A pilot study of maternal exposure to organophosphate pesticides and newborn neurodevelopment in Thailand

Susan Woskie^a, Pornpimol Kongtip^b, Wiroj Thanasanpaiboon^c , Napaporn Kiatdamrong^d ,
Nantha Charoonrungsirikul^e , Noppapun Nankongnab^b, Anu Surach^b and Akkarat Phamophon^b

^aDepartment of Work Environment, University of Massachusetts Lowell, Lowell, MA, USA; ^b Faculty of Public Health, Department of Occupational Health and Safety, Mahidol University, Bangkok, Thailand; ^cDepartment of Pediatrics, Sawanpracharak Hospital, Nakhonsawan, Thailand; ^dDepartment of Pediatrics, Amnatcharoen Hospital, Amnat Charoen, Thailand; ^eDepartment of Pediatrics, Paholpolpayuhasena Hospital, Kanchanaburi, Thailand

ABSTRACT

The objective of this study was to evaluate whether prenatal exposures to organophosphate (OP) pesticides from maternal agricultural use are associated with neonatal neurobehavioral effects. A pilot study conducted in three provinces in Thailand measured maternal urinary metabolites of OP pesticides in pregnant women at 7 months gestation and at birth. Within 3 days of birth, 82 newborns underwent neurobehavioral testing using the Brazelton Neonatal Behavioral Assessment Scale (NBAS). Comparison of the NBAS test results with maternal OP metabolite levels found the neonates NBAS Habituation cluster score increased with maternal dimethylphosphate (DMP) levels ($p = 0.04$) while the Range of State score increased with total diethylphosphate (DEP) levels ($p = 0.01$). The Number of Abnormal Reflexes in the neonate also increased as maternal urinary total DEP levels increased ($p = 0.06$). These preliminary findings suggest that pre-natal exposures to OP pesticides can impact newborn neurodevelopment and support the need for a longitudinal evaluation of childhood neurodevelopment in relation to pesticide exposures in Thailand.

ARTICLE HISTORY

Received 17 April 2014
Accepted 6 March 2018

KEYWORDS

Neurodevelopment;
pesticides; Thailand;
organophosphate; NBAS;
children

Background

Impacts of pesticide use on newborns

Most of the studies on the neurobehavioral effects of pesticide exposures in children have been conducted in Western countries, initially focusing on prenatal and neonatal exposure to organochlorine (OC) pesticides and how they negatively impact psychomotor development in infants 12 months and younger [1–4]. However, OC pesticides (like DDT) have now been banned in most countries. The neurodevelopmental impacts of the replacement organophosphate (OP) insecticides have been studied in the U.S. CHAMACOS cohort of largely Latina low-income women living in an agricultural community, and researchers found there was an increase in the number of abnormal reflexes in newborns, as maternal prenatal urinary OP metabolite levels increased [5]. The finding of a dose-related increase in abnormal neonatal reflexes in relation to increasing maternal OP metabolite levels was also found in a cohort of minority women in New York City, exposed primarily inside the home from pest control activities [6]. For U.S. children aged 1–3 years in urban and agricultural communities, there have also been reports of lower

performance on the Bayley mental development index and more maternally reported pervasive developmental disorders among children exposed to higher OP levels as measured in their cord blood at birth or by maternal OP metabolite levels in-utero [7,8]. Among 7-year-old children in the U.S. in urban and agricultural communities, urinary OP metabolite levels were associated with lower performance on some measures from the Wisconsin Card Sorting Test [9] while prenatal OP exposures were associated with deficits in IQ [10] as well as working memory [11]. Among U.S. children 6–9 years of age in New York City, whose mothers carried a specific PON1 genotype, prenatal OP exposures were associated with decrements in perceptual reasoning [12].

More recently, results from a general birth cohort in Brittany, France reported no evidence that prenatal OP exposures were associated with adverse neurodevelopment in 6-year olds using the WISC-IV test, although this cohort had a higher socioeconomic status than those studied in the U.S. [13]. On the other hand, there have been several studies of the general population in China that suggest that prenatal OP exposures are associated with decrements in neurodevelopment. A study of pregnant women in Shenyang China found that urinary OP

levels were significantly associated with poorer scores on a neonatal test, similar to the NBAS [14]. At nine months, umbilical cord levels of OPs were associated with poorer visual acuity [15] and at 24 months, prenatal OP exposures were negatively associated with developmental quotients from the Gesell Development Schedules [16,17].

To date, we have published the only Thai study of neurobehavioral effects of prenatal exposure to pesticides on infants (at 5 months of age). In that pilot birth cohort study, which is also reported here at an earlier age, we found that prenatal concentrations of maternal urinary OP metabolites were associated with reduced infant cognitive and motor development [18]. This is important since pesticide exposures are likely to be much higher in Thailand than in western countries where most of the research on pesticide exposures on child neurodevelopment has been done. This is, in part, because there is minimal regulation of pesticide use by farmers and little use of personal protective equipment during pesticide application [19].

