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Organophosphate Pesticide Exposure of Farmers in Chiang Mai Province, Northern Thailand

Surat Hongsibsong [1 +], Tanyaporn Kerdnoi [1], Watcharapol Polyiem [1], Vanvimol Patarasiriwong [2] and Tippawan Prapamontol [1]

[1] Environment and Health Rerearch Unit, Research Institute for Health Science, Chiang Mai University, Chiang Mai, 50200 Thailand

[2] Department of Environmental Quality Promotion, Ministry of Natural Resources and Environment, Bangkok, 10400 Thailand

Abstract. Organophosphate (OP) pesticides are the most commonly used pesticides among Thai farmers. Chiang Mai Province, in northern Thailand, substantially contributes agricultural produce to the markets.Due to the regular exposure to organophosphate pesticides, the farmers involved in the production run a considerable risk. The present study aimed to analyze biomarkers of exposure, i.e. cholinesterase and dialkylphosphate metabolites of organophosphate pesticides. The activities of cholinesterase enzymes were measured by a modified Elman's assay while DAP metabolites (DMP, DMTP, DMDTP, DEP, DETP, and DEDTP) were measured by GC-FPD. The results showed AChE and BChE activities ranging from 0.48 – 5.60 U/mL and 0.73 – 3.59 U/mL, respectively. All subjects had at least one of the DAP metabolite in their urine. Thus, farmers are exposed to organophosphate pesticides during the time they work on the farm, but also during the consumption of their produce. Showering or washing hands immediately after working in the farm, in particular after using organophosphate pesticides, will reduce the impact of possible exposure to pesticide residues.

Keywords: ChE, Dialkylphosphate metabolites, Organophosphate exposure.

1. Introduction

In Thailand, organophosphates (OPs) are applied in agriculture for pest control. OPs are organic esters of phosphoric acid, thiophosphoric acid, and dithiophosphoric acid. Popular OPs used in Thailand are chlorpyrifos, dichlorvos, dimethoat, oxydemeton-methyl, parathion, parathion-methyl, and pirimiphos-methyl [1]. Most OPs produce toxicity by inhibiting the acetyl cholinesterase (AChE) activity, which terminates the action of neurotransmitter acetylcholine (ACh) within nerve synapses, thus resulting in destruction of ChE. The effects, depending on route and exposure dose, vary from mild, such as salivation and tremor, to more serious such as convulsion, seizure, paralysis, and death. The AChE enzyme activity can be measured in plasma and erythrocyte [2]. Biomarkers of OP exposure are cholinesterase enzyme and dialkylphosphates (DAPs) as major metabolites (dimethylphosphate (DMP), dimethylthiophosphate (DMTP), dimethyldithiophosphate (DMDTP), diethylphosphate (DEP), and diethylthiophosphate (DETP), diethyldithiophosphate (DEDTP)) [3], [4].

Mae Taeng district in Northern Thailand covers an area 1,362.784 km2, and is located at 19° 7′ 19″ N 98° 56′ 37″ E. This district is an important fruits and vegetables production area for the markets around this province. One of the reasons for its productivity is the proximity of the Ping River as a main water resource, while another reason is the suitability of the soil enabling farmers to grow crops year round. Thus most people here are farmers or perform agriculture-related jobs. Therefore, the aim of the present study was

+ Corresponding author. Tel.: +66-539-42508 ext. 314; fax: +66-539-42528
E-mail address: surat.hongsibsong@cmu.ac.th.

to determine OP exposure by using plasma and erythrocyte cholinesterase enzymes and DAPs in urine as biomarkers.

2. Materials and Methods

2.1. Study Design

The present cross-sectional study is meant to determine the biomarkers of OP exposure by analyzing DAPs in urine and cholinesterase in plasma and erythrocyte samples collected from farmers, on December 2013. It involved one hundred and twenty volunteers who were farmers working in orchards and/or were growing vegetable crops around the Mae Taeng district. This study received ethical approval from the Office of Research Ethics of the Research Institute for Health Sciences, Chiang Mai University.

