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ORGANOPHOSPHATE PESTICIDE EXPOSURE AND DIALKYL PHOSPHATE URINARY METABOLITES AMONG CHILI FARMERS IN NORTHEASTERN THAILAND

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ABSTRACT

Background. Chlorpyrifos and profenofos are organophosphate pesticides (OPPs), we studied exposure and urinary metabolites in an agricultural area in the northeastern of Thailand during the chili-growing season (March - April) in 2012.
Objective. This study was designed to assess pesticide exposure concentration through dermal and inhalation pathways and to find and depict a relationship between urinary metabolites and means of exposure.
Materials and methods. To estimate the pesticides exposure concentration, dermal wipes (hand, face, and feet), dermal patches and air samples were collected from 38 chili farmers. The morning void of pre and post application urine samples was an indicator of biological monitoring in the study which derived from 39 chili farmers.
Results. Chlorpyrifos and profenofos residues were detected on dermal patches, face wipes, and hand wipe samples, while no significant residues were found on the feet. Using a personal air sampling technique, all air samples detected pesticide residues. However, significant correlation between dermal pesticide exposure concentration and inhalation was not found ($p>0.05$). For urinary metabolite levels, there was a relationship between the first pre application morning void and post application morning void ($p<0.05$); similar to the association between the first pre application morning void and the second post application morning void ($p<0.05$). The main relationship between pesticide exposure and urinary metabolite was found to have been relevant to dermal exposure ($r= 0.405$; $p<0.05$).
Conclusions. The results of this study could suggested that public health education training programs, including the use of appropriate personal protective equipment (PPE), should be offered for the chili growing farmers in order to improve their ability to properly use pesticides.

Key words*: pesticide exposure, chili farmers, urinary metabolites, organophosphate pesticides*

STRESZCZENIE

Wprowadzenie. Chlorpiryfos i profenofos należą do pestycydów fosforoorganicznych (OPPs) stosowanych w rolnictwie, dlatego narażenie na te związki badano w północnowschodniej Tajlandii w okresie uprawy chili (marzec – kwiecień) w 2012 roku.
Cel. Celem badania była ocena narażenia na pestycydy przez skórę i drogi oddechowe oraz zbadanie zależności pomiędzy stężeniami metabolitów w moczu a drogą narażenia.
Materiał i metoda. Do oceny wielkości narażenia wykorzystano wymazy z rąk, twarzy i stóp i naskórne plastry absorpcyjne oraz próbki powietrza pobierane za pomocą indywidualnych próbników u 39 rolników uprawiających chili.
Wyniki. Pozostałości chlorpiryfosu i profenofosu stwierdzano na plastrach absorpcyjnych, wymazach z twarzy i rąk, podczas gdy w wymazach ze stóp nie stwierdzano znaczących ilości tych pestycydów. We wszystkich próbkach powietrza stwierdzono obecność pestycydów. Jednakże, nie wykazano znaczącej korelacji pomiędzy wielkością narażenia przez skórę i drogi oddechowe ($p>0.05$). W przypadku poziomów metabolitów w moczu, wykazano zależność pomiędzy poziomami metabolitów w moczu przed i po pierwszym zabiegu wykonywanym rano ($p<0.05$). Zależność pomiędzy narażeniem na pestycydy a stwierdzanymi metabolitami w moczu wynikała z narażenia przez skórę ($r= 0.405$; $p<0.05$).
Wnioski. Wyniki niniejszych badań mogą sugerować potrzebę wprowadzenia programów edukacyjnych z zakresu zdrowia publicznego, uwzględniających stosowanie przez hodowców chili odpowiedniego sprzętu ochrony osobistej, w celu poprawy możliwości właściwego stosowania pestycydów.

Słowa kluczowe: *narażenie na pestycydy, hodowcy chili, metabolity w moczu, pestycydy fosforoorganiczne*

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INTRODUCTION

Because of the heavy usage of pesticides in agriculture, farmers are being exposed continuously to a large amount of chemicals [2]. Pesticides are a health risk for agricultural workers as they apply pesticides. Farmers are exposed to pesticides through many pathways as they work mainly dermal, inhalation and accidental ingestion. Dermal exposure has the potential to be the most serious exposure followed by inhalation of significant gas vapor pressure and accidental ingestion [5].