Pesticide exposures in Thailand

In Thailand, almost 40% of the population works in the agricultural sector [20] where pesticide use has been increasing steadily with the largest increases in the use of herbicides and insecticides [21]. Increasingly popular in Thailand are the herbicides 2,4-D, glyphosate, atrazine, and a variety of OP insecticides including methyl parathion, diazinon, methamidophos, and others [22]. In 2012, pesticide poisoning in Thailand were reported at a rate of 12.37 per 100,000 population [23], compared to 3–6 per 100,000 population in the U.S [24]. When the Thai Bureau of Occupational and Environmental Disease screened agricultural populations for cholinesterase levels, an indicator of exposure to OP insecticides, they found that ~30% of the population had “unsafe or risky levels” of cholinesterase inhibition [25]. A study of 85 Thai women from a farming community in northern Thailand found 36% had “risky or unsafe” cholinesterase levels, many without any obvious direct exposure to pesticides except through other family members [26]. Few studies of cholinesterase levels among farmers in Western countries have been reported, however, of the 215 participants of the Washington State Cholinesterase Monitoring Program in the United States, only 3% had “risky” levels of cholinesterase inhibition [27]. These findings suggest that OP exposures to farmers in Thailand are likely to exceed those of most western countries, like the United States. For example, a study of urinary pesticide metabolites in small-scale farmers in northern Thailand, found 97% of the farmers had measureable levels of urinary dialkylphosphate metabolites as well as metabolites of chlorpyrifos (76%) and parathion (99%), all OP insecticides [28]. In the birth cohort reported here, measureable levels of urinary dialkylphosphate

metabolites were found in 90–100% of the women measured prenatally, at birth and 2 months post-partum, even though only 27% reported working as farmers [29].

In this pilot birth cohort study, a collaborative effort between Mahidol University Faculty of Public Health in Bangkok Thailand and the University of Massachusetts Lowell in the USA, we examine whether prenatal exposures to OP pesticides from agricultural use, as measured by maternal urinary biomarkers, are associated with neonatal neurobehavioral effects.

Methods

Participants

The participants were pregnant women recruited during the 28th week of pregnancy from three government provincial hospitals in the provinces of Amnartcharoen, Karnjanaburi, and Nakorn Sawan. The pregnant women were eligible for recruitment into the study if: (1) they were adults in childbearing years (20 to 35 years old); (2) used the hospital for prenatal care and planned to use the hospital for follow-up infant care; (3) intended to breast feed their infants; (4) did not have factors known to impact neurodevelopment including hypertension, diabetes, thalassemia, HIV+ status; and (5) completed the informed consent procedure approved by Mahidol University Faculty of Public Health Ethical Committee and the University of Massachusetts Lowell Institutional Review Board. If the woman gave birth at another hospital or underwent caesarian section, they were then excluded from follow-up.

Data collection during pregnancy

In their 7th month of pregnancy, the women were interviewed about their general health, smoking, drug use, work and related toxic exposures, use of vitamins and iodine supplements, use of home pesticides and also, if they self-reported their occupation as an agricultural worker or they lived with an agricultural worker, the type of crops they raised and their use of pesticides. The mother was defined as an agricultural worker during pregnancy if she reported working in the fields 2–3 days/week or more in any trimester.

Birth and neonatal behavioral assessment scale (NBAS)

At birth, hospital nursing staff collected data on the newborn weight, height, length, head circumference, and gestational age along with APGAR scores at 1, 5, and 10 min after birth. Neonates less than the Thai Ministry of Public Health National Growth Reference Values for Children at birth were defined as low birth weight (<2.5 kg), height (<46 cm), or head circumference (<31 cm) for analysis. Before leaving the hospital,

the newborn underwent testing by the hospital pediatrician for that province, who had been trained and certified as a Brazelton Neonatal Behavioral Assessment Scale (NBAS) tester. The NBAS consists of 28 behavioral items, which measure the infant's behavioral capacities, and 16 reflex items, which measure the infant's neurological status [30]. The scored items involve some observations and some administered stimuli. Items can be clustered to describe the infant's functioning in seven key areas: Habituation – the infant's ability to shut out response to discrete visual and auditory stimuli while asleep or drowsy; Orientation – alertness and the newborn's ability to fixate on visual and auditory stimuli; Motor – spontaneous and elicited motor performance and movement, and the overall quality of motor maturity and tone; Range of State – intensity of response to stimuli, irritability and lability of state; Regulation of State – the infant's ability to regulate his/her state in the face of increasing levels of stimulation either by self-quieting or in response to consoling by the examiner; Autonomic Stability – response to stress related to the central nervous system such as tremors, skin color changes, or startle response. In addition, the NBAS examines the infant's reflexes, indicating when abnormal. Cluster scores for the key areas used the standard NBAS weighting algorithm developed as part of the testing system by Lester, Als, and Brazelton [31].

Urine sample analysis

Hospital nursing staff collected urine samples from the mothers in polyethylene containers at 7 months gestation and at birth and froze them at -45°C until analysis. Thawed specimens were pretreated using solid phase extraction and four urinary dialkyl phosphates (DAP) metabolites were derivatized with 2,3,4,5,6-Pentafluorobenzyl bromide, analyzed by Gas Chromatography-Mass Spectrometry (GC-MS) and concentrations reported in nmole/liter [32]. The detection limits of dimethylphosphate (DMP), diethylphosphate (DEP), diethylthiophosphate (DETP), and diethyldithiophosphate (DEDTP) in urine were 5.00, 0.034, 0.028, and 0.054 ng/ml [24]. The non-detectable urine samples were assigned the detection limit divided by 2 [33]. The total DEP represented the summation of DEP, DETP, and DEDTP. Because these were spot samples at two points in time, averaging the concentrations from the urine samples at 7 months gestation and birth helped to stabilize the estimates and also where only one sample was available, we utilized that value to maximize the number of neonatal NBAS test results that had paired maternal urinary metabolite measurements. The Pearson correlation coefficients and paired t-tests for the birth and 7 month samples ($n = 55$) were non-significant for all metabolites, so averaging enabled us to increase our sample size to $n = 72$. Like other studies, the distribution of urinary OP metabolite concentrations in the

Thai cohort was highly skewed, so the measurements were transformed into the $\log_{10}$ scale for the exposure response analysis [5,8].

