residues in blood at unsafety level. Since the ingestion of contaminated chilli is one of the possibility pesticide residues exposures, therefore, this study was focus on pesticide residue in fresh chili product and also the potential risk from chili consumption.

1.2 Hypothesis

Local people in Hua Rua, Ubonratchathani province, Thailand were at risk of organophosphate pesticide residue exposure from their chilli consumption.

1.3 Research Hypothesis

H_o: There was no significant difference of risk from organophosphate pesticide exposure from the chilli consumption of local people living in Hua Rua, Ubonratchathani province, Thailand.

H_1: There was significant difference of risk from organophosphate pesticide exposure from the chilli consumption of local people living in Hua Rua, Ubonratchathani province, Thailand.

1.4 Objectives

The main aim of this research was to assess the human health risks associated with the consumption of organophosphate pesticide residues on chilli fresh product. The specific objectives were as follows:

1.4.1 To analyze the residues of organophosphate pesticides in chilli products.

1.4.2 To compare the level of organophosphate pesticides residues on chilli with the national and international Maximum Residue Limits (MRLs).

1.4.3 To investigate the average daily dose (ADD) for the oral exposure pathway and evaluate the human health risk from organophosphate pesticide exposure from the chilli consumption of local people living in Hua Rua, Ubonratchathani province, Thailand.

1.5 Scope of Study

1.5.1 Chilli was collected from a chilli farm in Hua Rua, Ubonratchathani province, Thailand, and organophosphate pesticide residues in chilli was extracted by a multi-residue technique, i.e., the modified QuEChERS method.

1.5.2 Observation, face-to-face interviews and a structured questionnaire survey regarding chilli consumption were administered within the community of the chilli farm area.

1.5.3 Human health risk assessment associated with chilli consumption was assessed for local people living in Hua Rua, Ubonratchathani province, Thailand.

1.6 Research Outcomes

The three main desired outcomes were as follows:

1.6.1 The concentrations of organophosphate pesticide residues in chilli products in Hua Rua were analyzed and the concentrations were compared to the national and international Maximum Residue Limits (MRLs).

1.6.2 The human health risk of the local people who consume chilli in Hua Rua were evaluated

1.6.3 Information that can be applied to risk management and risk communication efforts to prevent or reduce the organophosphate pesticide residue risk to the local community.

CHAPTER II

LITERATURE REVIEW

2.1 Hua Rua sub-district, Ubonratchathani province

Chilli is one of vital product for Thais's daily life. Because of spiciness, Thais commonly use chilli as an ingredient in their cooking. Chilli was used as vegetable and seasoning. For its utilization in food transformed- and seasoning- industry, chilli would be used as dried chilli, chilli powder, curry paste, dired chilli sauce, and chilli sauce etc. In medical, chilli is one of the element in drug. For these reasons, chilli is become an important export products of Thailand such as fresh chilli, dried red chilli (Aksornsri, 2005).

Hua Rua sub-district, Muang district, Ubonratchathani province (Figure 2.1) is one of the largest areas of chilli cultivation in Thailand. It located at North-East of Thailand, where is the original of Hua Rua chilli (called Phrik Hua Rua).

Figure 2.1 Hua Rua sub-district, Muang district, Ubonratchathani province, Thailand

2.1.1 Background of Hua Rua's chilli

Over 20-30 years, farmers at Hua Rua sub-district had grown chilli. Aksornsri (2005) studied a career of local people in Hua Rua. The research found that 100% of participants were chilli growing farmer (*n* = 157) and 79.6% were rice growing famer (*n* = 125). As the research, the main career of local people was chilli growing farmer because it could generate better income and easier cultivation. Hua Rua area produces about 4,000 tons of chilli and had an annual income of 50 million baht from chilli in 2004-2005 (Cluster, 2009). The famous chilli in Hua Rua called "Phrik Hua Rua", which was selected due to resist insect high amount of product, and high chilli market demand.In general, chilli growing farmer would start planting chilli's seedling on August, than chilli sprouts would be planting on November. The chilli harvest would be continuously conducted since January until May (winter-summer season), then switched to rice growing on June (rainy season).

While planting, the chilli-growing farmers use a number of pesticides both pre- and post-harvest to control chilli pests, protect the crops from disease, and meet high production targets. At the beginning, chemicals for preventing chilli' plumule from aphides would be used when their sprouts were planted for 7 days. Pesticides would be sprayed increasingly every 7 days when chilli initially cropped and every 15 days when their crops matured. Later, chemicals for preventing them from disease and insects would be used after keeping the first generation of seeds and before gathering crops. Chilli cultivation at Hua Rau area was similarly reported the high amount of pesticide usage and over the recommendation dose. (Aksornsri, 2005; Norkaew, 2009; and Taneepanichskul, 2009). Norkaew 2009 reported the pesticide usage in Hua Rua, such as abamectin, selecron (profenofos), prodium 600 (chlorpyrifos), paraquat and lanate (carbamate). Aksornsi (2005) studied awareness of pesticide usage in chilli cultivation at Hua Rua. Chilli growing farmers (*n* = 157) would choose pesticides base on chemical efficiency, price, weed problem, and sprayer safety. The chemical efficiency is the main factor in choosing pesticide because chilli growing farmer did not need to spray pesticide several times, that would be increased cost investment. Moreover, most of them mixed more 2 types of pesticide because they believed that mixing several types of pesticide would increase the chemical efficiency and could eradicated diseases and insects in only one spraying. In addition, some of chilli growing farmer mixed pesticide over the recommendation dose because they believed that it is the way to suddenly increase pesticide efficiency. Seriously, the research found that chilli growing farmer would collect chilli before chemical breakdown when the chilli was in high price. And, Hua Rua also did not have pesticide residues test. Therefore, it is possible that local consumer might get risk of residues in chilli.

Moreover, the health information from Hua Rua Primary Healthcare Unit indicated the risk of pesticide exposure in local people at Hua Rua. Result of blood test of farmer in 2000, 2002 and 2009 were shown in Table 2.1. The acetyl cholinesterase test is used to measure the effect of exposure to certain or acetylecholin esterase-affected pesticides. The result showed increasing the number of people who got the risk level result.

Table 2.1 Acetylcholine Esterase enzyme test of local people in Hua Rua sub-district, Ubonratchathani

Acetylcholine Esterase Test	**Year**		
	2000* (n = 50)	**2002*** (n = 83)	**2009**** (n = 624)
Risk level [1]	39	67	383
Exceeded the acceptable level [2]	11	10	229
Normal level [3]	-	6	12

[1] There are chemical residues in blood at the level of no safety or severely chemical residues in blood..
[2] There are chemical residues in blood at the level of safety or moderately chemical residues in blood.
[3] There are no chemical residues in blood or slightly chemical residues in blood.
* Hua Rua Primary Healthcare Unit (2002)
** Hua Rua Primary Healthcare Unit (2009)

2.2 Organophosphate Pesticides

Organophosphate pesticides (OPPs) have been the most widely used type of pesticide for at least the past three decades, since the banning of organochlorine pesticides (such as DDT), based on their broad spectrum of pesticide activity, effectiveness, and non-persistence in the environment. However, the widespread of OPPs usage have led to the frequent exposure of human populations through multiple routes (Griffin, 1999; Jaipieam, 2009; Jirachaiyabhas, 2004; Petchuay, 2006; Siriwong, 2009; Taneepanichskul, 2009; Un Mei Pan, 2009).

2.2.1 Chemical Properties

OPPs are chemical substances originally synthesized by the reaction of phosphoric acid and alcohols. Over 100 OPP compounds representing a variety of chemical, physical, and biological properties are presently in commercial use. Most OPPs are slightly soluble in water and have high oil to water partition coefficient and low vapor pressure. These compounds consist of main elements i.e., carbon, nitrogen, phosphorus and oxygen or sulfur. The general chemical structure of OPPs is shown in figure 2.2

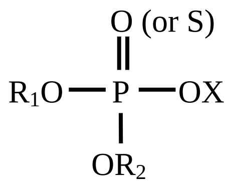


Figure 2.2 The general structure of organophosphate pesticide

A terminal oxygen or sulfer atom bond by a double bond to the phosphorus which can be called phosphates (P=O) or phosphorothioates (P=S). The alkyl groups (R_1, R_2) are usually simply alkyl or aryl groups and X is a leaving group, can be any one of a wide variety of substituted and branched aliphatic, aromatic or heterocyclic groups.

2.2.2 Classification of Organophosphate Pesticides

All OPPs can be classified by chemical structure together with the common names which are shown in Table 2.2 (IPCX Intox databank)

Table 2.2 Variation in the chemical structure of OPPs

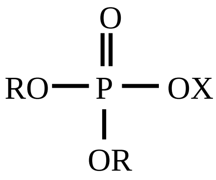
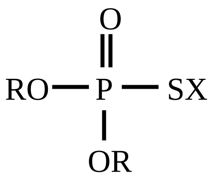
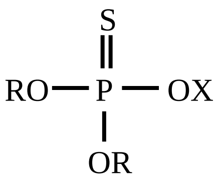
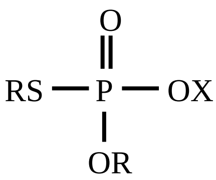
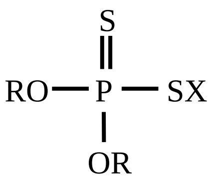
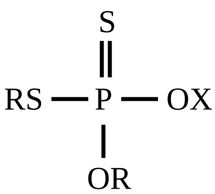
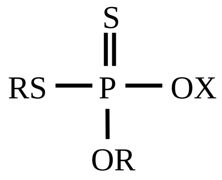
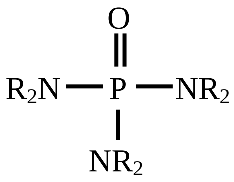
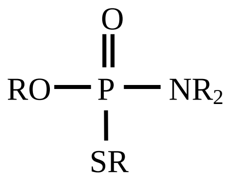
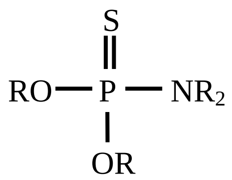
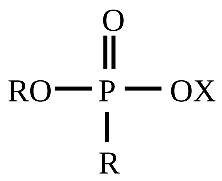
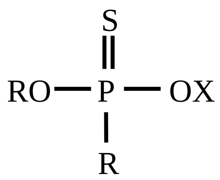
Phosphorus group	General structure	Common name
Phosphate		chlorfenvinphos, crotoxyphos, dichlorvos, dicrotophos, , heptenphos, mevinphos, monocrotophos, naled, phosphamidon, TEPP, tetrachlorvinphos
O-alkyl phosphorothioate		amiton, demeton-s-methyl, omethoate, oxydemeton-methyl, vamidothion
		azothoate, bromophos, bromophos-ethyl, chlorpyriphos, chlorpyriphos-methyl, coumaphos, diazinon, dichlofenthion, fenchlorphos, fenitrothion, fenthion, fensulfothion, iodofenphos, parathion, parathion-methyl, phoxim, pyrimiphos-ethyl, pyrimiphos-methyl, pyrazophos, sulfotep, emephos, thionazin
S-alkyl phosphorothioate		profenofos, trifenofos, prothiofos
Phosphorodithioate		amidithion, azinophos-ethyl, azinophos-methyl, dimethoate, dioxathion, disulfoton, ethion, formothion, malathion, mecarbam, menazon, methidathion, morphothion, phenthoate, phorate, posalone, phosmet, phothoate
S-alkyl phosphorodithioate		sulprofos

Table 2.2 Variation in the chemical structure of OPPs. *(continue)*

Phosphorus group	**General structure**	**Common name**
Phosphoroamindate		cruformate, fenamiphos, fosthistan, mephosfolan, phosfolan
Phosphorotriamindate		triamiphos
Phosphorothioamindate		methamidophos, acephate
		isofenphos
Phosphonate		butonate, trichlorfon
Phosphonothioate		cyanofenphos, EPN, leptophos, trichlornat

2.2.3 Chlorpyrifos

"O,O-diethyl- O-3,5,6-trichloro-2-pyridyl phosphorothioate" is a chemical name of Chlorpyrifos. Chlorpyrifos is organophosphate pesticide, which is classified as non-carcinogenic effect and moderately toxicity effect. (Figure 2.3)

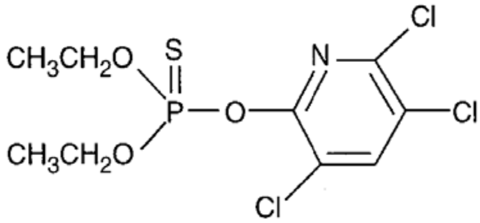


Figure 2.3 Structure of Chlorpyrifos

<u>Physical Properties</u>:

Appearance:	An amber to white crystalline solid with a mild sulfur odor
Chemical Name:	O,O-diethyl O-3,5,6-trichloro-2-pyridyl phosphorothioate
CAS Number	2921-88-2
Molecular Weight	350.62
Water Solubility	2 mg/L at 25ºC
Solubility in Solvents:	benzene, acetone, chloroform, carbon disulfide, diethyl ether, xylene, methylene chloride, methanol
Melting Point	41.5 - 44 ºC
Vapor Pressure:	2.5 mPa at 25 ºC
ADI:	0.01 mg/kg/day
RfD:	0.003 mg/kg/day

<u>Toxicological Effects</u>:

Acute toxicity: Chlorpyrifos is moderately toxic to humans. Poisoning from chlorpyrifos may affect the central nervous system, the cardiovascular system, and the respiratory system. It is also a skin and eye irritant. Symptoms of acute exposure to organophosphate or cholinesterase-inhibiting compounds may include the following: numbness, tingling sensations, incoordination, headache, dizziness, tremor, nausea, abdominal cramps, sweating, blurred vision, difficulty breathing or respiratory depression, and slow heartbeat. Some organophosphates may cause delayed symptoms beginning 1 to 4 weeks after an acute exposure which may or may not have produced immediate symptoms. In such cases, numbness, tingling, weakness, and cramping may appear in the lower limbs and progress to incoordination and paralysis. Improvement may occur over months or years, and in some cases residual impairment will remain (EXTOXNET, 1996). Toxicity data in the form of LD_{50} values of chlorpyrifos were shown in Table 2.3.

Table 2.3 Lethal Dose (LD_{50}) of chlorpyrifos

Route of Exposure	**LD_{50} of Chlorpyrifos (mg/kg)**					
	Rats	Mice	Rabbit	Chicken	Guinea pigs	Sheep
Oral	95-270	60	1000	32	500-504	800
Dermal	> 2000	-	1000-2000	-	-	-
Inhalation	> 0.2	-	-	-	-	-

Chronic toxicity: Repeated or prolonged exposure to organophosphates may result in the same effects as acute exposure including the delayed symptoms. Other effects reported in workers repeatedly exposed include impaired memory and concentration, disorientation, severe depressions, irritability, confusion, headache, speech difficulties, delayed reaction times, nightmares, sleepwalking, and drowsiness or insomnia. A measurable change in plasma and red blood cell cholinesterase levels was seen in workers exposed to chlorpyrifos spray. Human volunteers who ingested 0.1 mg/kg/day of chlorpyrifos for 4 weeks showed significant plasma cholinesterase inhibition.

Organ toxicity: Chlorpyrifos primarily affects the nervous system through inhibition of cholinesterase, an enzyme required for proper nerve functioning.

Metabolism:

Chlorpyrifos is readily absorbed into the bloodstream through the gastrointestinal tract if it is ingested, through the lungs if it is inhaled, or through the skin if there is dermal exposure. In humans, chlorpyrifos and its principal metabolites are eliminated rapidly. After a single oral dose, the half-life of chlorpyrifos in the blood appears to be about 1 day. Chlorpyrifos is eliminated primarily through the kidneys. Following oral intake of chlorpyrifos by rats, 90% is removed in the urine and 10% is excreted in the feces.

It is detoxified quickly in rats, dogs, and other animals. The major metabolite found in rat urine after a single oral dose is trichloropyridinol (TCP). TCP does not inhibit cholinesterase and it is not mutagenic Chlorpyrifos does not have a significant bioaccumulation potential. Following intake, a portion is stored in fat tissues but it is eliminated in humans, with a half-life of about 62 hours (EXTOXNET, 1996).

Environmental Fate: (EXTOXNET, 1996).

Breakdown in soil and groundwater: Chlorpyrifos is moderately persistent in soils. The half-life of chlorpyrifos in soil is usually between 60 and 120 days, but can range from 2 weeks to over 1 year, depending on the soil type, climate, and other conditions Adsorbed chlorpyrifos is subject to degradation by UV light, chemical hydrolysis and by soil microbes

Breakdown in water: The concentration and persistence of chlorpyrifos in water will vary depending on the type of formulation. For example, a large increase in chlorpyrifos concentrations occurs when emulsifiable concentrations and wettable powders are released into water. As the pesticide adheres to sediments and suspended organic matter, concentrations rapidly decline. Volatilization is probably the primary route of loss of chlorpyrifos from water. Volatility half-lives of 3.5 and 20 days have been estimated for pond water. It is unstable in water, and the rate at which it is hydrolyzed increases with temperature, decreasing by 2.5- to 3-fold with each 10ºC drop in temperature. The rate of hydrolysis is constant in acidic to neutral waters, but increases in alkaline waters. In water at pH 7.0 and 25 C, it had a half-life of 35 to 78 days.

Breakdown in vegetation: Chlorpyrifos may be toxic to some plants, such as lettuce. Residues remain on plant surfaces for approximately 10 to 14 days. Chlorpyrifos and its soil metabolites can accumulate in certain crops.

2.2.4 Profenofos

"(*RS*)-*O*-4-bromo-2-chlorophenyl *O*-ethyl *S*-propyl phosphorothioate" is a chemical name of profenofos. Profenofos is organophosphate pesticide, which is classified as non-carcinogenic effect and moderately toxicity effect. Profenofos is used to control cotton bollworm, aphids, spider, diamondback moth, and other insects when plant cotton and vegetables. (Figure 2.4)

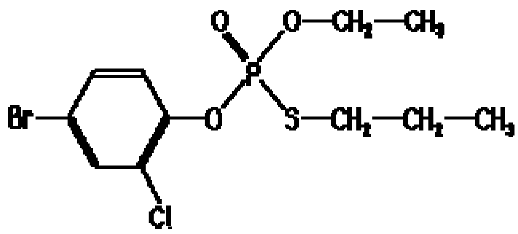


Figure 2.4 Structure of Profenofos

Physical Properties:

Appearance:	Pale yellow liquid with garlic-like odor
Chemical Name:	(*RS*)-*O*-4-bromo-2-chlorophenyl *O*-ethyl *S*-propyl phosphorothioate
CAS Number	41198-08-7
Molecular Weight	373.6
Water Solubility	28 mg/L at 20ºC
Solubility in Solvents:	Acetone, Xylene, Methanol
Boiling Point	110 ºC
Vapor Pressure:	2.53 mPa at 25°C
ADI:	0.01 mg/kg/day
RfD:	0.0001 mg/kg/day

Toxicological Effects:

Organophosphorus intoxication results from accumulation of acetylcholine at nerve endings. Symptoms of profenofos intoxication can include headache, nausea, blurred vision, papillary constriction, tiredness, giddiness, cramps, diarrhea, discomfort in the chest, nervousness, sweating, tearing, salivation, pulmonary oedema, convulsion, coma. If swallowed and aspirated into the lungs, chemical pneumonia can occur. Depending on severity of poisoning these symptoms become worse with the onset of vomiting, abdominal pain, diarrhea, sweating and salivation. Confusion, ataxia, slurred speech, loss of reflexes are some of the central nervous system effects may lead to misdiagnosis of acute alcoholism. Toxicity data in the form of LD_{50} values of chlorpyrifos were shown in Table 2.4

Table 2.4 Lethal Dose (LD_{50}) for profenofos

Route of Exposure	**LD_{50} for Profenofos (mg/kg)**					
	Rats	Mice	Rabbit	Chicken	Guinea pigs	Sheep
Oral	358	-	700	-	-	-
Dermal	> 2000	-	472	-	-	-
Inhalation	3	-	-	-	-	-

Organ toxicity: Profenofos primarily affects the nervous system through inhibition of cholinesterase, an enzyme required for proper nerve functioning.

<u>Environmental Fate:</u>

Available environment fate studies show that pH-dependent hydrolysis is the major route of dissipation for profenofos while aerobic and anaerobic metabolisms become important after the initial hydrolysis. Profenofos is not expected to volatilize from dry soil surfaces (SRC) based upon a vapor pressure of 9.00×10^{-7} mmHg. Profenofos metabolizes rapidly under alkaline aerobic conditions. In an alkaline (pH **7**.8) soil, profenofos degraded with a half-life of 2 days. The rate of metabolism is influenced by chemical hydrolysis and aerobic metabolism in neutral and acid soils is likely to be slower. Profenofos also metabolizes rapidly under alkaline anaerobic conditions. In alkaline (pH **7**.8) soil, profenofos degraded with a half-life of 3 days under anaerobic conditions. Profenofos hydrolyzes in neutral and alkaline solution, with half life of 104-108 days, 24-62 days at pH 7 and 7-8 hour at pH 9. Photolysis is not a major pathway in degradation of profenofos.

2.2.5 Toxic Effect of Organophosphate Pesticides

2.2.5.1 Cholinesterase inhibition reaction

For most OPP compounds, neurotoxicity is the most serious toxicological concern. The mechanism of acute toxicity occurs in vertebrates and invertebrates. Normally, (Green panel below) the acetylcholine (ACh) is a neurotransmitter, can be broken down to choline and acetic acid, and inactivated in milliseconds by acetylcholinesterase enzyme (AChE). (Yellow panel below) OPPs act as an irreversible AChE inhibitors. (Red panel below) Without this AChE results in the accumulation of ACh in the central and peripheral nervous systems, resulting in an acute cholinergic syndrome via continuous neurotransmission as shown in figure 2.5 (Chamber, 1992).

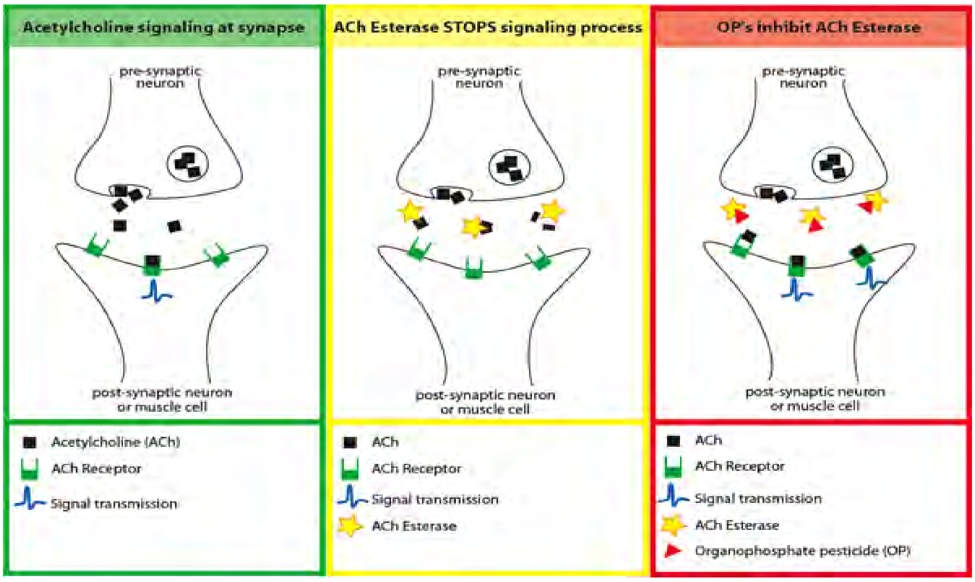


Figure 2.5 Mechanisms of acetylcholinesterase inhibition (Pediatric Environmental Health Specialty Unit, 2007).

