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Organophosphate Urinary Metabolite Levels during Pregnancy, Delivery and Postpartum in Women Living in Agricultural Areas in Thailand

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Abstract: Organophosphate Urinary Metabolite Levels during Pregnancy, Delivery and Postpartum in Women Living in Agricultural Areas in Thailand: Pornpimol KONGTIP, *et al*. Department of Occupational Health and Safety, Faculty of Public Health, Mahidol University, Thailand—Objective: Prenatal exposure to organophosphate pesticides can lead to developmental neurotoxicity. A longitudinal birth cohort was established to investigate pesticide exposures from different agricultural activities. Maternal urinary organophosphate metabolites were measured at 28 weeks of pregnancy (n=86), delivery (n=67) and 2 months postpartum (n=51). **Method:** Subjects were interviewed with questionnaires about work, home and behavioral factors potentially associated with pesticide exposures, and spot urine samples were also collected. The urine samples were analyzed for dimethyl phosphate (DMP), diethyl phosphate (DEP), diethyl thiophosphate (DETP) and diethyl dithiophosphate (DEDTP), using gas chromatography-mass spectrometry. **Results:** The urinary DMP and dialkyl phosphate (DAP) concentrations at 28 weeks of pregnancy and delivery were not significantly different, but the DMP and DAP concentrations at 28 weeks of pregnancy and DAP concentrations at delivery were significantly different (p<0.05) from those at 2 months postpartum. The factors influencing the urinary DAP concentrations at 28 weeks of pregnancy included insecticide used in the home, living close to agricultural farmland, frequency of agricultural field visits during the first and second trimesters of pregnancies, occupation of subjects, pesticide used and other agricultural activities. **Conclusions:** The urinary organophosphate metabolites, DMP, DEP, DETP, DEDTP, total DEP and DAPs, at 28 weeks of pregnancy, delivery and 2 months postpartum fluctuated depending on their pesticide exposures both at home and in agricultural fields.
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Key words: Agricultural activities, Organophosphate metabolites, Pesticides, Pregnant women

In Thailand, approximately 40% of the land is used for agriculture, and 38.7% of the total national workforce is employed in the agricultural sector[1]. The Ministry of Agricultural and Cooperatives reported that Thailand imported approximately 39,634 tons of pesticides in 1997 and that the volume increased to 117,815 tons in 2010[2]. A survey of intelligence level in Thai children found that the average IQ fell steadily over the years 1997 to 2009 from 91 to 88, which was lower than the WHO standard range of 90–110[3]. The results of the age-appropriate development survey in children under 5 years old conducted by the Department of Health concluded that development of Thai children decreased from 72% in 2004 to 67% in 2007[3]. The survey results suggest that poor environment, education and food contributed to the decreasing IQs.

One large birth cohort of primarily Latina women in an agricultural community in the United States was used to examine the association between organophosphate pesticide exposures during pregnancy and the effects on the neurodevelopment of infants and children. The pregnant woman may have been exposed to occupational pesticides or common household pesticides; however, significant impacts on child neurodevelopment were found[4–6]. As part of that study,

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Bradman *et al*. presented the urinary organophosphate metabolite levels of pregnant women in different periods (13 weeks of gestation, 26 weeks of gestation and 9 days after delivery)[7]. The level of organophosphate metabolites was increased during pregnancy and higher in the postpartum period.

Due to the neurotoxic vulnerability of the developing fetus, exposure of pregnant women to pesticides is of great concern[8]. Previous studies have shown that in Thailand, there are many cases of improper management of pesticides, inappropriate or nonexistent use of personal protective equipment, use of higher pesticide concentrations than specified on the product label and high volumes of pesticide use on farms[9, 10]. The aims of this study were to investigate the levels of exposure during pregnancy and postpartum and to identify the factors that increase the risk of higher levels of exposure among our cohort.

Subjects and Methods

Recruitment of pregnant women

Pregnant women who came for prenatal care at the three hospitals, Amnatchareon Hospital in Amnatchareon Province in the north east, Sawanpracharak Hospital in Nakhonsawan Province in the lower north and Paholpolpayuhasena Hospital in Kanchanaburi Province in the west of Thailand, were recruited during May 2011 to January 2012. To be recruited, the woman had to be in their 7th month of pregnancy, had to be 20–35 years of age, had to not have diabetes or hypertension and had to be planning to give birth and have follow-up infant care at the recruiting hospital. This study was approved by the Committee on Human Rights Related to Human Experimentation, Faculty of Public Health, Mahidol University, and the University of Massachusetts Lowell Institutional Review Board.