Thailand is one of the world's largest exporters of agricultural products. Forty percent of Thailand's land area is devoted to agricultural production [19]. More than half of the total national workforce is currently engaged in the agriculture sector [15, 22]. Furthermore, the main source of income of rural Thai people originates from agriculture, because of Thailand's geographic location and climate [23]. Unfortunately, the climate does not only support agricultural activities, it also supports insect and other crop pests. As a result, the fields experience significant populations of insects and other pests during growing season. To control the pests, Thai farmers use excessive amounts of chemicals and pesticides to protect their harvest and to increase productivity [31]. To ensure sufficient pest control products imported pesticides have dramatically increased from last decade [20].

Thai farmers are at high risk of pesticide poisoning because they have insufficient understanding of pesticide safety and pesticide exposure [32]; they may be exposed to pesticide residues through the common environment or their occupation [29]. The common misuses including the use of larger volumes or concentrations of pesticides lead to the chance of increased exposure. Thai farmers prefer to create their own pesticide "cocktails" by mixing several pesticides together and adding more than the label indicates, with little regard for proper use and label rates [22, 26].

Chili, which is mostly grown in the Northeastern of Thailand, is a famous agricultural product [35]. To produce attractive and high quality chili, large amounts of pesticides are applied, particularly those in the organophosphate (OP) group. Organophosphates lead to many adverse health effects in humans by inhibiting the function of acetylcholinesterase [13]. Chili farming starts around December and is worked until March/April each year, *Norkaew* et al. [19] reported insufficient knowledge and concern about pesticide usage among chili farmers in the study area. Risk assessment of chili consumption in this area demonstrated that the residue of Profenofos on chilies was higher than the acceptable level suggested by the hazard quotient (HQ > 1) [21]. However, multi-route of exposure to pesticides among chili farmers was not studied in this community. In this study it was sought to estimate pesticide exposure levels through dermal and inhalation pathway by measuring urinary metabolites as indicators of pesticide exposure in biological monitoring. This could provide understanding of the most relevant exposure route to be able and help farmers increase their awareness and to encourage personal protective equipment usage.

MATERIALS AND METHODS

Participants: selection and recruitment

The chili farmers were part of an agricultural health study in Hua rau sub-district, Muang district, Ubon Ratchathani province (Figure 1) during March - April 2012.

PS program (power and sample size calculation; Version 3.0.43) [8, 9] was used to calculate sample size. An independent t-test mode was chosen for cases and control sample size to find a difference between two independent groups (exposure vs. non-exposure) on the means of a continuous variable. The data for sample size calculation was derived from *Curwin* et al. [7], for which a difference in the mean of two groups (farmer and non-farmer) (δ) was equal to 2.6 μg/L. The variances of two groups were pooled in order to achieve the

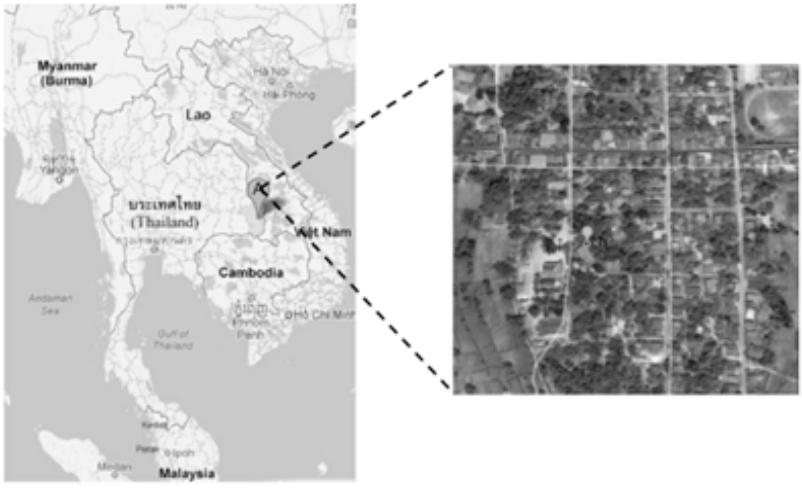

Figure 1. Study area location

best estimate of the (assumed equal) variances of the 2 populations [27] and the pooled variance was used to calculate the standard deviation ($\sigma = 3.26$) to fill in the program. The ratio (m), in this study, was equal to 1. This study was planned on a continuous response variable from independent control and experimental subjects with 1 control(s) per experimental subject. If the true difference in the experimental and control means was 2.6, we need to study 34 experimental subjects and 34 control subjects to be able to reject the null hypothesis with probability (power) 0.9. The Type I error probability associated with this test of null hypothesis was 0.05 (α); 10% of sample size was added. The total subject count was approximately 80 individuals, in order to be able and perform a personal in-depth monitoring for pesticide exposure. The subjects were separated into 2 groups; Chili-growing farmers (exposure; n=40) and reference group (non-exposure; n=40). However, this study was concentrated on the chili farmers only. Chili farmers in this study were selected by systemic sampling with every tenth individual from census records of three villages in the study because these villages were growing chili during the data collection period.

