

RISK ASSESSMENT OF ORGANOPHOSPHATE PESTICIDES FOR CHILI CONSUMPTION FROM CHILI FARM AREA, UBON RATCHATHANI PROVINCE, THAILAND

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ABSTRACT: A cross-sectional study of human health risk assessment related to chili consumption was done from October 2010 to February 2011 in Hua Rua agricultural community at Hua Rua sub-district, Muang district, Ubon Ratchathani province. Socio-demographic and dietary survey were completed by face-to-face questionnaire 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 ($\pm$ S.D.) was 57 ± 10 kg. The average daily intake of chili was 0.018 kg/day which was higher than the average of general Thais (0.005 kg/day). For pesticide residues analysis, thirty-three chili samples were collected from farm after the day 7th of pesticides application. Chili samples were extracted using QuEChERS technique and quantified by gas chromatography equipped with flame photometric detector (FPD). Chlorpyrifos and profenofos (organophosphate pesticide) were commonly detected in the range of < 0.010 - 1.303 mg/kg and 0.520 - 6.290 mg/kg, respectively. For exposure assessment, an 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. A potential health risk characterization was indicated that risk characterization of chlorpyrifos did not exceed an acceptable risk ratio (hazard quotient; HQ < 1.0), but risk characterization of profenofos exceeded an acceptable risk ratio (HQ > 1.0). In conclusion, the local people in this area might be getting non-carcinogenic adverse health effects from profenofos residues in chili. This study suggested that an appropriated risk communication for pesticide residues reduction should be recommended for the Hua Rua community.

Keywords: Organophosphate pesticide, Chili consumption, Consumption risk assessment

INTRODUCTION

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 [1].

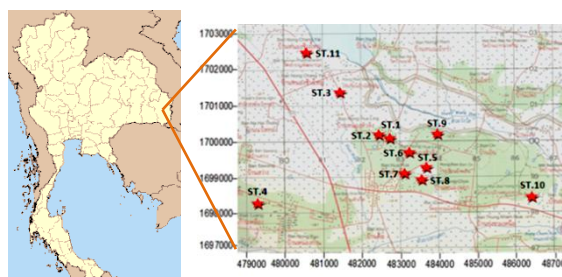
Because of the banning of organochlorine pesticides in Thailand in 1981 [2], organophosphate pesticides are now the most used because of their effectiveness, short half-life, and low price. However, organophosphate pesticides have higher acute toxicities than chlorinated pesticides. Organophosphate pesticides' 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 [3]. The WHO/FAO [4] estimated an annual worldwide total of 3 million cases of acute and severe pesticide poisoning, resulting in some 220,000 deaths. Recently, Ecobichon reported the largest proportion of human acute toxicity of pesticides due to organophosphates [5]. 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, fresh chili is one of important product for Thai people's daily life because Thai people, especially Northerners, prefer spiciness and use

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Figure 1 The 11 sampling stations (st), Hua Rua sub-district, Muang district, Ubon Ratchathani province (★)



chili as an ingredient in their daily cook. The Ministry of Public Health reported Thai's chili consumption of approximately 5 grams per day or 1 teaspoon. Hua Rua sub-district, Ubon Ratchathani province is indicated as one of the largest areas of chili production in Thailand. Chili-growing farmers applied many sort of organophosphate pesticides (OPPs) both pre- and post-harvest to control chili pests, protect the crops from disease, and meet high production targets. As well as, chili-growing farmers lack proper knowledge and awareness of pesticide usage, such as overdose use, early harvest, etc [6-8]. Therefore, this study was focus on pesticide residue in fresh chili product and also the potential risk from chili consumption consumption.

This research aims to (1) investigate the organophosphate pesticide residues in chili and (2) assess the human risks regarding the regular consumption of chili of local people in Hua Rua, Ubon Ratchathani province, Thailand.

MATERIALS AND METHODS:

Pesticide Standards and Chemicals

Hua Rua, one of the largest areas of chili production at Ubon Ratchathani province, was selected as a study area (Figure 1). Three samples from each of 11 chili farms were randomly collected 7 days after the last pesticide application in February 2011 for a total of 33 chili samples. Chili harvest in the Hua Rua area commonly occurs 7 days after the last pesticide application.

