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

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

Keywords: Chlorpyrifos, Risk assessment, Dermal exposure, Chilli-growing farmers

INTRODUCTION: Thailand is one of the most important countries in Southeast Asia to support agricultural products to the world. Thailand is the country's primary exports of agricultural goods. A half of population in the country is in agricultural sector and their mainly income to support their family also is earned from agricultural product.[1)] Agricultural workers are the largest occupational group in developing countries.[2)]

Pesticides can be classified according to the types of pests which they destroy. Use of pesticides, such as insecticides, fungicides, insecticides, herbicides etc., is after required to protect crop from pests.[3)] Thousand tons of pesticides are imported to Thailand in order to keep high crop yields. Because of the pressure of yield, the heavy loads of pesticides are applied to the farms. Many drugs can be purchased without prescription and pesticides are widely used.[4)] Farmers commonly use pesticides in the organophosphate group, as they are highly effective.[5)] Many pesticides have the potential to harm human health. The use of pesticides is steadily increasing. Pest resistance to pesticides in some developing countries, and aggressive marketing are among

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the causes for the growing use.[2] Thus, some residues are contaminate the environment, such as soil, water, and air, and affect the health of humans in the crop area.[6,7]

In Thailand, chilli is a famous agricultural product. Most of the products, including chilli, are grown in Northeast of Thailand. Most village households engage primarily in agriculture as their primary or secondary occupation.[8] Chilli is one of the crops that use a lot of pesticides. But chilli-growers lack knowledge to protect themselves from pesticide exposure. Chilli-growing farmers have risk because of lack of knowledge about pest control. Most of them frequently use pesticide with overdose applications. Due to pricing of pesticides, short reentry intervals, and inefficient sprayer maintenance, not only farmers but also their family are exposed to these agro-chemicals and they are at risk in this situation.[9]

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

This study was to estimate Chlorpyrifos (Organophosphate pesticide) exposure through dermal contact. The specific objectives are to measure residue of Chlorpyrifos on chilli-growing farmers on the hands and to assess human risk associated with dermal exposure to Chlorpyrifos in chilli-growing farmers.

MATERIALS AND METHODS: This study was approved by The Ethical Review Committee for Research Involving Human Research Subjects, Health Science Group, Chulalongkorn University, Thailand with the certified code number 013/2010. All participants in this research agreed with Participant Information sheet and signed an Informed Consent Form before they enrolled in this study.

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

Wipe Samples

Wipe samples were collected from chilli-growing farmers. The samples were collected after spraying pesticide immediately. Hand wipe method applied for sample collection to estimate residue of Chlorpyrifos. Both hands of each subject were wiped by moistened gauze with 40% isopropanal.

Gauze pads were kept in the box at 4°C until the GC-analysis process. Extraction and clean up samples in this research were developed from QuEChERs which stands for quick, easy, cheap, effective, rugged, and safe method. The method was a multiresidue method for analysis of pesticide residue in low-fat products and was a solid phase extraction. [34]

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

A calibration curve used external mixed standard. Quantitative recovery was 93% and Relative standard deviation (RSD) was 6.7%. Limit of detection (LOD) and limit of quantification (LOQ) were 0.050ng/mL and 0.100 ng/mL, respectively. All values were in the standard that AOAC (The Scientific Association Dedicated to Excellence in Analytical Methods) recommended.

Questionnaire

Chilli-growing farmers, who participated in this study, were interviewed face to face after finishing wipe sample step. The questionnaire was separated into 3 parts; General information (Gender, Body weight, Age and Height), Personal protective equipment

(PPE) data (Gloves and Frequency of washing or cleaning gloves) and field and pesticide use data (Formulate Product, Duration of application, Tank Condition and Frequency of spraying chlorpyrifos).[10] Information from this questionnaire was used to calculate Chlorpyrifos exposure assessment.

Hand Surface area

Hand Surface area of subjective in this study was calculated by the following equation.

