

ORGANOPHOSPHATES IN MECONIUM OF NEWBORN BABIES WHOSE MOTHERS RESIDED IN AGRICULTURAL AREAS OF THAILAND

Chompunut Onchoi¹, Pornpimol Kongtip^{1,3}, Noppanun Nankongnab^{1,3},
Suttinun Chantanakul^{1,3}, Dusit Sujirarat² and Susan Woskie⁴

¹Department of Occupational Health and Safety, ²Department of Epidemiology, Faculty of Public Health, Mahidol University, Thailand; ³Center of Excellence on Environmental Health and Toxicology (EHT), Thailand; ⁴Department of Public Health, University of Massachusetts Lowell, MA, USA

Abstract. Organophosphates (OPs) are widely used for pest and weed control in many countries including Thailand. In addition to causing environmental pollution, OPs affect human health by overstimulating neurotoxins, and OP exposure during pregnancy can lead to adverse health effects of mothers and their fetuses. Using gas chromatography-mass spectrometry with a dedicated extraction protocol to identify OPs in meconium of newborn babies ($n = 68$) from hospitals in Amnat Charoen, Kanchanaburi and Nakhon Sawan provinces, agricultural regions of Thailand, among ten OP types analyzed, eight were detected in 98% of meconium samples (chlorpyrifos (median $\pm$ interquartile range (IQR) 0.08 ± 0.03 - $0.16 \mu\text{g/g}$) in 32% of samples, demeton-s-methyl (0.35 ± 0.26 - $0.49 \mu\text{g/g}$) in 73%, dichlorvos (0.67 ± 0.58 - $0.71 \mu\text{g/g}$) in 38%, dimethoate (0.43 ± 0.09 - $1.56 \mu\text{g/g}$) in 50%, ethion (0.21 ± 0.19 - $0.26 \mu\text{g/g}$) in 12%, malathion (0.28 ± 0.15 - $0.52 \mu\text{g/g}$) in 50%, omethoate (5.63 ± 4.85 - $8.57 \mu\text{g/g}$) in 34%, and tolclofos-methyl (0.08 ± 0.03 - $0.10 \mu\text{g/g}$) in 41%). There are no significant differences in these parameters from babies whose mothers did and did not work in the agricultural or who lived near (within one km) and distant from farmland. The findings should be of benefit in developing programs to protect pregnant women and newborn babies from exposure to OP pesticides.

Keywords: GC-MS, meconium, organophosphate, pesticide, Thailand

INTRODUCTION

Thailand is an agricultural country, with approximately 35% of the population working in this sector (NSO, 2013).

Correspondence: Pornpimol Kongtip, Department of Occupational Health and Safety, Faculty of Public Health, Mahidol University, 420/1 Ratchawithi Rd, Rachathewi, Bangkok 10400 Thailand.

Tel.: +66 (0) 2644 4069; fax: +66 (0) 2354 8561.

E-mail: pornpimol.kon@mahidol.ac.th

Synthetic pesticides are widely used and their import has increased from 164,500 tons in 2013 to 198,300 tons in 2017, comprising 75% herbicides, 11% insecticides and 10% fungicide (OAE, 2017). Reported cases of pesticide poisoning totaled 2.0/100,000 population in 2006 and rose to 3.7/100,000 in 2015, when 2,421 cases of poisoning were reported: 27.5% from organophosphates (OPs) and carbamate, 25.0 from herbicide and fungicide, 2.9% from rodenticide,

2.1% from halogenated pesticides, and 42.5% from unidentified compounds (MOPH, 2015); however, no fatality was reported.

Pregnant women living in agricultural areas (and even those using common household pesticides) have higher levels of urinary OP metabolites than the general population and higher levels during immediate postpartum period (Bradman *et al*, 2005). As children organs are not fully developed until later in life, continual environmental exposures to OPs can result in lifelong adverse health effects, particularly in utero (Chalupka and Chalupka, 2010). Prenatal exposure to OP pesticides is associated with low head circumference, birth weight and body length of newborn babies, and adversely affects neurological development (Rauch *et al*, 2012; Naksen *et al*, 2015).