Sample Analysis

Sixteen organophosphate pesticide standards for Methamidophos, dichlorvos, acephate, omethoate, demeton-s-methyl, dimethoate, tolclofos-methyl, pirimiphos-methyl, malathion, chlorpyrifos, methidathion, prothiofos, profenofos, ethion, Azinphos methyl, Pyrazophos were obtained from Restek (USA).

A stock of the standard mixing containing 16 pesticides was prepared in 99% acetone at concentration of 100 µg/ml and stored at -4°C in a refrigerator. OPPRs in chili were extracted by using QuEChERS method (Quick, Easy, Cheap, Effective, Rugged and Safe) involving 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. A 10 g of the homogenized chili 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 MgSO₄ (4 g) were added, and the tube was vigorously shaken for 1 min and centrifuged at 5,000 rpm for 5 min. 1325 µl aliquot of the supernatant (Acetonitrile 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 stream for GC analysis [9-11].

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, USA). 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 220°C. The oven initial temperature was 80 °C for 1 min, the programmed to increase at 12°C/min to 195°C. Then, it increased at 2°C/min to 210°C, held for 3 min. It increased to 225°C at 15°C/min, held 2 min. The last temperature was 275°C which increased at 40°C/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.

A calibration curves using the external mixed standard was quantified at concentration of 0.1, 0.5, 1, 5, and 10 µg/ml. Correlation coefficients were greater 0.99. The 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 detections (LOD) and limit of quantifications (LOQ) was 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 this qualitative study was in the recommended standard level [12].

Dietary Survey

All of participants in this research were local people living in Hua Rua, Ubon Ratchathani province. These participants were chosen since the study focused on people who consume chili from Hua Rua area. Face-to face interview was conducted from October 2010 to February 2011. One hundred and ten ($n = 110$) subjects age 15 years or older were randomly selected for the questionnaire based

dietary survey. A face-to-face interview focused on the exposure frequency (number of times per day, week, month, or year), quantity of consumption, and body weight. A measuring cup and a balance were used during the interview to facilitate the quantification of food intake [13, 14].

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.

Exposure Assessment

An individual's exposure to OPPs from the chili consumption (mg/kg-day) (ADD) was estimated by multiplying the concentration of OPPs in chili (mg/kg) by mean daily consumption rate (kg/day) before dividing by the average body weight (kg) of surveyed populations [15]. (See Equation 1)

$$ADD = \frac{C \times IR \times EF \times ED}{AT \times BW} \quad (1)$$

Where C is the concentration in mg pesticide per kg chili, IR is the chili intake rate in kg chili per day, EF is the exposure frequency in days per year, ED is the exposure duration in years, BW is body weight in kg, and AT is the average time for non-carcinogenic effects, AT is equal ED in days.

The reasonable maximum exposure (RME) 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. 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. 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 statistical data are available, exposure time and exposure frequency are recommended to be described in terms of the 95th percentile [16, 17].

Risk Characterization

OPP is classified as non-carcinogenic pesticide. The criterion, that is the one used in non-carcinogen risk characterization, is the reference dose (RfD). An evaluation of non-carcinogenic toxicity of individual risks can be computed by using the hazard quotient (HQ) ratio (See Equation 2). This value indicates the degree of exposure (ADD), greater or less than the RfD. When the exposure exceeds the RfD, the exposure population may be at risk [15, 18].

$$\text{Hazard Quotient (HQ)} = \frac{ADD}{RfD} \quad (2)$$

Table 1 The concentration of OPPs residue in chili collected from Hua Rua sub-district, Ubon Ratchathani

OPPs (n = 33)	Concentration (mg/kg, ppm)		
	Range	Mean ± S.D.	95 th
Chlorpyrifos	< 0.01 ^a - 1.38	0.33 ± 0.39	1.30
Profenofos	0.52 - 6.29	2.53 ± 1.67	5.94

^a The concentration values were < LOD estimating to LOD 0.01 mg/kg.