Sample collection design and wipe sample

The sample collection design (Figure 2) was adapted from *Thomas* et al. [33]. Face-to-face interviews were conducted to collect socio-demographic data and farm descriptions information. Urine morning void samples were collected before pesticide application and after application for two days. During the pesticide application, applicators were required to collect air samples and dermal patch samples. After pesticide application, wipe samples were collected from the applicators; including hands, feet and face wipe samples. Hands and feet wipe samples were collected from chili farmers before washing or cleaning their hands and feet. If they wore glove or boots, they were recommended to remove them before sampling. The wipe method was modified from *Geno* et al. [11]. Briefly, two moistened patches with 40% isopropanol were used to wipe pesticide residue on each farmer's hand and foot. Hands and feet wipe samples were kept separately and labeled with the farmer's code on square foil. After hands and feet wipe samples were collected, the farmer was introduced to the wiping procedure so they could perform the wiping by themselves; a patch with approximately 10 mL of the surfactant solution (40% isopropyl alcohol/water) was given to the farmer. If they wore a mask, they were recommended to remove it before sampling. The farmer's face was thoroughly wiped with the moistened patch and they repeated the wiping again with the same patch. All wipe samples were closed, sealed, and frozen for transport to the laboratory for analysis [30].

Patch samples collection

The patches used were standard patches (10 cm × 10 cm) to collect exposure data via dermal route. There were 7 patches that were fitted to the operator's clothing with safety pins following *Johnson* et al. [14]. The 7 positions were as followed; Position 1: on the hat, as close as possible to the top of the head; Position 2: over the sternum, on the outside of normal clothing; Position 3: on the sternum, on the inside of normal clothing; Position 4: upper surface of the right forearm held with the elbow bent at right angles across the body, midway between elbow and wrist, on the outside of normal clothing.; Position 5: front of left leg, mid-thigh, on the outside of normal clothing; Position 6: front of left leg, above the ankle, on the outside of normal clothing;

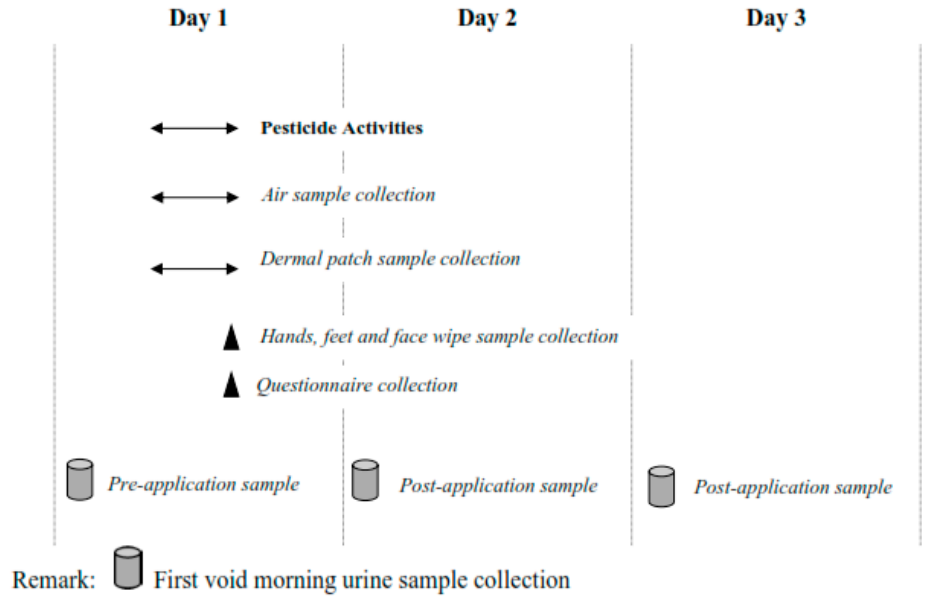

Figure 2. Sample collection protocol

Position 7: on the back between shoulder blades, on the outside of normal clothing. All 7 patches were combined together for laboratory analysis.