Fetus acquires OPs from maternal blood and OP pesticide residues are found in blood, hair, meconium, serum, and urine of the newborn (Barr and Needham, 2002). Six meconium is an ideal matrix to determine prenatal exposure to xenobiotics because of the ease of collection, noninvasiveness and the ability to detect a wide window of fetal exposure (Moore *et al*, 1998). In human fetus, meconium begins to accumulate in bowel at approximately 16 weeks of gestation and excreted after delivery (Bearer *et al*, 1999). Meconium is indicative of fetus intestinal content and consists of a complex mixture containing bile acids, blood group substances, cholesterol, enzymes, epithelial cells, lipids, mucopolysaccharides, proteins, residual amniotic fluid, salts, squamous cells, and sterol precursors (El-Baz *et al*, 2015). Xenobiotics enter the meconium as a sequence of bile excretion in intestines or of fetus swallowing amniotic fluid (Ortega García *et al*, 2006). OP metabolites in

meconium, namely, diethyldithiophosphate (DEDTP), diethylphosphate (DEP), diethylthiophosphate (DETP), dimethyldithiophosphate (DMDTP), dimethylphosphate (DMP), and dimethylthiophosphate (DMTP) are suggested as biomarkers of prenatal exposure OPs (Whyatt and Barr, 2001).

Gas chromatography-mass spectrometry (GC-MS) is often employed to analyze pesticides in meconium (Ostrea *et al*, 2002; Bielawski *et al*, 2005; Ostrea *et al*, 2008). A solid phase extraction method was modified to isolate multiple classes of parent pesticides from meconium using methanolic/hydrochloric acid methyl ester derivatization prior to liquid-liquid extraction (Bielawski *et al*, 2005). Methods were developed to determine parent OP pesticides using GC-MS, measure their concentrations in meconium of newborn babies whose mothers resided in three agricultural areas in Thailand and identify factor associated with level of OP concentrations in meconium of newborn babies.

MATERIALS AND METHODS

Analytical standards, chemicals and reagents

Pesticide Mix, containing chlorpyrifos, dichlorvos, dimethoate, demeton-s-methyl, ethion, malathion, omethoate, pirimiphos-methyl, pyrazophos, and tolclofos-methyl (100-1000 $\mu\text{g/ml}$ acetone), was from Restek (Bellefonte, PA); disodium hydrogen phosphate, sodium chloride, sodium phosphate monobasic, and triphenylphosphate (TPP) were from Sigma-Aldrich (St Louis, MO); acetone, acetonitrile, methanol, and toluene were from Merck (Darmstadt, Germany); and chemicals for clean-up method, magnesium sulfate anhydrous, primary

secondary amines (PSA), and octadecyl (C_{18}) were from Agilent Technology (Thailand) Co Ltd (Bangkok, Thailand).

Sample collection

Meconium samples ($n = 68$) were collected from newborns whose mothers (19-35 years of age) attended prenatal clinics in three agricultural areas in Thailand, namely, Amnat Charoen Hospital in Amnat Charoen Province, northeastern region; Sawanpracharak Hospital in Nakorn Sawan Province, lower northern region; and Paholpolpayuhasena Hospital in Kanchanaburi Province, western region. Samples were collected by nurses in polyethylene cassettes and stored at -45°C until used.

The study protocol was approved by the Ethics Committee on Human Rights Related to Human Experimentation, Mahidol University (MUPH 2011-098) and the University of Massachusetts Lowell Institutional Review Board (UML 10-129).

Preparation of stock standard mixture, calibration standards and quality control samples

Working OP standard solution was prepared by diluting Pesticide Mix in acetone to 150-9000 ng/ml and stored in a refrigerator until used. Calibration curves were developed using Pesticide Mix-spiked (0.2-12.0 $\mu\text{g/g}$) meconium samples ($n = 3$) from newborns of (OP pesticide-free) mothers living outside the study areas. For within-day assay, meconium samples spiked with chlorpyrifos, pirimiphos-methyl and tolclofos-methyl (0.38, 0.64 and 0.90 $\mu\text{g/g}$), dichlorvos and pyrazophos (1.92, 3.21 and 4.49 $\mu\text{g/g}$), dimethoate, demeton-s-methyl, ethion, and malathion (0.77, 1.28 and 1.79 $\mu\text{g/g}$), and omethoate (3.85, 6.41 and 8.97 $\mu\text{g/g}$) were analyzed in three replicates on the same day. For between-day assay,

meconium samples described above were analyzed on different days. Quality control charts were developed to monitor accuracy and precision of analysis of OPs in meconium samples.