Any pesticide that can bind, or inhibit cholinesterase, making it unable to breakdown acetylcholine, is called a "cholinesterase inhibitor," or "anticholinesterase agent." as biomarkers of exposure are important in toxicology. Biomarkers are used to assess the exposure (internal dose or the amount of chemical exposure that has resulted in absorption into the body) and elucidate cause-effect and dose-effect relationships in health risk assessment, in clinical diagnosis and for monitoring purposes. The two main classes of cholinesterase inhibiting pesticides are the organophosphates (OPPs) and the carbamates (CMs). For example, Gershon and Shaw (1961) described the anxiogenic effects of exposure to OPPs in humans, Marrs (1993), Minton and Murray (1988) reviewed the systemic toxicity caused by inhibition of cholinesterase activity and of treatment of acute poisoning from OPPs, and Katherine, *et. al* (2004) studied cholinesterase inhibition and neurobehavioral effects in rats exposed to fenamiphos or profenofos.

Overexposure to OPPs or CMs can result in cholinesterase inhibition. These pesticides combine with acetylcholinesterase at nerve endings in the brain and nervous system, and with other types of cholinesterase found in the blood. This allows acetylcholine to build up, while protective levels of the cholinesterase enzyme decrease. The more cholinesterase levels decrease, the more likely symptoms of poisoning from cholinesterase inhibiting pesticides are shown (EXTOXNET, 1993).

Although the signs of cholinesterase inhibition are similar for both CMs and OPPs poisoning, blood cholinesterase returns to safe levels much more quickly after exposure to CMs than after OPPs exposure. Depending on the degree of exposure, cholinesterase levels may return to pre-exposure levels after a period ranging from several hours to several days for CMs exposure, and from a few days to several weeks for OPPs.

Anyone exposed to cholinesterase-affected pesticides can develop lowered cholinesterase levels. The purpose of regular checking of cholinesterase levels is to alert the exposed person to any change in the level of this essential enzyme before it can cause serious illness. Ideally, a pre-exposure baseline cholinesterase value should be established for any individual before they come in regular contact with OPPs and CMs. Fortunately, the breakdown of cholinesterase can be reversed and cholinesterase levels will return to normal if pesticide exposure is stopped.

2.2.5.2 Cholinesterase test

Humans have three types of cholinesterase:

- Red blood cell (RBC) cholinesterase, called "true cholinesterase;"
- Plasma cholinesterase, called "pseudocholinesterase;"
- Brain cholinesterase.

Red blood cell cholinesterase is the same enzyme that is found in the nervous system, while plasma cholinesterase is made in the liver. When a cholinesterase blood test is taken, two types of cholinesterase can be detected. Physicians find plasma cholinesterase readings helpful for detecting the early, acute effects of organophosphate poisoning, while red blood cell readings are useful in evaluating long-term, or chronic, exposure (Paul, 1987)

The cholinesterase test is a blood test used to measure the effect of exposure to certain or cholinesterase-affected pesticides. Both plasma (or serum) and red blood cell (RBC) cholinesterase should be tested. These two tests have different meanings and the combined report is needed by the physician for a complete understanding of the individual's particular cholinesterase situation.

The interpretation of cholinesterase test results should be done by a physician. A 15 to 25 percent depression in cholinesterase means that slight poisoning has taken place (Normal). A 25 to 35 percent drop signals moderate poisoning (Exceeded the acceptable), and a 35 to 50 percent decline in the cholinesterase readings indicates severe poisoning (Risk level).

2.2.6 Signs and Symptom of Poisoning

The specific symptomology following exposure to an OPP compounds will vary with the species of the animal, dosage, route of exposure and chemical involved. Exposure by inhalation results in the fastest appearance of toxic symptoms, followed by the gastrointestinal route and finally the dermal route.

2.2.6.1 Acute toxicity

Generally, OPPs are acutely toxic occurring within 2-3 days after exposure. Acute poinsoning depends on amount of OPPs dosing. It is characterized by widespread muscarinic and nicotinic effects which are caused by inhibition of AChE at nerve ending, in ganglia, and in the brain. The earliest muscarinic symtoms, in eyes, are muosis, blurred vision and eye pain, in digestion system, are hypersalivation, nausea, vomiting, abdominal cramps, diarrhea and tenesmus, in inhalation system, are cough, expectoration of frothy secretions, pulmonary oedema, chest tightness, and wheeze, especially secretory effects (salivation, bronchorrhoea) are often seen. The nicotinic effects of poisoning include fasciculation, progressive flaccidity and weakness of proximal muscle groups, in particular the neck flexors but later extra-ocular muscles of respiratory. In serious cases, respiratory failure and death can occur

2.2.6.2 Chronic toxicity

The symptoms of chronic effect occur after exposure at a low concentration of OPPs for a long time which is known to cause a delayed neuropathy. The first symptoms are often sensory with tingling and burning sensations followed by weakness of arms and legs, cuff pain and paresthesia of tiphands and tiptoe, wasting of hand muscles. In serious cases, extensive flaccid paralysis can occur for several days.

2.2.7 Metabolism

The occurrence of poisoning depends on the rate at which the pesticide is absorbed. Breakdown mainly occurs by hydrolysis in the liver, and rates of hydrolysis widely vary from one compound to another. In the case of certain OPPs, they break down relatively slowly and may look for temporary storage in body and fat. Some OPPs such as methyl parathion and diazinon have significant lipid solubility, allowing for fat storage with delayed toxicity due to rate release. Many organothiophosphates readily undergo conversions from thios (P=S) to oxons (P=O). Conversion occurs in the environment by the influence of oxygen and light, and in the body mainly by the action of liver microsomes. Finally, both thios and oxons are hydrolyzed at the ester linkage, yielding alkyl phosphates and leaving groups, both of which are of relatively low toxicity. They are either excreted or further transformed in the body before excretion.

2.3 Environmental Health Risk Assessment

The environmental health risk assessment has been defined as “the systematic scientific characterization of potential adverse health effects resulting from human exposure to hazard agents or situations.” Since 1980s, most health, environmental, and even technological risk assessments have been largely consistent with the basic health risk assessment paradigm put forth by the National Academy of Sciences’ National Research Council (NRC). The paradigm describes a four-step process for analyzing data, drawing interferences from all available related information and then summarizing the implication in a risk characterization that others, including risk managers and the public, can easily follow and understand. For each step, the relevant and scientifically reliable information is evaluated. The four steps of risk assessment by the NRC are as follows: (1) hazard identification, (2) dose-response assessment, (3) exposure assessment, and (4) risk characterization. (Felicia and William, 2007)

2.3.1 Hazard Identification

Hazard identification refers to the review of key research to identify any potential health problems in humans that a chemical can cause. The method commonly used to identify hazards is the weight-of-the evidence: a qualitative scientific evaluation of a substance for a specific purpose. The method for the evaluation includes the chemical and physical properties of the substance, route and pattern of exposure to the substance; and metabolic, pharmacokinetic and toxicological data of the substance. Based on the evidence gathered, conclusions are then made regarding the capability of a particular substance to cause a particular health effect.

Profenofos and chlorpyrifos are OPP, which are classified as non-carcinogenic pesticide. EPA has determined that chlorpyrifos is not carcinogenic. There was no increase in the incidence of tumors when rats were fed 10 mg/kg/day for 104 weeks nor when mice were fed 2.25 mg/kg for 105 weeks. The classification of profenofos and chlorpyrifos are moderately hazard. The classification of OPPs toxicity is shown in Table 2.5.

Table 2.5 The classification of OPPs toxicity (Lunliu, 2006).

Class of pesticide toxicity		**Organophosphat pesticides**
Ia	**Extreamly hazard**	chlorfenvinphos, EPN, disulfoton, fonofos, mephosfolan, mevinphos, parathion, parathion methyl, phoxim, sulfotep
Ib	**Highly hazardous**	azinphos-ethyl, azinphos-methyl, bromophos-ethyl, carbophenothion, dichlorvos, dicrotophos, fenthion, isazophos, isofenphos, methamidophos, monocrotophos, omethoate, oxydemeton-methyl, thiometon, triazophos, vamidothion
II	**Moderately hazardous**	***chlorpyrifos***, diazinon, dimethoate, ethion, etrimphos, fenitrothion, formothion, methacrifos, naled, phenthoate, phosalone, phosmet, ***profenofos***, prothiophos, quinalphos, sulprofos
III	**Slightly hazardous**	acephate, azamethiphos, bromophos, malathion, pirimiphos-methyl, tetradifon, trichlorfon

2.3.2 Dose-Response Assessment

Dose response assessment is the relationship between the magnitude of a dose of a substance and the occurrence of a health effect. Typically, the relationship is presented as a plot between the dose and the probability of a selected toxic endpoint (e.g., the percent of mortality), or the probability that the endpoint will occur (e.g., the probability of having cancer). Human studies are preferred sources of information for developing dose-response relationships. However, they may not be available and a determination of the relationship may be based on studies done on animals. In such cases, extrapolation of the data from the animal to humans is required.

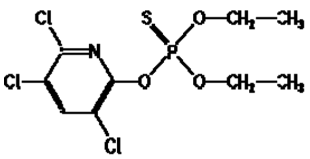
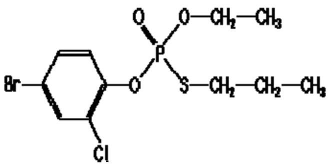
The level of health effect depends on the degree of OPPs exposure. Chronic or acute effects depend on the correlation between the cholinesterase inhibitor and exposure behavior. For instance, when inhibition occurs slowly and repeatedly, like chronic exposure, the correlation with illness may be low or nonexistent. On the other hand, if the correlation increases and the rate of inhibition are faster, there will be signs of acute intoxication. The association between the AChE inhibition, level of poisoning, and clinical symptoms are shown in Table 2.6.

Table 2.6 Severity and prognosis of acute organophosphate intoxication at different levels of AChE inhibition (Cattani, 2004).

% 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, diarreia, 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

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 0.003 mg/kg/day. The effect of chlorpyrifos is decreased plasma cholinesterase activity after 9 days and inhibited human cholinesterase activity in 20 days. The estimate value of profenofos RfD is 0.0001 mg/kg/day. The effect of profenofos is Inhibited cholinesterase activity in plasma and RBC's (Table 2.7).

Table 2.7 Quantitative estimate of non-carcinogenic risk from oral exposure

	Noncarcinogenic					
OPPs	**Experimental Doses (mg/kg·day)**		**UF**	**MF**	**Rfd (mg/kg-day)**	**Critical effects**
	LOEL	NOEL				
Chlorpyrifos [a] 	0.10	0.03	10	1	0.003	• Decreased plasma cholinesterase activity after 9 days. • Inhibited human cholinesterase activity in 20 days
Profenofos [b] 	ND	0.005	100	ND	0.0001	• Inhibited cholinesterase activity in plasma and RBC's

[a] Reference from website : http://www.epa.gov/iris/
[b] Reference from website http://www.epa.gov/oppsrrd1/reregistration/REDs/profenofos_red.pdf
[b] Jaipieam, 2009 (Oral reference dose of profenofos)
[ND] ND = No Data

2.3.3 Exposure Assessment

Exposure is the condition of a chemical contacting the outer boundary of a human. The chemical concentration at the point of contact is the exposure concentration. Exposure over a period of time can be represented by a time dependent profile of the exposure concentration. For example, ingestion of contaminated fruits and vegetables is a potential pathway of human exposure to toxic chemicals. Fruits and vegetables may become contaminated with toxic chemicals by several different pathways such as pesticide spraying, soil additives, and fertilizers. The scheme of dose and exposure via the oral route is shown in Figure 2.6

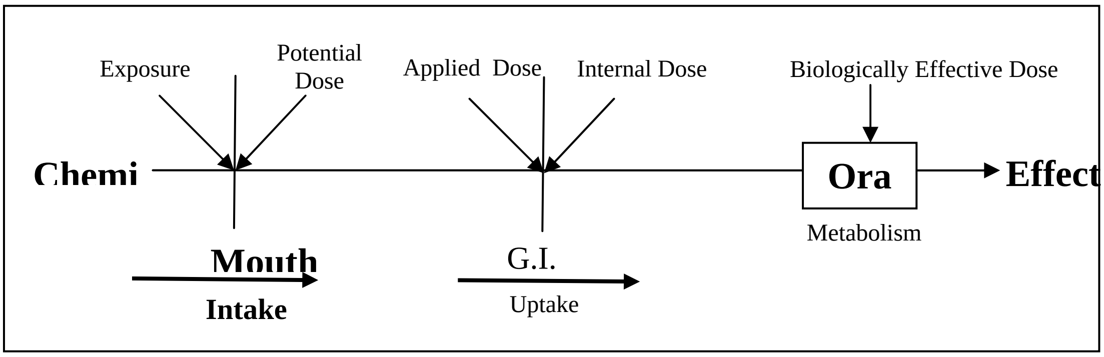


Figure 2.6 Schematic of dose and exposure via the oral route (US EPA, 1999a)

Average Daily Dose Calculations for the Intake Process via the Ingestion Route

Average exposures or doses over the period of exposure is sufficient for making an assessment. These averages are often in the form of average daily doses (ADD) as seen in the following equation (US EPA, 1992a).

$$ADD = \frac{C \times IR \times EF \times ED}{BW \times AT} \qquad \text{Equation 1}$$

Where;

ADDs = Exposure duration (mg/kg·day)

C = Pesticide concentration (mg/kg)

IR = Intake rate (e.g. kg chilli/day)

EF = Exposure frequency (days/year)

ED = Exposure duration (years)

BW = Body weight (kg)

AT = Average time (day); for non-carcinogenic effects, AT = ED in days

ADD of chlorpyrifos and profenofos could be calculated by using data from OPPRs analysis in chilli and questionnaire based dietary survey.

2.3.4 Risk Characterization

Risk characterization is the final step to assess human health risks; it summarizes both qualitative and quantitative findings. It is an instrument to communicate the findings of the risk assessment to the risk manager or decision makers. The risk characterization combines and uses the appropriate method to analyze the essential information from the hazard identification, dose-response assessment, and exposure assessment to make risk estimates for the exposure scenarios of interest.

Chlorpyrifos and Profenofos are OPP, which are classified as non-carcinogenic pesticide. The criterion, that is the one used in non-carcinogen risk characterization, is the reference dose (RfD). The RfD is defined as the daily oral dose of a chemical that is unlikely to cause adverse effects given a lifetime of exposure. An evaluation of non-carcinogenic toxicity of individual risks can be computed by using the hazard quotient (HQ) ratio. This value indicates the degree of exposure, greater or less than the RfD. When the exposure exceeds the RfD, the exposure population may be at risk (US EPA, 1986b and 1999b).

$$\text{Hazard Quotient (HQ)} = \frac{\text{Exposure}}{\text{RfD}} \qquad \textit{Equation 2}$$

Where;

	$HQ > 1$	Adverse non-carcinogenic effects of concern (risk)
	$HQ \leq 1$	Acceptable level (no concern)
Exposure	= Chemical exposure level or ADDs (mg/kg·day)	
RfD	= Reference dose (mg/kg·day)	

2.3.5 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, 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, 2009).

2.4 Regulatory Limit for Organophosphate Pesticides

The US Food and Drug Administration (FDA) currently provides assurance that any pesticide remaining in or on the food is within safe limits for minimizing the potential hazards to human health. OPPs are one of the most widely used and their residues are found on agriculture products. Preventive action aims to reduce the contamination as well as to prevent both acute and chronic adverse effects to consumers.

Several indices of pesticide levels can be used to predict pesticide residue intake. The Maximum Residue Limits (MRL) is one such index and represents the maximum concentration of a pesticide residue (mg/kg) that the Codex Alimentarius Commission recommends be legally permitted in food commodities and animal feeds. Factors that may be considered when choosing an index to be used in predicting pesticide residue intake include the residue levels found in practice, their distribution in the commodity, and the effect of the residues from the various processes used in the preparation of food. The MRL is not permanently fixed. The MRL is determined according to the best judgment of a group of internationally recognized experts on the data available to them at the time of the evaluation. As new data become available, the MRL may be reconsidered (WHO, 1997).

As OPPs are the significant adverse effects in human, the international organizations, i.e., FAO/WHO, Pesticide/action network (PAN), European Union (EU), Environmental Protection Agency (EPA) and governments in many countries as well as Thailand by Ministry of Agriculture and Cooperation of Thailand (2006) are interested in the pesticide residues on agricultural products and have established the MRLs in agricultural products for consumer's health protection. The example of MRLs of OPPRs in chilli by various organization and governments in many countries are shown in Table 2.8.

Table 2.8 Maximum Residue Limit (MRL) of organophosphate pesticide residues in chilli

Organophosphate Pesticides (OPPs)	**Chilli /Pepper MRL (mg/kg)**			
	Codex [a]	**EU** [b]	**ASEAN** [c]	**Thailand** [d]
Chlorpyrifos	0.5	0.5	-	0.5
Diazinon	0.5	-	-	-
Dimethoate	1.0	1.0	-	-
Fenitrothion	0.5	-	-	-
Malathion	0.5	0.5	0.5	0.5
Methamidophos	2.0	2.0	-	-
Mevinphos	0.1	-	-	-
Monocrotophos	0.2	0.2	-	0.2
Parathion-methyl	0.2	-	-	-
Profenofos	5.0	5.0	-	5.0
Triazophos	0.02	-	-	-

[a] Codex Alimentarius, 2010
[b] EU MRLs, 2004
[c] ASEAN Cooperation in Food, 2007
[d] National Bureau of Agricultural Commodity and Food Standard, 2008
Thai Agricultural Commodity and Food Standard (ACFS), TACFS 9002-2008 (Announcement in the Royal Government Gazette Vol. 125, Part 139D, Special issue, Dated 18th August B.E. 2551)

2.5 Multiresidue Analysis of Organophosphate Pesticides

Pesticide residues in fruits and vegetables are a significant health risk. Governments and international organizations have established the Maximum Residue Levels (MRLs), limiting the amounts of pesticides in foods. Regulatory authorities provide assurances that any pesticide remaining in or on the food is within safe limits through monitoring programmes of random sampling and the analysis of raw and processed food (Stajnbaher and Zupancic-Kralj, 2003) Pesticide monitoring programs have been established by governments in order to assess and control the quality of foods and thereby evaluate and enforce the proper usage of pesticides in agricultural practices (Martínez Vidal, 2002). Therefore, concern over pesticide residues in fruits and vegetables has led to the development of many multi-residue methods as the most cost-effective approach to residue analysis.

The conventional methods used to determine the multiresidues of pesticide in fruits and vegetables have three basic steps: solvent extraction, cleanup, and instrumental analysis. Theoretically, extraction strategies in multi-residue analyses are varied (Motohashi, 1996). Using of organic solvent extractions is preferred in routine laboratory analysis because of their simplicity, speed, and high recoveries for compounds in a wide range of polarity. The cleanup step is used to reduce the amount of interferences and their negative interferences on the selectivity of the analytical signal and maintenance of the instruments. To detect the large number of pesticides applied to crops typically requires the use of analytical separation techniques such as gas chromatography (GC) or high-performance liquid chromatography (HPLC) (Martínez Vidal, 2002). Both techniques have been widely used with class-selective detection methods, especially for GC with electron-capture (ECD) nitrogen-phosphorus (NPD), flame photometric (FPD) and mass spectrometry (MS) detection

In addition, gas chromatography equipped with FPD or MS detection has been wildly used for both multi-residue determinations and trace-level identifications of a wide range of OPPs. The GC-FPD in phosphorous mode is reliable because of its specificity for organic phosphorous present in OPPs regardless of the complexity of most plant matrixes. The GC-MS in the selective ion monitoring (SIM) mode is also a powerful analytical tool because it can provide confirmation by the selective monitoring of target and qualifier ions and their relative abundances and ratios specific to the analysis of interest. Then, the advantage of both specificity and selectivity in a GC-FPD and GC-MS/SIM can be complementary used to analyze OPPRs in complex plant matrixes (Wong, 2007).

The example of analysis techniques for determination of pesticides is Podhorniak *et al.* (2001) developed the first multi-residue analysis of OPPs in fresh fruits and vegetables at level down to 1.0 ppb using gas chromatography equipped with a pulsed flame photometric detector (GC-PFPD). The method used an acetone extraction-multiple liquid-liquid partition cleanup developed by Luke (Luke, 1975).

Then, the followed step was a tandem graphitized carbon black (GCB)/primary-secondary amine (PSA) solid-phase extraction (SPE) column cleanup, developed by Schenck and Howard-King, 1998. The method can be prepared in 1 working day for overnight instrumental analysis. The recovery data (recoveries ranged from 50-150%) showed that a daily column-cutting procedure used in combination with the SPE extract cleanup effectively reduces matrix enhancement at the ppb level for many OPPRs.

2.5.1 QuEChERS Method

Traditional methods such as Podhorniak *et al.* (2001), Gelsomino *et al.* (1997), Johnson *et al.* (1997), Lacassie *et al.* (1998), Štajnbaher and Zupancic-Kralj (2003), and Albero *et al.* (2005) for pesticide determination tend to be complicated by several disadvantages, namely, a large volume of the solvent requirement and wastes , labor intensive, time consuming and expensive. Therefore, “QuEChERS,” a newer method in sample preparation and measurement was developed (Wilkoaka and Biziuk, 2011).

In 2003, Anastassiades *et al.* (2003) introduced a multi-residue analysis of pesticides in fruits and vegetables called QuEChERS (“catchers”), which stands for “Quick, Easy, Cheap, Effective, Rugged, and Safe.” This method entailed using an acetonitile extraction, partitioning with a mixture of sodium chloride (NaCl) and magnesium sulfate ($MgSO_4$) for salting out extraction/partition, and then dispersive solid phase extraction (D-SPE) cleanup. Following this, the extract was mixed on a vortex mixer with a PSA SPE sorbent rather than eluting the extract through an SPE column. The recovery data (recoveries ranged from 85-101%) indicated that the D-SPE with PSA effectively removes many polar matrix components such as organic acids, certain polar pigments, and sugar from food extracts. The original QuEChERS study performed by Anastassiades *et al.* has been cited in the literature more than 210 times according to the ISI Web of Knowledge citation index (Lehotay, 2010).