Data collection

At the hospital, the women were interviewed by nurses regarding general health, diet, work exposures including agricultural work and use of pesticides at home and work at 28th weeks of pregnancy and 2 months postpartum. Spot urine samples were collected in polyethylene bottles at the 28th week of pregnancy, delivery and 2 months postpartum. The urine specimens were stored at −45°C until analysis.

Analysis of urine samples

The analysis of urinary dialkyl phosphates (DAPs) was modified from Alwis *et al*.[11]. Four DAPs were determined: including dimethyl phosphate (DMP) and three diethyl phosphates, diethyl phosphate (DEP), diethyl thiophosphate (DETP), diethyl dithiophosphate (DEDTP).

The urinary DAP analysis method

Two milliliters of urine was placed in a 10-m*l* screw-cap tube and spiked with 25 μl of the internal standard (dibutyl phosphate, 5 mg/*l*), acidified with 3 M hydrochloric acid (50 μl) and mixed. SPE cartridges (Bond Elut PPL 500 mg/3 m*l*, Varian, Palo Alto, CA, USA) were placed on Visiprep™ DL Vacuum Manifolds (Supelco, Bellefonte, PA, USA). SPE cartridges were conditioned with acetonitrile (4 m*l*) followed by 0.1 mol/*l* HCl (4 m*l*). The urine sample was loaded onto the SPE cartridge at a flow rate of 0.25 m*l*/min. The cartridge was dried using nitrogen gas (~30 psi) for 10 minutes, washed with 0.1 mol/*l* HCl (1 m*l*), and then dried again for 5 minutes. Elution was accomplished with acetonitrile (6 m*l*) at a flow rate of 0.5 m*l*/min into a 10-m*l* screw-cap tube containing potassium carbonate (~25 mg). The eluate was evaporated to dryness in a Reacti-Therm™ III Heating Module (Thermo Fisher Scientific Inc., Waltham, MA, USA) with high purity nitrogen for approximately 2 hours. The dried residue was resuspended in 2 m*l* of acetonitrile. Potassium carbonate (~25 mg) and 2, 3, 4, 5, 6-Pentafluorobenzyl bromide (30 μl) were added to the tube, which was then placed in an oven at 60°C for 4 hours. After heating, the sample tubes were allowed to cool to room temperature. The top layer was carefully transferred using Pasteur pipettes to 10-m*l* centrifuge tubes without disturbing the sediment at the bottom of the tubes. The solutions were evaporated to dryness in a Reacti-Therm™ III Heating Module (Thermo Fisher Scientific Inc., Waltham, MA, USA) with nitrogen for 30 minutes. The residue was dissolved with 150 μl of toluene and transferred to an auto-sampler vial for analysis by gas chromatography-mass spectrometry (GC-MS) (Agilent Technologies, Santa Clara, CA, USA). An aliquot (1 μl) of the sample was injected in the splitless mode onto a DB-5MS ([5%-phenyl]-methylpolysiloxane) capillary GC column (30 m, 0.25-mm i.d., 0.25 μm) using the autosampler. The carrier gas used was helium. The injection port and transfer line were set at 250°C and 280°C, respectively. Constant flow mode was used with a flow rate of 1.2 m*l*/min. The temperature of the GC oven was initially set at 90°C for 1 minute, increased at 4°C/min to 150°C and then increased at 50°C/min to 270°C. The final temperature of 270°C was held for 5 minutes. The MS was operated in electron impact ionization mode at 70 eV, and ions were monitored in the selected ion monitoring mode.