2.2. Standard Chemicals and Reagents

2.2.1. Cholinesterase measurement

Substrates reagents used, i.e. acetylthiocholine iodide (ATCH), butyrylcholine iodide (BTCH), the color reagent 5, 5' -dithiobis-2-nitrobenzoic acid (DTNB) were all obtained from Sigma-Aldrich.

2.2.2. Dialhkylphosphates (DAPS) analysis by Gas Chromatography-Flam Photometric Detection (GC-FPD)

All organic solvents, i.e. acetone, ethyl acetate, acetonitrile, hexane, and toluene were of analytical grade, purchased from J.T. Baker (PA, USA). PFBBr (99%) was purchased from Sigma–Aldrich (MO, USA). The standard chemicals used were DMP (98%), purchased from ACROS Organic (NJ, USA), DEP (99.5%) purchased from Chem Service (PA, USA). DMTP and DMDTP (>90%, 1000 g/mL) purchased from Cerilliant (TX, USA). DETP (98%) and DEDTP (90%) were purchased from Sigma–Aldrich.

2.3. Determination of Cholinesterase Activities by Elman's Assay

Whole blood was collected by using heparinized tube as anticoagulant and then centrifuged at 800g for 15 minutes to achieve plasma separation. The Erythrocyte was washed two times using a phosphate buffer saline (pH 7.0). The Cholinesterase activities were measured by using ATCH as substrate for acetyl cholinesterase activity in erythrocyte and BTCH as substrate for butyryl cholinesterase activity in plasma, in compliance with a method of Gonzalez and collages [5].

2.4. DAP Analysis in Urine

During the first morning void urine samples were used as these are suggested to provide the best representative measurement of daily OP exposure when using spot samples (Kissel et al., 2005). Thereafter, these samples were brought to the Toxicology laboratory, Research Institute for Health Science, Chiang Mai University, on ice and then kept in a freezer at -20 ℃ prior to analysis. Sample extraction and DAP analysis using the GC-FPD methods were in accordance with the method of Prapamontol and colleagues [6].

3. Results and Analysis

3.1. Study Participants

A total of 118 farmers joined the study, representing 88 males (74.6%) and 30 females (26.4%). Most subjects ranged in age between 40 - 60 years (71%), and had worked in agriculture either between 21 – 30 years (30.2%) or 31- 40 years (27.6%).

3.2. Cholinesterase Enzymes Activities

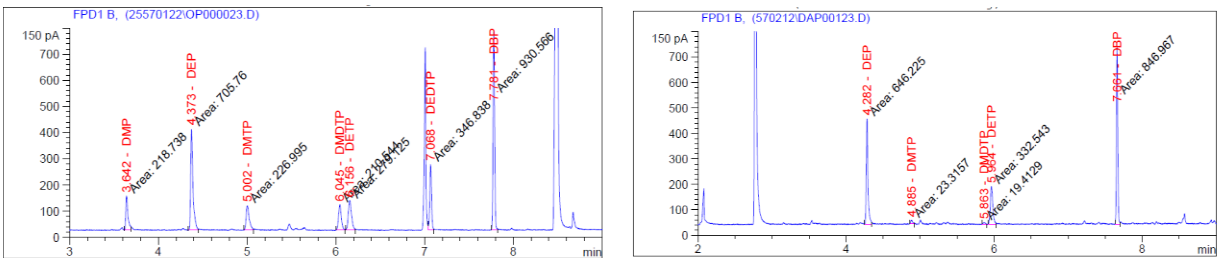
The results of acetylcholinesterase in erythrocyte and butyrylcholinesterase in plasma are presented in Table I. The means of AChE and BChE were 3.43 and 1.99 U/mL, respectively. The results show a significant difference between AChE and BChE at $p < 0.05$. The means of AChE and BChE were higher than of farmers in a previous study [3]. The BChE can be inhibited by OPs or carbamate pesticides for long time exposure [7].