RESULTS AND DISCUSSION

Concentration of OPPs in Chili

Three samples from each of 11 chili farms were randomly collected 7 days after the last pesticide application in February 2011 for a total of 33 chili samples. Chlorpyrifos and profenofos are OPPs that were found in 33 chilli samples. The OPPs concentration values in chili (mg/kg) are shown in Table 1.

The Codex Alimentarius (FAO/WHO) 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. The concentration of chlorpyrifos was <0.01 - 1.38 mg/kg. The mean and 95th percentile concentrations were 0.33 and 1.30 mg/kg, respectively. Furthermore, of 27% chlorpyrifos contaminated samples which were higher than the MRL. 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, of 15% profenofos contaminated samples were higher than the MRL which is considered only the concentration of pesticide residue.

The Office of Agriculture Regulation, Ministry of Agriculture and Cooperatives, Thailand has approved the use of chlorpyrifos, profenofos and ethion for Thai chili cultivation. Similarly, Lunliu [19], Norkaew [7] and Jansong [20] indicated that podium 400 (chlorpyrifos) and selecon (profenofos) were commonly sold and used in chili cultivation since the significant problem of chili-growing farmer in Hua Rua area was worm, aphid and plant louse. Furthermore, during sampling on February 2011, the chili growing farmers reported that aphid was seriously presenting problem and most of them used the mixed of profenofos and chlorpyrifos due to they believed an increase in effectiveness resulted from the combination pesticides. Therefore, chlorpyrifos and profenofos were detected. However, Phumongkutchai, et al. [21] studied the profenofos degradation of chili to indentify MRL value by using pesticide followed the recommendation dose (40 ml profenofos/20 ml water). They concluded that all profenofos residues on chili were lower than MRL value after pesticide spraying for 3 days. On the other hand, in this study, chili samples were collected and analyzed on 7 days after the last pesticide spraying, but the

Table 2 Health risk assessment of organophosphate pesticide residues (OPPRs) due to chili consumption for the people ($n = 110$) in Hua Rua sub-district, Ubon Ratchathani, Thailand from October 2010 to February 2011

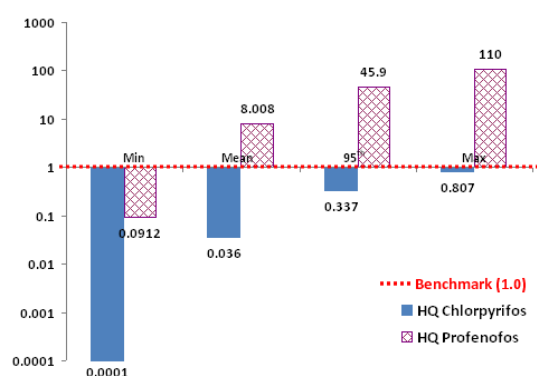
OPPs		ADD ^a	Oral RfD ^{a,b,c}	HQ
Chlorpyrifos	Mean	1.07×10^{-4}	0.003	0.04
	95 th	1.01×10^{-3}	0.003	0.34
	Max	2.42×10^{-3}	0.003	0.81
Profenofos	Mean	8.00×10^{-4}	0.0001	8.01
	95 th	4.59×10^{-3}	0.0001	45.90
	Max	1.10×10^{-2}	0.0001	110.00

^a Unit: mg/kg-day

^b IRIS, 1988 (Oral reference dose of chlorpyrifos)

^c Jaipieam, 2009 (Oral reference dose of profenofos)

Figure 2 Non-cancer hazardous quotient (HQ) of OPPs for daily chili consumption by local people in Hua Rua sub-district, Ubon Ratchathani



pesticides were still detected in 5 chili samples at high concentration exceeded the MRL. This may be result from Hua Rua' chili-growing farmers applied the pesticides over the recommendation dose or the pesticides persist longer in environmental condition in the area. Kamrin [22] described that chlorpyrifos residues remain on plant surfaces for approximately 10-14 days and lost primarily by volatilization. The results from Norkaew [7] and this study indicated that chili growing farmer at Hua Rua area used over the recommendation dose, and commonly collected the chili on 7 days after the last pesticide spraying. Additionally, they sometimes collected chili earlier than 7 days if the chili market is in high price [6]. It resulted in chlorpyrifos and profenofos concentrations were detected higher than the MRL.

