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Neurobehavioral effects of exposure to organophosphates and pyrethroid pesticides among Thai children

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Abstract

The use of pesticides for crop production has grown rapidly in Thailand during the last decade, resulting in significantly greater potential for exposure among children living on farms. Although some previous studies assessed exposures to pesticides in this population, no studies have been conducted to evaluate corresponding health effects. Twenty-four children from a rice farming community (exposed) and 29 from an aquaculture (shrimp) community (control) completed the study. Participants completed a neurobehavioral test battery three times at 6 month intervals: Session I: preliminary orientation; Session II: high pesticide use season; Session III: low pesticide-use season. Only sessions II and III were used in the analyses. High and low pesticide use seasons

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were determined by pesticide use on rice farms. Urinary metabolites of organophosphates (OPs) and pyrethroids (PYR) were analyzed from first morning void samples collected the day of neurobehavioral testing. Rice farm participants had significantly higher concentrations of dialkylphosphates (DAPs) (common metabolites of OPs) and TCPy (a specific metabolite of chlorpyrifos) than aquaculture farm children regardless of season. But, TCPy was significantly higher during the low rather than the high pesticide use season for both participant groups. Rice farm children had significantly higher DCCA, a metabolite of PYR, than aquaculture participants only during the high exposure season. Otherwise, no significant differences in PYR metabolites were noted between the participant groups or seasons. No significant adverse neurobehavioral effects were observed between participant groups during either the high or low pesticide use season. After controlling for differences in age and the Home Observation for Measurement of the Environment (HOME) scores, DAPs, TCPy, and PYR were not significant predictors of adverse neurobehavioral performance during either season. Increasing DAP and PYR metabolites predicted some relatively small improvement in latency of response. However, due to the small sample size and inability to characterize chronic exposure, any significant differences observed should be regarded with caution.

Keywords

neurobehavior; Behavioral Assessment and Research System; organophosphates; pyrethroids; pesticides; Thailand; children

Agriculture accounts for 8.4% of the gross domestic product in Thailand and employs the largest sector of the labor force (39.6%) (Bank of Thailand 2013). Major crops include rice, cassava, rubber, coconut, cotton, sugar cane and oil palm. Since 2000, Thailand has experienced an approximate four-fold increase in pesticide use (Aiemsupasit 2005) with organophosphates (OPs) and pyrethroids (PYR) as the major pesticide classes used for crop protection (Panuwet et al. 2012). High levels of OP exposures have been objectively documented in ambient air samples breathed by farmers (Jirachaiyabhas et al. 2004) as well as in their urine (Panuwet et al. 2008). Children who lived in farm areas were also found to be exposed to a significantly higher amount of OPs than non-farm reference children (Panuwet et al. 2009; Petchuay et al. 2008). Unfortunately due to limited resources, the health consequences of these exposures, especially for children, have not been adequately characterized.

OPs and PYR are neurotoxicants known to disrupt neurologic development. For example, even in the absence of acetylcholinesterase (AChE) inhibition, animal studies show loss of hippocampal dopamine particularly if OP exposure occurs during pregnancy (Aldridge et al. 2005). Children may be more vulnerable than adults to the effects of pesticides because of the potential for increased exposure through proportionally higher intake of food, water and air relative to body weight, along with immaturity in neurologic development and detoxification pathways (Costa 2006; National Research Council 1993; Eskenazi et al. 1999; Grandjean and Landrigan 2014). As a result of concerns about neurodevelopmental toxicity, four major US birth cohort studies were initiated to determine the health effects of pre and post-natal exposure to OPs. Thus far, these birth cohort studies have shown a negative

association between indices of maternal OP exposure and Bayley mental (MDI) and motor development (PDI) scores among the children at ages 2–3 and measures of intelligence at age 7 (Bouchard et al. 2011; Engel et al. 2011; Rauh et al. 2011). More specifically, maternal indicators of OP exposure, measured in urinary metabolites and cord blood, predicted reductions in tasks of perceptual reasoning and working memory among 6–9 year old children of exposed mothers (Bouchard et al. 2011; Engel et al. 2011; Rauh et al. 2011). Bouchard et al. (2011) also reported reductions in the domains of processing speed and verbal comprehension. Studies of Ecuadorian children (5–8 y.o.), reported that maternal exposure to OPs was predictive of reduced visuomotor skills, impaired fine motor coordination, and slowed response speed (Grandjean et al. 2006; Handal et al. 2008; Harari et al. 2010).

Numerous cross sectional, descriptive studies have evaluated the effects of childhood pesticide exposure on neurobehavior, but results have been inconsistent due to differences in exposure assessment methods. Unlike organochlorines, OP and PYR pesticides do not persist in human tissue and have a relatively short half-life (Barr et al. 1999). Therefore, measurement of chronic exposure relies on historical reconstruction based on questionnaires and/or measurement of recent exposure using biomarkers such as urinary metabolites and AChE inhibition. Several cross-sectional studies have shown that, relative to unexposed children, OP pesticide exposed children had significant decrements in one or more of the following neurobehavioral functions: latency of response, fine and gross motor skills, visuomotor problem solving, short term memory, and attention (Eckerman et al. 2007; Rohlman et al. 2005; Ruckart et al. 2003). However, exposure was determined either by questionnaire, parental occupation, or was not concurrent with performance measurement. Thus, it is difficult to attribute performance deficits to OP pesticide exposure. Bouchard et al. (2011) found inconsistent or only marginal associations between OP urinary metabolites at age 5 and reduced scores for measures of working memory and perceptual reasoning at age 7. This result may not be surprising given the time frame between biomarker assessment and neurobehavioral evaluation. In contrast, lower acetylcholinesterase (AChE) activity measured at the time of neurobehavioral performance revealed that adolescent male pesticide applicators from Egypt performed significantly worse than controls on tests of visuomotor speed, immediate memory and general information. (Abdel Rasoul et al. 2008). Adolescent subjects who worked more days during the pesticide application season performed more poorly on general information, timed math skills, conceptual thinking, visuomotor problem solving, speed of response and memory. Moreover, Lizardi et al. (2008) also reported that higher OP metabolite concentrations at the time of testing were significantly associated with compromised performance on a test of mental flexibility and conceptual thinking among 7 year old children.