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

Where:

SA	surface area (m^2)
H	height (cm)
W	weight (kg)
a_0, a_1, a_2	constant values (US EPA)[11]

Average Daily Dose (ADD)

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

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

Where:

Cs	Concentration of pesticide on both hands (mg/kg)
SA	Surface area (cm^2)
DAevent	absorbed dose per event (mg/cm^2-event) (456×10^{-6} (mg/cm^2-h)[12])
EV	Event frequency (hour/day)
ED	Exposure duration (years)
EF	Exposure frequency (day/year)
BW	Body weight (kg)
AT	Averaging time (days) for non-carcinogenic effects (ED x 365 days)

Non-Carcinogenic Risk Estimation

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

Hazard Quotient (HQ) = Exposure / RfD

Exposure	chemical exposure level, or intake (mg/kg-day)
RfD	reference dose (mg/kg/day) (0.0015 mg/kg-day)[13]

Where:

HQ > 1	adverse non-carcinogenic effect concern
HQ ≤ 1	acceptable level (no concern)

RESULTS AND DISCUSSIONS:

Questionnaire Information

The proportion between men and women who enrolled in this study were 74.2% and 25.7% respectively. The results showed that age of male and female in the study was in the range of 41 – 50 years old (table 1). The results show that the average age of farmers in Thailand were in the middle age group. The majority of chilli-growing farmers were males as same as other farmers.[14,15]

The average age and weight of the two groups were similar. The average age and weight of this population were 56.34 (±11.11) years and 44.29 (±11.08) kilograms, respectively. But the average height of the studied groups were different between men and women. The average height for the subjects in this study was 161.31 (±7.89) centimeters, rank between 161 and 170 centimeters. About 60% of participants in the study did not use gloves as protective equipment during spraying period. Glove use was associated with the hand and total dermal exposure levels.[16] Gloves were mostly worn, followed by a spraying suit and breathing protection. However, only a few of chilli-growing farmers, who used gloves during farm period, did not reuse their gloves. Result showed that most of the farmers reusing the gloves had never washed their gloves. The result included farmers who did not use gloves during farm period, so it was showed high percentage (79.4%). In this study, most chilli-growing farmers (65.7%) mixed Chlorpyrifos in an appropriate tank condition, so there was some little leaking of pesticide from the tank. Other research showed that some farmers used leaking spray tanks and they were exposed to pesticide through wet clothes.[10] The farmers mixed Chlorpyrifos ratio of Chlorpyrifos (formulated product) 21-30 ml per 20L of water. The average volume was 30.49 mL. Chilli-growing farmers (85.7% of participants) sprayed pesticide every per week while, some of them sprayed pesticide one time per two weeks. They also spent their time in the field to work approximately one hour including spraying pesticide and other agricultural activities. The half-day shift worked by the large majority of the sprayers indicates that pesticide spraying was usually only performed over certain hours of the day and it did not expose them for the whole day.[18] The duration of chilli cultivation in this area was 5 month per year approximately.

However, there were some farms in this area growing chilli for either longer (about 6 months) or shorter (about 3 months) periods because the periods depended on kinds of chilli, such as red, green, or black chilli.

Table 1: General Information of chilli-growing farmers Hua-ruea sub-district, Muang district, Ubon Ratchathani province, Thailand