Meconium extraction and clean-up procedure

In the extraction step, meconium sample (0.15 g) was added to 5 ml of 0.1 M phosphate buffer pH 7: methanol (25:75) solution, vortexed for three minutes and then 200 μl aliquot of internal standards was added, followed by 1.6 g of NaCl and solution vortexed for two minutes. A 5 ml aliquot of acetonitrile was added, solution vortexed for three minutes and centrifuged at 10,000 g for 30 minutes. Upper layer was extracted with 5 ml of acetonitrile twice. In the clean-up step, 250 mg of magnesium sulfate anhydrous, 300 mg of primary secondary amines (PSA) and 300 mg of C_{18} were added to 9 ml aliquot of extract, which was vortexed for one minute, centrifuged at 10,000 g for 10 minutes. Supernatant was evaporated to dryness under nitrogen gas, dissolved in one ml of toluene, filtered using a PTFE syringe filter (Science Integration, FilTrex, Singapore) and subjected to GC-MS.

GC-MS procedure

GC separation was performed in a gas chromatograph (GC6890N; Agilent Technologies, Santa Clara, CA) equipped with a temperature programming capability, a pulsed splitless 1.0 μl injector (kept at 250°C), a capillary column (30 m $\times$ 0.25 mm ID $\times$ 0.25 μm film thickness); helium as the carrier gas at a flow rate of 1.5 ml/minute. GC-MS operating conditions were as follows: initial temperature of column oven of 50°C held for 10 minutes; then temperature raised at $25^{\circ}\text{C}/\text{min}$ to 175°C , at $5^{\circ}\text{C}/\text{minute}$ to 225°C and at $20^{\circ}\text{C}/\text{minute}$ to 290°C and held for 5

minutes. MS separation was performed by mass spectrometry (MS5975, Agilent Technologies, Santa Clara, CA) with operating conditions as follows: source temperature 220°C, SIM mode, Electron Impact (EI), positive ionization. The total analysis time was 33 minutes.

Evaluation of method reliability

Detection limit. Standard OP mixture solutions, prepared by diluting stock standard mixture solution with acetone to 50-175 ng/ml, were added to 0.15 g of meconium sample and analyzed as described above. Lowest of the calibration concentration was used to define the detection limit of the method. The standard error of estimate for regression (SEE) and LOD were calculated using the equation: $LOD = 3 \times SEE / \text{slope}$ (Keith *et al*, 1983; Taylor, 1984).

Accuracy and precision. Mixed OP standard solutions were prepared at three concentrations (0.3-7.0 µg/ml) and added to 0.15 g of control meconium sample and analyzed as described above. Accuracy and precision are reported as percent

recovery and relative standard deviations (%RSD) of three replicate determinations.

Statistical analysis

Data were collected, verified and analyzed using SPSS statistical package (Version 24). The results are reported as median ± interquartile range (IQR), mean ± SD, number and percentages. Chi-square test was used to determine significance of categorical variables and Mann-Whitney U test for non-parametric continuous variables.

RESULTS

Validation of OP analysis method

Retention times of OPs ($n = 10$) varied from 14.6 to 28.5 minutes and detection limit from 0.02 to 0.48 µg/g (Table 1). Recovery of OPs varied from 96.36 to 102.20% with relative standard deviation (RSD) <4.55% (Table 2) at OP concentrations ranging from 0.38 to 8.97 µg/g. The optimized method presented good linearity with correlation coefficient (r^2) of 0.995-0.999. Detection limit of the 10

Table 1
Retention time, range of calibration, limit of detection (LOD) and regression coefficient of standard organophosphate pesticides in spiked meconium control samples analyzed by gas chromatograph-mass spectrometry.