Although a number of different neurobehavioral functions have shown significant reductions among pesticide-exposed children, these differences include both lower order functions such as motor speed and higher order cognitive processing involved in problem solving and memory. Increasing evidence from animal and human studies indicates that numerous brain regions may be affected by pre- and post-natal exposure to OPs and that the behavioral consequences of OP exposure depends on the timing as well as the level of exposure (Colborn 2006; Slotkin 2004). Moreover, the behavioral manifestations may be not be

immediately obvious, but could become detectable as the child develops and is required to perform increasingly complex cognitive tasks.

In contrast to OPs, the neurobehavioral effects of PYR exposure have not been adequately evaluated and are poorly understood. One Canadian study reported a positive association between the PYR metabolite, *cis*-DCCA (3-(2,2-dichlorovinyl-2,2-dimethylcyclopropane carboxylic acid), and increased total difficulties score as reported by parents on the Strengths and Difficulties Questionnaire (Oulhote and Bouchard 2013), but the authors did not measure behavioral performance. Horton et al. (2011) found that air samples of piperonyl butoxide, a synergist to potentiate the insecticidal action of PYR, collected during the 3rd trimester of pregnancy, was associated with significantly lower Bayley Mental Development Index scores at age 3. However, Bayley mental and psychomotor development were not significantly associated with the specific PYR metabolites, *cis*- or *trans*-permethrin measured in maternal/cord plasma. Therefore, the neurobehavioral effects observed cannot clearly be attributed to PYR.

The purpose of the current study was to evaluate the neurobehavioral effects of OP and PYR exposures among 6–8 year old Thai children living in the central farming region of Thailand where pesticide exposure opportunities significantly exceed those seen in the developed world. In previous studies, we documented that Thai children had higher urinary metabolites of OP and PYR than US children in the National Health and Nutrition Examination Survey (NHANES) (Barr et al. 2010; Panuwet et al. 2009; Rohitrattana et al. 2014a; Rohitrattana et al. 2014c). Based on the existing literature and the concentrations of OP and PYR metabolites among our sample, we hypothesized that these exposures would predict decrements in latency of response, motor speed, and higher order cognitive functions of visuomotor coordination, attention, and working memory.

Method

Participants

Fifty-four, 6–8 year old, healthy male and female Thai children were randomly selected from 200 volunteers recruited from rice (N=25) and aquaculture farming (i.e., shrimp farms) (N= 29) regions outside of Bangkok, Thailand (hereinafter designated RICE and AQUA). If more than one child from a family was eligible, only one child was selected at random to participate. The study sites were selected because they were easily accessible from Chulalongkorn University in Bangkok and our Thai collaborators had working relationships with community leaders. The levels of exposures to pesticides among the RICE farms were expected to vary seasonally because of a unique farming schedule. The two seasons are hereinafter referred to as HIGHUSE and LOWUSE season. Children from aquaculture farming were selected to participate in this study as a control group. Shrimp farming requires little or no use of OP pesticides. On the other hand, PYR was used in both RICE and AQUA households for mosquito control as well as on RICE farms.

Prior to recruitment, an introductory meeting was organized by the Chulalongkorn University collaborators and the study protocol was communicated to a community advisory board comprised of community leaders, teachers, parents, and medical personnel from the

community clinic. These community leaders informed local parents and children about the project and asked them to contact our collaborators to volunteer for the study. Based on exclusion criteria, children with significant developmental delay, mental retardation, diabetes, neurologic disorder, significant head trauma, or lung, kidney or cardiac disease were excluded from participating in this study. After screening, one child from the rice farming group with signs of autism was excluded, resulting in a total of 24 participants from rice farms. The Rutgers University Robert Wood Johnson Medical School and Chulalongkorn University IRBs reviewed and approved the study.

Neurobehavioral Tests

The computerized neurobehavioral test system, the Behavioral Assessment and Research System (BARS), has been adapted and augmented for use with children, age 5 and above (Rohlman et al. 2007a; Rohlman et al. 2007b; Rohlman et al. 2008). The battery is relatively inexpensive to administer, requires limited language and educational abilities, and has acceptable test-retest reliability under repeated test administration conditions (0.47 to 0.88 test-retest correlations) (Rohlman et al. 2007a). It has been translated into a number of different languages, and was translated into Thai and piloted by our research team prior to use in this study (Rohitrattana et al. 2014b). The battery includes computerized tests administered to each child individually by a trained examiner on a personal computer with a 9 button response system as opposed to a standard computer keyboard. Additional tests adapted from the Pediatric Environmental Neurobehavioral Test Battery (Amler et al. 1996) were also administered individually by a trained examiner. All examiners were trained in administration by the Thai and US collaborators during two separate training sessions. All test stimuli and standardized instructions were translated into Thai and back-translated by a bi-lingual co-investigator (P Suttiwan). Adjustments to the test stimuli were made to insure familiarity with the materials (e.g., object memory items). To reduce practice effects and familiarize participants with our procedures, the tests were given once 6 months prior to study initiation (Rohlman et al. 2000). This first administration (Session I) was also used to assess equipment and stimuli and to ascertain the integrity of administration by the examiners. The data from the first administration was not included in the final analysis. Tests and the functions assessed are listed in Table 1.

Home Environment and Parental Ability

Home environment and parental education and intelligence have an impact on a child's cognitive function and were assessed during the initial evaluation. The Home Observation for Measurement of the Environment (HOME) scale has been translated and used in rural Thailand to evaluate mothers and children 13 to 35 months old (Williams et al., 2003). Together with our collaborating Thai developmental psychologist (P. Suttiwan), we translated and modified this scale building on what was learned by Williams and colleagues regarding cultural differences in rural home environments. We used the total HOME score in our analyses. Maternal education (highest grade completed) and a Thai vocabulary test developed by our Thai collaborator were used to assess the mother's cognitive ability.