Air sample collections

Air samples were collected near the farmers' breathing zone by using personal sampling pumps connected to the sampler (Solid sorbent tube; OVS-2 tube: 13 mm quartz; XAD-2, 270 mg/140mg). The collected methods followed NIOSH 5600. The pump was set at a flow rate of 2 L/min and the sampler was set to connect along the calibrated personal sampling pump with tube and clipped to the applicator's collar, which was in the farmer's breathing zone. Pump flow-calibration checks were performed before and at the end of the sampling period. At the completion of sampling, the sampler was capped at both ends with plastic caps and packed for shipment. After collection, the samples were labeled and frozen in an icebox to be sent to the laboratory.

Urine sample collections

The subjects were familiarized with the process of urine sample collection and given the instructions by the researcher. A morning void urine sample was collected from each chili-growing farmer. Labeled polyethylene bottles with urine sample collection instructions were provided for all farmers. Urine samples were collected from chili farmers 3 times/ farmer. Before the spraying day, farmers were asked to collect the first void of urine for that day. The second urine sample was collected on the day after they farmed. On the third day, farmers were asked for urine samples again. All urine samples were first void morning urine. This research planned to collected urine 3 times due to the urinary half-lives, following dermal dose, of 30 hours for dialkylphosphate (DAP) metabolites [34]. Samples were transferred to zip-lock bags, labeled and kept in a cooler with frozen ice packs for transportation to the laboratory.

Laboratory analysis

A single researcher and standard laboratory were obtained for the analysis to control inter and intra observer variation. In each analysis, a calibration plot was constructed by 5 different calibration concentrations and repeated 5 times for each concentration. Correlation coefficients of calibration plot were greater than 0.99. Percent recovery of residual sample was found within the limits (80-120%) and percent relative standard deviation did not exceed the recommended guideline (Association of Official Agricultural Chemists (AOAC) Peer Verified Methods Program).

OP pesticides in wipe samples and patch samples

A modified liquid-liquid extraction (LLE) technique from QuEChERS (stands for Quick Easy Cheap Effective Rugged Safe) method and *Taneepanichskul* et al. [30] was used. First, the gauze pad was weighted; 1 gram approximately into a 50 mL centrifuge tube, to which 5 g NaCl, 10 mL Acetonitrile (HPLC Grade), 2 g $MgSO_4$ and 10 mL de-ionize water were added. Then, the tube was centrifuged for 10 minutes at 3,000 U/min, 5ºC. A supernatant was taken; 5 mL were added to new tube and then were evaporated until the volume was less than 1 mL. After that, Acetonitrile HPLC grade was added to adjust volume to 1 mL, and 0.5 g $MgSO_4$ and 0.5 g PSA (Primary-Secondary Amine) were added. The tube was shaken via a vortex mix machine for 1 min and centrifuged for 3 minutes at 3,000 U/min, 5ºC. Then, the supernatant was dispersed through a Syring filter Nylon (0.2 µm diameter). Finally, it was injected to a Gas Chromatography with Flame Photometric Detector (GC-FPD). An Agilent 6890N GC with Flame Photometric Detector was use to analyze concentration of wipe samples. The capillary column, which uses to separate compound, was DB-1701 (30.0 m length, 0.248 mm i.d., 0.25 µm film thickness) coated with 14% Cyanopropylphenyl and 86% methyl polysiloxane (J&W Scientific). External standards were used to perform sample quantification. A 2 µL of sample was injected into GC. The initial temperature of injection was 200ºC. The oven's initial temperature was 80 ºC for 0 min and it was programmed to increase at 12ºC/min to 195ºC. Then, it increased at 2ºC/min to 210ºC, which was held for 7 min. It increased to 225ºC at 15ºC/min, held for 15 min. The last temperature was 275ºC which increased at 35ºC/min and was held for 13 min; the total run time was 50.51 min.

OP pesticides in air samples

Samplers were used and the solvent was extracted and analyzed by GC-FPD. First, the mixed toluene and acetone (9:1, v/v) were added to air sampler. The solvent was evaporated by using an evaporator rotary with a acetone program at 65 ºC until the volume was less than 1 mL. After that, the volume was adjusted to 1 mL by ethyl acetate and injected to GC-FPD. The same GC-FPD conditions as for the wipe samples analysis were applied for the analysis of air samples.