	Retention time (min)	Range of calibration (µg/g)	LOD (µg/g)	Regression coefficient (r^2)
Chlorpyrifos	21.7	0.06-1.28	0.02	0.995
Demeton-s-methyl	17.5	0.13-2.56	0.05	0.997
Dichlorvos	14.6	0.32-6.41	0.20	0.997
Dimethoate	18.5	0.13-2.56	0.07	0.995
Ethion	26.0	0.13-2.56	0.04	0.999
Malathion	21.5	0.13-2.56	0.06	0.999
Omethoate	17.2	1.92-12.82	0.48	0.997
Pirimiphos-methyl	21.2	0.06-1.28	0.04	0.995
Pyrazophos	28.5	0.32-6.41	0.14	0.996
Tolclofos-methyl	20.6	0.06-1.28	0.03	0.996

Table 2
Percent recovery and relative standard deviation (RSD) of standard organophosphate (OP) pesticides in spiked meconium control samples analyzed by gas chromatography-mass spectrometry.

	OP concentration ($\mu\text{g/g}$)	Percent recovery	Percent RSD
Chlorpyrifos	0.38-0.90	97.16-99.87	0.46-3.38
Demeton-s-methyl	0.77-1.79	96.36-99.56	0.35-1.96
Dichlorvos	1.92-4.49	99.14-100.07	0.68-1.48
Dimethoate	0.77-1.79	98.49-99.64	0.78-2.89
Ethion	0.77-1.79	97.62-101.11	0.99-2.69
Malathion	0.77-1.79	98.51-102.20	1.41-4.55
Omethoate	3.85-8.97	98.59-100.39	0.49-1.66
Pirimiphos-methyl	0.38-0.90	96.40-99.78	0.34-2.29
Pyrazophos	1.92-4.49	97.52-99.93	0.63-2.44
Tolclofos-methyl	0.38-0.90	98.28-100.58	0.29-2.63

OPs in spiked meconium samples ranged from 25.1 to 481.8 ng/g and recovery of each OP analyzed from 96.36 to 102.20%. Between-day difference in detection precision of standard OPs (0.38-8.97 $\mu\text{g/g}$) ranged from 0.29 to 4.55%.

OPs in meconium samples

Mothers residing in the three provinces (Amnat Charoen, Kanchanaburi and Nakhon Sawan) had an average age of 26 years, average family monthly income of 11,291 THB (one THB is approximately USD 0.032) (69% <10,000 THB monthly income), junior high school or lower (63%) education, occupation in agricultural sector (47%), and residence near farmland (53%) (Table 3). Most families used pesticides within than outside their homes.

Meconium samples from newborn babies ($n = 68$) were analyzed for 10 OP pesticides. Eight OPs were detected by GC-MS (Fig 1) in meconium samples ranging from ethion in 12% to demeton-s-methyl in 73% of samples, with amounts

(median $\pm$ IQR) ranging from $0.08 \pm (0.03-0.16)$ $\mu\text{g/g}$ for chlorpyrifos to $5.63 \pm (4.85-8.57)$ $\mu\text{g/g}$ for omethoate (Table 4).

Amounts of total OP pesticides in meconium of babies with mothers working in agricultural and non-agricultural sector are not significantly different (Table 5); similar observations for each OP pesticide were obtained for meconium of babies with mothers living near (within 1 km) and distant from farmland (Table 3).

DISCUSSION

OP pesticides are widely used in Thailand and can be found in the environment, *ie* air, water, soil, and food sources (Ostrea *et al*, 2008). Exposure of pregnant women to pesticides remains a major concern because many pesticides are neurotoxicants (Barone *et al*, 2000; Ostrea *et al*, 2008). In humans, abnormal reflexes were found in infants whose mothers were exposed to OPs during pregnancy (Young *et al*, 2005). Meconium is accepted as the most sensitive matrix

Table 3
Demographics of mothers and relationship with their newborn meconium organophosphate (OP) pesticides concentration determined by gas chromatography-mass spectrometry.