Procedure

On the day before each neurobehavioral evaluation session, research technicians, developmental psychologists, and our Thai collaborator (WS) visited each child's home to explain the project to the parents and to obtain informed consent. Trained technicians instructed parents in procedures for collection of urine samples, collected environmental samples, and administered the HOME (Session I only). Each child and a parent were given a morning appointment on the next day at the clinical center. Upon arrival, parents gave the nurse from the community health center the urine sample which was labeled with the subject ID and logged into the sample collection spread sheet. The nurse then performed a screening physical exam to ascertain the health of each child according to the previously outlined inclusion/exclusion criteria. Parents completed a questionnaire about their child's activity and potential for pesticide exposure (Petchuay et al. 2006), and completed a Thai vocabulary test (Session I only). The study was designed to capture exposure and behavioral performance during the rainy or high pesticide use (HIGHUSE) and the dry or low pesticide (LOWUSE) use seasons. Participants completed Session II (HIGHUSE) and III (LOWUSE) testing sessions 6 months and one year after the initial testing session.

Urine Samples and Analysis

On the morning of the neurobehavioral appointment, parents collected first morning void urine in the pre-washed, labelled screw top container provided by the technician. Urine samples were stored in the home refrigerator until the time for the test appointment (see Rohitrattana et al. 2014c for specific analytic details). The urine samples were stored at −40 °C in a freezer and were shipped on dry ice to the Research Institute for Health Sciences (RIHES), Chiang Mai University, Chiang Mai, Thailand for the analysis of class-specific dialkylphosphate (DAP) metabolites indicating OP exposures. The six common DAP metabolites were measured including dimethylphosphate (DMP), diethylphosphate (DEP), dimethylthiophosphate (DMTP), dimethyldithiophosphate (DMDTP), diethylthiophosphate (DETP), and diethyldithiophosphate (DEDTP). Briefly, the urine samples were saturated with salt, acidified, and then extracted with acetone: ethyl acetate. The extract was derivatized with pentaflurobenzyl bromide to form the PFB phosphate esters of the DAPs. The DAPs were analyzed using gas chromatography-nitrogen phosphorus detection (GC-NPD) and were cross-validated against the gas chromatograph with tandem mass spectrometric analysis (Prapamontol et al. 2014).

In order to combine all six DAP metabolites into a molar summed unit, the reported concentrations (C) were divided by molecular weight of each metabolite using the following equations. This allowed three summed metabolite concentration to be created: ΣDEAP which was the sum of DEP, DETP and DEDTP; ΣDMAP which was the sum of DMP, DMTP and DMDTP; and ΣDAPs which was the sum of all six metabolites.

DMP (nM)	=	C (μg/L)/0.126 (μg/nmol)
DMTP (nM)	=	C (μg/L)/0.142 (μg/nmol)
DMDTP (nM)	=	C (μg/L)/0.158 (μg/nmol)

DEP (nM)	=	C (μg/L)/0.154 (μg/nmol)
DETP (nM)	=	C (μg/L)/0.170 (μg/nmol)
DEDTP (nM)	=	C (μg/L)/0.186 (μg/nmol)

For the specific metabolites of chlorpyrifos, 3,5,6-trichloropyridinol (TCPy) was measured using a minor modification of a method previously published (Olsson et al. 2004). The TCPy analysis was performed at the Department of Environmental Health, Rollins School of Public Health (RSPH), Emory University, Atlanta, Georgia. Briefly, TCPy in urine was hydrolyzed to liberate its glucuronide and sulfate bound conjugates. The hydrolysate was extracted using solid phase extraction and analyzed by high-performance liquid chromatography-tandem mass spectrometry. For pyrethroid metabolites, two metabolites, 3-phenoxybenzoic acid (3PBA), a non-specific metabolite of many pyrethroids, and cis/trans-2,2-(dichloro)-2-dimethylvinylcyclopropane carboxylic acid (DCCA), a more specific PYR metabolite, were analyzed at the same laboratory using the same protocol. The units of adjusted DAPs and PYR metabolite concentrations are presented in microgram per gram creatinine (ug/g Cr) and sum molar concentration of DAPs are presented in micro mole per gram creatinine (umol/g Cr).

Statistical Analysis

Data from Session II (HIGHUSE) and III (LOWUSE) were used in the analyses. Descriptive univariate statistics and histograms were examined. When appropriate, log-transformations were used to stabilize variances and correct for skewness. In all subsequent models, age, sex, family income, maternal education (years), home environment (HOME), and mother's vocabulary score were considered as potential confounders. Preliminary correlational analysis identified that age and HOME total score were significantly correlated ($p \leq 0.10$) with neurobehavioral measures during one or more testing sessions and with OP and/or PYR metabolites for one or more testing sessions. Therefore, all subsequent analyses included age and HOME as covariates. Analyses were performed using SAS version 9.4 for Windows.

To account for multiple comparisons, variables were initially grouped into the following domains: latency of response, accuracy of response, motor speed, and learning. Overall multivariate analyses were performed initially to determine effects for each domain followed by univariate analyses for each variable within the domain.

Mixed linear multivariate models were applied to test the effects of pesticide exposure. To evaluate whether effects of exposure existed, all data were included in a mixed linear model with a random effect for individual subjects to account for correlation between repeated measures within subject. This model included an across-subject factor indicating whether the participant was from the rice or aquaculture farm group (variable FARM) and a within subject factor indicating high or low pesticide use season (variable SEASON) as well as an interaction between the two (FARM × SEASON).

Two sets of regression analyses were conducted examining the effect of urinary metabolites on neurobehavioral performance. First, within SEASON regression models examined

whether variation in neurobehavioral performance can be explained by variation in the urinary markers for pesticides. Second, regression models examined the effect on neurobehavioral performance of (1) subject-level pesticide metabolite concentrations averaged over high- and low-pesticide-use seasons in order to determine longer-term effects across subjects; and, (2) changes in pesticide metabolite concentrations between high and low pesticide use seasons in order to examine whether changes in pesticide metabolites across seasons are reflected in neurobehavioral performance within subject.

Results

Participants

Selected demographic variables are shown in Table 2. Results indicated the two groups were comparable with the exception of HOME total score which was significantly greater for aquaculture families.

Urinary OP and PYR Metabolites (Table 3)

Group main effect (FARM): RICE participants had significantly higher concentrations of ΣDAP, DEAP, and TCPy, but not DMAP metabolites than AQUA participants regardless of season (FARM main effect). RICE participants also had significantly higher concentrations of DCCA, a metabolite of PYR, than AQUA participants only during the HIGHUSE season.