Dietary Survey

One hundred and ten ($n = 110$) of participants completed the dietary survey. 45 participants (37.8%) were male and 65 participants (54.6%) were female. The average age ($\pm$ S.D.) was 47 ± 14 years (in range of 15–79 years) and the average body weight ($\pm$ S.D.) was 57 ± 10 kg (in range of 32–86 kg). Most of them ($n = 83$, 69.7%) graduated in elementary school or below and were farmer ($n = 86$, 72.3%). The average consumption (frequency) and duration of chili consumption were 365 days/year and 41 years, respectively. The average daily chili consumption ($\pm$ S.D.) was 0.018 ± 0.015 kg/day. Additionally, the average daily chili consumption of

local people in Hua Rua was 0.018 kg/day more than 3 times the average of general Thai (0.005 kg/day).

According RME, the statistical calculation regarding chili consumer interviews ($n = 110$), the 95th percentile of exposure time was 63 years and exposure frequency was 365 days/year. Furthermore, the body weight of chili consumer obtained from the exposure survey was 57 kg using the 50th percentile values, instead of the 95th percentile values. This is because body weight are strongly correlated and 50th percentile values are most representative of average weight which is assumed all other exposure pathways [23].

Health Risk Assessment

Health risk assessment 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 2.

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

Non-cancer hazardous quotient (HQ) of OPPs for daily chili consumption by Hua Rua' consumers was illustrated in Figure 2. The result indicated that all of chlorpyrifos HQ were less than the acceptable level 1.0, suggesting that the chili consumer may not get higher risk from consuming contaminated chili with chlorpyrifos. Since, we considered the RME at 95th percentile; it also showed HQ lower than 1.0. On the other hand, HQ of profenofos value was greater than the benchmark, 1.0. Severely, RME at 95th percentile and maximum level showed the highly concerned result that HQ were greater than 45- and 110- times profenofos RfD, respectively. Therefore, consuming fresh chili as early as seven days after applying profenofos may result in decreasing cholinesterase activity because of high profenofos contamination and increasing health risk [24].

Additionally, it is concerned that children and aging individuals are susceptible groups and more sensitive to the adverse health effects than adult. Normally, children do not consume chili.

Therefore, the chili consumption behaviors of adult and aging were evaluated. 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. The average daily chili consumption was not significantly difference between adult and aging people ($p \geq 0.05$). The average daily chili consumption of adult people ($\pm$ S.D.) was 0.019 ± 0.016 kg/day. The average daily chili consumption of aging people ($\pm$ S.D.) was 0.016 ± 0.010 kg/day. The ADD and HQ for chlorpyrifos and profenofos in adult and aging people were not significantly difference ($p \geq 0.05$). It was similar result. All HQ of chlorpyrifos values were lower than the acceptable level 1.0. HQ of profenofos residue value was greater than the acceptable level 1.0. Therefore, adult and aging consumers of chili contaminated with profenofos were at risk regarding non-carcinogenic effect.

CONCLUSION

This study is a preliminary risk assessment of OPPs through chili consumption in Hua Rua sub-district, Ubon Ratchathani, Thailand. Chlorpyrifos and profenofos residues were detected. Of 27% chlorpyrifos and 15% profenofos contaminated samples exceeded Codex' MRL. The risk characterization indicated that chili consumer in Hua Rua area may have higher risk potential of profenofos pesticide residue on chili. According to the higher risk potential of non-carcinogenic from pesticide residue on chili, household should wash chili thoroughly before cooking and/or consuming and chili-growing farmer should collect chili follow the recommendation (14 days). Moreover, environmental monitoring, health surveillance and the appropriated risk management should be applied to help local communities avoid and protect themselves from OPPs.

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