Data analysis

Questionnaire data were entered twice, results compared and discrepancies resolved. Statistical analysis was done using SAS version 9 (SAS, Cary, NC). To evaluate the relationship between pesticide exposures and neonatal performance on the NBAS, a separate model was fit for each of the NBAS cluster scores. Pesticide exposure potential was examined as: (1) a dichotomous variable where the mother was defined as an agricultural worker, or not; and (2) as the individual OP metabolite concentration or as the total of all DEP metabolite levels for each woman NBAS scores and urinary metabolites were examined for non-linearity using Lowess smoothing (Proc Lowess). Potential covariates were selected based on previous work examining the relationship of NBAS outcomes to pesticide exposures and other neurotoxins [4–6,30,34]. In univariate analyses, the potential predictors of each NBAS cluster score that were examined, and included in the multiple regression analysis if $p \leq 0.15$, were: maternal education (junior high education vs higher (1/0), income (self-reported income sufficiency (0/1), maternal age, ever smoked during pregnancy (0/1), ever drank alcohol during pregnancy (0/1), ever used any of a series of illegal or over the counter drugs during pregnancy (0/1), ever drank caffeinated beverages during pregnancy (0/1), parity (parity > 1 (0/1) or parity = 0 (0/1), child gender, NBAS tester/province. For all NBAS clusters, except for the reflex cluster, linear regression was used (Proc GLM). Since the reflex cluster is a count of abnormal reflexes, a negative binomial regression model was used (Proc Genmod). Logistic regression was used to examine the association of low birth weight (0/1) with maternal urinary pesticide metabolite levels. Covariates were included in the low birth weight logistic regression with the exposure variable if they had a predictive power of $p \leq 0.15$ in univariate logistic regression models.

Results

We interviewed 113 women during their 7th month of pregnancy. Nineteen delivered at other hospitals, 12 delivered Caesarian, pre-term (<37 weeks) or with complications leaving 82 newborns that underwent NBAS testing. The average age of these women was 25.7 (SD 4.3 years). The demographics of those participants are reported in Table 1. During pregnancy, 61% ($n = 50$) of the participants reported that chemical insecticides were applied in their home and of those, 74% reported being the applicator of the insecticide. During pregnancy, 18% ($n = 15$) reported that their house was treated for termites, of which 67% reported being the applicator of the

Table 1. Characteristics of Thai women who participated in the study during their 28th week of pregnancy ($N = 82$).^a

Characteristic	<i>N</i>	% ^b
Maternal education completed		
Elementary school	21	26
Junior high school	32	39
Senior high school or vocational certificate	20	24
Bachelor's degree or higher vocational certificate	9	11
Income		
Sufficient with savings	36	45
Sufficient without savings	38	48
Insufficient	6	7
Self-reported occupation		
Agricultural worker (family farm or plantation)	34	42
Housewife	16	20
Temporary worker	15	18
Own business	7	8
Private company or government worker	4	5
Other	6	7
Agricultural work during pregnancy ^c		
Yes	21	26
No	61	74
Parity		
1st child	26	32
2nd child	46	56
3rd + child	10	12
Married		
Yes	79	96
No	3	4
Caffeine use during pregnancy		
Yes	23	29
No	56	71
Alcohol use during pregnancy		
Yes	5	6
No	75	94
Use of cough medicine during pregnancy		
Yes	11	14
No	69	86
Smoked cigarettes during pregnancy		
Yes	1	1
No	81	99
Child gender		
Male	46	56
Female	36	44

^aIncludes 10 participants whose urine samples were lost in the 2011 flooding of Nakon Sawan Hospital in Thailand but whose children were NBAS tested.

^bWhere data missing % calculated from participants who answered question.

^cAgricultural work during pregnancy defined as working in the field 2–3 days/week or more in any trimester.

pesticide. About half (46%) of the participants did not live near farmland ($n = 38$) and about half (48%) lived near farmland where they reported that pesticides were sprayed ($n = 39$). During their pregnancy, 42% ($n = 34$) women reported their occupation as agricultural worker, however only 26% ($n = 21$) reported working in the fields 2–3 days per week or more in any trimester (Table 1). During their pregnancy, 11 participants reported treating seeds with pesticide before planting, 2 reported using pesticides to preserve seeds, and 34 reported washing clothes that had been worn during mixing or applying pesticides. There were 21 participants who reported mixing pesticides for application during their pregnancy with a frequency ranging from 1–4 days ($n = 15$) to 5–10 days ($n = 4$) to over 10 days ($n = 2$). There were 27

participants who reported applying pesticides to crops during their pregnancy with a frequency ranging from 1–4 days ($n = 16$) to 5–10 days ($n = 4$) to over 10 days ($n = 7$). Twenty women reported applying pesticides to animals during their pregnancy with a frequency ranging from 1–4 days ($n = 13$) to 5–10 days ($n = 3$) to over 10 days ($n = 4$). Evaluation of the association between these potential usage patterns and urinary OP metabolite levels is reported elsewhere [29].