Diethyl DAPs in urine samples

We measured 3 diethyl DAP metabolites (DEP, DETP and DEDTP) because the parent compounds in this study were Chlorpyrifos and Profenofos. Profenefos itself did not produce any DAP metabolites and Chlorpyrifos produced only diethyl DAP metabolites [4]. Total diethyl DAP was calculated by the following equation:

$$[\text{Diethyl DAP}] = [\text{DEP}] + [\text{DETP}] + [\text{DEDTP}]$$

The analyzed procedure was modified from *Prapamontol* et al. [25]. Briefly, a 5 mL of urine sample was pipetted into a 25 ml glass tube and spiked with 50 µL DBP (IS) solution (1.25 ppm). Subsequently, 2 grams of NaCl and 1 mL of HCl were added to the solution. Liquid-liquid extraction was done by ethyl acetate: acetone (1:1, v/v) solvent. The tube was shaken for 5 minutes and centrifuged for 5 minutes at 2000 U/min, respectively. The supernatant was transferred into the new tube which contained 20 mg of K_2CO_3. The sample volume was then reduced to 0.5 mL by evaporation. The 20 mg of K_2CO_3, 3 mL of Acetonitrile and 50 µL of PFBBr were added and heated at 50°C for 15h to covert the phosphate acids to their pentafluorobenzyl (PFB) esters. The derivatives were re-extracted by liquid-liquid extraction by 4 ml of H_2O and 5 mL of Hexane. The supernatant volume was then reduced to be able and dry via N_2 gas. After that, Toluene 200 µL was added to adjusted the last volume for injection into the GC-FPD.

Statistical analysis

In this study data analysis was done using an SPSS program V.17 for Windows. The *Kolmogorov–Smirnov* test was used to check the distribution of analyzes concentrations.

The negative asymmetric distribution ($p>0.05$) was found; so non-parametric statistic was used for analysis.

In term of statistical difference among farmers' urinary metabolite levels in different days, *Wilcoxon* signed ranks test were used to explain the urinary metabolite statistical difference of each pair of urine samples (Before mixing, loading and applying pesticide (MLA) - 1st post MLA; before MLA - 2nd post MLA; 1st post MLA - 2nd post MLA). In addition, *Spearman's* rank correlation was used to find the association between route of exposure and biological monitoring. Statistical significance was set at $\alpha=0.05$.

Ethical consideration

This study was approved by the Institutional Review Boards (IRB) of Ethical Committee of College of Public Health Sciences, Chulalongkorn University (ECCU group 1) (COA No. 038/2555; Date of approval: 9 March 2012).

RESULTS

Participants' characteristics

Table 1 depicts the participants' socio-demographic and farm operation descriptions. Both male (65%) and female (35%) chili-growing farmers participated in this study. The majority of chili-growing farmers were 30 to 39 years of age while an average age (±SD) of all was 40.95 (±6.12) years old. Average BMI (±SD) was 23.18 (±4.48). Farmers who completed elementary school (75%) were in more abundance than those who had completed high school (25%); mostly non-smokers. Chili farmers with over 10 years of experience of pesticide application were the majority of participants (85%), only few farmers had lower than 5 years of experience. The average working period of pesticide application was two hours per application; this was due to the fact that applications were performed in small cultivation areas (average = 2.05 rai). An annual pesticide application was around 16 times. Average total annual income of chili crop after cutting off capital was around 30,000 THB (995 USD).

Table 1. Chili farmers' socio-demographic and farming characteristics

	Chili farmers
Gender, n (%)	
Men	26 (65%)
Women	14 (35%)
Age groups, n (%)	
20 – 29	2 (5%)
30 – 39	18 (45%)
40 – 49	16 (40%)
50 or more	4 (10%)
Mean ±SD	40.95 (±6.11)
BMI (mean ±SD)	23.18 (±4.48)
Education, n (%)	
Illiteracy	-
Elementary School [a]	30 (75%)
High School [b]	10 (25%)
Smoking Status, n (%)	
Non-Smokers	30 (75%)
Smokers	10 (25%)
Agricultural works and farming characteristics	
Area of cultivation (rai[c]) (mean ± SD)	2.05 (±0.71)
Duration of application/time[d] (Hrs) (mean ± SD)	2.0 (±0.3)
Years of using pesticides (n (%))(mean ± SD)	14.40 (±6.53)
< 10 years	6 (15 %)
10 – 19 years	18 (45%)
≥ 20 years	16 (40%)
Frequency of pesticide applications (times) (mean ± SD)	15.90 (±4.06)
Annual income (USD) (mean ±SD)	995 (±673)