Season main effect: No significant differences in DAP or PYR metabolites were observed between HIGHUSE and LOWUSE for RICE or AQUA participants. Contrary to expectations, both RICE and AQUA participants had significantly greater TCPy metabolites during the LOWUSE vs. HIGHUSE- season.

Neurobehavioral Measures (Table 4)

RICE vs. AQUA comparisons (FARM main effect)—There was no significant reduction in performance for RICE vs. AQUA children during either the HIGHUSE or LOWUSE seasons, even though urinary metabolites of OPs were significantly greater among RICE vs. AQUA children during both seasons. Table 4 gives individual and multivariate test results for both FARM groups by SEASON. RICE children exhibited significantly faster motor speed (PEG), faster latency of response (CPT), and more efficient learning (OM) than AQUA children during LOWUSE, but not during HIGHUSE season. The interaction of FARM × SEASON was significant for PEG, both hands test. Specifically, during the HIGHUSE season AQUA participants performed better than RICE participants, but during the LOWUSE season, RICE participants performed better than AQUA participants ($p = .008$). Interaction terms for all other variables were non-significant (data not shown).

OP and PYR urinary metabolites as predictors of neurobehavioral performance (age and HOME controlled)—Within SEASON Models: Separate regression models within SEASON revealed an overall significant association of DAPs for latency of response ($p = 0.03$) and accuracy of response during LOWUSE season only ($p = 0.03$). No significant association of DAPs were observed for the motor speed or learning

domains for LOWUSE season or for any domain during HIGHUSE season (data not shown). Specific findings for latency of response and accuracy of response within the LOWUSE season were as follows: increased ΣDAPs predicted significant improvement in CPT false alarm latency ($p = 0.003$) and percent CPT false alarms ($p = 0.004$). These findings correspond to partial correlations, after adjusting for age and HOME scores of −0.37 and −0.41, respectively. No significant effects were observed in any domain or SEASON for TCPy. Separate regression models within SEASON revealed significant overall effects for the PYR metabolites, DCCA and 3PBA, for the learning domain during LOWUSE season only (DCCA learning: $p = 0.04$; 3-PBA learning: $p = 0.005$). Specific findings for learning within the LOWUSE season were as follows: increasing DCCA and 3-PBA each predicted lower OMT recognition scores (DCCA: $p = 0.0001$; partial $r = −0.15$) (3-PBA: $p = 0.001$; partial $r = 0.08$). However, the distribution of values for OMT recognition memory were highly skewed left with most values at the maximum (16), thus violating testing assumptions. DCCA and 3-PBA were not significant predictors for the domains: latency of response, accuracy of response, or motor speed.

Across SEASON Models: Using regression models controlling for age and HOME scores, increasing average ΣDAPs predicted significant improvement in average latency (SDT: $p = 0.008$; partial $r = 0.10$) and accuracy (MTS: $p = 0.05$; partial $r = 0.09$) of response (data not shown). Increasing average TCPy across SEASON was associated with increasing motor speed (PEG non-preferred: $p = 0.03$; partial $r = 0.01$) and improved learning (CPT false alarms: $p = 0.05$; partial $r = −.20$). Increasing average PYR metabolites across SEASON were associated with improved average latency of response only (SDT: DCCA $p = 0.04$; partial $r = −0.03$). Average 3-PBA across SEASON did not predict neurobehavioral performance.

Within Participant Regression Models: Within participant changes in ΣDAPs across SEASON did not predict significant changes in neurobehavioral performance. Increasing TCPy within subjects was a significant predictor of increased motor speed (PEG both hand: $p = 0.01$; partial $r = 0.15$). However, within participant increases in DCCA were associated with significantly lower scores on recognition memory (OMT: $p = 0.02$; partial $r = −0.34$) but no other indices of OMT (data not shown). Within participant changes in 3-PBA did not predict changes in neurobehavioral performance.

Finally, to determine if the effect of performing work in different farming operations could influence neurobehavioral performance and perhaps overcome effects of OP or PYR exposure, analyses were repeated controlling for FARM. However, this approach did not alter the results. Creating high and low exposure groups based on a median split for total DAPs also did not reveal any significant differences between groups (data not shown).

Discussion

Although urinary metabolite concentrations revealed that rice farm participants had significantly greater OP and PYR exposure than children living on aquaculture farms, their neurobehavioral performance was not adversely affected by this exposure. Group comparisons suggested some performance improvement for rice relative to shrimp farm

participants during the “low pesticide use season”. This result could be interpreted to suggest that when pesticides were used in greater quantities (HIGHUSE) the neurobehavioral performance of RICE participants was suppressed. However when exposure was quantified with urinary metabolites in regression analyses, increasing biomarkers of exposure did not predict compromised neurobehavioral performance. Overall, any associations between metabolite concentrations and performance were not consistent between pesticide use seasons or across similar measures of performance. The only specific finding of compromised performance was the association between increasing metabolites of PYR and lower recognition memory scores (OMT). However, showing a reduction in recognition memory in the absence of any adverse effect of pesticide exposure on immediate or delayed recall is not typical in the literature. Generally, recognizing a previously presented object is easier than recalling that object from memory, and therefore, we would expect recognition memory to be less sensitive to disruption of learning, not more sensitive. In some regression analyses, improved performance such as shorter latency of response and fewer false alarms (CPT) was associated with pesticide exposure specifically during the LOWUSE season and in analyses averaging DAP and PYR metabolites across SEASON. However, these improvements were relatively modest (e.g., 2/3 standard deviation for PEG motor speed) and were not consistently observed across measures of the same functional domain (e.g., motor speed).