Due to massive flooding in Thailand from July to December 2011, 10 urine samples were lost when the hospital in Nakorn Sawan province lost electricity for over a week. Urinary metabolites of the OP pesticides were found in the urine of most participants with the lowest number of non-detectable values found for DMP which also had the highest detection limit (Table 2). DETP and DEDTP were the metabolites with the most non-detectable values (28 and 29%, respectively).

The birth data for the cohort are shown in Table 3. Among the newborns, only two had APGAR scores in the fairly low range during the first minute (score of 4 and 6), but all were in the normal range by five minutes. Based on the Thai Ministry of Public Health, National Growth Reference Values for Children two children had head circumference less than the reference range of 31–35 cm, five children had weights less than the reference range of 2.5–4.0 kg, and two had birth heights less than the reference value of 46–50 cm. The five children with low birth weight included the two low head circumference babies and one of the low birth height babies; however, low birth weight was not related to low APGAR at 1 min. To examine if pesticide exposure was associated with the probability of experiencing low birth weight (0/1), we used logistic regression models that included parity (0/1 for parity > 1), since it was the only covariate from univariate models with $p \leq 0.15$. When the maternal concentrations of each of the OP urinary metabolites were added to the models, none were significant predictors of low birth weight ($p > 0.05$), nor was agricultural work during pregnancy (0/1) ($p > 0.05$).

The NBAS testing (Table 3) was completed an average of 1.8 days (SD 1.1) after birth, with 91.5% being tested within 3 days of birth, and 8.5% tested on the 4th day after birth. The NBAS system does not publish a set of “norms” since in clinical settings it is used to give individualized feedback on supportive parenting for infants, while in research settings, population comparisons are conducted. Although the NBAS system does not provide normative data to identify infants with “abnormal” test scores, lower scores indicate poorer performance, except for the reflex score where higher numbers indicate more abnormal reflexes were observed.

No NBAS data have been published for the general Thai or Asian neonate population for comparison. We did compare the NBAS results of this cohort of neonates from agricultural areas in Thailand with those from a

Table 2. Urinary metabolites of organophosphate (OP) pesticides (nmole/liter) and creatinine corrected (nmole/g creatinine) averaged across 7-month prenatal and birth samples from mothers of newborns tested with the newborn behavioral assessment scale (NBAS).

Metabolite	N	% samples detectable	Median nmole/liter (5–95%ile)	Median nmole/g creatinine (5–95%ile)
Diethylphosphate (DEP)	72	89	18.7 (0.1–48.4)	29.7 (0.2–150.1)
Diethylthiophosphate (DETP)	72	72	12.5 (0.07–59.4)	19.0 (0.1–246.0)
Diethyldithiophosphate (DEDTP)	72	71	6.4 (0.1–337.0)	13.1 (0.1–600.2)
Total diethylphosphates* (total DEP)	72	100	63.0 (5.6–375.6)	128.2 (14.5–702.1)
Dimethylphosphate (DMP)	72	93	35.9 (19.8–78.8)	69.1 (23.2–297.4)

*Total DEP = DEP + DETP + DEDTP.

Table 3. Birth outcomes and newborn behavioral assessment scale (NBAS) testing results.

	Mean ^a	SD	Range
<i>NBAS cluster scores</i>			
Habituation	7.3	1.2	3.2–9.0
Orientation	6.1	1.5	1.0–8.6
Motor performance	5.7	0.8	3.6–7.2
Range of state	3.9	0.5	2.3–5.2
Regulation of state	5.2	1.7	1.5–8.2
Autonomic stability	6.1	1.1	3.7–7.7
Number of abnormal reflexes	1.8	2.3	0–8
<i>Birth outcomes</i>			
Gestational age (weeks)	38.9	1.2	37–41
Birth weight (kg)	3.1	0.4	2–4
Birth height (cm)	51.7	2.3	45–57
Birth head circumference (cm)	33.3	1.4	30–38
APGAR 1 min	8.9	1.0	4–10
APGAR 5 min	9.6	0.6	8–10
APGAR 10 min	9.8	0.4	9–10

^aIncludes 10 subjects whose urine samples were lost in the 2011 flooding of Nakon Sawan Hospital in Thailand.

farming community in California [5]. We found statistically significant differences ($p < 0.003$) for all but Motor Performance and Number of Abnormal Reflexes. On average, the Thai infants did more poorly on Orientation, Regulation of State and Autonomic Stability, while the U.S. infants did more poorly on Habituation and Range of State.

The results of exposure response modeling for each NBAS cluster score with the maternal OP urinary metabolite concentrations and maternal status as an agricultural worker during pregnancy are shown in Table 4. Being an agricultural worker who worked in the fields during pregnancy for 2–3 days per week or more ($n = 21$) or having a self-reported occupation as agricultural worker ($n = 34$) was not a statistically significant predictor of any NBAS cluster score. For each NBAS cluster, the covariates from univariate models that had $p \leq 0.15$ were included in the models used to evaluate whether maternal urinary pesticide metabolite levels were significantly associated with the neurobehavioral outcomes. We found a significant association between an increase in the concentration of maternal OP metabolite DMP and an increase in the NBAS Habituation cluster score ($p = 0.04$). There was also a significant increase in the NBAS Range of State cluster score associated with an increase in maternal DEP metabolite levels ($p = 0.05$), as well as with increased concentrations of the total of all

diethylphosphate metabolites (total DEP) ($p = 0.01$). In addition, we found that increases in the maternal urinary concentrations of the total of all diethylphosphate metabolites (total DEP) and DEDTP were associated with an increase in the Number of Abnormal Reflexes in the newborns, although these increases were not statistically significant ($p = 0.06$ and 0.07 , respectively). When the statistically significant models were run with only the 7 month spot urine results ($n = 66$), the Range of State findings remained significant but the Habituation model with DMP became non-significant, highlighting the limitations of a small sample size.