The QuEChERS (Quick, Easy, Cheap, Effective, Rugged and Safe) method involves microscale extraction using acetonitrile and purifying the extract using dispersive Solid Phase Extraction (D-SPE) technique for extracting the multiresidues of pesticides from fruits and vegetables. The QuEChERS method is described as follows:

Quick : Typically, 8 samples can be prepared in just under 30 min.

Easy : It requires less handling of extracts than other techniques.

Cheap : Less sorbent material and less time is need to process samples.

Effective : The simple technique gives high and accurate recovery levels for a range of different compound types, e.g., polar pesticides and pH dependent compounds.

Rugged : The method can detect a large range of pesticides including pH dependent and polar pesticides.

Safe : Unlike other techniques, it does not require any chlorinated solvents. Extraction is typically done using acetonitrile, which is both GC and LC amenable.

The QuEChERS procedure involves two simple steps. Firstly, the homogenized samples are extracted and partitioned using an organic solvent and salt solution to enhance extraction efficiency and protect sensitive analyzes. Secondly, the supernatant is further extracted and cleaned using D-SPE technique. By the combination of bulk drying salts and SPE adsorbent packing removes excess water and unwanted contaminants from the sample extracts. After a brief agitation and centrifugation, the cleaned extracts are then prepared for analysis (QuECHERS, 2006).

The QuEChERS method is very flexible and can be modified depend on the analyze properties, matrix composition and analytical technique available in the lab. The different ration, types of sample size, type and amount of solvent, salt and sorbent are used in modification to achieve high recoveries. For example, Lehotay *et al.* (2005) and AOAC Official Method (2007) modified the original method by using 1% acetic acid-acetonnitrile extraction and buffering to improve the stability of certain base-sensitive pesticides. Wong *et al.*, 2003 modified the method by using both GCB and PSA for the D-SPE cleanup. The use of different sorbent in D-SPE cleanup tube makes the multiresidue pesticide analysis to achieve a high degree of cleanup without reducing recoveries for some pesticides. (Lehotay *et al.*, 2010) Recently, Schenck *et al.* (2009) publiched a modified QuEChERS method based on procedures developed by Podhorniak *et al.*, 2001, Anastassiades *et al.,* 2003 and Wong *et al.,* 2007 for determination of 102 OPPs in fresh fruit and vegetables at level down to 1 µg/kg (ppb) levels using gas chromatography equipped with a pulsed flame photometric detector (GC-PFPD). The procedure entailed extraction of pesticides from the sample with acetonitrile, salting out with $MgSO_4$ and NaCl. Then the extract was cleaned using PSA/GCB/$MgSO_4$. Recoveries ranged from 63-125%. This modified method is significant of saving time, cost and also reduce approximately 90% of solvent usage and hazardous production compared to traditional methods.

The QuEChERS methods have been routine determined for extracting multiresidues of pesticides from various foods such as tomato, spinach, cabbage, orange and grape. The literature reviews of QuEChERS methods for determinating pesticides in fruits and vegetables are shown in Table 2.9. However, no publication has documented the QuEChERS methods for multiresidue pesticide analysis in chilli. Therefore, This analytical research aim to developed and modified QuEChERs method for the quantification of OPPs from fresh chilli based on procedures developed by Anastassiades *et al.,* 2003, AOAC Official Method, 2007, Schenck *et al.,* 2009.

Table 2.9 The literature reviews of Q uEChERS methods in the analyses of pesticides in fruit and vegetable samples.

Sample	Extraction solvent	Clean-up	Detector	Recovery	Reference
Tomato, Apple, Zucchini	Acetonitrile	PSA/$MgSO_4$ - SPE	GC-MS	85 – 101%	Anastassiades *et al.*, 2003
Avocado	Acetonitrile	PSA/$MgSO_4$ - SPE	GC-MS	> 95%	Lehotay *et al.*, 2005
Orange, Lettuce, Paprika	Acetonitrile (HAc 0.1%)	GCB/PSA/$MgSO_4$ - SPE	GC-FPD	70 – 110%	Okihashi *et al.*, 2005
Dried Ground Ginseng Root	Acetonitrile	GCB/PSA/$MgSO_4$ - SPE	GC-FPD	70 – 120%	Wong *et al.*, 2007
Cucumber	Acetonitrile (HAc 1%)	PSA/$MgSO_4$ - SPE	GC-MS	No reported	Garrido Frenich *et al.*, 2008
Grape, Lemon, Onion, Tomato	Acetonitrile	PSA/$MgSO_4$ - SPE	GC-MS	70 – 110%	Lesueur *et al.*, 2008
Cabbage, Radish	Acetonitrile (HAc 0.5%)	PSA/$MgSO_4$ - SPE	GC-MS	80 – 115%	Nguyen *et al.*, 2008
Mangosteen	Acetonitrile	GCB/PSA/SAX - SPE	GC-FPD	56 – 133%	Sukeium, 2008
Bananas	Acetonitrile	PSA/$MgSO_4$ - SPE	GC-NPD	67 – 118%	Hernández-Borges *et al.*, 2009
Pepper	Acetonitrile	PSA/$MgSO_4$ - SPE	GC-MS	85 – 98%	Mezcua *et al.*, 2009
Grape, Orange, Tomato, Spinach	Acetonitrile	GCB/PSA/$MgSO_4$ - SPE	GC-FPD	62 – 125%	Schenck *et al.*, 2009
Korean herbs	Acetonitrile (HAc 0.5%)	GCB/PSA/$MgSO_4$ - SPE	GC-MS	62 – 119%	Nguyen *et al.*, 2010

PSA: Primary Secondary Amine, GCB: Graphitized Carbon Black, SAX: Strong Anion Exchange, SPE: Solid Phase Extraction, HAc: Acetic acid

2.6 Related Articles

Jansong, *et al.* (2010) studied on pesticide residues in 150 chilli samples from lower Northeastern agricultural area followed the Good Agricultural Practice (GAP). 73 chilli samples were detected pesticide residue and 10 chilli samples exceeded the MRL. They detected chlorpyrifos, profenofos, cypermethrin and endosulfan. The study areas are Ubonratchathani and Srisakate provinces. The chilli farms at Ubonratchathani found chlorpyrifos (0.095 –1.097 mg/kg, *n* = 12) and 3 chilli samples exceeded the MRL. The chilli farms at Srisakate found profenofos (0.110 – 3.400 mg/kg, *n* = 23) and chlorpyrifos (0.088 – 2.105 mg/kg, *n* = 17). The 3 chlorpyrifos contaminated samples exceeded the MRL.

Hua Rua Primary Care Unit (2009) conducted the acetylchlorine esterase enzyme test for local people who living in 16 villages in Hua Rua sub-district. The 624 of respondents had the acetylchlorine esterase enzyme test. 61.4% of respondents had risk of cholinesterase level. 61.4% of respondents had risk of cholinesterase level. 36.7% and 1.9% of respondents had normal and safe of cholinesterase level, respectively.

Norkaew (2009) studied knowledge, attitude and practice (KAP) of using Personal Protective Equipment (PPE) for chilli-growing farmers in Hua Rua sub-district, Ubonrachathani province, Thailand. The 330 chilli-growing farmers were interviewed by face to face interviewing. The result showed that 77.2% of chilli-growing farmers had low knowledge level, 54.5% of the farmer attitudes were not concerned about pesticides use and exposure and 85.0% of farmer had fair practices level. The conclusion is “Most of the respondents had Low knowledge, Not concern attitude and Fair practice”. Moreover, 40.9% of chilli-growing farmer agreed that they used pesticides more than the recommendation dose to increase their crop yield. 9.1% of respondents had abnormal of cholinesterase. 1.5% respondents had abnormal of cholinesterase and have health effect. In addition, the researcher reported the common pesticide uses in chilli farm area, including abamectin 47%, selecron (profenofos) 23.3%, prodium 600 (chlorpyrifos) 14.9%, paraquat 9.1% and lanate (carbamate) 3%.

Taneepanichskul (2009) studied risk assessment of OPPs (Chlorpyrifos) associated with dermal exposure in chilli-growing farmers during growing season (December 2009 to January 2010) at Hua Rua sub-district, Ubonrachathani province, Thailand. Chlorpyrifos residues on 35 chilli-growing farmers' hands after spraying were collected using hand-wiping technique. The average daily dose (ADD) of farmers was 2.51×10^{-9} $mg.kg^{-1}$·day. The ADD of male farmers was higher than female farmers. For risk characterization, both of the hazard quotient (HQ) for male and female farmers were lower than acceptable level. Even if, the conclusion was not at risk with non-carcinogenic effects from dermal exposure, the researcher suggested that inhalation and oral exposure routes should be evaluated risk assessment because the farmer had mentioned on acute and repeated or prolonged effects of OPPs after their application.

Un Mei Pan (2009) investigated the dermal exposure of OPPs (Chlorpyrifos and Profenofos) and assessed health risk in rice farmers at the Rangsit Agriculture Area, Prathumthani province, Thailand. The 29 rice farmers were interviewed to understand their characteristics of the rice farmer and pesticide use. Some of respondents had neurological signs and symptoms that could be related to OPPs existed. The respondents agreed that they used more than 1 type of pesticide and often mixed more amount of pesticides than recommended. Health risk of chlorpyrifos and profenofos exposure of 14 rice farmers were assessed. Hazard Index (HI) assumes the effects of the different compounds and effects are additive. HI of chlorpyrifos and profenofos exceeds unity ($HI > 1$). Theses rice farmer may be at risk and may get chronic adverse health effects.

Darko and Akoto (2008) studied dietary intake of OPPRs in vegetables, including tomato, eggplant and pepper, from Kumasi market, Ghana. OP pesticides detected in vegetables are methyl-chlorpyrifos, ethyl- chlorpyrifos, dichlorvos, dimethoate, malathion, monocrotophos, omethioate, methyl-parathion and ethyl-parathion. Levels of malathion in tomatoes (0.120 ± 0.101 $mg.kg^{-1}$) and pepper (0.143 ± 0.042 $mg.kg^{-1}$) exceeded the MRLs of 0.1 $mg.kg^{-1}$. Health risk indices of the residual were computed using the research data and food consumption assumption of Ghana from FAO 2003. Health risks were found to be associated with omethioate, methyl-parathion and ethyl-parathion in tomato and omethioate, methyl-parathion, ethyl-parathion, dichlorvos and monocrotophos in eggplant. No health hazard was found associated with the consumption of pepper.

Phumongkutchai, *et al.* (2008) studied the profenofos degradation of chilli to identify MRL value by using pesticide followed the recommendation dose (40 ml profenofos/20 ml water). They concluded that all profenofos residues (3.96 mg/kg) on chilli were lower than MRL value (5 mg/kg) after pesticide spraying for 3 days. They also randomly investigated the pesticide residues on 60 chilli samples from Nakhonpathom and Kanjanaburi provinces. Chlorpyrifos (0.010 – 0.820 mg/kg, n = 15) and profenofos (0.010 – 4.600 mg/kg, n = 15) were mainly detectable but the concentration did not exceed the MRL.

Bhanti and Taneja (2007) studied the residual concentration of selected OPPs (methyl parathion, chlorpyrifos and malathion) in vegetables grown of different seasons (summer, rainy and winter) in northern India. The winter vegetables are the most contaminated followed by summer and rainy vegetables. The analysis of health risk estimates indicated that the methyl parathion has been found to poses some risk to human health, but chlorpyrifos and malathion were found to be under safe limit. In addition, the researcher suggested that the consumer was exposure only to the lower concentration of pesticides that may cause chronic diseases. The concentration of various pesticides were well below the set maximum residue levels (MRLs), but continuous consumption of such vegetables even with moderate contamination level can accumulate in the receptor’s body and may prove fatal for human population in the long term.

Bai *et al.* (2006) investigated the OPPRs in market foods in the Shaanxi area of China. In order to providing off-season fresh fruits and vegetables to countries, the greater volumes of OPPs are frequently used during the growing season in the area and result in serious food contaminations The 60 samples of cereals, 80 samples of vegetables and 60 samples of fruit were determined. In 18 of 200 samples, five OPPs, including dichlorvos, dimethoate, methyl-parathion, methyl-pirimiphos and parathion, were found in concentrations ranging from 0.004 to 0.257 $mg.kg^{-1}$. Dimethoate in fruits and parathion in vegetables exceeded the MRLs.

Lunliu (2005) investigated preliminary risk management of OPPs, including in chilli consumers from Amphoe Sadao, Songkhla province, Thailand. 30 samples from Good Agricultural Practice (GAP) plots and 30 samples from farmer plots were analyzed for quality and quantity. No pesticide residue was detected in the GAP plots. 7 OPPs (Chlorpyrifos, profenophos, methamidophos, parathion-methyl, triazophos, diazinon, dimethoate) were detected. The levels of 3 chlorpyrifos comtaminated samples (0.7445, 0.7532 and 0.7668 $mg.kg^{-1}$) and 1 triazophos contaminated sample (0.0573 $mg.kg^{-1}$) were higher than MRLs, 0.5 $mg.kg^{-1}$ and 0.02 $mg.kg^{-1}$ of chlorpyrifos and triazophos, respectively. HQ is calculated for risk assessment. HQ values ranged from 0.0002-0.0375 (HQ<1), indicating that chilli samples Amphoe Sadao, Songkhla province were safety for consumption. For risk management, using GAP method was the most effective method to reduce the risk.

Office of Agricultural Research and Development Region 8 (1999) studied pesticide residues in chilli. The chilli were collected 40 samples from various sources such as market foods from Amphoe Hat- yai, Songkhla province, farmer plots from Phatthalung, Nakon Si Thammarat, and Songkhla province. The 16 of 23 samples were mostly detected the residues of OPPs in chilli products. The concentrations ranging of 5 parathion methyl (0.01 to 0.08 $mg.kg^{-1}$), 5 chlorpyrifos (0.01 to 0.49 $mg.kg^{-1}$), 4 methamidophos (0.07 to 2.46 $mg.kg^{-1}$) and 2 dimethoate (0.47 to 0.48 $mg.kg^{-1}$) were found. The levels of methamidophos contaminated sample (2.46 $mg.kg^{-1}$) was higher than MRLs, 2 $mg.kg^{-1}$ of chlorpyrifos.

CHAPTER III

RESEARCH METHODOLOGY

3.1 Study design

The research design was "experimental and observational" as shown in Figure 3.1. Experimental design was analysis of the residues of OPPs in chilli by using QuEChERS method. Observational design was dietary intake survey of chilli for local people who consumed chilli in Hua Rua. The integration of OPPRs analysis data and information from the questionnaire-based dietary survey were used in estimating the population's potential risk and providing a plausible worst-case scenario. A cross-sectional study was done from October 2010 to February 2011. 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 No. 082.1/2010. All participants signed a consent form prior to participation in this study.

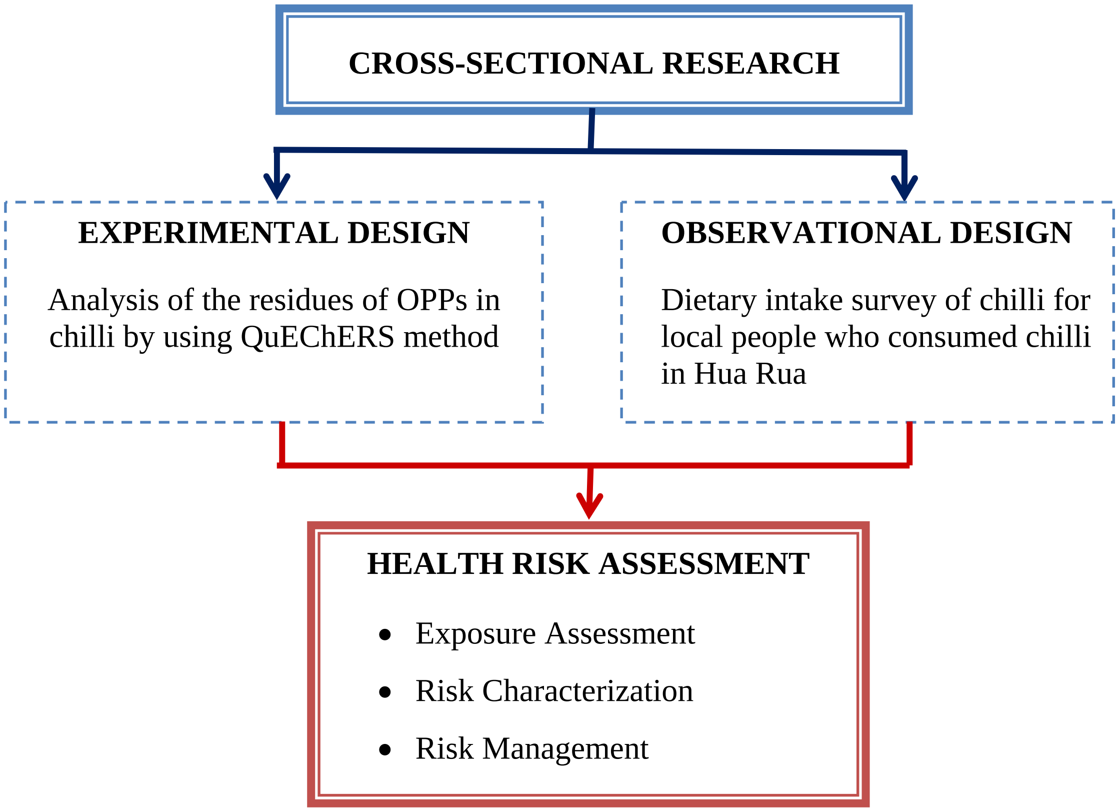


Figure 3.1 Cross-sectional research

3.2 Study area and sampling sites

Hua Rua, is one of the largest areas of chilli cultivation where located at Ubonratchathani province, was selected to be the study area. (Figure 3.2)

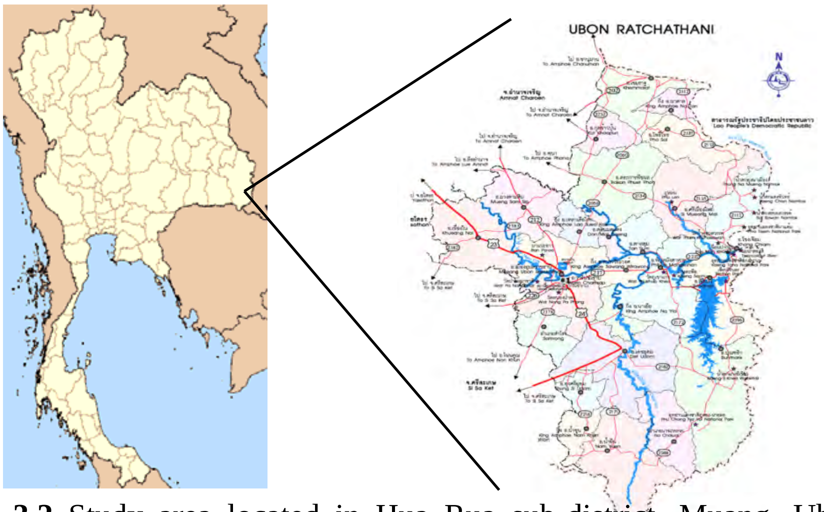


Figure 3.2 Study area located in Hua Rua sub-district, Muang, Ubonratchathani, Thailand

The dietary survey was conducted from October 2010 to December 2010 and the chilli sampling was conducted on February 2011. Thirty-three Chilli samples (n = 33) were collected from 11 chilli farm areas in Hua Rua after pesticide spraying for 7 days. (Figure 3.3) At 7 day, it is the commonly day of chilli harvest in Hua Rua area. The analyzed chilli would not be washed in order to provide a plausible worst-case scenario.

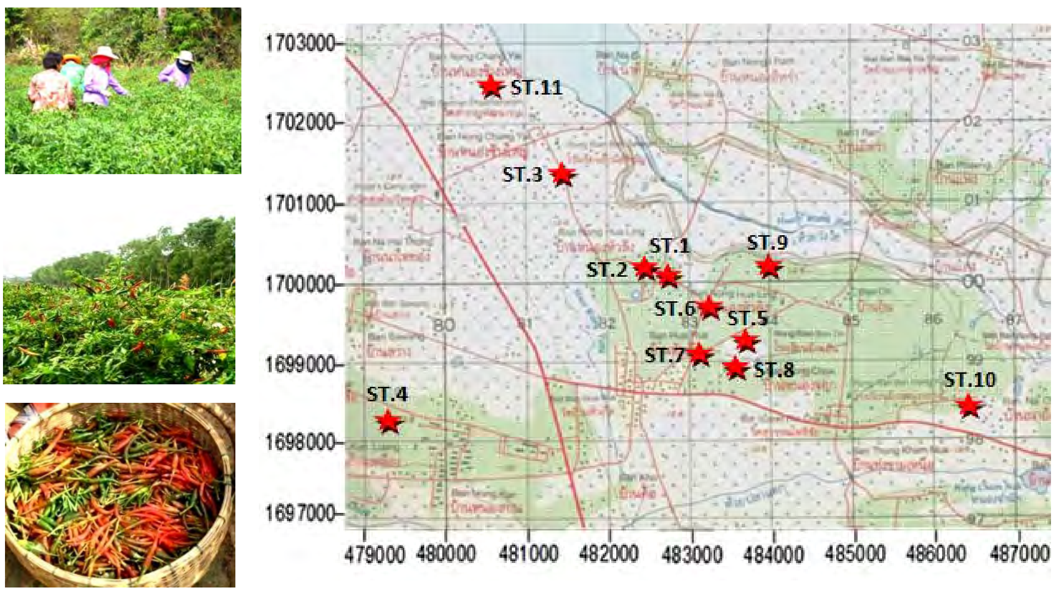


Figure 3.3 Location of sampling sites in Hua Rua [Chilli sampling sites (★), n = 33]

3.3 Dietary survey

All of participants in this research were local people living in Hua Rua, Ubonratchathani province. This research was concentrated on local people who consume chilli from Hua Rua area. One hundred and ten (*n* = 110) in raging age of 15 years old or above were randomly participated for a questionnaire-based dietary survey. A face-to-face interview (between the researcher and community members) focused on the frequency (number of times per day, week, month, or year), the quantity of consumption, and the body weight of the participant (Figure 3.4). A measuring cup and a balance were used during the interview to facilitate the quantification of food intake (Siriwong, 2006; 2008). The daily consumption (kg/day) of chilli was evaluated for each individual. The interview questionnaire study was consisted of three parts:

Figure 3.4 Face-to-face interview between the research and Hua Rua' local people from October to December 2010

In part 1: **the general information and personal background** of the local people who consumed chilli in Hua Rua area, were obtained, namely, the age, body weight, gender, education level, occupation.

In part 2: **the consumption behavior** of the local people who consumed chilli in Hua Rua area, namely, chilli consumption behavior, frequency and quantity of consumption.

In part 3, **information on health problems** was obtained to assess any health problems potentially associated with exposure to OPPs, including signs and symptoms that occurred throughout the locals' history of health, and their general health status.

The structure of the questionnaire was modified based on questions which were established by Siriwong (2006), Norkaew (2009), Taneepanichskul (2009), and Un Mei Pan (2009). Form of a questionnaire-based dietary survey is in Appendix A.