The metabolite concentrations for both OPs and PYR documented among Thai children are clearly higher than those seen among children of a similar age in the U.S. general population (Barr et al. 2005; Barr et al. 2010; Barr et al. 2011) and our ΣDAP levels are almost twice as high as levels among 5 year old Mexican American farm children from Salinas Valley, California, who have greater exposure than the general U.S. population (GM = 147; CI: 124.2–173.0) (Quiros-Alcala et al. 2011). Dimethyl-substituted OPs were larger contributors to the ΣDAP levels of the Mexican-American children than the diethyl-substituted OPs in our Thai population. Because urinary DAPs only reflect relatively recent OP exposure (usually within 24–48 hours), the observed lack of adverse effects cannot fully address potential health effects from chronic pesticide exposure. However based on our understanding of farming practices (Rohitrattana et al. 2014c) and our observation of similar or even small improvements in neurobehavioral performance for RICE relative to AQUA children, chronic neurobehavioral effects were also not suggested by the current study.

The design of this study is similar to several other cross-sectional studies comparing performance on the BARS or other neurobehavioral measures between exposed and unexposed groups of children (Eckerman et al. 2007; Rohlman et al. 2005; Ruckart et al. 2003). While several of these studies documented performance differences between the exposed and unexposed, some relied almost solely on location or questionnaires as means to assess exposure. The current study differs from the previous literature because biomarkers of OP and PYR exposure were collected immediately prior to behavioral assessment. Thus, the acute exposure-response relationship could be determined. Results similar to ours were observed when child exposure biomarkers were collected among children followed in birth cohort studies. That is, prenatal exposure to OP pesticides predicted reductions in mental development (MDI), but concurrent OP exposure biomarkers were not associated with reduced mental development. Eskenazi et al. (2007) reported improved performance among

children with higher concurrent OP exposure while Bouchard et al. (2011), did not find any consistent relationship between cumulated OP exposure from ages 6 months through age 5 and cognitive performance. Our results appear to be consistent with these previous studies.

Among those studies in which biomarkers of OP exposure were collected, Abdel Rasoul et al. (2008) observed significant adverse neurobehavioral effects, but these effects were associated with lower AChE activity and neurologic symptoms indicative of acute toxicity (e.g., dizziness, blurred vision) among their occupationally exposed adolescent sample. Moreover, the Abdel Rasoul et al. (2008) study used relatively less sensitive neurobehavioral measures (e.g., Wechsler Adult Intelligence Scale - Information) than in the current study, substantiating the significance of the neurologic health effects observed. Although Lizardi et al. (2008) also observed significant effects on a test of problem solving and planning (Wisconsin Card Sort), the association with urinary DAPs was only observed when 2 of the 48 participants with outlier DAP concentrations were included in the analysis. Thus, it appears that these extreme values were driving the observation of adverse neurologic effects on one of several measures (e.g., Short form Wechsler Intelligence Scale for Children). In addition, their exposure as indicated by the metabolite with the highest concentration was appreciably higher than that of our participants (i.e., Lizardi DMP mean = 65.5 (SD= 78) mcg/L vs. 7.89 (SD= 14.51) mcg/L).

Our study had several limitations that include a relatively small convenience sample in close proximity to Bangkok and the participating Thai University, and short-term biomarkers of OP and PYR exposure that may also be derived from environmental exposures to the preformed metabolites. The AQUA families showed slightly greater maternal education, HOME score, and higher family income, but these differences would be expected to bias towards finding a performance decrement among the more highly exposed RICE participants.

Like many other studies we had no appropriate biomarker of longer term exposure. RICE participants had higher OP biomarker concentrations than AQUA participants, suggesting that they probably also have more chronic exposure. However, comparisons of performance simply based on farm location also failed to show significant decrements when pesticide use was at its highest. DAP and TCPy metabolite concentrations were higher among RICE vs. AQUA participants in both SEASONs, while DCCA, as a metabolite of pyrethroids, was higher among RICE relative to AQUA participants but only during the HIGHUSE season. Pyrethroids such as permethrin or cypermethrin are used for public health purposes to control mosquitos that transmit dengue and therefore, are commonly used in households. We observed several large values among AQUA participants during the LOWUSE season, suggesting recent home use during the time frame we labeled as LOWUSE based on the rice farming community agricultural practices. These findings further illustrate the impact of a few outliers on the results in the context of a relatively small sample size and spot collection of metabolites as indicators of exposure.

Although we took care to adapt the neurobehavioral measures for use with Thai children, it is possible our tests may not have been as sensitive to performance differences in this population. Future cross sectional studies will need to establish better indicators of chronic

exposure (Rohlman et al. 2011) and include larger samples to determine potential effects. For example in the current study when significant differences were observed, partial correlations were generally small suggesting a relatively weak effect that would require larger samples to clarify. However, accumulating evidence suggests that prenatal exposure may be more predictive of adverse neurologic effects. Therefore cross sectional studies are unlikely to adequately assess health effects unless better markers of both prenatal and early childhood exposure are available.

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Highlights

- 6–8 year old Thai children have significantly higher exposures to OPs than US children of similar age and a sample of US farm children
- Rice farm children have significantly greater exposure to OP pesticides than aquaculture children
- Metabolites of OP and PYR exposures did not predict adverse neurobehavioral performance in any domain of function

Table 1

Description of neurobehavioral tests and functions for BARS

Test Description	Function	Variables
Finger tapping (TAP) • Right and left hand taps for 20 seconds; 2 trials/ hand	Response speed and coordination	• Average number of taps each hand*
Divided attention (DAT) • Tap while reciting nursery rhyme (Chang song)	Divided attention	• Average number of taps each hand while singing*
Purdue pegboard (PEG) • Number of small pegs placed in holes during two 30 second trials each hand • Preferred, non-preferred, and both hand trials	Dexterity	• Average number of pegs placed: preferred, non-preferred, both*
Visual motor integration (VMI) • Copied line drawing	Hand-Eye coordination	• Total score for correct segments*
Digit span (DST) • Spoken presentation of number sequences ◦ Forward and reverse recall	Memory and attention	• raw score forward, backward, total*
Object memory test (OMT) • Show and name 16 objects • Immediate and delayed recall • Recognition of target and non-target items	Recall and recognition memory	• Immediate recall; delayed recall; recognition*
Symbol-Digit (SDT) ◦ Match number and symbol from key	Information processing speed	• Average latency (ms) of response for correct match#
Match-to-Sample (MTS) • 15 stimuli shown for 3 seconds • Identify target from 3 choices ◦ Delay between presentation and choice varies from 1 to 8 seconds	Visual memory	• Average latency (ms) for correct choice# • Number correct*
Continuous performance (CPT) • Different shapes shown rapidly for 4 min in original version and 7 min in alternate version • Press key when target (original = circle; alternate = triangle) shown	Sustained attention	• Percent correct* • Average latency (ms) for correct response (hit)# • Average latency (ms) for false alarms# • D-Prime*

Adapted in part from Rohlman et al. (2007b).