Discussion

This pilot study was part of a project to develop methods to look at environmental exposures and their impact on neurobehavioral development of children. Strengths of the project include that it was successful in recruiting hospital staff in three provinces to participate. This willingness to be trained in the neurobehavioral testing methods and to add a complex data collection protocol to their hospital duties reflected the deep interest of the Thai pediatricians and nursing staff in participating in this research project, which we hope will eventually evolve into the establishment of a longitudinal birth cohort. Our project continued during the unprecedented flooding that covered 15 provinces, causing over \$45 billion in damages and impacting over 64 million people for six months in 2011. The flooding resulted in the evacuation of one of our hospitals, causing the loss of some of our samples and the displacement of some of the women recruited during pregnancy. Nevertheless, we were able to collect data on the babies of 82 out of the original 113 recruited women. Limitations of the study include some of these same factors: loss of samples and participants due to the flooding, the heavy work burden placed on the hospital staff, measurement of only four of the six possible OP metabolites due to lack of available standards, collection of only 2 spot urine samples to represent pregnancy pesticide exposure levels, no data on consumption of fruits and vegetables that could have significant pesticide residue levels, focus on OP pesticides when many other types are also in use in Thai agriculture, and lack of NBAS data on the general population in Thailand or Asia for comparison.

Table 4. Association of maternal urinary organophosphate metabolites (log10 nmole/liter) and NBAS scores for neonatal behavior.

NBAS cluster	N ^a	Ag work during pregnancy β [95%CI]	Diethylphosphate (DEP) β [95%CI]	diethylthiophosphate (DETP) β [95%CI]	Diethyldithiophosphate (DEDTP) β [95%CI]	Total diethylphosphates (total DEP) β [95%CI]	Dimethylphosphate (DMP) β [95%CI]
Habituation ^b	71	−0.21 [−0.74–0.31]	−0.09 [−0.44–0.26]	−0.03 [−0.25–0.19]	0.07 [−0.12–0.27]	0.22 [−0.20–0.64]	1.73 [#] [0.11–3.35]
Orientation ^c	72	−0.43 [−1.18–0.33]	−0.26 [−0.86–0.34]	−0.06 [−0.37–0.26]	−0.21** [−0.48–0.07]	−0.35 [−0.96–0.25]	0.58 [−1.8–3.0]
Motor performance ^d	70	−0.20 [−0.51–0.12]	−0.04 [−0.25–0.17]	0.03 [−0.11–0.16]	−0.05 [−0.16–0.07]	0.005 [−0.25–0.26]	0.87 ⁺ [−0.09–1.83]
Range of State ^e	70	0.20** [−0.05–0.46]	0.16 [#] [0.003–0.31]	0.06 [−0.04–0.15]	0.04 [−0.05–0.13]	0.23 [#] [0.05–0.41]	−0.25 [−0.99–0.49]
Regulation of State ^f	71	0.08 [−0.75–0.90]	0.12 [−0.40–0.65]	−0.21* [−0.53–0.11]	0.02 [−0.27–0.31]	0.14 [−0.48–0.77]	−0.31 [−2.81–2.18]
Autonomic stability ^g	70	−0.15 [−0.70–0.40]	−0.15 [−0.40–0.10]	0.04 [−0.12–0.19]	0.11** [−0.03–0.25]	0.22* [−0.09–0.53]	−0.22 [−1.43–0.99]
Number of Abnormal Reflexes ^h	70	0.02 [−0.58–0.61]	0.22* [−0.13–0.69]	−0.12 [−0.34–0.10]	0.20 ⁺ [−0.01–0.43]	0.46 ⁺ [−0.05–1.06]	0.44 [−1.31–2.18]

^aN for urine metabolite models.^bAdjusted for NBAS tester, parity > 1 (0/1).^cAdjusted for NBAS tester, parity = 0 (0/1).^dAdjusted for NBAS tester, self-reported income sufficiency (0/1).^eAdjusted for NBAS tester, junior high education vs higher (1/0), married (0/1) alcohol use (0/1), cough medicine use (0/1).^fAdjusted for NBAS tester, married (0/1), alcohol use (0/1), maternal age.^gAdjusted for NBAS tester, married (0/1), cough medicine use (0/1).^hNegative binomial model adjusted for NBAS tester, caffeine use(0/1).* $p \leq 0.2$; ** $p \leq 0.15$; + $p \leq 0.10$. # $p \leq 0.05$.