3.4 Analysis of organophosphate pesticide residues in chilli

3.4.1 Pesticide standard and chemicals

Thirteen organophosphate pesticide standards for dichlorvos, acephate, omethoate, demeton-s-methyl, dimethoate, tolclofos-methyl, pirimiphos-methyl, malathion, chlorpyrifos, methidathion, prothiofos, profenofos, ethion were obtained from Restek. A stock of the standard mixing containing 13 pesticides was prepared in 99% acetone at concentration of 100 µg/ml and stored at -4ºC in a refrigerator.

Analytical solvents such as 99% acetone and 99% acetonitrile were pesticide grade solvents purchased from Labscan Analytical Science. All Chemical reagents were purchased from Carlo Erba i.e. Sodium Chloride, Magnesium sulfate. 99% Acetic acid were purchased from Merck. The 2 ml disposable polypropylene centrifuge tubes, 50 mg GCB, 50 mg PSA, and 150 mg $MgSO_4$ were purchased from Agilent. All Chemical used in this study are listed in Table 3.1

Glassware was well-cleaned with laboratory detergent, and then sequentially rinsed with distilled water and acetone. Finally, washed glassware was baked in oven at 300ºC for overnight.

Table 3.1 Chemical lists using for OPPs analysis

Chemicals	Supplier/ Grade
European Organophosphate pesticide Mix	RESTEK/ Pestiscan grade
Acetone (CH_3COCH_3)	Labscan Analytical Science/ Pestiscan grade
Acetonitrile (CH_3CN)	Labscan Analytical Science/ Pestiscan grade
Acetic acid (HAc)	Merck/ HPLC grade
Sodium Chloride (NaCl)	Carlo Erba/ Analytical grade

Table 3.1 Chemical lists using for OPPs analysis *(continue)*

Chemicals	Supplier/ Grade
Magnesium sulfate ($MgSO_4$) – Anhydrous powder	Carlo Erba / Analytical grade
QuEChERS D-SPE tube (2 ml disposable polypropylene centrifuge tubes, 50 mg GCB, 50 mg PSA, and 150 mg $MgSO_4$)	Agilent/ Analytical grade

3.4.2 Sample extraction and clean up

Weight 10 g of the homogenized chilli sample and add it into a 50 ml centrifuge tube.
Add 10 ml of cold acetonitrile-HAc 0.1%; shake for 1 min
↓
Add 1 g NaCl; Add 4 g anhydrous $MgSO_4$;
↓
shake vigorously for 1 min
↓
Centrifuge at 5000 rpm for 5 min
↓
Transfer 1325 µl of supernatant to a 2 ml QuEChERS D-SPE tube
↓
vortex mix for 1 min
↓
Centrifuge at 13,000 rpm for 3 min
↓
825 µl transfer to an autosampler
↓
Evaporate to 0.6 ml under N_2 stream
↓
GC analysis

Figure 3.5 Flow chart of the modified QuEChERs method for extraction of chilli

A 10 g of the homogenized chilli was placed into a 50 ml disposable polypropylene centrifuge tube and cold acetonitrile containing hydrochloric acid of 0.1% (10 ml) was added. The centrifuge tube was capped and shaken for 1 min. Then, NaCl (1 g) and anhydrous $MgSO_4$ (4 g) were added, and the tube was vigorously shaken for 1 min and centrifuged at 5000 rpm for 5 min. 1325 µl aliquot of the supernatant (ACN extract) was transferred into a QuEChERS D-SPE tube. The tube was capped and mixed in a vortex mixer for 1 min, and then centrifuged at 13,000 rpm for 3 min. An 825 µl aliquot of the extract was transferred to a GC vial. The extract was evaporated to 0.6 ml under nitrogen gas (N_2) stream for GC analysis. A schematic of the extraction and cleanup procedure is shown in Figure 3.5.

3.4.3 Gas chromatography analysis

Table 3.2 The condition for analysis of OPPs

Capillary Column	DB-1701 (30.0 m length, 0.25 mm i.d., 0.25 µm film thickness) coated with 14% Cyanopropylphenyl and 86% methyl polysiloxane			
Carrier Gas	Helium (He)			
Flow rate of He	0.75 ml/min			
Type of Injection	Splitless			
Injection volume	1 µL			
Injector Temperature	220 ^{o}C			
Detector	Flame Photometric Detector (FPD)			
Detector Temperature	250 ^{o}C			
Oven Ramp	ºC/min	Next ºC	Hold (min)	Run Time (min)
Initial		80	1.00	1.00
Ramp 1	12.00	195	0.00	10.58
Ramp 2	2.00	210	3.00	21.08
Ramp 3	15.00	225	2.00	24.08
Ramp 4	40.00	275	10.00	35.33

An Agilent 6890N GC equipped with Flame Photometric Detector (FPD) was used for quantification. Compounds were completely separated using 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). Sample quantification was performed using multiple external standards. A 1 µL of sample was injected into GC on splitless mode. The initial temperature of injection was 220ºC. The oven initial temperature was 80 ºC for 1 min, the programmed to increase at 12ºC/min to 195ºC. Then, it increased at 2ºC/min to 210ºC, held for 3 min. It increased to 225ºC at 15ºC/min, held 2 min. The last temperature was 275ºC which increased at 40ºC/min and held for 10 min. Total run time was 35.33 min. The helium gas was used as a carrier gas with a flow rate at 0.75 mL/min. The optimum condition could be obtained as, shown in Table 3.2.

3.5 Health risk assessment

Before human health risk assessment in this study, the planning should be done in order to find the important information. The following questions are asked for finding the answers regarding the purpose, scope and technical approaches that were used in the process of risk assessment as shown in Table 3.3.

Health risk estimations were done based on an integration of OPPRs analysis data and information from the questionnaire-based dietary survey. Moreover, the individual exposure (mg/kg·d) was calculated using the upper boundary of 95% confidence interval (C.I.) for the OPPRs mean for the local people. The information was useful in estimating the population's potential risk and providing a plausible worst-case scenario. If statistical data was available, exposure time and exposure frequency were recommended to be described in terms of the 95th percentile (US EPA, 1989). All of the data were analyzed by using *license SPSS for Windows* (Version 17.0).

3.5.1 Hazard identification

A preliminary survey of pesticide used in chilli farm from pesticide and chemical stores and from several literatures found that OPPs, such as chlorpyrifos, profenofos, Ethyl-O-(p-nitrophenyl) phenylphosphonothionate (EPN), abamectin, paraquat and carbamate, were the common pesticide usage in Hua Rua chilli farm area. (Taneepanichskul and Norkaew, 2009) OPP is classified as non-carcinogenic pesticide. It functions an irreversible AChE inhibitors, thereby affecting neuromuscular transmission.

3.5.2 Dose–response assessment

The relevant oral reference dose (RfD) was obtained from the US EPA's Integrated Risk Information System (IRIS), available on their website : http://www.epa.gov/iris/. (Table 2.7)

3.5.3 Exposure assessment

An individual's exposure to OPPRs from the ingesting of chilli (mg/kg·day) was estimated by multiplying the concentration of OPPs in chilli (mg/kg) by mean daily consumption rate (kg/day) before dividing by the average body weight (kg) of surveyed populations (US EPA, 1992a) (See *Equation 1*)

The worst-case scenario defined as the reasonable maximum exposure (RME) that is reasonably expected to occur at a site. RMEs are estimated for individual pathways. If a population is exposed via more than one pathway, the combination of exposures across pathways also must represent an RME. Therefore, the upper confidence (95^{th} percentile) on the arithmetic average concentrations was used to estimate the RME because the uncertainty associated with any estimate of exposure concentration might occur in this situation. If statistical data are available, exposure time and exposure frequency are recommended to be described in terms of the 95^{th} percentile (US EPA, 1989 and Siriwong 2009). Based on the statistical calculation regarding chilli consumer interviews (n = 110), the 95^{th} percentile of exposure time was 63 years and exposure frequency was 365 days/year. Furthermore, the body weight of chilli consumer obtained from the exposure survey was 57 kg using the 50^{th} percentile (mean or central estimate) values, instead of the 95^{th} percentile values.

3.5.4 Risk characterization

The reference dose (RfD) is used as a criterion in non-carcinogen risk characterization. The non-carcinogenic toxicity of individual risk estimation was estimated by calculating the Hazard Quotient (HQ) (See *Equation 2*). The interpretation HQ is equal to or greater than 1 ($\geq$ 1 exposure exceeds the RfD, probable risk), the exposed populations may be at risk.

Table 3.3 The essential information in the step of planning before health risk assessment

Questions	The Important Information
Who / What / Where is at risk?	Local people who consume contaminated chilli with OPPs from Hua Rua sub-district, Ubonratchathani province.
What is the environmental hazard of concern?	OPPs are concerned.
Where do these environmental hazards come from?	OPPs come from the chemical-pesticides that chilli-growing farmers mostly used in their chilli cultivation.
How does exposure occur?	Local people expose through ingestion pesticide residues in/on chilli.
What does the body do with the environmental hazard?	OPPs mainly breakdown hydrolysis in the liver. Some OPPs breakdown relatively slow and may look for temporary storage in body and fat. Human body has the ability to break down OPPs and be converted to a non-toxic chemical which is excreted in the urine.
What are the health effects?	OPPs inhibit AChE, an enzyme critical to the control of nerve impulse transmission from one cell to another. When the enzyme is inhibited, there is overstimulation and then paralysis of the secondary cell. The character, duration, and degree of the resulting physiologic effect are directly related to the amount and rate of enzyme inhibition at certain receptor sites in the central and peripheral nervous systems. Some critical amount of enzyme must be inactivated before the signs and symptoms of poisoning are evident.
How long does it take for an environmental hazard to cause a toxic effect? Does it matter when in a lifetime exposure occurs?	OPPs are acutely toxic occurring within 2-3 days after exposure. It is characterized by widespread muscarinic and nicotinic effects which are caused by inhibition of AChE at nerve ending, in ganglia, and in the brain. The symptoms of chronic effect occur after exposure at a low concentration of OPPs for a long time which is known to cause a delayed neuropathy.

CHAPTER IV
RESULTS AND DISCUSSION

4.1 Dietary survey

All of participants in this research were local people living in Hua Rua, Ubonratchathani province. These participants were chosen since the study focused on people who consume chilli from Hua Rua area. Face-to face interview was conducted from October to December 2010. The data were illustrated in Table 4.1 and Table 4.2.

One hundred and ten (n = 110) of participants were attended. 45 participants (40.9%) were male and 65 participants (59.1%) were female. The average age (±standard deviation; ±S.D.) was 47 ± 14 years (in range of 15–79 years) and the average body weight was 57.29 ± 10 kg (in range of 32–86 kg). Most of them (n = 83, 75.5%) graduated in elementary school or below and were farmers (n = 86, 78.2%).

All of them consumed chilli from Hua Rua area. The interview showed that participants of 65.5% consumed chilli from their farms and of 18.2% consumed chilli from local market in which pesticide were applied. Additionally, all of participants were the Northeastern people who normally consumed spicy food. The result also showed that 46.4% could consume about 4-6 chilli per menu. 35.5% consumed very spicy food and could consume more than 7 chilli per chilli menu. The average daily chilli consumption was 0.018 ± 0.015 kg/day. The average consumption (frequency) and duration of chilli consumption were 365 days/year and 41 years, respectively.

For information on cooking practices, 93.6% of participants always wash their hands and 6.4% of participants sometimes wash their hands before cooking. For chilli cooking, of 73.6% of participants always wash chilli and of 16.4% sometimes of participants wash chilli before cooking. Participants who never washed chilli before cooking were 10.0%. Therefore, the chilli consumer might get higher risk from contaminated chilli consumption if they sometimes (16.4%) or never (10.0%) wash chilli before cooking.

Participants had checked a level of cholinesterase in blood in the last 12 months. 41.8% of them were in a normal level and 2.7% of them were over the acceptable level of cholinesterase in blood. 4.5% of participants were in risk level. However, the participants who did not check the cholinesterase in blood test in last 12 months were 45.5%. The cholinesterase blood test from local people living in Hua Rua area, was reported by Hua Rua Primary Healthcare Unit (PCU) (2009) and Norkaew (2009). Hua Rua Primary Healthcare Unit had surveyed the cholinesterase in blood for 624 of Hua Rua people in 2009. It was reported that 61.3% were at risk level, 36.7% were over the acceptable of cholinesterase in blood, and 1.9% were normal level. Norkaew (2009) also surveyed 330 chilli-growing farmers about cholinesterase in blood test in last 12 months by using face-to-face interview. It was found that 1.5% was at risk, 9.1% were over the limitation of cholinesterase in blood, and 48.5% were normal level. However, 34.8% of the farmers did not check a cholinesterase in blood test in last 12 months similar to the results of this research. Based on prior studies and this study, it was obvious that people in this area potentially got health problem from pesticide resulting about one-fifth of people attending blood test presented problem about cholinesterase in blood. However, based on percentage of people did not attend blood test from the studies (prior studies and this study), the problem in this case is people ignore the health care and monitoring. Therefore it is suggested that the Provincial Agricultural Extension Office and Provincial Public Health Office of this local area should have an appropriated strategies concerning their health service throughout.

The interview also covered health problems related to pesticide exposure but no participant noted any evidence. This might be because they could not recall for long time. However, the continuous consumption of chilli even with pesticide contamination can accumulate and affect the receptor’s body in long term. (David and Richards, 2001)

Moreover, this study surveyed on pesticide use of chilli-growing farmers in Hua Rua (*n* = 11). All of them have similarly used chemical pesticides, such as Podium 600 (chlorpyrifos), Selecon (profenofos) and EPN (Ethyl-O-(p-nitrophenyl) phenylphosphonothionate), in their chilli cultivation. Norkaew (2009) also reported that the common pesticide usage in Hua Rua were abamectin (47.00%), profenofos (23.30%), and chlorpyrifos (14.9%). The farmers sprayed pesticide every 7 days by using portable pump. Most of them agreed that they mixed more than 2 pesticides and applied higher doses of pesticide application depending on type and number of insect pests on the field at that time as observed by farmers. Most of the chilli-growing farmers collected chilli after 6 – 7 days of pesticide application. All chilli farmers did not concern and test pesticide residues in chilli. They mainly focused on the quantity and the price of chilli. They agreed that they had to apply pesticide for all cultivation processes or apply higher doses of pesticide to protect their products. If they did not handle, they would not get chilli. Even if they consumed by themselves or sold to the local market, they still applied pesticide in the same application. Aksornsri (2005) studied pesticide use in chilli cultivation at Hua Rua. Most of farmers used several chemical pesticides instead of biological pesticide because the biological pesticide is slow and less efficiency to eradicate insects. Regarding mixing more than 2 pesticides, farmers believed that mixing of several kinds of chemicals would increase the chemical efficiency and could eradicate more diseases and pests when they sprayed in only one time. Aksornsri (2005) similarly reported that the residue test of chilli in Hua Rua area has not been conducted. Moreover, the study reported that chilli-growing farmer usually collected chilli before the chemical degrade without concerning about consumer safety regarding the toxic residue because they thought that consumers need to wash vegetables before consuming. Therefore, not only Hua Rua consumers might get some risks of pesticide residues in chilli, but the people living out of this area might get risk due to consumption of Hua Rua's chilli without well washing.

Table 4.1 Socio-demography and general information of study population and chilli consumption at Hua Rua sub-district, Ubonratchathani

Characteristics	**Hua Rua sub-district, Ubonratchathani province**	
	Number *(n = 110)*	**Percentage (%)**
Sex		
Female	65	59.1
Male	45	40.9
Mean age (range 15-79 years)	47 ± 14 years	
age (range 15-59 years)	84	76.4
age (range 60-79 years)	26	23.6
Mean body weight (range 32-86 kg)	57.29 ± 10 kg	
Mean body weight (age range 15-59 years)	57.26 ± 10 kg	
Mean body weight (age range 60-79 years)	57.38 ± 12 kg	

Table 4.1 Socio-demography and general information of study population and chilli consumption at Hua Rua sub-district, Ubonratchathani. *(continue)*

Characteristics	**Hua Rua sub-district, Ubonratchathani province**	
	Number ***(n = 110)***	**Percentage (%)**
Education		
≤ Elementary school	83	75.5
Secondary school	20	18.2
Bachelor's degree	4	3.6
Diploma	3	2.7
Occupation		
Agriculture/Farmer	86	78.2
Student	11	10.0
Business owner	7	6.4
Government official/ Officer	6	5.5
Respondents consume chilli from Hua Rua area		
Yes	110	100
No	0	0
Source of chilli consumption		
Own chilli farm; Used pesticide	72	65.5
Local market; Used pesticide	20	18.2
Own chilli farm; No pesticide usage	16	14.5
Local market; Organic product	2	1.8
Spicy level/Chilli quantity		
Very spicy (≥ 7 chilli/menu)	39	35.5
Moderate spicy (4 - 6 chilli/menu)	51	46.4
Less spicy (1 - 3 chilli/menu)	20	18.2
No	0	0

Table 4.1 Socio-demography and general information of study population and chilli consumption at Hua Rua sub-district, Ubonratchathani. *(continue)*

Characteristics	**Hua Rua sub-district, Ubonratchathani province**	
	Number (*n = 110)*	**Percentage (%)**
Washing hand before cooking		
Always	103	93.6
Sometime	7	6.4
Never	0	0
Washing chilli product before cooking		
Always	81	73.6
Sometime	18	16.4
Never	11	10.0
Cholinesterase check within the last 12 months		
Never checked	50	45.5
Checked, but I don't know the results	6	5.5
Checked, result was normal	46	41.8
Checked, result exceeded the acceptable	3	2.7
Checked, result was at risk level	5	4.5

Table 4.2 Average consumption frequency (days/year), consumption duration (years) and daily consumption (kg/day)

Exposure variable	**Value (±S.D.)**
Average consumption frequency (days/year)	365
Average exposure duration (year)	41 ± 14
Average chilli consumption (kg/day)	0.018 ± 0.015
Average chilli consumption* (chilli/day)	13

*1 chilli = 1.32 ± 0.05 g

4.2 Extraction of chlorpyrifos and profenofos residues in chilli

4.2.1 Chlorpyrifos and profenofos retention time

Thirty-three chilli samples (*n* = 33) were collected from 11 chilli farming areas at Hua Rua in February 2011 after pesticide spraying for 7 days and maintained below 4°C during transportation to the laboratory where they were processed immediately. The unwashed chilli samples were homogenized using a food processor and stored at –4 °C until analysis. OPPRs in chilli were extracted by using QuEChERS method and analyzed by GC-FPD.

Chlorpyrifos and profenofos are OPPs that were found in 33 chilli samples as illustrated in Figure 4.1. Chlorpyrifos were indentified at 16.79 min and profenofos were indentified at 22.13 min.

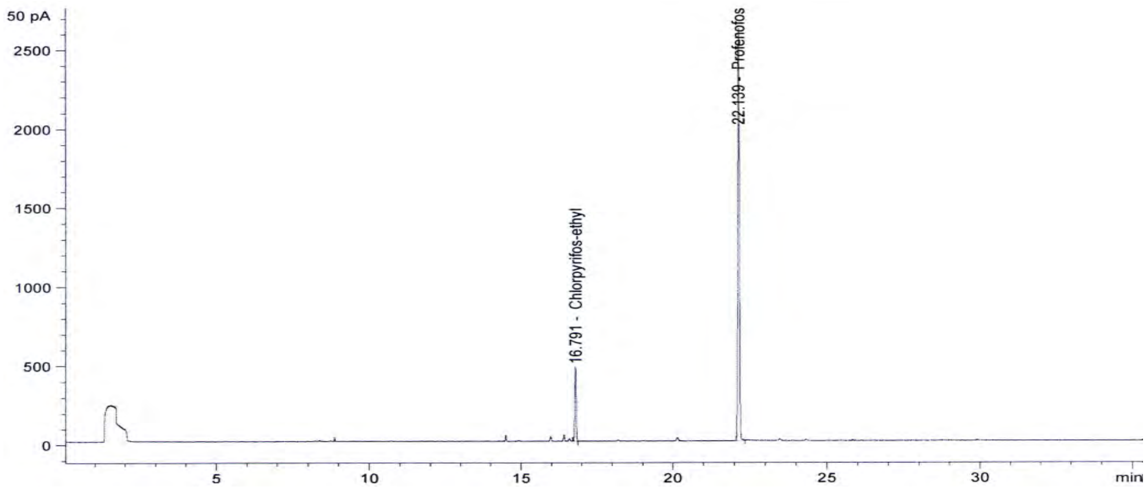


Figure 4.1 The chromatogram of OPPs in chilli samples using DB-1701 (30.0 m length, 0.25 mm i.d., 0.25 μm film thickness) coated with 14% cyanopropylphenyl and 86% methyl polysiloxane.

4.2.2 Quality control

All samples in this study were analyzed using Agilent 6890N gas chromatography equipped with Flame Photometric Detector (FPD); 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). A calibration curves using the external mixed standard was quantified at concentration of 0.1, 0.5, 1, 5, and 10 μg/ml (Appendix B). Correlation coefficients were greater than 0.99. A calibration standard was run every 10 samples and all measurements were performed in the ranges of linearity found for each compound. In Appendix C, validation data showed essentially quantitative recovery in the range of 82–104 % with relative standard deviation (RSD) lower than 10% for all compounds in the concentration range of 0.5–2.0 mg/kg. The limit of detection (LOD) and limit of quantification (LOQ) were 0.01 µg/ml and 0.02 µg/ml, respectively. According to the Scientific Association Dedicated to Excellence in Analytical Method (AOAC), all quality control values showed that this qualitative study was in the recommended standard level (AOAC Peer Verified Method Program, 1993).

4.2.3 Concentration of chlorpyrifos and profenofos

The mean, minimum, maximum, 95th percentile concentration of chlorpyrifos and profenofos in chilli (mg/kg, ppm) were shown in Table 4.3. In addition, the upper confidence 95th percentile on the concentration was used to estimate the reasonable maximum exposure (RME) for the worst-case scenario to protection and prevention of high oral exposure consumer. Chlorpyrifos and profenofos concentration in 33 chilli samples were compared with MRL as shown in Figure 4.2 and Figure 4.3, respectively.

The Codex Alimentarius sets limitation of pesticide residue remaining on food or the Maximum Residue Limits (MRL) to protect consumer health. The MRL of chlorpyrifos is 0.5 mg/kg and the MRL of profenofos is 5 mg/kg. (Table 4.3)

Table 4.3 The concentration of OPPs residue chilli collected from Hua Rua sub-district, Ubonratchathani.

OPPs	**MRL** [a]	**Concentration (mg/kg, ppm)**			
***n* = 33**	**(mg/kg)**	**Mean ± S.D.**	**Minimum**	**95th**	**Maximum**
Chlorpyrifos	0.5	0.339 ± 0.392	< 0.010 [b]	1.303	1.380
Profenofos	5	2.536 ± 1.669	0.520	5.940	6.290

[a] Codex Alimentarius, 2010
[b] Limit of Detection (LOD); The concentration values were < LOD estimating to LOD; 0.010 mg/kg.