*Higher number indicates better performance

#Lower number indicates better performance

Table 2

t tests comparing demographics for rice and aquaculture groups

Demographic	**RICE (N=24)**	**AQUA (N=29)**	
	Mean (*SE*)	**Mean (*SE*)**	***p***
Child Age in Months	82.0 (1.8)	82.7 (1.8)	.77
Education of Mother in years	7.7 (0.7)	9.0 (0.8)	.25
Monthly Income (baht)	11,445.0 (1468.2)	18,571.4 (3366.7)	.10
Vocab Score of Mother	4.7 (0.4)	4.3 (0.3)	.51
HOME Total (max score = 59)	37.1 (1.2)	42.4 (1.1)	.002
	***n/N* (%)**	***n/N* (%)**	***Chi-square***
Gender (male)	16/24 (66%)	15/29 (52%)	.27

Table 3

OP and PYR means, geometric means and 95% confidence intervals: rice vs. aquaculture and pesticide use season comparisons

	RICE						AQUA* *N=27 for Pyrethroids						RICE to AQUA ratio of LOW to HIGHUSE (95%CI)
	LOWUSE (N=23)		HIGHUSE (N=24)		HIGH vs. LOW USE	Ratio LOW to HIGHUSE GMs (95%CI)	LOWUSE (N=28)		HIGHUSE (N=28)		HIGH vs. LOW USE	Ratio LOW to HIGH USEGMs (95%CI)	
	Mean (S.E.)	G.M. (C.I.)	Mean (S.E.)	G.M. (C.I.)	*p*-value		Mean (S.E.)	G.M. (C.I.)	Mean (S.E.)	G.M. (C.I.)	*p*-value		
Organophosphates													
DAP[1] (umol/g Cr)	365.5 (78.1)	235.5 (148.0, 374.6)	646.3 (186.3)	270.6 (154.4, 474.5)	.70	0.87 (0.42, 1.81)	145.9 (31.9)	96.4 (68.3, 136.1)	191.9 (50.6)	101.2 (65.5, 156.3)	.80	0.95 (0.64, 1.41)	0.90 (0.42, 1.97)
DEAP[2] (umol/g Cr)	237.7 (50.2)	127.7 (81.8, 231.8)	454.3 (134.8)	182.0 (100.9, 328.5)	.43	0.97 (0.36, 1.57)	107.9 (26.8)	57.4 (36.8, 89.3)	138.2 (47.2)	47.2 (26.8, 83.2)	.43	1.21 (0.73, 2.01)	0.61 (0.26, 1.43)
DMAP (umol/g Cr)	147.7 (61.0)	45.9 (25.4, 83. 0)	192.1 (110.6)	46.7 (26.8, 81.2)	.96	0.98 (0.45, 2.15)	38.0 (8.0)	26.6 (19.5, 36.1)	53.8 (9.3)	37.5 (26.8, 52.4)	.09	0.71 (0.47, 1.06)	1.38 (0.16, 3.13)
TCPy[3] (ug/g creatinine)	14.08 (2.62)	9.59 (6.29, 14.63)	8.85 (2.15)	6.06 (4.29, 8.55)	.02	1.60 (1.10, 2.32)	4.85 (0.48)	4.31 (3.57, 5.20)	3.78 (0.61)	2.84 (2.06, 3.90)	.003	1.52 (1.12, 2.05)	0.95 (0.60, 1.51)
Pyrethroids													
DCCA[4] (ug/g creatinine)	1.97 (0.67)	1.11 (0.75, 1.65)	2.53 (0.67)	1.46 (0.97, 2.19)	.41	0.78 (0.58, 1.07)	15.95 (8.99)	1.73 (0.88, 3.40)	1.81 (0.45)	1.28 (0.95, 1.71)	.07	1.37 (0.70, 2.68)	1.78 (0.83, 3.83)
3-PBA (ug/g creatinine)	2.92 (0.44)	2.24 (1.59, 3.16)	3.02 (0.88)	1.74 (1.13, 2.67)	.14	1.30 (0.84, 2.04)	10.80 (5.88)	2.26 (1.26, 4.00)	2.46 (0.38)	1.80 (1.29, 2.51)	.07	1.26 (0.64, 2.48)	0.97 (0.41, 2.32)

Rice vs. Aquaculture Comparisons

[1] Rice > Aquaculture: DAP Low Season p=0.002; High Season p=0.006

[2] Rice > Aquaculture: DEAP Low Season p=0.01; High Season p=0.001

[3] Rice > Aquaculture: TCPy Low Season p=0.006; High Season p=0.0003

[4] Rice > Aquaculture: DCCA Low Season p=0.45; High Season p=0.04

Table 4

Unadjusted and adjusted (for age and HOME) means, standard error, range, and 95% confidence intervals for rice and aquaculture high and low pesticide use seasons. NOTE: Overall multivariate analyses for each domain presented in the footnote..