Using the babies from Latina women living in an agricultural community in California, USA (the CHAMACOS cohort) [5] for comparison, we found that on average, NBAS scores for our Thai cohort (Table 3) were higher (more optimal) for the clusters of Habituation and Range of State and lower (less optimal) for the Orientation, Autonomic Stability, and Regulation of State clusters. When the maternal urinary OP pesticide metabolite levels reported for this cohort are compared to the 26-week prenatal maternal urinary OP levels reported for the CHAMACOS cohort, this Thai cohort had 3–20 times higher median metabolite concentrations except DETP (which was the same) [30] (Figure 1). In the CHAMACOS cohort, 43% worked in agriculture during their pregnancy and 89% lived with one or more farm workers. While in our study, 42% of the pregnant women reported their occupation as agriculturists and 69% had family members who worked in agricultural fields. This suggests that, on average, exposures to OP pesticides during pregnancy may be higher in Thailand than in the more regulated US farming communities. When compared to other studies of general population (not agricultural communities), other patterns arise. Urine samples were collected from 249 mostly urban pregnant women in Shenyang China during their pregnancy [14]. Levels of all metabolites except DEDTP were much higher than our Thai cohort, or other cohorts of pregnant women from developed countries (Figure 1). Only 13% of these women lived rurally but they had significantly higher total DEP levels than urban women. The authors attributed the high levels of OP exposure to the daily consumption of fresh vegetables and fruits contaminated with high OP residue

levels [14]. Samples collected from pregnant women in the general population (not agricultural workers) in the Netherlands [36] and Israel [37] had lower levels of DEP, DETP, and DEDTP than the Thai, Chinese, or U.S. women but higher levels of DMP than the Thai or U.S. women (Figure 1). The authors speculate that this was due to higher consumption of fruits and vegetables and use of pesticides in the home that are metabolized to DMP [36,37]. Currently there are no data on urinary concentrations of the OP pesticides in the general Thai population for comparison of the overall population risk. However, we previously reported that the women in this cohort who reported living close to fields sprayed by pesticides had significantly higher urinary OP metabolite levels than women who did not [29]. Also, the women who reported working as agriculturists or living with agricultural workers had significantly higher OP metabolite levels than those who did not [29].

Although 6% of the babies in our cohort had a birth weight below the Thai reference range, we found no association between maternal urinary metabolites of OP pesticides and low birth weight. However, the sample size of our cohort was small and may have been underpowered to identify this relationship. A previous study of residential pesticide exposures to chlorpyrifos and diazinon among a cohort of minority New York City women and their babies found a significant negative correlation between the cord blood concentrations of the OP pesticides and the birth weight and birth length of the neonates [38]. On the other hand, no relationship was found between maternal urinary OP pesticide metabolites and fetal weight, height or head circumference in the CHAMACOS cohort of low-income, Latina women

Figure 1. Comparison of urinary organophosphate pesticide metabolite concentrations (nmole/L) during pregnancy from several countries including China [14], Thailand (this study), U.S. [35], Netherlands [36], and Israel [37].

living in an agricultural community in California [39]. A study in Thailand did report a negative correlation between maternal total DEP and newborn head circumference and birthweight for mothers with low maternal PON1 activity [40].

In this study, we found a borderline significant positive relationship between the Number of Abnormal Reflexes and maternal urinary total diethyl phosphate metabolite levels (total DEP) ($p = 0.06$) and one of the component DE metabolites DEDTP ($p = 0.07$). Previous studies in the U.S. that have investigated the impact of OP pesticide exposures on newborn NBAS results have found a significant positive association between prenatal urinary total dimethyl and/or diethyl phosphate metabolite levels and the Number of Abnormal Reflexes in the newborn [5,6]. A study of 3-day-old infants in China found a negative relationship between primary reflexes as well as other test categories (behavior, passive tone, active tone, and summary score) with urinary OP metabolite levels [14]. We have previously published work examining the neurobehavioral effects of prenatal exposure to pesticides on infants at 5 months of age from this cohort. That study used the Bayley Scales of Infant and Toddler Development-III (Bayley-III) to examine the association of maternal urinary OP metabolite levels and child development. Higher prenatal total DEP levels were

significantly associated with reduced motor composite scores and reduced cognitive composite scores [18].

We also found a significant positive relationship between maternal urinary DMP levels and the NBAS Habituation cluster score and a significant positive relationship between either total diethyl phosphate metabolite levels (total DEP) or the component DE metabolite DEP and the NBAS Range of State cluster score. These positive associations between exposures and NBAS scoring were not anticipated and have not been found previously [5,6]. However, a recent study suggested that higher habituation scores at three days old were associated with externalizing problems at age six [41]. Externalizing behaviors, including aggression, attention deficit hyperactivity disorder (ADHD), and disruptive behaviors, have been associated with children on the autism spectrum [42]. A positive association was identified between prenatal and postnatal urinary total dialkylphosphate pesticide metabolite levels (includes all diethyl and dimethyl metabolites) and the risk of pervasive developmental disorders as identified by the Achenbach Child Behavior Checklist (CBCL) collected at age two [8]. In addition, prenatal maternal urinary total dialkylphosphate levels were associated with attention problems and ADHD at age five, particularly among boys [43].

Conclusion

This pilot study has found evidence of statistically significant effects on newborn neurodevelopment associated with maternal exposures to OP pesticides. Although the sample size of this study was small, the findings are consistent with studies in Western countries and China, perhaps due to the higher exposures in this cohort. In this cohort at age five months, and in other studies, these deficits have been found to continue into childhood. Thus, we believe it is time for a longitudinal birth cohort in Thailand that can, in part, investigate whether prenatal and childhood exposures to the many pesticides widely used in Thailand are associated with developmental and/or behavioral disorders including autism and attentional deficits during childhood.