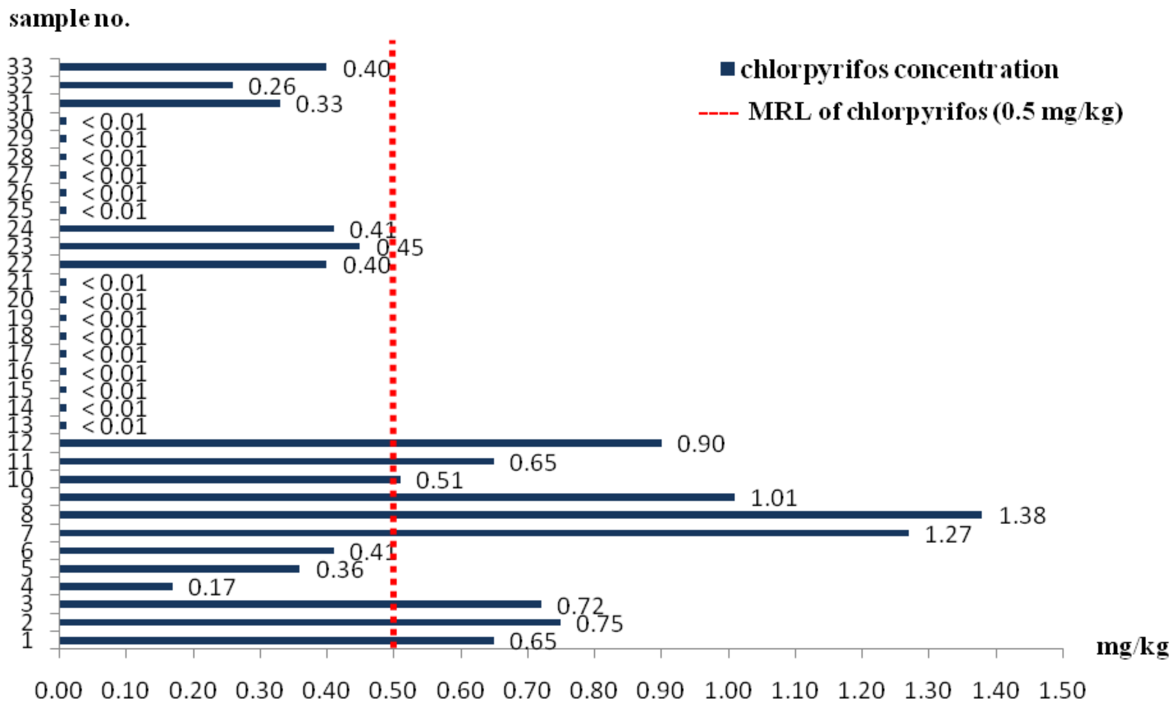


Figure 4.2 Chlorpyrifos concentration in 33 chilli samples (MRL reference: Codex Alimentarius, 2010)

The concentration of chlorpyrifos was <0.010 – 1.380 mg/kg. The mean and 95th percentile concentrations were 0.339 and 1.303 mg/kg, respectively. Furthermore, the level of 9 chlorpyrifos contaminated samples which were higher than the MRL of 0.5 mg/kg ranged from 0.51 to 1.38 mg/kg (Codex Alimentarius, 2010).

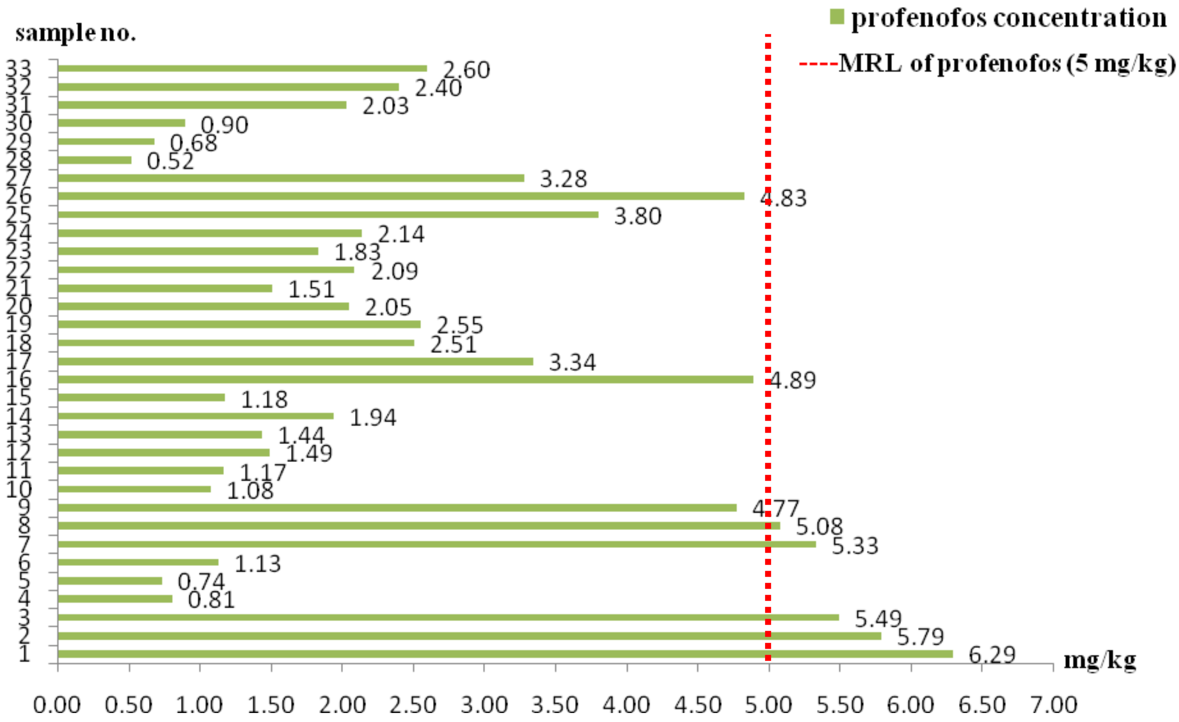


Figure 4.3 Profenofos concentration in 33 chilli samples (MRL reference: Codex Alimentarius, 2010)

The concentration of profenofos was 0.520 – 6.290 mg/kg. The mean and 95th percentile concentrations were 2.536 and 5.940 mg/kg, respectively. Moreover, the level of 5 profenofos contaminated samples (5.08, 5.33, 5.49, 5.79, and 6.29 mg/kg) were higher than the MRL (5 mg/kg). Phumongkutchai, *et al.* (2008) studied the profenofos degradation of chilli to identify MRL value by using pesticide followed the recommendation dose (40 ml profenofos/20 ml water). They concluded that all profenofos residues on chilli were lower than MRL value after pesticide spraying for 3 days. On contrarily, in this study, chilli samples were collected and analyzed after pesticide spraying for 7 days, but the pesticides were still detected in 5 chilli samples at concentration exceeded MRL. The result may be because Hua Rua' chilli-growing farmer used the pesticide over the recommendation dose or the pesticide could resist in environmental condition in the area. Kamrin (1997) described that chlorpyrifos residues remain on plant surfaces for approximately 10-14 days and lost primarily by volatilization. Radwan (2005) recommended that the consumable safety time were found to be 10 days on sweet pepper and 14 days on hot pepper and eggplant fruits after profenofos spraying. The results from Norkaew (2009) and this study indicated that chilli growing farmer at Hua Rua area used over the recommendation dose, and normally collected the chilli after pesticide spraying 7 days. Additionally, they sometimes collected chilli earlier than 7 days if the chilli market is in high price (Aksornsri, 2005). It is result in chlorpyrifos and profenofos concentrations higher than the MRL were detected.

This pesticide residues result is similar to previous works. The pesticide residues left over the MRL were detected in some chilli samples. A good illustration is Lunliu's work. Lunliu (2006) studied on pre-risk management of OPPs in chilli consumers from Sadao, Songkhla province, Thailand. 7 OPPs including chlorpyrifos (0.010 – 0.766 mg/kg, n = 25) and profenofos (0.010 – 0.065 mg/kg, n = 17) residues on chilli were detected. It was found that 3 chlorpyrifos contaminated samples (0.744, 0.753, and 0.766 mg/kg) were higher than the MRL. Another example is the work by Phumongkutchai, *et al.* (2008). They randomly investigated the pesticide residues on 60 chilli samples from Nakhonpathom and Kanjanaburi provinces. Chlorpyrifos (0.010 – 0.820 mg/kg, n = 15) and profenofos (0.010 – 4.600 mg/kg, n = 15) were mainly detectable but the concentration did not exceed the MRL.

Jansong, *et al.* (2010) studied on pesticide residues in chilli in lower Northeastern agricultural area followed the Good Agricultural Practice (GAP). They detected chlorpyrifos, profenofos, cypermethrin and endosulfan. The study areas are Ubonratchathani and Srisakate provinces. The chilli farms at Ubonratchathani found chlorpyrifos (0.095 –1.097 mg/kg, n = 12) and 3 chilli samples exceeded the MRL. The chilli farms at Srisakate found profenofos (0.110 – 3.400 mg/kg, n = 23) and chlorpyrifos (0.088 – 2.105 mg/kg, n = 17). The 3 chlorpyrifos contaminated samples exceeded the MRL.

The Office of Agriculture Regulation specified chlorpyrifos, profenofos, and ethion allowing for Thai chilli cultivation. Similarly, Lunliu (2006), Norkaew (2009), Jansong (2010) and this result indicated that Selecon (profenofos), and Podium 600 (chlorpyrifos) were commonly used in chilli cultivation. Profenofos (23.3%) and chlorpyrifos (14.9%) were mostly OPPs used since the significant problem of chilli-growing at Hua Rua area was worm, aphid, and plant louse (Norkaew, 2009). Taneepanichskul (2009) detected 6.25 mg/kg of chlorpyrifos residues on two hands of chilli growing farmer. Furthermore, during sampling on February 2011, the chilli growing farmer reported that aphid was seriously presenting problem. They preferred to use the mixed of profenofos and chlorpyrifos due to their effectiveness. Therefore, chlorpyrifos and Profenofos could be detected in this study.

Moreover, Bhanti and Taneja (2007) studied OPPRs in vegetables in different seasons at Northern India. The OPPRs concentrations in vegetables of different seasons shows that the winter vegetables are the most contaminated followed by summer and rainy vegetables. This research did not compare with different seasons because the chilli cultivation at Hua Rua had been done 1 times per year from August to May. The chilli harvest would be starting on January until May that was in the period of winter and summer, then farmer would switch to rice growing.

4.3 Health risk assessment

Health risk estimation was done based on an integration of OPPRs analysis data and information from the questionnaire-based dietary survey. An evaluation of non-carcinogenic risk to the local people of Hua Rua was summarized in Table 4.3.

The average daily dose (ADD) was used to estimate the exposure for non-carcinogenic following *Equation 1*. An evaluation of non-carcinogenic toxicity of individual risks can be computed by using the hazard quotient (HQ) ratio. When the calculation of local people non-cancer risk ratio is greater than 1.0 or RfD, the exposure population may be at risk (US EPA, 1986b; US EPA, 1999b). Furthermore, the benchmarks of non-cancer risk are set the value 1.0 shown in Figure 4.5, Figure 4.6 and Figure 4.7. The relevant reference dose (RfD) determined by US EPA's Integrated Risk Information System (IRIS) for chlorpyrifos and profenofos are 0.003 and 0.0001 mg/kg-day, respectively.

The average daily dose (ADD) of chlorpyrifos from chilli consumption in this area was 1.07 x 10^{-4} mg/kg-day. The average daily dose of profenofos from chilli consumption in this area was 8.00 x 10^{-4} mg/kg-day. Regarding chili consumption, local people had high exposure to profenofos than chlorpyrifos. (Figure 4.4).

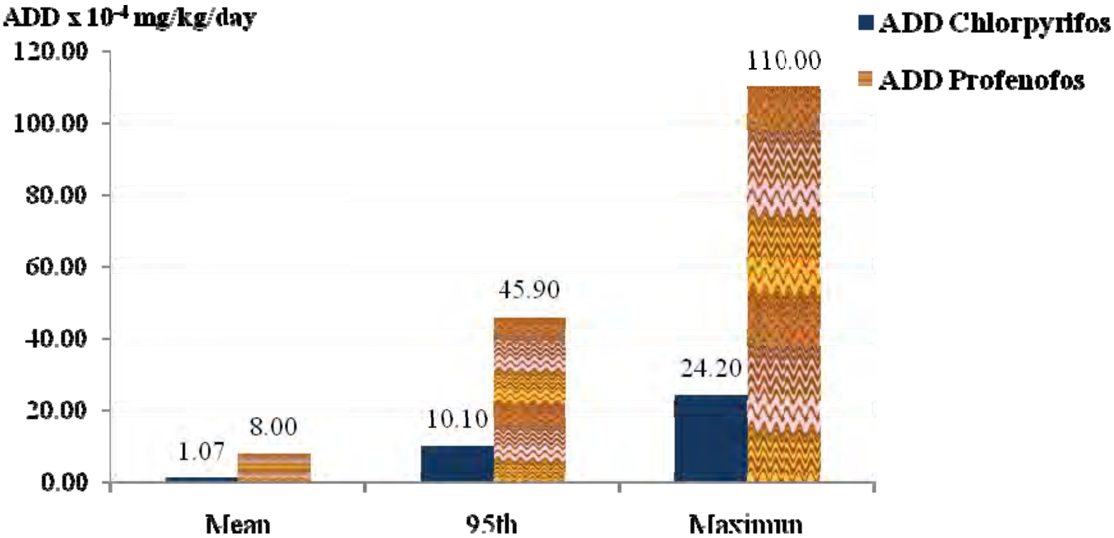


Figure 4.4 Average Daily Dose of OPPs for chilli consumption by local people in Hua Rua sub-district, Ubonratchathani

Table 4.4 Health risk assessment of organophosphate pesticide residues (OPPRs) due to chilli consumption for the people (*n* = 110) in Hua Rua, Ubonratchathani, Thailand from October 2010 to February 2011

OPPs pesticides		**Concentration (mg/kg) *n* = 33**	**Exposure Assessment (*n* = 110)**						**Oral RfD[a,b] (mg/kg-day)**	**Risk Characterization**
			IR[*] (kg/day)	**EF (days/yr)**	**ED (yrs)**	**BW[**] (kg)**	**AT (days)**	**ADD (mg/kg-day)**		**Hazard quotient**
Chlorpyrifos	**Min**	< 0.010[c]	0.001	365	8	57	2920	1.75×10^{-7}	0.003	**0.0001**
	Mean	0.339	0.018	365	41	57	14,965	1.07×10^{-4}	0.003	**0.0360**
	95th	1.303	0.044	365	63	57	22,995	1.01×10^{-3}	0.003	**0.3370**
	Max	1.380	0.100	365	72	57	26,280	2.42×10^{-3}	0.003	**0.8070**
Profenofos	**Min**	0.520	0.001	365	8	57	2920	9.12×10^{-6}	0.0001	**0.0912**
	Mean	2.536	0.018	365	41	57	14,965	8.00×10^{-4}	0.0001	**8.0080**
	95th	5.940	0.044	365	63	57	22,995	4.59×10^{-3}	0.0001	**45.900**
	Max	6.290	0.100	365	72	57	26,280	1.10×10^{-2}	0.0001	**110.000**

[a] IRIS, 1988 (Oral reference dose of chlorpyrifos)
[b] Jaipieam, 2009 (Oral reference dose of profenofos)
[c] Limit of Detection (LOD); The concentration values were < LOD estimating to LOD; 0.010 mg/kg.
[*] 1 chilli = 1.32 ± 0.05 g
[**] Average weight (US EPA, 1989 and Siriwong, 2009)

Table 4.5 Individual risk assessment of organophosphate pesticide residues (OPPRs) due to chilli consumption in Hua Rua, Ubonratchathani, Thailand from October 2010 to February 2011

Average Concentration (mg/kg)	**Individual Exposure Assessment**	**Individual Exposure Assessment**						**Oral RfD** (mg/kg-day)	**Risk Characterization**
		IR* (kg/day)	**EF** (days/yr)	**ED** (yrs)	**BW**** (kg)	**AT** (days)	**ADD** (mg/kg-day)		**Hazard quotient**
Chlorpyrifos	**Min**	0.001	365	40	65	14,600	5.22×10^{-6}	0.003[a]	**0.001**
0.339	**Max**	0.100	365	23	55	8,395	6.16×10^{-4}	0.003[a]	**0.210**
Profenofos	**Min**	0.001	365	40	65	14,600	3.90×10^{-5}	0.0001[b]	**0.390**
2.536	**Max**	0.100	365	23	55	8,395	4.61×10^{-3}	0.0001[b]	**46.11**

[a] IRIS, 1988 (Oral reference dose of chlorpyrifos)
[b] Jaipieam, 2009 (Oral reference dose of profenofos)
* 1 chilli = 1.32 ± 0.05 g
** Average weight (US EPA, 1989 and Siriwong, 2009)

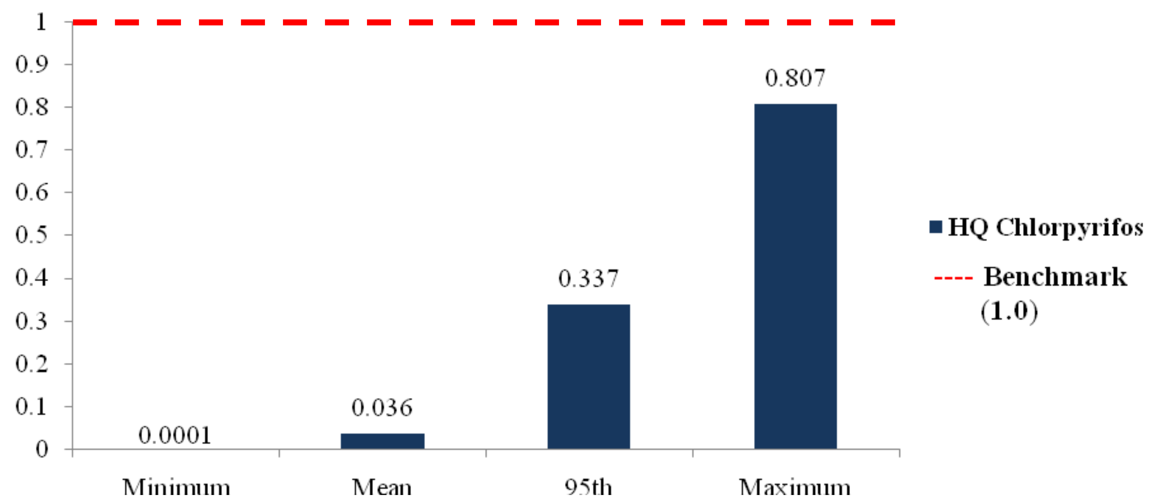


Figure 4.5 Non-cancer HQ of chlorpyrifos for daily chilli consumption by local people in Hua Rua sub-district, Ubonratchathani

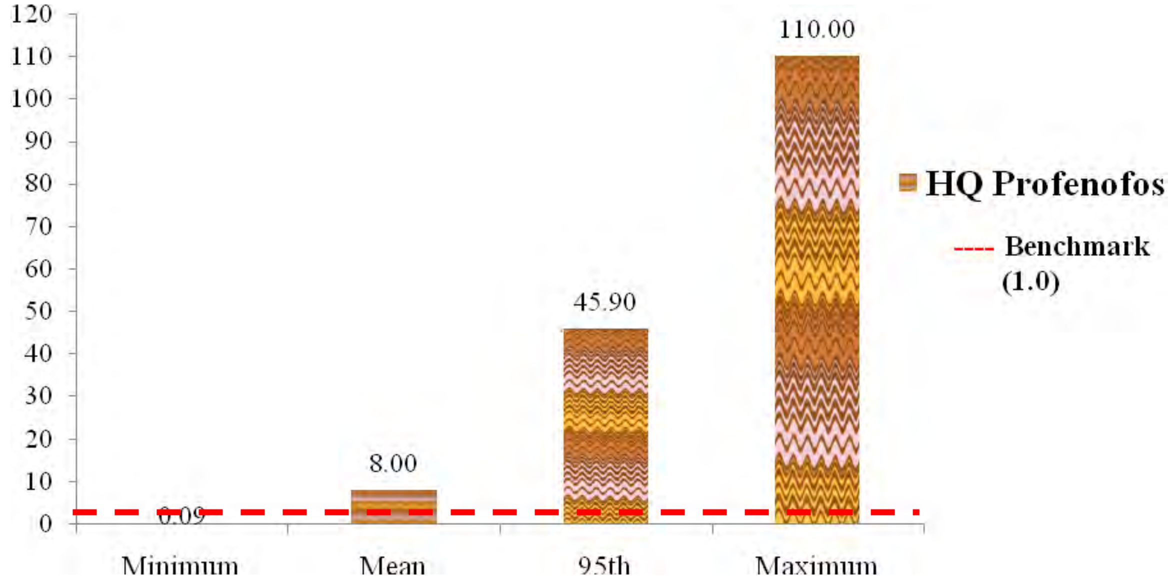


Figure 4.6 Non-cancer HQ of profenofos for daily chilli consumption by local people in Hua Rua sub-district, Ubonratchathani

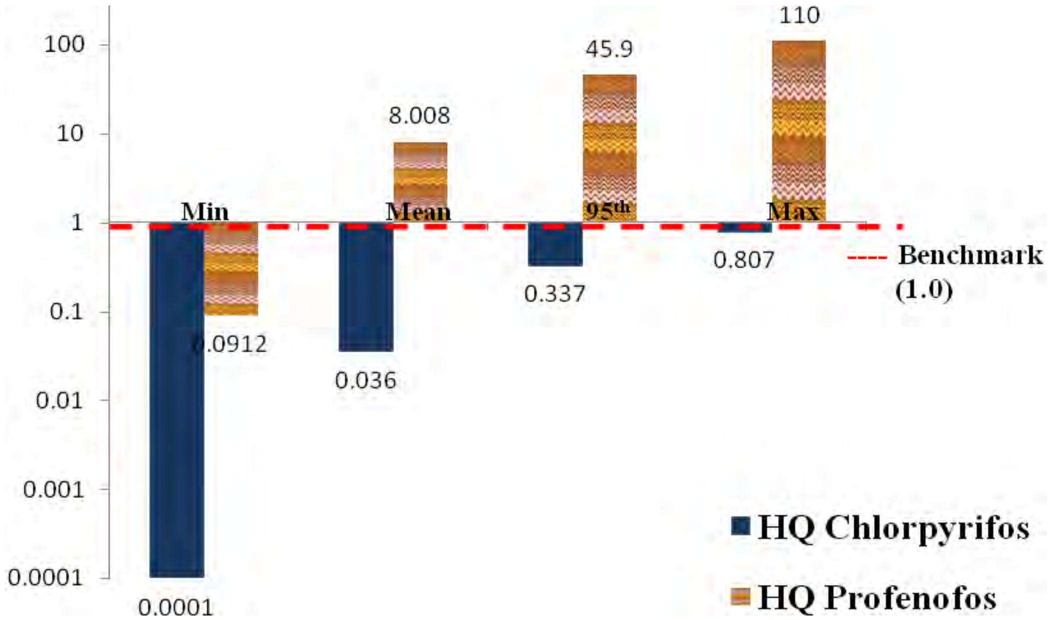


Figure 4.7 Non-cancer HQ of OPPs for daily chilli consumption by local people in Hua Rua sub-district, Ubonratchathani

The result indicated that all HQ of chlorpyrifos residue values were lower than the acceptable level 1.0 suggesting that the chilli consumer may not be risk by consuming chlorpyrifos contaminated-chilli. Although we considered at RME level (95^{th} percentile), it also showed the low HQ value.