The calibration curves of four dialkyl phosphate (DAP) metabolites were prepared at concentrations of 1, 5, 10, 25, 50, 75 and 100 ng/m*l*. The ranges of urinary DMP, DEP, DETP and DEDTP in field samples were not detectable (ND)-90.9, ND-64.1,

ND-96.7 and ND-95.0 ng/ml, respectively. The average recoveries of the DAP analysis method ranged from 93.64 to 99.92% at DAP concentrations of 10 and 75 ng/m*l*. The between-day assay coefficients of variation were in the range of 0.59 to 6.45%. The quality control urine samples containing the four DAP metabolites (10 and 75 ng/m*l*) were analyzed together with urine samples. The detection limits of the four DAPs were analyzed according to the methods of the National Institute for Occupational Safety and Health (NIOSH)[12]. The detection limits of DMP, DEP, DETP and DEDTP in urine were 5.00, 0.034, 0.028 and 0.054 ng/m*l*. Concentrations were reported in nmol/*l*, and DEP, DETP and DEDTP were summed as total DEP concentration. Adding DMP to the total DEP resulted in four DAP concentrations. Values below the limit of detection were substituted with zero; the detection limits of DEP, DETP and DEDTP were almost zero[13].

All urine samples were analyzed for creatinine by automated clinical chemistry analyzers (Hitachi 917 analyzer SN.0967-09, Japan). The creatinine-adjusted DAP concentration was calculated as nmole/g creatinine. The comparisons of urinary organophosphate metabolite levels used the creatinine-adjusted organophosphate metabolite concentrations due to different quantities of urine excreted in spot urine samples.

Classification of the agricultural worker group

The pregnant women were classified in to three groups: (G1) higher risk agricultural workers referred to pregnant women who visited the farmland on 4–7 days in the first trimester and at least 2–3 days in the second trimester; (G2) moderate/lower risk agricultural workers referred to pregnant women who visited farmland less or had family members in the same house working in a field; and (G3) no agricultural work referred to pregnant women who did not perform work related to an agricultural field.

Data analysis

The descriptive statistics were calculated using SPSS (version18; PASW Statistics Base 18, Serial no. 5082368, ID no. 5071846) from SPSS (Thailand) Co., Ltd., Thailand. Since exposures were highly skewed, concentrations were reported as the median, range, and interquartile range (IQR). For comparisons, nonparametric analyses were used including the Mann Whitney U test, Kruskal-Wallis test and all pairwise comparisons (Dwass-Steel-Chritchlow-Fligner).

Results

Demographic characteristics

Urine samples were collected from 86 women at 28 weeks of pregnancy, 67 at delivery and 51 at 2 months postpartum. The eighty-six 28 weeks pregnant women were 26 years old on average (SD 4.2). Eighty-six percent had some education at the secondary school or high school level. Their occupations included agriculturist (27%), housewife (20%) and self-employed (13%). Agriculturists grew rice, corn, vegetables, flowers, etc. In their homes, 62.8% of family members of pregnant women worked in agricultural fields. The planting season differed depending on the types of plants. There was at least one growing season at the farm; the growing seasons differ depending on the types of plants. Only one pregnant women smoked cigarettes; she smoked two cigarettes/week. Five pregnant women reported drinking beer; one drank 2–4 times/week, and four drank less than once a month.

Agricultural activities of women at 28 weeks of pregnancy

The pesticide exposures information collected by the questionnaires at 28 weeks of pregnancy and 2 months postpartum is shown in Table 1. During pregnancy, 62% of pregnant women reported using insecticide in their homes. Of those using insecticides, 72% applied the insecticide in their homes, and the most common brands reported were Bigon (55%), Shieldtox (9%), Aswin (7%), Art and Shell (5%) and 16% were other brands. Forty-five percent of the women reported living near farmlands that were sprayed with pesticides.

The pregnant women were classified by the amount of time they reported working in agricultural fields during pregnancy. Group 1 (22%) was presumed to be at higher risk. Group 2 (42%) was presumed to be at moderate to low risk. Group 3 (36%) had no agricultural work performed by the women or family members.

Pregnant women performed several agricultural activities; they grew plants (23.3%), applied chemical fertilizer or manure or compost (19.2%), applied pesticide (10.5%), eliminated weeds (9.0%) and hand-picked crops, or plants or flowers (24.4%).

Agricultural activities of women at 2 months postpartum

At 2 months postpartum, 55% of the women reported using and applying insecticides in their homes. Fifty-one percent reported living near agricultural areas sprayed with pesticides, and 22% worked outside their home. In Thailand, mothers who deliver infants can take a leave from work for 45 days to take care of their infants at home. The postpartum mothers reported fewer agricultural activities: 2% grew plants, 8% applied chemical fertilizer, 2% eliminated weeds, and 2% hand-picked crops, plants or flowers.