General Information	Chilli-growing farmers Male		Female		Total	
	N	%	N	%	N	%
Gender	26	74.3	9	25.7	35	100
Age (years)						
≤ 30	4	11.4	-	-	4	11.4
31 – 40	5	14.3	2	5.7	7	20.0
41 – 50	10	28.6	5	14.3	15	42.9
51 – 60	5	14.3	2	5.7	7	20.0
≥ 61	2	5.7	-	-	2	5.7
Body Weight (kilograms)						
≤ 50	7	20.0	4	11.4	11	31.4
51 – 60	9	25.7	4	11.4	13	37.7
61 – 70	6	17.7	1	2.9	7	20.0
≥ 71	4	11.4	-	-	4	11.4
Height (centimeters)						
≤ 150	1	2.9	4	11.4	5	14.3
151 – 160	10	28.6	3	8.6	13	37.1
161 – 170	14	40.0	2	5.7	16	45.7
≥ 171	1	2.9	-	-	1	2.9
Use of Personal Protective Equipment						
Use	6	17.1	1	2.9	7	20.0
Use (damage)	3	8.6	4	11.4	7	20.0
Not use	17	48.6	4	11.4	21	60.0
Wash or clean gloves						
Not reuse	4	11.8	-	-	4	11.8
Once a week	3	32.2	1	2.9	4	35.1
Once a month	-	-	-	-	-	-
Never	19	55.9	8	23.5	27	79.4
Formulated product (ml per 20 L water)						
≤ 20	4	11.4	-	-	4	11.4
21 – 30	22	62.9	6	17.1	28	80.0
31 – 40	-	-	-	-	-	-
≥ 41	-	-	3	8.6	3	8.6
Tank Condition						
Good	17	48.6	6	17.1	23	65.7
Average	9	25.7	3	8.6	12	34.3
Leaking	-	-	-	-	-	-
Frequency of spraying Chlorpyrifos						
For a week						
Once /two week	2	5.7	2	5.7	4	11.4
Once /week	23	65.7	7	20.0	30	85.7
Two time /week	1	2.9	-	-	1	2.9
For a year						
3 months /year	3	8.6	-	-	3	8.6
4 months /year	-	-	-	-	-	-
5 months /year	22	62.9	7	20.0	29	82.8
6 months /year	1	2.9	2	5.7	3	8.6

Personal Monitoring (Hand Wiping samples)

A hand wipe sampling and analysis procedure was developed for the measurement of dermal contact to pesticides.[19] The mean, maximum and minimum of chlopyrifos concentrations (mg/kg) dose estimates, values are shown in table2. The mean of residue Chlorpyrifos concentration on chilli-growing farmers' hands is 6.95 (±18.24) mg/kg. Curwin et al. (2005) studied on the Chlorpyrifos concentration of hand wipe sample and found that the concentration was between 0.36 and 19 ng/cm^2 [20]. Jaipieam (2008) studied on vegetable growers in Thailand and found that residue of chlorpyrifos on hands was 0.070 mg/both hands. [13] The rank between minimum and maximum of chlorpyrifos concentration were 0.10 and 98.59 mg/kg, respectively. The reasonable maximum exposure (RME) at the 95th percentile was estimated for protection and prevention of high dermal exposure farmers. Other difference percentiles (25th, 50th and 75th) were also calculated.

For the quality control (QC), all samples in this study were analysed by the standard laboratory. Limit of detection (LOD) in this study was 0.050 ng/mL. Curwin et al. (2005) also studied on wipe sample and found that LOD was 0.12 ng/cm^2 [20]. A limit of quantitation (LOQ) was 0.10 ng/mL. Another study of chlorpyrifos concentration analysis showed that LOQ was 8 µg/L.[21] Method Detection Limit (MDL) was 30.95 ppb. The relative standard deviation and recovery of this analysis was 6.7% and 93%, respectively. According to the Scientific Association Dedicated to Excellence in Analytical Methods (AOAC), all QC values showed that the quality of this study was according to the recommended standard level.

Hand Surface Area

The results (table 3) showed that the average hand surface areas of both male and female of this study were 0.088 m^2 and 0.075 m^2, respectively. These values were used to calculate the ADD equation.

The direct calculation of hand surface areas was similar to the US Environmental Protection Agency default values for both males and females (table 3). There were some differences in values in the equation, such as weight and height, thus the values of area was not the same as the default values. Skin is the most exposed organ while

Table 2: Dose estimate of Chlopyrifos Concentration on chilli-growing farmers' hands (mg/kg)

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

spraying the pesticide on fields.[22] The frequent skin contact with pesticides was on the hands and face. About 30% of farmers had hand dermatitis, and more than two thirds had pigmentation and thickening on the hands.[23] Moreover, the highest concentrations of Chlorpyrifos were found at the wrist and hand.[24]