However, HQ of profenofos value was higher than the benchmark of 1.0. It was found that the RME (95^{th} percentile) and maximum levels were 45- and 110-time higher than the RfD, respectively. Therefore, consumption of chilli contaminated with profenofos was at risk. The participants could get effect of the pesticide to cholinesterase activity in plasma (or serum) and red blood cell (US EPA, 2006).

Furthermore, individual risk assessment of local consumers in Hua Rua area was calculated (Appendix E). Table 4.5 showed the HQ of chlorpyrifos and Profenofos at minimum and maximum values. The individual HQ of chlorpyrifos at minimum and maximum were lower than the acceptable level 1.0. Consequently, chilli consumer, who was the minimum and maximum exposure, may not be risk from consumption chilli contaminated with chlorpyrifos. The individual HQ of Profenofos at minimum was lower than the acceptable level 1.0. However, the individual HQ of profenofos at maximum value was 46- time higher than the RfD. Therefore, chilli consumer who was maximum exposure was at risk from consumption chilli contaminated with profenofos.

Lunliu (2006) assessed health risk for chilli consumer with OPPs contamination at Sadao, Songkhla. 11 OPPs including chlorpyrifos and profenofos were studied. The mean ADD of chlorpyrifos was 0.00001 mg/kg-day lower than this result (0.00017 mg/kg-day). HQ of chlorpyrifos was 0.0014, which was also lower than this result (HQ of chlorpyrifos = 0.0360). Profenofos were found and calculated ADD of profenofos which was 0.00001 mg/kg-day while the value from this result was 0.00080. HQ of profenofos was 0.0016, which was much lower than this result (HQ of profenofos = 8). The result indicated that chilli consumer from Hua Rua might get much higher risk than chilli consumer from Songkhla due to the average daily dose (ADD) and the pesticide residue concentration on chilli were higher.

The several researches and the pesticide use survey indicated that chilli growing farmer at Hua Rua applied over the recommendation dose and collected the chilli before the chemical degradation resulting in high values of pesticide residues detectable. Additionally, the chilli consumers at Hua Rua have the high average daily chilli consumption. Therefore, the result showed the high health risk of consumers from chilli consumption. The exposure of profenofos caused greater health risk than that of chlorpyrifos because profenofos was found higher concentration of pesticide residue and is more hazardous than chlorpyrifos. The research also showed the higher health risk than the result of Lunliu (2006). It was caused by several factors. For example, Sadao, Songkhla province is located in tropical monsoon climate, where has rain almost all year. Therefore, the observed result showed lower pesticide residue concentration. The lower average daily chilli consumption of Sadao people also was a factor of lower health risk.

Additionally, it is concerned that children and aging individuals are susceptible groups and more sensitive to the adverse health effects than general adult. Typically, children do not consume chilli or just little dose; therefore, the chilli consumption behaviors of adult and aging were evaluated and illustrated in Table 4.6. The 84 participants (76.40%) were adult who aged in range of 15-59 years old and 26 participants (23.60%) were aging who were 60 years old or above. For adult, the average daily chilli consumption (±S.D.) was 0.019 ± 0.001 kg/day. The average consumption frequency and duration of chilli consumption were 365 days/year and 35 years, respectively. For aging, the average daily chilli consumption (±S.D.) was 0.016

± 0.002 kg/day. The average consumption frequency and duration of chilli consumption were 365 days/year and 58 years, respectively. The data were illustrated in Appendix D.

Table 4.6 Consumption information of local people in Hua Rua sub-district, Ubonratchathani and general Thai population

Characteristics	**Susceptible group in Hua Rua sub-district, Ubonratchathani province — Average value (±S.D.)**			
	Total [a] *n* = 110, 100%	**Adult [b] *n = 84*, 76.40%**	**Aging [c] *n = 26*, 23.60%**	**General Thai [d]**
Consumption frequency (days/yr)	365	365	365	ND
Consumption duration (yrs)	41 ± 14	35 ± 11	58 ± 5	ND
Chilli consumption (kg/day)	0.018 ± 0.015	0.019 ± 0.001	0.016 ± 0.002	0.005

[a] The range of age 15-79 years old
[b] The range of adult age 15-59 years old
[c] The range of aging age 60-79 years old
[d] The Ministry of Public Health, Thailand
[ND] ND = No Data reported

The average daily chilli consumption was not significantly difference between adult and aging people ($p \geq 0.05$). In addition, the average daily chilli consumption of local people in Hua Rua was 0.018 kg/day. It was 3 times more than the average of general Thai people consumption (0.005 kg/day). The result indicated that chilli is one of the most consumed spices in Hua Rua, so people consuming large amount of these contaminated chilli may get risk. An evaluation of non-cancer risk to the adult and aging group of Hua Rua was summarized in Table 4.7 and Table 4.8. Comparison of risk estimated for all ages was showed in Figure 4.8 and Figure 4.9.

Table 4.7 Health risk assessment of organophosphate pesticide residues (OPPRs) due to chilli consumption for adult (*n* = 84) in Hua Rua, Ubonratchathani, Thailand from October 2010 to February 2011

OPPs pesticides		Concentration (mg/kg) *n* = 33	ADULT Exposure Assessment [d] (*n* = 84) IR* (kg/day)	EF (days/yr)	ED (yrs)	BW** (kg)	AT (days)	ADD (mg/kg-day)	Oral RfD[a,b] (mg/kg-day)	Risk Characterization Hazard quotient
Chlorpyrifos	**Min**	< 0.010[c]	0.001	365	8	57	2,920	1.75×10^{-7}	0.003	**0.0001**
	Mean	0.339	0.019	365	35	57	14,965	1.13×10^{-4}	0.003	**0.0377**
	95th	1.303	0.048	365	53	57	22,995	1.10×10^{-3}	0.003	**0.3658**
	Max	1.380	0.100	365	71	57	26,280	2.42×10^{-3}	0.003	**0.8070**
Profenofos	**Min**	0.520	0.001	365	8	57	2,920	9.12×10^{-6}	0.0001	**0.0912**
	Mean	2.536	0.019	365	35	57	14,965	8.45×10^{-4}	0.0001	**8.4533**
	95th	5.940	0.048	365	53	57	22,995	5.00×10^{-3}	0.0001	**50.0211**
	Max	6.290	0.100	365	71	57	26,280	1.10×10^{-2}	0.0001	**110.3509**

[a] IRIS, 1988 (Oral reference dose of chlorpyrifos)
[b] Jaipieam, 2009 (Oral reference dose of profenofos)
[c] Limit of Detection (LOD); The concentration values were < LOD estimating to LOD; 0.010 mg/kg.
[d] The range of adult age 15-59 years old
* 1 chilli = 1.32 ± 0.05 g
** Average weight (US EPA, 1989 and Siriwong, 2009)

Table 4.8 Health risk assessment of organophosphate pesticide residues (OPPRs) due to chilli consumption for aging people (*n* = 26) in Hua Rua, Ubonratchathani, Thailand from October 2010 to February 2011

OPPs pesticides		Concentration (mg/kg) *n* = 33	AGING Exposure Assessment [d] (*n* = 26)							Risk Characterization
			IR* (kg/day)	EF (days/yr)	ED (yrs)	BW** (kg)	AT (days)	ADD (mg/kg-day)	Oral RfD[a,b] (mg/kg-day)	Hazard quotient
Chlorpyrifos	**Min**	< 0.010[c]	0.004	365	46	57	2,920	7.02×10^{-7}	0.003	**0.0002**
	Mean	0.339	0.016	365	58	57	14,965	9.52×10^{-5}	0.003	**0.0317**
	95th	1.303	0.040	365	71	57	22,995	9.14×10^{-4}	0.003	**0.3048**
	Max	1.380	0.040	365	72	57	26,280	9.68×10^{-4}	0.003	**0.3228**
Profenofos	**Min**	0.520	0.004	365	46	57	2,920	3.65×10^{-5}	0.0001	**0.3649**
	Mean	2.536	0.016	365	58	57	14,965	7.12×10^{-4}	0.0001	**7.1186**
	95th	5.940	0.040	365	71	57	22,995	4.17×10^{-3}	0.0001	**41.6842**
	Max	6.290	0.040	365	72	57	26,280	4.41×10^{-3}	0.0001	**44.1404**

[a] IRIS, 1988 (Oral reference dose of chlorpyrifos)
[b] Jaipieam, 2009 (Oral reference dose of profenofos)
[c] Limit of Detection (LOD); The concentration values were < LOD estimating to LOD; 0.010 mg/kg.
[d] The range of aging age 60-79 years old
[*] 1 chilli = 1.32 ± 0.05 g
[**] Average weight (US EPA, 1989 and Siriwong, 2009)

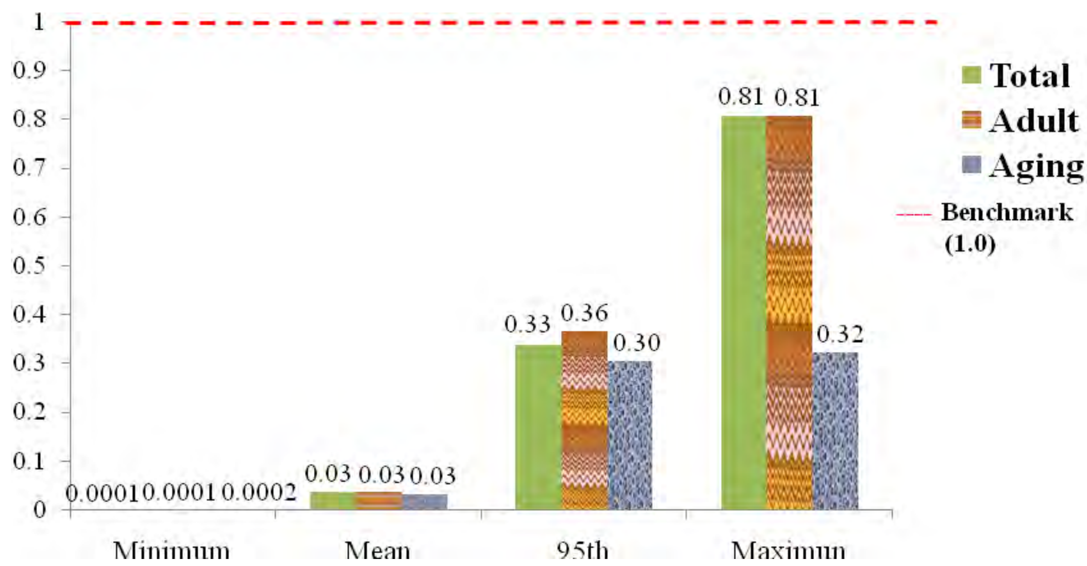


Figure 4.8 Non-cancer HQ of chlorpyrifos of adult and aging people in Hua Rua, Ubonratchathani

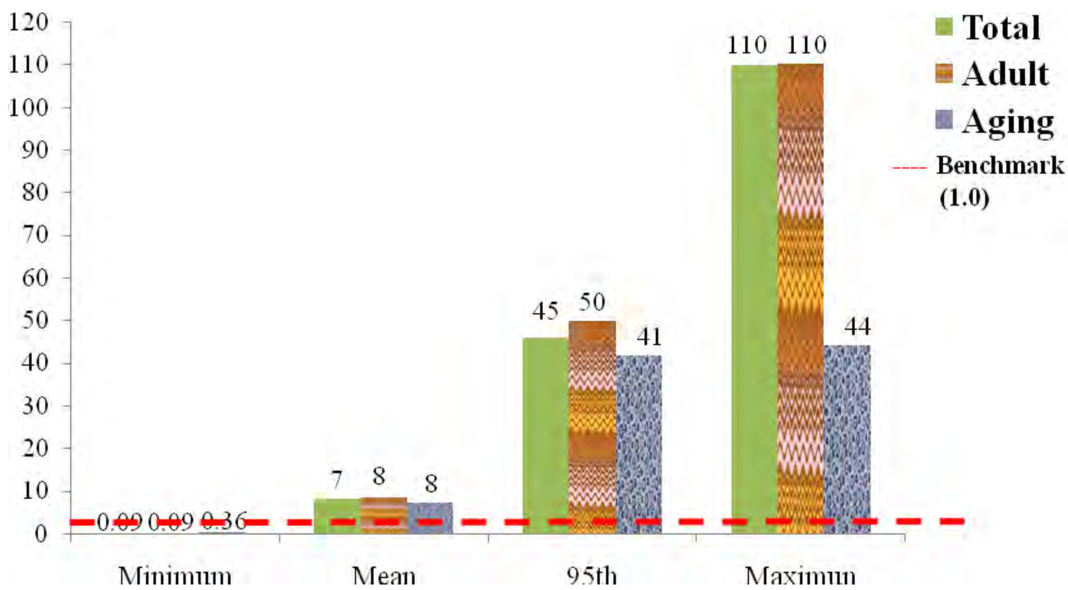


Figure 4.9 Non-cancer HQ of profenofos of adult and aging people in Hua Rua, Ubonratchathani

The ADD and HQ for chlorpyrifos and profenofos in adult and aging people were not significantly different ($p \geq 0.05$). It was similar result. All HQ of chlorpyrifos values were lower than the acceptable level 1.0, suggesting that the chilli consumer might not be at risk from consuming chlorpyrifos contaminated chilli. Although we considered at RME level (95th percentile), it also showed the low HQ value. On the other hand, HQ of profenofos residue values was greater than the acceptable level 1.0. Therefore, consumption of chilli contaminated with profenofos were at risk and can affect cholinesterase activity in plasma (or serum) and red blood cell (US EPA, 2006).

4.5 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). According to the risk assessment, the result indicated that chilli consumer in Hua Rua area were higher risk potential of non-carcinogenic from pesticide residue on chilli. Therefore, the appropriated risk management recommendations to help local communities avoid and protect themselves from OPPs are listed in the following sections.

1. **Communities awareness**

Local government should provide the risk assessment information and communication to local communities. Free publishing material, including poster, signboard, and leaflet, should be made to inform people who consume chilli from Hua Rua about the risk of OPPs and how to minimize the hazard to contribute consumer awareness.

Realizing the important of safe food for consumer, the good agricultural practices (GAP) are the best way for chilli cultivation without pesticide residues. Lunlin (2006) did not detect pesticide residues on chilli from GAP farm. GAP are a collection of principles to apply for on-farm production and post-production processes, resulting in safe and healthy food, while taking into account economical, social and environmental sustainability (FAO, 2003). To reduce the amount of exceeded pesticide concentration at Hua Rua area, GAP should be recommended.

Good agricultural practices for crops, Ministry of Agriculture and cooperatives manages all manufacturing process in the safety production, pesticides free and get the quality of consumers' requirements. GAPs are included choosing water source, using hazardous materials in agriculture, storing, transporting and recording product. GAP would manage all processes to achieve the quality in harvest and post harvest. (MASCI, 20).

Phaengchan, *et al*. (2008) recommended the integrated management for chilli cultivation. This knowledge integrated the GAP with local wisdom focusing on biological pesticide instance chemical pesticide. The result reported that it decreased the production costs and no pesticide residues problem that increased the product value at least 15%. For example, using of integrated management in chilli farm at Loei gave high yield 965 kg/rai and got profit 13,512 baht/rai comparing to local farm gave yield only 545 kg/rai and got profit only 7,367 bath/rai. The higher profit of cultivation is very well motivation to chilli growing farmer to change their original cultivation

Furthermore, Boss-2000 is the commercially biological detergent reducing pesticide residues on fruit and vegetable, which was recommended for spraying before chilli harvest or washing chilli before consumption by Lunlin (2006). The result showed that the drenched chilli with Boss-2000 for 5 min can reduce 55.14% of chlorpyrifos and 41.08% of profenofos residues on chilli. Moreover, the drenched chilli with Boss-2000 for 10 min can reduce 59.9% of chlorpyrifos and profenofos residues on chilli. Using Boss-2000 is one alternative that can use at Hua Rua area to reduce pesticide residues.

2. Individual household awareness

Residue levels in prepared chilli often get reduced substantially when the raw commodity is subjected to trimming, washing, and cooking. Therefore, household should wash raw commodities thoroughly before cooking and/or consuming. Cooking with heat is also recommended to reduce the OPPRs.

Department of Agriculture, the Ministry of Agriculture and Cooperative (2001) and the Folk Doctor book recommended several ways the pesticide residues reduction for example,

- washing with 1 tablespoon sodium bicarbonate in warm water (15 - 20 liters) for 15 minutes was the 90 – 95% pesticide residues reduction,

- washing with 0.5%Vinegar in water (4 liters) and drenching in water for 15 minute reduced 60 – 84% of pesticide residues,
- flowing moderate water through vegetable for 2 minutes reduced 54 – 63% of pesticide residues,
- boiling vegetables with hot water was the 50% pesticide residues reduction,
- drenching with 20 – 30 potassium permanganate in water (20 liters) for 10 minutes then rinse with water again reduced 35 – 43% of pesticide residues,
- washing with 1 tablespoon salt in water (20 liters) for 10 minutes then rinse with water again reduced 29 – 38% of pesticide residues,
- drenching with water from washing rice for 10 minutes then rinse with water again reduced 29 – 38% of pesticide residues,
- drenching with soaking solution for 10 minute then rinse with water again reduced 22 – 36% of pesticide residues,

Additionally, Radwan (2005) recommended the removal way of profenofos residues from hot and sweet pepper fruits, such as washing with potassium permanganate (95.75% reduction), sodium chloride solution (79.89% reduction), acetic acid solution and tap water (53–65%). Blanching and frying of pepper and eggplant fruits resulted in great reduction to almost completely removed (100%) of the deposited profenofos. In addition, pickling process removed 92.58 and 95.61% from hot pepper fruit after one week and after two weeks, respectively.

The dietary survey found that participants sometimes (15.1%) or never (9.2%) wash chilli before cooking. Therefore, the earlier ways should be conducted to protect consumer safety.

3. National government agencies concern

State and local responsibility in Thailand such as the Ministry of Public Health, the Ministry of Natural Resources and Environment and National Bureau of Agricultural Commodity and Food Standard (ACFS) should be launched for legal decisions to prevent, control and minimize health risk.

CHAPTER V
CONCLUSIONS AND RECOMMEDATIONS

5.1 Conclusions

This study assessed human health risk of organophosphate pesticide residues for chilli consumer in Hua Rau area, Ubonratchathani province, Thailand. The results were concluded as following;

1. For dietary survey (*n* = 110), one hundred and ten of participants were 45 males and 65 females. The age and weight of participants were in range of 15 – 79 years and 32-86 kg, respectively. The average age (±S.D.) was 47 ± 14 years. The average body weight (±S.D.) was 57 ± 10 kg. Most of them graduated in elementary school or below and were agricultureres i.e. chilli-growing farmers and paddy field farmers. All of them consumed chilli from Hua Rua area that of 65.5% consumed chilli from their farms and of 18.2% consumed chilli from local market in which pesticide were applied.

2. For pesticide usage survey (*n* = 11), all participants were chilli-growing farmer that have used chemical pesticides, such as profenofos, chlorpyrifos, EPN, in their chilli cultivation. They sprayed pesticide every 7 days by using portable pump and kept chilli after 6-7 days of pesticide application. Most of them agreed that they mixed more than 2 pesticides and applied higher doses of pesticide recommendation. All chilli farmers did not concern and test pesticide residues in chilli.

3. The average daily chilli consumption of people in this area was 0.018 kg/day higher than the average of general Thai people (0.005 kg/day).

4. A multiresidue method based on dispersive solid phase extraction sample preparation or QuEChERS and gas chromatography with flame photometric detection was used to analyze OPPRs on chilli. The recoveries for all the pesticides studies were from 82–104 % with relative standard deviation lower than 10% for all compounds in the concentration range of 0.5–2.0 mg/kg. Limit of qualification (LOQ) was 0.02 µg/ml. Limit of detection (LOD) was 0.01 µg/ml.

5. Thirty-three chilli samples (*n* = 33) were extracted by using QuEChERS method and analyzed by GC-FPD. Chlorpyrifos (<0.010 – 1.380 mg/kg) and profenofos (0.520 – 6.290 mg/kg.) were OPPs detectable. The mean concentration of

chlorpyrifos (±S.D.) was 0.339 ± 0.392 mg/kg. The mean concentration of profenofos (±S.D.) was 2.536 ± 1.669 mg/kg. The 9 chlorpyrifos contaminated samples were higher than the MRL, 0.5 mg/kg. The 5 profenofos contaminated samples were higher than the MRL, 5 mg/kg.

6. The average daily dose (ADD) of chlorpyrifos for chilli consumer in this area was 1.07 x 10^{-4} mg/kg-day. The ADD of profenofos for chilli consumer in this area was 8.00 x 10^{-4} mg/kg-day. The results indicated that chilli consumer get more risk from profenofos exposure than that from chlorpyrifos exposure

7. The HQ for chlorpyrifos of people in this area was in the acceptable risk level (HQ<1.0) suggesting that the chilli consumer may not be at higher risk from consuming chlorpyrifos contaminated chilli. While the HQ for profenofos was over the acceptable level (HQ>1.0). The RME (95^{th} percentile) and maximum level were 45- and 110-time higher than the RfD, respectively. Therefore, consumption of chilli contaminated with profenofos was higher risk that can affect cholinesterase activity in plasma (or serum) and red blood cell (US EPA, 2006).

8. The individual HQ of chlorpyrifos at minimum and maximum were lower than the acceptable level 1.0. So, chilli consumer, who was the minimum and maximum exposure, may not be risk from consumption chilli contaminated with chlorpyrifos. The individual HQ of Profenofos at minimum was lower than the acceptable level 1.0. However, the individual HQ of profenofos at maximum value was 46- time higher than the RfD. Therefore, chilli consumer who was maximum exposure was at risk from consumption chilli contaminated with profenofos.

9. Focus on susceptible group, the evaluation, including the average daily chilli consumption and hazard quotient, were no significant difference between adult and aging people ($p \geq 0.05$). Therefore, the similar results were showed as HQ of chlorpyrifos was lower than 1.0 at both mean and RME level and HQ of profenofos values were greater than 1.0, suggesting that the adult and aging consumer in this area were higher risk from consuming profenofos contaminated chilli.