Table 1. Agricultural activities of women at 28 weeks of pregnancy and 2 months postpartum

Activities of pregnant women	28 weeks of pregnancy (n=86)	2 months postpartum (n=51)
	Number (%)	Number (%)
Application of insecticide in your home		
Yes	53 (61.6)	28 (54.9)
No	33 (38.4)	22 (43.1)
You applied insecticide in your home		
Yes	38 (44.2)	28 (54.9)
Never	48 (55.8)	22 (43.1)
You live next to farmland where pesticides are sprayed		
Not near farmland	43 (50.0)	24 (47.1)
Near farmland, don't know if pesticides are sprayed	4 (4.7)	1 (2.0)
Near farmland where pesticide are sprayed	39 (45.3)	26 (51.0)
How often you visit agricultural fields		
Group 1 Agricultural work (Higher risk)	19 (22.1)	13 (21.7)
Group 2 Agricultural work (Moderate/low risk)	36 (41.9)	24 (24.5)
Group 3 No Agricultural work	31 (36.1)	14 (33.2)
Working outside your home		
Yes	41 (47.7)	11 (21.6)
No	44 (51.2)	39 (76.5)
Your occupation		
Agriculturist	23 (27.1)	9 (17.6)
Other occupation	62 (72.9)	41 (80.4)
You do farm work related to growing plants		
Never	58 (67.4)	49 (96.1)
Yes	20 (23.3)	1 (2.0)
You apply chemical fertilizer, manure or compost		
Never	63 (80.8)	46 (90.2)
Yes	15 (19.2)	4 (7.8)
Applied pesticides		
Never	69 (80.2)	50 (98.0)
Yes	9 (10.5)	None
You apply herbicide to control weeds		
Never	71 (91.0)	48 (94.1)
Yes	7 (9.0)	1 (2.0)
Hand-pick crops, plants or flowers		
Never	57 (66.3)	49 (96.0)
Yes	21 (24.4)	1 (2.0)

Urinary DAP metabolites

The unadjusted and creatinine-adjusted urinary DAP metabolites at the 28th week of pregnancy, delivery and 2 months postpartum show variability in exposures over time by metabolite (Table 2). For the creatinine-adjusted DAP, the creatinine levels in urine were very low in a few subjects during the delivery period, but the creatinine levels went back to the normal range at 2 months postpartum. The detection frequency of DMP decreased slightly from 78% at 28 weeks of pregnancy to 72% at delivery and then to 67% at 2 months postpartum. The DEP metabolites were detected in 67, 72 and 63% of the urine samples at 28 weeks of pregnancy, delivery and 2 months postpartum, respectively.

Significant differences were found for the median creatinine-adjusted DMP at 28 weeks of pregnancy (48.9 nmole/g) vs. 2 months postpartum (25.6 nmole/g;

Table 2. Unadjusted and creatinine-adjusted urinary dialkyl phosphate (DAP) metabolite levels at three time points: 28 weeks of pregnancy, delivery and 2 months postpartum

	Detection frequency (%)	Unadjusted DAP (nmole/*l*)		Creatinine-adjusted DAP (nmole/g creatinine)	
		50th percentile	Range	50th percentile	Range
28 weeks of pregnancy (n=86)					
DMP	77.9	37.7	ND–121.5	48.9	ND–450.9
DEP	67.4	14.6	ND–72.0	20.8	ND–325.7
DETP	47.7	ND	ND–464.4	ND	ND–956.3
DEDTP	44.2	ND	ND–476.8	ND	ND–1199.5
Total DEP	89.5	45.2	ND–613.2	84.1	ND–1332.9
DAPs	95.4	90.1	ND–688.8	160.9	ND–1783.7
At delivery (n=67)					
DMP	71.6	36.4	ND–166.0	50.5	ND–829.8
DEP	71.6	14.4	ND–102.8	18.8	ND–376.0
DETP	52.2	2.4	ND–100.5	6.5	ND–755.8
DEDTP	53.7	2.3	ND–505.4	4.5	ND–2066.2
Total DEP	94.0	64.9	8.1–569.8	87.4	ND–2066.2
DAPs	100.0	108.9	11.0–597.2	190.8	ND–2442.8
2-month postpartum (n=51)					
DMP	66.7	38.0	ND–720.3	25.6	ND–637.3
DEP	62.8	17.3	ND–416.2	14.8	ND–743.3
DETP	41.2	ND	ND–301.3	ND	ND–240.8
DEDTP	49.0	ND	ND–510.0	ND	ND–1399.2
Total DEP	80.4	46.7	ND–717.4	56.1	ND–1546.4
DAPs	90.2	92.1	ND–805.1	101.8	ND–1546.4