[a] *Elementary School: finished grade 6*
[b] *High School: finished grade 12*
[c] *Change Unit: 1 rai = 0.4 acre*
[d] *Duration of application including mixing and loading*

Chili farmers' pesticide exposure

Pesticide exposure concentration was measured via dermal and inhalation measurements from chili farmers during and after their pesticide application (MLA). From 40 interviewed chili farmers, 38 of them agreed to provide wipe samples and air samples. The exposure to pesticides are shown in Table 2. Feet wipe samples did not show Chlorpyrifos or Profenofos while during

Table 2. Percentage of positive samples and pesticide concentration (mg/kg of wipe sample)

	Detected samples[d] (%)	Mean	SD	Range
Hands[a]				
Chlorpyrifos	10.53	0.043	0.068	<LOD - 0.240
Profenofos	26.32	0.087	0.132	<LOD - 0.450
Face[a]				
Chlorpyrifos	26.32	0.044	0.050	<LOD - 0.210
Profenofos	31.58	0.513	1.542	<LOD - 9.750
Body[a]				
Chlorpyrifos	78.95	2.179	4.199	<LOD - 15.95
Profenofos	78.95	2.151	5.221	<LOD - 22.80
Feet[a]				
Chlorpyrifos	n/d[c]	-	-	-
Profenofos	n/d[c]	-	-	-
Air samples[b]				
Chlorpyrifos	100.0	0.004	0.004	<LOD - 0.010
Profenofos	21.05	0.001	0.002	<LOD - 0.006

[a] LOD <0.02 mg/kg
[b] LOD <0.001 mg/kg
[c] n/d: not detected
[d] The percentage of samples with detected concentration higher than LOD

the hand wipe samples both Chlorpyrifos (10.53%) and Profenofos (26.32%) were present. The pesticides were detected in thirty percent of face wipes and in almost eighty percent of body wipes. All personal air samplers detected Chlorpyrifos, but only 21.05% of them had Profenofos. The concentration of Chlorpyrifos and Profenofos greatly varied in body patch samples (Chlorpyrifos: < Limit of detection (LOD) - 15.95 mg/kg and Profenofos: <LOD - 22.80 mg/kg). However, air samples were found to not have much variation in concentration among each sample. The study also found that there was no statistical relationship between dermal and inhalation exposure (Spearman rho's test; r = 0.155, p> 0.05).

Chili farmers' urinary metabolite levels

The first morning void was collected from Chili farmers before MLA and 2 following morning voids after MLA. 39 (97.5%) out of 40 chili farmers participating had completely provided 3 morning void urine samples (Pre-MLA, 1st Post-MLA and 2nd Post-MLA). Around 2 mL of the urine samples were sent to the laboratory for creatinine analysis. The average creatinine concentration for chili farmers was 128 mg/dL.

For DAP metabolite, this could be detailed before (Diethylphosphate (DEP), Diethylthiophosphate (DETP) and Diethyldithiophosphate (DEDTP)), the 1st morning void following MLA had the highest detected frequencies. The change of DAP level was dramatically increased from pre-MLA to 1st MLA and then decreased from 1st MLA to 2nd MLA. Only diethyl DAP metabolite should be detected due to Chlorpyrifos exposure. The Chlorpyrifos methyl was not found in this study area, so the dimethyl DAP metabolites were not produced [25]. Moreover, Profenofos did not produce any DAP metabolite [4]. On the pre-MLA day, the highest detected metabolite was DETP (41.7%). On the 1st post-MLA, DETP (85.7%), DEP (65.7%) and DEDTP (25.7%) were detected. Diethyl DAP metabolite was found on DETP (48.5%), DEP (42.4%) and DEDTP (12.1%) on the last day of data collection.

On the pre- MLA day (day -1), the geometric means were; DEP 0.33 µg/L (0.29 µg/ g cre.), DETP 0.46 µg/L (0.40 µg/ g cre.) and DEDTP 0.15 µg/L (0.13 µg/g cre.). In the 1st post-MLA (day 0) all diethyl DAPs metabolite concentrations were higher than those of day -1 because of Chlorpyrifos exposure. The geometric mean of each metabolite concentration could be detected as followed: DEP 1.04 µg/L (0.92 µg/ g cre.), DETP 4.39 µg/L (3.88 µg/ g cre.) and DEDTP 0.28 µg/L (0.25 µg/ g cre.). Day +1 (the 2nd following MLA), all previous metabolites were detected and they were higher than in day-1. The geometric mean concentration of each die-