Characteristic	Number (%) (n = 68)	Total OP pesticides ($\mu\text{g/g}$) Median (IQR)	p-value
Age (years)			
Mean (SD)	25 (4)		
Average monthly income (THB)			
≤10,000	40 (69)		
10,001-30,000	15 (26)		
30,001-50,000	3 (5)		
Mean (SD)	11,300 (9, 500)		
Education			
Junior high school or lower	41 (63)		
Senior high school or Vocational Certificate	17 (26)		
Diploma or Higher Vocational Certificate	7 (11)		
Occupation			0.147
Agricultural sector	32 (47)	16.00 (4.23-30.97)	
Nonagricultural sector	36 (53)	6.52 (3.20-24.38)	
Home			0.614
Near farmland farm (within 1 km)	36 (53)	6.78 (1.99-32.08)	
Not near farmland	32 (47)	9.89 (4.73-27.03)	
Insecticide used in home			0.685
Yes	40 (59)	11.47 (3.39-28.56)	
No	28 (41)	7.65 (3.71-27.43)	
Insecticide used outside home			0.769
Yes	28 (41)	15.02 (3.39-30.89)	
No	40 (59)	8.25 (3.87-27.98)	

IQR: interquartile range; US1 is approximately 31 THB.

to analyze fetal exposure to pesticides compared to cord blood and infant hair (Ostrea *et al*, 2008).

Ostrea *et al* (2002) analyzed OPs in meconium by HPLC-MS, while Bielawski *et al* (2005) employed GC-MS in which meconium is extracted with methanol, derivatized by heating

with methanolic/hydrochloric acid before analysis (Bielawski *et al*, 2005). In the present study, a simpler extraction protocol was employed without any derivatization prior to GC-MS, with clear discrimination of the ten test OP peaks, and acceptable dynamic range and inter-day precision. Recovery yields were