Acknowledgments

The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health or the Eunice Kennedy Shriver National Institute of Child Health and Human Development. We would like to thank the nurses, doctors, and participants who participated in this project from Amnartcharoen hospital in Amnartcharoen province, Paholpolpayuhasena hospital in Karnjanaburi, and Sawanpracharak hospital in Nakorn Sawan province.

Disclosure statement

No potential conflict of interest was reported by the authors.

Funding

This work was supported by the National Institutes of Health Fogarty International Center [grant number R21HD060520] and the Eunice Kennedy Shriver National Institute of Child Health & Human Development.

ORCID

Wiroj Thanasanpaiboon <http://orcid.org/0000-0002-9960-4802>

Napaporn Kiatdamrong <http://orcid.org/0000-0003-0351-2856>

Nantha Charoonrungrasirikul <http://orcid.org/0000-0001-7893-0556>

Anu Surach <http://orcid.org/0000-0001-7198-3567>

Akkarat Phamonphon <http://orcid.org/0000-0002-6276-7675>

References

- [1] Ribas-Fito N, Cardo E, Sala M, et al. Breastfeeding exposure to organochlorine compounds and neurodevelopment in infants. *Pediatr*. 2003;111:e580–e585.
- [2] Eskenazi B, Marks A, Bradman A, et al. In utero exposure to dichlorodiphenyltrichloroethane (DDT) and dichlorodiphenyldichloroethylene (DDE) and neurodevelopment in young Mexican American children. *Pediatr*. 2006;118:233–241.
- [3] Torres-Sánchez LT, Rothenberg S, Schnaas L, et al. p, p'-DDE exposure and infant neurodevelopment: a perinatal cohort in Mexico. *Environ Health Persp*. 2007;115:435–439.
- [4] Fenster L, Eskenazi B, Anderson M, et al. In utero exposures to DDT and performance on the Brazelton neonatal behavioral assessment scale. *Neurotox*. 2007;28:471–477.
- [5] Young J, Eskenazi B, Gladstone E, et al. Association between in utero organophosphate pesticide exposures and abnormal reflexes in neonates. *Neurotox*. 2005;26:199–209.
- [6] Engel S, Berkowitz G, Barr D, et al. Prenatal organophosphate metabolite and organochlorine levels and performance on the Brazelton neonatal behavioral assessment scale in a multiethnic pregnancy cohort. *Am J Epidemiol*. 2007;165:1397–1404.
- [7] Rauh V, Garfinkel R, Perera FP, et al. Impact of prenatal chlorpyrifos exposure on neurodevelopment in the first 3 years of life among inner-city children. *Pediatr*. 2006;118:e1845–e1859.
- [8] Eskenazi B, Marks A, Bradman A, et al. Organophosphate pesticide exposure and neurodevelopment in young Mexican-American children. *Environ Health Persp*. 2007;115:792–798.
- [9] Lizardi PS, O'Rourke MK, Morris RJ. The effects of organophosphate pesticide exposure on Hispanic children's cognitive and behavioral functioning. *J Pediatr Psychol*. 2008;33(1):91–101.
- [10] Bouchard MF, Chevrier J, Harley KG, et al. Prenatal exposure to organophosphate pesticides and IQ in 7-year-old children. *Environ Health Persp*. 2011;119(8):1189–1195.
- [11] Rauh V, Arunajadai S, Horton M, et al. Seven-year neurodevelopmental scores and prenatal exposure to chlorpyrifos, a common agricultural pesticide. *Environ Health Persp*. 2011;119(8):1196–1201.
- [12] Engel SM, Wetmur J, Chen J, et al. Prenatal exposure to organophosphates, paraoxonase 1, and cognitive development in childhood. *Environ Health Persp*. 2011;119(8):1182–1188.
- [13] Cartier C, Warembourg C, Le Maner-Idrissi G, et al. Organophosphate insecticide metabolites in prenatal and childhood urine samples and intelligence scores at 6 years of age: results from the mother-child PELAGIE cohort (France). *Environ Health Persp*. 2016; 124(5):674–680.
- [14] Zhang Y, Han S, Liang D, et al. Prenatal exposure to organophosphate pesticides and neurobehavioral development of neonates: a birth cohort study in Shenyang China. *PLoS one*. 2014;9(2):e88491.
- [15] Silver MK, Shao J, Ji C, et al. Prenatal organophosphate insecticide exposure and infant sensory function. *Int J Hyg Environm Health*. 2018 Feb 2;17:S1438–S4639. Epub ahead of print DOI:10.1016/j.ijeh.2018.01.010
- [16] Wang Y, Zhang Y, Ji L, et al. Prenatal and postnatal exposure to organophosphate pesticides and childhood neurodevelopment in Shandong China. *Environ Int*. 2017;108:119–126.
- [17] Liu P, Wu C, Chang X, et al. Advers associations of both prenatal and postnatal exposure to organophosphorous pesticides with infant neuro development in an agricultural area of Jiangsu province China. *Environ Health Perspect*. 2016;124(10):1637–1643.
- [18] Kongtip P, Techasaensiri B, Nankongnab N, et al. The impact of prenatal organophosphate pesticide