Table 3: Average hand surface areas

Sex	Average height (cm)	Average weight (kg)	Hand surface area[a] (m^2)	Hand surface area[b] (m^2)
Male (n= 26)	163.42	59.27	0.088	0.084
Female (n=9)	155.22	51.00	0.075	0.075

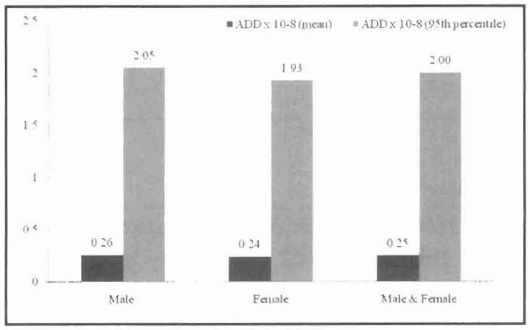
[a] Direct Calculation
[b] US EPA,1997[11]

Exposure Estimation

Men's average daily dose (ADD) in this area was 2.57 x 10^{-9} mg/kg-day which is higher than women ADD (2.41 x 10^{-9} mg/kg-day) (figure 1). Krieger et al. (2001) showed that fathers whose family was living in the spraying insecticide area were exposed to the chemical more than mothers. In contrast, the study showed that male children exposed to chemical were fewer than female children.[25] However, the ADD for all participants in this study was 2.51 x 10^{-9} mg/kg-day. Jaipieam (2009) found that ADD of dermal exposure to chlorpyrifos in vegetable growers in Thailand was 3.23 x 10^{-5} mg/kg-day.[14] The reasonable maximum exposure (RME) at 95th percentile was also estimated in this calculation. ADD for men and women were 2.05 x 10^{-8} and 1.93 x 10^{-8} mg/kg-day, respectively. The results also showed that men in this area also expose to Chlorpyrifos more than women. The ADD for both of them at RME level was 2.00 x 10^{-8} mg/kg-day.

Aggregate daily exposures for chlorpyrifos (inhalation, dietary and dermal) ranged from 13.5ng/day to 12,821.0 ng/day, with a mean daily aggregate exposure of 1,390.0 ng/day.[2]

mean level and 95th percentile level by chilli-growing farmer in Hua-rua sub-district, Muang district, Ubon Ratchathani province, Thailand



Non-Carcinogenic Hazard Quotient

Hazard Quotients at mean level were 1.71 x 10^{-6} for male and 1.61 x 10^{-6} for women (table 4). For both males and females, HQ at mean was 1.67 x 10^{-6}. However, the results of HQ showed that chilli-growing farmers in this area were not getting risk from dermal exposure through their hands because HQ values were lower than 1, which is an acceptable level.

The RME at the 95th percentile also

$$\text{Hazard Quotient (HQ)} = \frac{2.51 \times 10^{-9} \text{ mg/kg-day}}{0.0015 \text{ mg/kg-day}} = 1.67 \times 10^{-6}$$

showed the low HQ (Male = 1.37 x 10^{-5}, Female = 1.29 x 10^{-5} and Male & Female = 1.33 x 10^{-5}). HQ at RME level was higher than mean level. However, the HQ values were also lower than acceptable level (HQ < 1). It indicated that chilli-growing farmers in this area of study area did not get risk from dermal exposure (by hands) to Chlorpyrifos, although we considered at the RME level. Essumang et al. (2008) found that the risk assessment showed cancer risk for adults and children due to the presence of endosulfan and chlopyrifos through oral route.[27] The hazard index for chlorpyrifos was greater than 1, which is a sign of contamination by chlorpyrifos. But, there were other routes of exposure which the farmers are at risk, such as inhalation. During the chlorpyrifos spray season