DMPs: dimethyl phosphate. DEP: diethyl phosphate. DETP: diethyl thiophosphate. DEDTP: diethyl dithiophosphate, ND: not detectable.

at p=0.011) and the median creatinine-adjusted total DEP at delivery (87.4 nmole/g) vs. 2 months postpartum (56.1 nmole/g; at p=0.027). There were also a significant differences for the median creatinine-adjusted DAPs at 28 weeks of pregnancy (160.9 nmole/g) vs. 2 months postpartum (101.8 nmole/g) and delivery (190.8 nmole/g) vs. 2 months postpartum (101.8 nmole/g; p=0.047 and 0.013, respectively).

Pesticide exposure at their homes

The urinary DMP and DAP concentrations of pregnant women who reported that they lived near agricultural areas sprayed with pesticides were significantly higher urine concentrations than those that did not in analyses with the Kruskal-Wallis test and all pairwise comparisons (Dwass-Steel-Chritchlow-Fligner; p=0.011 and 0.021, respectively; Table 3).

Agricultural work risk groups

Regarding the agricultural work groups in the women at 28 weeks of pregnancy, Group 1 (higher risk agricultural workers) had a higher total DEP concentration in their urine compared with Group 2 (moderate/low risk agricultural workers, p=0.043) and Group 3 (no agricultural work, p=0.021) in analyses with the Kruskal-Wallis test and all pairwise comparisons (Dwass-Steel-Chritchlow-Fligner) (Table 4). Women in Group 1 (higher risk agricultural workers) also had concentrations of DAPs higher in their urine compared with Group 2 (moderate/low risk agricultural workers, p=0.033) and Group 3 (no agricultural work, p=0.019) (Table 4).

Pesticide exposure from agricultural work activities in the 28th week of pregnancy

Those who worked outside their homes had significantly higher urinary total concentrations of DEP and DAPs than those worked at home (Mann-Whitney U test p=0.030 and 0.015, respectively) (Table 5). Pregnant women who reported their occupation as agriculturists had significantly higher urinary DEDTP concentrations than those working in other occupations (Mann-Whitney U test p=0.047). Pregnant women who grew plants, applied chemical fertilizer,

Table 3. Creatinine-adjusted urinary organophosphate metabolites (nmole/g) of women in the 28th week of pregnancy categorized by potential for home exposure to pesticides from nearby farmland

Parameter		DMP	DEP	DETP	DEDTP	Total DEP	DAPs
Do you live next to farmland?							
C1. Not near farmland (n=43)	Median (IQR)[a]	37.6 (ND[b]–78.1)	16.3 (ND–38.9)	ND (ND–38.9)	ND (ND–22.7)	70.6 (16.3–148.5)	108.7 (49.1–235.4)
C2. Near farmland but don't know if pesticides are sprayed (n=4)	Median (IQR)	88.5 (55.8–126.9)	39.5 (5.2–67.2)	54.7 (ND–139.1)	ND (ND–5.7)	113.4 (33.1–164.8)	201.9 (88.9–291.7)
C3. Near farmland where pesti cides are sprayed (n=39)	Median (IQR)	67.0 (40.3–124.7)	30.9 (9.6–60.3)	15.0 (ND–70.2)	1.5 (ND–34.4)	94.3 (47.2–243.5)	202.1 (94.9–317.8)
p-value for Kruskal-Wallis test and all pairwise comparisons (Dwass-Steel-Chritchlow-Fligner)		0.006* C1 vs. C2=0.128 C1 vs. C3=0.011* C2 vs. C3=0.680	0.106	0.629	0.352	0.148	0.026* C1 vs. C2=0.631 C1 vs. C3=0.021* C2 vs. C3=0.890

[a] IQR: interquartile range. [b] ND: not detectable. DMP: dimethyl phosphate. DEP: diethyl phosphate. DETP: diethyl thiophosphate. DEDTP: diethyl dithiophosphate, DAP: dialkyl phosphate. *significant at $p<0.05$.