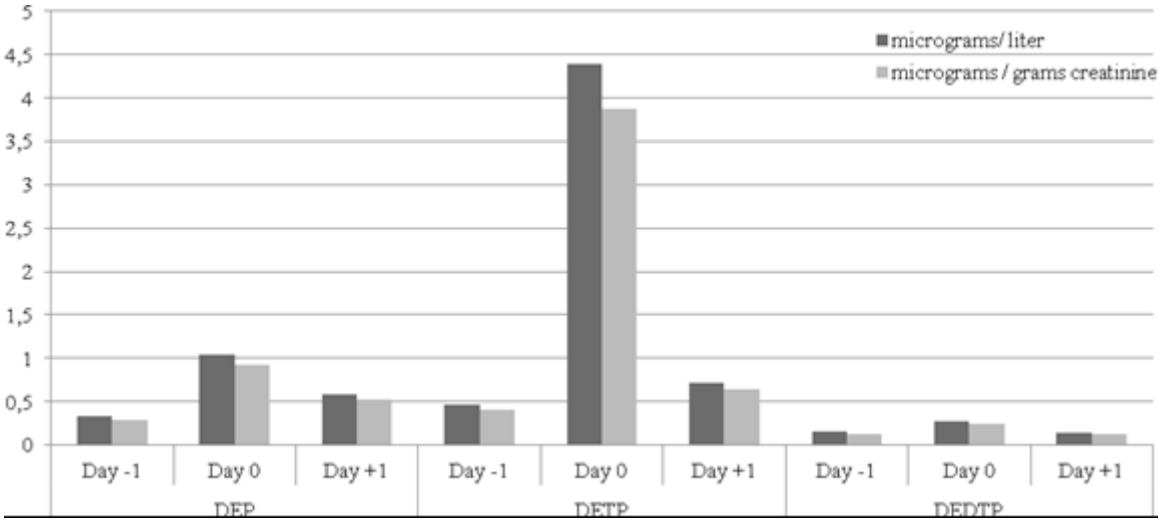

Figure 3. DEP, DETP and DEDTP metabolites concentration

thyl DAP metabolite were: DEP 0.59 μg/L (0.52 μg/g cre.), DETP 0.72 μg/L (0.64 μg/ g cre.) and DEDTP 0.14 μg/L (0.12 μg/ g cre.). Additionally, the diethyl DAP geometric mean of pre-MLA, 1st post-MLA and 2nd post-MLA were 7.45 nmol/L (6.58 nmol/g cre.), 56.1 nmol/L (49.5 nmol/g cre.) and 9.60 nmol/L (9.37 nmol/g cre.). The range of diethyl DAP concentrations are presented in Figure 3.

Comparison of urinary DAP metabolite levels for pre- and two post- application

There was difference of 3 urinary metabolite samples (day-1, day0 and day+1) found (*Friedman* test, $p<0.001$). The diethyl DAP urinary metabolite of chili farmers in the 1st post application (day 0) was different from the day before application (day -1) and 2nd post application (day +1). However, the urinary metabolite levels for the day before application was not different from the 2nd post application day (Table 3). The metabolite was decreased from 1st post application day to 2nd post application statistical significantly.

Table 3. Pre and post application urinary metabolite statistical differences

	P-value*
Day 0 - Day -1	< 0.001
Day 0 - Day +1	< 0.001
Day -1 - Day +1	0.131

Day -1: Morning void urine before MLA
Day 0: 1st Morning void urine following MLA
Day +1: 2nd Morning void urine following MLA

Association between exposure route and urinary metabolite level

Spearman correlation was used to analyze correlation between route of pesticide exposure and urinary metabolite. Dermal and Inhalation routes were identified as the route of pesticide exposure in this study and the urinary metabolite concentrations were separated to 1st post MLA and 2nd post MLA for analysis. The metabolite of pre-MLA was excluded from this part of analysis. The correlation between dermal exposure and 1st post MLA urinary metabolite was found at moderate levels of correlation with statistical significant levels ($p<0.05$) (Table 4). Other correlations were not statistically significant in this analysis.

Table 4. Urinary metabolites as related to exposure route

Exposure Route	Urinary metabolite	r_r
Dermal[a]	1st post MLA (Day 0)	0.405*
	2nd post MLA (Day +1)	0.205
Inhalation[b]	1st post MLA (Day 0)	0.108
	2nd post MLA (Day +1)	0.175

[a] Dermal route : summation of Chlorpyrifos concentration of hand wipe, face wipe and body patch
[b] Inhalation route : Chlorpyrifos concentration of air sampler
* Correlation is significant at the 0.05 level (2-tailed)

DISCUSSION

Most chili farmers in this study were male, with a small percentage of women participants 17, 24], agricultural activities in Thailand are typically performed by men. Body mass index (BMI) of both chili farmers and non-chili farmers were classified in the normal range (18.5 – 24.9) by WHO definition [34].