$$ADD_{mean} = \frac{6.95 \text{ mg/kg} \times 10^{-6} \text{ kg/mg} \times 456 \times 10^{-6} \text{ mg/cm}^2\text{/h.} \times 1 \text{ h/day} \times 44.19 \text{ years} \times 19.22 \text{ days/year} \times 8.2 \times 10^2 \text{ cm}^2}{54.6 \text{ kg} \times 44.19 \text{year} \times 365 \text{ days/year}} = 2.51 \times 10^{-9} \text{ mg/kg-day}$$

measurable values were found in the air over a 28 day periods.[28] The HQs for the median of children's acute exposures to chlorpyrifos via inhalation were 4.0 and 0.8, respectively.[29] Inhalation of indoor air contaminated with Chlorpyrifos accounted for 76.1% of the aggregate exposure to the population. However, chlorpyrifos is not very volatile.[26]

Table 4: Hazard Quotient (HQ) of study population

	Male	Female	Male & Female
HQ	1.71×10^{-6}	1.61×10^{-6}	1.67×10^{-6}
HQ (95th percentile)	1.37×10^{-5}	1.29×10^{-5}	1.33×10^{-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.[30,31] In this study, the result shows that chilli-growing farmers in this area may be not at risk from chlorpyrifos exposure. However, some evidence shows that the population in this study area still had some effect from pesticide exposure, such as disability and mortality. Therefore, other routes of exposure, such as inhalation and oral, should be considered as an important route. To assess risk for other routes (inhalation and oral) is recommended for the future studies. Moreover, other kind of pesticide should be investigated.

Regarding risk management in this study showed that personal care and personal manner of work, including the manner in which gloves are used, are other factors that affect dermal exposure as identified by some researches. It is highly probable that the correct use of gloves leads to lower dermal exposure than insufficient use of gloves.[32]

Inhalation exposure is the main route of exposure to pesticide which should raise a great concern.[26] Using appropriate personal protective equipment (PPE) is an alternative to protect farmers' health. Marin et al. (2004) also found the need to wear PPE, including a respirator for the application of pesticides.[33] Exposure to pesticides can be reduced by wearing PPE.[17] Prevention includes giving knowledge to exposed workers with regard to the safe handling of chemicals.[29] However, farmers must recognize and be concerned of the risk that pesticides may pose to their health and that of their families, but their decision to use exposure control practices may need to be negotiated through the cultural and practice norms of their community as well as the particular constraints of their own farming operation.[17]

CONCLUSION: The participants in this study were both males and females. Most of them were men; their age was between 41 and 50 years old. The weight and height of participants were in the range of 51-60 kilograms and 161-170 centimeters. Most chilli-growing farmers in the area of study did not use gloves as protective equipment during spaying period. Spray mix condition of their pesticide was 21-30 mL per 20 L of water with good tank condition. The cultivation duration in this area was around 5 months in each year. The farmers also sprayed Chlorpyifos once a week during the cultivation crop period. Due to hand surface area calculation, the values of hand areas in this population were close to US EPA default values. Male and female hand surface areas of this population were 0.088 and 0.075, respectively by direct calculation. The Average daily dose (ADD) for chilli-growing farmer in the area of study was equal to 2.51×10^{-9} mg/kg/day. Male chilli-growing farmers' average daily dose (2.57×10^{-9}mg/kg/day) was higher than female (2.41×10^{-9}mg/kg/day) indicating that men may be exposed to Chlorpyrifos more than women. Hazard Quotient (HQ) of study population was not greater than 1.0 at both mean and RME 95th percentile level. Therefore, the value shows that chilli-growing farmers in this area of study may not be at risk due to exposure to Chlorpyrifos through hands (dermal exposure). Risk management was suggested to address other routes of exposure. Inhalation is another route which is recommended to be evaluate for the risk of exposure because there are some studies showing that farmers could be exposed to pesticide during spraying period.

Further studies should determine other routes of exposure other organophosphate, such as profenofos and herbicide and should increase the number of participants of the study. The chilli-growing farmers might be at risk from other agricultural activities, such as mixing and loading pesticide.

These further studies should be concern about other activities. Children and older people who are susceptible in the area study research should be included in further studies. Both children and older people can be indirect exposure group from pesticides and agrochemical.

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