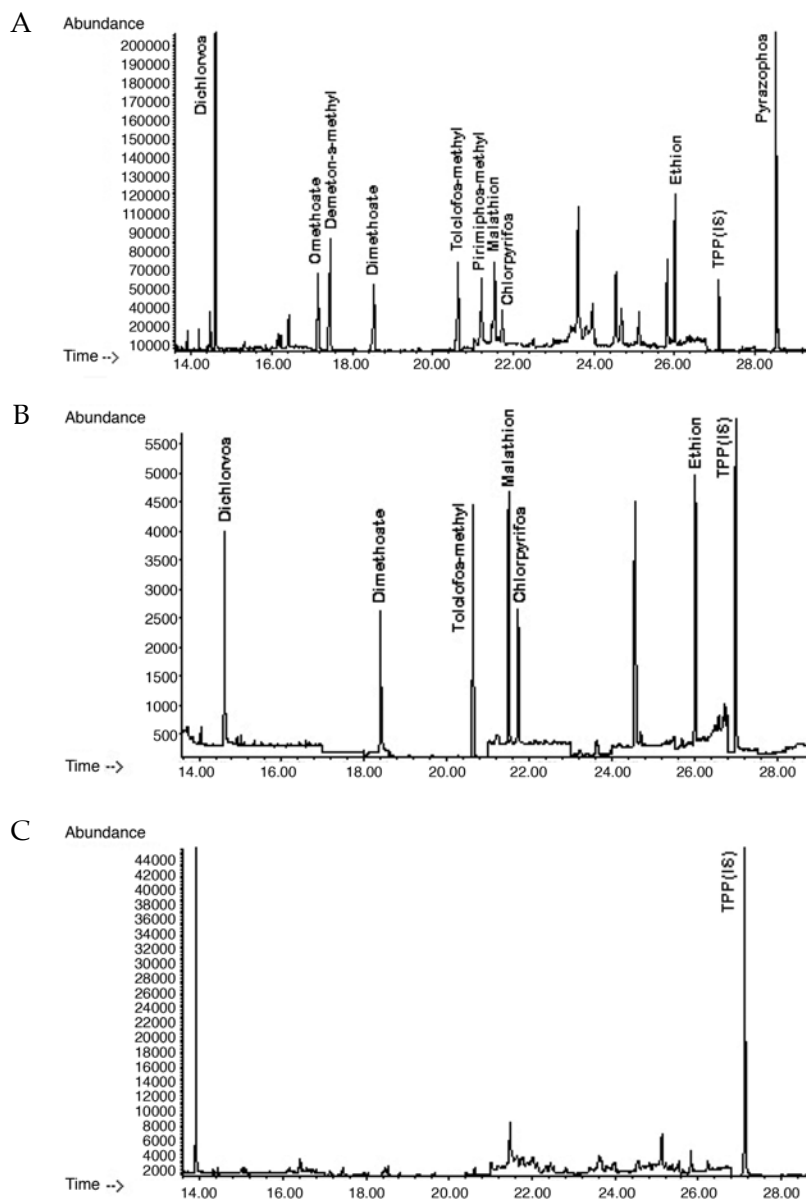


Fig 1-Gas chromatography separation of organophosphate pesticides. Organophosphate (OP) pesticides were extracted from meconium sample with acetonitrile and separated by gas chromatography (GC6890N; Agilent Technologies, Santa Clara, CA) using the following conditions: column oven of 50°C held for 10 minutes; then temperature raised at 25°C/min to 175°C, at 5°C/minute to 225°C and at 20°C/minute to 290°C and held for 5 minutes; total analysis time was 33 minutes. Peaks were subsequently identified by mass spectrometry (MS5975, Agilent Technologies, Santa Clara, CA). A: Typical gas chromatogram of control meconium sample spiked with a mixture of ten OP pesticides and triphenylphosphate (TPP) (internal standard); B: Typical gas chromatogram of OP pesticides extracted from test meconium sample; C: Typical gas chromatogram of control meconium sample.

Table 4
Organophosphate (OP) pesticides detected in meconium samples by
gas chromatography-mass spectrometry.

OP pesticide	OP pesticide concentration ($\mu\text{g/g}$)			
	Number of samples (%) ($n = 68$)	Median (IQR)	Min	Max
Chlorpyrifos	22 (32)	0.08 (0.03-0.16)	ND	0.36
Demeton-s-methyl	50 (73)	0.35 (0.26-0.49)	ND	0.83
Dichlorvos	26 (38)	0.67 (0.58-0.71)	ND	70.29
Dimethoate	34 (50)	0.43 (0.09-1.56)	ND	26.60
Ethion	8 (12)	0.21 (0.19-0.26)	ND	0.39
Malathion	34 (50)	0.28 (0.15-0.52)	ND	1.27
Omethoate	22 (32)	5.63 (4.85-8.57)	ND	10.18
Tolclofos-methyl	28 (41)	0.08 (0.03-0.10)	ND	0.21
Total	67 (98)	8.60 (3.65-28.46)	ND	320.43

IQR: interquartile range; Max: maximum; Min: minimum; ND: no detection

Table 5
Relationship of organophosphate (OP) pesticides in meconium samples
with mother occupation.

OP pesticide	Agricultural sector ($n = 32$)		Nonagricultural sector ($n = 36$)		p -value
	Number of samples (%)	OP concentration ($\mu\text{g/g}$) median (IQR)	Number of samples (%)	OP concentration ($\mu\text{g/g}$) median (IQR)	
Chlorpyrifos	8 (25)	0.06 (0.02-0.12)	14 (39)	0.11 (0.03-0.17)	0.328
Demeton-s-methyl	25 (78)	0.30 (0.26-0.44)	25 (69)	0.39 (0.32-0.60)	0.088
Dichlorvos	12 (37)	0.69 (0.60-0.86)	14 (39)	0.65 (0.59-0.70)	0.256
Dimethoate	14 (44)	1.03 (0.09-2.86)	20 (55)	0.36 (0.12-1.49)	0.078
Ethion	4 (12)	0.20 (0.19-0.34)	4 (11)	0.24 (0.20-0.26)	0.714
Malathion	15 (47)	0.27 (0.16-0.48)	19 (53)	0.29 (0.14-0.65)	0.675
Omethoate	12 (37)	5.61 (4.67-8.38)	10 (28)	5.4 (4.88-9.47)	0.722
Tolclofos-methyl	12 (37)	0.08 (0.04-0.09)	16 (44)	0.08 (0.03-0.10)	0.512
Total	31 (97)	16.0 (4.23-30.97)	36 (100)	6.93 (3.31-25.66)	0.147

IQR: interquartile range

higher than those reported by Bielawski *et al* (2005), probably as the latter protocols are optimized for detection of not only organophosphates but also pyrethroids and organochlorines.