Table 4. Creatinine-adjusted urinary organophosphate metabolites (DMP, DEP, DETP, DEDTP, Total DEP and DAPs in nmole/g) of 28 weeks of pregnant women from agricultural field visits during the first and second trimester of pregnancy

Parameter		DMP	DEP	DETP	DEDTP	Total DEP	DAPs
How often you visit the farmland?							
Group 1 (G1) Higher risk agricultural worker (n=19)	Median (IQR)[a]	59.3 (36.0–125.9)	21.7 (13.5–63.1)	21.4 (0–138.6)	ND[b] (ND–128.5)	201.7 (60.6–268.1)	292.6 (91.3–346.2)
Group 2 (G2) Moderate/ lower risk agricultural workers (n=36)	Median (IQR)	46.2 (ND–98.4)	20.0 (ND–44.8)	ND (ND–67.7)	0.8 (ND–22.7)	87.6 (27.0–167.4)	162.4 (82.6–273.0)
Group 3 (G3) No agricultural work (n=31)	Median (IQR)	48.7 (22.7–98.4)	20.8 (ND–41.5)	ND (0–34.9)	ND (ND–22.7)	65.6 (17.1–120.0)	126.3 (65.2–198.4)
p-value for Kruskal-Wallis test and all pairwise comparisons (Dwass-Steel-Chritchlow-Fligner)		0.527	0.245	0.240	0.596	0.027* G1 vs. G2=0.043* G1 vs. G3=0.021* G2 vs. G3=0.968	0.021* G1 vs. G2=0.033* G1 vs. G3=0.019* G2 vs. G3=0.990

[a] IQR: interquartile range. [b] ND: not detectable. DMP: dimethyl phosphate. DEP: diethyl phosphate. DETP: diethyl thiophosphate. DEDTP: diethyl dithiophosphate, DAP: dialkyl phosphate. *significant at $p<0.05$.

applied pesticide, eliminated weeds and picked crops/ plants/flowers by hand had significantly higher urinary pesticide metabolites (DEDTP, total DEP or DAPs) than those who did not ($p<0.05$) (Table 5).

Pesticide exposure from agricultural work activities at 2 months postpartum

The urinary DETP metabolite concentrations of the women who reported their occupation as agriculturist were significantly higher than those who reported other occupations (Mann-Whitney U test $p=0.013$) (Table 6). Women who reported agricultural activities at 2 months postpartum had significantly higher DAP concentrations than those that did not (Mann-Whitney U test $p=0.027$).

Discussion

In our cohort, 27% of the women reported their occupation as agriculturist, and more than half of them had family members in the same house who worked in agriculture. These women may help with some of the agricultural activities both at home and in the farm fields. Some of them grew plants for family use and to sell. Some of these women may be exposed to pesticides at home, since they reported using insecticides at their homes and some lived near agricultural fields where pesticides were sprayed.

Regarding creatinine-unadjusted urinary metabolites, the organophosphate metabolites DMP, DEP, DETP, DEDTP, and total DEP and DAPs were not significantly different when comparing women at 28 weeks of pregnancy, delivery and 2 months postpartum. Although the women reduced their agricultural activities after giving birth, the unadjusted-urinary metabolites tended to slightly increase at 2 months postpartum. Bradman *et al*. also found an upward shift of

Table 5. Creatinine-adjusted urinary organophosphate metabolites (nmole/g) from agricultural activities among women in the 28th week of pregnancy