3.3. The Level of DAP Metabolites in the Urine

Results using the GC-FPD method show that the chromatograms of standard spiked urine had good separations, without interfering peaks, and short run times (Fig. 1).The limit of detection ranged from 0.10 - 2.5 ng/mL. The correlation coefficients of the calibration curve for all analyses exceeded 0.99. Intra-batch and inter-batch precisions were reported in percentages of relative standard deviations (RSDs). The percentages RSD of intra-batch and inter-batch precisions for all compounds were 2.15 – 13.91%, and 5.30 – 19.03%. The method recoveries ranged from 92.5 – 119.13%.

Table I: Means, Median and Min – Max of cholinesterase enzymes (ChE) activities in erythrocyte (AChE) and plasma (BChE) of the farmers

Cholinesterase	Activity (U/mL)		
	Mean ±SD	Median	Min-Max
Acethylcholinesterase (AChE)	3.43 ±0.77	3.51	0.48-5.60
Butyrylcholinesterase (BChE)	1.99 ±0.57	1.90	0.73-3.59



(a) (b)

Fig. 1: Standard peaks in urine spiked DMP = 75 µg/L, DEP = 20 µg/L, DMTP = 8 µg/L, DMDTP = 4 µg/L, DETP = 4 µg/L, DEDTP = 8 µg/L, DBP as internal standard = 12.5 µg/L (a), and detected peaks in urine samples (b).

The results of DAP metabolites in urine samples are shown in Table II. Subject farmers had at least one DAP metabolite in the urine. Urinary DAP levels of farmers ranged from 0.00 - 45.53 µmole/mole creatinine, with a median level of 3.58 µmole/ mole creatinine. The order of DAP metabolites detection was DEP, DETP, DEDTP, DMTP, DMDTP, and DMP, respectively; these give evidence that the subjects were exposed to organophosphate compounds. The order of detected DAP metabolites was different from another study, which had a high DMP detection percentage [8], [9] perhaps due to a different kind of organophosphate pesticide used. The present study had results of DAPs levels lower than farmers whose sprayer from previous study [4].

Table II: DAPs metabolite levels (µmole/mole creatinine) in subjects' urine samples

DAPs metabolite group	% detected	DAPs levels (µmole/mole creatinine)		
		Mean ±SD	Median	Min-Max
DMP	5.10	8.34 ±6.67	4.84	3.21 - 17.26
DEP	97.50	3.36 ±3.82	9.6	0.39 - 24.19
DMTP	14.40	1.33 ±0.73	1.35	0.52 - 3.12
DMDTP	7.60	0.35 ±0.22	0.26	0.12 - 0.84
DETP	88.10	2.20 ±3.16	1.03	0.17 - 24.58
DEDTP	32.20	0.69 ±0.40	0.36	0.19 - 1.94
Total DAPs	100	5.92 ±7.17	3.58	0.00 – 45.53

There are 2 kinds of DAP metabolites from organophosphate insecticides, i.e. molecular structure of the methyl group, or the ethyl group. Most of the DAP metabolites in the farmers' urine samples were metabolites of the ethyl group (Table III) that had significant different levels at $p < 0.05$.

Table III: Dimethyl- and diethyl- DAPs metabolite levels (µmole/mole creatinine) in subject urine samples

DAP metabolites	Levels (µmole/mole creatinine)		
	% detection	Mean±SD Median	P*
Dimethyl group Metabolites	16.10	3.52 ± 1.10	< 0.05
Diethyl group Metabolites	98.45	7.65 ± 1.84	

* Significant difference by *t-test* at $p<0.05$

4. Conclusions

The present study, the first assessment in Chiang Mai, Thailand, aimed to determine the concentration of dialkylphosphate metabolites in blood and urine of farmers working in Mae Taeng district. The study found AChE and BChE activities ranging from 0.48 – 5.60 U/mL and 0.73 – 3.59 U/mL, respectively. Analysis of DAPs in the urine of the farmers' blood detected that all subjects had at least one DAP metabolite, suggesting that contamination with organophosphate pesticides is a health problem for these farmers indeed, but also those consuming the agricultural products from this area.

5. Acknowledgements

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