10. An appropriated risk management should be hastily implemented in Hua Rua area to protect chilli consumer due to pesticide residues exposure. For example, Free

publishing material, including poster, signboard, and leaflet, should be made to inform people who consume chilli from Hua Rua about the risk of OPPs and how to minimize the hazard to contribute consumer awareness, consumer should wash raw commodities thoroughly before cooking and/or consuming, and cooking with heat is also recommended to reduce the OPPRs.

11. 5.2 Recommendations

1. Since, the risk of pesticide exposure from chilli consumer is higher than the acceptable level, various risk management decision may have to be made by the regulatory authorities in Thailand such as the Ministry of Public Health, the Ministry of Natural Resources and Environment and National Bureau of Agricultural Commodity and Food Standard (ACFS) as well as for legal decisions to prevent, control and minimize health risk.
2. The literature review and the result from this study indicated that chilli-growing farmers are lack of awareness regarding safe use of pesticides and consumer safety, so public education should be organized for improving their pesticide spraying knowledge, attitude and practice of pesticide use on agricultural farms of Hua Rua. For example, the Good Agricultural Practices (GAP) was recommended.
3. This study assessed the health risk related to oral exposure. Taneepanichskul (2009) studied risk assessment related to dermal exposure. Therefore, inhalation exposure should be conducted to complete information for all exposure routes. Moreover other kinds of reported pesticide in Hua Rua should be concerned, for example, abamectin and EPN.
4. Determination of blood and urinary OPPs metabolite in local population would be needed in the future study because these data could be used as reference data to compare with OPPs exposure.
5. An established dietary database for the local populations of Hua Rua should be completely conducted for other risk assessment with different contaminants in future work.

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APPENDIX A

Interviewer name__________.   Questionnaire no. ______ (____/____/____)

Center of Excellence for

Environmental and Hazardous Waste Management

Chulalongkorn University

Questionnaire for consumption exposure of pesticide residues on/in chilli within the chilli farming area of Hua Rau sub-district, Muang district, Ubonratchathani Thailand

Please answer the questions in the blanks provided and/or add a ✓ in the appropriate circle.

PART 1: GENERAL INFORMATION

1. Name-Surname ____
2. Address____
3. Gender   O Male   O Female
4. What is your highest level of education?

   O Elementary education   O Secondary education

   O Diploma   O Bachelor's degree
5. Age______________________ years
6. Body weight _______________kg
7. What is your main occupation?

   O Government official/ Officer

   O Business

   O Agriculturist

   O Student

PART 2: CONSUMPTION BEHAVIOR

1. How hot (i.e., spicy) do you like your food?

   O Very spicy ($\geq$7)

   O Moderately spicy (4-6)

   O A little spicy (1-3)

   O None

2. Do you consume chilli from Hua Rua farm O Yes O No

3. The sources of chilli you consume

   O Own chilli form; No pesticide

   O Own chilli farm; Used pesticide

   O Local market; No pesticide

   O Local market; Used pesticide

4. Do you wash your hands before cooking?

   O Always O Sometimes O Never

5. Do you wash the chilli before cooking with it?

   O Always O Sometime O Never

6. Do you wash your hand before cooking?

   O Usually O Sometime O Never

7. What types of spicy food do you like? List the main dishes, side dishes, dips/sauces, and/or condiments containing chilli that you most often consume.

   1.)__________________________________2.)___________________________

   3.)__________________________________4.)___________________________

8. The amount of fresh chilli side dishes and the chilli's composition food (without cook by heat) you consume are ________________chilli/menu

9. Family members ________________ people

10. The frequency of your fresh chilli side dishes consumption and the chilli's composition food (without cook by heat) are _________meal/day_______times/week

11. How old are you when you started the fresh chilli side dishes consumption and the chilli's composition food ? _________________ years

PART 3: HEALTH INFORMATION

1. Have you got the toxicity symptom after you consume fresh chilli as fresh chilli's composition or chilli side dishes?

Symptoms	Always	Sometime	Never
Headache, Dizziness			
Flabbiness			
Nausea/Vomiting			
Stomach cramps			
Diarrhea			
Blurs vision			
Chaste pain/ Difficulty of breathing			
Unconscious			
Other ________________________			

2. Have you had a cholinesterase test within the last 12 months?

O No

O Yes, but I don't know the results

O Yes, and my results were normal

O Yes, and my results were not normal

O Yes and my results were not normal and showed health effect

Interviewer name__________.Questionnaire no. _______ (____/____/____)

Center of Excellence for

Environmental and Hazardous Waste Management

Chulalongkorn University

Questionnaire the pesticide use of chilli-growing farmers

At Hua rau sub-district, Meaung district, Ubon Ratchathani province, Thailand

Please answer the question and/or mark ✓ in the blank

PART 1: GENERAL INFORMATION

1. Name-Surname ____
2. Address____
3. Gender O Male O Female
4. What is your highest level of education?
   - O Elementary education O secondary education
   - O diploma O Bachelor's degree
5. Age______________________ years
6. What is your main occupation?
   - O Government official O Officer
   - O Business O Agriculturist
   - O Student O Employee
   - O Unemployed O Other _________________________

PART 2: PESTICIDE USE

1. What time do you have chilli cultivation?

   O All year

   O Month ___________until____________

2. Do you use pesticides on your chilli plants? O Yes O No

3. What instruments and application methods do you generally use when you apply pesticides? (Can check more than one)

   O Boom on tractor, Truck, or Trailer

   O Hand spray gun

   O Backpack gun

   O Mist blower/fogger

   O Other (Please indicate_______________________)

4. How do you use pesticide for your chilli farm?

   O Single pesticide type

   O Mix pesticides

   O Other (Please indicate_______________________)

5. How do you usually mix or prepare pesticide?

   O According to the instructions O I add more than the instructions recommend

6. How often do you use pesticide for your chilli farm? (Can check more than one)

   O Before chilli cultivation i.e. prepare area for cultivation

   O During chilli cultivation i.e. chilli young plant

   O Before chilli harvest

   O After chilli harvest

   O Spray on chilli before sale or transport

   O Other (Please indicate_______________________)

7. Do you have any insect, weed, plant disease or animal problems after using pesticide?

   O No (Go to question no.9)

   O Yes (Go to question the next question)

8. How do you do to solve the problem?

   O Increase the amount of pesticide

   O Increase the frequency of using pesticide

   O Stop using pesticide and use other ways

   O Nothing

9. How much time do you allow after you spray your plants with pesticide before you harvest the chilli?

   O Immediately

   O Follow recommendation (Please indicate______________________)

   O Other (Please indicate______________________)

8. Do the source of chilli have pesticide test?

   O Yes; The result is negative

   O Yes; The result is positive

   O No; Not know the result

9. Please indicate the names of the pesticides that you use.

   1.)__________________________________

   2.)__________________________________

   3.)__________________________________

   4.)__________________________________

PART 3: GENERAL HEALTH INFORMATION

1. During the last 12 months have you experienced any of the following while and/or after applying pesticide?

   O Never

   O Few symptoms (Headache, fatigue, dizziness, tearing, sweating, throat irritation and stomach cramps)

   O Moderate symptoms (Nausea, vomit, blurs vision, shivering, constriction, cramp, excessive sweating and hyperventilation)

   O Nervous symptoms (Difficulty of breathing, chaste pain, Contracted pupils of the eye weakness in your arms or legs, involuntary twitches in your arms or legs and unconscious)

2. Have you had a cholinesterase test within the last 12 months?

   O No

   O Yes, but I don’t know the results

   O Yes, and my results were normal

   O Yes, and my results were not normal

   O Yes and my results were not normal and showed health effect

APPENDIX B

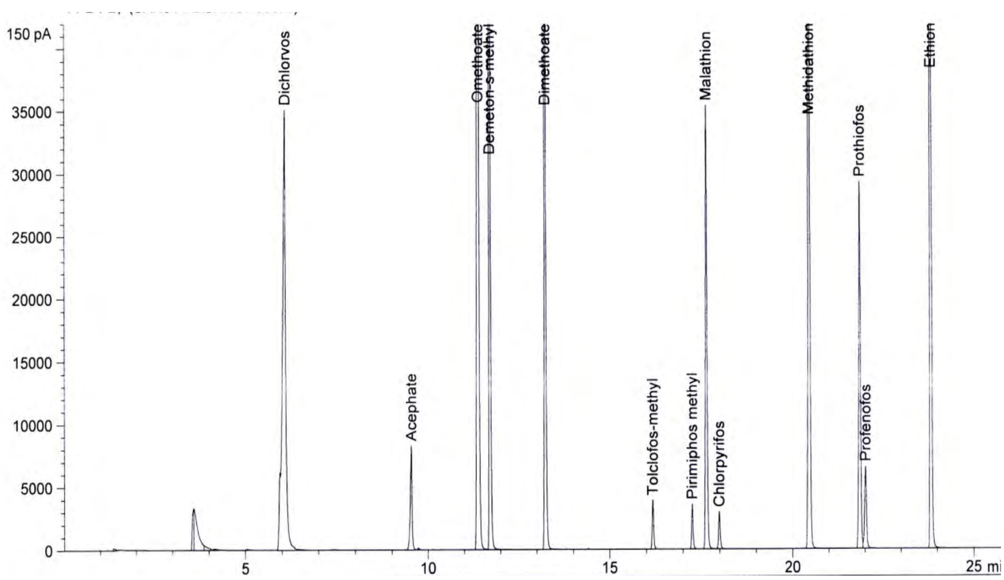

Figure B-1 The Chromatogram of organophosphate pesticide standard 1μL
Agilent 6890N gas chromatography equipped with Flame Photometric Detector; DB-1701 (30.0 m length, 0.25 mm i.d., 0.25 μm film thickness) coated with 14% Cyanopropylphenyl and 86% methyl polysiloxane.

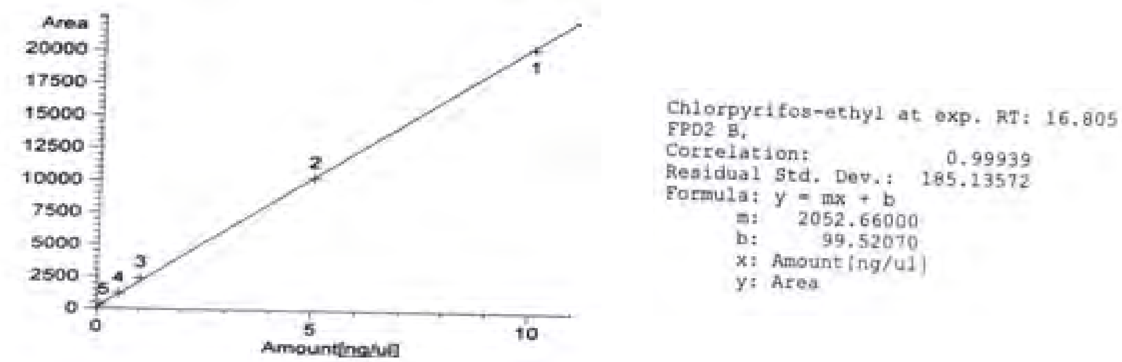

Figure B-2 The calibration curve of Chlorpyrifos

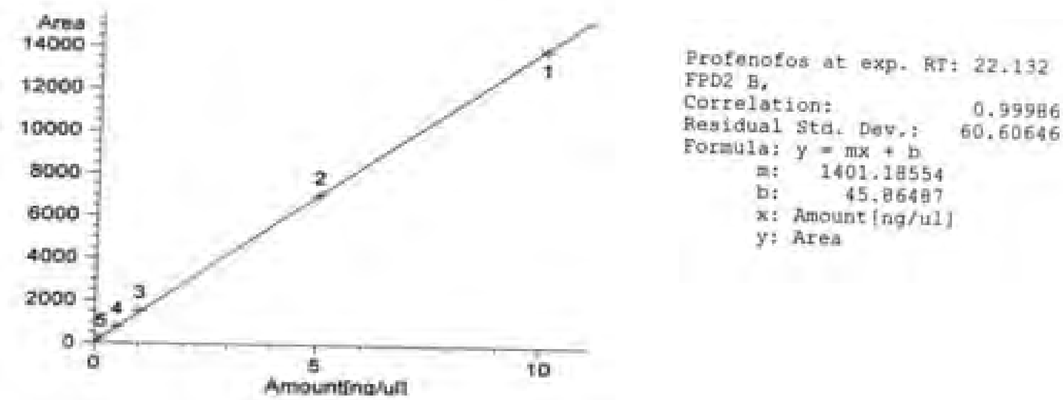

Figure B-3 The calibration curve of Profenofos

APPENDIX C

QUALITY CONTROL

Table C-1 Validation data of pesticides in chilli samples

OPPs	Equation	Determination coefficient (r^2)	Retention time (min)	LOD (µg/ml)	LOQ (µg/ml)	% Recovery ± RSD, Spiked concentration of OPPs on chilli (mg/kg), *n* =7	
						0.5	**2.0**
Chlorpyrifos	y = 2052.66x + 99.52	0.99939	16.80	0.01	0.02	87 ± 8	82 ± 6
Profenofos	y = 1401.19x + 45.86	0.99986	22.13	0.01	0.02	104 ± 9	94 ± 4

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 (at 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, a peak with a height that is at least twice or three times that of 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 being 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 an exact measurement. The LOD and LOQ can be calculated by the equations below (Siriwong, 2006):

$$\text{LOD} = \frac{3\ (\text{Signal})}{\text{Noise}} \qquad \textit{Equation C1}$$

$$\text{LOQ} = \frac{10\ (\text{Signal})}{\text{Noise}} \qquad \textit{Equation C2}$$

Assessment of method precision

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

$$\%RSD = \frac{100\,(SD)}{Mean}$$

Equation C4

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

Table C-2 Analyzed concentration versus precision acceptance (relative standard deviation, RSD) recommended by the AOAC

% Analyze	Analyze 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}	10 ppb	21
0.0000001	10^{-9}	1 ppb	30

Assessment of method accuracy

The method's accuracy is assessed by calculating the percent of recovery from an analysis of the reference materials or laboratory control samples. The percent recovery is calculated by equation below.

$$\% \text{ Recovery} = \frac{M_s - M_u}{T_s} \times 100 \qquad \textit{Equation C5}$$

Where;

M_s = Measured concentration of the target analysis in the spiked sample

M_u = Measured concentration of the target analysis in the unspiked sample

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

The concentration should cover the range of concern and should include one concentration close to the quantitation limit. Table 2-B shows the estimated recovery data, as recommend by AOAC

Table C-3 Recovery at different concentrations by the AOAC

% Active Ingredient	Analyze 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}	10 ppb	60 – 115
0.0000001	10^{-9}	1 ppb	40 – 120

APPENDIX D

Table D-1 General information of study population and chilli consumption at Hua Rua sub-district, Ubonratchathani

Characteristics	**Hua Rua sub-distric, Ubonratchathani province**					
	Total (*n* = 110)		**Adult (*n* = 84)**		**Aging (*n* = 26)**	
	Number	**%**	**Number**	**%**	**Number**	**%**
Sex						
Female	65	59.1	52	61.9	13	50
Male	45	40.9	32	38.1	13	50
Mean age	47 ± 14		41 ± 10		65 ± 5	
Mean body weight	57.29 ± 10		57.26 ± 10		57.38 ± 12	
Education						
≥ Elementary school	83	75.5	60	71.4	23	88.5
Secondary school	20	18.2	18	21.4	2	7.7
Bachelor’s degree	4	3.6	3	3.6	1	3.8
Diploma	3	2.7	3	3.6	0	0
Occupation						
Agriculturist/Farmer	86	78.2	66	78.6	20	76.9
Student	11	10.0	9	10.7	2	7.7
Business owner	7	6.4	5	6.0	2	7.7
Government official/ Officer	6	5.5	4	4.8	2	7.7

Table D-1 General information of study population and chilli consumption at Hua Rua sub-district, Ubonratchathani. *(continue)*

Characteristics	**Hua Rua sub-distric, Ubonratchathani province**					
	Total (*n* = 110)		**Adult (*n* = 84)**		**Aging (*n* = 26)**	
	Number	**%**	**Number**	**%**	**Number**	**%**
Do you consume chilli from Hua Rua area?						
Yes	110	100	84	100	26	100
No	0	0	0	0	0	0
The source of chilli usage						
Own chilli farm; Used pesticide	72	65.5	58	69.0	14	53.8
Local market; Used pesticide	20	18.2	13	15.5	7	26.9
Own chilli farm; No pesticide usage	16	14.5	13	15.5	3	11.5
Local market; No pesticide usage	2	1.8	0	0	2	7.7
Spicy level/Chilli quantity						
Very spicy (≥ 7 chilli/menu)	39	35.5	30	35.7	9	34.6
Moderately (6 - 4 chilli/menu)	51	46.4	42	50.0	9	34.6
Little (1 - 3 chilli/menu)	20	18.2	12	14.3	8	30.8
No	0	0	0	0	0	0
Wash your hand before cooking						
Always	103	93.6	79	94.0	24	92.3
Sometime	7	6.4	5	6.0	2	7.7
Never	0	0	0	0	0	0

Table D-1 General information of study population and chilli consumption at Hua Rua sub-district, Ubonratchathani. *(continue)*

Characteristics	**Hua Rua sub-distric, Ubonratchathani province**					
	Total (*n* = 110)		**Adult (*n* = 84)**		**Aging (*n* = 26)**	
	Number	**%**	**Number**	**%**	**Number**	**%**
Wash chilli before cooking						
Always	81	73.6	66	78.6	15	57.7
Sometime	18	16.4	13	15.5	5	19.2
Never	11	10.0	5	6.0	6	23.1
Have you had a cholinesterase test within the last 12 months?						
Never	50	45.5	37	44.0	13	50
Yes, but I don't know the results	6	5.5	5	6.0	1	3.8
Yes, My result was normal	46	41.8	36	42.9	10	38.5
Yes, over limit level	3	2.7	2	2.4	1	3.8
Yes, My result was in risk level	5	4.5	4	4.8	1	3.8
Average exposure frequency (days/yr)	365		365		365	
Average exposure duration (yrs)	41 ± 14		35 ± 11		58 ± 5	
Average chilli consumption[*] (kg/day)	0.018 ±0.015		0.018 ± 0.016		0.016 ± 0.010	

[*] 1 chilli = 1.32 ± 0.05 g

APPENDIX E

Table E-1 Individual risk assessment of organophosphate pesticide residues (OPPRs) due to chilli consumption in Hua Rua, Ubonratchathani, Thailand from October 2010 to February 2011.

Average Concentration (mg/kg)	Individual Exposure Assessment	Individual Exposure Assessment						Oral RfD[a,b] (mg/kg-day)	Risk Characterization
		IR[*] (kg/day)	EF (days/yr)	ED (yrs)	BW[**] (kg)	AT (days)	ADD (mg/kg-day)		Hazard quotient
Chlorpyrifos 0.339	1	0.001	365	40	65	14600	5.215×10^{-6}	0.003	0.0017
	2	0.004	365	46	76	16790	1.784×10^{-5}	0.003	0.0059
	3	0.004	365	61	67	22265	2.024×10^{-5}	0.003	0.0067
	4	0.005	365	40	80	14600	2.119×10^{-5}	0.003	0.0071
	5	0.005	365	57	78	20805	2.173×10^{-5}	0.003	0.0072
	6	0.005	365	47	70	17155	2.421×10^{-5}	0.003	0.0081
	7	0.005	365	64	65	23360	2.608×10^{-5}	0.003	0.0087
	8	0.004	365	57	50	20805	2.712×10^{-5}	0.003	0.0090
	9	0.007	365	39	86	14235	2.759×10^{-5}	0.003	0.0092
	10	0.004	365	13	48	4745	2.825×10^{-5}	0.003	0.0094
	11	0.005	365	30	60	10950	2.825×10^{-5}	0.003	0.0094
	12	0.004	365	56	45	20440	3.013×10^{-5}	0.003	0.0100
	13	0.005	365	45	54	16425	3.139×10^{-5}	0.003	0.0105
	14	0.005	365	17	52	6205	3.260×10^{-5}	0.003	0.0109
	15	0.007	365	21	69	7665	3.439×10^{-5}	0.003	0.0115
	16	0.005	365	28	47	10220	3.606×10^{-5}	0.003	0.0120
	17	0.004	365	53	37	19345	3.665×10^{-5}	0.003	0.0122
	18	0.006	365	40	50	14600	4.068×10^{-5}	0.003	0.0136
	19	0.007	365	31	57	11315	4.163×10^{-5}	0.003	0.0139
	20	0.008	365	56	65	20440	4.172×10^{-5}	0.003	0.0139

Table E-1 Individual risk assessment of organophosphate pesticide residues (OPPRs) due to chilli consumption in Hua Rua, Ubonratchathani, Thailand from October 2010 to February 2011. *(continue)*

Average Concentration (mg/kg)	Individual Exposure Assessment	Individual Exposure Assessment						Oral RfD[a,b] (mg/kg-day)	Risk Characterization
		IR[*] (kg/day)	EF (days/yr)	ED (yrs)	BW[**] (kg)	AT (days)	ADD (mg/kg-day)		Hazard quotient
Chlorpyrifos 0.339	21	0.005	365	36	40	13140	4.238×10^{-5}	0.003	0.0141
	22	0.009	365	63	68	22995	4.487×10^{-5}	0.003	0.0150
	23	0.008	365	32	57	11680	4.758×10^{-5}	0.003	0.0159
	24	0.008	365	31	57	11315	4.758×10^{-5}	0.003	0.0159
	25	0.009	365	39	62	14235	4.921×10^{-5}	0.003	0.0164
	26	0.008	365	35	52	12775	5.215×10^{-5}	0.003	0.0174
	27	0.008	365	55	51	20075	5.318×10^{-5}	0.003	0.0177
	28	0.012	365	25	75	9125	5.424×10^{-5}	0.003	0.0181
	29	0.008	365	72	50	26280	5.424×10^{-5}	0.003	0.0181
	30	0.010	365	40	61	14600	5.557×10^{-5}	0.003	0.0185
	31	0.011	365	30	65	10950	5.737×10^{-5}	0.003	0.0191
	32	0.010	365	49	59	17885	5.746×10^{-5}	0.003	0.0192
	33	0.009	365	48	53	17520	5.757×10^{-5}	0.003	0.0192
	34	0.008	365	55	47	20075	5.770×10^{-5}	0.003	0.0192
	35	0.012	365	48	70	17520	5.811×10^{-5}	0.003	0.0194
	36	0.010	365	38	58	13870	5.845×10^{-5}	0.003	0.0195
	37	0.010	365	28	56	10220	6.054×10^{-5}	0.003	0.0202
	38	0.013	365	53	70	19345	6.296×10^{-5}	0.003	0.0210
	39	0.013	365	34	70	12410	6.296×10^{-5}	0.003	0.0210
	40	0.012	365	50	63	18250	6.457×10^{-5}	0.003	0.0215

Table E-1 Individual risk assessment of organophosphate pesticide residues (OPPRs) due to chilli consumption in Hua Rua, Ubonratchathani, Thailand from October 2010 to February 2011. *(continue)*