Parameter		DMP	DEP	DETP	DEDTP	Total DEP	DAPs
Do you work outside your home?							
No (n=44)	Median	49.6	20.4	ND	ND	61.7	105.7
	(IQR)[a]	(ND[b]–96.9)	(ND–50.5)	(ND–49.0)	(ND–15.1)	(16.1–150.8)	(54.4–243.1)
Yes (n=41)	Median	48.3	20.8	21.4	2.5	117.5	195.3
	(IQR)	(33.9–104.4)	(ND–46.0)	(ND–75.8)	(ND–42.6)	(51.5–234.1)	(105.1–323.2)
p-value		0.519	0.957	0.135	0.068	0.030*	0.015*
What is your occupation?							
Agriculturist	Median	50.5	23.4	ND	15.2	113.9	281.2
(n=22)	(IQR)	(26.4–129.8)	(9.8–60.7)	(ND–64.0)	(0–147)	(31.9–266.1)	(80.5–351.5)
Other occupation	Median	49.6	20.8	ND	ND	70.4	148.0
(n=62)	(IQR)	(17.1–97.8)	(ND–44.2)	(ND–57.9)	(ND–15.0)	(20.6–156.6)	(68.9–246.7)
p-value		0.465	0.287	0.956	0.047*	0.196	0.100
During your pregnancy, did you do any of the following agricultural work ···· grow plants?							
Never (n=58)	Median	49.6	20.9	ND	ND	75.8	150.0
	(IQR)	(23.7–98.5)	(ND–44.2)	(ND–37.8)	(ND–16.7)	(24.1–156.6)	(75.2–242.6)
Yes (n=20)	Median	56.2	20.8	10.7	18.7	183.5	281.2
	(IQR)	(37.0–136.6)	(2.9–62.8)	(0–120.6)	(0–144.7)	(50.9–270.4)	(119.0–337.1)
p-value		0.275	0.519	0.360	0.095	0.078	0.025*
During your pregnancy, did you do any of the following agricultural work ···· apply chemical fertilizer, manure or compost?							
Never (n=63)	Median	50.2	20.9	ND	ND	81.1	156.7
	(IQR)	(22.7–98.7)	(ND–46.0)	(ND–43.7)	(ND–16.6)	(21.7–156.7)	(75.1–243.9)
Yes (n=15)	Median	59.4	16.6	16.5	24.5	201.7	292.6
	(IQR)	(44.1–135.1)	(ND–51.6)	(ND–138.6)	(0–202.5)	(57.8–272.0)	(95.6–367.4)
p-value		0.184	0.862	0.320	0.043*	0.033*	0.010*
During your pregnancy, did you do any of the following agricultural work ···· apply pesticides?							
Never (n=69)	Median	50.2	20.9	ND	ND	87.0	159.1
	(IQR)	(25.6–98.6)	(ND–45.5)	(ND–49.4)	(ND–16.8)	(23.3–166.8)	(9.3–164.4)
Yes (n=9)	Median	59.4	39.4	21.4	30.6	201.7	311.5
	(IQR)	(44.9–218.5)	(6.76–57.3)	(ND–389.1)	(12.6–126.7)	(61.3–467.2)	(183.9–544.6)
p-value		0.142	0.460	0.306	0.009*	0.062	0.023*
During your pregnancy, did you do any of the following agricultural work ···· eliminate weeds?							
Never (n=71)	Median	50.2	20.9	ND	ND	81.1	159.1
	(IQR)	(27.2–101.5)	(ND–46.0)	(ND–43.7)	(ND–22.7)	(24.87–165.3)	(83.4–278.9)
Yes (n=7)	Median	59.4	39.4	70.2	24.5	206.7	285.9
	(IQR)	(47.0–109.9)	(ND–63.1)	(ND–138.6)	(ND–202.5)	(140.7–374.5)	(273.6–421.5)
p-value		0.511	0.677	0.245	0.101	0.034*	0.038*
During your pregnancy, did you do any of the following agricultural work ···· hand-pick crops/plants/flowers?							
Never (n=57)	Median	48.7	20.9	ND	ND	87.0	156.7
	(IQR)	(25.0–98.6)	(ND–44.5)	(ND–49.4)	(ND–20.0)	(23.3–156.6)	(78.7–243.0)
Yes (n=21)	Median	59.4	17.9	20.6	2.5	165.3	291.1
	(IQR)	(41.0–130.7)	(6.5–56.8)	(ND–136.1)	(ND–89.7)	(52.5–281.4)	(88.4–373.6)
p-value		0.188	0.457	0.225	0.297	0.082	0.019*

[a] IQR: interquartile range. [b] ND: not detectable. DMP: dimethyl phosphate. DEP: diethyl phosphate. DETP: diethyl thiophosphate. DEDTP: diethyl dithiophosphate. Total DEP=DEP + DETP + DEDTP. DAPs=DMP + Total DEP. *significant at $p<0.05$.