The frequencies of chlorpyrifos and

malathion detected in meconium were similar to a study in Egypt (35.8 and 49.5% respectively) (El-Baz *et al*, 2015); while the two studies in the Philippines revealed the frequencies of chlorpyrifos and malathion as 0 and 1.2% (Bielawski *et al*, 2005), and 11

and 53% (Ostrea *et al*, 2008), respectively. The plausible explanation was that the studied babies were all borne from pregnant women in agricultural families or living in agricultural areas.

Interestingly, there is no significant difference in amounts of OP pesticides, both total and individual, from meconium of babies whose mothers lived near or distant from farmland. Ostrea *et al* (2009) reported the major source of pesticide exposure among pregnant women and their fetus, even mothers residing in an agricultural environment, was from home. The present study observed higher proportion of pesticide was in homes. Whyatt *et al* (2003) and Whyatt *et al* (2004) noted high exposure rates to home pesticides among pregnant women and their infants residing in urban areas of Columbia. Pesticide labels do not, in general, contain warnings that the product should not be used by pregnant woman or provide explicitly instructions on the most appropriate way of use and time duration before re-use (Ostrea *et al*, 2009). In addition, pregnant women could have acquired pesticides through eating fruits and vegetables, which often are contaminated with pesticide residues, as have been reported in Thailand (Wanwimolruk *et al*, 2015; Wanwimolruk *et al*, 2017). The Thai Pesticide Alert Network (Thai-PAN) tested 296 samples of fruits and vegetables in 2016 and reported 51.4% were contaminated with pesticide residues above the maximum residue limit (Ousap, 2016). This may help to explain, in part, the lack in difference of meconium OPs amounts between agricultural and nonagricultural households.

In summary, using a dedicated extraction protocol for organophosphate pesticides detection by gas

chromatography-mass spectrometry, eight types of organophosphate pesticides are shown to be present in meconium of babies whose mothers resided in three different agricultural regions of Thailand. Although the frequency of detection and amount in meconium varied among individual pesticides, there are no significant differences between mothers who did and did not work in the agricultural sector or who resided near or distant from farmland. It was noticeable the majority of families used pesticides within rather than outside their homes. Babies exposed to organophosphate should be followed up to determine possible toxigenic effects on their growth and mental development.

ACKNOWLEDGEMENTS

The authors thank the Center of Excellence on Environmental Health and Toxicology (EHT) for laboratory facilities. Funding for the project was provided by NIH Fogarty International Center, National Institutes of Environmental Health Sciences, the Center for Disease Control under Award Numbers U01 TW010091.

REFERENCES

- Barone S, Das KP, Lassiter TL, White LD. Vulnerable processes of nervous system development: a review of markers and methods. *Neurotoxicology* 2000; 21: 15-36.
- Barr DB, Needham LL. Analytical methods for biological monitoring of exposure to pesticides: a review. *J Chromatogr B Analyt Technol Biomed Life Sci* 2002; 778: 5-29.
- Bearer CF, Lee S, Salvator AE, Minnes S, Swick A, Yamashita T, Singer LT. Ethyl linoleate in meconium: a biomarker for prenatal ethanol exposure. *Alcohol Clin Exp Res* 1999; 23: 487-93.