Average Concentration (mg/kg)	Individual Exposure Assessment	Individual Exposure Assessment						Oral RfD[a,b] (mg/kg-day)	Risk Characterization
		IR[*] (kg/day)	EF (days/yr)	ED (yrs)	BW[**] (kg)	AT (days)	ADD (mg/kg-day)		Hazard quotient
Chlorpyrifos 0.339	41	0.012	365	45	63	16425	6.457×10^{-5}	0.003	0.0215
	42	0.012	365	66	62	24090	6.561×10^{-5}	0.003	0.0219
	43	0.012	365	48	62	17520	6.561×10^{-5}	0.003	0.0219
	44	0.012	365	54	60	19710	6.780×10^{-5}	0.003	0.0226
	45	0.010	365	22	48	8030	7.063×10^{-5}	0.003	0.0235
	46	0.012	365	23	57	8395	7.137×10^{-5}	0.003	0.0238
	47	0.013	365	34	61	12410	7.225×10^{-5}	0.003	0.0241
	48	0.015	365	33	70	12045	7.264×10^{-5}	0.003	0.0242
	49	0.015	365	32	70	11680	7.264×10^{-5}	0.003	0.0242
	50	0.010	365	60	46	21900	7.370×10^{-5}	0.003	0.0246
	51	0.012	365	38	53	13870	7.675×10^{-5}	0.003	0.0256
	52	0.016	365	17	70	6205	7.749×10^{-5}	0.003	0.0258
	53	0.010	365	53	43	19345	7.884×10^{-5}	0.003	0.0263
	54	0.011	365	8	47	2920	7.934×10^{-5}	0.003	0.0264
	55	0.012	365	47	50	17155	8.136×10^{-5}	0.003	0.0271
	56	0.013	365	32	54	11680	8.161×10^{-5}	0.003	0.0272
	57	0.016	365	34	65	12410	8.345×10^{-5}	0.003	0.0278
	58	0.019	365	19	77	6935	8.365×10^{-5}	0.003	0.0279
	59	0.013	365	41	52	14965	8.475×10^{-5}	0.003	0.0283
	60	0.013	365	44	52	16060	8.475×10^{-5}	0.003	0.0283

Table E-1 Individual risk assessment of organophosphate pesticide residues (OPPRs) due to chilli consumption in Hua Rua, Ubonratchathani, Thailand from October 2010 to February 2011. *(continue)*

Average Concentration (mg/kg)	Individual Exposure Assessment	Individual Exposure Assessment						Oral RfD[a,b] (mg/kg-day)	Risk Characterization
		IR[*] (kg/day)	EF (days/yr)	ED (yrs)	BW[**] (kg)	AT (days)	ADD (mg/kg-day)		Hazard quotient
Chlorpyrifos 0.339	61	0.012	365	45	47	16425	8.655×10^{-5}	0.003	0.0289
	62	0.020	365	58	78	21170	8.655×10^{-5}	0.003	0.0289
	63	0.016	365	54	58	19710	8.692×10^{-5}	0.003	0.0290
	64	0.020	365	47	71	17155	9.352×10^{-5}	0.003	0.0312
	65	0.016	365	50	55	18250	9.549×10^{-5}	0.003	0.0318
	66	0.020	365	35	65	12775	9.862×10^{-5}	0.003	0.0329
	67	0.016	365	35	52	12775	1.043×10^{-4}	0.003	0.0348
	68	0.015	365	38	48	13870	1.043×10^{-4}	0.003	0.0348
	69	0.010	365	21	32	7665	1.059×10^{-4}	0.003	0.0353
	70	0.023	365	61	72	22265	1.059×10^{-4}	0.003	0.0353
	71	0.016	365	27	50	9855	1.083×10^{-4}	0.003	0.0361
	72	0.020	365	31	60	11315	1.085×10^{-4}	0.003	0.0362
	73	0.020	365	26	59	9490	1.130×10^{-4}	0.003	0.0377
	74	0.020	365	44	58	16060	1.149×10^{-4}	0.003	0.0383
	75	0.020	365	22	57	8030	1.169×10^{-4}	0.003	0.0390
	76	0.016	365	71	45	25915	1.189×10^{-4}	0.003	0.0396
	77	0.015	365	43	42	15695	1.205×10^{-4}	0.003	0.0402
	78	0.020	365	71	55	25915	1.211×10^{-4}	0.003	0.0404
	79	0.020	365	62	55	22630	1.233×10^{-4}	0.003	0.0411
	80	0.012	365	45	47	16425	1.233×10^{-4}	0.003	0.0411

Table E-1 Individual risk assessment of organophosphate pesticide residues (OPPRs) due to chilli consumption in Hua Rua, Ubonratchathani, Thailand from October 2010 to February 2011. *(continue)*

Average Concentration (mg/kg)	Individual Exposure Assessment	Individual Exposure Assessment						Oral RfD[a,b] (mg/kg-day)	Risk Characterization
		IR[*] (kg/day)	EF (days/yr)	ED (yrs)	BW[**] (kg)	AT (days)	ADD (mg/kg-day)		Hazard quotient
Chlorpyrifos 0.339	81	0.026	365	58	70	21170	1.259×10^{-4}	0.003	0.0420
	82	0.020	365	32	53	11680	1.279×10^{-4}	0.003	0.0426
	83	0.016	365	38	42	13870	1.291×10^{-4}	0.003	0.0430
	84	0.017	365	30	43	10950	1.340×10^{-4}	0.003	0.0447
	85	0.020	365	25	50	9125	1.356×10^{-4}	0.003	0.0452
	86	0.026	365	46	65	16790	1.356×10^{-4}	0.003	0.0452
	87	0.024	365	12	60	4380	1.356×10^{-4}	0.003	0.0452
	88	0.020	365	55	45	20075	1.507×10^{-4}	0.003	0.0502
	89	0.020	365	48	42	17520	1.614×10^{-4}	0.003	0.0538
	90	0.024	365	47	48	17155	1.695×10^{-4}	0.003	0.0565
	91	0.040	365	53	78	19345	1.738×10^{-4}	0.003	0.0579
	92	0.030	365	57	57	20805	1.784×10^{-4}	0.003	0.0595
	93	0.032	365	33	60	12045	1.808×10^{-4}	0.003	0.0603
	94	0.026	365	46	48	16790	1.836×10^{-4}	0.003	0.0612
	95	0.030	365	52	54	18980	1.883×10^{-4}	0.003	0.0628
	96	0.026	365	60	46	21900	1.916×10^{-4}	0.003	0.0639
	97	0.040	365	34	70	12410	1.937×10^{-4}	0.003	0.0646
	98	0.036	365	41	55	14965	2.219×10^{-4}	0.003	0.0740
	99	0.040	365	30	61	10950	2.223×10^{-4}	0.003	0.0741
	100	0.040	365	54	56	19710	2.421×10^{-4}	0.003	0.0807

Table E-1 Individual risk assessment of organophosphate pesticide residues (OPPRs) due to chilli consumption in Hua Rua, Ubonratchathani, Thailand from October 2010 to February 2011. *(continue)*

Average Concentration (mg/kg)	Individual Exposure Assessment	Individual Exposure Assessment						Oral RfD[a,b] (mg/kg-day)	Risk Characterization
		IR[*] (kg/day)	EF (days/yr)	ED (yrs)	BW[**] (kg)	AT (days)	ADD (mg/kg-day)		Hazard quotient
Chlorpyrifos 0.339	101	0.040	365	30	56	10950	2.421×10^{-4}	0.003	0.0807
	102	0.040	365	40	56	14600	2.421×10^{-4}	0.003	0.0807
	103	0.048	365	52	64	18980	2.543×10^{-4}	0.003	0.0848
	104	0.040	365	24	48	8760	2.825×10^{-4}	0.003	0.0942
	105	0.040	365	13	45	4745	3.013×10^{-4}	0.003	0.1004
	106	0.060	365	30	66	10950	3.082×10^{-4}	0.003	0.1027
	107	0.048	365	47	47	17155	3.462×10^{-4}	0.003	0.1154
	108	0.040	365	58	35	21170	3.874×10^{-4}	0.003	0.1291
	109	0.075	365	25	54	9125	4.708×10^{-4}	0.003	0.1569
	110	0.100	365	23	55	8395	6.164×10^{-4}	0.003	0.2055

Table E-1 Individual risk assessment of organophosphate pesticide residues (OPPRs) due to chilli consumption in Hua Rua, Ubonratchathani, Thailand from October 2010 to February 2011. *(continue)*

Average Concentration (mg/kg)	Individual Exposure Assessment	Individual Exposure Assessment						Oral RfD[a,b] (mg/kg-day)	Risk Characterization
		IR[*] (kg/day)	EF (days/yr)	ED (yrs)	BW[**] (kg)	AT (days)	ADD (mg/kg-day)		Hazard quotient
Profenofos 2.536	1	0.001	365	40	65	14600	3.902×10^{-5}	0.0001	0.3902
	2	0.004	365	46	76	16790	1.335×10^{-4}	0.0001	1.3347
	3	0.004	365	61	67	22265	1.514×10^{-4}	0.0001	1.5140
	4	0.005	365	40	80	14600	1.585×10^{-4}	0.0001	1.5850
	5	0.005	365	57	78	20805	1.626×10^{-4}	0.0001	1.6256
	6	0.005	365	47	70	17155	1.811×10^{-4}	0.0001	1.8114
	7	0.005	365	64	65	23360	1.951×10^{-4}	0.0001	1.9508
	8	0.004	365	57	50	20805	2.029×10^{-4}	0.0001	2.0288
	9	0.007	365	39	86	14235	2.064×10^{-4}	0.0001	2.0642
	10	0.004	365	13	48	4745	2.113×10^{-4}	0.0001	2.1133
	11	0.005	365	30	60	10950	2.113×10^{-4}	0.0001	2.1133
	12	0.004	365	56	45	20440	2.254×10^{-4}	0.0001	2.2542
	13	0.005	365	45	54	16425	2.348×10^{-4}	0.0001	2.3481
	14	0.005	365	17	52	6205	2.438×10^{-4}	0.0001	2.4385
	15	0.007	365	21	69	7665	2.573×10^{-4}	0.0001	2.5728
	16	0.005	365	28	47	10220	2.698×10^{-4}	0.0001	2.6979
	17	0.004	365	53	37	19345	2.742×10^{-4}	0.0001	2.7416
	18	0.006	365	40	50	14600	3.043×10^{-4}	0.0001	3.0432
	19	0.007	365	31	57	11315	3.114×10^{-4}	0.0001	3.1144
	20	0.001	365	40	65	14600	3.121×10^{-4}	0.0001	3.1212

Table E-1 Individual risk assessment of organophosphate pesticide residues (OPPRs) due to chilli consumption in Hua Rua, Ubonratchathani, Thailand from October 2010 to February 2011. *(continue)*

Average Concentration (mg/kg)	Individual Exposure Assessment	Individual Exposure Assessment						Oral RfD[a,b] (mg/kg-day)	Risk Characterization
		IR[*] (kg/day)	EF (days/yr)	ED (yrs)	BW[**] (kg)	AT (days)	ADD (mg/kg-day)		Hazard quotient
Chlorpyrifos 0.339	21	0.005	365	36	40	13,140	3.170×10^{-4}	0.0001	3.1700
	22	0.009	365	63	68	22,995	3.356×10^{-4}	0.0001	3.3565
	23	0.008	365	32	57	11,680	3.559×10^{-4}	0.0001	3.5593
	24	0.008	365	31	57	11,315	3.559×10^{-4}	0.0001	3.5593
	25	0.009	365	39	62	14,235	3.681×10^{-4}	0.0001	3.6813
	26	0.008	365	35	52	12,775	3.902×10^{-4}	0.0001	3.9015
	27	0.008	365	55	51	20,075	3.978×10^{-4}	0.0001	3.9780
	28	0.012	365	25	75	9,125	4.058×10^{-4}	0.0001	4.0576
	29	0.008	365	72	50	26,280	4.058×10^{-4}	0.0001	4.0576
	30	0.010	365	40	61	14,600	4.157×10^{-4}	0.0001	4.1574
	31	0.011	365	30	65	10,950	4.292×10^{-4}	0.0001	4.2917
	32	0.010	365	49	59	17,885	4.298×10^{-4}	0.0001	4.2983
	33	0.009	365	48	53	17,520	4.306×10^{-4}	0.0001	4.3064
	34	0.008	365	55	47	20,075	4.317×10^{-4}	0.0001	4.3166
	35	0.012	365	48	70	17,520	4.347×10^{-4}	0.0001	4.3474
	36	0.010	365	38	58	13,870	4.372×10^{-4}	0.0001	4.3724
	37	0.010	365	28	56	10,220	4.529×10^{-4}	0.0001	4.5286
	38	0.013	365	53	70	19,345	4.710×10^{-4}	0.0001	4.7097
	39	0.013	365	34	70	12,410	4.710×10^{-4}	0.0001	4.7097
	40	0.012	365	50	63	18,250	4.830×10^{-4}	0.0001	4.8305

Table E-1 Individual risk assessment of organophosphate pesticide residues (OPPRs) due to chilli consumption in Hua Rua, Ubonratchathani, Thailand from October 2010 to February 2011. *(continue)*

Average Concentration (mg/kg)	Individual Exposure Assessment	Individual Exposure Assessment						Oral RfD[a,b] (mg/kg-day)	Risk Characterization
		IR[*] (kg/day)	EF (days/yr)	ED (yrs)	BW[**] (kg)	AT (days)	ADD (mg/kg-day)		Hazard quotient
Profenofos 2.536	41	0.012	365	45	63	16425	4.830×10^{-4}	0.0001	4.8305
	42	0.012	365	66	62	24090	4.908×10^{-4}	0.0001	4.9084
	43	0.012	365	48	62	17520	4.908×10^{-4}	0.0001	4.9084
	44	0.012	365	54	60	19710	5.072×10^{-4}	0.0001	5.0720
	45	0.010	365	22	48	8030	5.283×10^{-4}	0.0001	5.2833
	46	0.012	365	23	57	8395	5.339×10^{-4}	0.0001	5.3389
	47	0.013	365	34	61	12410	5.405×10^{-4}	0.0001	5.4046
	48	0.015	365	33	70	12045	5.434×10^{-4}	0.0001	5.4343
	49	0.015	365	32	70	11680	5.434×10^{-4}	0.0001	5.4343
	50	0.010	365	60	46	21900	5.513×10^{-4}	0.0001	5.5130
	51	0.012	365	38	53	13870	5.742×10^{-4}	0.0001	5.7419
	52	0.016	365	17	70	6205	5.797×10^{-4}	0.0001	5.7966
	53	0.010	365	53	43	19345	5.898×10^{-4}	0.0001	5.8977
	54	0.011	365	8	47	2920	5.935×10^{-4}	0.0001	5.9353
	55	0.012	365	47	50	17155	6.086×10^{-4}	0.0001	6.0864
	56	0.013	365	32	54	11680	6.105×10^{-4}	0.0001	6.1052
	57	0.016	365	34	65	12410	6.242×10^{-4}	0.0001	6.2425
	58	0.019	365	19	77	6935	6.258×10^{-4}	0.0001	6.2577
	59	0.013	365	41	52	14965	6.340×10^{-4}	0.0001	6.3400
	60	0.013	365	44	52	16060	6.340×10^{-4}	0.0001	6.3400

Table E-1 Individual risk assessment of organophosphate pesticide residues (OPPRs) due to chilli consumption in Hua Rua, Ubonratchathani, Thailand from October 2010 to February 2011. *(continue)*

Average Concentration (mg/kg)	Individual Exposure Assessment	Individual Exposure Assessment						Oral RfD[a,b] (mg/kg-day)	Risk Characterization
		IR[*] (kg/day)	EF (days/yr)	ED (yrs)	BW[**] (kg)	AT (days)	ADD (mg/kg-day)		Hazard quotient
Profenofos 2.536	61	0.012	365	38	47	13870	6.475×10^{-4}	0.0001	6.4749
	62	0.012	365	45	47	16425	6.475×10^{-4}	0.0001	6.4749
	63	0.020	365	58	78	21170	6.503×10^{-4}	0.0001	6.5026
	64	0.016	365	54	58	19710	6.996×10^{-4}	0.0001	6.9959
	65	0.020	365	47	71	17155	7.144×10^{-4}	0.0001	7.1437
	66	0.016	365	50	55	18250	7.377×10^{-4}	0.0001	7.3775
	67	0.016	365	35	52	12775	7.803×10^{-4}	0.0001	7.8031
	68	0.020	365	35	65	12775	7.803×10^{-4}	0.0001	7.8031
	69	0.015	365	38	48	13870	7.925×10^{-4}	0.0001	7.9250
	70	0.010	365	21	32	7665	7.925×10^{-4}	0.0001	7.9250
	71	0.023	365	61	72	22265	8.101×10^{-4}	0.0001	8.1011
	72	0.016	365	27	50	9855	8.115×10^{-4}	0.0001	8.1152
	73	0.020	365	31	60	11315	8.453×10^{-4}	0.0001	8.4533
	74	0.020	365	26	59	9490	8.597×10^{-4}	0.0001	8.5966
	75	0.020	365	44	58	16060	8.745×10^{-4}	0.0001	8.7448
	76	0.020	365	22	57	8030	8.898×10^{-4}	0.0001	8.8982
	77	0.016	365	71	45	25915	9.017×10^{-4}	0.0001	9.0169
	78	0.015	365	43	42	15695	9.057×10^{-4}	0.0001	9.0571
	79	0.020	365	71	55	25915	9.222×10^{-4}	0.0001	9.2218
	80	0.020	365	62	55	22630	9.222×10^{-4}	0.0001	9.2218

Table E-1 Individual risk assessment of organophosphate pesticide residues (OPPRs) due to chilli consumption in Hua Rua, Ubonratchathani, Thailand from October 2010 to February 2011. *(continue)*

Average Concentration (mg/kg)	Individual Exposure Assessment	Individual Exposure Assessment						Oral RfD[a,b] (mg/kg-day)	Risk Characterization
		IR[*] (kg/day)	EF (days/yr)	ED (yrs)	BW[**] (kg)	AT (days)	ADD (mg/kg-day)		Hazard quotient
Profenofos 2.536	81	0.026	365	58	70	21170	9.419×10^{-4}	0.0001	9.4194
	82	0.020	365	32	53	11680	9.570×10^{-4}	0.0001	9.5698
	83	0.016	365	38	42	13870	9.661×10^{-4}	0.0001	9.6610
	84	0.017	365	30	43	10950	1.003×10^{-3}	0.0001	10.0260
	85	0.024	365	12	60	4380	1.014×10^{-3}	0.0001	10.1440
	86	0.020	365	25	50	9125	1.014×10^{-3}	0.0001	10.1440
	87	0.026	365	46	65	16790	1.014×10^{-3}	0.0001	10.1440
	88	0.020	365	55	45	20075	1.127×10^{-3}	0.0001	11.2711
	89	0.020	365	48	42	17520	1.208×10^{-3}	0.0001	12.0762
	90	0.024	365	47	48	17155	1.268×10^{-3}	0.0001	12.6800
	91	0.040	365	53	78	19345	1.301×10^{-3}	0.0001	13.0051
	92	0.030	365	57	57	20805	1.335×10^{-3}	0.0001	13.3474
	93	0.032	365	33	60	12045	1.353×10^{-3}	0.0001	13.5253
	94	0.026	365	46	48	16790	1.374×10^{-3}	0.0001	13.7367
	95	0.030	365	52	54	18980	1.409×10^{-3}	0.0001	14.0889
	96	0.026	365	60	46	21900	1.433×10^{-3}	0.0001	14.3339
	97	0.040	365	34	70	12410	1.449×10^{-3}	0.0001	14.4914
	98	0.036	365	41	55	14965	1.660×10^{-3}	0.0001	16.5993
	99	0.040	365	30	61	10950	1.663×10^{-3}	0.0001	16.6295
	100	0.040	365	30	56	10950	1.811×10^{-3}	0.0001	18.1143

Table E-1 Individual risk assessment of organophosphate pesticide residues (OPPRs) due to chilli consumption in Hua Rua, Ubonratchathani, Thailand from October 2010 to February 2011. *(continue)*

Average Concentration (mg/kg)	Individual Exposure Assessment	Individual Exposure Assessment						Oral RfD[a,b] (mg/kg-day)	Risk Characterization
		IR[*] (kg/day)	EF (days/yr)	ED (yrs)	BW[**] (kg)	AT (days)	ADD (mg/kg-day)		Hazard quotient
Profenofos	101	0.040	365	40	56	14600	1.811×10^{-3}	0.0001	18.1143
2.536	102	0.040	365	54	56	19710	1.811×10^{-3}	0.0001	18.1143
	103	0.048	365	52	64	18980	1.902×10^{-3}	0.0001	19.0200
	104	0.040	365	24	48	8760	2.113×10^{-3}	0.0001	21.1333
	105	0.040	365	13	45	4745	2.254×10^{-3}	0.0001	22.5422
	106	0.060	365	30	66	10950	2.305×10^{-3}	0.0001	23.0545
	107	0.048	365	47	47	17155	2.590×10^{-3}	0.0001	25.8996
	108	0.040	365	58	35	21170	2.898×10^{-3}	0.0001	28.9829
	109	0.075	365	25	54	9125	3.522×10^{-3}	0.0001	35.2222
	110	0.100	365	23	55	8395	4.611×10^{-3}	0.0001	46.1091

APPENDIX F

Table F-1 Chlorpyrifos and Profenofos concentrations on 33 chilli samples from Hua Rua, Ubonratchathani province

Sample No.	Chlorpyrifos* (mg/kg)	Profenofos* (mg/kg)
1	0.65	6.29
2	0.75	5.79
3	0.72	5.49
4	0.17	0.81
5	0.36	0.74
6	0.41	1.13
7	1.27	5.33
8	1.38	5.08
9	1.01	4.77
10	0.51	1.08
11	0.65	1.17
12	0.90	1.49
13	< 0.01	1.44
14	< 0.01	1.94
15	< 0.01	1.18
16	< 0.01	4.89
17	< 0.01	3.34
18	< 0.01	2.51
19	< 0.01	2.55
20	< 0.01	2.05
21	< 0.01	1.51
22	0.40	2.09
23	0.45	1.83
24	0.41	2.14
25	< 0.01	3.80
26	< 0.01	4.83
27	< 0.01	3.28
28	< 0.01	0.52
29	< 0.01	0.68
30	< 0.01	0.90
31	0.33	2.03
32	0.26	2.40
33	0.40	2.60
Minimum	< **0.01**	**0.52**
Maximum	**1.38**	**6.29**
Mean (±S.D.)	**0.34 (± 0.39)**	**2.66 (± 1.67)**

* LOD = 0.01 µg/ml

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- Proceeding publication (volume 2/Session 1) as topic of "Dietary Intake of Chilli for Local People Living in Chilli farm Area, Ubonratchathani Province, Thailand"
- Oral presentation at 2nd International Graduate Students Conference on Population and Public Health Sciences, College of Public Health Sciences, Chulalongkorn University, 18 May 2011, Thailand