Session		HIGHUSE (II)					LOWUSE (III)				
Test	**Variables (Test-retest *r* scores)[2]**	**RICE (*N*=24)**		**AQUA (*N*=27)[1]**			**RICE (*N*=24)**		**AQUA (*N*=28)**		
		Unadjusted Mean (SE) Range	***Adjusted Mean (95%CI)***	**Unadjusted Mean (SE) Range**	***Adjusted Mean (95%CI)***	***Difference in Adjusted Means (95% CI) p***	**Unadjusted Mean (SE) Range**	***Adjusted Mean (95%CI)***	**Unadjusted Mean (SE) Range**	***Adjusted Mean (95%CI)***	***Difference in Adjusted Means (95% CI) p***
TAP	Right hand* (0.71)	71.8 (1.9) 59.5–92.5	72.5 (68.5–76.5)	70.6 (1.8) 52.5–93.5	70.0 (66.1–73.9)	2.5 (−3.4, 8.3) 0.40	73.4 (2.2) 54.5–96.5	73.9 (69.1–78.6)	75.4 (2.0) 52.5–95.0	75.3 (70.8–79.9)	−1.4 (−8.3, 5.5) 0.68
	Left hand* (0.72)	63.0 (2.3) 46.0–83.5	64.5 (60.1–68.9)	61.9 (2.2) 38.5–86.0	59.6 55.3, 63.9	4.9 (−1.6, 11.3) 0.14	66.7 (2.7) 47.5–86.5	68.9 (64.0–73.4)	64.1 (2.1) 45.0–83.0	62.3 (57.7–66.9)	6.5 (−0.5, 13.6) 0.07
DAT: song	Tap right average* (0.67)	48.0 (1.7) 32.0–64.0	48.5 (44.2–52.8)	50.4 (1.8) 27.0–70.0	50.0 45.5, 53.9	−1.2 (−7.5, 5.1) 0.70	51.4 (1.6) 35.0–69.0	52.1 (48.6–55.5)	56.2 (1.5) 40.0–72.0	55.3 (52.0–58.7)	−3.3 (−8.3, 1.8) 0.20
	Tap left average* (0.77)	44.3 (1.8) 29.0–62.0	44.6 (40.8–48.4)	61.9 (2.1) 38.5–86.0	45.0 41.3, 48.7	−0.4 (−5.9, 5.2) 0.90	49.3 (1.8) 35.0–65.0	50.4 (46.8–53.9)	49.5 (1.7) 26.0–66.0	48.5 (45.1–51.9)	1.9 (−3.3, 7.1) 0.46
PEG	Preferred hand* (0.72)	13.0 (0.3) 9.0–15.0	13.2 (12.6–13.8)	13.4 (0.3) 10.0–17.0	13.2 12.6, 13.8	−0.1 (−1.0, 0.8) 0.90	14.3 (0.3) 12.0–17.0	14.7 (14.2–15.2)	14.0 (0.3) 10.5–17.0	13.7 (13.1–14.2)	1.0 (0.3, 1.8) 0.01
	Non-preferred hand* (0.71)	12.4 (0.3) 8.0–15.0	12.6 (11.9–13.3)	12.3 (0.3) 8.5–16.0	12.0 11.3, 12.6	0.6 (−0.3, 1.6) 0.20	12.9 (0.3) 11.0–15.5	13.3 (12.8–13.8)	12.8 (0.3) 9.5–17.0	12.5 (12.0–13.0)	0.8 (0.1, 1.6) 0.04
	Both hands† (0.71)	18.2 (1.1) 8.5–26.0	18.9 (17.2–20.7)	21.0 (0.6) 14.0–28.0	20.3 18.6, 22.0	−1.3 (−3.9, 1.2) 0.30	22.5 (0.4) 19.0–27.0	23.3 (22.3–24.2)	21.9 (0.6) 16.0–28.0	21.4 (20.5–22.3)	1.9 (0.5, 3.2) 0.01
VMI	Total Correct* (0.64)	15.3 (0.4) 2.0–18.0	15.3 (14.6–16.1)	15.8 (0.3) 12.0–18.0	15.6 14.9, 16.4	−0.3 (−1.4, 0.8) 0.59	16.1 (0.3) 12.0–18.0	16.2 (15.6–16.8)	15.9 (0.2) 13.0–18.0	15.9 (15.3–16.4)	0.3 (−0.5, 1.2) 0.45
DST	DigSPF* (0.41)	9.5 (0.5) 6.0–16.0	9.6 (8.7–10.5)	10.1 (0.4) 6.0–15.0	9.9 9.0, 10.7	−0.2 (−1.6, 1.1) 0.70	10.0 (0.3) 8.0–14.0	10.1 (9.2–11.0)	10.9 (0.4) 6.0–15.0	10.8 (10.0–11.6)	−0.6 (−1.9, 0.6) 0.31