- exposures on Thai infant neurodevelopment. *Int J Res Pub Health*. 2017;14(6):570–582.
- [19] Panuwet P, Siri Wong W, Prapamontol T, et al. Agricultural pesticide management in Thailand: status and population health risk. *Environ Sci and Policy*. 2012;17:72–81.
 - [20] National Statistical Office of Thailand [Internet]. The survey results of job characteristic in Thai people. Bangkok; 2015 Feb [cited 2015 Dec 2]. Available from: <http://service.nso.go.th/nso/nsopublish/themes/files/lfs58/reportFeb.pdf>
 - [21] Office of Agricultural Economics [Internet]. Bangkok: Ministry of Agricultural and Cooperatives; 2010 [cited 2010 Oct 10]. Available from: http://www.oae.go.th/ewt_news.php?nid=146&filename=index
 - [22] Thapinta A, Hudak P. Pesticide use and residual occurrence in Thailand. *Environ Monitor Assess*. 2000;60:103–114.
 - [23] Ministry of Public Health [Internet]. Bureau of epidemiology, Department of disease control report. Bangkok; 2010 [cited 2010 Aug 7]. Available from: http://epid.moph.go.th/Annual/Annual49/.../52_PesticidePoisoning.doc
 - [24] Center for Disease Control [Internet]. Pesticide illness & injury surveillance. Atlanta (GA); 2016 [cited 2016 Sep 23] Available from: <https://www.cdc.gov/niosh/topics/pesticides/>
 - [25] Itsaraphan, P. Health risks of agriculturists and the general population from pesticide toxicity. *Proceedings of the Conference on Chemical Pesticides*; 2012 Nov 15–16 [cited 2014 Jun 23]. Available from: http://www.thaipan.org/sites/default/files/conference2555/conference2555_1_02.pdf
 - [26] Merrill, V. Pesticide exposures among women farmers in Mae Wang, Thailand. *American Public Health Association Annual Meeting and Exposition*; 2006 Nov 4–8; Boston, MA [cited 2014 Jun 23]. Available from: https://apha.confex.com/apha/134am/techprogram/paper_141856.htm
 - [27] Strelitz J, Engel LS, Keifer MC, et al. Blood acetylcholinesterase and butyrylcholinesterase as biomarkers of cholinesterase depression among pesticide handlers. *Occ Environl Med*. 2014;71(12):842–847.
 - [28] Panuwet P, Prapamontol T, Chantara S, et al. Concentrations of urinary pesticide metabolites in small-scale farmers in Chiang Mai Province, Thailand. *Sci Total Environ*. 2008;407(1):655–668.
 - [29] Kongtip P, Nankongnab N, Woskie S, et al. Organophosphate urinary metabolite levels during pregnancy, delivery and postpartum in women living in agricultural areas in Thailand. *J Occ Health*. 2013;55:367–375.
 - [30] Brazelton T, Nugent J. Neonatal behavioral assessment scale. London: Mac Keith Press; 2011.
 - [31] Lester B, Als H, Brazelton T. Regional obstetric anesthesia and newborn behavior: a reanalysis toward synergistic effects. *Child Dev*. 1982;53:687–692.
 - [32] De Alwis GK, Needham LL, Barr DB. Measurement of human urinary organophosphate pesticide metabolites by automated solid-phase extraction derivation and gas chromatography-tandem mass spectrometry. *J Chrom B*. 2006;843(1):34–41.
 - [33] Hornung R, Reed L. Estimation of average concentration in the presence of nondetectable values. *App Occ Environl Hyg*. 1990;5:46–51.
 - [34] Rogan W, Gladen BC, McKinney JD, et al. Neonatal effects of transplacental exposure to PBBs and DDE. *J Ped*. 1986;109:335–341.
 - [35] 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 Persp*. 2005;113(12):1802–1807.
 - [36] Ye X, Pierik FH, Hauser R, et al. Urinary metabolite concentrations of organophosphorous pesticides, bisphenol A and phthalates among pregnant women in rotterdam the Netherlands: the generation R study. *Environ Res*. 2008;108:260–267.
 - [37] Berman T, Hochner-Celnikier D, Barr DB, et al. Pesticide exposure among pregnant women in Jerusalem Israel: results of a pilot study. *Environ Internat*. 2011;37:198–203.
 - [38] Whyatt RM, Rauh V, Barr DB, et al. Prenatal insecticide exposures and birth weight and length among an urban minority cohort. *Environ Health Persp*. 2004 Jul;112(10):1125–1132.
 - [39] Eskenazi B, Harley K, Bradman A, et al. Association of in utero organophosphate pesticide exposure and fetal growth and length of gestation in an agricultural population. *Environ Health Persp*. 2004 Jul;112(10):1116–1124.
 - [40] Naksen W, Prapamontol T, Mangklabruks A, et al. Association of maternal organophosphate pesticide exposure and PON1 activity with birth outcomes in SAWASDEE birth cohort, Thailand. *Env Res*. 2015;142:288–296.
 - [41] Canals J, Hernández-Martínez C, Fernández-Ballart JD. Relationships between early behavioural characteristics and temperament at 6 years. *Inf Behav Dev*. 2011;34(1):152–160.
 - [42] Masse JJ, McNeil CB, Wagner SM, et al. Pparent-child interaction therapy and high functioning autism: a conceptual overview. *J Early Int Behav Interv*. 2007;4(4):714–735.
 - [43] Marks AR, Harley K, Bradman A, et al. Organophosphate pesticide exposure and attention in young Mexican-American children: the CHAMACOS study. *Environl Health Persp*. 2010;118(12):1768–1774.