DAP metabolites after delivery (9 days postpartum) compared with prenatal levels. The urinary total DAP levels in the prenatal period in the current study were similar to those in the study of Bradman *et al.*, but the urinary DAP levels at 2 months postpartum were considerably lower than those in the study of Bradman *et al.* at 9 days postpartum[7]. The urinary DMP levels in the current study were much higher than those in the study of Bradman *et al.*[7]. This may be because Thai people apply more insecticides to prevent mosquitoes and insects at home. The insecticides used in the home (such as ARS, Shell, Bigon

Table 6. Creatinine-adjusted urinary organophosphate metabolites (nmole/g) from agricultural activities among women at 2 months postpartum

Parameter		DMP	DEP	DETP	DEDTP	Total DEP	DAPs
Since your baby was born, what is your occupation?							
Agriculturist (n=9)	Median	27.9	4.4	24.8	11.1	64.7	195.3
	(IQR)[a]	(ND[b]–91.9)	(0–22.6)	(5.1–62.5)	(ND–180.7)	(27.0–247.9)	(76.4–293.2)
Other occupation (n=41)	Median	25.6	15.1	ND	ND	31.7	84.8
	(IQR)	(ND–60.8)	(ND–39.2)	(ND–22.4)	(ND–59.3)	(5.1–103.6)	(23.0–207.3)
p-value		0.739	0.413	0.013*	0.311	0.265	0.098
Since your baby was born, did you do any agricultural work?							
No (n=46)	Median	25.6	15.0	ND	ND	37.7	84.7
	(IQR)	(ND–59.1)	(ND–37.0)	(ND–24.8)	(ND–55.4)	(13.0–102.7)	(36.9–207.2)
Yes (n=4)	Median	49.2	2.2	30.1	90.8	213.1	264.7
	(IQR)	(ND–248.6)	(ND–103.7)	(ND–103.0)	(2.8–185.8)	(48.8–256.5)	(204.2–347.3)
p-value		0.689	0.607	0.381	0.251	0.290	0.027*

[a] IQR: interquartile range. [b] ND: not detectable. DMP: dimethyl phosphate. DEP: diethyl phosphate. DETP: diethyl thiophosphate. DEDTP: diethyl dithiophosphate. Total DEP=DEP + DETP + DEDTP. DAPs=DMP + Total DEP. *significant at $p<0.05$.

and Shieldtox) contain 0.5% dichlovos together with pyrethroid or carbamate insecticides[14]. ARS contains 0.2% permethrin or 0.07% Neo-Pynamin. Bigon contains 0.3% tetramethrin or 1% Propoxur. Sheldtox contains 0.1% bioallethrin. Dichlorvos can metabolize to DMP in the body[15].

Regarding creatinine-adjusted organophosphate metabolites, the DMP and DAPs metabolite concentrations during the prenatal and delivery periods were similar, but the DAPs were significantly lower at 2 months postpartum. This may be the result of the reduced exposure of postpartum mothers or the physical changes postpartum in the mother's metabolism and efficiency of toxin clearance as well as blood volume and body weight[16]. The observation of higher exposures during the prenatal period may reflect the report by the women that they continued to go to the fields up to delivery, and since the organophosphate metabolites have a half-life of 15–30 hours, this would show up in urine samples[17]. A previous study reported median urinary creatinine-adjusted concentrations of DMP, DEP, DETP and DEDTP in small scale farmers of ND, ND, 0.88 μg/g creatinine and ND, respectively[10]. The current study reported median urinary creatinine-adjusted concentrations of DMP, DEP, DETP and DEDTP in pregnant women of 48.87 nmole/g creatinine, 20.82 nmole/g creatinine, ND and ND; the exposure of pesticides in pregnant women was probably lower than those in the small-scale farmers.

Regarding those working as agriculturists, there were higher concentrations of metabolites in the DEP group (particularly DEDTP and total DEP), for activities related to farming (Table 5). The types of organophosphate and carbamate pesticides reportedly used on the farms of 48.39% subjects and family members included profenofos (3.3%), methyl parathion (6.6%), acephate (3.3%), methomyl (20.0%), etc.

The results of the current study clearly presented that pregnant women were exposed to organophosphate pesticides and that this was dependent on living close to agricultural fields, use of insecticides to prevent mosquitoes and insects at home, use of pesticides in their work as agriculturists, field visits and various agricultural activities. To reduce organophosphate pesticides exposure, pregnant women and postpartum mothers should reduce pesticide exposure to as low as possible to prevent developmental neurotoxicity in their children.

The limitation of this study was that the numbers of urine samples from women at 28 weeks of pregnancy, delivery and 2 months postpartum were low (86, 67 and 51, respectively); some urine samples were lost at Sawanpracharak Hospital in Nakornsawan Province due to terrible flooding in Thailand last year.

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