- Bielawski D, Ostrea E Jr, Posecion N Jr, Corrion M, Seagraves J. Detection of several classes of pesticides and metabolites in meconium by gas chromatography-mass spectrometry. *Chromatographia* 2005; 62: 623-9.
- Bradman A, Eskenazi B, Barr DB, *et al.* Organophosphate urinary metabolite levels during pregnancy and after delivery in women living in an agricultural community. *Environ Health Perspect* 2005; 113: 1802-7.
- Chalupka S, Chalupka AN. The impact of environmental and occupational exposures on reproductive health. *J Obstet Gynecol Neonatal Nurs* 2010; 39: 84-102.
- El-Baz MAH, El-Deek SEM, Nsar AY, El-Maali NA, AbdelHafez FF, Amin AF. Prenatal pesticide exposure: meconium as a biomarker and impact on fetal weight. *J Environ Anal Toxicol* 2015; 5: 3.
- Keith LH, Crummett W, Deegan J, Libby RA, Taylor JK, Wentler G. Principles of environmental analysis. *Anal Chem* 1983; 55: 2210-8.
- Ministry of Public Health (MOH). Pesticide poisoning. Annual epidemiological surveillance report 2015 [cited 2018 Nov 21]. Available from: URL: <http://www.boe.moph.go.th/Annual/AESR2015/aesr2558/Part%201/09/pesticide.pdf> [in Thai]
- Moore C, Negrusz A, Lewis D. Determination of drugs of abuse in meconium. *J Chromatogr B: Biomed Sci Appl* 1998; 713: 137-46.
- Naksen W, Prapamontol T, Mangklabruks A, *et al.* Associations of maternal organophosphate pesticide exposure and PON1 activity with birth outcomes in SAWASDEE birth cohort, Thailand. *Environ Res* 2015; 142: 288-96.
- National Statistical Office (NSO), Ministry of Information and Communication Technology. Survey of Thai population employment 2013 [cited 2018 Nov 21]. Available from: URL: <http://service.nso.go.th/nso/nsopublish/themes/files/lfs56/reportJan.pdf> [in Thai]
- Office of Agricultural Economics (OAE). Quantity and value of pesticide import during 2014 – 2017 [Cited 2018 Nov 21]. Available from: URL: <http://www.oae.go.th/view/1/ปัจจัยการผลิต/TH-TH> [in Thai]
- Ortega García JA, Carrizo Gallardo D, Ferris i Tortajada J, García MM, Grimalt JO. Meconium and neurotoxicants: searching for a prenatal exposure timing. *Arch Dis Child* 2006; 91: 642-6.
- Ostrea EM, Bielawski DM, Posecion NC, *et al.* A comparison of infant hair, cord blood and meconium analysis to detect fetal exposure to environmental pesticides. *Environ Res* 2008; 106: 277-83.
- Ostrea EM, Bielawski DM, Posecion NC, *et al.* Combined analysis of prenatal (maternal hair and blood) and neonatal (infant hair, cord blood and meconium) matrices to detect fetal exposure to environmental pesticides. *Environ Res* 2009; 109: 116-22.
- Ostrea EM, Morales V, Ngoumgna E, *et al.* Prevalence of fetal exposure to environmental toxin as determined by meconium analysis. *Neurotoxicology* 2002; 23: 329-39.
- Ousap P. Situation of pesticide import, use and residue in food products. Annual Thai Pesticide Alert Network Conference; 2016 Oct 7 [cited 2018 Jan 20]; Chulabhorn Research Center, Bangkok. Available from: URL: http://www.thaipan.org/sites/default/files/conference2559/pesticide_conference_2559_3.6.pdf
- Rauch SA, Braun JM, Barr DB *et al.* Associations of prenatal exposure to organophosphate pesticide metabolites with gestational age and birth weight. *Environ Health Perspect* 2012; 120: 1055-60.
- Taylor JK. Quality assurance of chemical measurements. Gaithersburg, MD: Center for Analytical Chemistry, National Bureau of Standards; 1984.
- Wanwimolruk S, Kanchanamayoon O, Phopin

- K, Prachayasittikul V. Food safety in Thailand 2: Pesticide residues found in Chinese kale (*Brassica oleracea*), a commonly consumed vegetable in Asian countries. *Sci Total Environ* 2015; 532: 447-55.
- Wanwimolruk S, Duangsuwan W, Phopin K, Boonpangrak S. Food safety in Thailand 5: The effect of washing pesticide residues found in cabbages and tomatoes. *J Consum Prot Food Saf* 2017; 12: 209-21.
- Whyatt RM, Barr DB. Measurement of organophosphate metabolites in postpartum meconium as a potential biomarker of prenatal exposure: a validation study. *Environ Health Perspect* 2001; 109(4): 417-20.
- Whyatt RM, Barr DB, Camann DE, *et al.* Contemporary-use pesticides in personal air samples during pregnancy and blood samples at delivery among urban minority mothers and newborns. *Environ Health Perspect* 2003; 111: 749-56.
- Whyatt RM, Rauh V, Barr DB, *et al.* Prenatal insecticide exposures and birth weight and length among an urban minority cohort. *Environ Health Perspect* 2004; 112: 1125-32.
- Young JG, Eskenazi B, Gladstone EA, *et al.* Association between *in utero* organophosphate pesticide exposure and abnormal reflexes in neonates. *Neurotoxicology* 2005; 26: 199-209.