Session		HIGHUSE (II)					LOWUSE (III)				
Test	**Variables (Test-retest *r* scores)[2]**	**RICE (*N*=24)**		**AQUA (*N*=27)[1]**			**RICE (*N*=24)**		**AQUA (*N*=28)**		
		Unadjusted Mean (SE) Range	***Adjusted Mean (95%CI)***	**Unadjusted Mean (SE) Range**	***Adjusted Mean (95%CI)***	***Difference in Adjusted Means (95% CI) p***	**Unadjusted Mean (SE) Range**	***Adjusted Mean (95%CI)***	**Unadjusted Mean (SE) Range**	***Adjusted Mean (95%CI)***	***Difference in Adjusted Means (95% CI) p***
	DigSPB* (0.48)	2.3 (0.4) 0.0–6.0	2.6 (1.9–3.2)	2.9 (0.3) 0.0–6.0	2.7 2.1, 3.4	−0.2 (−1.2, 0.3) .53	3.1 (0.3) 0.0–6.0	3.3 (2.5–4.1)	4.0 (0.4) 0.0–9.0	4.0 (3.3–4.7)	−0.7 (−1.8, 0.4) 0.21
	Digtot	11.8 (0.7) 6.0–19.0	12.2 (10.9–13.5)	13.1 (0.6) 6.0–18.0	12.6 11.4, 13.9	−0.4 (−2.3, 1.5) 0.66	13.2 (0.6) 9.0–18.0	13.4 (11.9–14.9)	14.9 (0.7) 6.0–22.0	14.8 (13.4–16.1)	−1.3 (−3.5, 0.8) 0.21
OMT	Immediate recall* (0.32)	7.8 (0.4) 5.0–12.0	8.1 (7.3–9.0)	7.9 (0.5) 0.0–12.0	7.6 6.7, 8.4	0.6 (−0.7, 1.8) 0.37	9.6 (0.4) 7.0–13.0	9.9 (9.1–10.8)	8.9 (0.4) 6.0–14.0	8.7 (7.9–9.4)	1.3 (0.1, 2.5) 0.04
	Delay recall* (0.16)	5.2 (0.7) 0.0–10.0	5.6 (4.3–7.0)	6.6 (0.6) 0.0–11.0	6.2 (5.0–7.5)	−0.6 (−2.5, 1.3) 0.53	8.0 (0.6) 2.0–12.0	8.3 (7.1–9.5)	8.1 (0.5) 2.0–14.0	7.8 (6.7–8.9)	0.5 (−1.2, 2.1) 0.56
	Recognition* (0.35)	14.6 (0.5) 6.0–16.0	14.9 (13.8–16.2)	14.8 (0.6) 1.0–16.0	14.4 (13.2–15.6)	0.6 (−1.2, 2.4) 0.51	15.9 (0.1) 15.0–16.0	16.1 (15.1–17.0)	15.3 (0.6) 0.0–16.0	15.2 (14.3–16.1)	0.9 (−0.5, 2.3) 0.19
SDT	Latency *(ms) (0.83)	4962.8 (236.6) 3689.0–7340.0	4626.8 (3924.9–5328.7)	5015.3 (491.7) 2602.3–13017.3	5228.4 (4556.6–5900.2)	−601.6 (−1618.5, 415.3) 0.24	4528.5 (271.0) 3288.0–8708.0	4350.0 (3787.0–4913.0)	4587.9 (292.7) 2418.0–9357.0	4723.4 (4207.6–5239.2)	−373.4 (−1171.6, 424.7) 0.35
MTS	Latency * (ms) (0.63)	4116.8 (143.9) 2710.0–5647.0	4033.0 (3757.5–4328.5)	3899.7 (135.8) 2485.0–5056.0	3944.8 (3662.0–4227.6)	88.2 (−339.9, 516.3) 0.68	3714.0 (118.5) 2827.0–4816.0	3617.3 (3356.5–3878.0)	3755.0 (140.0) 2489.0–6249.0	3818.1 (3579.3–4057.0)	−200.9 (−570.5, 168.8) 0.28
	Correct* (0.11)	0.75 (0.0) 0.53–1.00	0.77 (0.71–0.84)	0.76 (0.0) 0.13–0.93	0.74 (0.68–0.87)	0.03 (−0.06, 0.13) 0.45	0.84 (0.0) 0.60–0.93	0.89 (0.81–0.91)	0.85 (0.0) 0.40–1.00	0.82 (0.78–0.87)	0.03 (−0.03, 0.10) 0.30
CPT	Percent Hits* (0.78)	0.75 (0.1) 0.31–1.00	0.78 (0.68–0.87)	0.80 (0.0) 0.17–1.00	0.78 (0.68–0.87)	0.00 (−0.14, 0.14) 0.98	0.82 (0.0) 0.28–1.00	0.82 (0.74–0.91)	0.86 (0.0) 0.16–1.00	0.84 (0.77–0.92)	−0.02 (−0.14, 0.10) 0.71
	HitLatency * (ms) (0.65)	505.3 (23.3) 309.7–764.7	495.9 (453.6–538.2)	517.6 (19.1) 360.5–809.9	528.4 (487.9–568.9)	−32.5 (−93.8, 28.8) 0.29	416.9 (21.5) 249.4–647.2	399.0 (355.5–422.6)	478.0 (20.7) 294.4–702.1	495.1 (455.3–535.0)	−96.1 (−157.8, –34.4) 0.003
	Percent False Alarms* (0.77)	0.06 (0.0) 0.00–0.34	0.09 (0.04–0.014)	0.12 (0.0) 0.02–0.57	0.13 (0.09–0.18)	−0.04 (−0.11, 0.03) 0.23	0.12 (0.0) 0.01–0.34	0.12 (0.07–0.17)	0.14 (0.0) 0.02–0.53	0.15 (0.10–0.20)	−0.03 (−0.10, 0.04) 0.38

Session		HIGHUSE (II)					LOWUSE (III)				
Test	**Variables (Test-retest *r* scores)[2]**	**RICE (*N*=24)**		**AQUA (*N*=27)[1]**			**RICE (*N*=24)**		**AQUA (*N*=28)**		
		Unadjusted Mean (SE) Range	***Adjusted Mean (95%CI)***	**Unadjusted Mean (SE) Range**	***Adjusted Mean (95%CI)***	***Difference in Adjusted Means (95% CI) p***	**Unadjusted Mean (SE) Range**	***Adjusted Mean (95%CI)***	**Unadjusted Mean (SE) Range**	***Adjusted Mean (95%CI)***	***Difference in Adjusted Means (95% CI) p***
	FALatency* (ms) (0.33)	453.2 (40.2) 0.0–987.8	441.1 (362.1–520.2)	453.9 (31.1) 254.5–820.6	476.7 (401.0–552.4	−35,6 (−150.1, 79.0) 0.53	469.6 (34.5) 279.3–866.4	427.9 (331.6–524.3)	569.6 (51.8) 261.1–1305.5	601.2 (513.0–689.5)	−173.3 (−309.9, −36.7) 0.01
	Correct D-Prime* (0.81)	2.4 (0.2) 0.1–5.3	2.5 (2.0–3.0)	2.5 (0.2) 0.2–4.4	2.3 (1.8–2.8)	0.1 (−0.6, 0.9) 0.71	2.4 (0.2) 0.4–3.9	2.5 (2.0–2.9)	2.6 (0.2) −0.3–4.5	2.5 (2.0–2.9)	0.0 (−0.67, 0.67) 0.99

[1] One subject was unable to complete testing due to local flooding during HIGHUSE season

[2] Test-Retest Values taken from Tables 2 and 3 in Rohitrattana et al. (2014a).

* Test of Group × Pesticide use season interaction, N.S.

† Test of Group × Pesticide use season interaction, p = 0.008

NOTE: MANOVAS; Wilks' Lambda test statistic

Latency of Response (SDT, MTS, CPT): HIGHUSE season: p=.61; LOWUSE season: p = .005.

Accuracy of Response (MTS, CPT): HIGHUSE season: p = .71; LOWUSE season: p = .42.

Motor Speed (TAP, DAT, PEG): HIGHUSE season: p=.10; LOWUSE season: p = .01.

Learning (OMT, VMI, DST): HIGHUSE season: p = .84; LOWUSE season: p = .03.