The higher pesticide exposure was related to the lack of education. Elementary level educated individuals were the majority of the study population. The safety instructions on pesticide containers are often written in unfamiliar language for the farmers and difficult to follow [10]. This confusion may lead to the unintentional pesticide exposure due to difficulties in understanding the instructions and safety procedures on the labels.

No feet wipe residues were detected for detected Chlorpyrifos and Profenofos. We noted in the field that chili farmers wore socks together with boots during MLA. Farmers reported that they felt comfortable with wearing socks and boots because the humidity; socks might absorb the sweat on their feet. Farmers normally washed their clothes after pesticide application. In 30% of face wipe samples both Chlorpyrifos and Profenofos were detected. Similar to *Schneider* et al. [28] and *Aprea* et al. [1] the pesticide (Azinphosmethyl) residues on face/neck wipe were studied and the concentration for the face/neck wipes was <0.002-0.05 mg.

Hand wipe samples detected lower concentrations than face wipe samples. Similar to *Curwin* et al. [7] study, the study conducted on farmers in Iowa (US) found that most of the hand wipe samples were showed no detectable residues. However, the concentration of Chlorpyrifos in the study was less than the previous study of *Taneepanichskul* et al. [30]. Higher concentrations of pesticide residues could be found from hand contact more than other parts of the body because of the exposure to the concentrated pesticide formulation while loading to the sprayer equipment.

Profenofos and Chlorpyrifos were detected on chili farmers <0.001 – 0.010 mg/m^3 and <0.001 – 0.006 mg/m^3, respectively. Chili farmers exposure levels did not exceed the time-weighted average (TWA) limit of the American Conference of Governmental Industrial Hygienist (ACGIH) recommendation value of 0.02 mg/m^3 which is similar to the findings of *Kongtip* et al. [16] who found that the average occupational chlorpyrifos exposure among rice farmers in Phatthalung Province was 0.062 ± 0.092 mg/m^3. Air samples contained more Chlorpyrifos (around 80%) than Profenofos due to the solubility of the chemical property. Chlorpyrifos has higher solubility than Profenofos, so this property could affect the process of laboratory analysis. Profenofos has limited solubility in water at 20 ppm but is completely

soluble in organic solvents (ethanol, acetone, toluene, n-octanol, and n-hexane).

Urinary metabolite levels in this study suggested that geometric concentration of pre-MLA, 1st post--MLA and 2nd post-MLA were 6.58 nmol/g creatinine, 49.5 nmol/g creatinine and 9.37 nmol/g creatinine, respectively. The highest detected frequency was the 1st post-MLA, 2nd post-MLA and pre-MLA. From the results, it could be explained that the urinary half-lives for dialkylphosphate metabolites through dermal dose was 30 hours [33]. The diethyl DAP was found in this study because of the parent compound (Chlorpyrifos) exposure. The Chlorpyrifos methyl was neither widely used nor found in this study area.

In Thailand, urinary DAP study with farmers suggested average levels (geometric mean) of 51.1 mg/g for vegetable farmers and 122.2 mg/g for fruit farmers [12]. Moreover, *Panuwet* et al. [22] assessed exposure pesticides of male farmers in Chiang Mai Province, Thailand and pointed out that no significant differences in metabolite concentrations of two farmer groups with differential topographical areas. *Blair* et al. [3] demonstrated that correlations of urinary levels with kilograms of active ingredient used, duration of application, or number of acres treated were lower.

In this study, the correlation between dermal exposure and 1st post MLA urinary metabolite was found at a statistically significant level ($p<0.05$), similar to *Curwin* et al. [7] which suggested that most hand wipe samples were non-detectable. Moreover, *Curl* et al. [6] found an association between dimethyl DAP levels in adult farmers urinary metabolite and exposure, however, the dimethyl DAP metabolite could posed to not only agricultural product exposure but also variety of OPPs. Additionally, dermal exposure on day one correlated with total metabolites collected the following morning and total metabolites collected after 48 hours were far less correlated. Some limitations of this study could be pointed out. Only diethyl DAP was analyzed in this study.

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